THE ECOLOGY OF INTESTINAL HELMINTH PARASITES OF THE FISH OF AFON TERRIG, NORTH WALES

A THESIS FOR THE DEGREE OF PH.D.

IN THE FACULTY OF SCIENCE

BY

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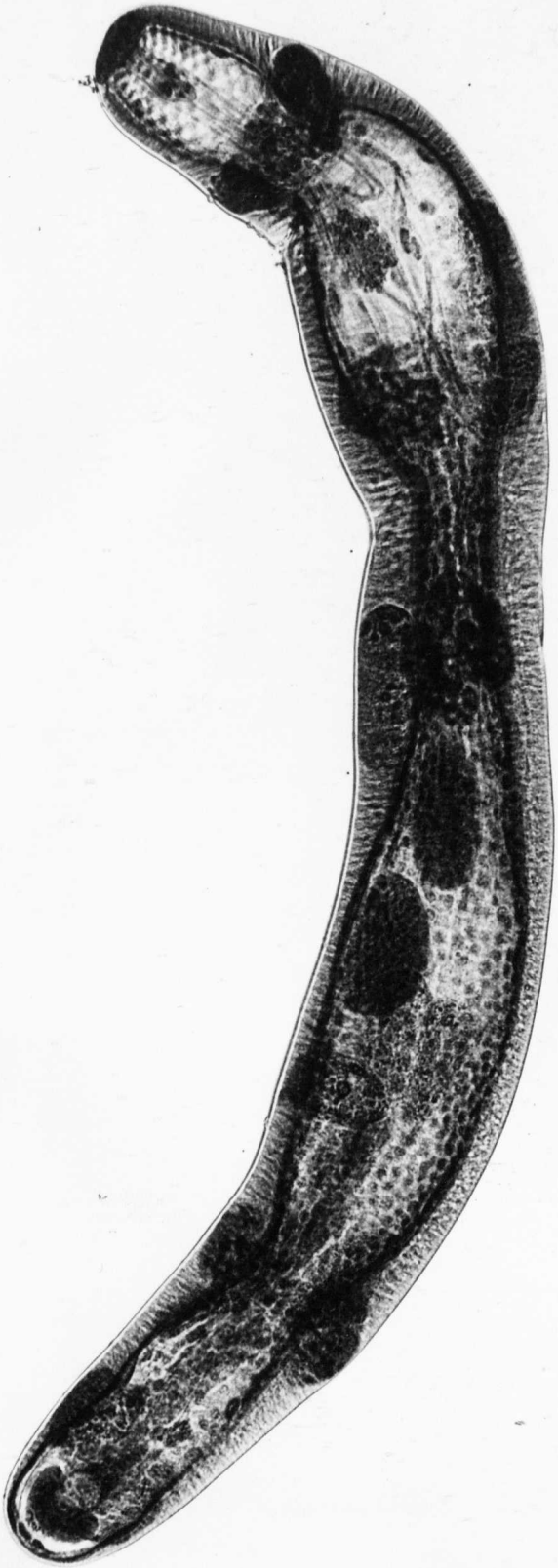
FRONTISPIECE

FORTYNINE-DAY-OLD ACANTHELLA OF

ECHINORHYNCHUS TRUTTAE FROM *G.PULEX* (X120)

(See Chapter II)



C O N T E N T S

PART I

GENERAL INTRODUCTION

CHAPTER I

GENERAL INTRODUCTION

1. THE AFON TERRIG

The Afon Terrig is a small fast-flowing stream which forms part of the Alun-Dee drainage system. Its upper reaches, 300 - 350 meters above sea level, trace the boundary between Flintshire and Denbighshire across a tract of open moorland as far as Rhydtalog. In this section, the stream is narrow and reaches a width of 2 meters in places (Plate 1 Fig.1). Dense patches of Callitriche are common (Hynes 1955). For the rest of its course, about 14 kilometers, the stream, shaded by trees and shrubs, flows through Flintshire (Plate 1 Figs. 2 and 3). It joins the Afon Alun near Pontblyddyn. The altitude here is just over 100 meters. In the middle and lower stretches, the width varies and is up to 6 meters at the widest points. The stream is generally shallow (Plate 1 Figs. 1-3). Except when flooded, the depth varies from a few centimeters to 1.5 meters (at the deepest pools along its course).

PLATE 1.

The Afon Terrig - features of the stream at the various stretches sampled.

Fig. 1 - Station I (Rhydtalog)

Fig. 2A - Station II (upstream where there is a ford)

Fig. 2B - " (further downstream)

Fig. 3 - Station III (Caegwydd)

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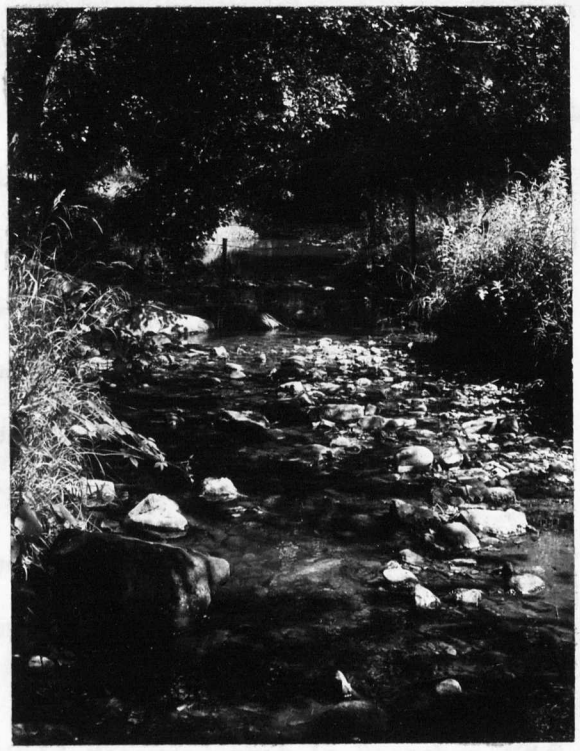

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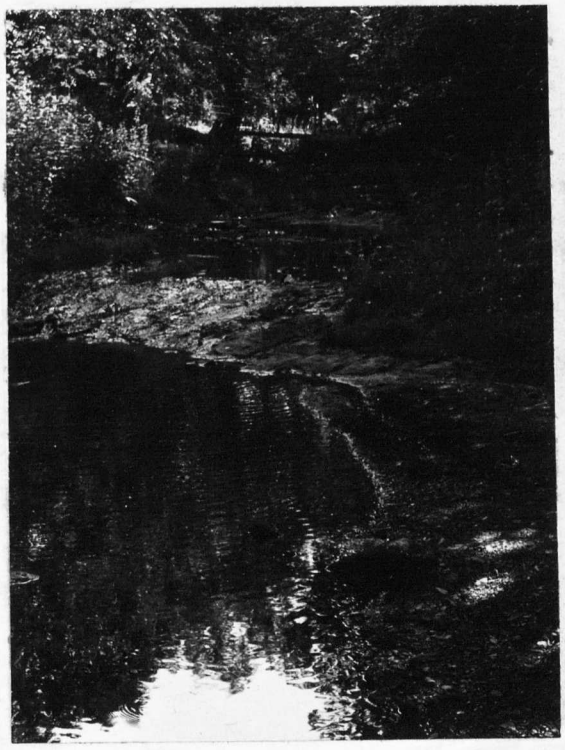

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PLATE 1

The bottom is stony with a variable amount of silt. When the present investigation started in October 1961, there was very little silt. By June 1963, however, the bottom had become generally silted. Silting became particularly severe and widespread after early spring floods. It seems that the present situation with regard to silt may be connected with the rather severe floods consequent on the unusually long and very cold winters of 1962 and 1963. Added to this is the fact that the stream has received more polluted effluents since 1961. The bottom becomes progressively gritty as the confluence with the River Alun is approached. There is no marked accumulation of vegetable debris anywhere along the stream.

The water is hard. Hynes (1955) recorded that in Deeember 1949 the water contained 11 mg/l.of Chloride, 14 mg/l. of Calcium, and a slight trace of Magnesium.

2. COMPOSITION OF THE FAUNA

Hynes (1955) gave the fauna of the stream at Rhydtalog. The organisms listed hereunder are those met with in all parts of the stream, during the present study. The list is, therefore, by no means exhaustive.

Faunal List.

INVERTEBRATA

Platyhelminthes
Turbellaria
Polycelis felina Dalyell

Nematomorpha
Gordius sp.

Annelida
Oligochaeta
Eiseniella sp.
Other oligochaetes

Arthropoda

Crustacea

Gammarus pulex pulex L.

Insecta

Ephemeroptera

Ecdyonurus torrentis Kimmins 1942
Baëtis rhodani Pictet 1844
Rithrogena semicolorata Curtis 1834
Ephemera danica Müller 1764
Ephemerella ignita Poda 1761
Paraleptophlebia submarginata Stephens 1836

Plecoptera

Amphinemura standfussi Ris.
A. sulcicollis Stephens
Nemoura cambrica "
N. cinerea Retzius
N. erratica Classen
Protonemura praecox Morton
P. meyeri Pictet
Brachyptera risi Morton
Perla bipunctata Pictet
Perlodes microcephala "
Chloroperla torrentium "
C. tripunctata Scopoli
Capnia bifrons Newman
Leuctra fusca L.
L. hippopus Kempny
L. inermis "
L. moselyi Morton
L. geniculata Stephens
L. nigra Olivier

Hemiptera

Velia caprai
Microvelia sp. ?

Neuroptera

Sialis lutaria L.

Trichoptera

Agapetus fuscipes Curtis
Rhyacophila sp.
Hydropsyche sp.
Halesus sp.
Stenophylax sp.

Other Limnephilidae
Plectrocnemia sp.
Odontocerum albicorne Scopoli
Philopotamus sp.
Silo sp.
Polycentropidae

Diptera
Simulium ornatum Meigen 1818
S. latipes " "
S. aureum Fries 1824
S. monticola Friederichs 1920
Tipula sp.
Medetera sp.
Hemerodromia sp.
Empididae
Stratiomys sp.
Leptidae (Rhagionidae)
Orthocladiinae
Tanytarsinae
Tanypodinae

Coleoptera
Helmis maugettii Bedel
Helodes sp.
Helophorus sp.
Agabus sp.
Platambus sp.
Dytiscus sp.
Esolus sp.
Chrysomelidae

Arachnida
Acarina
Watermites

Mollusca
Gastropoda
Limnaea pereger Müller
Hydrobia (Potamopyrgus) jenkinsi Smith
Ancylastrum fluviatile Müller

Lamellibranchiata
Pisidium sp. (probably casertanum Poli)

VERTEBRATA
Chordata
Pisces
Teleostei

Salmo trutta L.
Cottus gobio L.
\+ Anguilla anguilla L.
* Nemacheilus barbatula L.

Practically all the above animals showed regional fluctuations in their occurrence and/or abundance in the stream. The dominant vertebrate was the brown trout Salmo trutta L. The bullhead, Cottus gobio L. occurs in some numbers in the lower half of the stream while the common loach, Nemacheilus barbatula L. was found in area of about 30 meters adjacent to the confluence of the stream with the Afon Alun and were probably migrants from the latter. Only one specimen of the eel, Anguilla anguilla L., taken near the bridge at Caegwydd in April, 1962, was found.

Of the invertebrates, G.pulex was abundant in the upper half of the stream particularly around Rhydtalog. In the lower half, on the other hand, this species was common but its distribution tended to be patchy. The Trichoptera, (particularly encased species larvae), Ephemeroptera (especially Ecdynurus torrentis), and Plecoptera (particularly Leuctra fusca) were abundant all over the stream. The distribution of molluscs calls for special comment. While the fresh-water 'limpet' Ancylastrum fluviatile was found throughout the entire course of the stream, Pisidium sp. and Limnaea pereger were exceedingly

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\+ Only one specimen taken
* Confined to the area near the confluence-with Afon Alun and are probably migrants from the latter.

rare upstream around Rhydtalog. L. pereger was found in good numbers in the middle portion of the stream and only occasionally taken further downstream. Pisidium sp. was sparsely distributed downstream (below Caegwydd) where the bottom is more gritty.

3. THE INTESTINAL PARASITES OF FISH

The parasites taken from the intestinal tract (including the pyloric caeca) of the fish of the Afon Terrig may be listed as follows:-

(i) Salmo trutta L. - brown trout.

Acanthocephala: Echinorhynchus truttae Schrank 1788

Trematoda
Digenea : Crepidostomum metoccus Braun 1900, (Braun 1900)
C. farionis Müller 1784 (Lühe 1909)

Cestoda : Cyathocephalus truncatus Pallas 1781

Nematoda : Capillaria sp.

(ii) Cottus gobio L - bullhead.

Acanthocephala : E. truttae (See Chapter VII)

4. A NOTE ON THE SYSTEMATIC POSITION OF THE ACANTHOCEPHALAN ECHINORHYNCHUS TRUTTAE SCHRANK 1788

Echinorhynchus truttae is a member of the family Echinorhynchidae (Cobbold 1879) in the order Palaeacanthocephala (Meyer 1933).

Recently there have been varying views on the systematic position of the genus Echinorhynchus O.F. Müller 1776. Petrochenko (1956) revised the genus and erected in its place three new genera, on the basis of the disposition of the cement glands (Van Cleave 1949b). These were as follows:-

1. Echinorhynchus:- six cement glands in a single row, one after the other.

Type species: Echinorhynchus gadi - Müller 1776

2. Pseudoechinorhynchus: six cement glands in three pairs, placed one pair after the other.

Type species: Pseudoechinorhynchus clavula - Dujardin 1845.

3. Metechinorhynchus: six cement glands arranged partly in pairs and partly one after the other.

Type species: Metechinorhynchus salmonis - Müller 1776.

Golvan (1960) while recognising the differences outlined above, preferred to leave the genus Echinorhynchus O.F. Müller 1776 intact; and gave Petrochenko's divisions the subgeneric status. Suffice it to state here that the acanthocephalan found in trout from the Afon Terrig belongs to the 'group' Metechinorhynchus Petrochenko 1956. In this thesis however, the commonly acknowledged name Echinorhynchus truttae Schrank 1788 ((= E. fusiformis Rudolphi 1809 = E. clavula Hamman 1892,= E. clavula (Dujardin in Von Linstow 1895)) is adopted.

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PART II

STUDIES ON *ECHINORHYNCHUS TRUTTAE* SCHRANK 1788 (ACANTHOCEPHALA).

C H A P T E R II

DEVELOPMENT AND LIFE HISTORY OF E. TRUTTAE (ACANTHOCEPHALA)

1. INTRODUCTION

Although there are many reports on the incidence of the Acanthocephala in the literature, comparatively few studies have been made on the life cycle of these parasites. Still less is known on the details of their development and life history in both natural and experimentsl infections. This is particularly surprising when it is considered that the first real study of acanthocephalan life cycle began in 1862. Leuckart (1862) observed the juvenile stages of Echinorhynchus proteus (probably Pomphorhynchus proteus (Hyman 1951)) in the amphipod Gammarus. He followed this up with an experimental investigation of the life cycle. He fed the eggs of the parasite to Gammarus and was able to follow the development of the larval stages. Ward (1940) provided a comprehensive review of acanthocephalan life cycle studies made from 1862. In a similar historical review covering the period 1940 - 1954, Hopp (1954) dealt with the more detailed studies on development. Thus his review covered the investigations of Ward (1940) on Neoechinorhynchus cylindratus Van Cleave 1913, Yamaguti and Miyata (1942), Moore (1946a) on Moniliformis dubius Meyer 1933,

Moore (1946b) on Macracanthorhynchus ingens Meyer 1933, DeGiusti (1949) on Leptorhynchoides thecatus Linton and Reisch (1950) on Polymorphus kenti Van Cleave 1947. The rather detailed description of the development of Macracanthorhynchus hirudinaceus Pallas in the green June beetle grub Cotinus nitidus and several grubs of May beetles of the genus Phyllophaga by Kates (1943) was, however, omitted. Several other less critical studies outlined hereunder were also left out. Miller (1943) followed the development and determined the growth rate of M. hirudinaceus in the Japanese beetle larva, Popillia japonicum. Jaczo (1943) found that in Hungary, Carinogammarus röeseli serves as intermediate host for Polymorphus minutus Goeze 1782. Sita (1949) traced the life cycle of Moniliformis moniliformis Bremser 1811, in Blaps mucroneta, Periplaneta americana and rats. Gupta (1950) described a couple of larval Centrorhynchus while Das (1954) made a similar study of larval Centrorhynchus batrachus Das 1954 from Rana tigrina (Daud) from India. The reference of the latter investigator to Schneider's (1871) work on Echinorhynchus gigas (= M. hirudinaceus Pallas) as the first investigation of an acanthocephalan life history is inaccurate as indicated above. It is perhaps the first report for the species recorded in the literature. Other accounts of studies on the same species were given by Kaiser (1893), and Meyer (1931, 1938a, b.).

More recent contributions to the knowledge on acanthocephalan life cycles and development include the following. The life history of P. minutus has been studied by Petrochenko (1956), Hynes and Nicholas (1957),

Kovalenko (1960), Nicholas and Hynes (1958). Petrochenko (1958) carried out a similar investigation on P. magnus. Stycznska (1958) studied the development and bionomics of the larvae of Filicollis anatis Schrank in Asellus aquaticus L. Ketelnikov (1959) followed the life cycle of the same parasite in both A. aquaticus and the final host, domestic ducks. Rayski and Garden (1961) described the life cycle of Profilicollis botulus in Britain. This parasite infests the eider duck Somatria mollissima L and the crab Carcinus moenas serves as intermediate host. Moore (1962) determined, by experimental infections, the life history and development of Mediorhynchus grandis Van Cleave 1916, in various intermediate and final hosts.

It is pertinent to point out that most of the Acanthocephala mentioned in the above reviews have either avian or mammalian final or definitive hosts. This is in spite of the fact that a large number of known Acanthocephala are parasites of fish and that the earliest known member of this phylum came from a fish (Hyman 1951). There are apparently in the literature only two detailed studies of fish Acanthocephala in both experimental and natural infections. These are those of Ward (1940) on N.cylindratus in the ostracod Cypria globula and the large mouthed bass, Huro salmoides, and De Giusti (1949) on L. thecatus in the amphipod, Hyalella azteca,and the rock bass, Ambloplites rupestris.

The information available on E. truttae is scanty and limited to reports on the incidence of the parasite in its hosts. It has been reported to occur in a number of fish. Baylis (1939) gave the brown

trout Salmo trutta L., grayling Thymallus thymallus L., eel Anguilla anguilla L., roach Rutilus rutilus L., and dace Leuciscus leuciscus L., as recorded hosts of this parasite in Britain. Petrochenko (1956) listed the final hosts on a world-wide basis. These are Salmo fario, Salmo erythraeus, Salmo trutta L., Thymallus thymallus L., Coregonus lavaretus, Esox lucius and probably two Australian fishes, Pomadasys hustate and Sparus berda. The commonest final host from the point of view of incidence and intensity of infection is S. trutta. Various reports in the literature show that G. pulex is the usual intermediate host (Lühe 1911, Meyer 1933, Scheer 1934, Steinstrasser 1936, Lestage 1938, Bauer 1953, Hoffmann 1954, Petrochenko 1956 etc.) In the R. Terrig, E. truttae occurs in its usual hosts G. pulex and S. trutta.

An opportunity was taken of the present broader investigation of the ecology of the intestinal helminths of fish, to study the details of the post-embryonic developmental history of E. truttae in both its hosts and thus determine how it compares with what is known of the other members of the phylum.

2. MATERIALS AND METHODS.

(a) The Intermediate Host. G. pulex used for laboratory experiments were, except otherwise stated, collected from Shotwick stream and two Raby ponds (Cheshire). In both localities E. truttae either does not occur or is exceedingly rare, as none has been recovered from several hundreds of shrimps examined. The shrimps were kept in the laboratory

for three to four weeks before use. As a rule only half-grown shrimps were used in life history studies. Hynes (1955), Hynes and Nicholas (1957) pointed out that these were easier to handle and had a longer expectation of life. Suitable shrimps were isolated and starved for 24 - 48 hours before being infected. Mature eggs of the parasite used for infecting shrimps, were obtained by dissecting female worms taken from the intestine of the brown trout from river Terrig.

The feeding procedure is a modification of that described by Hynes and Nicholas (1957). Twenty shrimps were transferred to an enamel dish, 30 cm. long and 25 cm. wide, containing hard water from the river. Into each dish were put three to four thoroughly soaked autumn-shed elm leaves. A thin suspension of eggs was then squirted onto these leaves. All the dishes were then covered with glass plates and left undisturbed for 18 - 24 hours. After this period, the shrimps were washed to free them from adhering eggs and then kept in fresh hard water. The use of several elm leaves, (the number used depended on their size), minimised the cases of gross over-infection common when single leaves were used.

In the first experiment, two to four shrimps were dissected and examined daily under the binocular microscope. In later experiments, however, shrimps were autopsied every two or three days. Dissection and examination were carried out in 0.6% saline as very young larvae exploded in tap or distilled water. All parasites recovered were measured and studied as far as possible in saline mounts. They were then fixed and stained for later critical study.

The fixation was done in alcohol-formol-acetic, (A.F.A.), (Van Cleave 1953). Acetic stains described by Chubb (1961) were used for whole mounts. Dehydration was done in graded mixtures of Glacial Acetic acid and Methyl salicylate. Worms were then cleared in pure Methyl salicylate and mounted in balsam. Sections of worms were, on the other hand, stained in Erhlich's haematoxylin and Eosin and dehydrated in alcohol as usual.

In later stages of development, it was found necessary to relax all the larvae in tap water for 10 - 24 hours before measurement and fixation. Although in the field, as many as five cystacanths have been recovered from a full-grown male G.pulex, only those shrimps with one to three larvae were used for the study of the development of the parasite. The cultures were kept at room temperature. This varied from 10 - 21°C with a mean of about 17°C.

(b) Nomenclature of larval Acanthocephala.

In the following account, the terms proposed by Van Cleave (1935, 1937, 1946) and later critically reviewed (Van Cleave 1947, 1953) are adopted to describe the various larval stages of E. truttae. These are the Acanthor, for the young larva hatching out of the egg; the Cystacanth, for the infective stage, and the Acanthella for all the other stages of the larva between the Acanthor and the cystacanth. As the so-called acanthocephalan eggs are really acanthors enclosed by shells and membranes, the term Acanthor is frequently used in the text to describe the egg of the parasite. Suitable qualifying words are also

employed in discriminating various sub-stages in preference to fixed sub-divisions of the Acanthor and Acanthella stages adopted by Kates (1943) in describing the larvae of M. hirudinaceus.

(c) The definitive Host.

The brown trout used for laboratory investigations were obtained from the Chirk fisheries. The parasite has not been taken from fish from this source. It is likely that either E. truttae does not occur or is rare in hatchery fish.

On arrival in the aquarium, temperature 15 - 20°C, each fish was transferred to a separate glass tank measuring 30 cms. long, 30 cms. wide and 30 cms. deep. The fish were then settled for three to four weeks in strongly aerated tap water. They were fed on minced meat and liver. During the first two weeks most of the fish refused to eat and,what is more, the population of the cestode, Proteocephalus sp. inhabiting the pyloric caeca of hatchery trout often in large numbers, was drastically reduced. These were discharged with the faeces probably as a result of starvation. Neo-echinorhynchus rutili Müller 1780 is occasionally found in hatchery fish but in no case was it recovered from fish used for studies on development. By the fourth week after their arrival most of the trout were used to their new environment and ate normally.

The length of the fish at the beginning of the experiments was 9 - 12 cms. The choice of the size of the trout and the existence of a 'settling period' between the time the fish were received in the laboratory and the commencement of investigations, minimised the chances of complications

that might have been introduced by interspecies competition in the intestine of trout. Holmes (1961) established that there was such a competition between the cestode Hymenolepis diminuta Rudolphi 1819 and the Acanthocephalan M. dubius. Also by keeping each fish in a separate tank, it was possible to follow the fluctuations in the number and sex of all the parasites established in the gut throughout the experimental period.

Infected shrimps were collected from the River Terrig. This supply was, whenever possible, supplemented by shrimps infected in the laboratory. With the experience gained during the study of the development of the worm in G. pulex, it was possible to isolate, with some accuracy, those shrimps with infective larvae.

The brown trout were infected by feeding them naturally on infected shrimps. Each fish was starved for 48 hours prior to infection. Fifteen infected G. pulex were then added to each tank at the usual feeding time. All the shrimps were usually ingested in three to six hours after addition. When cystacanths were lost to the water during ingestion, as they often were, they were replaced by adding an equivalent number of infected shrimps.

Because of the limitation of available space and more important still, the difficulties encountered in keeping trout under the above described laboratory conditions, it was not possible to autopsy normally more than one fish a week for the experimental period of sixteen weeks. In exceptional circumstances, however, when, for instance, only members of one sex were present in the intestine, a second fish was killed. In some cases, a fresh infection was started to fill the gap.

The Acanthocephala recovered after each week of development were relaxed in water for 18 - 24 hours, and then measured. For subsequent study they were treated as described for the larvae from G. pulex except that, in many cases and for most purposes, it was not necessary to stain the adult parasites. Whole worms cleared and stored in pure Creosote were also used for study. To follow the trends in the development of the acanthors (cf. Table 2.1) female worms had to be dissected.

Faecal examination for whole worms was done daily. Four weeks after feeding fish on infected shrimps, daily microscopical examination of the faeces for eggs was started. Egg counts were not made as it was felt that the result would be too inaccurate to be of any use. This is because the strong aeration provided in each tank continually agitated the faeces and would thus lead to the loss of a considerable number of eggs passed out with the faeces.

3. DEVELOPMENT IN G. PULEX.

The mature egg or shelled acanthor is similar to that of L. thecatus described by De Giusti (1949). It is 0.11 - 0.14 mm. long and 0.023 - 0.027 mm. wide. The ensheathed embryo is a Syncytium made up of a centrally located embryonic nuclear mass surrounded by a sparsely granular peripheral region. Anteriorly, a number of larval spines are distinguishable (Plate 2 fig.1). The sheath is made up of three envelopes. These are the membranous outer envelope, the thick middle shell and a delicate inner covering of the embryo.

Mature eggs ingested by G. pulex hatch in 1 - 20 hours usually in

TABLE 2. 1

The Stage of sexual maturity of female parasites recovered after various periods in trout.

Days after infection on which trout was killed.	No. of male parasites	No. having everted bursa.	No. of female parasites.	No. having copulatory cap.	Stage of sexual maturity of females				
					No. (%) having ovarian balls only.	No. (%) having ovarian balls & few free immature acanthors	No. (%) having ovarian balls & numerous immature Acanthors	No. (%) having ovarian balls & Immature & Mature Acanthors ca. 4 : 1	No. (%) having ovarian balls & Immature & Mature 1 : 1	No. (%) having ovarian balls & Immature & Mature 1 : 1
1	5	4	3	1	3 (100.0)	0	0	0	0	0
2	4	0	8	1	8 (100.0)	0	0	0	0	0
3	9	0	5	4	5 (100.0)	0	0	0	0	0
7	0	-	2	0	2 (100.0)	0	0	0	0	0
7	4	2	5	3	5 (100.0)	0	0	0	0	0
14	2	2	3	0	3 (100.0)	0	0	0	0	0
14	5	0	3	0	2 (66.7)	1 (33.3)	0	0	0	0
14	7	1	7	3	3 (42.8)	2 (28.6)	2(28.6)	0	0	0
14	2	1	6	0	2 (33.3)	4 (66.7)	0	0	0	0
16	7	3	11	0	1 (9.1)	3 (27.3)	7(63.6)	0	0	0
21	0	0	1	0	0	1 (100)	0	0	0	0
21	4	1	7	0	0	3 (42.8)	4(57.2)	0	0	0
28	1	0	1	0	0	1 (100)	0	0	0	0
35	3	1	3	0	1 (33.3)	1 (33.3)	1(33.0)	0	0	0
42	1	1	3	1	0	0	3(100)	0	0	0
49	5	4	2	0	0	0	2(100)	0	0	0
56	1	1	6	0	0	0	5(83.3)	1(16.7)	0	0
63	3	0	5	0	0	1 (20.0)	1(20.0)	1(20.0)	1(20.0)	1(20.0)
70	1	1	4	0	0	0	3(75.0)	1(25.0)	0	0
98	0	0	1	0	0	0	0	*1(100)	0	0

* Contained degenerating ovarian balls, immature and mature acanthors.

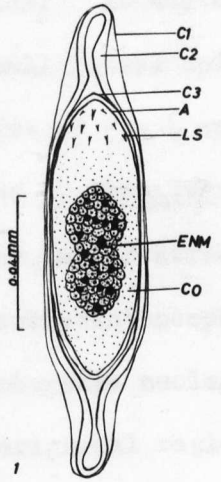
in the intestine. Thus twenty hours after feeding shrimps with a heavy dose of eggs, the following observations were made. A mobile acanthor and many eggs, with one or both ends of their shells clipped off, were present in the gastric mill. In the fore- and mid-intestine were numerous empty shells, many mobile acanthors and a few acanthors wriggling out of their shells. It would appear, therefore, that hatching is achieved by the action of three factors viz: the gastric mill, the digestive juices and the movements of the acanthor itself, operating in that order. Hynes and Nicholas (1957) suggested that the peculiar shape of the eggs may be an adaptation against damage during the passage through the gastric mill of shrimps. This appears to be so in E. truttae.

The newly hatched acanthor crawls actively along the intestinal wall of the shrimp. The method of movement is by the protrusion and withdrawal of the anterior extremity of the body coupled by the shortening and lengthening of the larva (Hynes and Nicholas 1957). This movement of the anterior extremity of the body was also observed in acanthors mounted in 0.6% Saline.

Soon after hatching, the acanthors begin to migrate through the intestinal epithelium. The exact time taken to accomplish this is not known. However, eleven to twenty hours after infection, acanthors have been recovered lying between the intestine and the digestive caeca in the haemocoel.

The structure of the acanthor at this stage is essentially the same as that of the shelled embryo given above. It is an elongated structure

IP - Invaginated proboscis
LB - lemniscal bud
Lg - ligament
LM - lemniscus
LRN - neck or lemniscal ring nucleus
LS - acanthor spine
NR - neck retractor muscle
O - ovarian mass
OB - ovarian ball
OL - orange liquid
P - proboscis
PH - proboscis hook
PHP - proboscis hook primordium
POS - retracted hind-end
PP - proboscis primordium
PR - proboscis retractor muscle
PS - proboscis sheath
PT - posterior testis
RA - remains of acanthor
RMN - proboscis retractor nucleus
RMP - retractor muscle primordium
RO - rostellum
S - Saefftigen's pouch
SC - subcuticula
SFT - Saeffligen's pouch primordium
SPH - sphincter
ST - transverse striation
T - testis
U - uterus
UB - uterine bell
V - vagina
VD - sperm duct
VDP - primordium of sperm duct
WB - body wall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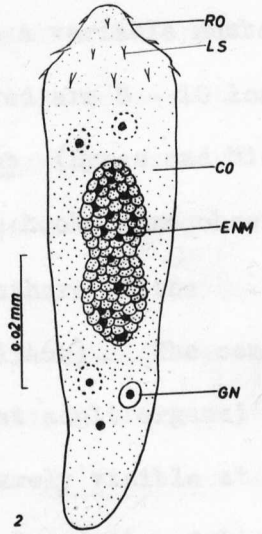
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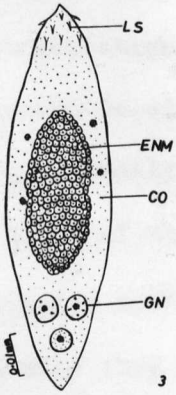
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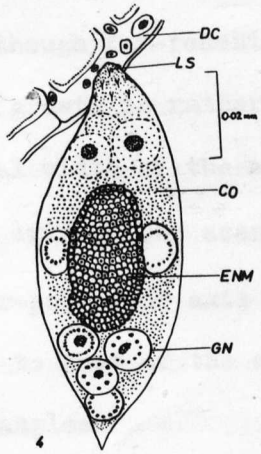
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with blunt extremities and measures 0.07 - 0.08 mm. long and 0.02 mm. wide. At the anterior end is a rostellum with a variable number of small larval spines. The usual numbers observed are 8 - 10 longitudinal rows of 3 - 4 spines per row. As in P. minutus (Hynes and Nicholas 1957) and L. thecatus (De Giusti 1949), no blade-like hooks were observed. The latter structure has been found in the acanthors of the Archiacanthocephala (Meyer 1936, Moore 1946a, 1946b). The central embryonic nuclear mass is the primordium of most adult organs, while the peripheral region, in which a few nuclei are barely visible at this stage, is the primordium of the cuticula and subcuticula of the adult worm. (Plate 2 fig. 2).

After a brief period of mobile life, most acanthors become attached by means of their spines to either the intestine or the digestive caeca. These secondary attachments are often deep-seated. By 24 hours after infection, most of the acanthors cease to move. Then follows a period of 30 - 32 days, when the acanthor is slowly and gradually transformed into the Acanthella. Although far-reaching differentiations are taking place in the larva, growth is rather slow. Increases in size occur mainly along the original width of the acanthor which gradually becomes the future longitudinal axis of the acanthella and hence of the adult worm. Thus the anterior-posterior axis of the developing acanthella is invariably at an angle to that of the acanthor. Typically they are apparently mutually at rightangles.

Before proceeding further, it should be pointed out that there were considerable variations in the size of the larvae recovered on each

day. Even for larvae of the same age, recovered from the same shrimp, the size and often the stage of development attained differed. Moore (1946a) gave four reasons for a similar observation made on the larvae of M. dubius. These were:- "1. Variation in the time required for hatching; 2. the length of time required to penetrate the gut wall; 3. abundance of food materials available in the portion of the body cavity where the larva is located, and 4. individual differences of larvae as to their ability to assimilate available nourishment". The first two reasons are probably less important in E. truttae where most of the larvae hatch and completely penetrate the intestinal wall in 11 - 20 hours after infection. In M. dubius complete penetration of the gut is achieved in 10 - 12 days (Moore 1946a). The considerable variation in the size of the eggs, as well as the third and fourth reasons given above, may account for the differences in E. truttae.

During the first six days of larval development, changes in the length of the acanthor are hardly perceptible. The width on the other hand has increased from about 01020 mm. after 24 hours to 0.022 mm. on the sixth day. The peripheral or cortical region is more granular and two to three giant nuclei are apparent at the polar regions. An apparent proliferation of the embryonic nuclear mass has begun (Plate 2. fig.3).

Twelve days after ingestion the larva is a broad spindle shape and measures 0.088 mm. long and 0.033 mm. wide. The cortex is now highly granular and in it, 8 - 12 vesicular giant nuclei, 0.008 - 0.012 mm. in diameter, are distinguishable. These are more or less irregularly arranged (Plate 2. fig. 4). The central nuclear mass is oval and has increased in

size. Differentiation of the embryonic nuclear mass appears to have started, for the outermost nuclei are larger than inner ones.

The period 13 - 16 days of development is characterised by the first appearance, in the cortex, of minute specks of orgnge-coloured matter. These are first apparent in the polar areas and were considered to be fatty in nature by Hynes and Nicholas (1957). As the acanthella increases in age, these orange particles become larger and give the worm its characteristic pale orange colour. The larvae of P. minutus which share the same intermediate host in nature are, on the other hand, of bright orange colour. It also appears that the amount of orange fat present in the haemocoel of the shrimp host and the intensity of its colour bear a direct relation to the colour of larval E. truttae.

Eighteen days after infection, considerable thickening of the larva has taken place. It is now oval in shape, 0.093 mm. long and 0.066 mm. wide. Its anterior and posterior ends are attenuated (Plate 3. fig.5). The central embryonic nuclear mass is distinctly oval. The number of apparent nuclei in the latter is further increased and it is differentiated into a central area of ovoid nuclei surrounded by three to four layers of rather elongated nuclei. The cortical region is sharply delimited from the embryonic nuclear mass. The giant nuclei, about 0.016 mm in diameter, are arranged around and close to the embryonic nuclear mass in a single layer. In well pigmented fresh specimens, the position of some of these giant nuclei are seen as minute paler patches under the microscope.

DEVELOPMENTAL STAGES OF E.TRUTTAE (Continued)

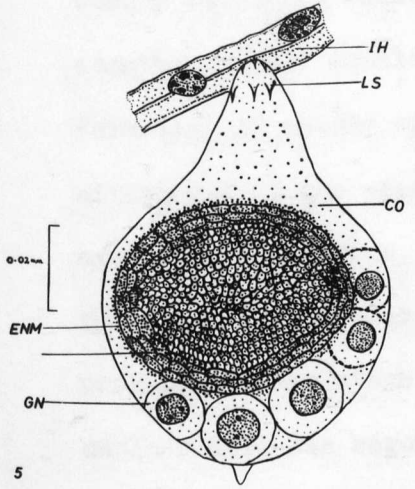
PLATE 3

Fig. 5 - Eighteen-day acanthella

Fig. 6 - Twentyfour-day acanthella

Fig. 7 - Thirtytwo-day acanthella

Fig. 8 - Thirtyseven-day acanthella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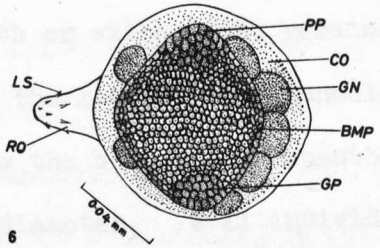
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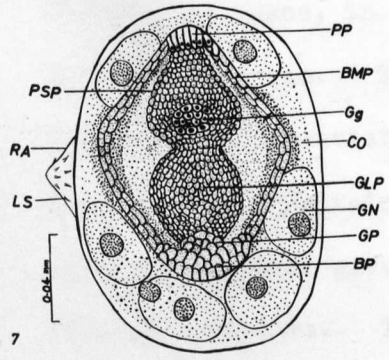
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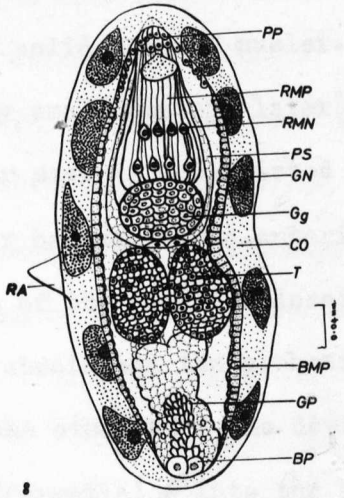
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8

From 19 - 24 days, the larva continues to increase in size but at such a rate that about 25 days after infection, the original width of the acanthor either equals or supersedes its length. This results in the formation of nearly spherical acanthellae with or without the remains of either end of the acanthor. When present, the latter are peduncular and are, sooner or later, completely merged into the body of the acanthella. Spherical acanthellae average 0.116 mm. in diameter. Oval individuals present at this stage are 0.121 mm. long and 0.110 mm. wide. The embryonic nuclear mass has begun to elongate along the acanthella axis and again has assumed a broad spindle shape. Its anterior and posterior ends are distinguishable by the positions of the primordia of the proboscis and the genital apparatus (Plate 3. fig. 6).

Although growth continues to be slow from 26 - 32 days, more internal changes take place. By the 32nd day, the acanthella is 0.192 mm. long and 0.168 mm. wide and shows the following features. (Plate 3. fig.7). In the embryonic nuclear mass, the primordium of the muscle layer of the body wall has become separated from a central solid mass of nuclei. The intervening space, in which are suspended many small nuclei, later forms the main body sac. The central solid nuclear mass is constricted at the middle to differentiate anterior and posterior halves. The anterior region, in addition to containing the oval primordium of the ganglion located posteriorly, will give rise to the proboscis sheath and the proboscis retractor muscles. The posterior half, on the other hand, is divisible into a large proximal portion which later differentiates into the gonads, the ligament and proboscis sheath retractors. The smaller distal portion

is the primordium of the genitalia. In the cortex, the giant nuclei have become granulated and begun to elongate. They are 0.040 mm. long and 0.028 mm. wide.

Between 32 - 37 days, the sex of an acanthella is recognisable. Acanthellae, 37 days old, are 0.440 mm. long and 0.192 mm. wide. The primordia of most organs are blocked out. Anteriorly in the central or medulla region, the nuclei forming the proboscis primordium are followed by a rather elongated primordium of the retractor muscles of the latter. The primordium of the ganglion still lies close to the posterior border of the proboscis sheath at this stage. In male acanthellae (Plate 3. fig. 8) the testes lie side by side immediately behind and abutting on the posterior border of the proboscis sheath. The various elements of the genital ligament primordium except the bursa, are not clearly demarcated at this stage. In the female worms also, the genital ligament is in a similar poor state of differentiation. The cortical giant nuclei, 0.048 - 0.080 mm long and 0.032 - 0.040 mm. wide, are arranged in four longitudinal rows of usually five nuclei in each row. After this stage the remains of the acanthor are not usually found. Moore (1946) found that the remains of the acanthor (the spines) of M. dubius persisted till late in the 'pre-acanthella stage'. In P1 minutus where the acanthor spines disappear earlier in development, their absence was taken by Hynes and Nicholas (1957) as marking the end of the acanthor stage and the beginning of the acanthella.

From 38 - 40 days, the giant cortical nuclei become larger and are positioned in a characteristic manner. There is an anterior apical nucleus

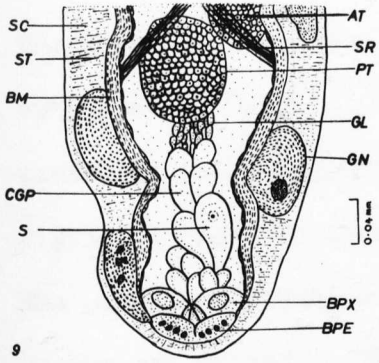
which later divides into four and contributes to the formation of the proboscis. Behind this, is a ring of four giant nuclei - the lemniscal ring - marking the posterior limit of the proboscis. This is followed by three groups of four nuclei each, not necessarily lying on the same circumference on the body at this stage. Finally there is a posterior group of four nuclei, one of which later becomes positioned at the extremity of the body and contributes to the formation of the eversible bell of the bursa. In the cortex also, striations are apparent. Their presence indicates the initiation of the cortical lacunal or canal system. (Kates 1943). Both sexes show a better differentiation of the genitalia. In male worms (Plate 4. fig.9), the disposition of the testes is changed, one lies partially anterior to the other. The primordia of the vasa deferentia, cement glands, Saefftigen's sac and the bursa are easily recognised. In the females the uterine bell and uterus are discernible. The proboscis sheath is at an advanced state of differentiation and is attenuated posterior to the position of the ganglion in it. The latter structure has become longer than broad and lies two-thirds down the length of the proboscis sheath. The various retractor muscles are formed and the insertion of the proboscis retractor muscles about the middle of the acanthella is easily detected. (Plate 4, fig. 9).

The period 41 - 56 days, is marked by the rapid elongation of the acanthella coupled with a more gradual disintegration of the cortical giant nuclei. Because the lengthening of the latter does not keep pace with the general elongation of the body, their positions in the subcuticula, are seen externally in both fresh and stained specimens, as small swellings on the body of the larva. (Plate 5. fig.14).

DEVELOPMENTAL STAGES OF E.TRUTTAE (Continued)

PLATE 4.

Fig. 9. - Posterior half of male acanthella, 39 days after infection.
Fig.10. - Anterior end of male acanthella, 49 days after infection.
Fig.11. - Acanthella, 73 days after infection.
Fig.12. - Cystacanth, 85 days after infection.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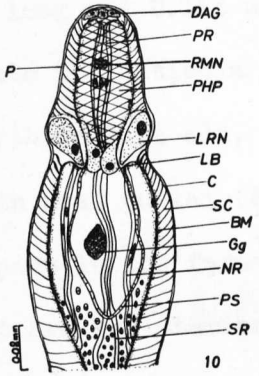
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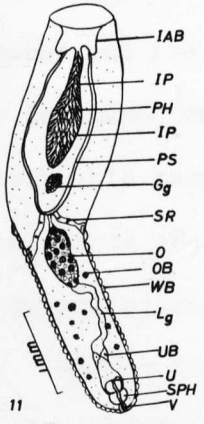
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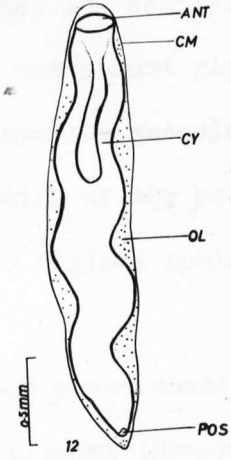
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Fortyone days after infection, the anterior apical and the posterior terminal giant cortical nuclei have taken their respective places (Plate 5. fig.13). The acanthella is 0.495 mm. long and 0.143 mm. wide. The cortex is narrower but its differentiation into the cuticula and subcuticula has begun. In female acanthellae fortythree days old, the centrally located genital ligament is studded with oval nuclei (Plate 5. fig.14). Some of these nuclei will later take part in the formation of the ovary. Continuous with the genital ligament are the uterine bell, uterus and vagina. The anterior apical nucleus has divided into four and the ganglion takes a position about three-fourths down the length of the proboscis sheath. In some specimens, a pair of short slender nerves running out diagonally from the posterior border of the ganglion are observed.

After fortyfive days of development, the acanthellae are 0.836 mm. long and 0.220 mm. wide. From this stage onwards, female worms are generally longer than male worms at the same stage of development. (Plate 5 figs.15 and 16). In the male acanthella, the testes are completely separated and lie in tandem. The vasa deferentia and cement glands are fully delimited while the bursa can be separated into the muscular cap and the eversible component. With the general elongation of the body, the various parts of the female genitalia including the vaginal sphincters have become distinct.

The proboscis of the species develops in the erect condition unlike those of L. thecatus (De Giusti 1949) and P. minutus (Hynes and Nicholas 1957). Fortyfive days after infection, its main nuclear

DEVELOPMENTAL STAGES OF E.TRUTTAE (Continued)

Photomicrographs.

PLATE 5

Fig. 13 - Male acanthella, 41 days after infection (X77)

Fig. 14 - Female acanthella, 43 days after infection (X53)

Fig. 15 - Male adanthella, 45 days after infection (X53)

Fig. 16 - Female acanthella, 45 days after infection (X53)

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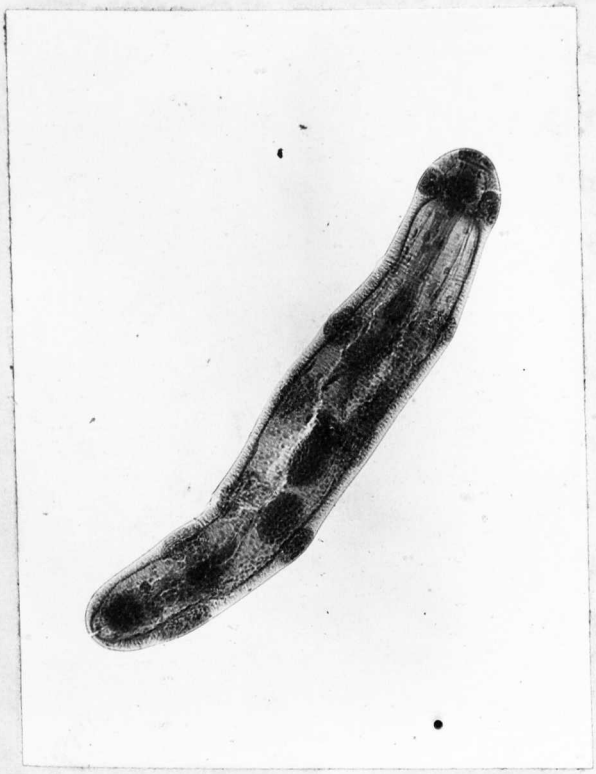

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PLATE 5

primordium is organised into a central solid body extending from the apical nuclei to the lemniscal ring. In fortysix days old acanthellae, the primordia of the hooks appear as closely packed protuberances or knobs below the sub-cuticula of the developing proboscis (Plate 6. fig.17). The acanthella at this stage is 1.012 mm. long and 0.209 mm. wide.

Fortynine days after infection, the female acanthella is 1.848 mm long and 0.198 mm. wide. The definitive proboscis is narrower and distinctly marked off from the rest of the body. It measures 0.154 mm. long and 0.110 mm. wide. In male larvae, the overall size is 1.617 mm. long and 0.198 mm. wide, while the proboscis is comparatively shorter and measures 0.132 mm. long and 0.110 mm. wide. The developing hooks have begun to make their way through the cortex. In the latter region of the proboscis, the onset of transverse striations probably indicates also the initiation and extension of the lacunal system. (Plate 6. fig.18).

The acanthellae recovered from fifty to fiftyfive days after infection were much paler in colour. This is attributable to a more sparse distribution of the orange coloured fat in the rapidly elongating larvae. During this period also, a very thin elastic envelope is observed. The latter is light orange and membranous and is closely applied to the cuticula. Its formation was observed, in various worms, on 52 - 54 days. Female acanthellae, 54 days old, attain a length of 2.77 mm. and taper posteriorly. Due to the fact that this tapering is rather distinct, the width of the body varies from 0.198 mm. at the widest point to 0.121 mm. near the posterior extremity. The proboscis is 0.286 mm. long and 0.143 mm. wide. Male larvae are, as indicated

PHOTOMICROGRAPHS OF DEVELOPMENTAL STAGES OF E. TRUTTAE.
(Continued)

PLATE 6.

Fig. I7 - Fortysix-day male acanthella (x53).

Fig. I8 - Fortynine-day male acanthella (x40).

Fig. I9 - Fiftyfour-day female acanthella (x28).

Fig. 20 - Fiftysix-day male acanthella, anterior two-thirds. Note invaginated proboscis (x58).

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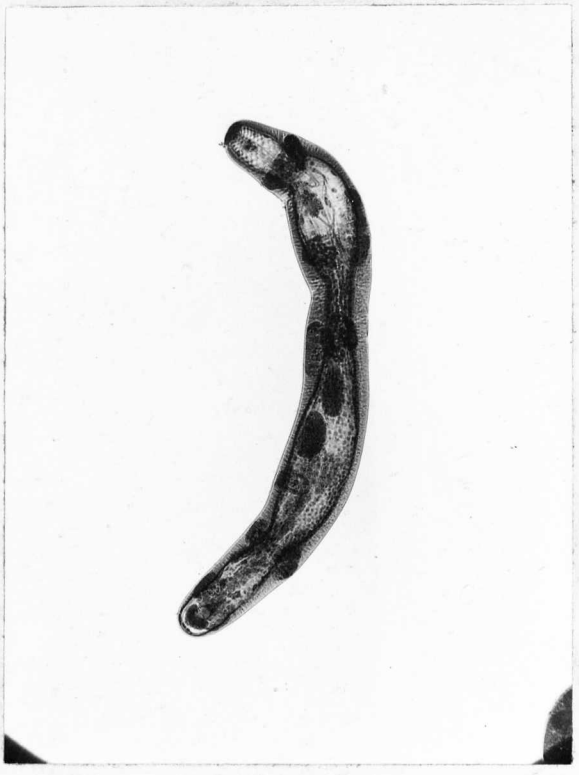

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PLATE 6

above, slightly smaller but have a more uniform body width of 0.176 mm. In the cortex, the giant nuclei are narrow and in some cases have begun to disappear. Female acanthellae of this age are characterised by the aggregation of nuclei of the genital ligament in small groups along the length of the latter. These will later coalesce to form the ovary. (Plate 6. fig.19).

Acanthellae 55 days old are about 3 mm. long. Their muscles have become active. In some worms the early stages in the initial retraction or inversion of the proboscis are observed. Fiftysix days after infection the proboscis is fully invaginated within the proboscis sheath in a good number of individuals recovered on this day (Plate 6, fig.20). With the invagination of the proboscis the body becomes shorter. Thus, while the female acanthella with retracted proboscis is about 2.5 mm. long, that with unretracted proboscis is 3.2 mm. long. The formation of the lemnisci takes place at the same time as the initial retraction of the proboscis. Observations on acanthellae with their proboscis at various stages of invagination indicate that the invagination of the basal portion of the proboscis initiates the simultaneous migration of the four neck giant nuclei to form the definitive lemnisci. Each lemniscus derives from two giant nuclei (Plate 7, figs. 22 and 23). Sooner or later, after the invagination of the proboscis, the neck and the anterior part of the main body are also retracted. The body becomes wrinkled. This wrinkling of the body is caused by the contraction of the muscles of the body wall. (Plate 4, fig.11; Plate 7, fig. 21). The invagination of the proboscis, retraction of the fore body, and the wrinkling of the body are responsible for the apparent gradual shortening

of the acanthella with age. It is important to emphasise that this decrease in size is only apparent, for acanthellae continue to grow though more gradually. This is demonstrated by measuring relaxed worms. It may be added in this connection that larvae of about the age under consideration are usually not fully stretched on relaxation. Other features of interest shown by the acanthella are as follows. The sub-cuticula contains the remains of the evanescent giant nuclei in the lacunae. The genitalia, in both sexes, are fully laid down but have not acquired an opening to the outside world. The larval membrane is thick and brown, and is freed from the body of the larva. In fresh specimens it encloses an orange liquid in which are enclosed fatty globules.

Further migration, lengthening, and separation of the lemniscal components from the neck region continue from 57 - 60 days. The cuticula and sub-cuticula are more adult in form. By the 60th day unrelaxed male and female acanthella are 2.0 mm. and 2.5 mm. long respectively. On relaxation in water the proboscis is not caused to evert. Male worms are 2.5 mm. long while females are about 3 mm. long. Acanthellae of this age are usually very soft and lightly pigmented. When relaxed in tap water for 24 hours, they lose most of their pigments and the lacunal system with disintegrating remains of the cortical giant nuclei can be demonstrated by suitable staining (Plate 7, fig. 23). Recurved hooks are observed in the middle of the retracted proboscis. This is an indication that they have penetrated the cuticle.

From 60 - 75 days after infection, the eversion of the proboscis is not caused either by the normal relaxation methods or by the application

PHOTOMICROGRAPHS OF DEVELOPMENTAL STAGES OF E.TRUTTAE (Continued)

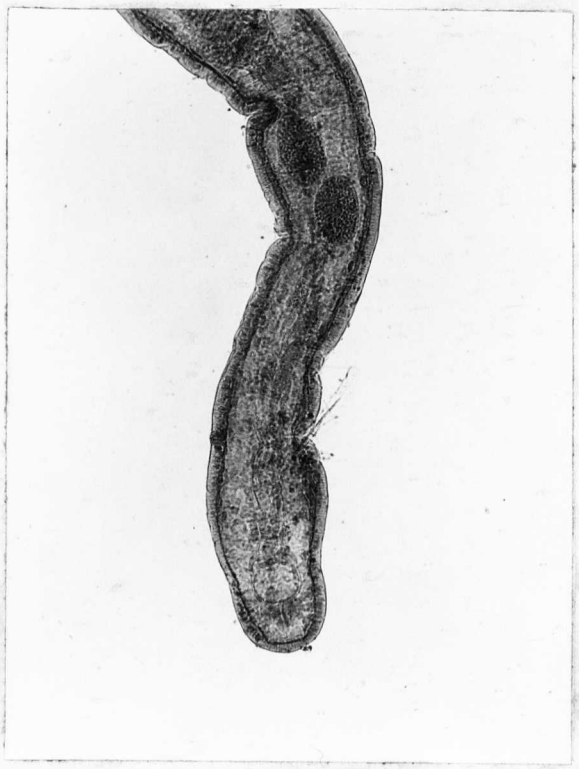
PLATE 7

Fig.21 - Fiftysix-day male acanthella, posterior region (X58)

Fig.22 - Fiftyseven-day male acanthella, anterior half (X78)

Fig.23 - Sixty-day male acanthella, anterior half (X78)

Fig.24 - Sixtyeight-day female acanthella, mid-body (X78)



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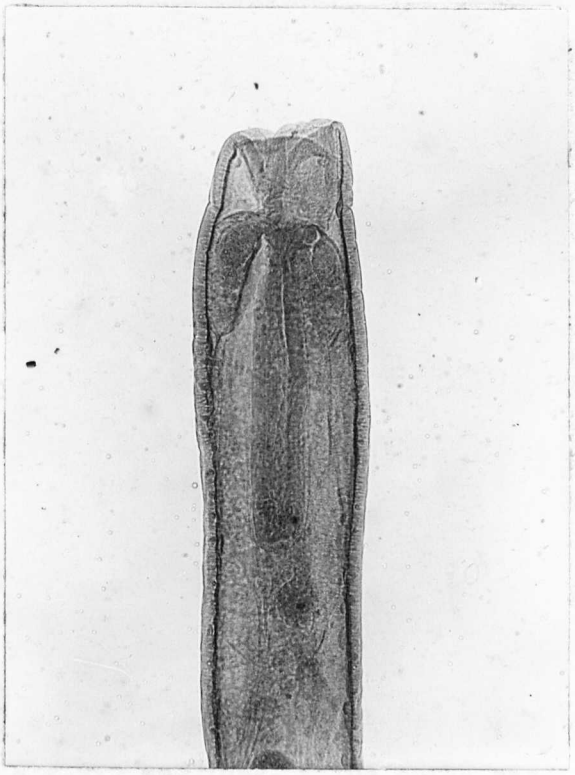

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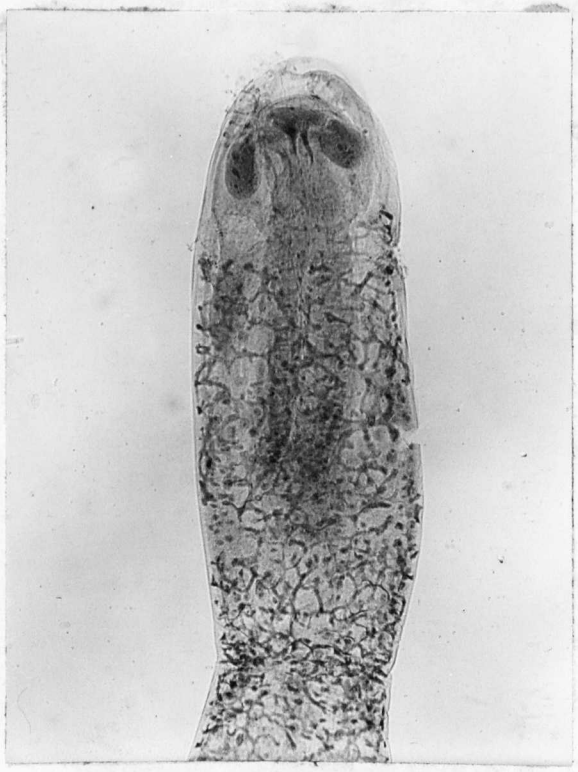

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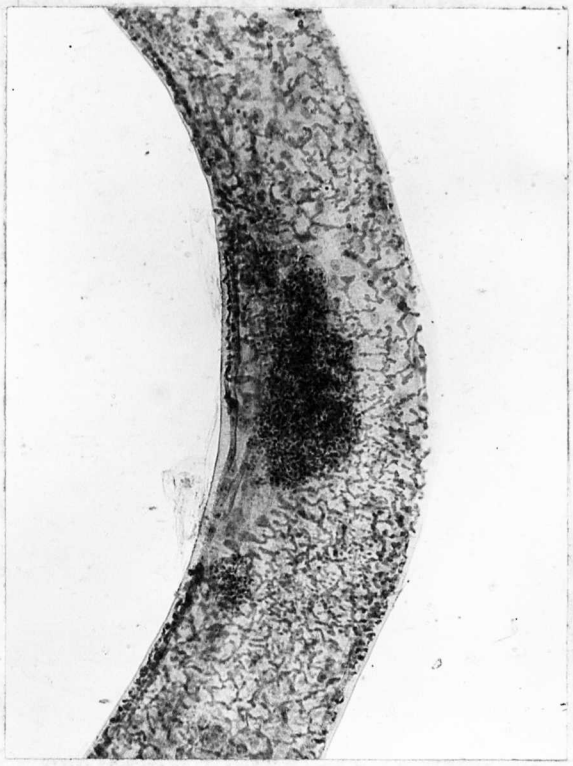

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PLATE 7

of mechanical pressure. The probable significance of this in the life cycle of this parasite will be discussed later.

On the 68th day, the female acanthella is about 4.2 mm. long (unrelaxed it is 3 mm. long). The small groups of nuclei in the genital ligament, observed about the 54th day, have coalesced to form an ovary or ovarian mass about the middle of the body. (Plate 7, fig. 24). This ovary is rather temporary for it later breaks up. The vagina has acquired a terminal opening to the exterior. This occurs in various worms between 65 and 68 days.

Eightyone days after infection, the proboscis of some larvae are everted on relaxation in water. Male worms are 5.5 mm. long. The bursa has acquired a subterminal connection to the outside world but is not everted when left in water. The cement glands are active and deposits, probably from this source, are found in the bursa. The testes are mature and are partially telescoped in the unrelaxed natural condition (Plate 8, fig.25). The cortex is much thickened and the cuticula is delimited from the subcuticula. Female acanthellae are about 6.5 mm. long (unrelaxed they are ca. 2 mm. long) and are either spirally coiled or longitudinally folded to accommodate them within the smaller ensheathing larval membrane. The ovary is partly disintegrated to give ovarian balls (Plate 8, fig. 26). The breaking up of the ovarian mass begins about the 70th day of development (Plate 4, fig.11), and is completed by the time the larva becomes infective.

Eightytwo days after infection, the larva becomes infective. The resulting cystacanth (Plate 4, fig.12, and Plate 8, fig.27) is dark orange

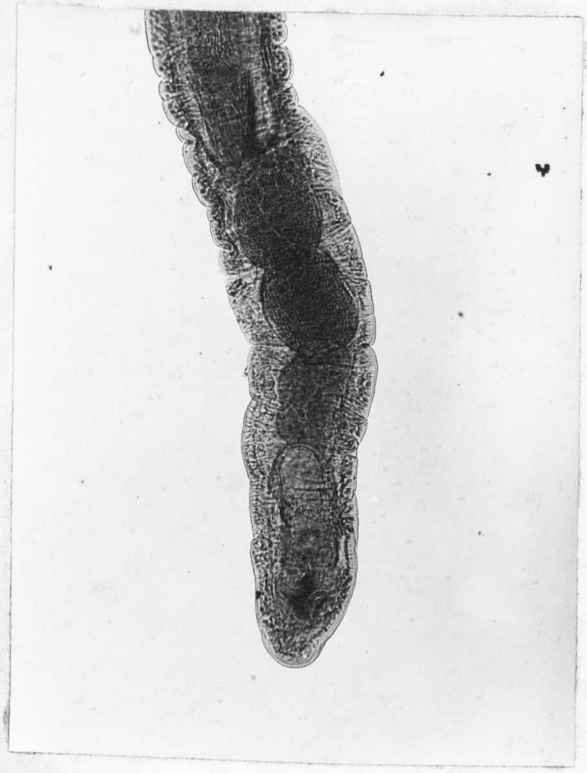
PHOTOMICROGRAPHS OF DEVELOPMENTAL STAGES OF E.TRUTTAE (continued)

PLATE 8

Fig. 25 - Posterior two-thirds of male worm, 81 days after infection - unrelaxed (X58)

Fig. 26 - Posterior two-thirds of female worm , 81 days after infection - unrelaxed (X58)

Fig. 27 - Male cystacanth 82 days after infection (X16)



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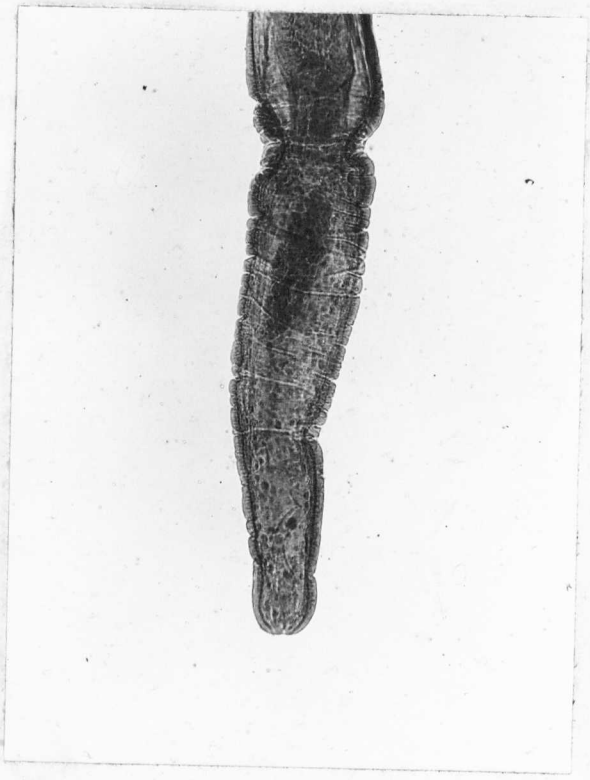

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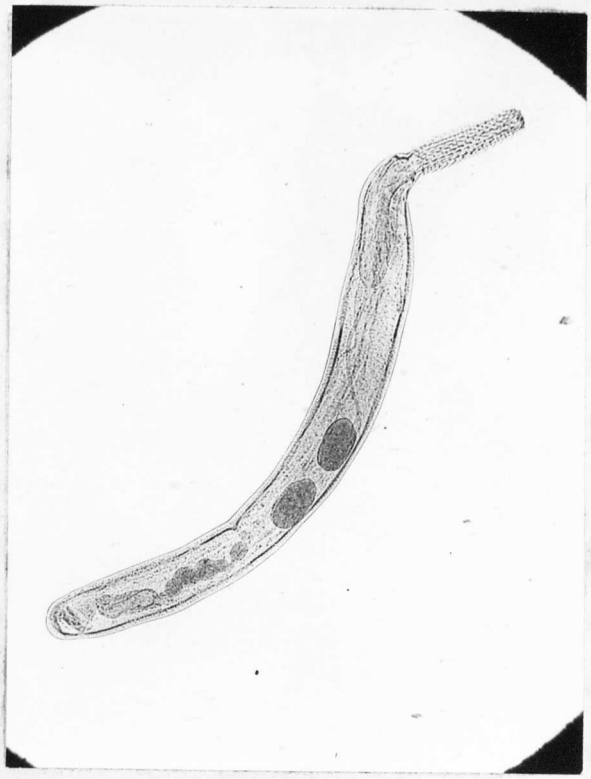

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PLATE 8

in colour, sexually mature and in every way morphologically similar to the young adult worms taken from the intestine of fish. In various laboratory cultures, this stage was reached after 80, 81, 82 and 84 days. It was also observed that neither by extended relaxation in water nor by keeping in physiological saline was the eversion of the bursa of male cystacanths procured. In female cystacanths the ovary has disappeared leaving developing ovarian balls suspended within the body cavity.

Since it was observed that the infectivity of the larva was associated with the ability to evert the proboscis, the latter was adopted in all later experiments as the criterion for determining when the cystacanth stage was reached. The normal form of the cystacanth in the haemocoel is as follows. It is flat, wrinkled with the proboscis and the fore body retracted. Movement is very slow and the body is folded to varying degrees (Plate 4, fig.12). It is pertinent to point out that this slow activity of worms within the haemocoel commences with the initial invagination of the proboscis. Larvae kept under regular observation for prolonged periods were found to shorten and lengthen and even change their position within the haemocoel.

The life span of cystacanths appears to be geared to the life expectation of their hosts at the time of infection. In one experiment, at room temperature, 9 - 22°C, cystacanths were first taken after 80 days. One shrimp of this lot containing one cystacanth died on the 197th day. If it is assumed, as seems reasonable, that this larva attained the infective stage at the same time as the others, the life span would be approximately 116 days. In another culture kept under the same conditions, one G. pulex lived till 311 days after infection. Cystacanths were first

recovered after 84 days. Calculated on the above basis, the approximate age of the cystacanth is about 7.5 months. This evidence suggests that a cystacanth can live as long as its host.

In conclusion, it may be said that the cystacanth of E. truttae is a fully mature worm, requiring only a change of environment to start on the next phase of its life history in the final host.

4. DEVELOPMENT IN S. TRUTTA.

On ingestion, the cystacanths are freed, usually in the cavity of the stomach, by the digestion of the tissues of the shrimp. Larvae recovered from the stomach twenty hours after infection, remained flat, wrinkled, and inactive. Their proboscis and forebody were still in the retracted condition. The conditions in the cavity of the stomach do not seem therefore to be suitable for the active existence of the parasite.

By the end of the second day, most of these young Acanthocephala have entered the pyloric region of the intestine. Worms observed in the latter region at the end of the first and second days after infection were actively attached to the mucosa of the intestine. Their bodies remained flat, wrinkled and were at intervals, swayed slowly across the narrow lumen of the pyloric intestine. The probosces were invariably lodged in the crypts between the villi. In some cases, a few individuals migrated into the pyloric caeca. A closer examination of these parasites, in the attached position, showed that some of the male parasites had their bursa in the everted position. Also a few female worms had soft

cream-coloured copulatory caps attached terminally to their body. These observations confirm that the worms are stimulated and become sexually active, when they enter the pyloric region of the intestine.

From the third day onwards, these worms begin a gradual, though often irregular movement down the intestinal tract. This conclusion is based on observations of the position of the majority of worms in the intestine, during the fourteen weeks' period of development. It was found that as the parasites aged, they tended to occupy a more posterior position in the intestine. Thus, while during the first week of development most of the worms were taken in the pyloric region, they were more common in the lower intestine after eight weeks of development. The observation made throughout the present investigation, that active and, to all appearances normal, worms were often found free or attached to the intestinal contents of experimental trout dissected immediately after killing, further attests to this movement of worms. The view is held that such worms found free in the intestine, with mobile erect probosces, are in the process of changing their location in the gut .

During the migration down the intestine, members of both sexes are frequently found attached in close proximity in groups of three to eight. This phenomenon is more frequent in the upper than in the lower intestine and no doubt facilitates copulation. Most female, and occasionally male, worms recovered three days after infection bore the characteristic conical copulatory caps. Since some male parasites from the field often bore these copulatory caps, it is concluded that mistaken copulation occurs in nature and that sex recognition may be poor in this species.

In the preceding account of the development of the parasite in its intermediate host, it was pointed out that sexual dimorphism appeared quite early in the life history of this parasite. It was also noted that larvae of the same sex and age, recovered from the same host, varied in size. As the cystacanths fed to fish came mainly from the field they were, as expected, of widely different ages and sizes. Measurement of ~~our~~ fifty cystacanths from the Afon Terrig, showed that while the males were 4 - 7 mm. long, the females ranged in length from 6 - 11 mm.

It is not surprising, therefore, that after the first week of development in fish, it is not easy to determine to what extent the parasites had grown. The male worms measure 5 - 7 mm. long while the females are 8 - 14 mm. long. In the latter, increase in length is appreciable. On opening up their body cavity only ovarian balls are present. These are in much the same state of differentiation as those in cystacanths.

Two weeks after infection, male worms are 7 - 9 mm. long. As for the larvae, the size of the proboscis is variable. In individuals 8mm in length, the proboscis is 0.75 mm. long and 0.22 mm. wide. The recurved hooks, measured from the anterior limit of the root to the tip of the hooks, are as follows in size. The anterior ones are 0.055 mm. long while the smaller posterior hooks are 0.044 mm. long. The female parasites at this stage, attain the length of 10 - 13 mm. Elliptical ovarian balls bearing characteristic boat-shaped developing embryos peripherally are found in the body-cavity. These ovarian balls

are 0.132 - 0.156 mm. long and 0.088 - 0.110 mm. wide. The developing eggs are also variable in size and are 0.072 - 0.077 mm. long and 0.016 - 0.022 mm. wide. In some female worms, the formation of the outer membrane of the egg has begun. This membrane is first laid down as crescentic protuberances around the apices of the egg. During the next four weeks it is gradually delimited around and then separated off from the egg. After about six weeks, in most eggs, a wide space separates them. For the details of the gradual attainment of functional sexual maturity of females see Table 2.1.

After three weeks of development, male worms are 8 - 10 mm. long. The everted ovid bursa of males 10 mm. long, is about 0.55 mm. long and 0.50 mm. wide. The lemnisci are 1.26 mm. long. Female parasites on the other hand, are 10 - 14 mm. long. In their body cavity, more immature acanthors are released. By the end of four weeks large numbers of immature acanthors are present.

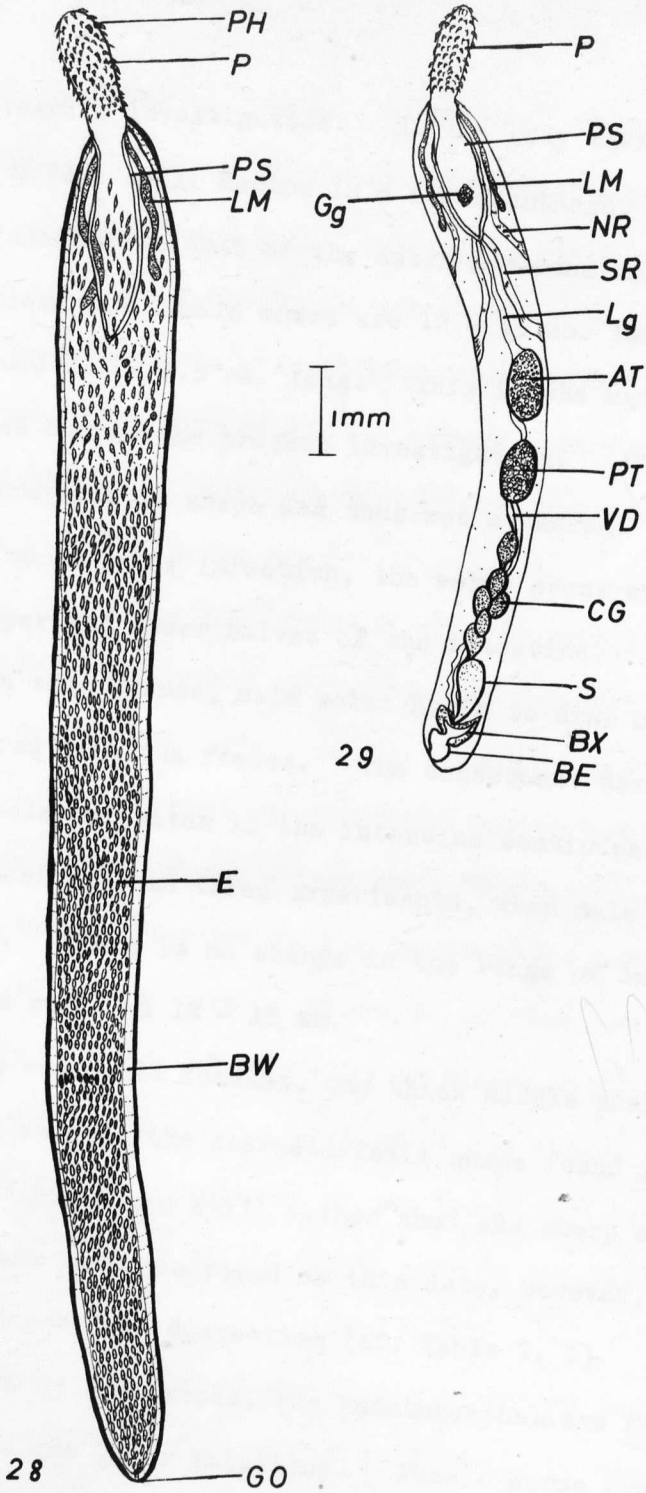
Five weeks after infection, most parasites are still found in the upper half of the small intestine. Males (Plate 9, fig.29) are 9 - 11.5mm. long and some have their bursa in the everted condition. No further increased in size over the above range are observed after this stage. The testes are oval and commonly subequal and measure 0.6 - 1.0 mm.long and 0.3 - 0.5 mm. wide. The female worms are 12 - 14.5 mm. long with their proboscis averaging 1.09 mm. long and 0.30 mm. wide. The copulatory caps are either cream-soloured or dark brown. The latter colour is probably indicative of an earlier copulation. The chemical process involved in this change of colour and the possible effects on the

PLATE 9

DIAGRAMS OF ADULT E. TRUTTAE FROM THE FINAL HOST.

Fig. 28 - Gravid female worm

Fig. 29 - Mature male worms. Note arrangement of cement glands partly in pairs and partly one after the other - characteristic of Metechinorhynchus. (cf. Chapter I)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spermatozoa require investigation. In the body cavity, the formation of the thick middle shell begins in a few acanthors. The mode of formation is similar to that of the outer membrane described above.

Six weeks old female worms are 12 - 15 mm. long and their lemnisci measure 1.4 - 1.5 mm. long. This is the maximum length of this organ recorded during the present investigation. After this stage, they are usually irregular in shape and thus not measured.

Seven weeks after infection, the worms occur with equal frequency in both the upper and lower halves of the intestine. By the 45th day, or much earlier in some fishes, male worms begin to drop out of the intestine and are recovered with the faeces. The consequent decrease in the population of male parasites in the intestine continues till the 82nd day. This is the latest day, in three experiments, when male worms are taken with the faeces. There is no change in the range of length of female worms. This is still at 12 - 15 mm.

In eight weeks old females, the thick middle shell is fully laid down but has not assumed the characteristic shape found in mature acanthors. The apices are still rather short and sharp and not elongated and blunt. In one worm recovered on this date, however, a few mature acanthors were observed on dissection (cf. Table 2. 1).

From eight to nine weeks, the Acanthocephala are found to be attached mainly to the lower intestine. Female worms are now 13 - 16 mm. long and have either vestigial or no copulatory caps. Some males, on the other hand, have their bursa everted and are probably still sexually active. At this stage, all the worms are, for the most part, of different

shades of grey or rarely pale orange in colour. This gradual change of colour, from the dark orange of cystacanths and young adults to grey, is observed as the worm grows older. The relationship between the colour of acanthocephalans and their age is not always easy to define. In the field, it has been found that the colour varies with the type of food eaten by the final host (Ekbaun 1938). Where, however, as in the present instance, the food of the host is standardised, the colour of these beasts serves a rough guide to age.

Females, ten weeks old (Plate 9, fig.28), are 14 - 16 mm. long and are gravid. The latter length is the maximum recorded for experimental female worms. During the period, mature acanthors are taken with the faeces. The shedding of eggs appears to be active but irregular and is continued for about three weeks - from about the 64th to the 85th day. Female parasites taken after the latter date contain degenerating ovarian balls, immature and mature eggs. It may be pointed out that the observed irregularity in the presence of acanthors in faeces might be due, in part at least, to the effect of strong aeration of the water. For the details of a quantitative assessment of the transition from the ovarian ball stage to the production of infective eggs in females reference may be made to Table 2. 1.

Eleven weeks after infection, female parasites begin to leave the intestine. The earliest record of a gravid female in the faeces was made on the 69th day of development. As found for the male worms, this loss of females continues intermittently, and by the end of fourteen weeks after

infection, the intestine of fish is free of all Acanthocephala.

Although the observations made here and elsewhere in this thesis, show that there is a gradual overall decrease in the number of male parasites with the age of infection, the proportion of the male to female worms, recovered after each week of development, varies and does not show any definite trends except in the case of one experiment (cf. Fig.2.1). The problem of the irregularly fluctuating sex ratio, during development under experimental conditions, will be discussed.

It is shown, therefore, that the developmental history of E. truttae in its final host consists essentially of copulation, and the gradual maturation of eggs accompanied by increase in size. Under the above laboratory conditions, full grown adult male worms are 8 - 11.5 mm. long and 0.9 - 1.2 mm. wide, while the females are 14 - 16 mm. long and about the same width. Their probosces are shown to vary from 0.9 - 1.1 mm long.

5. EFFECTS OF ENVIRONMENTAL FACTORS ON DEVELOPMENT.

A study of the influence of environmental factors on the development of a parasite whose hosts are both poikilothermous, was found to be of considerable interest. During the experiments on the life history of E. truttae, there were indications that the temperature and the degree of parasitic infestation had some influence on the growth rate and the attainment of the relevant final stage in both the intermediate and final hosts. The following investigation was thus undertaken to ascertain to what extent these factors affected the course of development. All the experiments were conducted in as near the natural conditions as possible.

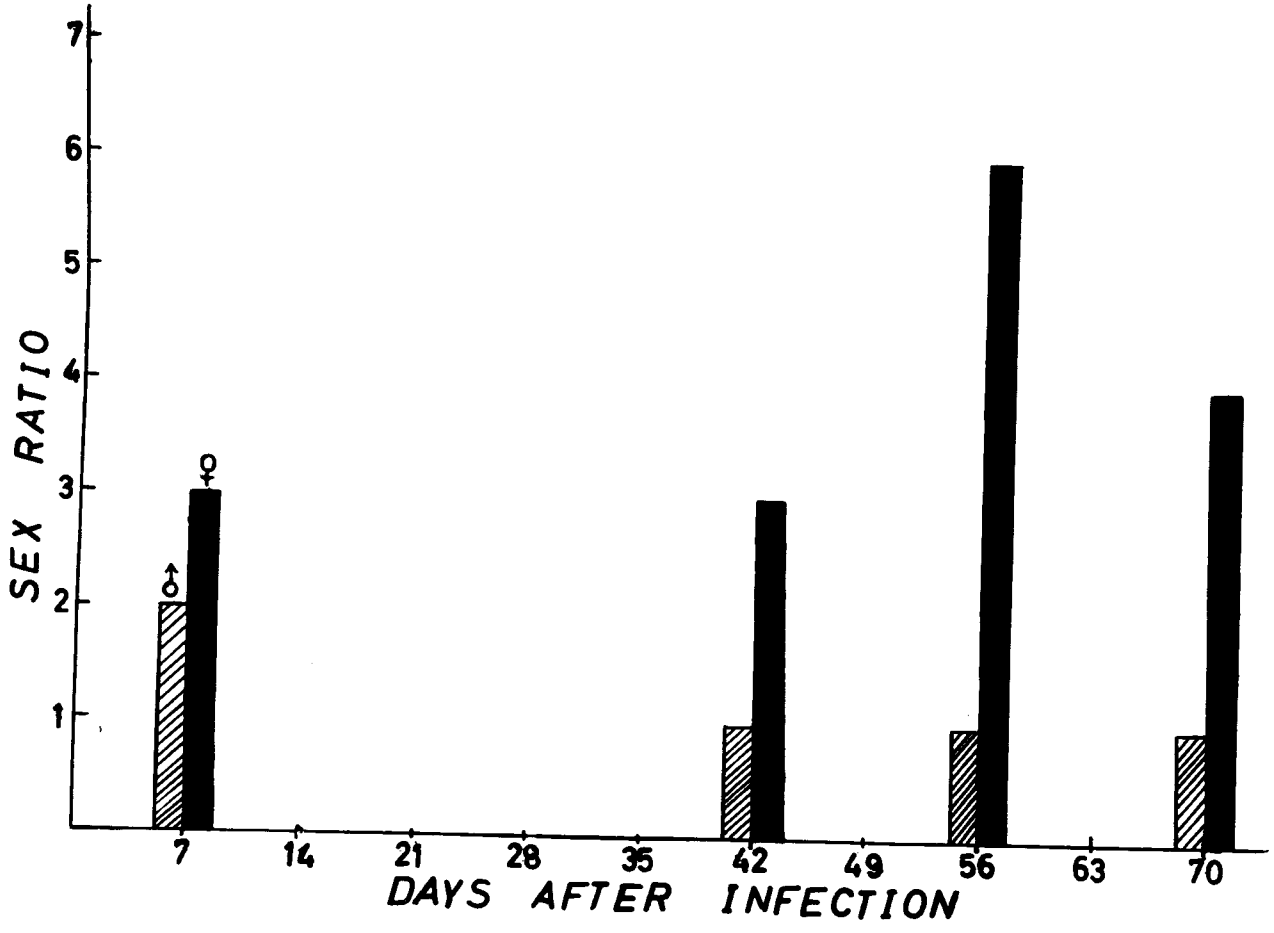


FIG 2·1 THE RATIO OF MALE TO FEMALE WORMS RECOVERED FROM ONE EXPERIMENT.

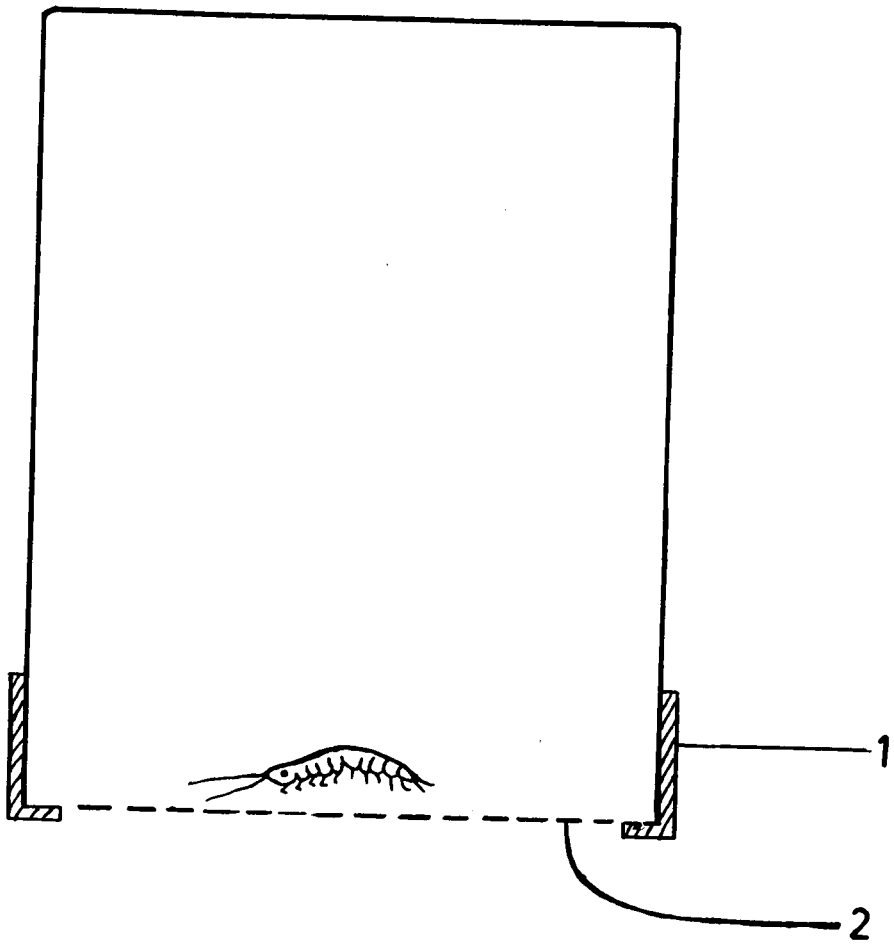
(a) Temperature.

The development of the parasite in G. pulex, at various temperatures, was determined as follows. Cultures of infected shrimps were kept at room temperature and the summer and winter temperatures of the stream, at the appropriate season, in a 'cold room'. The temperature and the lighting conditions in the latter were regulated to simulate natural conditions in the river. Some of the shrimps were kept in enamel dishes as described before. Others were maintained in bottles (Hanson's bottles, Fig.2.2) placed in a trough of running stream water. These bottles are simply 125 mls. specimen bottles, in each of which the bakelite screw cap is cut off, leaving a sufficient ledge to hold a very fine mesh on to the top of the bottle. Stream water, sufficient to make the bottle float in an upright position was poured in. Infected shrimps and an elm leaf were then introduced. The bottle was then stoppered and inverted so that it floated with the stoppered end in water. This arrangement allowed sufficient aeration for the shrimps. The water in the trough was circulated by a conventional pump. Both the pump and the trough were made of perspex. The use of the latter material was necessary as it has been found (Hynes pers. comm.) that traces of metal in water were lethal to G. pulex under laboratory conditions. It was also found necessary to change the leaves and water in the bottles weekly, to avoid clogging the fine mesh. The trough was emptied and the water replaced at irregular intervals. It should be pointed out that the above set up was suitable for low temperature work only. At the higher summer temperatures, the water in the circuit tended to be fouled rather easily. The introduction of a centrifugal pump to increase the rate of flow and hence aeration, brought many difficulties. The most important of these was that the temperature tended to be much

Fig. 2. 2. Hanson's bottle - position in perspex trough with circulated stream water.

1. - bakelite screw cap

2. - fine mesh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higher than required. During the summer therefore, infected cultures were maintained in enamel dishes only. Shrimps were dissected at convenient intervals and the earliest day after infection, on which cystacanths were taken was recorded. As indicated earlier, the larvae were regarded as having attained the infective stage, if on relaxation their probosces were everted.

The temperature and the corresponding date after infection, on which cystacanths were recovered, are given hereunder:

1. 17°C ..... 82 days
2. 13 - 15°C ..... 80 days
3. 10 - 14°C (Summer tamperature of stream 1962) 96 days
4. 3 - 14°C ..... 196 days
5. 2 - 10°C ..... 240 days.

The last two results and the details of the variation of temperature during the experimental period, require further comment. The investigation at 3 - 14°C was carried out from late February till September 1962. The necessary changes in the temperature and length of day were made usually at fortnightly intervals. The observations made at autopsy were as follows: After 63 days at 3°C, only transparent acanthors about 80 microns long were recovered. The temperature was then altered and after a further 19 days at 9 - 10°C, spherical acanthellae about 110 microns in diameter were taken. The temperature then rose to 13 - 14°C, and 142 and 196 days after infection, acanthellae with invaginated probosces and cystacanths were respectively obtained on dissection. The fifth experiment, conducted at 2 - 10°C, lasted from September 1962 to the end of May 1963,. The rather low temperature over the period reflects the long hard winter of 1962/1963. At the start of the investigation, the temperature was 8°C.

This soon dropped to 2 - 3°C by the end of October and remained so till the middle of March 1963 when it went up to 7 - 8°C again. By mid-April it was changed up to 10°C. Under these conditions, cystacanths were taken on the 240th day after infection. It should be noted, however, that shrimps were not dissected between 226 and 240 days.

In yet another experiment carried out while the temperature was at its lowest range 2 - 4°C (31.10.62. - 16.3.63) it was observed that after 136 days of development, all the parasites were only at the spherical or earlier stages. It will be recalled that the spherical stage is reached after 20 - 24 days at room temperature.

It seems safe to conclude that at the low winter temperature of 2 - 4°C, the development of this parasite in G. pulex is extremely slow. Above this temperature, growth progresses at a rate which is related to the temperature. What the exact relationship is requires further investigation. From the data available, it is clear that growth and differentiation at 13 - 15oC proceed at much the same rate as at room temperature.

For a similar study on the final host, infected fish were kept in glass tanks in the cold room. The following results were got when trout were kept at 4 - 14°C for 113 days (20 days at 4°C and 93 days at 9 - 14°C).

a. After 14 days all the female worms had ovarian balls only. No copulatory caps were found on them.

b. Both male and female worms were present after 113 days.

c. In all the female worms recovered on the latter date, there was a preponderance of mature over immature acanthors.

c. The average length of these gravid females was 12.5 mm.

In another experiment in which infected fish were kept at 5 - 10°C it was observed that the state of sexual maturity of female worms, as judged by acanthor development, was about the same as for those recovered after 28 days at 12 - 16°C. Although it was not possible to conduct these experiments for long periods at fixed low temperatures, some general and tentative conclusions may be reached. It would appear that at the lowest temperature which trout has to endure naturally, the development of the parasite proceeds at a slower rate but is not as greatly retarded as found in the shrimps. The time at which both sexes leave the intestine may thus depend on the temperature of the environment. At low temperatures, this may be much later than that found at room temperature. This indication that at the low temperatures normally experienced in nature, the development of E. truttae in the brown trout is not greatly retarded, may be due to the relatively higher level of activity of the latter in comparison with G. pulex.

(b) Crowding.

(i) G. pulex.

Before undertaking the study of the effects of crowding or the intensity of infection on development in G. pulex, it was necessary to establish the following:

1. the maximum number of the larvae that can complete their development in one shrimp under natural conditions;
2. the degree of variation in size and differentiation expected when such a number of parasites of the same age developed in the same host.

Field studies (Chapter VII) have shown that as many as five cystacanths can develop in a shrimp 9 mm. long. During the experimental study of the life history, the degree of variation possible when 2 - 5 parasites developed simultaneously in one shrimp, was noted.

To obtain high and varying intensities of infection, shrimps were fed on a suspension of acanthors in petri dishes for periods varying from 6 - 8 hours. They were then washed free of adhering eggs and then kept at 3 - 4 °C and 16 - 20°C. Dissection and examination were done at suitable intervals.

During the first 40 days at 16 - 20°C, there was nothing unusual in the observed variations in growth and differentiation. In the next 42 days, however, markedly wider differences than would be expected were apparent. These variations in the size and the stage of differentiation of the larvae were found to be greater at higher intensities of infection. Thus a shrimp 8 mm. long, autopsied 56 days after infection contained: 2 transparent acanthors about 80 microns long

6 spherical acanthella ca. 100 microns in diameter

6 oval acanthella ca. 341 microns long

4 elongated acanthella 1.97 - 2.46 mm. long

2 wrinkled acanthella with retracted probosces.

It has been shown earlier that after 56 days of development, at the same temperature, most larvae had either withdrawn their proboscis or were at various stages of doing so. For an outline of the other results see Table 2. 2. It was also observed that disturbances in the development of the parasite in shrimps, which were less than 8 mm. long, occurred at

Table 2. 2

Summary of the effect of crowding on the development of E.truttae in G.pulex.

Temp. °C	Length of G.pulex mm.	Days after which shrimps killed	Total No.of parasites recovered	No.of parasites in each stage of development					
				Acanthor	Spherical Acanthella	Oval Acanthella	Elongated Acanthella	Acanthella with retracted proboscis	Cystacanths
3-4	8	81	40	40	0	0	0	0	0
"	8.5	136	25	0	25	0	0	0	0
2-8	10	226	13	0	0	0	7	6	0
"	12	226	23	0	0	1	13	9	0
"	9	226	33	0	3	2	10	18	0
2-10	10	240	9	0	0	0	0	0	9
"	10	240	14	0	0	0	0	2	12
"	9	240	28	0	0	0	0	12	16
17	8	56	20	2	6	6	4	2	0
"	9	56	63	0	7	10	46	0	0
"	8.5	86	12	0	0	0	3	0	9
"	9.5	86	14	0	0	1	3	0	10
"	10	86	8	0	0	3	0	0	5
"	8.5	87	17	0	4	2	2	0	9

intensities of infection lower than 5 larvae in a shrimp. The extent of the disturbance appeared to depend on the size of the shrimp at the time of infection. The whole question of the relationship between E. truttae and its shrimp host will be dealt with in a later chapter of this thesis.

The observations made at low temperatures are also outlined in Table 2. 2. It is seen that after 136 days at the winter temperature of 3 - 4°C all the 40 larvae recovered from one shrimp were at about the same stage of development. This is not surprising as the larvae were still so small that marked variations would seem unlikely. In an 8 mm. G. pulex therefore, an intensity of infection of up to 40 at 3 - 4°C, did not produce any marked effect on development of the parasite, probably because the growth rate was very slow. When the temperature was raised in the course of development, disturbances in growth and differentiation became apparent. Thus after 226 days at 2 - 8°C, three larvae were still at the spherical stage (See Table 2. 2) while in 18 others, the prosbosces had been invaginated.

Other more qualitative effects of crowding on the larvae during the late stages of development were as follows: Elongated acanthellae and cystacanths were smaller and more slender than those developing in less crowded conditions. In some cases, the whole body of the parasite became malformed and irregular in shape. Of more common occurrence were malformed and globose probosces which were incapable of retraction. Some of the latter had a few well developed recurved hooks jutting out irregularly from them. In a few males there was a single testis instead of two. However, there was no conclusive evidence to suggest that this was

due to the intensity of infection (crowding).

It seems likely that these disturbances in the growth and differentiation of larval acanthocephalans, consequent on crowding, may be due to intraspecific competition for space and nutriments in the haemocoel of shrimps. In spite of this competition, it was observed that as many as 16 cystacanths can develop within a shrimp 9 mm. long.

(ii) S. truttae.

During the course of experiments designed to study the effects of initial heavy infection of the brown trout on the host-parasite relation, (Chapter V), observations were made on the possible effects of the degree of parasitic infestation, on development in the final host.

Each brown trout was fed with 30 infected shrimps. Control fish were fed 15 infected shrimps. At autopsy after 14 days, the Acanthocephala recovered were relaxed, measured and females examined for the state of maturity of the eggs. Worms recovered with faeces during the period of investigation were similarly treated. For the purposes of the present study, two brown trout fed with 30 infected shrimps were left for 6 weeks at 12 - 18°C. Since it has been shown that male worms begin to leave the intestine before the end of 6 weeks in some cases, it was considered that no useful purpose would be served by continuing the experiment beyond this period.

The number and sex of the parasites recovered during the experimental period, at 4 - 9°C and 12 - 16°C, are given in Table 2. 3. Observations relevant to the present study were as follows:

Table 2. 3

The effect of initial heavy invas-ion of *S.trutta* by *B.truttae*.

Expt. No.	Fish No.	Temp. °C.	Days after infection. 1 M.	F.	Total	2 M.	F.	Total	3 M.	F.	Total	4 M.	F.	Total	5 M.	F.	Total	6 M.	F.	Total	7 M.	F.	Total	8 M.	F.	Total	9 M.	F.	Total	10 M.	F.	Total	11 M.	F.	Total	12 M.	F.	Total	13 M.	F.	Total	14 M.	F.	Total	Total after 14 days M.	F.	Total
1	1	9-12	14	6	20	1	-	1			0			0			0			0			0			0			0			0			0			0			0	6	2	8	21	8	29
	2	"			0	2	2	4			0			0			0			0			0			0			0			0			0			0	1	0	1	10	9	19	13	11	24
	Control 1	"	2	1	3			0			0			0			0			0			0			0			0			0			0			0			0	5	3	8	7	4	11
	Control 2	"			0	0	1	1			0			0			0			0			0			0			0			0			0			0			0	7	7	14	7	8	15
2	1	4-9			0			0			0	1	1	2	1	0	1	1	0	1			0			0			0			0			0			0			0	17	12	29	20	13	33
	2	"			0			0			0	2	0	2	1	1	2			0			0			0			0			0			0			0	1	0	1	18	7	25	22	8	30
	Control 1	"			0			0	1	2	3			0			0			0			0			0			0			0			0			0			0	1	0	1	2	2	4
	Control 2	"			0			0			0	0	1	1			0	0	1	1			0			0			0			0			0			0			0	8	8	16	9	10	19
3	1	12-16	2	1	3	3	5	8	3	5	8			0	1	0	1	1	0	1			0			0	1	0	1	0	1	1	0	1	0	1	0	1	0	1	0	1	5	5	10	18	16	34
	2	"	14	8	22			0	2	2	4	1	1	2			0			0			0			0	1	0	1	0	1	2	1	3	0			0			0	8	2	10	30	17	47
4	1	12-18	-		-	-		-	-		-	-		-	-		-	-		-																						42 Days 2	6	8			
	2	"	-		-	-		-	-		-	-		-	-		-	-		-																						0	5	5			

1. There was a tendency for a high proportion of the parasites to be lost during the first few days after infection. This loss was more at higher temperatures.

11. Many males and females taken with faeces during the first week had everted bursa and copulatory caps respectively.

111. No ill effects or abnormalities were noticed in established parasites, recovered at autopsy, after 14 and 42 days.

The high intensity of infection does not, therefore, appear to have a deleterious effect on the growth rate of established parasites in the trout. It would in fact, seem to have a beneficial effect on development. By increasing the changes of earlier copulation, acanthors may mature earlier than would appear possible at low infections.

6. DEVELOPMENT IN UNUSUAL HOSTS.

(a) Intermediate Hosts.

The development of *E. truttae* was studied in three other *Gammarus* spp. occurring in Britain but not, as far as is known, recorded as intermediate hosts for this parasite. These are *Gammarus lacustris* Sars, *G. duebeni* Lilljeborg, and *G. tigrinus* Sexton. *G. tigrinus* is a recently introduced North American species while the others are native species (Hynes 1955, Hynes & Nicholas 1958). The possibility of *Asellus aquaticus* L. serving as intermediate host was also investiaged.

G. lacustris were collected from Llyn Llywenan (Anglesey), *G. duebeni* came from the Wirral (Cheshire) and the Isle of Man, and *G. tigrinus* from brackish ponds near the Ribble estuary. *A. aquaticus* were taken from Shotwick stream. The feeding procedure was the same as

described for G. pulex and the experiments were done at 3 - 4°C and 14 - 20°C. G. lacustris were autopsied every other day. In the other cases the crustaceans were dissected weekly.

The results are summarised as follows. In G. lacustris a good number of the larvae were destroyed. The rate of growth and differentiation in the established parasites, was much the same as that in G. pulex e.g. 56 days after infection at room temperature, some of the larvae recovered had their probosces in the retracted state. Also, cystacanths that were taken after 87 days of development, were infective to S.trutta. In G. duebeni, G. tigrinus and A. aquatics, the parasite failed to develop.

G. lacustris is thus shown to be a potential intermediate host capable of playing an important role in the future spread of E.truttae in freshwater lakes and ponds of Britain. The reactions of this and the other crustacean species dealt with above, will be more fully covered in Chapter IV.

(b) Final or Definitive Host.

The occurrence of E. truttae in the rainbow trout, S.gairdneri Richardson, has been reported on the European continent (Dorier 1932, Reichenbach-Klinke 1954) and in various parts of the Soviet Union (Petrochenko 1956). No such record has been found in the literature for Britain. As the rainbow trout is of relatively recent introduction to this country, it was decided to ascertain if E. truttae would establish and develop successfully in its intestine.

Hatchery trout were used, and the method followed was the same as that for experiments on the development of the parasite in brown trout. Fewer fish were, however, involved and thus autopsy was carried out at longer intervals. The experiment was conducted from October to February in an unheated aquarium. The temperature was thus rather low for the greater part of the period. It was 5 - 10°C for the first 96 days and for the rest of the experimental period the range was 5 - 15°C.

The observations were as follows: The parasites recovered 30 days after infection, compared favourably in their size and state of development with those from the brown trout of about the same period (28 days), after infection. There were six male worms 6 - 10 mm. long and four females 12 - 15 mm. long. The latter bore free immature acanthors, in some of which the outer egg membrane had started to develop. The last fish, killed 126 days after infection, harboured seven parasites - 3 males and 4 females, measuring 10 - 12.5 mm. long and 16 - 17.7 mm. long respectively. Mature acanthors from one of the female worms were infective to G. pulex. The other three females contained immature acanthors at various stages of development. In some of the latter the central nuclear mass had been differentiated and the thick middle shell laid down, but had not assumed the characteristics of the mature form.

In most of the experimental rainbow trout, N. rutili was present at autopsy. It appears, therefore, that N. rutili is more common in this species than in brown trout from the same source. It may also be added that rainbow trout behaved rather well in the laboratory. They fed well

and their condition, judged by the external form and the deposition of adipose tissue on the viscera, may be said to be very good.

It is concluded that, under experimental conditions, E. truttae can complete its developmental cycle in the rainbow trout without apparent adverse effects on the latter. The rate of development may be expected to be much the same as in the usual host, the brown trout.

7. DISCUSSION.

A comparison of the details of the developmental cycle of E. truttae with those of the other Acanthocephala so far studied, reveals interesting differences and similarities. On the whole, as might be expected, this parasite agrees more with the two other Palaeacanthocephala so far described - L. thecatus and P. minutus. It, however, differs from them in a number of respects. In some of these, it agrees more with the members of the Archi- and Eo-acanthocephala (Meyer 1938, Ward 1940, Kates 1943, Moore 1946 and Hopp 1954).

During the early stages of the development of E. truttae in shrimps, the basic architecture of the worm is laid down as in the other members of this phylum. The axis of the acanthella and hence the adult worm, however, is shown in this parasite to be invariably at an angle and typically at rightangles to the acanthor axis. Even allowing for the possible effects of temperature, it would appear that this phase of development is a rather long one. It takes about 32 days. Moore (1946b) described a similar phase of 30 - 35 days, during which the acanthor of M. ingens penetrated the intestinal wall of its intermediate host.

The acanthor of E. truttae penetrates the intestinal epithelium completely as in the other species except L. thecatus (De Giusti 1949) but it usually acquires a secondary attachment to the intestine or the digestive caeca. The larva, thus, develops while attached as in L. thecatus. Unlike the latter, however, the rostellar end, which is peduncular during the first 30 - 40 days of development, is later completely merged into the body of the acanthella.

The proboscis of E. truttae begins its development in the erect position. Here it differs from P. minutus and L. thecatus and is in agreement with the Archi- and Eo-acanthocephala.

Van Cleave (1920) observed encysted acanthocephala in the mesentery of fish. He explained that juvenile acanthocephala, swallowed by abnormal final hosts, penetrated the intestine and became established in the mesentery probably because they were unable to establish themselves in the intestine. De Giusti (1949) also recorded the incidence of encysted L. thecatus in the mesentery of abnormal fish hosts. In an atempt to account for this observation, he fed young juveniles at all stages of development to the normal fish host. His finding was that the worms which had developed for 26 - 28 days only, could not establish themselves within the intestine of the normal fish host, but penetrated the wall and encysted in the mesenteries. This ability of juvenile Acanthocephala to penetrate the intestine of normal or abnormal final hosts, has been recorded also for N. cylindratus (Ward 1940), M. ingens (Moore 1946b) and N. emydis (Hopp 1954). In these cases, the penetration may be supposed to have been an active process involving, perhaps, the use of the

proboscis. Acanthellae of E. truttae of various ages were found to be uninfective in any form. When shrimps with acanthellae, 56 - 75 days after infection, were fed to trout, the larvae not only failed to establish in the intestine, but were apparently unable to penetrate the wall. No encysted forms were taken from the mesentery or viscera. In other experiments, partially digested juveniles have been found in the intestine. These observations may well be due to the inability of non-infective larvae of this parasite to evert their proboscis. It has already been pointed out that neither relaxation in water, nor the application of mechanical pressure, procured the eversion of the probosces of juveniles. To these may be added the observation that juveniles with retracted proboscis, from the field did not protrude their probosces in pepsin/trypsin solutions used for hatching metacercaria of trematodes, whereas the cystacanth of E. truttae and P. minutus did. These findings indicate that the presence of a transport host in the life cycle of E. truttae is unlikely.

In E. truttae the migration of the lemniscal nuclei of four (2 + 2) to form the lemnisci, appears to be initiated by, and is simultaneous with, the initial retraction of the proboscis. There is no correlation between these events in the other described species. It should be pointed out, however, that in several cases of abnormal development, where the proboscis is malformed and not invaginated at any stage, the migration of the lemniscal nuclei takes place but invariably only stumpy lemnisci result.

In the development of its ovary, this parasite differs from all the other species. A compact ovary or ovarian mass is formed, about

68 days after infection, in the intermediate host. This ovary is rather temporary in its existence for it soon begins to break up into a number of segments, each liberating ellipsoidal ovarian balls with developing oocytes. By the time the acanthellae are infective, there is usually no remains of the ovary. In all the other Acanthocephalan species, the formation of the ovarian balls takes place in the final host. In L. thecatus, even the ovary is formed in the rock bass (De Giusti 1949).

The cystacanth in the other species has been either described or regarded by other investigators as a quiescent stage, awaiting a transfer to the definitive host, to continue its growth and development. Observations and measurements of the cystacanths of E. truttae, of various ages in shrimps, have shown that they not only continue to grow but gradually move and even change their positions within the haemocoel, very slowly. The final size attained appears to be related to the size of the shrimp host and hence the amount of nutriment available to the parasite. In spite of this gradual increase in size with age, all the cystacanths and late acanthellae, as pointed out earlier, show an apparent decrease in size. Kates (1943) ascribed this phenomenon in M.hirudinaceus to the withdrawal of the proboscis. Moore (1946a) on the other hand, explained that the development of flange-like lateral folds of the hypodermis accounted for a similar feature in M. dubius. In addition to the above two reasons, the shortening of the body in E. truttae is further effected as follows. The part of the body immediately behind the proboscis is withdrawn. In both sexes, but more often in female worms, the flat and wrinkled body is either thrown into longitudinal folds

or becomes spirally coiled within the ensheathing membrane. The hind end of females is, more often than not, tucked in, in older cystacanths. Hynes and Nicholas (1957) recorded a similar intucking of the hind tip of the body in all the cystacanths of Pl minutus. Since the larval membrane is laid down when acanthellae are 1.5 - 2 mm. long, and is elastic to a very limited extent, the various shortening devices help the young juveniles to grow and differentiate, and yet accommodate themselves within the smaller larval membrane.

The origin of this membrane, where it has been observed, appears to be problematical. In M. hirudinaceus (Meyer 1938), M. dubius and M. ingens (Moore 1946a, 1946b), the envelope is considered to be of the parasite origin. De Giusti (1949) observed the envelope only in larval L. thecatus whose growth was retarded at low temperatures and thought it was of host origin. The envelope was observed in P. minutus, about 35 days after infection, by Hynes and Nicholas (1957) but they were unable to come to a conclusion about its origin. This is not surprising as it was observed, during the course of experiments involving P. minutus described elsewhere in the thesis, that even the late acanthellae of the latter parasite had a comparatively heavy investment of host cells. In E.truttae, the mode of formation of the envelope given earlier in the text, would suggest that it is laid down by the worm. Little or no investment of the parasite by host cells is observed during the formation of the membrane in G.pulex. It is also likely that its formation at a time when the larva is undergoing rapid elongation and is consequently soft and fragile, serves to provide some protection.

In E. truttae the cystacanths are shown to be sexually mature. Copulation takes place in the final host as soon as the environment is suitable. In the other species, a period of physiological adjustment in the final host appears to be a necessary prelude to copulation. It was pointed out earlier on, that the cystacanths of this parasite remain flat, wrinkled, and inactive in the stomach of fish but become activated soon after entering the pyloric region of the intestine. The determination of the physico-chemical characters which evoke the above reactions from the worms, as well as those which render the intestine of trout suitable for their establishment, calls for investigation. It seems likely that the difference in the hydrogen ion concentration between the stomach and the intestine, and the presence of bile in the latter region, may have some influence on the observed behaviour of the parasite in the pyloric intestine. Read (1950) Smyth (1962), noted that the osmotic pressure, p^H, viscosity, partial pressure of gases and the oxidation-reduction potential of the gastro-intestinal tract were the main physico-chemical factors which influence the vertebrate intestine as an environment for intestinal helminths. The type of food and the secretions poured into the tract from various sources, determine the chemical nature of the environment. Read further pointed out that comparative evidence from the literature showed that the points of discharge into the intestine, of the bile and pancreatic juices, had some relation to the location of helminths along the intestine. The latter consideration appears to be relevant to E. truttae only as far as the initial activation is concerned, for the parasite inhabits the entire intestinal tract including the caeca.

The relation between the life span of the parasite and that of its intermediate host calls for more comment. Experimental evidence has been adduced to show that the life span of the parasite in shrimp is geared to the life expectation of the latter, at the time of infection. If the eggs are ingested by shrimps early in their life, the resulting cystacanths may live from a few days to well over six months depending on the environmental temperature. Eggs ingested by adult shrimps have very little chance of completing their development and would thus be wasted. It will be shown later in this work, that in nature, this waste is minimised by the rather close correspondence of the peak incidence of the young stages of the parasite and its shrimp host.

During development in S. trutta, it was noticed that male worms often bore copulatory caps terminally or subterminally. Similar observations have been made on worms from the field. This state of affairs is most likely the result of 'mistaken' copulation between males. Also, grey female worms 15 - 18 mm. long, recovered from the hind intestine in both experimental and natural infections, were found, on dissection, to be unfertilised. Thus, in spite of their age (experimental specimens were taken 8 - 10 weeks after infection), they were filled with ovarian balls only. Since in the trout harbouring these individuals, male worms and fertilised females were also present, and as such old, unfertilised females were of frequent occurrence in nature, it may be concluded that sex recognition is poor and the sexual process rather inefficient in this species.

It was expected that the sex ratio in experimental infection would show interesting features, since male parasites disappear earlier from

the intestine. As noted above, except in the case of one experiment (cf. fig. 2.1), the ratios were rather irregular and did not show any definite trends. This may be partly due to the fact that, in some fishes, there was considerable loss of parasites with the faeces during all stages of development. Such parasites recovered soon after expulsion with faeces, were found to be quite normal in appearance compared to those observed in the attached condition in the intestine. They were flat, wrinkled and active but with their probosces attached to the faeces. As it has been shown earlier in the text, that E. truttae occupies a more posterior position with age, it is suggested that these losses occur during the movement of the parasite in the intestine. It is quite possible that during the change of position (Burlingane & Chandler 1941, Kates 1944, Van Cleave 1952) a detached proboscis may become mistakenly embedded in the faeces and thus expelled with the latter. In both experimental and naturally infected fish dissected immediately after killing with a blow on the head, worms with their probosces attached to faeces or the remains of shrimps, were often found. As worms in such situation were more frequent in experimental infections, it may be that the type of food eaten has a bearing on the extent of loss. It is pertinent to remark in this connection that in natural infection, where the intestine contained remains of earthworms or smaller trout, the number of worms with their proboscis attached to faecal matter was high. Another factor which might have contributed to the irregularity of the sex ratio in experimental infections, is the consideration that some of the shrimps fed to fish may have contained late acanthellae and not

cystacanths. In Experiment No.8, however, the ratio showed the expected preponderance of female over male worms as the latter left the intestine. After 14, 42, 56 and 70 days of development the ratio of males to females were 2:3, 1:3, 1:6 and 1:4 respectively. It may also be mentioned that the loss of parasites discussed above, made it difficult to determine in some fishes when the natural depletion of male worms commenced.

The environmental factors, temperature and crowding, are shown to have far-reaching effects on development in the intermediate host. It was found that at the low winter temperature of 2 - 4°C, development was retarded. The relation between this finding and the seasonal occurrence of worms, in trout, will be dealt with in Chapter VII. It was also observed that at 2 - 4°C some acanthors and spherical acanthellae were killed. Small round or oval bodies, mainly brown in colour, resulted and were attached either to the intestine or the caeca. Similar structures were observed by Miller (1943), DeGiusti (1949) and Hynes and Nicholas (1958). The explanation given by De Giusti appears to be applicable to E. truttae. It appears that at low temperatures, the development of this parasite is so slow that host reaction is able to kill some of them. At higher temperatures, on the other hand, growth is much faster and thus fewer individuals are destroyed. The walling off of the larva by a 'chitinous-like' membrane before its death, reported by De Giusti (1949) was not observed in G.pulex and G.lacustris, in which E. truttae developed. Nor was it found in G.duebeni and G.tigrinus which were not successfully invaded by the parasite. In A.aquaticus, however, such membranes were

often found around dead parasites.

In G.pulex, crowding is shown to produce wide variations in growth and differentiation particularly at later stages of development. In sume cases, great retardation or even malformation of some acanthellae resulted. It is explained that the small size of shrimps leads to competition, among the acanthella, for the limited available space and food. This is in contrast to the brown trout in which the effects of the intensity of infection was hardly apparent in established worms. Holmes (1962) found that M. dubius, under crowded conditions, extended their linear intestinal distribution only slightly. All the acanthocephalans were attached to the anterior part of the small intestine. There is no evidence from the present study to show that established parasites suffered any ill-effects from crowding within the intestine. Thus, in experimental infections, as many as 25 and 29 parasites were recovered from trout, about 12 cm. and 13 cm. respectively, two weeks after feeding each with 30 infected shrimps. Compared with the worms in the control fish, which were fed 15 infected shrimps, they were normal in both their size and the state of differentiation reached. As for M. dubius above, their range of distribution in the intestine was extended. This extension was, unlike that in M.dubius, rather marked. In the two fishes cited above, there were approximately 52% and 62% of the worms in the lower intestine. It is likely that competition for space had influence on this rather early spread of E. truttae over the entire intestinal cavity. The finding that, under natural conditions, as many as 49 E.truttae of different ages were recovered from a brown

trout of about the same length as the experimental ones, underlines the relative unimportance of the ill effects of crowding in the development of the parasite in its final host. Larger fish were found to be capable of supporting a very heavy parasite burden, e.g. in June 1962 203 parasites were recovered from a single fish, 22.1 cm. long, taken from the River Terrig.

Crowding may even have beneficial effects on development in the final host. A greater number of female worms than usual, was found to have copulatory caps early in development, in infections of 30 shrimps to one fish. In a parasite such as this, where sex recognition among worms is apparently poor, crowding increases the chances of fertilisation (Chubb in press) and hence the continuity of the species. It may, of course, lead to an earlier maturation of the acanthors.

Finally, the observations on the development of this species in S. gairdneri, indicate that this fish, which is a relatively new arrival in Britain, is a potential final host and may well, in future, be instrumental in the spread of E. truttae in British freshwaters.

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C H A P T E R III

THE ACANTHOR OF E. TRUTTAE

1. INTRODUCTION

Apart from morphological accounts, information on the acanthocephalan egg is scanty and generally limited to cursory remarks made in life cycle studies. The exact stage in its development at which the acanthor becomes infective to the intermediate host, has not been ascertained. Hynes and Nicholas (1957) observed that the eggs of P. minutus which had been 16 days in the duck were uninfective, while those recovered after 32 days produced infections in G. pulex. Viability tests have also been largely limited to trials with the eggs of the more common epecies, e.g. M. hirudinaceus. ((Spindler and Kates (1940), Kates (1942)). The result of these studies would indicate that the acanthocephalan egg is very resistant to changes in external conditions. Manter (1928), Moore (1942), have also shown that the eggs of some Acanthocephala hatch on being dried and rewetted.

In the present investigation, the infectivity of five stages in the development of the acanthor of E. truttae is studied. Viability experiments are carried out on the infective acanthor under various environmental condntions and attempts are made to induce hatching in the

infective eggs of the parasite.

2. INFECTIVITY

Observations during the experimental study of the life cycle of the acanthocephalan in fish, have shown that the developmental cycle of the acanthor is divisible into five distinct stages on the basis of the number and stage of differentiation of the three embryonic envelopes. These are as follows: i. The oval embryo in which the outer membrane (vitelline membrane) is either not visible or just becoming apparent under low megnifications (Plate 10, figs. 1a and 1b).

ii. Second stage acanthor - in which the outer membrane is fully delimited and widely separated from the embryo (Plate 10, fig.2).

iii. Third stage acanthor - where the thick middle envelope or shell is laid down apically only. The concentration of nuclear elements centrally to form the inner nuclear mass has begun (Plate 10, fig.3).

iv. Fourth stage acanthor - has all the three envelopes laid down in outline. The thick middle shell is elongated apically and encloses granules between its inner surface and the embryo (Plate 10, figs. 4a and 4b). In P. minutus some of these granules stained with Feulgen and Carmine while others were unstainable (Nicholas and Hynes 1963).

v. Fifth stage acanthor - in which the middle shell has assumed the characteristic rolling pin shape (Meyer 1933, Hyman 1951 interalia). Eggs shed with the faeces of trout are of this

PLATE 10

STAGES OF DEVELOPMENT OF THE SHELLED EMBRYO OR ACANTHOR OF ECHINORHYNCHUS TRUTTAE. (Not drawn to scale)

Figs. 1a, b. - Stage I acanthor

Fig. 2 - Stage II acanthor

Fig. 3 - Stage III acanthor

Figs. 4a, b. - Stage IV acanthor

Fig. 5 - Stage V or mature acanthor,

Legends.

C.1. - Outer envelope of acanthor
C.2. - thick middle shell of acanthor
C.3. - inner envelope of acanthor
CO. - Cortical region
ENM. - Central embryonic nuclear mass
GN. - giant nucleus.

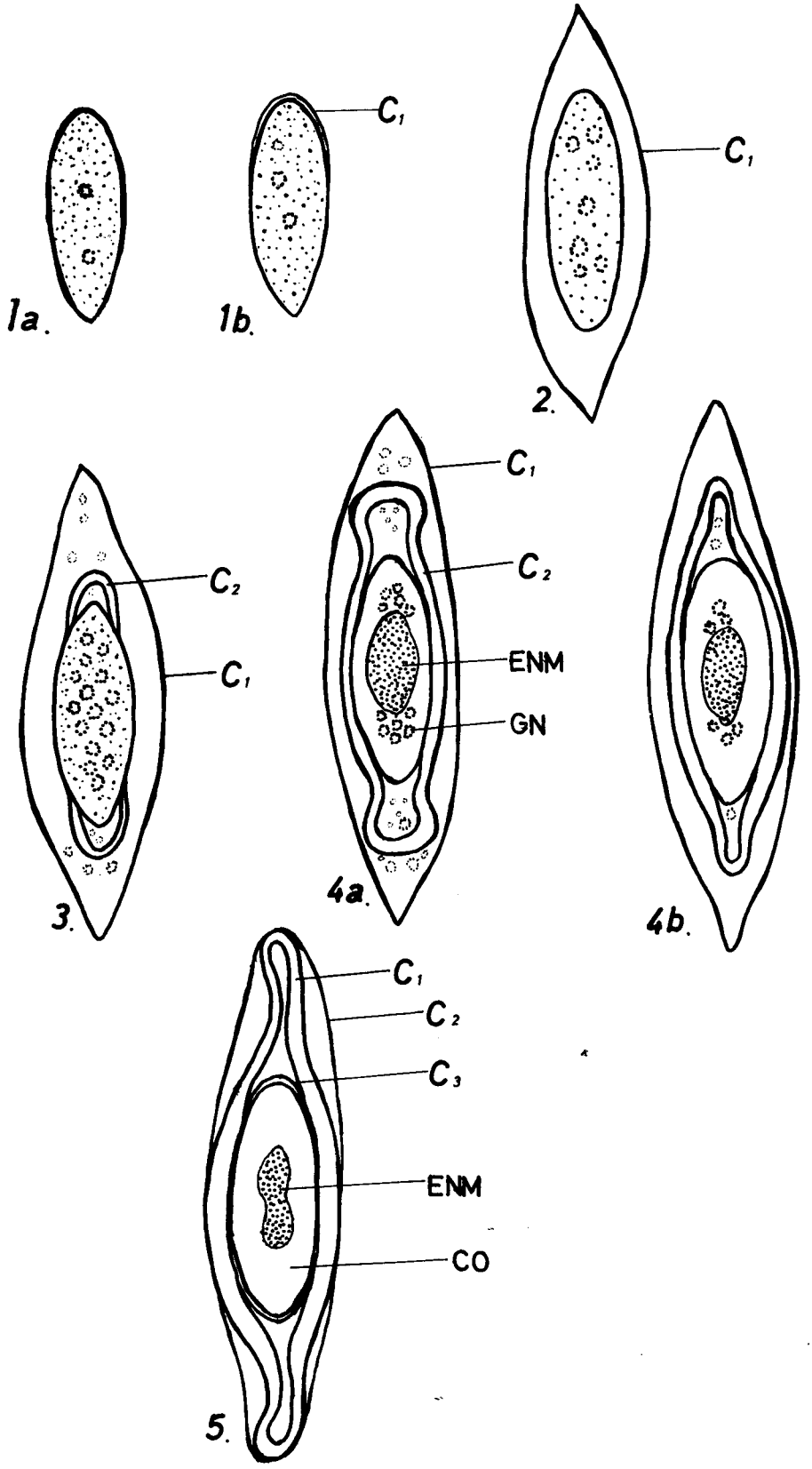

PLATE 10

form. (Plate 10, fig.5).

It was decided, therefore, to find out at which of these stages the acanthor became infective to the intermediate host.

(a) Materials and Methods.

On various occasions during the analysis of female worms obtained from the field for their state of sexual maturity, it was possible to obtain large numbers of acanthors at each of the above five stages of development. The acanthors at each stage were then divided into two lots. One lot was administered to G. pulex by the Elm-leaf method, the other was transferred to a petri dish and shrimps allowed to feed on them for 12 hours. The shrimps were then transferred to glass-covered enamel dishes in which they were maintained at room temperature. Dissection and examination of shrimps for infection, were done after 30, 38 and 46 days. These tests were repeated six times and on each occasion only acanthors freshly recovered from female worms were used.

(b) Observation.

No developing larva of the parasite was found in shrimps fed with acanthors at stages i - iv. However, in about 50% of the shrimps fed stage iv eggs, many very small black spots were observed to be attached to the intestine and its caeca. These 'black spots' represent parasites destroyed soon after penetrating the intestinal wall and were similar to those found in G. pulex at low temperatures (Chapter II). Even if these black spots were of stage iv acanthor origin, it is certain that the invasion was unsuccessful. Stage v acanthors on the other hand were

found to be definitely infective. In shrimps allowed to feed on these in petri dishes, there were many cases of gross over-infections.

3. ARTIFICIAL HATCHING.

Hereunder are given the three experiments which were designed to procure the hatching of the eggs of E. truttae. Stage v or mature acanthors only were used.

(a) Drying and Rewetting.

The acanthors were smeared on glass slides and left in a dry state for 5, 10, 20 minutes, 1 hour, 3, 12, 16 and 24 hours at 3 - 4°C, 13 - 14°C and at room temperature (18 - 23°C). They were then rewetted with stream or distilled water and examined under the binocular microscope.

No hatching was observed. Acanthors left in a dry state for 3 hours and over were found to be abnormal in form. The outer membrane was shrivelled and the central nuclear mass shrank into an amorphous mass, displaced from its usual position in the acanthor. Prolonged soaking in water did not lead to the recovery of the normal shape of the acanthors.

(b) Pepsin Solution.

The solution was made up as described by Meyer and Vik (1961): 7 grams of pepsin and 4 ml. of Hydrochloric acid in a litre of warm water. The fresh solution was poured into petri dishes. A thick suspension of acanthors was then transferred to these dishes which were incubated at

37°C. They were examined at hourly intervals.

When, after 6 hours, no eggs were hatched, incubation was continued overnight. Some petri dishes containing acanthors in the solution were also left overnight at 13 - 14°C and room temperature. It was observed that after 30 hours at the two latter temperatures and 18 hours at 37°C, hatching did not occur. The acanthors in the solution at 13 - 14°C and 18 - 20°C but not those at 37°C, remained infective to shrimps.

(c) Pepsin/Trypsin cycle.

In the third test, a method described by Erasmus (1962) for the excystation of the metacercaris of Holostephanus luhei Szidat 1936, was tried on the eggs of E. truttae. Two solutions were involved. There was a digest solution made up of 0.5 gm. pepsin, 0.75 ml. Hydrochloric acid (conc.) and 100 ml. of 0.6% Sodium Chloride. The other solution was the hatching solution and consisted of 100 ml. 1% Sodium bicarbonate, 1 gm. of trypsin and 0.5 gm. Sodium tauroglycocholate. The acanthors were first placed in petri dishes containing the digest solution and incubated at 37°C for $\frac{1}{4}$ - 1 hr. They were then transferred by means of a fine pipette to the hatching solution at 37°C and examined at intervals of 15 minutes during the first hour and thereafter at convenient intervals.

At the end of 3 hours the eggs failed to hatch but most of them had median constrictions of the outer and thick middle membranes. The dishes were thus returned to the incubator. After a further three hours, hatching did not take place nor was the position improved by leaving the eggs overnight at 37°C. Acanthors subjected to the pepsin/trypsin cycle

at 10 - 12°C and 18 - 20°C also failed to hatch.

Since none of the solutions dissolved any of the egg membranes and no movements of the acanthor were seen, it is patent that acanthors, per se, are unable to initiate the hatching process.

4. VIABILITY AND ENVIRONMENTAL FACTORS.

E. truttae has an entirely aquatic life cycle. Its acanthors would not, therefore, be subjected to extreme environmental conditions as those of other Acanthocephala with terrestrial life cycles. However, as noted earlier, the stream temperature varies from 2 - 4°C in winter to 10 - 15°C in summer. The marginal water of the stream has been observed to freeze in winter, while in summer parts of the bottom of the wider stretches are exposed but not entirely dried out. As far as the parasite is concerned, therefore, temperature and some degree of dryness may be considered relevant factors in connection with the viability of the acanthors. Experiments were thus carried out to study the effects of the following on viability:

i. Alternate drying and wetting.

ii. Continuous drying at various temperatures.

iii. Temperature on eggs in water.

iv. Freezing in stream water and in a dry state.

(b) Materials and Methods.

The eggs used in these experiments were obtained by dissecting female worms from naturally infected trout. Only freshly recovered eggs were employed. To determine the effect of continuous drying, some eggs

were smeared on glass slides while others were left in open petri dishes to dry. They were left in this state for various periods viz. 10, 20, 30 minutes, 1 hour, 3, 6, 12 and 24 hours at 3 - 4°C and room temperature. After each period the eggs were examined microscopically and then fed to shrimps in a petri dish. In one experiment at room temperature, acanthors dried for 5 minutes were subjected to alternate wetting and drying for a three-hour period and then fed to shrimps.

Egg smears were also dry-frozen at -8°C for 3 and 4 days. Eggs and whole gravid female worms in shallow water in separate dishes, were also frozen at -8°C for 5, 26 and 46 days. The eggs were then thawed at room temperature and shrimps allowed to feed on them for 12 hours. In the case of whole females, the parasite was teased before shrimps were introduced.

In tests made to find out how long acanthors remained viable at various stream temperatures as well as at room temperature, eggs were kept in petri dishes half-filled with stream water. They were uniformly dispersed in each petri dish and in tests which lasted for a long period, the water was replenished at intervals. The experiments were done at 2 - 4°C, 2 - 10°C, 4 - 8°C, 13 - 14°C and 18 - 20°C. Similar but less extensive tests were made with tap and distilled water at 3 - 5°C and 13 - 14°C. At weekly periods, some eggs were withdrawn with a fine pipette and fed to shrimps. The shrimps were autopsied after about 30 days. At this period acanthellae are easily recognised and most infected shrimps were alive. When, however, because of high room temperature or

otherwise, shrimps began to die earlier, the date of killing and examination was advanced as necessary.

(c) Observations.

The acanthors of E. truttae were unable to withstand drying lasting 5 minutes and over. Shrimps fed on acanthors dried for these periods were uninfected. As observed earlier, on drying, the protoplasmic matter of the embryo became granulated and the outer membranous envelope was shrivelled. Acanthors with this form may thus be regarded as dead.

All the forms of freezing investigated were also found to be lethal to the eggs. None of the shrimps fed on the frozen eggs was infected. Except in the case of dry-frozen specimens, egg membranes remained normal in form after freezing at -8°C. A closer examination showed that the embryo was granular all over and indistinguishable into the central nuclear mass and the peripheral cortical region. The latter region is normally non-granular at this stage under low magnification.

The results from viability tests at temperatures above freezing point were interesting. At room temperature 16°- 22°C, acanthors in stream water remained infective to shrimps for 7 days.only. In distilled and tap water, on the other hand, the eggs were viable after 36 and 25 days respectively at the same temperature. Repeated critical experiments to determine exactly when acanthors lost their viability at room temperature were unsuccessful. Most shrimps used in the tests died too early for observations made on them to be conclusive. The lower viability in stream water compared to tap and distilled water under experimental

conditions, may be due to high organic content of stream water. Microbial activity was evident in the latter after a few days at room temperature.

Viability under the stream conditions imitated in the cold room was as follows: At 2 - 10°C, acanthors were viable (remained infective to shrimps) for 185 days. At the lowest winter temperature of 2-4°C, the eggs were viable for 137 days. Because of the continual changes of the temperature of the cold room in line with those of the stream, it was not possible to expose the eggs to these temperatures for longer periods. At 13 - 14°C, the summer temperature of the stream, eggs were found to be viable for a maximum period of 64 days. The viability period in distilled water at 2 - 5°C was the same as that in stream water at 2 - 4°C. It was also observed in all the viability tests, that the number of acanthors remaining infective decreased with the period of exposure to a particular temperature. Thus, in the experiment in which acanthors were kept in stream water at 2 - 4°C, it was found that after the first two weeks about 80 - 90% of the shrimps used for viability tests were infected with 8 - 15 parasites per shrimp. After 14 weeks, less than 40% of the shrimps fed with about the same amount of eggs were infected with 1 - 3 parasites per shrimp.

5. DISCUSSION.

In Acanthocephala with terrestrial intermediate hosts, the acanthor is typically enclosed by four envelopes (Meyer 1936, Kates 1943) including two thick embryonic shells. The outer of these is thicker,

granular or striated and often brown in colour. Von Brand (1939a, 1940) has shown that in M. hirudinaceus, the envelope immediately enclosing the acanthor is as thick as the inner shell and has swelling properties. It was suggested that these swelling properties are responsible for the cracking open of the outer shell during hatching. On the basis of these findings, the differential effects of 'drying and wetting' in inducing hatching among the eggs of various species of Acanthocephala are understandable. Thus, while Manter (1928), Moore (1942) recorded successful hatching with the method in M. hirudinaceus, M. ingens, M. dubius, Mediorhynchus grandis and Hamanniella turtosa, it failed with the eggs of N. cylindratus, N. emydis and Centrorhynchus sp. Hynes and Nicholas (1958) were unable to hatch the eggs of P. minutus by the method. In the present study, the method has not only failed to induce hatching in E. truttae but proved fatal to the eggs. It is significant to note that all the Acanthocephala in which eggs were not hatched, have aquatic intermediate hosts. Their acanthors have three coverings and the envelope immediately ensheathing the acanthor is membranous and not thick. The latter, in E. truttae, has not been observed to swell in water. In E. truttae also, the hatching solutions pepsin, pepsin/trypsin are shown to be unable to dissolve the shells. The failure of the acanthors of this parasite to hatch artificially under the conditions tested, finds some explanation in a consideration of the probable chemical nature of the egg membranes (Monné and Hönnig 1954, a, b, c, Monné 1955, 1956). Monné and Hönnig (1954b) have shown that in the closely related species P. minutus and P. botulus, the inner membrane consists chiefly of chitin

with some keratin-like protein. The middle envelope is two-layered, the inner layer being proteinaceous but with a certain amount of chitin, while the outer layer is fibrillar and made up of keratin-like protein without chitin. The outer membranous envelope consists of non-keratinised protein. In this connection it is interesting to note that Von Brand (1940) demonstrated the presence of chitin in the innermost membranes of M. hirudinaceus.

Monné and Hönnig also found that the various ensheathing components exhibited different solubility properties. A similar observation is recorded for the acanthors of E. truttae. Using 0.5 - 10% solution of Sodium hypochlorite at 18°C it was found that the membranes were dissolved in this order, a. the inner envelope and the inner layer of the middle envelope; b. the fibrillar outer layer of the middle envelope and c. the outer membranous envelope, the latter being frequently still present when the other coats were dissolved. Sodium hypochlorite was, however, found to be lethal to the acanthors of E. truttae at all concentrations. Acanthor spines were also dissolved.

In nature, the resistant outer membrane and the thick middle envelope appear to be dealt with mechanically during the hatching process. Meyer (1933) regarded the characteristic elongated rolling-pin shape of the middle shell as an adaptation for ingestion by aquatic anthropods. It probably serves to protect the eggs from damage during their passage through the gastric mill. The results recorded earlier (Chapter II) and observations made by Hynes and Nicholas (1957) indicate that in nature, the action of the gastric mill of the crustacean intermediate hosts may

be essential in the hatching process. The ends of the middle shell which are variously curved in mature eggs, were observed to be clipped off by the gizzard. DeGiusti (1949), Hynes and Nicholas (1957) observed that some acanthors emerged while still enveloped by the inner membranous sheath. The opening of the thick middle shell thus brings the acanthor in touch with the environment within the intestine and hence provides the initial impetus for hatching.

The eggs of certain thorny-headed worms have been shown to be remarkably resistant to changes of the environment. Spindler and Kates (1940) found that in Beltsville, U.S.A., the eggs of M.hirudinaceus mixed with soil and pig faeces and given various exposures to the sun remained viable (infective to beetle grubs) for as long as $3\frac{1}{2}$ years. Kates (1942) recorded that the acanthors of the same species recovered from female worms, survived continuous exposures in water to temperatures up to 45°C, and freezing in water and in a dry state at -10oC to -16°C, for 140 days when the experiment was terminated. Eggs in water were, however, destroyed by instantaneous exposure to 70°C. No appreciable reduction in the number of viable eggs was observed in dry preparations exposed for 50 days at 5 - 9°C and 37 - 39°C and for 265 days at 21 - 26°C. Alternate wetting and drying at 37 - 39°C destroyed the viability of eggs mixed with soil in 368 days, but eggs subjected to the same treatment at 2 - 5°C were still viable after 551 days. More modest results are recorded for worms with aquatic life cycles. De Giusti (1949) reported that the eggs of L. thecatus recovered from the body cavity of female worms and stored in water in a refrigerator at 4°C, were viable after 9 months. Petrochenko (1956) showed that the eggs of P.magnus lived no

less than 6 months in water at 10 - 17°C. The loss of infectivity of the eggs of P. minutus from female worms, occurred between the 9th and 16th day in water at about 17°C (Hynes and Nicholas 1957). In the present study it is shown that the eggs of E. truttae in water are sensitive to abnormally high and low temperatures. Thus, while some acanthors remained viable after 185 days at 2 - 10°C (the prevailing autumn, winter and early spring temperature range of the stream in 1962/63), viability has not been demonstrated after 8 days at 19 - 22°C. At the normal summer stream temperature of about 14°C, the eggs remained viable for 64 days. Freezing at -8°C proved fatal to the acanthors.

Although more work with the acanthors of other acanthocephalan species is called for, it appears from available data that the eggs of these worms with aquatic life cycles may not show anything like the resistance to extreme environmental changes exhibited by M.hirudinaceus. Simple hatching methods have so far failed with these eggs and it is possible that they may have special artificial hatching requirements.

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CHAPTER IV

STUDIES ON E. TRUTTAE IN SOME CRUSTACEAN HOSTS

1. INTRODUCTION.

G. pulex and other gammarids have been widely recorded as intermediate hosts of acanthocephalan parasites (Leuckart 1862, Greef 1864, Lühe 1911, Nybelin 1923, 1924, Dorier 1931/32, Scheer 1934b, Steinstrasser 1936, Jaczo 1943, Rašin 1949, Bauer 1953, Schäperclaus 1954, Hoffmann 1954, Hynes 1955, Lucasovics 1959, Kovalenko 1960 'inter alia'). However, observations on host-parasite relations between the Acanthocephala and their gammarid or other amphipod hosts are scanty. Le Roux (1933), Hynes (1955), have noted that female Gammarus infected with the cystacanths of P.minutus did not reproduce normally. Male shrimps, on the other hand, appeared to be unimpaired in their reproductive functions by the presence of cystacanths. Hynes and Nicholas (1957, 1963) recorded further that while P.minutus caused reproductive disturbances in G.pulex, the much larger cystacanths of E.truttae appeared to exert no effect on the reproductive capability of shrimps. Munro (1953) found that there was intersexuality in the isopod A.aquaticus parasitised by a polymorphid acanthocephalan. These findings indicate that in some cases acanthocephalan parasites may have far-reaching consequences on the life of their intermediate hosts. Freshwater Acanthocephala are known to have

either annual or no definite life cycles (Van Cleave 1916, 1920, Sternstrasser (1936), De Giusti (1949), Komarova 1950, Bauer 1953, Shulman and Shulman-Albova (1953), Akmerov 1959, Bullock 1962, Chapter VII). It is apparent, therefore, that if these parasites can effectively interfere with the reproduction of their essential hosts as shown above, the very existence of the parasites would be gravely endangered.

Preliminary observations on the relationship between E.truttae and Gammarus spp. showed that the parasite developed in G.lacustris and G.pulex but not in G.duebeni and G.tigrinus (Chapter II) All these species are known to occur in Britain (Hynes 1951, 1954 a, b, 1955a). E.truttae also failed to develop in the isopod A.aquaticus often found together with the natural intermediate host G.pulex. It was also noted that at low temperatures 2 - 4°C, a good number of acanthors did not successfully establish in G.pulex. Eggs at a late stage of development (Stage iv) apparently invaded shrimps but were unable to establish in them (Chapter III).

In the present work, some aspects of the above observations on host-parasite relations are considered. More evidence on parasitic castration has been gathered, while experimental studies are made to elucidate field and laboratory observations on the intra- and inter-species relations of P.minutus and E.truttae in their common natural intermediate host G.pulex.

2. THE EFFECT OF PARASITISATION ON THE GROWTH OF G.PULEX.

(a) Field Observations.

While going through monthly samples taken in connection with an

investigation of the population dynamics of larval E.truttae in G.pulex in the R.Terrig (Chapter VII), it was noticed that infected shrimps 4 - 6 mm. long also harboured the cystacanth stage (Table 4.1). Usually shrimps with cystacanths were mature forms of normal adult size. Hynes (1955) has shown that adult G.pulex are usually above 8 mm. in length. Thus at Shotwick (Cheshire), the percentage of male G.pulex above 9 mm. in length varied from 2% in June to 46% in September and November. In the River Terrig it was noticed that male shrimps were considerably above 10 mm. towards the fall.

It has been shown experimentally (Chapter II) that in summer, the cystacanth of E.truttae is formed in about three months depending on the prevailing temperature of the stream. At 14 - 15°C it takes 80 days, while at 10 - 14°C cystacanths are recovered after 96 days of development. Hynes (1955) showed that young G.pulex appearing in March were mature in July (3 - 4 months). Since infected shrimps less than 5 mm. long were observed in the stream in late summer and the fall, it seems reasonably certain that these shrimps belong to either the spring or early summer brood which, by the autumn, are above 8 mm. long. This would suggest that parasitisation by E.truttae has a deleterious effect on the growth of shrimps. Experiments were thus conducted to verify this observation.

(b) Laboratory experiments.

Shrimps collected from Shotwick stream (Cheshire) were sorted into two size groups viz: about 3 mm. and 6 mm. long respectively. They were then infected with the acanthors of E.truttae using the Elm-leaf

TABLE 4. 1

Showing the occurrence of G.pulex retarded in their growth as a result of parasitisation by E.truttae in the Afon Terrig. All shrimps with cystacanths below were retarded.

Month	Total no.of shrimps <6 mm. examined.	No.infected with cystacanths.	% of cystacanths of of total no.infected.
Jan.	360	4	57.2
Feb.	240	2	28.6
Mar.	390	0	-
April	360	1	25.0
May	360	4	80.0
June	360	0	-
July	360	0	-
Aug.	360	0	-
Sep.	360	0	-
Oct.	360	2	28.6
Nov.	360	6	66.7
Dec.	360	6	60.0

method and cultures maintained at room temperature. Control shrimps were kept under similar conditions.

After 85 days only 5 infected shrimps were alive in the 6 mm. size group, while 9 remained in the 3 mm. group. As expected, they all bore cystacanths. Shrimps in the former size group were 8 - 9 mm. long and those in the latter group 5 - 6 mm. long. Only two control shrimps of the initial 6 mm. size group remained on the 85th day. Both measured 9 mm. long. All the 20 control shrimps in the other size group died after 40 days. The cause of this mass mortality was unknown. A thin film of mouldy growth was, however, observed to cover the surface of water in which the shrimps were kept.

In another experiment, 60 G.pulex 3 - 5 mm. in length were used. After 65 days, it was noted that a substantial number of shrimps had died. A sample of 5 infected and 5 control shrimps were killed with Ethyl acetate and measured. Both infected and uninfected shrimps were 5 - 7 mm. long with a mean length of 6.5 mm. After 80 days, the only surviving infected shrimp was 6 mm. long.

To circumvent the possible adverse effects of the wide daily fluctuation of room temperature on shrimps, the experiment was repeated under the more natural conditions in the cold room. Most shimps were kept in enamel dishes while some were maintained in Hanson's bottles in a current of circulated stream water (Chapter II). Since development has been shown to be slow at low temperatures (De Giusti 1949 Chapter II) shrimps were left as long as possible before examination (January - July 1962 at 4 - 14°C). It was also necessary to keep shrimps for a long

time so that the effect of the parasite on growth, if any, could be differentiated from natural variation in their size. After 171 days cystacanths were formed but most shrimps were dead. The remaining shrimp in the 3 - 5 mm. size group was 6 mm. long and bore one cystacanth. The three shrimps in the other size group measured 7, 8 and 8.5 mm. respectively.

(c) Conclusions.

Although only a few shrimps were left at the end of each experiment because of the difficulty of maintaining G. pulex for a long period under laboratory conditions (Appendix I, Tables 1 and 2), the available data are nevertheless interesting. Shrimps infected while still very young, about 3 mm. long, were 5 - 6 mm. long after 85 days. At the latter period, they would normally be expected to attain the adult size. On the other hand, shrimps infected when 6 mm. and above in length, were shown to attain the adult size of 8 - 9 mm. after 85 days, under similar experimental conditions. It is suggested, therefore, that shrimps infected while very young may not be able to attain the normal adult size. Normal infection at later stages of the life cycle does not appear to influence the growth of G.pulex.

3. PARASITIC CASTRATION.

From November 1961 to January 1963, many thousands of G.pulex from Afon Terrig were examined and observations made on the condition of the gonads and stage of sexual maturity of infected shrimps. This was done in order to verify the earlier recorded observations (Hynes and Nicholas 1957, 1963) on the effect of E.truttae on the reproductive cycle

of G.pulex. Wherever possible, the gonads of infected specimens were compared with those uninfected shrimps of similar size taken simultaneously from the stream. Although shrimps with larval Acanthocephala at all stages of development were met, a special note was made of those shrimps with cystacanths during the breeding season. It is patent that any adverse effects, both mechanical and humoral, exerted by the parasite would be expected to be apparent in shrimps by the time the parasite attained the cystacanth stage.

It was found that in over 300 adult G.pulex in which cystacanths were present in the haemocoel, no lesions or abnormality of form in the gonads of male and female shrimps were apparent. Infected ovigerous female shrimps were taken at the appropriate time of the year. Similar observations were made in experimental shrimps. Cultures of G.pulex infected in the laboratory in January and February, and maintained at the prevailing stream and room temperatures, bred in Spring. During this period, the shrimps bore either late acanthellae or cystacanths depending on the temperature.

It seems conclusive, therefore, that this parasite has no apparent effect on the reproductive cycle of its intermediate host, G.pulex.

4. THE RESISTANCE OF SOME AMPHIPOD AND ISOPOD SPECIES TO INFECTION BY E. TRUTTAE.

During experimental studies of the development of E.truttae in G.pulex (Chapter II) it was noted that in some cases, especially at 2 - 4°C, infected shrimps had in addition to larval Acanthocephala small brown or black bodies attached to their intestine. Hynes and Nicholas (1958)

observed a similar phenomenon while dealing with P.minutus. They further showed that the 'black spots' were, in fact, dead acanthors. It was also noted (Chapter II) that G.lacustris exposed to infection by the Elm-leaf method for the usual period contained destroyed parasites in the form of brown or darker spots at both low and high temperatures. Some acanthellae had reached the late oval stage before they were killed. There is little doubt that in these cases fewer parasites, than would otherwise, succeeded in establishing themselves in G.pulex and G.lacustris. Hynes and Nicholas (1958) have found that each of the three British Gammarus species showed resistance to cross-infection with acanthors of P.minutus originating from the other two. There were thus three strains of P.minutus corresponding to the three intermediate hosts investigated. In E.truttae, the formation of strains may be precluded since it appears that G.pulex may be the only natural intermediate host in Britain.

'Black spots' have been observed in naturally infected specimens. As might be expected, these black spots in nature would arise from various sources. Some of these spots in G.pulex from R.Terrig were definitely not of acanthocephalan origin. They were neither of the form observed in earlier laboratory investigations on E.truttae (Chapters II and III) nor did they resemble those described for L.thecatus and P.minutus (De Giusti 1949, Hynes and Nicholas 1958). Records were kept of the number of shrimps in which there were many black spots (>5 per shrimp) of apparently acanthocephalan origin. These showed that dead parasites occur throughout the year. The highest incidence was in

December where 106 out of the 1200 shrimps examined had numerous black spots (Fig.4. 1). Since the incidence of P.minutus in shrimps during the period of investigation (November 1961 - January 1963) was very low, it is assumed that Fig. 4. 1 gives some indication of the reaction of G.pulex to E.truttae in nature.

To throw some light on the nature and extent of this host reaction to the establishment of E.truttae, it was decided to study the relative reactions of four species of Gammarus and two of Asellus to invasion by this ananthocephalan.

(b) Materials and Methods.

In the first three experiments of the series, the reactions of G.pulex, G. lacustris and G.duebeni were studied. Adult shrimps only were used in the first and third experiments. G.pulex came from the usual sources (Shotwick stream and Raby ponds, Cheshire). G. lacustris and G.duebeni were kindly supplied by Dr.Hynes of this department. The former species was collected from Llyn Llywenan (Anglesey) and the latter from the Isle of Man. Both were maintained in the laboratory (cold room), for 6 - 7 months before use. To determine if there was 'age effect' on the reactions of these species, only young shrimps were used in the second experiment. G.pulex used were about 4 mm. long and the other two species about 5 mm. in length. Young G.lacustris came from Hatchmere (Cheshire) and G.duebeni from brackish ponds near the sea coast of Wallasey (Wirral). Acanthors were administered to groups of 20 shrimps by the Elm-leaf method. In each experiment shrimps were maintained at room temperature and the prevailing stream temperature provided in the

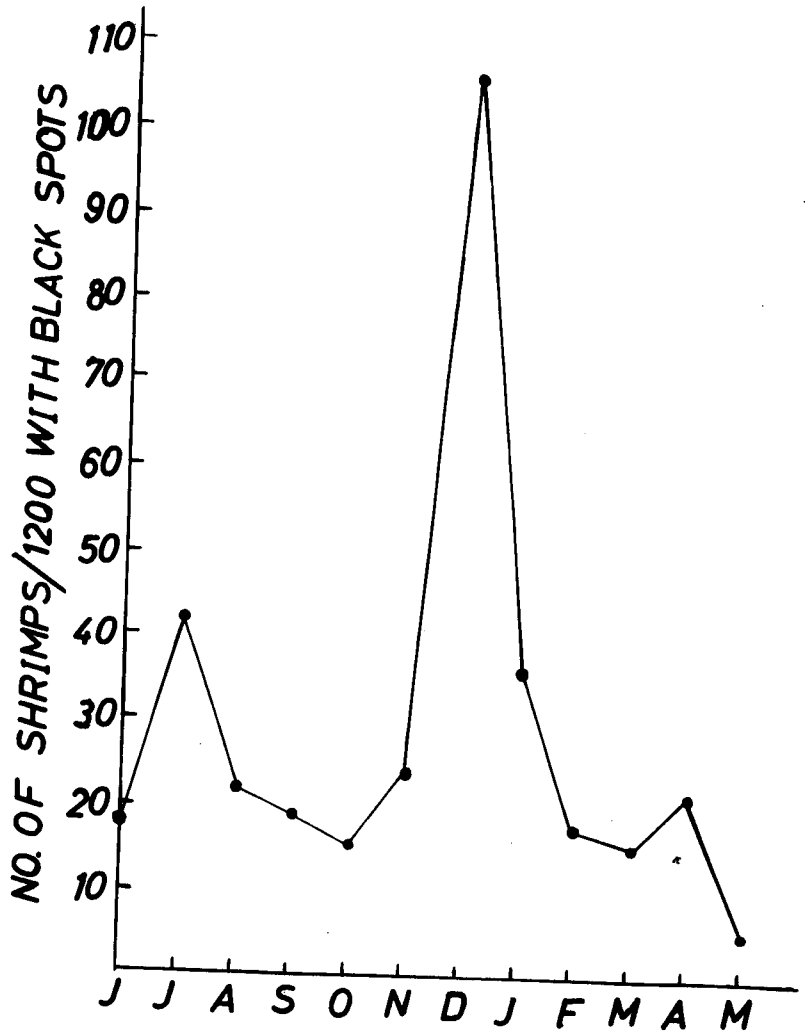

FIG. 4·1 SEASONAL VARIATION IN NO. OF SHRIMPS WITH MANY BLACK SPOTS.

cold room viz: 8 - 2oC and 11°C. Because of the difficulty experienced earlier in maintaining cultures of G.pulex in still water, the experiments involving adult shrimps were terminated after 34 and 35 days and those with young shrimps, after 48 days. Both living and dead acanthellae are easy to recognise after these periods of development.

In the fourth experiment, G.tigrinus collected from salt marshes on the estuary of River Ribble (Lancashire) were allowed to feed on acanthors in petri dishes for 24 hours at 18°C. They were then kept in brackish water at 4°C and 18 - 20°C. The isolation of this experiment from those for the other Gammarus spp. was necessitated by the smaller numbers of G.tigrinus available.

In the fifth experiment a mixture of A.aquaticus L. and A. meridianus Racovitza 1919 from Llyn Llywenan were fed acanthors of E.truttae as described in the fourth experiment. In these two latter experiments, autopsy was done after 38 days.

(c) Observations.

The results are outlined in Tables 4. 2 - 4. 4. It was found that the number of shrimps with dead parasites as well as the number of dead parasites increased at low temperatures as far as both G.pulex and G.lacustris were concerned. Thus in G.pulex at 2 - 8°C, 72.2% of adult shrimps contained dead Acanthocephala (Table 4. 3) as opposed to 25.0% and 28.1% at room temperature (Table 4. 2). There was also no marked difference between the resistance of young and adult G.pulex at the temperatures investigated.

TABLE 4. 2

Showing the details of results obtained when three Gammarus spp. were infected by E.truttae at room temperature.

Exp. No.	Temp. °C.	Months when expt. performed.	Day on which shrimps killed	Species Investigated	Initial No. of Shrimps	No. Surviving	No.(%) normally infected.	No. (%) with black spots only	No. (%) with both parasites and blackspots.	No. (%) un-infected
1	16-21	April May	35	G.pulex	44	36	26 (72.2)	0	9 (25)	1 (2.8)
				G.lacustris	40	24	1 (4.2)	1 (4.2)	22 (91.6)	0
				G.duebeni	40	32	0	30 (93.7)	0	2 (6.3)
2 *	16-23	May June July	48	G.pulex	40	31	18 (58.1)	0	13 (41.9)	0
				G.lacustris	40	32	4 (12.5)	3 (9.4)	25 (4) + (78.1)	0
				G.duebeni	40	40	0	38 (95)	0	2 (5)
3	9-20	November December	34	G.pulex	44	32	23 (71.9)	0	9 (28.1)	0
				G.lacustris	40	22	0	5 (22.7)	17 (77.3)	0
				G.duebeni	40	26	0	24 (92.4)	1 + (3.8)	1 (3.8)

* Young shrimps used.

\+ Shrimps in which larval parasites were in an unhealthy condition.

Table 4. 3

Showing the details of results obtained when three Gammarus spp. were infected by E.truttae under imitated natural environmental conditions.

Expt. No.	Temp. °C.	Months when expt. performed	Day on which shrimps killed	Species Investigated	Inition No. of shrimps	No. Surviving.	No.(%) normally infected.	No.(%) with black spots only	No.(%) with both parasites and black spots	No. (%) un-infected
1	11	April May	35	G.pulex	44	36	17 (47.2)	0	18 (50)	1 (2.8)
				G.lacustris	40	31	2 (6.5)	5 (16.1)	23 (74.2)	1 (3.2)
				G.duebeni	40	40	0	38 (95)	1 (2.5)	1 (2.5)
2 *	11	May June July	48	G.pulex	40	38	13 (34.2)	0	24 (63.2)	1 (2.6)
				G.lacustris	40	38	10 (26.3)	1 (2.6)	26(2) + (68.5)	1 (2.6)
				G.duebeni	40	38	0	38 (100)	0	0
3	2-8	November December	34	G.pulex	40	36	5 (13.9)	2 (5.5)	24 (66.7)	5 (13.9)
				G.lacustris	40	40	0	28 (70)	0	12 (30)
				G.duebeni	40	38	0	37 (97.4)	0	1 (2.6)

* Young shrimps used

\+ Number of shrimps out of the total in which larval parasites were in an unhealthy condition

In G. lacustris there was a strong reaction to the presence of the parasite in their body cavity. All developing larvae were surrounded to varying extents by host cells. This covering of larva by host cells was invariably thick in the case of acanthors and oval acanthellae. Elongated forms had a very thin and discontinuous investment except at the ends of their bodies where host cells tended to be amassed. This is contrast to G.pulex in which oval and elongated forms were rarely seen to have an investment of host cells. Dead parasites recovered after 35 days from G.lacustris were brown spherical bodies. These were found in practically all infected shrimps at room temperature but as indicated above, the greater number of brown spots per shrimp as well as their smaller size showed that the reaction of G.lacustris was stronger at low temperatures. It was further observed that the dead parasites got after the same experimental period were black (black spots), As in G.pulex , young G.lacustris were not less resistant to infection.

The available data show that G.lacustris offers more resistance to the parasite than G.pulex. Thus, at room temperature, approximately 98% of adult G.lacustris showed resistance compared to 26% of G.pulex.

Apart from one dubious case at 11°C (Table 4. 3), the resistance of G.duebeni to the establishment of acanthors is shown to be complete. When a similar case of pale spherical acanthellae was found in Experiment No.3 at room temperature, the parasites were fixed in Alcohol-formol-acetic and stained in Acetic haematoxylin for critical study. This showed that the acanthellae had been dead when found. It was also observed that in G.duebeni the dead parasites were distinctly black at both temperatures. While at

room temperature most black spots tended to be large and oval, at 2 - 8°C they were generally smaller with a spherical or spindle shape. Another interesting observation was that many black spots were found at room temperature whereas at 2 - 8°C dead parasites were comparatively few viz: 1 - 5 per shrimp. This is either due to a low number of acanthors having been ingested under 'cold room' conditions or to the failure of many ingested acanthors to penetrate the intestinal wall. Such a difference was not found in G.lacustris and G.pulex infected under similar conditions.

The results of the experiments with G.tigrinus are given hereunder:

Expt. No.4:

Temperature	4°C	18 - 20°C
Initial no. of G.tigrinus	40	40
No. left after 37 days	37	8
No.normally infected	0	0
No. with larvae and black spots	0	0
No. with Black Spots only	6	6
No. uninfected	20	2
No. of black spots per shrimp	2 - 3	20 - 30

Table 4. 4 showing the resistance of G.tigrinus to infection by acanthors of E.truttae.

Repetition of the above experiment with a smaller number of shrimps kept at room temperature for 60 days gave a similar result - no parasite was successfully established.

It would appear, therefore, that G.tigrinus is very resistant to E. truttae. Not only were no parasites living on the 37th day but it was also observed that the dead parasites were black and rod-like - 'black rods'. As in G.duebeni, there were fewer dead parasites at 4°C than at room temperature. It was also interesting to note that female shrimps

kept at room temperature were ovigerous by the 37th day showing that any reactions which may have been consequent on the unsuccessful invasion by this parasite did not disturb the reproductive cycle of G.tigrinus.

The investigation with Asellus spp. was ended after 38 days at room temperature. No Asellus bore developing acanthellae. In about 33% of the shrimps, however, dark-brown hyaline cysts with dead parasites at their centre were found within the haemocoel, covered with a thick investment of host cells. There were usually 1 - 2 such cysts in each Asellus. Both species were unsuccessfully invaded. As in G.duebeni and G.tigrinus, it is apparent that only a few acanthors got into the body cavity.

A number of supporting tests were made, i) to determine if a breakdown of resistance occurs in the more resistant Gammarus species when a large number of acanthors were fed to a host; ii) to verify the observed occurrence of a small number of black spots in the more resistant species.

As G.duebeni was easily obtained in good numbers and because this species kept reasonably well under experimental conditions, it was used in the experiments. The effect of exposing G.duebeni to heavy infection for a brief period was first investigated. Adult shrimps were allowed to feed on a high concentration of acanthors in petri dishes for 5 hours at 17°C. The infection was done at this temperature to ensure the ingestion of as large a number of acanthors as possible. Shrimps were kept at 3 - 4°C. A control was set up to help distinguish any 'black spots' which may have been initially present in shrimps. They were killed and

examined after 38 days and the results were as follows:

Expt. No.6.

	Infected shrimps	Control
Total no. of shrimps dissected	40	40
No. with E.truttae only	0	0
No. with black spots only	30	9*
No. with black spots and E.truttae	0	0
No. with 1 - 5 black spots	5	8
No. with 6 - 30 black spots	25	1

Table 4. 5. Showing details of experiment in which G.duebeni was exposed to heavy infection for a short period.

* Black spots not of acanthocephalan origin. Dead acanthors and acanthella of E.truttae were distincly different from naturally occurring brown and dark spots which were, in many cases, mere specks.

To investigate the effect of prolonged exposure to infection on the resistance of G.duebeni, shrimps were infected as described above for 48 hours. After this period both the shrimps and acanthors were transferred to enamel dishes so that eggs were accessible to shrimps for a longer time. They were maintained at 14 - 15°C in the first test (Expt. No.7) and at 3 - 7°C and 16 - 20°C in the second test (Expt. No.8). In the latter experiment, because of the high mortality of shrimps recorded at 14 - 15°C, autopsy was carried out after 16 days at room temperature and after 44 days at 3 - 7°C. The results are given hereunder:

Experiment No.7.

Initial no. of shrimps	40
No.left after 38 days	10
No. with E.truttae only	0
No. with E.truttae and black spots	1
No. with black spots only	9
No. of black spots per shrimp	20 - 200

Table 4. 6. Showing the details of experiment in which G.duebeni was exposed to heavy infection for an extended period at 14 - 15°C.

Experiment No.8.

Temperature	16 - 20°C	3 - 7°C
Initial No. of shrimps	47	47
No. at autopsy	4	34
No. with E.truttae only	0	0
No. with E.truttae and black spots	0	0
No. with black spots only	4	29
No. of black spots per shimp.	14 - 163	5 - 15

Table 4. 7 Showing the details of experiment in which G.duebeni was exposed to heavy infection for an extended period at both low and high temperatures.

It was noticed that all black spots were surrounded by a dense mass of host cells. In one of the experiments in which shrimps were exposed to heavy infection for a long period (Expt. No.7), the five spherical acanthellae in the only shrimp showing successful invasion after the experimental period, had very thin coverings of host cells and were normal in form. Earlier observations (Chapter II) have shown that this parasite failed to establish in G.duebeni when left for a long period at room temperature. It seems, therefore, that the five early acanthellae which escaped destruction for 38 days may have been killed on being left in the shrimp for a longer time. It would, however, appear that while a brief exposure to heavy infection had no obvious effect on the

resistance of this species, continuous exposure may lead to a prolongation of the period during which all parasites were killed. It also seems, from the results, that at low temperatures a comparatively much lower number of acanthors got through the intestinal wall barrier.

(d) Summary.

The results of these series of investigations are in accord with observations on host-parasite relations made in earlier studies (Chapters II and III) and in the field, and may be summarized as follows:
The parasite failed to develop in G.duebeni, G. tigrinus, A.aquaticus and A. meridianus because of the strong reaction of these species to the presence of this parasite in their body cavities. Brief or continuous exposure to heavy invasion did not appear to be capable of altering the resistance barrier permanently in G.duebeni. It is suggested that this may be true of the other three species which ordinarily showed more resistance than G.duebeni. G. pulex and G.lacustris, while showing resistance to the parasite, were unable to arrest the development of a substantial number of invading parasites. As would be expected, many more acanthors established and developed normally in the natural intermediate host G.pulex than in G.lacustris.

5. CO-INVASION OF G.PULEX BY P.MINUTUS AND E.TRUTTAE.

Although there are numerous records of heterologous and analogous co-invasion of hosts by helminths involving the Acanthocephala, attempts at ascertaining the relations between these parasites either experimentally or otherwise are not many. Cross (1934a, b) reported

the existence of an inverse relationship in the numbers of the cestode Proteocephalus exiguus La Rue 1911 and Neoechinorhynchus invading ciscoes of the Trout Lake region, North Wisconsin, U.S.A. Beck (1951) while studying the effects of various factors on egg production of single worm infections of H.diminuta, observed some cases of accidental infections by the thorny-headed worm Moniliformis dubius. Although he concluded that there was no discernible influence on the trend in egg production by the tapeworms, he suggested that there might be some crowding effect and competition. Holmes (1961, 1962a) applied statistical methods to determine the effects of concurrent infection on H.diminuta and M.dubius in rats. He found that the general effects on the parasites were comparable to crowding. There appears to be no record of any study of the interspecies relations of two acanthocephalans in their arthropod intermediate host.

In the River Terrig, P.minutus and E.truttae share the same intermediate host - G.pulex. Field observations made over a period of 15 months show that though both acanthocephalans were common in the lower half of the stream, they were only occasionally found together in the same shrimp. Of the many thousands of shrimps dissected and examined under the binocular microscope during the period, only three had both parasite species in their haemocoel. The larvae thus recovered were apparently healthy but at different stages of development. In none of the three cases were infective stages of the parasites present. The low incidence of concurrent invasion by both species may be due to the generally low incidence of P.minutus in the stream. It was also observed

that while P.minutus was frequently taken in the lower half of the stream, it was very scarce upstream. The reverse was the case with E.truttae.

Experimental studies were thus made with the following aims - 1. to investigate the relations of these two acanthocephalans in their intermediate host; 2. to find out if their relationship in G.pulex has any effect on their distribution in the stream. The details of development and considerable information on host-parasite relations in normal single species infection of these worms, which are a necessary prelude to this type of study, are available. The works of Petrochenko (1956), Hynes and Nicholas (1957, 1958) provide such information for P.minutus, while in this thesis (Chapters II, III and IV), a similar background knowledge on E.truttae has been supplied.

(b) Materials and Methods.

Uninfected shrimps used in these experiments came mainly from the stock raised in the laboratory. This was supplemented by shrimps from the field. The eggs of E.truttae were obtained as described before, by dissecting female worms recovered from trout taken from the River Terrig. To obtain the eggs of P.minutus the procedure described by Nicholas and Hynes (1958) was adopted. Three one-month old Aylesbury ducks were fed with shrimps containing the cystacanths of P.minutus collected from a small stream at Wistanswick (Shropshire). As above, mature acanthors were obtained by dissecting female worms recovered from ducks at autopsy on the 37th and 47th day after infection.

To secure simultaneous co-invasion, a mixture consisting of equal

volumes of the acanthors of both parasites was fed to shrimps. Two methods were employed. Some shrimps were fed by the Elm-leaf method except that in this case, the period of exposure to infection was limited to 12 hours. The other shrimps were allowed to feed on a thin suspension of acanthors in petri dishes for $\frac{1}{4}$, $\frac{1}{2}$, 1, 3 and 12 hours respectively. This was done to obtain various intensities of infection involving both parasites.

Experiments aimed at ascertaining the relationships in successive co-invasion effected either early or very late in the development of one of the parasites were also performed. In the first series of the experiments, shrimps with obvious cases of infection with cystacanths of either parasite were used. These were collected from the field and kept in the laboratory at low temperatures for 3 - 8 weeks before use. Those with the cystacanths of E.truttae were fed the acanthors of P.minutus and vice versa. Both methods of administering acanthors were employed Since it appeared from earlier experiments that large-scale penetration of the intestine for a long period had adverse effect on the life-span of shrimps (Table 4. 6 and 4. 7) the period of exposure of adult infected shrimps to infection in petri dishes was limited to a maximum of one hour. In the second series, one lot of uninfected shrimps were fed eggs of E.truttae and five days later, the latter were challenged with the acanthors of P.minutus. With the other lot, P.minutus eggs were challenged by those of E.truttae after an interval of five days. After each feeding period shrimps were washed to free them from adhering eggs and then kept as usual in glass-covered enamel dishes in groups of twenty.

Experiments involving intra-specific challenges as well as control single-species infections, were set up. As above, shrimps were infected in such a way as to produce normal and heavy infestation by each parasite species. In order to follow the trends in the relationship of both species at all stages of development, three to six shrimps from each experimental group were dissected and examined at weekly intervals.

(c) Observations.

(i) Simultaneous co-invasion.

Both E.truttae and P.minutus were found to establish simultaneously in G.pulex without any apparent deleterious effects on either species. Growth and differentiation of these species proceeded at the same rate as in the control specimens. P.minutus was observed to develop at a faster rate than E.truttae. Thus, after 39 days of development, oval acanthellae of E.truttae about 231 microns long and 157 microns wide were recovered, while most P.minutus larvae were at the late acanthellae stage, in which the regional differentiation of the body into fore, middle and hind sections had begun. These are in agreement with the findings of Hynes and Nicholas (1957) and those recorded earlier (Chapter II) in this thesis. Other observations on the size of acanthellae after various periods in simultaneous concurrent infection are given in Table 4. 8. Only cases where shrimps contained 1 - 2 larvae of P.minutus and one of E.truttae and vice versa are given. This allows a wide margin for crowding effects, since shrimps taken from Wistanswick bore up to seven cystacanths of P.minutus. It has also been shown experimentally (Chapter II) that the

TABLE 4. 8

Showing growth and development relations of E.truttae and P.minutus in Simultaneous Co-invasion. Data based on infections comprising altogether 2-3 larvae of both Acanthocephala.

Days after infection	E. truttae			P.minutus		
	Stage of development.	Size: diameter or length (mm)	Control (mm)	Stage of development.	Size: diameter or length(mm)	Control (mm)
25	Spherical		0.116	Spherical	0.165	
25	Oval	0.133	0.132	Oval	0.198	0.231
32	'Stalked' Spherical	0.176	0.165	Elongated: proboscis differentiated	1.320	1.397
39	Oval	0.231	0.275	Late Acanthellae *	?	?
46	Elongated	1.210	1.012	Late Acanthellae	?	?
53	Elongated: proboscis differentiated.	2.365	2.244	Soft cystacanths and cystacanths	?	?
60	*Retracted proboscis	?		"	?	?
81	Cystacanth	?		"	?	?

* Not measured. Body wrinkled. Proboscis and fore-body retracted.

critical intensity of infection in half-grown shrimps is about 3 larvae of E.truttae.

Both species attained the infective stages at the normal time in both simultaneous co-invasion and control specimens. The cystacanths of P.minutus were first taken after 53 days while those of E.truttae were recovered after 81 days.

(ii) Successive co-invasion.

(1) Acanthor challenges.

Shrimps in which the larvae of one acanthocephalan were allowed to establish for five days before the eggs of the other species were administered, were shown to be successfully invaded by the latter. The development of both species was normal and their growth rate compared favourably with those of control infections. All the landmarks in the development of either species were arrived at at approximately the usual time. Initial heavy infestation by one species did not seem to affect the establishment of the other. For instance, 32 days after a concurrent infection in which the eggs of P.minutus preceded those of E.truttae, the following recovery was made from one shrimp, viz: 12 acanthellae of P.minutus consisting of 2 spherical, 3 oval and 7 elongated forms; and 2 oval acanthellae of E.truttae. In shrimps heavily parasitised by both species, retardation in growth and differentiation in some of the acanthellae was observed. The retarded larvae belonged to both species. Since acanthellae in control shrimps as well as those in single-species challenges exposed to heavy infection, showed the same order of retardation, it is

concluded that the distrubances in growth and development in co-invasion involving many acanthellae of these two Acanthocephala, were due to the effects of crowding (Chapter II).

(2) Cystacanth - Acanthor challenges.

In infections in which the cystacanths of E.truttae were challenged by the eggs of P.minutus and vice versa, development from the acanthor stage was found to take place normally. Thus, in the former challenge, the only infected shrimp left on the 49th day contained one cystacanth of E.truttae and 2 soft cystacanths of P.minutus. Similar observations on the development of larvae were made in experiments where the cystacanth of P.minutus was challenged with the eggs of E.truttae. After 46 days, shrimps containing 2 - 3 cystacanths of P.minutus also bore healthy acanthellae of E.truttae at the early stage of elongation (550 x 220 microns). Shrimps dissected on the 81st day were found to harbour the cystacanth of E.truttae in addition to those of P.minutus present at the beginning of the experiment. Also in single-species cystacanth-acanthor challenge, the development of the acanthor to the infective stage was accomplished in the normal period.

6. DISCUSSION.

Smyth (1962) explained that "the basis for resistance to animal parasite infections is essentially the same as for viruses, bacteria or non-living materials.... If a parasite has an opportunity to become established in a host and fails to do so, never having previously come in contact with a particular host, it is said in a general way to possess a

natural resistance to infection by this species of parasite. The mechanisms preventing the establishment of a parasite in a host may be extremely complex and frequently difficult to define in precise terms". Smyth went further to distinguish between 'susceptibility' and 'resistance' (Chandler 1932, Schneider 1951, Read 1958). Susceptibility refers to that state in which a host is theoretically capable of being infected by a parasite and implies that there are no adverse physiological conditions which would eliminate the parasite before it had an opportunity to become established in the host. Resistance, on the other hand, is defined as a physiological response by the host to a previous or present contact with the parasite, the nature of the response being such that it is directed against the establishment and survival of the parasite.

In the light of the above consideration, the results obtained from the present study are interesting. All the crustaceans dealt with may be regarded as susceptible. It was nevertheless found that all of them including the natural host G.pulex, possessed natural resistance to E.truttae. In all the species, but to varying extents, the initial entry of acanthors into the body cavity elicited host reactions which were first manifest in the form of an investment of young parasites by host cells. Parasites overcome by host reactions were left as variously coloured spots attached to the intestine and caeca of shrimps. Salt (1955, 1956) showed that the presence of the eggs or larvae of the ichneumon fly Nemeritus canescens in the body cavity of abnormal hosts such as lepidopteran hosts or the stick insect Carausius morosus produced similar defence reactions. He further showed that the dark material deposited on the

investment of haemocytes was probably melanin as in experiments where melanin reaction was inhibited by Phenylthiourea, no darkening occurred.

Hynes and Nicholas (1958) drew attention to the evidence that the blood of crustaceans darkened on exposure (Maluf 1939, Roeder 1953) and further observed that this was the case in G.pulex, G.lacustris and G.duebeni. They also established histochemically using a variant of the Masson-Fontana test for melanin (Pearse 1953) that the blackening of dead P.minutus larvae was due to the deposition of melanin. The probable reactions involved in the darkening of dead parasites were discussed. Observations on dead acanthors of E.truttae showed that as in P.minutus, melanin deposition occurs within the parasites and is connected with their death. Thus, in G.lacustris at room temperature, unhealthy parasites were pale coloured, while in dead parasites the colour varied through various shades of brown to black, depending on the age of the infection. In this connection, it was significant to note that a few elongated acanthellae recovered after 81 days at about 17°C had 4-5 black spots lodged within their subcuticula. Late acanthellae with such dark centres in their body had an investment of host cells outside the larval membranous envelope but, unlike the cases reported by Salt, no black spots occurred outside the larval membrane. There were indications that the above acanthellae were dying or moribund. Their probosces were remarkably short and narrow for larvae of their age. No hook promordia were apparent in those with erect proboscis. Others with retracted proboscis failed to evert them on relaxation. It may be added that larvae which escaped immune reaction of G.lacustris everted their proboscis on relaxation after 80 days and were infective to trout.

On the basis of the size and shape of dead parasites, hence the stage of development at which they were killed, and judged by the colour of dead parasites after similar experimental periods, it is patent that the species investigated, offered different degrees of resistance to E. truttae. Since approximately the same volumes of eggs were administered, the number of parasites killed under similar environmental conditions provided additional proof of the relative resistance of the crustacean species. Thus, while after 35 - 48 days at room temperature, in G.pulex, dead parasites were very few, pale coloured, spherical and oval bodies, in G.lacustris they were of the same size but of mostly brown colour. Host reaction appears to be stronger in the latter species. In G.duebeni, resistance seems even greater. Numerous black spindle-shaped spots (20 - 200 per shrimp) were recovered 38 days after infection. The reaction of G.tigrinus to invasion is shown to form a definite barrier to the establishment of this acanthocephalan. The rod-like form of the 'black spots' found indicates that acanthors were destroyed soon after penetrating the gut wall. The resistance of the Gammarus spp. was found to increase at low temperatures. While, however, this increase in resistance was accompanied in G.pulex and G.lacustris by an increase in the number of black spots and a definite decrease in the number of established parasites, in G.duebeni and G.tigrinus greater resistance seems to result in a low number of black spots and thus an actual decrease in the number of acanthors that got through the intestinal wall. The possibility that this was due to the ingestion of a small number of eggs at low temperature seems unlikely in the light of results obtained when G.duebeni infected at room

temperature were kept at 3 - 7°C (Table 4. 7).

Read (1958) put forward the view that socio-physiological stresses such as crowding, may produce physiological changes in animals resulting in the lowering of tissue responses related to the development of resistance. It is shown here that though exposure of shrimps to brief but heavy infection produced no such effect, prolonged exposure may lower, at least temporarily, the resistance of G.duebeni. As pointed out earlier, all larval E.truttae were killed when infected shrimps were left for 80 - 84 days - the parasite's normal developmental period at room temperature (Chapter II).

A.aquaticus and A.meridianus which are often found in the same habitat as G.pulex were found to offer complete resistance to E.truttae. The resulting dead parasites were structurally different from the 'black spots' found in shrimps, but similar to dead M.hirudinaceus and L.thecatus observed by Miller (1943) and DeGiusti (1949) in Popillea japonicum and Hyalella azteca respectively. De Giusti suggested that the 'chitin-like structures' which encircled dead larvae were apparently formed by the hardening of a protoplasmic sheath spread over a parasite by investing amoeboid cells.

It seems reasonably certain that the resistance exhibited by these various species to Acanthocephala is humoral in character. The differences in arthropod reactions pointed out above may well be due to likely differences in their physiology. Thus the low incidence (ca.33%) and intensity (1 - 2) of unestablished infection in Asellus spp. may be

connected with their feeding behaviour (Williams 1960) or the action of digestive juices which are important factors in the determination of host specificity.

The marked differences in the natural resistance of the four Gammarus spp. find an explanation in their phylogeny, history, ecology and distribution in Britain. It is shown that while G.lacustris, a species occurring in freshwater ponds and lakes and often together with G.pulex , can serve as intermediate host for the parasite, G.duebeni and G.tigrinus, on the whole coastal and brackish water types in Britain, resist the establishment of E.truttae in their haemocoel. Taxonomically G.lacustris is more closely related to G.pulex than either G.duebeni and G.tigrinus to the latter (Hynes and Nicholas 1958). Also it is shown that G.tigrinus which is of relatively recent introduction to Britain, appeared to show greater resistance than the other three native species. It seems reasonable to suggest that the present confinement of G.duebeni to salt marshes and ponds off the British coast and estuaries (Hynes 1954, 1955) may have limited the contact of this species with Salmonid fishes and hence its rather strong reaction to infection by E.truttae.

Sandground (1929) has pointed out that age resistance as expounded by Looss (1911) and Fülleborn et al (1928) does not appear to be of general occurrence in helminth infections nor is the extent of its occurrence known. The results of the experiments made with young shrimps show no evidence of age resistance in the shrimps studied. Hynes and Nicholas (1957) noted that G.pulex of all ages were infected with P.minutus.

The findings from the investigations on concurrent infections involving 40 different cultures of G.pulex appear to be conclusive. Simultaneous and successive infection with one or both species were found to be possible. The studies of Michajlow (1953, 1958) and Kisielewska (1957) led to a similar conclusion for two cestodes, Triaenophorus lucii Müller 1776 and Drepanidotaenia lanceolata Bloch 1782, in their natural copepod intermediate hosts - Cyclops strenuus strenuus Fischer and C.vicinus Ulj.. Neither facilitation in the penetration of the intestinal wall nor an increase in resistance was evident in successive infections with one or both acanthocephalan species. Nor had the order of precedence any effect on the establishment and development of the parasites as found by Michajlow (1958) for the cestodes. The attainment of the relevant final stages of the parasites in all the forms of co-invasion studied not only confirms field observations that these palaeacanthocephalans can develop side by side within the same shrimp, but also shows conclusively that the formation of their cystacanths in such shrimps is possible. Hynes (pers.comm.) informs me that he has recovered the cystacanths of both parasites together in nature. Since both species established in good numbers and developed to the infective stage in single- and two-species cystacanth-acanthor challenges, it is concluded that premunition does not develop in infections involving either or both E.truttae and P.minutus.

Michajlow (1958) noted that in combined parasito-coenosis of T.lucii and D.lanceolata larvae, there was some degree of 'predominance' in successive co-invasion. He remarked that "the prevalence of one species over the other may be decided by the degree of its adaptation to the host and characteristic features of various development stages (more

intense growth and organogenesis at some of its stages)". A comparison of the developmental relationships of P.minutus and E.truttae in the forms of co-invasions studied with control data, does not reveal any of the features outlined above for the tapeworms. Even in heavily infected shrimps, the disturbance in growth and development was of the same order in single- as in two-species successive infections, allowing for the innate differential in the developmental rates of both species (Hynes and Nicholas 1958, Chapter II).

The above findings further show that the greater incidence of E.truttae and P.minutus in the upper and lower stretches of Afon Terrig respectively, is not connected with any reactions arising from the relationship between the two parasites in G.pulex but is likely due to external environmental factors. The relative more frequent occurrence of P.minutus downstream is probably due to the accessibility of the stream to ducks from a farm situated about midway down the stream. Hynes (pers.comm.) has observed that P.minutus was common upstream, while flocks of duck were kept at Rhydtalog for experimental purposes. During the period he took at least 6 shrimps in which both parasites were present. In a small stream at Wistanswick (Shropshire) a very high incidence of P.minutus in shrimps has been recorded nearer a duck farm than further away from it (Hynes and Nicholas 1963). The factors influencing the incidence of E.truttae in the stream will be dealt with later (Chapter VII).

Finally, it has been shown experimentally that E.truttae, unlike P.minutus, does not interfere with egg-production in female G.pulex. This lends support to the view that parasitic castration in female shrimps may

be humoral and not mechanical (Pflugfelder 1956). Available experimental evidence also indicates that parasitisation of young G.pulex by E.truttae may retard development and growth, and hence the attainment of the adult size. It is suggested that trophic factors may be involved in this effect of the parasite on young shrimps.

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C H A P T E R V

STUDIES ON E.TRUTTAE IN THE FINAL HOST

1. INTRODUCTION

Field investigation on the seasonal incidence of E.truttae in trout, has shown that a large number of the parasite could establish in this host. In a number of cases, the intestine was found to be virtually covered by the parasite, just over 200 worms having been recovered in one case. Similar reports of the establishment of a large number of Acanthocephala in their final hosts, are available in the literature. Wolffhügel (1900) found as many as 1000 P.minutus in the intestine of a duck. Ball (1930) recorded 1154 Corynosoma strumosum Rudolphi in the California Harbour seal Phoca richardii. Perry (1942) took 1482 Filicollis altmani Perry, 1942 from a Scoter (marine bird) of the genus Melanitta. As would be expected, larger species occur in comparatively smaller numbers. Thus Meyer (1933) noted that up to 70 M.hirudinaceus may be found in hogs.

By sorting the population of parasites in heavy infections and arranging them in groups on the basis of size and the state of sexual maturity of females, it was found that, allowing for variation in development (Chapter II), the worms were established at different times.

Infections of more than the fourth order were apparent. Although as noted earlier (Chapter II) younger worms in these infections tended to occupy the more anterior positions in the intestine, some of them were dispersed unevenly throughout the lower half often attached side by side with the older and gravid parasites. No retardation in the size of young worms was observed in these cases of natural concurrent infections.

In the light of the above observations, the conclusion reached by Baer (1952) that "all animals, whether vertebrate or invertebrate, possess a normal mechanism that protects them from superinfestation by parasites that are already harboured either in the larval or in the adult stages", may require re-examination as far as some Acanthocephala are concerned. In E.truttae as indicated above, extensive superinfestation appears possible in nature.

It has been shown in an earlier work (preceding chapter) that single-species concurrent infection is experimentally possible in shrimps and does occur in nature. The present study deals with experimental work carried out to ascertain some aspects of host-parasite relations in the definitive host - the brown trout. Thus the effects of initial heavy infection, superimposed infection, host starvation as well as the possibility of infections establishing in young trout, have been investigated.

2. THE EFFECT OF INITIAL HEAVY INFECTION.

Brown trout 120 - 150 mm. long from Chirk fishery were used. As explained earlier (Chapter II) these were preferred to larger ones for they

were only lightly infected by the cestode Proteocephalus. On starvation in the laboratory, most of these worms were shed thus preventing possible complications from interspecies competition (Holmes 1957, 1961, 1962a, b).

Experiments (Chapter II) have shown that 15 E.truttae can establish almost simultaneously and develop normally without apparent adverse effect on the fish host of the size-range given above. In the present study, therefore, infection of trout with 15 cystacanths is taken as standard. The effects of intensities of infection greater than this number are then determined.

Trout were kept separately in glass tanks and first starved for 24 hours before being infected. Thirty infected shrimps were then introduced to the tanks at the usual feeding time. They were all ingested in 1 - 3 hours. Any loss of cystacanths during ingestion was made up by the administration of the same number of infected shrimps. Although results from earlier experiments (Chapter II) were available for comparison, controls in which 15 infected shrimps were fed, were set up as a further check. To determine if there was a seasonal factor in the relationship being investigated, the experiments were performed in both summer and winter 1962 and summer 1963, in an unheated aquarium where the environmental factors fluctuated with those of the outside world. Due to the limitation of material, especially infected shrimps, the number of fish employed was necessarily small. During the experimental period, the fish were fed daily on minced meat and liver. Before feeding, each tank was carefully examined for parasites. Faeces were also collected and later teased out for the same purpose. Records were kept of the sex of all the parasites

recovered each day, in an attempt to find out if members of one sex benefited or suffered under the conditions investigated. As earlier studies (Chapter II) have shown that this acanthocephalan is established in 1 - 3 days after an infective meal, and that male worms begin to drop out of the intestine earlier than females but after a variable period, the experiments were terminated after two weeks to offset the effect of the latter on the result.

The details of the result of the experiments are set out in Table 5. 1. It was found that there was a tendency for an unusually large number of parasites to be ejected with faeces during the first few days after infection at high temperature. Thus, in fish No.1A, all the 21 worms shed were taken on the first two days after infection while in another fish (No.3B), 26 of 37 were taken during the same period. All the recovered worms were normal in form. Male parasites had their bursa in the everted condition, while some females bore copulatory caps terminally. This shows that the worms were attached to intestinal mucusa for some time, for it has been observed that attachment of these parasites in close proximity is a necessary pre-requisite for successful copulation (Chapters II and VI). About 50% of the expelled worms had their probosces lodged in faecal matter while the others were free in the tank. There was no indication of the loss being more among members of one sex.

At low temperatures 4 - 9°C, the number of worms expelled was much less and what loss there was, did not take place as early. Control fish also showed some delay in the time worms were first recovered with droppings. So also did fish infected with 15 shrimps, used in other investigations (Chapter II). The consequence was that in the latter cases, the number

Table 5. 1

Showing the effect of initial heavy infection of trout by E.truttae

Expt. No.	Fish No.	Temp °C.	Days after infection 1 M	1 F	1 Total	2 M	2 F	2 Total	3 M	3 F	3 Total	4 M	4 F	4 Total	5 M	5 F	5 Total	6 M	6 F	6 Total	7 M	7 F	7 Total	8 M	8 F	8 Total	9 M	9 F	9 Total	10. M	10. F	10. Total	11. M	11. F	11. Total	12. M	12. F	12. Total	13. M	13. F	13. Total	14. M	14. F	14. Total	Total Recovered. M	Total Recovered. F	Total Recovered. Total
1	1A	9-12	14	6	20	1	0	1			0			0			0			0			0			0			0			0			0			0	6	2	8	21	8	29
	1B	"			0	2	2	4			0			0			0			0			0	1	0	1			0			0			0			0	10	9	19	13	11	24
	1A Control	"	2	1	3			0			0			0			0			0			0			0			0			0			0			0	5	3	8	7	4	11
	1B	"			0	0	1	1			0			0			0			0			0			0			0			0			0			0	7	7	14	7	8	15
2	2A	4-9			0			0			0	1	1	2	1	0	1	1	0	1			0			0			0			0			0			0	17	12	29	20	13	33
	2B	"			0			0			0	2	0	2	1	1	2			0			0			0			0			0			0	1	0	1	18	7	25	22	8	30
	2A Control	"			0			0	1	2	3			0			0			0			0			0			0			0			0			0	1	0	1	2	2	4
	2B	"			0			0			0	0	1	1			0	0	1	1			0	1	0	1			0			0			0			0	8	8	16	9	10	19
3	3A	12-16	2	1	3	3	5	8	3	5	8			0	1	0	1	1	0	1			0			0	1	0	1	1	0	1			0	1	0	1	5	5	10	18	16	34
	3B	"	14	8	22	2	2	4	2	2	4	1	1	2			0			0			0	1	1	2	0	1	1	2	1	3			0			0	8	2	10	30	17	47

of parasites recovered at autopsy was generally higher than in 30-shrimp infections at higher temperatures. It may also be pointed out that under the higher summer temperature (Experiments Nos. 1 and 3) the total number of worms present in the intestine after 14 days was of the same order in both heavily infected and control fish. In the experiment performed in winter (4 - 9°C) on the other hand, the parasites in the intestine of control fish were, as expected from the number of shrimps administered, about half the number in heavily infected speciments. It seems clear, therefore, that at lower temperature most of the cystacanths were able to establish in trout. In fact, Experiment No.3 was undertaken to confirm observations made in Experiment No.1. The discrepancy between the total number of infected shrimps fed to fish and the total number of worms recovered including those shed during the experimental period, is probably due to some of the larvae in shrimps not being infective. Such uninfective larvae, as shown earlier (Chapter II), are incapable of everting their proboscis and are digested in the gut.

It was also noticed that the established parasites taken at autopsy in all the experiments, showed no ill effects such as stunting. Measurements showed that they were of about the same size as in control fish. It was, however, noted that for infections of that age, the parasites were markedly spaced out in the intestine. In all the experimental fish there were approximately equal numbers of worms in both halves of the intestine. One other interesting observation was made in connection with the stage of development of worms recovered at the end of the experiments. Most female worms from the intestine of heavily infected fish were found

on dissection, to bear young developing acanthors free in their body cavity. Comparison with control worms and other available comparable data (Chapter II) showed that the initial presence of a large number of parasites was indeed beneficial to the parasites as it ensured earlier fertilisation.of eggs. Chubb (in press) noted that the production of shelled acanthors in the female worms may be correlated with the degree of concentration of Echinorhynchus clavula Duj 1845 in the fish intestine, thus in the eel where the maximum concentration was found, maximum production of shelled acanthors was seen.

On the part of the host, no loss of form was apparent in trout subjected to heavy intestinal infection. Nor was any sign of gross mechanical damage of the intestinal mucosa obvious under the binocular microscope.

3. THE EFFECT OF SUPERIMPOSED INFECTION.

It would appear from the results recorded above that when trout ingested a large number of cystacanths (up to 30) within a few hours in summer, a mechanism was called into play whereby a substantial number of the parasites was expelled. The nature of such a mechanism, its origin and mode of operation was not clear. It was thus decided to study the effect of concurrent infection in trout in the hope that knowledge of the events in the latter would help to understand the reaction or reactions involved in the former.

As in the preceding investigation only a modest number of fish could be used, as the number of available infected shrimps was limited. In the

first experiment at 5 - 11°C, two brown trout were employed and the standard infective meal of 15 shrimps administered in two stages. Each was first fed with 10 infected shrimps and after one week with another 5 infected shrimps. The number of worms lost with faeces during the experimental period was recorded as before. The fish were killed on the 8th day after the second infection. Controls in which 15 infected shrimps were fed to fish in a single meal were also examined.

In the intestine of the first fish there were 8 worms - 4 males and 4 females. Of these, 5 were attached to the upper intestine and the other three to the intestinal content. The second fish bore 10 parasites - 6 males and 4 females. Three were attached to the upper intestine, 5 in the upper half of the lower intestine and the other 2 in the latter region were unattached. One control fish harboured only one parasite and this was attached to the upper intestine. The other had 16 worms (8 males and 8 females). Of these 7 were attached to the upper intestine and 9 were lodged in the contents of the intestine.

In the second experiment, the ingestion of 30 infected shrimps by each fish was spread over 4 weeks as follows. Each fish was initially fed with 10 infected shrimps and thereafter with 5 infected shrimps at weekly intervals. Twentyfour hours after the last infective meal, the fish were killed. In fish No.1 there were two free, healthy but inactive cystacanths (Chapter II) in the stomach; whereas in the intestine there were 16 (8 males and 8 females) attached worms. Of the worms in the latter region, half were in the upper half of the intestine and the other half in the lower. Fish No.2 had 4 free cystacanths and one disintegrating late acanthella in its stomach. The intestine contained 10 worms

(7 males and 3 females) in the upper, and 7 (3 males and 4 females) in the lower intestine.

In the third experiment at 5 - 11°C, an attempt was made to determine the effect of a more gradual acquisition of yet smaller numbers of the parasite on the number of parasites that establish in the intestine. A total of 20 infected shrimps were administered to fish in four equal meals at intervals of 3 days. The fish were killed 7 days after the last infection. Losses were, as usual, recorded. The fish bore 7 and 12 parasites respectively at autopsy.

The above results are summarized in Table 5. 2. From the number of parasites recovered both at autopsy and with faeces during the experimental period, it seems reasonably certain that within the range of intensity of infection tested, concurrent infection occurred and that there was no appreciable failure of parasites to become established. Thus the total number of worms lost during the experimental period was generally of the same order as that attributable to 'accidental' loss (Chapter II). It was also observed that the total number of parasites established in concurrent infection involving a total of 30 infected shrimps (Expt. No.2) tended to be higher than in infections with a total of 15 or 20 shrimps. As indicated above, the low percentage of parasites found established at autopsy was partly due to the fact that some of the shrimps must have borne non-infective larvae. Since the periods after which fish were dissected were rather short, no attempt was made to sort the parasites recovered according to the age of infection. Measurements and dissection of female worms

Table 5. 2

Summary of results obtained in various superimposed infections of trout by E.truttae

Expt. No.	Fish No.	Total No. of infected shrimps ingested.	No. (%) of parasites recovered at autopsy.	Total No. taken with faeces	Total No. (%) accounted for.
1	1A	15	8 (53.3)	0	8 (53.3)
	1B	15	10 (66.7)	2	12 (80)
	Control 1	15	8 (53.3)	2	10 (66.7)
	Control 2	15	16 (106.7)	3	19 (126.7)
2	2A	30	16 (53.3)	7	23 (76.7)
	2B	30	17 (56.7)	3	20 (66.7)
3	3A	20	7 (35)	4	11 (55)
	3B	20	12 (60)	2	14 (70)

showed that they were of the expected size and stage of development.

4. THE EFFECT OF HOST STARVATION.

During the course of the study of the incidence of the acanthocephalan in trout in the stream, it was observed that a fish taken from the field and kept in the aquarium refused to feed for 5 days and yet did not shed any worms. At autopsy, however, it was found to harbour some parasites. It was thus decided to begin the study of the effect of host starvation with naturally infected fish.

Five brown trout were taken from the stream. On arrival in the laboratory they were kept in separate tanks and were not fed. The tanks were examined daily for parasites. After various periods of starvation (7 - 24 days), they were killed and examined. The results are outlined below.

Fish ref. no.	Days of Starvation	No. of parasites expelled	No. at autopsy.
1	7	0	3
2	10	0	11
3	18	0	8
4	18	0	5
5	24	0	7

Table 5. 3. The effect of starving fish host on naturally acquired infection.

As seen from the table, no parasites were expelled. It may be added that in all the above cases, the stomach and intestine were completely empty at autopsy.

In the next series of experiments, the effect of subjecting trout with infections of known age to one long period or a number of shorter

periods of starvation was investigated.

1. Three brown trout of the usual size were fed 15 infected shrimps. The infection was allowed to establish for 14 days. The fish were then starved for 3 days. They were fed for the subsequent 3 days and then subjected to another 3 days of starvation. After feeding them for a further 4 days, they were killed. The results are summarized as follows:-

Fish ref. no.	No. of shrimps ingested	Loss at first starvation	Loss during interposed feeding period	Loss at second starvation	Loss at subsequent feeding period	No. at autopsy
1	15	0	0	0	0	7
2	15	0	1	0	0	7
3	15	0	0	2	0	15

Table 5. 4 Showing the details of the experiment in which trout were starved for two short periods.

2. Fifteen infected shrimps were administered to two brown trout. Fourteen days after infection they were starved for 7 days. No worms were taken with faeces during the latter period. Trout were fed for one day and then killed. One fish contained 4 parasites, all in the upper intestine. The other bore 11 parasites, 6 of which were attached to the upper and 5 in the lower half of the intestine.

3. In the third experiment, one fish which ingested 12 infected shrimps was not fed for the subsequent three weeks. No parasites were expelled during the period. At autopsy on the 22nd day after infection, only one parasite was present in the intestine. It is patent here that the 'infective meal' contained largely uninfective larvae.

From the results of the above experiments it seems reasonable to

to conclude that starvation may not lead to the expulsion of *E. truttae* from trout. It has been shown that neither prolonged starvation following immediately on infection, nor shorter or longer periods of starvation on longer-established infections was able to produce any noticeable expulsion of worms from the intestine. In the single case where loss occurred, it was not greater than what is known to result 'accidentally' at irregular intervals under normal conditions (Chapter II). The fact that in naturally infected trout no losses whatever occurred in spite of the fact that the gut contained no food for the greater part of the starvation period, and the association of a good number of expelled parasites with remains of food observed throughout these experiments, would indicate that the presence of a large quantity of food in the gut may expose this parasite to a greater risk of being expelled from the intestine than host starvation. In this connection it is recalled that it was pointed out (Chapter II) that the nature of food ingested bears some relation to the number of worms with their probosces lodged in intestinal contents.

5. THE SIZE OF TROUT AND PARASITISATION

It was next decided to find out if there is a lower limit to the size of trout that can become infected. Field and laboratory investigations were consequently undertaken.

Brown trout less than 100 mm. long were taken from both the upper and lower stretches of the stream in December and January. In the December sample, 14 fish representing 85.5% of the total number taken upstream around

Rhydtalog, were infected. The intensity of infection was 1-11 worms per fish. In about 50% of the infected fish, mature acanthors were present in female worms. The smallest infected individual was 51 mm. in length. None of the 12 fish taken from the lower stretches of the stream was infected. In January, no trout of suitable size was taken from the latter region. All the 12 fish taken around Rhydtalog bore the acanthocephalan, the smallest infected individual being 57.5 mm. long. All the fish examined were within the range 49 - 97 mm. long. Examination of their scales showed that they were all in the first year of life i.e. 0 + age group (Ball 1957). This is, of course, expected as Jones (pers.comm.) has observed that there is considerable variation in the time the winter cheek or rings appear on the scales of trout.

To verify if the infection of young trout is experimentally possible 4 small trout in the 0+ age group measuring 70-83 mm. long from Chirk fishery were fed on infected shrimps 5 - 6 mm. long. Each ingested 5 shrimps. On dissection 5 - 9 days after infection, 1 - 5 worms were found attached to the intestine.

It seems conclusive that there is no lower limit to the size of trout that can become infected. Evidence has been adduced to show that worms established in young fish were normal in size and the performance of adult functions.

6. DISCUSSION

Experimental studies on M. dubius in its definitive host by Burlingame and Chandler (1941) have shown that in heavy infection of rats with 40 - 200 parasites, the percentage survival was within the same range

as in 20- worm infections. Only a moderate decrease in growth was noted. Nicholas and Hynes (1958) arrived at a similar conclusion for P.minutus by comparing the results of 30- worm and 120- worm infections. The results of the present study would indicate that the position in E.truttae in its final host is different. Thus in heavy infections involving 30 parasitised shrimps at 14 - 15°C, only a third of the administered worms were established, the others having been expelled within the first couple of days after infection. Established worms showed no apparent ill-effects. Since trout of comparable size and age groups (1+ (2) years) as the experimental ones, have been observed to harbour up to 49 worms in nature, and in the light of the results obtained in superimposed infections discussed below, it is suggested that the initial expulsion of a large number of parasites was due to mechanical reaction on the part of the host. It is plausible that the expected almost simultaneous attachment on the intestinal mucosa of a large number of probosces would stimulate the intestine to violent peristaltic contractions. This would result in the expulsion of a large number of worms within the first few days, as observed in the experiments. The possibility that competition for available space in the comparatively much shorter intestine of trout, may have been contributory, is not ruled out. It seems more likely that the observed spacing out and the early extension in the linear distribution of worms may have been due either to an initial crowding effect or host reaction or both. The fact that this reaction of the host was exhibited markedly at a higher temperature (the maximum summer temperature of the stream of 14 - 15°C) is probably correlated with a higher general metabolic activity of the poikilothermous final host, and lends

support to the nature of the resistance of trout to initial heavy infection. It may also be pointed out that this contrasts with the situation in the intermediate host where resistance was found to be more effective at low temperature. It is noted, however, that while resistance in G.pulex was humoral in character, in trout it appears to be purely mechanical.

Crowding effect in secondary infections has been shown by two species of the Acanthocephala in their final hosts (Burlingame and Chandler 1941, Nicholas and Hynes 1958, Holmes 1961, 1962a). A similar phenomenon is known in some cestodes occupying a similar environment (Chandler 1939a), Read 1951, 1955, 1959, Read and Phifer 1959 inter alia). Burlingame and Chandler, Nicholas and Hynes have found that the establishment of secondary infections of M.dubius and P.minutus respectively tended to be inhibited by the presence of worms. This was attributed to competition for suitable sites of attachment. The relation of worms in concurrent infections of E.truttae appears to be different. Within the range of the degree of parasitisation investigated, the establishment of worms in secondary, tertiary infections etc. was shown to be little affected by the presence of parasites established in earlier infections. There was no falling off in the number established as in P.minutus and M.dubius. That the result might be different if infections involved a larger total number of E.truttae administered over a longer period than investigated, seems improbable in the light of intensities of infection encountered in nature. It has been noted above that for fish of the same size and age, considerably heavier infections result from concurrent infections in nature. The difference between the behaviour of E.truttae and M.dubius in secondary infections, is probably due

to the normal extents of their attachment in the intestine. M.dubius has a definite 'zone of viability'. Worms move forward during the first 7 weeks from the initial site of establishment into this region, the females further forward than males. Those which fail to establish themselves in the zone or move into it early in development fail to survive (Burlingame and Chandler 1941). Nicholas and Hynes (1958) showed that P.minutus becomes established in a zone of the intestine which is rather sharply defined anteriorly and less so posteriorly. This zone lies a little more than half way down the intestine. Observations on the movement of E.truttae during development (Chapter II) showed that in contrast to M.dubius and P.minutus there is no zone of viability. Young E.truttae are primed in the pyloric region where they become first established and then occupy a more posterior position with age. After periods varying from about 5 - 8 weeks upwards, worms generally occur along the entire area of the intestine. It is also shown in the present study that this range of attachment is extended earlier in both superimposed infection and single heavy infection. Thus while in M.dubius and P.minutus competition for the limited zone of viability leads to the failure of many worms in secondary infection becoming established, in E.truttae no such situation exists as the entire gut is apparently suitable for worm development.

Burlingame and Chandler have also shown that the susceptibility of rats to secondary infections of M.dubius is proportionate to the primary infection, the rats with more than an average number of primary worms harbouring more than an average number of secondary worms etc. The results

from comparatively heavy primary infection point to the improbability of the above relation obtaining in infections with E.truttae. Heavy infections, primary or secondary, would elicit strong host reaction in the form of mechanical expulsion of worms.

Starvation of albino rats for 2 days expelled a large number of M.dubius (Burlingame and Chandler 1941). Read and Rothman (1958b) were able to show a dramatic loss in the body weight of the same acanthocephalan when the carbohydrate intake of the host was curtailed. Nicholas and Hynes (1958) found that host starvation was not as effective in expelling P.minutus. The starvation of ducks for periods of one or two days, they said, may lead to the elimination of P.minutus from the gut, although it cannot be relied on to do so. The finding for E.truttae is that host starvation for 1 - 24 days or for consecutive brief periods, has practically no effect on worms established in nature or under experimental conditions. Even starvation following immediately on infection appears to have no effect on the establishment of worms.

Robertson (1953) found that only trout greater than 14.5 cm. in length were infected by E.truttae. This was taken to indicate that there is a lower limit to the size of trout that could become infected. The latter situation, she felt, would arise from a corresponding limit to the size of trout feeding on the intermediate host, Gammarus. It has been conclusively shown here, from both field and experimental infections, that there is no lower limit to the size of actively feeding trout infected. Young trout as small as 51 mm in length were shown to be infected in nature. Since, however, fish less than 100 mm. in length from the lower half of the stream, where the incidence of the parasite in shrimps is known to be low

(Chapters IV and VII) were uninfected, it is apparent that any limit to the size of trout infected in any environment would depend largely on the incidence of infective larvae in the intermediate host. Infected shrimps have been shown (Chapter IV) to be of all sizes, as shrimps infected at a tender age are retarded and remain subnormal in size. Added to this, examination of stomach contents has shown that trout 51 mm. in length are able to ingest full-grown shrimps. The question of the size of shrimps constituting a barrier to the infection of young trout does not, therefore arise.

Finally, it may be noted that in the acanthocephalan species mentioned above, and in which experimental studies have been made on the relation between the parasites and their final hosts, there is no evidence of humoral immunity either natural or acquired. Premunition where it occurs is limited, as in M.dubius and P.minutus. Resistance is shown to be of environmental nature in the three species. It is also interesting to note that adult intestinal cestodes exhibit premunition which has nothing to do with immune reactions (Chandler 1939a, Smyth 1962). On the basis of experimental and field studies (Chapter VII) there appears to be no mechanism that protects trout from superinfestation by E.truttae. Baer's conclusion of the existence of a normal mechanism against superinfestation in animals may not, therefore be of general application.

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CHAPTER VI

IN VITRO STUDIES

1. INTRODUCTION

The value of 'In vitro' studies in the understanding of various aspects of the life of a parasitic organism is generally recognised. During the experiments on the development of E.truttae described earlier in this thesis (Chapter II) it became apparent that a study of this parasite in vitro would most profitably be made at the cystacanth-adult phase of the life cycle. It seemed that if conditions similar to those 'in vivo' (pyloric intestine) are provided 'in vitro', the worms would become active, mate, and that fertilised females might live long enough to produce viable eggs. The difficulties to be overcome in achieving this end are by no means small. Dougherty et al (1959) reviewed at some length, the problems associated with axenic culture of invertebrate metazoa. Earlier, Smyth (1955) had dealt with the problems to be solved in the 'in vitro' cultivation of pseudophyllidean cestodes from the egg to the adult stage. These were broadly grouped under the following headings: 1. Establishment and maintenance of Asepsis;
2. Development and maintenance of suitable environmental conditions - pH, Oxygen tension, temperature,

Osmotic pressure and positional pressure;
3. Development of satisfactory criteria for 'growth' (in vitro) as opposed to mere survival; and
4. Development of highly nutrient media for the development of those stages in which tissue synthesis is necessary before differentiation can take place.

It seems reasonable to assume that these problems would apply generally to helminths and particularly to the Acanthocephala which are, in many ways, similar to the Cestoda.

Although extensive 'in vitro' studies have been made on the nematodes, and on the cestodes and trematodes to a lesser extent, the Acanthocephala have received rather scanty treatment, and for them, practically all the four problems outlined above are untouched. Smyth (1962) observed that experimental work on this group is exceedingly meagre.

Von Brand (1940) wasable to maintain M.hirudinaceus in saline for only 20-30 hours at 37°C under aerobic conditions. He remarked that the sluggishness of the worms coupled with the fact that they did not keep well 'in vitro', was a major hindrance to studies designed to gain definite knowledge about their type of energy production. Gettior (1942) found that at 20°C, in the dark, N.emydis survived for the longest period in 0.5% saline. He also demonstrated that the addition of 0.02% Calcium Chloride had a beneficial effect on survival. It increased the average period of survival from 13 to 20.3 days, and the maximum individual survival from 20 to 25 days.

Working on the same species, Van Cleave and Ross (1944) arrived at the conclusion that 'in vitro' solutions of 0.8 - 0.85% Sodium Chloride were essential for the maintenance of the normally flattened form of the worm. At lower concentrations, the body wall could not function normally as a membrane to control the passage of liquids. Lal (1947) found that

1% Saline appeared most suitable for the survival of E.truttae. Following the successful use of Streptomycin in experiments with Ascaris by Fairbairn and Reesal (1950), Ward (1951) showed that the antibiotics, Penicillin and Streptomycin, by reducing the growth of micro-organisms in culture media, increased the longevity of M.hirudinaceus from just below 48 hours in ordinary media, to 8 days. Dunagan (1962) succeeded in maintaining adult N.emydis and N.pseudemys aseptically in various media for extended periods, during which there was no indication of growth but the worms copulated and eggs were produced. In Tyrode-balanced salt solution (T-BSS) with Sodium Chloride concentration of 0.9% and pH 8.2, the range of survival time, as determined by motility was 14 - 26 days, with an average of 21 days. In T-BSS, pH 8.0, 0.1% glucose, and 11% turtle serum on the other hand, the range was 65 - 85 days and the average was 75 days. He, however, found that the juveniles recovered from snails, failed to survive as long as mature specimens. He expressed surprise at this, for such young worms would be expected to survive longer than the old specimens.

In the present investigation, the survival and behaviour of the juvenile (cystacanth) and adult stages of E.truttae in water and salt solutions were studied. Also, preliminary attempts at the 'in vitro' cultivation of the cystacanth stage in various media, have been made. Except where stated in the latter experiments, generally no attempt was made to secure asepsis. This was partly because the precaution was found unnecessary for most purposes. Added to this, aseptic conditions would have been most difficult to maintain under the available working conditions.

2. THE LIFE SPAN OF CYSTACANTHS AND ADULTS IN WATER

In this and other subsequent experiments 'in vitro' both stages of the parasite were recovered by dissecting freshly killed infected shrimps and trout from the field. Where possible, they were supplemented with material from laboratory-infected hosts. The worms were freed, as much as possible, from the micro-fauna, flora and debris adhering to them by rinsing them in two changes of tap or stream water, as appropriate. In the various series of tests, groups of 2 - 5 worms, depending on the total number available, were transferred to petri dishes, 75 mm. in diameter. These were half filled with either tap or stream water. The tests were conducted at the various seasonal temperatures of the stream provided in the cold room, where the lighting conditions were also controlled so as to simulate those of the stream all the year round, and at room temperature. A total of 25 worms were employed in tests with each medium at each temperature. Examination of worms for survival and behaviour was done at two-hourly intervals under the binocular microscope.

The definition of what constitutes normal form and behaviour of the adult parasite, which lives in the intestine of fish, is difficult. However, from observations made on parasites attached to the intestinal wall and disturbed as little as possible on opening the gut of freshly killed fish, the following criteria were adopted. Normal adult worms are flat, wrinkled and can move their bodies at short but irregular intervals. The proboscis is either everted and mobile, or attached. If withdrawn, it is capable of continued active protraction and retraction. In male worms, the bursa in the everted condition, is associated with readiness for copulation

(Chapter II). Observations made in the latter chapter, on cystacanths which lived for over 7 months in their shrimp host, show that the cystacanth is normal, flat, wrinkled and capable of slow movements. The proboscis is not everted nor is the bursa of male cystacanths. The bursa is also not everted in water, hypotonic and hypertonic salt solutions (Chapter II).

Both stages of the parasite were regarded as dead if they failed to move when slightly depressed between two metal seekers.

It was observed that during the first few hours in stream water, the worms retained their normal form and responded to a strong beam of light (and perhaps radiant heat from the source of light). As they lost vitality they became swollen and, at death, they were turgid and difficult to depress.

At 2 - 4°C, adult worms retained their normal form for 6 hours and lived for 10 - 20 hours in a partially swollen and definitely abnormal condition. The average life span was 13.6 hours. These figures dropped with increase in temperature. Thus, at room temperature 18 - 20 °C, adult worms were only able to maintain their normal body form for a maximum of 2 hours. The range in the period of survival was 4 - 6 hours, while the average life span dropped to 4.8 hours.

The cystacanths showed similar trends of survival in stream water at the various temperatures at which experiments were conducted. At lower temperatures, they survived for appreciably longer periods. At 2 - 4°C for instance, cystacanths retained their normal body form for 10 hours, while their range and average of the survival period were 19 - 54 hours and 26.5 hours respectively.- The last figure being almost double the average adult survival time of 13.6 hours. At 8°C the corresponding data are 4 hours, 8 - 24 hours and 16.8 hours respectively. At temperatures ranging from

12 - 20°C, all the cystacanths lost their normal form in 1 - 2 hours and were dead in 7 - 10 hours. No appreciable differences from survival in stream water were observed in the survival of both stages in tap water.

3. SALINE REQUIREMENTS OF THE CYSTACANTH AND ADULT STAGES

As a necessary preliminary to any attempt at 'in vitro' cultivation, the saline concentration in which a parasite would survive for the longest time, in the normal condition, had to be determined. In the following experiments, saline solutions ranging from 0.1 - 1.0% made up at 0.1% intervals and one at 1.5% were used (Gettier 1942). Widmann (1935) recorded that the osmotic pressure (O.P.) of G.pulex, expressed in Sodium Chloride equivalent was 265 m M/litre in February. Beadle and Cragg (1940) found that in April the O.P. and the Chloride equivalent of the blood of G.pulex were 160 and 120 m M/litre respectively. Tests for survival were thus carried out in salines containing 120, 160 and 265 m M/litre to find which of these three solutions gave optimal results.

Adult worms were detached as carefully as possible from the intestine of freshly killed brown trout. They were then examined and sorted in 0.7% saline, according to sex and age, under the binocular microscope. Damaged parasites and those that were full grown, grey and thus apparently too old for the purposes of this experiment, were rejected (Chapter II). The worms were then rinsed free of as much adhering matter as possible, with the appropriate saline. They were then kept in groups of five in each petri dish. Care was taken to ensure that both sexes were represented in each dish in order to determine if any difference exists in their survival periods 'in vitro'. It will be recalled that during the development of this worm

in trout, it was found that males disappear from the intestine earlier than the females. The experiments were conducted from January to May in the cold room, under normal lighting conditions and in the dark. The temperature of the cold room during the period varied from 3 - 10°C. Due to the limitation in the supply of adult worms, fewer experiments were done at room temperature 18 - 20°C.

A similar procedure was followed for the determination of the 'in virto' survival of cystacanths in saline. No tests were, however, made in the dark as this was found unnecessary from experiments with adult worms. In any case, cystacanths are in nature exposed to light. They are visible as pale orange streaks on either side of the body of infected G.pulex. As the haemocoel of G.pulex is rather rich in micro-fauna, the cystacanths were rinsed two or three times in the appropriate saline, before being transferred to petri dishes in which they were kept. Cystacanths were, in addition, tested for survival.throughtout the year in 120, 160 and 285 mM/litre salines, in the cold room. This was to determine if there were seasonal differences in survival, in these salines, as might be expected if there were seasonal fluctuations in the O.P. of shrimps (Widmann 1935).

Both the adult and juvenile worms were examined daily under the binocular microscope in transmitted light. The media were changed only when contamination became apparent. These changes were more frequent in experiments with cystacanths probably because blood is a richer medium for micro-organisms than the gut contents.

Observations.

As found in the experiments on survival in water, all adult worms retaining the normal wrinkled and flattened form reacted spontaneously to a strong beam of light by moving actively. These movements were, in most cases, accompanied by the eversion and withdrawal of the proboscis. As the worms lost their normal condition, with the onset of swelling, they were no longer sensitive to light. They moved only if their bodies were slightly depressed. As before, the parasites were regarded as dead when they failed to respond to the latter stimulus.

The survival periods of the cystacanths and adult worms are given in Tables 6. 1, 6. 2 and 6. 3. No appreciable difference was seen either in the range of, or the average life span of, adult worms kept in the dark and under normal day and night conditions. Salines 0.1% - 0.5% in strength were shown to be hypotonic and lethal to adult worms. The survivals in 0.6 - 1.0% saline were broadly similar. However, 0.8% gave the best results. Worms in this medium, in the dark, retained their normal form for 20 - 24 days. The average life span was 22.2 $\pm$ 2.4 days while the range in longevity was 8 - 34 days. 1.5% saline and that containing 265 mM/litre, were definitely hypertonic, for the worms were, for the most part, either abnormally flat or shrunken and distorted in these media, and even when dead, were not swollen. In the other salines, swelling set in as the parasites gradually lost their vitality, and at death they were generally turgid. and resistant to depression. The saline concentration of 160 mM/litre gave results very similar to those of 0.6 - 1.0% saline. Experiments with 0.6 - 0.8% saline at temperatures greater than 10°C indicated that the period of survival in simple salines was markedly reduced at high temperatures.

Table 6. 1

Survival of adult E.truttae in simple salines in the dark (February - May)

No.of worms	Conc. of $_n$ NaCl Sol^n.	Period when normal form maintained (days)	Life Span (Days) Range	Mean	S.E.
10	0.1%	< 1	2	2.0 ±	0
10	0.2%	< 1	2 - 4	2.4 ±	0.1
10	0.3%	< 1	2 - 8	3.9 ±	0.6
10	0.4%	2	2 -16	7.6 ±	1.3
10	0.5%	4	2 -16	8.2 ±	1.4
9	0.6%	8	8 -20	14.2 ±	1.5
10	0.7%	10 - 24	8 -46	20.6 ±	4.1
10	0.8%	20 - 24	8 -34	22.2 ±	2.4
9	0.9%	16 - 24	10 -32	18.0 ±	2.1
10	1.0%	16	6 -28	18.4 ±	2.4
9	1.5%	? 6	10 -32	16.9 ±	2.4
9	120mM/l	10	6 -20	11.3 ±	1.3
9	160mM/l	18	6 -40	17.3 ±	3.6
10	265mM/l	? 6	6 -34	16.0 ±	2.5

Table 6. 2

Survival of Adult E.truttae in simple salines under normal day and night conditions. (February - May)

No.of worms	Conc.of NaCl Sol^{n}.	Life Span (Days) Range	Mean	S.E.
10	0.1%	1 - 2	1.7 ±	0.1
10	0.2%	2 - 3	2.5 ±	0.1
9	0.3%	2 -14	6.6 ±	1.5
10	0.4%	2 -36	9.8 ±	3.0
10	0.5%	2 -18	9.8 ±	1.5
26	0.6%	8 -28	14.2 ±	2.2
11	0.7%	8 -24	15.8 ±	2.0
9	0.8%	10 -22	16.9 ±	1.6
10	0.9%	8 -24	15.0 ±	1.7
10	1.0%	4 -32	14.8 ±	2.5
10	1.5%	6 -36	16.4 ±	2.7
17	120mM/l	6 -26	13.7 ±	1.3
15	160mM/l	6 -28	15.2 ±	1.9
18	265mM/l	6 -18	10.7 ±	0.8

Table 6. 3

Survival of Cystacanths of E.truttae under normal day and night conditions. (May and June)

No.of worms	Conc. of NaCl Sol$^{n.}$	Period when normal form maintained (days)	Life Span (Days) Range	Mean	S.E.
5	0.1%	< 1 (5 hrs.)	3 - 4	3.7 ±	0.3
5	0.2%	<1 "	3 - 6	4.4 ±	0.7
5	0.3%	< 1 "	8 - 12	11.2 ±	0.8
5	0.4%	1	16	16.0 ±	0
5	0.5%	8 - 12	12 - 24	20.4 ±	2.4
5	0.6%	12 - 18	16 - 20	18.0 ±	0.6
5	0.7%	18 - 28	18 - 36	26.4 ±	3.0
5	0.8%	20 - 32	20 - 36	28.8 ±	2.7
5	0.9%	18 - 28	18 - 36	26.8 ±	2.6
5	1.0%	4 - 24	4 - 28	20.0 ±	4.3
5	1.5%	? 4	4 - 14	11.6 ±	1.9
5	120mM/1	4 - 28	4 - 32	20.4 ±	5.3
5	160mM/1	18 - 30	20 - 36	31.2 ±	2.9
5	265mM/1	? 1 - 6	8 - 14	10.0 ±	1.2

Thus, at 18 - 20 °C, the range in the period of survival was 4 - 6 days in 0.6% and 0.7% saline, compared to 8 - 28 days and 8 - 46 days respectively under similar conditions at 3 - 10°C.

The survival of juvenile worms in simple salines followed much the same pattern as that exhibited by the adult worms. However, the survivals were more uniform and hence the range in the survival period tended to be generally narrower. In the more suitable salines, 0.6 - 0.9% and 160mM/litre, the range was remarkably narrow. There was also a consequent general improvement of the period of survival in all salines. In 160mM/litre saline, for example, the mean survival period (31.2 ± 2.9 days) was found to be almost double that (16.3 ± 2.8 days) of adult worms. 0.5% saline is shown not to be as lethal to cystacanths as to adult worms. At this concentration, the cystacanths maintained their normal form for 8 - 12 days, while the mean and range of life span were 20.4 ± 24 and 12 - 24 days respectively. Comparable figures for adult worms are 4, 9.8 ± 1.5 and 2 - 18 days respectively. It was also observed that 0.1 - 0.4%, 1.5% and 265mM/litre salines were not more lethal to juveniles than to adult worms. The overall best periods of survival and maintenance of the normal body form were obtained in 0.8% saline, though it should be noted that there was little or no difference in the behaviour and survival of juveniles in 0.7 - 0.9% and 160 mM/litre salines, asindicated earlier. In 0.8% saline, juveniles maintained their normal body form for 20 - 32 days, survived for 20 - 36 days with a mean of survival period of 28.8 ± 2.7 days. The monthly survival tests with 120, 160 and 265 mM/litre salines showed that 160 mM/litre saline gave optimal survival of cystacanths all

the year round.

The results of the experiments on the effects of temperature on the survival of juvenile worms showed that, as for the adults, the life span was markedly shortened at higher temperatures. It was found, for example, that in 160 mM/litre saline, the survivals were as follows: At 10°C, the cystacanths retained their normal form for 18 - 30 days, their range in longevity was 20 - 36 days and the average life span was 31.2 days. At 14.5°C, the corresponding figures were 3 - 6, 5 - 9 and 8.2 days while at 18 - 20°C, the periods were further reduced to 1 - 2, 2 - 6 and 3.2 days respectively. This fall in the period of survival with increased temperature will be discussed later.

4. THE EFFECT OF THE EXCLUSION OF AIR, pH, GLUCOSE AND CALCIUM CHLORIDE, ON THE SURVIVAL OF CYSTACANTHS IN SIMPLE SALINES.

Having established that in 0.8% saline optimal survival was achieved, it was decided to determine whether the above factors altered survival in simple salines.

To determine the behaviour and survival of worms under anaerobic conditions, the following procedure was followed. Groups of five worms were introduced into 4 ml. vials with bakelite screw-cap covers. These were filled to the brim with 0.8% saline and then carefully corked so that all the air was expelled. They were kept in the cold room at 10 - 14°C and the medium changed every two days. The worms were found to retain their normal body form for 16 - 22 days while the longevity and the average life span were 16 - 24 and 20.7 days respectively. It would appear that

under anaerobic conditions, survival is appreciably less.

The next series of tests were conducted to verify if the provision of a suitable pH, would improve the survival of the cystacanths as shown for the adults of other species (Fenwick 1939, Dunagan 1962 et al). The cystacanths were kept in groups of five in both 4 ml. vials and petri dishes half filled with 0.9% saline buffered at about pH 8.0. A few drops of 0.1% phenol red were added as indicator (Dunagan 1962). As in the preceding experiment, the medium was changed every two days. The results showed that under aerobic conditions, the provision of a suitable hydrogen ion concentration, had a beneficial effect on the survival of cystacanths. In one experiment, the normal form was maintained for a maximum of 42 days at 10 - 14 ^{o}C. This is the longest period during which the worms remained normal in the present 'in vitro' study of the species.

By keeping worms in vials and petri dishes containing 0.9% saline pH 8.0, 1% glucose and phenol-red as indicator, it was found that the addition of glucose improved the survival of worms in simple salines. Experiments on survival in 0.8% saline to which 0.02% Calcium Chloride was added (Gettier (1942)) showed that the presence of Calcium ions in the medium served to improve the suitability of the latter for survival. The average period during which the normal form of the body was maintained, was improved. It should be added that in this experiment, it was found necessary to rinse the cystacanths initially with a solution of 0.8% Sodium Chloride and 0.02% Calcium Chloride containing Penicillin and Streptomycin (see next section). This was because micro-organisms appeared to thrive and were difficult to get rid of in the presence of Calcium.

5. ATTEMPTS TO CULTURE CYSTACANTHS IN RELATIVELY SIMPLE MEDIA

In the foregoing experiments in which juvenile and adult worms were kept in simple and glucose salines, the behaviour of worms was distinctly abnormal and could not be compared with that within the intestinal lumen of the final host. The worms were, for the most part, inactive in salines except when stimuli in the form of heat, light and touch were provided. The following preliminary investigations were, therefore aimed at finding a medium or media in which worms behaved normally and in which they could thus be cultured. The criteria for the normal form and behaviour have been given earlier in the text. The following experiments were ended when worms ceased to be active or became abnormal in form. Cystacanth membranes were carefully dissected away before the commencement of each experiment.

(a) Medium I.

Since it has been shown (Chapter II) that the cystacanths are immobile within the stomach of trout but become active soon after entering the pyloric region of the intestine, it was decided first to try a medium consisting of fresh trout serum and bile extracted in 0.8% saline. Both the cystacanths and the 4 ml. vials in which they were kept were rinsed twice in a solution comprising 0.8% Sodium Chloride, 0.02% Calcium Chloride, 200 units /ml. Benzyl penicillin and 1 mg./ml. Streptomycin hydrosulphate (partly after Dunagan 1962). Nine to ten worms were kept at 18 - 20°C in vials about half filled with the culture medium. The latter was changed every two days.

Within one hour of their introduction into the medium, the worms

became active, everting and withdrawing their probosces without the usual stimuli being provided. The vials were then positioned to bring the worms together in the hope that copulation might occur. After two days all the worms were normal in both appearance and activity. There was no evidence of copulation, but most of the males had their copulatory bursa in the everted position - an indication that they were ready to mate (Chapter II). On the sixth day, the worms remained flat and wrinkled and no contamination was apparent but none of them exhibited the characteristic movements when the vials were shaken. After two further days the experiment was terminated as the worms showed only very slow bending movements, on being depressed. Similar observations were made on the repetition of the experiment.

Normal active life in this saline-serum-bile medium is found to be of a rather short duration. It appears, however, that the medium contains at least some of the ingredients necessary to activate the cystacanths to assume adult functions. What the chemical nature of these ingredients are, requires a biochemical investigation. The eversion and retraction of the proboscis and bursa are adult activities. The movements of the proboscis were very frequent during the first few hours of existence in the medium and may well indicate a search for a surface of attachment. It has also been shown that sex recognition in this species is poor. The absence of a surface for the attachment of the proboscis, and hence the non-existence of suitable positional relationships between male and female worms would, therefore, further decrease the chances of copulation. Apart from the rather short existence of E.truttae in the medium, the provision of

the latter in sufficient quantities posed a great problem. In the next experiment, another medium was tried.

(b) Medium II .

Fish extract (including extracts of liver, muscle, serum and bile) to which 1% glucose was added, was used. Compressed cotton wool was introduced into the medium, to find out if the provision of a simple anchoring material would enhance copulation and improve on the period of normal and active life of the parasites. As before, the cystacanths were bathed in a solution containing antibiotics and then transferred to petri dishes in which they were kept at 12 - 18°C. In these dishes the worms were encircled by a wall of the compressed cotton wool. The medium was changed daily and on each occasion the worms were rinsed in the solution given above.

Almost 50% of the worms became active a few minutes after being introduced to the medium. After 20 hours, it was observed that most of these worms had secured attachments to the cotton wool and that a few had disappeared into the latter. Their body form and movements were apparently normal. On the second day, male worms had their bursa everted and unattached worms were rejected. After one week, most of the worms were alive and normal in form and behaviour. On the ninth day, however, pathological conditions set in. This was probably because of the unsuitability of the eighth medium change which, in addition to containing an unusually large amount of fat, was apparently contaminated. The investigation was thus discontinued. The use of the last and apparently unsuitable

medium for the 8th change was necessitated by the absence of something more suitable. It was not possible to repeat the experiment because of the difficulty of procuring and storing for any length of time, sufficient quantities of the medium.

The results, nevertheless, are of some value as useful inferences can be made. Cotton wool, while providing worms with something in which to embed their probosces, did not solve the problem of providing a suitable positional relationship between the sexes. As shown above, the worms simply disappeared into it in different directions. Since some worms were normal in form and behaviour for well over a week, in the medium, it is suggested that experiments with more easily available extracts and glucose might produce interesting results.

(c) Medium III.

The difficulties experienced in using the more natural media given above, led to the devising of a relatively simple chemical medium for the culture of E.truttae. A chemical medium has a very important advantage over natural ones in that it can be provided always in sufficient quantity. The formula for such a medium has to take into consideration the physical and chemical factors of the environment in which the parasite lives (Read 1950). These factors have not apparently been determined for the intestine of S.truttae. Most of the available evidence relate to the mammalian intestine (Smyth 1962). On the basis of the observations made on the parasite in the above two media, and during its life history in the intestine of trout, it appeared that in an artificial medium, the

provision of a suitable pH, isotonic saline, glucose, essential amino-acids, and bile components, would enable worms to be cultured 'in vitro'. A medium made up of 0.9% saline buffered at pH 8.0, 1% glucose and a few drops of dilute Sodium tauroglycocholate solution, was tested. The number of cystacanths kept in each vial was increased to 20 to give the male worms a better change of coming in contact with the females. The medium was changed every 24 hours and the experiment ended if worms failed to maintain their normal form or behaviour.

Within an hour of their introduction into the medium, the worms were moving actively. The vial was then tilted to bring the worms together. On the second day the contents of the vial were emptied into a petri dish and examined. All the male worms had their bursa in the everted condition and two freshly deposited copulatory caps were found floating in the medium. On closer examination, one male and one female worm were observed to have newly-deposited copulatory caps on the hind third but not terminally on their body. That on the male worm was almost terminal in position. After the sixth day the worms were still reasonably flat but were inactive. Depression between two metal seekers elicited only very slow reactions. The experiment was thus terminated.

In another experiment with the same medium in petri dishes, cotton wool was introduced as described above. The behaviour of the worms was similar to that in Medium II. Some of the worms became actively attached to the cotton wool. Unlike in the latter medium, however, all the worms began to lose their normal form after two days. It seems,

therefore, that although worms are activated in this medium, it is inadequate to maintain the parasite for an extended period.

6. DISCUSSION.

Van Cleave and Ross (1944) reported that adult N.emydis in tap water, lost their normal body form within an hour of their introduction and that all the parasites were dead after 6 hours. The results of the present work on the juvenile and adult stages of E.truttae show that under starvation conditions, both stages retained their normal body form and survived for a longer time at low temperatures (3 - 8°C.) At higher temperatures 18 - 20°C, the findings approximate to those given for N.emydis.

In various laboratory experiments in which the brown trout was naturally fed on infected shrimps, it was observed that not only were cystacanths lost during ingestion, but that worms established in the intestine were occasionally, and perhaps accidentally, lost with the faeces. There has been evidence to show that the latter state of affairs results when the proboscis is mistakenly embedded in the intestinal contents as a worm changes its position of attachment in the mucosa (Chapter II). As it is likely that similar losses would occur in nature, the longevity of worms in stream water would have some ecological significance in determining the chances of survival of worms released accidentally into the external environment.

Survival experiments have shown that in the hard stream water from Afon Terrig at 3°C, cystacanths retained their normal form for 10 hours, while their average and maximum life span were 26.5 and 54 hours respectively.

At 8°C the corresponding periods dropped to 4, 16.8 and 24 hours respectively. Van Cleave and Ross (1944) have shown that turgid but living worms recover their normal form when placed in isotonic saline. It is suggested, therefore, that during the winter months when the temperature of the stream varies from 1 - 4°C, cystacanths liberated from shrimps during ingestion, have some chance of becoming established in the intestine if they are picked up by trout within two days. The chances of the latter happening are enhanced by the fact that not only are trout limited to feeding on bottom fauna in winter, but also G.pulex is abundant and forms a major component of the food of the brown trout all the year round in the R.Terrig. In summer, on the other hand, the chances of accidentally freed cystacanths being reingested are negligible for the low average period of survival coupled with the tendency of trout to feed partly on aerial fauna at this season, would negative any advantages accruing from the increased feeding activity of the fish. Adult parasites, with their lower average period of survival and maintenance of the normal body form, have comparatively little chance, if any, of being re-ingested, if lost with faeces.

The saline requirements of the cystacanth and adult forms are remarkably similar. Both have a wide range of saline tolerance, viz: 0.5 - 1.0% for juveniles, and 0.6 - 1.0% for adults. In both cases 0.8% saline is shown to be optimal for survival. This isnot unexpected since both stages parasitise cold-blooded animals whose physiological states are known to fluctuate. Added to this is the observed fact that the cystacanths of E.truttae are sexually mature and very similar to the adults.

They have further been shown to require little or no period of physiological adjustment before performing adult functions in the intestine of trout. (Chapter II).

The optimal simple saline for survival, under conditions of starvation of 0.8% for E.truttae agrees closely with the result of Van Cleave and Ross (1944). They reported that for N.emydis 0.8 - 0.85% saline was essential for the maintenance of the normally flattened body form. In 0.8 and 0.85% salines, the normal form was maintained for 10 and 12 - 14 days respectively. E.truttae is shown to maintain its normal form in 0.8% saline for 20 - 24 days (Table 6. 1). Gettier (1942) found, however, that adult N.emydis in the dark, survived for the longest period (20 days) in 0.5% saline - a somewhat hypotonic solution. His result appears to agree with Stoll's finding on Haemonchus contortus Rudolph (1803) (Stoll (1940)). It has been conclusively demonstrated, in the present investigation, that there is no appreciable difference between survival in the dark and that under normal day and night conditions. The difference between Gettier and Stoll's results on the one hand, and those of Van Cleave and Ross and the present investigation on the other, are difficult to explain. It may be due to likely differences in the physiology of these parasites. Lal (1947) found that 1.0% saline appeared most suitable for the survival of E.truttae. The parasites lived for about two weeks. It is explained that the difference between Lal's optimal saline and that recorded here for the same parasite, is probably due to the fact that his tests were rather limited. Only 0.75%, 1.0%, 1.5% and 2.0% saline were used

in his experiments.

Fenwick (1939) for Ascaris suum Goeze 1782 and Gettier (1942) for N.emydis, have shown that the introduction of Calcium ions to simple salines improved the survival of worms by providing antagonism and reducing the toxicity of Sodium ions. A similar result has been obtained with E.truttae. The addition of 0.02% Calcium Chloride to 0.8% saline is shown to improve the survival of juvenile worms.

Glucose is also observed to improve simple salines for the survival of worms. This quality of glucose has been shown by various investigators (Lewis 1922, Fenwick 1939 et al). The mode of operation of glucose to improve longevity requires further investigation and elucidation. Ward (1952) established more that 'in vitro' female M.hirudinaceus utilised more endogenous glycogen under anaerobic than aerobic conditions. Laurie (1957) showed that the acanthocephalan M.dubius, 'in vitro' was able to ferment exogenous glucose anaerobically. Alkaline phosphatase detected in the outer layer of the subcuticula of the trunk region of the same worm, was presumed to be associated with absorption or secretion from the body (Bullock 1958). On the basis of these findings, it would appear that the addition of glucose would affect the life span by providing an external energy source on which worms would draw, thus preventing earlier deaths due to the depletion of internal energy reserves. This view finds support in the results of various workers on cestodes in similar environmental situations. Hopkins (1952) demonstrated that Schistocephalus solidus Mueller 1776 absorbed nutriment from various media only when glucose was added. He further observed that there was a correlation between longevity

and the exhaustion of glycogen reserves. Phifer (1960) found that the absorption of glucose by H.diminuta was not accounted for by simple diffusion and suggested that a more complicated process, probably involving metabolic process, was involved.

One of the most striking features exhibited by both juvenile and adult worms in their survival under starvation conditions, is the wide range of the survival period. This is attributable to the operation of certain variable factors which could not be controlled, viz: age of worms, their physiological states as well as those of their hosts of origin, and the expected individual differences of worms in their reaction to saline. Changes of media and handling are also contributory factors (Van Cleave and Ross 1944). It is also probable that individual differences in the rate of depletion of endogenous glycogen may, in part, be responsible for the wide range of the period of survival (Hopkins 1952). In spite of the latter, the fact that cystacanths and adult worms have about the same period of survival under identical treatment suggests that their metabolism in non-nutritive saline proceeds at about the same rate.

It is also interesting to note that the range in survival time is comparatively smaller among cystacanths. This is accounted for by the fact that cystacanths are not only at the same stage of development but also are of a more uniform age, and hence the greater uniformity in their survival in saline. The wider, even if slight, saline tolerance of juvenile worms is not unexpected because Widmann (1935) has demonstrated a seasonal variation in the osmotic pressure of the blood of G.pulex which bathes these worms in nature. Widmann's data showed that the osmotic

pressure of the blood of G.pulex, determined by the depression of freezing point technique and expressed in Sodium Chloride equivalent, varied from 118 mM/litre in August to 265 mM/litre in February. It should be pointed out, however, that the best results for survival throughout the year were obtained in 160 mM/litre saline. This is the osmotic pressure equivalent obtained for the blood of G.pulex in April, by Beadle and Cragg (1940).

In all the experiments with simple and glucose salines, despite occasional contamination of worms by fungi and other microorganisms, usually after 20 days, no difference was observed between male and female worms in their longevity or the period of maintenance of the normal body form. The survival of this parasite in saline, as in water, is shown to be markedly better at and below 10 - 14°C than at higher temperatures. This is probably due to the fact that the worms are not usually exposed to such high temperatures in nature. Both hosts are cold-blooded and the summer temperature of the stream varies from 10 - 15oC.

The present attempts to culture E.truttae, it must be emphasised, were primarily undertaken as a follow-up on the observations made on the behaviour of the parasite in various salines and during its developmental history. Cultures involving trials with standard sera or chemically defined media such as Medium 199, Eagle's, NCTC 109, etc. (Morgan et al 1950, Eagle 1959, McQuilkin et al 1957 respectively) were not contemplated, However, it would be interesting to evaluate the results obtained. Smyth (1946) outlined four criteria for the assessment of the suitability of a medium for artificial cultivation of cestodes 'in vitro'. These were viability, normality of behaviour, growth and development.

These criteria would appear to be of general application to helminths. Smyth (1946) found that peptone broth satisfied the above criteria except growth for S.solidus. Ligula intestinalis L. cultured singly from procercoids in horse serum, produced eggs, 6% of which were fertile. It would appear, therefore, that at least in some cestodes, all the above criteria, except growth, have been satisfied under starvation conditions.

In the present investigation three media have been tested for suitability for 'in vitro' cultivation of the cystacanths of E.truttae with rather limited, but interesting, results. In all the media, the worms behaved normally as long as they lived but their life span was relatively short. Growth and development would, thus, not be expected to be observed. Medium II, of fish extract and glucose, gave the best overall results. Here the worms lived and behaved normally for 9 days in spite of the fact that there was incomplete asepsis. No successful copulation was observed but it seems reasonable to assume that in the presence of glucose nutriment was absorbed, and that perhaps there was some growth. No attempt was, however, made to measure growth.

Stunkard (1932) found that the fresh serum of Necturus was definitely toxic to the cestode Crepidobothrium löennbergi (Fuhrm), which inhabits the intestine of the newt. In contrast, the acanthocephalan E.truttae is shown to behave normally in fresh trout serum and bile. The latter medium is, however, inadequate for long-term culture purposes. The provision of either Medium I or II for artificial cultures presents a formidable problem. It is in this context that the results obtained with the relatively simple chemical medium (Medium III) is considered interesting.

In this medium, the worms apparently lived a normal life and there was evidence of attempted copulation. Copulatory caps were found attached to the bodies of worms of both sexes. The deposition of copulatory caps by males on other male worms also occurs in nature (Chapter II). It seems therefore, that the failure to achieve a successful copulation in these cultures, is partly due to the absence of the right positional relationships between the worms. Smyth (1955) solved the problem of the positional condition in S.solidus by using seamless cellulose tubing in his culture media. This imitated the gut wall, pressure against which was required before insemination could take place. Observations of E.truttae in its natural environment have shown that these worms copulate mainly in the upper intestine where they are attached together in groups, with their hind ends in close proximity. A similar relationship between worms in artificial cultures, could be achieved by the provision of a matrix perhaps tubular in form. It must be such that worms can secure effective attachment of their probosces without going through easily. Its position in the culture medium should also ensure that worms can change their points of attachment without being eliminated from the environment. Compressed cotton wool is shown to be an unsuitable matrix as worms disappear into it. A cognate problem yet to be solved is that of removing metabolites from the culture. In the present experiments, the media were changed every day. For a parasite which is shown to require about 8 - 10 weeks for the eggs to mature, daily changes are hardly suitable. Perhaps a method whereby the medium is continuously shifted such as that described by Berntzen (1961) would be applicable.

Although no valid conclusions can be reached from these preliminary trials, it is hoped that the results may be of some use to future investigators of the 'in vitro' cultivation of this parasite. The results indicate that if asepsis and the right positional relationship are secured, a medium of fish extract and glucose would be suitable for the culture of E.truttae from the cystacanth stage. It also seems from observations in Medium III, that this parasite may not be exacting in its physio-chemical requirements. Thus the difficulty of providing a suitable medium in large quantities could be overcome by the development of a relatively simple but nutritive chemical medium along the lines pointed out above. The recent isolation of a growth factor for parasitic nematodes from horse liver (Sayre et al 1961) is a step in the right direction. Silverman (1963) has noted that even the standard chemical media, when unsupplemented with serum or tissue extracts, have proved less suitable than simple balanced salt solution for 'in vitro' cultivation.

Temperature is also shown to be an important physical factor to be taken into consideration in planning critical and more extensive 'in vitro' cultivation of this parasite. It is probable that the comparatively low survival periods were partly due to the abnormal temperatures under which the cultures were kept. A similar temperature effect was noted for worms in simple salines. Smyth (1946) showed that at 40°C (the body temperature of the avian final host) the plerocercoids of S.solidus developed rapidly to sexual maturity in 2 - 3 days in isotomic media. Below that temperature the plerocercoids failed to develop. E.truttae is unlikely to tolerate temperatures much above 15°C.

Dougherty (1959) proposed this species as a possible candidate for 'in vitro' cultivation studies. He, however, noted that as a member of one of the groups of the less known lower invertebrate metazoa which are obligately parasitic, its axenic cultivation posed special problems. Some of these have been dealt with above for the ex-sheathed cystacanth-adult phase of the life cycle. Considerably more problems have to be faced in planning axenic cultivation from the egg to the unsheathed cystacanth stage. These include (i) the development of simple and sterile media for hatching the acanthors and exsheathing the cystacanths. No success has been achieved in hatching the acanthor by alternate drying and wetting (Manter 1928, Moore 1942). Incubation in pepsin (Vik and Meyer 1961) or in pepsin-trypsin cycle (Erasmum 1962) also produced negative results (Chapter III);

(ii) the provision of a suitable isotonic and highly nutritive medium for the growth and development of the acanthor and acanthella stages;

(iii) the development of a suitable method for the removal or dilution of by-products of metabolism. This is particularly important as the acanthor and acanthella stages are stationary. Stoll (1959) showed that even for such active nematodes as Neoaplectana glaseri Steiner 1929 for which special precautions were not needed, even slight agitation of the culture vessels improved results markedly.

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C H A P T E R VII

SEASONAL VARIATION IN THE OCCURRENCE OF E.TRUTTAE

1. INTRODUCTION

Definite periodicity of development and occurrence has been recorded in only a few of the many known Acanthocephala of fish, viz: + Neo. echinorhynchus gracilisentis Van Cleave 1913 and * N.longirestris Van Cleave 1913 from the gizzard shad Dorosoma cepedianum Le Sueur from the Illinois River System (Van Cleave 1916); Neochinorhynchus rutili Müller 1780 in the rainbow trout Salmo irideus Gibbons (Steinstrasser 1936); Leptorhynchoides thecatus Linton 1891 which occurs as a widespread parasite of freshwater fish of the United States (De Giusti 1949): Acanthocephalus lucii Müller 1776 in perch Perca fluviatilis L.of the River Dnepr (Komarova 1950); Echinorhynchus gadi Müller 1776 in the fishes of the White Sea (Shulman and Shulman-Albova 1953).

Van Cleave (1916) dealing with the factors that determine seasonal distribution of Acanthocephala pointed out that "the mere fact that a parasite is present in its final host for the greater part of, or even for

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+ Gracilisentis gracilisentis Van Cleave 1913 by Van Cleave 1919 (ex Petrochenko 1958)

* Tanaorhamphus longirostris Van Cleave 1913 by Ward 1918 " " "

the entire year, is not proof that there is no periodicity in its occurrence. One generation of parasites might overlap another generation, yet if conditions for reinfestation were such that larvae could enter the final host only at restricted periods it would be possible to detect a periodicity in the infestation upon the basis of the distinctions between immature and mature individuals. On the other hand, if the intermediate host of the parasite constitutes a part of the food of the final host throughout the year, the chances for constant reinfestation make it impossible to recognise distinct cycles of infestation",

In the present investigation a detailed study has been made of the occurrence and development of E.truttae in both its hosts in Afon Terrig, North Wales. An attempt has also been made to ascertain to what extent various and varying ecological factors influenced the incidence, degree of parasitisation and the development of the worm in nature.

2. MATERIALS AND METHODS

Samples of the intermediate host Gammarus pulex pulex L., and the final host the brown trout Salmo trutta L., were taken monthly at three points or stations where the stream is readily accessible. The sampling stations were located so as to span the length of the stream where fishing rights had been obtained. Station I was at Rhydtalog - as far upstream as practicable. Station III was sited at the opposite end of the stream around Caegwydd, while the second Station was about mid-way between the above sampling points. To ensure that shrimps and trout were examined as soon as possible after capture, a period of about two weeks was allowed

between the sampling trips for both hosts.

The intermediate host.

Within the first week of each month from November 1961 to January 1963, shrimps were taken from over a stretch of about 30 meters at each station. Although it was planned to continue sampling till March 1963, the severe winter of 1962-63 made sampling very difficult and thus no shrimps were taken after January 1963. The stream was not only frozen over, but was also inaccessible. It may be noted here that failure to sample in February and March 1963 had no effect on the results as, fortunately, sampling had gone on for more than a year before the freeze.

After the first three months of preliminary work, the following procedure was adopted as standard for sampling and examining shrimps. G.pulex was collected with a hand net. The shrimps were taken alive to the laboratory in large breffitt bottles. On arrival in the laboratory the unsorted samples, except those examined on the same day, were deep-frozen. During the first three months, it was observed that adult shrimps and particularly infected ones, tended to die earlier on being kept in glass-covered enamel dishes, at imitated stream temperature, pending examination. By deep-freezing shrimps the inaccuracies which might have arisen from the above observation were eliminated. Frozen shrimps were thawed as they were required and the examination of all shrimps was accomplished in 6 - 10 days after sampling.

About 400 shrimps from each station were dissected and examined under the binocular microscope for the larval stages of the parasite. To enable comparison to be made of the occurrence of the worm in both young

and adult intermediate host, separate records were kept for shrimps below and above 6 mm. in length. The number of worms at each of the following developmental categories was also recorded, viz:

1. Acanthellae at spherical and earlier stages.
2. Acanthellae at oval to the positioning of giant cortical nuclei stages.
3. Acanthellae at rapid elongation to erect proboscis differentiation stage
4. Acanthellae with invaginated probosces and later stages of development including the cystacanth. (See Table 7. 1 - 7. 3).

Several of the dominant elements of the invertebrate fauna of the stream especially Ephemeroptera and Plecoptera nymphs as well as Diptera larvae, were also examined monthly for the parasite.

The definitive host.

Fish were sampled usually during the third week of each month. The capture was by electric fishing and was done between 11 a.m. and 3 p.m. To avoid an early depletion of the fish population of the small stream (Chapter I), the number of trout taken monthly from each of the three stations was fixed at about seven. It was also decided after the first three months to take regularly only trout above 100 mm. in length, as these would give a better picture of the incidence of the parasite in the stream. Fishing was not possible in January and February 1963 because, as noted above, most stretches of the stream were frozen-over. As samples had been taken over a year by December 1962, it was not considered necessary to continue regular sampling after the cold spell. Added to this was the fact that trout of the right size was becoming scarce and thus it was difficult to take the required number at each station.

Table 7. 1.

The occurrence and developmental stages of *E.truttae* in *G.pulex* from Afon Terrig, upstream at Rhydtalog (Station I)

Month	Jan.	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.
No.of shrimps >6 mm. examined	280	270	280	270	280	280	280	280	280	280	280	280
No. " " <6 mm. examined	120	110	120	130	120	120	120	120	120	120	120	120
No.(%) shrimps >6 mm. infected	47(16.8)	26(9.6)	23(8.2)	21(7.8)	26(9.3)	18(6.4)	22(7.9)	10(3.6)	33(11.8)	41(14.6)	29(10.4)	52(18.6)
No. " " <6 mm. infected	10(8.3)	7(6.4)	3(2.5)	4(3.1)	4(3.3)	1(0.8)	0	0	0	2(1.7)	6(5.0)	9(7.5)
Total no. of worms found	79	37	27	31	35	22	24	17	40	46	41	76
No.(%) acanthella in spherical and earlier stages	7(8.8)	4(10.8)	7(25.9)	9(29.0)	5(14.2)	1(4.6)	1(4.2)	1(5.8)	9(22.5)	6(13.0)	5(12.2)	4(5.3)
No.(%) acanthella in oval to positioning of cortical nuclei stages	12(15.2)	9(24.3)	3(11.1)	1(3.2)	8(22.9)	5(27.3)	6(25.0)	2(11.8)	9(22.5)	6(13.0)	5(12.2)	4(5.3)
No.(%) acanthella in elongation to erect proboscis differentiation stages.	18(22.8)	6(16.2)	7(25.9)	6(19.4)	8(22.9)	2(9.1)	4(16.6)	6(35.3)	10(25.0)	17(37.0)	14(34.1)	20(26.3
No.(%) acanthella in invaginated proboscis and later stages.	42(53.2)	18(48.7)	10(37.1)	15(48.4)	14(40.0)	13(59.1)	13(54.2)	8(47.1)	12(30.0)	17(37.0)	17(41.5)	48(63.1)

Captured fish were taken to the laboratory, dissected and examined within 24 hours. Most fish samples except those taken during the warmest months, were alive on arrival in the laboratory and were killed with a sharp knock on the head just before dissection. Each trout was examined as follows. Observation was first made on the general condition of the fish. It was then weighed, measured for length and scales taken for age determination at a later date. The length of the fish was taken from the tip of the snout to the middle of the spread-out tail fin. On opening the fish, the sex condition (stage of maturity) was noted. The stomach was then cut off (see below) and preserved in 70% Alcohol for later study.

The intestine was then slit longitudinally and thoroughly searched for Acanthocephala. Observations were made on the distribution of the parasite within the intestinal tract according to the size, colour, number of worms in each region, as well as their attachment condition (free or attached to faeces or gut mucosa at recovery). The worms were relaxed in cold water for 12 - 18 hours and then sorted according to sex and a note made of the number with copulatory caps and everted bursa as applicable. Female worms were dissected and assessed for their stage of sexual maturity on the basis of the development of the acanthors. Each was allocated to one of the following four categories:

I Females with ovarian balls only

II Females with ovarian balls and immature acanthors

III Females with ovarian balls, immature and mature acanthors

IV Females with mainly mature acanthors.

In females in the third group, a record was kept of the ratio of immature to mature acanthors. Male worms as well as the remains of females from each fish were preserved in Alcohol-formol-acetic (Van Cleave (1953) for future reference. Where, as in some cases, some worms appeared outstanding in length, they were measured before being processed.

Stomach Contents.

Hynes (1950) has reviewed the methods that have been used in the study of the food of fishes. He showed by recalculating and comparing the results of these methods that for a fish with a generalised diet, the methods would give about the same result provided that a large number of stomachs were examined.

From a parasitological point of view, it was felt that the occurrence and number methods in conjunction with records of the degree of stomach fullness would provide the relevant information. The volume method appears to be best for assessing the nutritive values of various food organisms (Ball R.C. (1948), Ball J. N. 1961).

Only the contents of the "standard" Stomach (Ball 1961, Graham and Jones 1962) were examined. To obtain the latter, a cut was made across the pylorus and continued across the adjacent cardiac limb of the stomach to give a U-shaped stomach. As all the fish were caught between 11 a.m. and 3 p.m., the effect of diurnal variation in feeding activity was eliminated. The degree of fullness of the standard stomach was estimated visually in the

fresh condition and points allocated as follows:

Degree of stomach fullness	Points
Empty	0
$\frac{1}{4}$ full	1
$\frac{1}{2}$ "	2
$\frac{3}{4}$ "	3
Full	4

From the above information, the mean number of points per stomach for each monthly sample (fullness index) was calculated (Ball 1957, 1961). This gives some indication of the seasonal fluctuation in the amount of food eaten.

To determine the occurrence and number of organisms, the preserved stomach was opened in a petri dish. All the food organisms were identified as far as possible, under the binocular microscope and records made of the number of each organism or a group of food organisms constituting a 'food type'. It was thus possible to calculate the percentage representation by occurrence and number of each food type in all the stomachs examined each month.

Scale reading.

The procedure adopted in scale preparation and reading was adapted from the works of Jones (1949) Ball (1957, 1961). Scales were taken from an area on the left-hand side above the lateral line and in front of the dorsal fin. This was to offset, as much as possible, inaccuracies likely to arise from the variation in the number of rings on scales in different

regions of the body (Esdiale 1912, Bhatia 1931b). The scales were cleaned by rubbing them between the palm of one hand and a finger of the other. About 10 scales were then picked with a blunt scalpel and mounted in Glycerine jelly or Aquamount. Reading for age was done under the low power of the microscope.

Other fishes:

In addition to the brown trout, the bullhead Cottus gobio L. which is the only other fish occurring in some numbers in the stream (lower stretches only) was examined for E.truttae as described above. No attempt was made to determine the age of this fish.

3. THE OCCURRENCE OF THE PARASITE IN THE INTERMEDIATE HOST.

The main features of the occurrence of the parasite in G.pulex are given in Fig.7.1.

(a) The incidence and intensity of infection.

It may be seen (Fig.7. 1a) that the pattern of incidence in shrimps of both size groups was similar. The peak infection occurred during the winter months of December and January. There was a drop in February and this fall in percentage infection continued during the warmer months till September when an upward trend in incidence may be noticed. It may also be noted that throughout the year the incidence was appreciably higher in shrimps 6 mm. and above in length than in those less than 6 mm., the range in incidence being 3 - 7% in the former and 0 - 3.6% in the latter. Also while the percentage infection in the former size group varied within a range from February to September, the lowest percentage of about 3% being

recorded in June, in the latter size-group, no parasites were taken in July - September. The drop in percentage infection among the larger size group in November may be due to sampling deficiency as a similar fall was absent in shrimps less than 6 mm. in length.

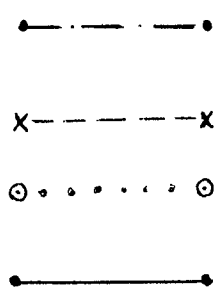
The intensity of infection traces a similar annual pattern as the incidence and follows closely on the percentage infection in shrimps 6 mm in length and above. It is pointed out that the lowest infection rate and degree of parasitisation recorded in summer (June - August) may be in part explained by considering the life cycle of G.pulex. Adult shrimps born in early spring breed and perish during the said period (Hynes 1954, 1955). Most shrimps would belong to the early summer brood and parasitisation of this group would be expected to be lower. It will also be shown hereunder that during the period, comparatively fewer acanthors were being released into the stream. This would further account for the low infection in shrimps.

(b) Changes in the composition of the population of larval stages.

The histogram (Fig. 7. 1b) shows the population dynamics of larval E.truttae in shrimps. All the four major groupings of the stages of development were present all through the year, though in varying proportions, indicating that infection occurred throughout the year. Although no dramatic changes seem obvious, certain trends in the pomposition of the population may be noted. Acanthellae at the spherical and earlier stages of development tended to increase as the temperature fell and constituted a relatively high proportion of the worm population from February to May

Fig. 7. 1

Fig. 7. 1a. The Occurrence of Echinorhynchus truttae in Gammarus pulex (Afon Terrig)



Incidence (% infection) in shrimps > 6 mm. in length.

Incidence in shrimps < 6 mm. in length.

Intensity of infection of shrimps by worm.

Temperature of stream.

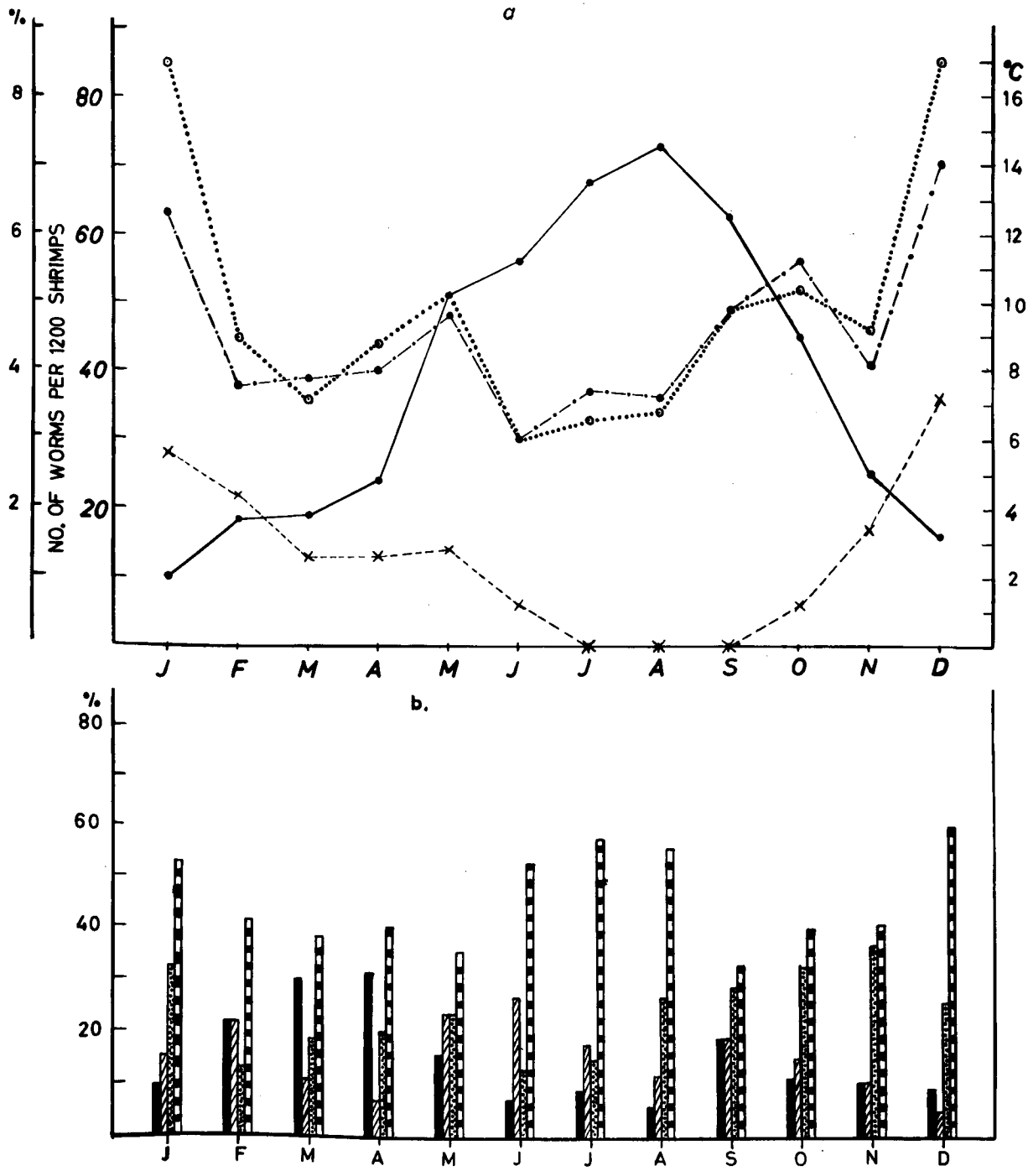
Fig. 7. 1b. Seasonal Trends in the Dynamics of the Population Structure of the Larvae of E.truttae in G.pulex.

Acanthellae at spherical and earlier stages.

Acanthellae at oval to positioning of nuclei stages.

Elongated acanthellae to proboscis differentiation stages.

Acanthellae with invaginated proboscis and later stages, including cystacanths.


a
%
8
6
4
2
80
60
40
20
NO. OF WORMS PER 1200 SHRIMPS
°C
16
14
12
10
8
6
4
2
J F M A M J J A S O N D
b.
%
80
60
40
20
J F M A M J J A S O N D

with a maximum of 31.8% in April. The probable factors responsible for this trend will be discussed later. Acanthellae at oval to positioning of cortical nuclei category attained a peak which followed naturally on that of the preceding and younger stage, the highest percentage of total (26.7%) being observed in June. Elongated acanthellae reached the highest proportions from August to January with a maximum percentage composition of 36.7% in November. A slower rate of development with decreasing temperature (Chapter II) of acanthors ingested in late summer may be partly responsible for this. The percentage of worms with invaginated proboscis was generally high throughout the year. This is not unexpected, as this group is the final developmental 'stage' which remains in shrimps until their death (Chapters II and IV). It is significant, therefore, that they showed peaks, even if slight, in summer (June, July, August) and winter (December-February). The summer peak is probably due to the fact that many larvae including those overwintering in juvenile shrimps (see Discussion) especially those at the 'Elongated Stage', would attain the infective stage about this period, with increasing temperature. The winter peak may find an explanation in the probability that with decreasing temperature, a high proportion of larvae ingested in late summer and autumn would have attained the invaginated proboscis stage of development by December. It may be recalled that it was experimentally shown (Chapter II) that the cystacanth of this parasite is formed in 80 - 240 days depending on environmental temperature. The corresponding figures for the initial invagination of the proboscis are 56 - 220 days. It is also noted

that the relative proportions and smaller peak composition of the first three stages may be correlated with their corresponding durations during development (Chapter II).

The details of the results at the three stations sampled are given in Tables 7. 1 - 7. 3. It may be observed that there were regional differences in the incidence and intensity of infection of shrimps by the worm, these being markedly higher upstream (Table 7. 1) than downstream (Table 7. 3). Thus, while upstream the percentage infection in shrimps above 6 mm. varied from 6% in June to 18.6% in December, the incidence at Stations II and III were much lower, and irregular, the highest figures being 3.2% and 3,6% respectively. The incidence of 3.5% recorded at Rhydtalog (Station I) in August may well be due to errors in sampling as in July and September the percentage infections were 7.8% and 11.8% respectively.

It may be added here that the parasite was not recovered from any other arthropod in the stream.

4. THE OCCURRENCE OF THE PARASITE IN ITS FINAL HOST.

(a) The Distribution of E.truttae in the Intestine of Trout.

From observations made on the parasite on opening the intestine of each fish, it may be noted as follows: The worm was established all over the entire length of the intestinal tract including the pyloric caeca. No established worms were found in the stomach. There was a tendency for a number of worms to be attached in close proximity particularly in the upper intestine where younger worms were concentrated. It was noted earlier

Table 7. 2

The occurrence and developmental stages of E.truttae in G.pulex from Afon Terrig at Station II.

Month	Jan	Feb.	Mar.	April	May	June	July	August	September	October	November	December
No.of shrimps >6 mm.examined	280	300	260	270	280	280	280	280	280	280	280	280
No.of shrimps <6 mm. examined	120	120	100	130	120	120	120	120	120	120	120	120
No.(%) shrimps >6 mm. infected	5(1.8)	3(1.0)	3(1.2)	5(1.9)	4(1.4)	5(1.8)	4(1.4)	9(3.2)	3(1.1)	3(1.1)	3(1.1)	4(1.4)
No.(%) shrimps <6 mm. infected	0	0	0	0	0	0	0	0	0	0	0	0
Total no.of worms found	5	5	3	5	4	5	4	9	3	3	3	4
No.(%) acanthella in spherical and earlier stages	1(20.0)	4(80.0)	1(33.3)	1(20.0)	1(25.0)	0	2(50.0)	0	0	0	0	0
No.(%) acanthella in oval to positioning of cortical nuclei stages	1(20.0)	1(20.0)	0	1(20.0)	1(25.0)	2(40.0)	0	0	0	1(33.3)	0	0
No.(%) acanthella in elongation to erect proboscis diffentiation stages	1(20.0)	0	0	1(20.0)	1(25.0)	1(20.0)	0	2(22.2)	2(66.7)	0	2(66.7)	1(25.0)
No.(%) acanthella in invaginated proboscis and later stages.	2(40.0)	0	2(66.7)	2(40.0)	1(25.0)	2(40.0)	2(50.0)	7(77.8)	1(33.3)	2(66.7)	1(33.3)	3(75.0)

Table 7. 3

The occurrence and developmental stages of E.truttae in G.pulex from Afon Terrig, downstream at Caegwydd (Station III).

Month	Jan.	Feb.	March.	April	May	June	July	August	September	October	November	December
No.of shrimps > 6 mm. examined	280	250	240	270	280	280	280	280	280	280	280	280
No.of shrimps < 6 mm. examined	120	130	80	130	120	120	120	120	120	120	120	120
No.(%) shrimps > infected	1(0.4)	2(0.8)	4(1.7)	6(2.2)	10(3.6)	2(0.7)	5(1.9)	7(2.5)	5(1.9)	3(1.1)	2(0.7)	3(1.1)
No.(%) shrimps < infected	0	1(0.8)	0	1(0.8)	1(0.8)	1(0.8)	0	0	0	0	0	2(1.7)
Total no.of worms found	1	3	6	8	11	3	5	8	5	3	2	5
No.(%) acanthella in spherical and earlier stages	0	2(66.7)	3(50.0)	4(50.0)	2(18.2)	1(33.3)	0	1(12.5)	0	0	0	4(80.0)
No.(%) acanthella in oval to positioning of cortical nuclei stages	0	0	1(16.7)	1(12.5)	3(27.3)	0	0	2(25.0)	0	1(33.3)	0	0
No.(%) acanthella in elongation to erect proboscis differentiation stages	0	0	0	2(25.0)	3(27.3)	1(33.3)	1(20.0)	1(12.5)	2(40.0)	0	1(50.0)	1(20.0)
No.(%) acanthella in invaginated proboscis and later stages	1(100)	1(33.3)	2(33.3)	1(12.5)	3(27.3)	1(33.3)	4(80.0)	4(50)	3(60.0)	2(66.7)	1(50.0)	0

(Chapter II) that this phenomenon would increase the chance of copulation. Chubb (1963) has reported that the production of shelled acanthors by female worms may be correlated with the degree of concentration of Echinorhynchus clavula Dujardin 1845 in the fish intestine, thus in the eel, where the maximum concentration was found, maximum production of shelled acanthors was found. There did not seem to be any relationship between the intensity of infection and occurrence of E.truttae in the caeca. Thus the pyloric caeca bore parasites at intensities of 1 - 75 parasites per fish, while in one fish with 201 worms, none was established in the caeca.

It was also observed that although worms at different stages of development were irregularly interspersed, females with mature eggs were rarely taken in the region of the pyloric caeca. Ekbaum (1938) found that Echonorhynchus gadi (Zöega) Müller recovered from the lower part of the intestine of Salmonids (Onchorhynchus spp.) were at a more advanced stage of development than those found elsewhere. Remarkable grad-ations in the size, colour and stage of maturation of worms were shown in many heavily parasitised intestines. In such intestines, the population varied from smaller dark-orange worms in the pyloric region representing recent infections, to mostly adult-sized dark-grey females near the anal end. It was also interesting to find that in some cases, these dark-grey and adult-sized females, on dissection, bore no mature acanthors, indicating that they were not fertilised. In two such cases, unusually large ovarian balls were found. It may also be added that in a few intestines, dark parasites were found attached near the middle of the intestine.

Free but otherwise apparently normal worms with protracted or retracted proboscis, as well as worms attached to faecal matter, were taken frequently in all parts of the intestine of freshly killed fish. This is interpreted to indicate that such worms may have been in the process of changing their places at the autopsy of the host. It may, nevertheless, be pointed out that the number of worms attached to intestinal contents was more in cases where soft-bodied beasts e.g. earthworms, slugs and fish, were ingested.

Records of the occurrence of the everted bursa and copulatory caps in male and female worms respectively, showed irregular fluctuation through the year. They were also commonly observed on smaller and younger worms in the upper intestine than in worms in the lower. Only in rare cases (4 out of a total of 2096 females) were freshly deposited cream-coloured copulatory caps found on worms containing mature acanthors. Male worms often bore copulatory caps attached terminally or subterminally.

From the above observations, the following conclusions may be reached for the parasite:

1. That E.truttae establishes in all parts of the intestine;
2. That worms activated on entering the pyloric region tend to move gradually down the intestine with age, though the movement of some older individuals may be often irregular;
3. That the extent and rapidity of this movement and the dispersion of worms in the intestine in nature, appears to be influenced by the occurrence and extent of concurrent or superimposed infection, which is shown to be common in natural infections by the presence of worms of

widely varying sizes and stages of development and hence age, in practically all the fish examined;

4. That the correlation between size and stage of sexual maturity (acanthor development) of female worms, on the one hand, and their colour on the other, indicates that the colour of this parasite may serve as rough guide to the age of worms and stage of maturation of the acanthors;
5. That the sexual process may not be very efficient and may depend to a good degree on the intensity of infection. Repeated insemination of females is possible, though whether or not this affects the egg-producing potential is not known.

It should be pointed out that the above findings have been confirmed experimentally (cf. Chapters II and V).

(b) The Incidence and Intensity of Infection in Trout.

All the fish examined were in the size range 100 - 375 mm. Fig.7. 2 summarises the occurrence of the worm in this fish. It may be seen that the proportion of trout infected was high throughout the year, 76.2 - 100%. The mean number of parasites per infected fish appears to show a seasonal trend (Robertson 1953) being higher during the summer months (March - October). The highest mean of 30.8 was recorded in June. The sharp mid-summer drop in July as well as the cyclical changes in the degree of parasitisation of trout may be a reflection of the changing food habits and feeding intensity of the fish. This will be dealt with later.

The relative proportion of male and female worms is indicated by the sex ratio. This was calculated monthly, by dividing the total number of females taken by the total number of males. It may be noticed that except

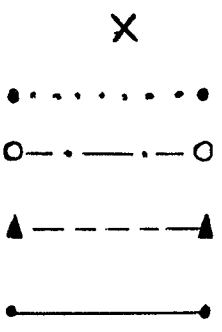
in June (0.9), female parasites outnumbered males throughout the year. This would be expected for it has been shown (Van Cleave 1953, Nicholas and Hynes 1958, Chapter II) that male worms tend to disappear earlier from the intestine than females. That females tended to be present in comparatively higher proportions during the colder months (sex ratio of 2.2 in December and January) than in summer (sex ratio of 0.9 in June), may be correlated with the expected differential in the rate of development of worms during the two periods. Experimental evidence (Chapter II) has shown that at low temperature, the development of the parasite proceeded at a slower rate with the consequent extension of the time of disappearance of both sexes especially the female. It seems plausible to suggest, therefore, that low environmental temperature would lead to a building-up of a population of female worms at various stages of development with a peak around December and January as shown in the graph. It is pertinent to remark that the temperature of the stream during the very cold winters of 1961/62 and 1962/63 was much lower (1-3°C) than that investigated in the laboratory (4 - 10°C) and thus should produce a more marked effect.

(c) Changes in the Composition of the Population of Adult Worms.

The structure of the fluctuations in the population of female worms at different stages of maturation is shown in Fig. 7. 2b. From the histogram, it may be noted that females at all stages of development were found throughout the year. There is little doubt that this was due to the observed occurrence of the cystacanth stage through the year. It seems significant that in both cases (January and May) when no worms with

Fig. 7. 2 Summarising the Occurrence and Seasonal trends in the Composition of the Population of Echinorhynchus truttae in the Brown trout (S. trutta) of Afon Terrig.

Fig. 7. 2a Occurrence of E. truttae in Trout.



Ordinate for sex ratio

Incidence

Intensity of infection

Sex ratio (No.of female worms) / (No.of male worms)

Temperature.

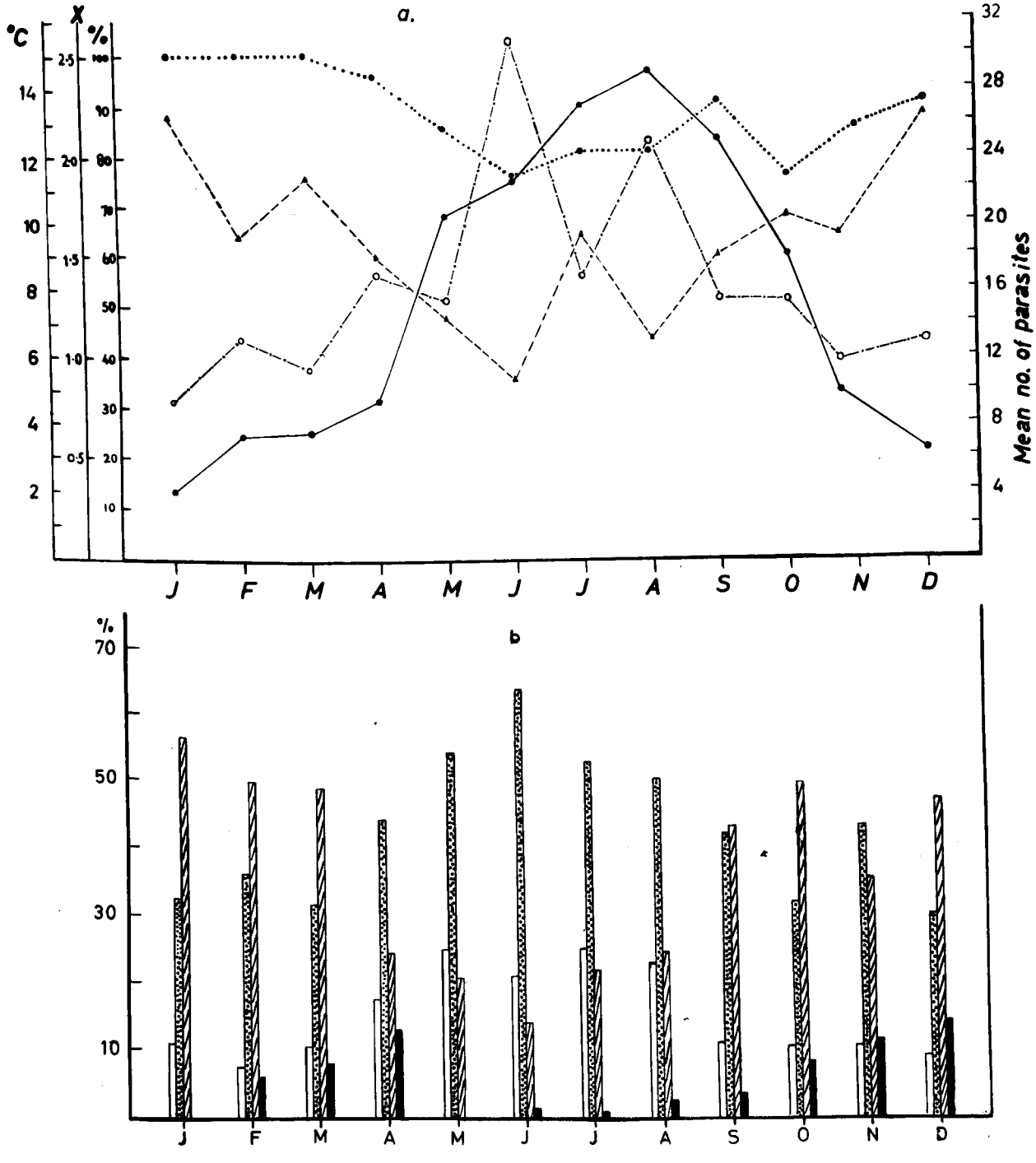
Fig.7. 2b. Stages of Sexual Maturity of Female Worms.

Ovarian balls only

Ovarian balls and immature acanthors

Immature and mature acanthors

Mainly mature acanthors


a.
°C
X
%
Mean no. of parasites
J F M A M J J A S O N D
b
%
J F M A M J J A S O N D

mainly mature acanthors were taken, their absence was immediately preceded by a peak percentage of worms in that category. It also seems apparent that whereas the proportions of female worms with (a) ovarian balls only (b) ovarian balls and immature acanthors, were appreciably higher during the summer (May - August), those of worms containing mature acanthors (comprising (a) females with immature and mature acanthors (b) females with mainly mature acanths), were higher from September to March. It is suggested that the effect of temperature on development pointed out above and the variation in the feeding activity of trout with increasing or decreasing day length and temperature (see Fig. 7. 6) may account for the observed trends. Thus the summer higher proportion of worms with immature acanthors and earlier stages, representing recently acquired parasites, reflects a higher feeding intensity on the part of trout. The acanthors in female worms, acquired in late summer and autumn, depending on the time of ingestion, would gradually attain maturity during the colder months. It is not surprising therefore, that a peak percentage of females with mature acanthors was found in December. The occurrence of a second peak of worms in this category in April is likely due to the maturation of worms acquired in late autumn and winter, with increasing temperature. Other aspects of the relation between the dynamics of the developmental stages in both hosts will be discussed. The various ratios of immature to mature acanthors calculated monthly showed no systematic trend in the turn-over of immature to mature eggs.

Tables 7. 4 - 7. 6 give the details of the occurrence of the parasite at each of the sampling stations. From the tables it may be seen

Table 7. 4

The occurrence of E.truttae and developmental stages of the female parasite in the brown trout of Afon Terrig, upstream at Rhydtalog (Station I).

Month	Jan.	Feb.	March	April	May	June	July	August	September	October	November	December
+No.of trout examined	7	7	7	7	7	7	7	7	7	7	7	7
No.of trout infected	7	7	7	7	6	6	7	7	7	7	6	7
% trout infected	100	100	100	100	85.7	85.7	100	100	100	100	85.7	100
Total no.of parasites found	67	108	125	128	135	234	140	301	149	160	101	118
Mean no.parasites/infected fish	9.6	15.4	17.9	18.3	22.5	39.0	20.0	43.0	21.3	22.8	16.9	16.9
No.of male parasites	17	43	37	53	60	116	50	143	66	60	51	48
No.of female parasites	50	65	88	75	75	118	90	158	83	100	50	70
No.(%) with ovarian balls only	6(12.0)	2(3.1)	3(3.4)	22(29.3)	17(22.7)	18(15.3)	21(23.3)	39(24.7)	13(15.7)	9(9.0)	6(12.0)	12(17.1)
No.(%) with ovarian balls and immature acanthors only	25(50.0)	20(30.8)	29(32.9)	26(34.7)	36(48.0)	78(66.1)	36(40.0)	76(48.1)	41(49.4)	27(27.0)	30(60.0)	26(37.2)
No.(%) with immature and mature acanthors	19(38.0)	37(56.9)	49(55.7)	19(25.3)	22(29.3)	20(16.9)	32(35.6)	39(24.7)	25(30.1)	59(59.0)	13(26.0)	29(41.4)
No.(%) with mature acanthors mainly	0	6(9.2)	7(7.9)	8(10.7)	0	2(1.7)	1(1.1)	4(2.5)	4(4.8)	5(5.0)	1(2.0)	3(4.3)

+ All the fish examined were above 100 mm. in length.

Table 7. 5

The occurrence of E.truttae and developmental stages of the female parasite in the brown trout of Afon Terrig, at Station II.

Month	Jan.	Feb.	March.	April	May	June	July	August	September	October	November	December
+No.of trout examined	7	7	7	7	7	7	7	7	7	7	7	7
No.of trout infected	7	7	7	7	6	4	7	6	5	4	7	6
% trout infected	100	100	100	100	85.7	57.1	100	85.7	71.4	57.1	100	100
Total no.of parasites found	106	96	79	180	104	235	144	97	58	54	89	101
Mean no. parasites/infected fish	13.3	13.7	11.3	25.7	17.3	58.8	20.6	16.2	11.6	13.5	12.7	16.8
No.of male parasites	31	40	35	71	52	135	58	42	14	25	27	18
No.of female parasites	75	56	44	109	52	100	86	55	44	29	62	83
No.(%) with ovarian balls only	7(9.3)	7(12.5)	6(13.6)	11(10.1)	16(30.8)	28(28.0)	21(22.4)	8(14.5)	2(4.6)	1(3.5)	6(9.6)	3(3.6)
No.(%) with ovarian balls and immature acanthors	17(22.7)	25(44.6)	17(38.6)	57(52.3)	32(61.5)	62(62.0)	58(67.4)	30(54.6)	10(22.7)	17(58.6)	21(33.9)	19(22.9)
No.(%) with immature/and mature acanthors	51(68.0)	22(39.3)	20(45.5)	23(21.1)	4(7.5)	9(9.0)	7(8.2)	16(29.1)	32(72.7)	6(20.7)	21(33.9)	40(48.2)
No.(%) with mature acanthors mainly	0	2(3.6)	1(2.3)	18(16.5)	0	1(1.0)	0	1(1.8)	0	5(17.2)	14(22.6)	21(25.3)

+ all the fish examined were above 100 mm. in length

Table 7. 6

The occurrence of E.truttae and developmental stages of the female parasite in the brown trout of Afon Terrig, downstream at Caegwydd (Station III).

Month	Jan	Feb.	March	April	May	June	July	August	September	October	November	December
+No.of trout examined	7	7	7	7	7	7	7	7	7	7	7	7
No.of trout infected	7	7	7	6	6		3	4	7	5	5	6
% trout infected.	100	100	100	85.7	85.7	5.7	42.9	57.1	100	71.4	71.4	85.7
Total no.of parasites found	24	74	38	32	44	8	4	25	87	32	24	c 26
Mean no.parasites/infected fish	3.4	10.6	5.4	5.3	7.3	4.0	1.3	6.3	12.4	6.4	4.8	4.3
No.of male parasites.	11	25	11	13	17	8	1	14	36	7	3	9
No.of female parasites	13	49	27	19	27	16	3	11	51	25	21	17
No.(%) with ovarian balls only	2(15.4)	4(8.2)	8(29.6)	3(15.8)	6(22.2)	3(18.8)	3(100)	4(36.4)	5(9.8)	6(24.0)	2(9.5)	0
No.(%) with ovarian balls and immature acanthors	3(23.1)	17(34.6)	5(18.5)	7(36.8)	15(55.6)	9(56.2)	0	6(54.5)	24(47.1)	5(20.0)	6(28.6)	6(35.3)
No.(%) with immature and mature acanthors	8(61.5)	26(53.1)	9(33.3)	8(42.1)	6(22.2)	4(25.0)	0	0	20(39.2)	11(44.0)	13(61.9)	11(64.7)
No.(%) with mature acanthors mainly	0	2(4.1)	5(18.5)	1(5.3)	0	0	0	1(9.1)	2(3.9)	3(12.0)	0	0

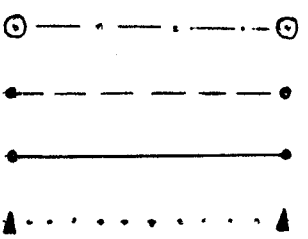
+ All the fish examined were above 100 mm. in length

that, as in the intermediate host, there was some regional variation in the incidence of the parasite in trout. Almost all the fish examined from upstream around Rhydtalog were infected, while at Station III the percentage infection varied from 42.9% to 100%. It is also pointed out that the intensity of infection was markedly higher upstream than downstream (see Fig. 7. 3). The higher rate and degree of infection upstream is correlated with the corresponding higher occurrence of the parasite in G.pulex noted above. It may also be pointed out that, as observed earlier, there was a drop in the degree of parasitisation of trout in all three Stations in July.

(d) The Relationship between the Length and Age of Trout, and the Occurrence of the Parasite.

From Figs. 7. 4 and 7. 5 it may be observed that the incidence and intensity of infection of trout by E.truttae increased with the length and age of fish. Thus in Fig. 7. 4, a substantial number of fish in the 49 - 140 mm. size range was uninfected. The highest incidence and intensity occurred in those 150 - 240 mm. in length, while in fish above 250 mm. long, the degree of parasitisation tended to fall off. Fig. 7. 5 shows that not only did the mean number of parasites per infected fish increase markedly with the age of fish, but that there was a dramatic rise in the maximum infection per fish from 11 in 0+ year group to 201 in the 4+ trout. No trout in its sixth year was taken, but the single representative of the 6+ group contained only 41 parasites. The above observations may find a probable explanation in the variations in the food and feeding intensity of trout with size and age, to be considered later.

Fig. 7. 3. Seasonal Variation in the Intensity of Infection of brown trout by E.truttae in different stretches of Afon Terrig.



Station I (Rhydtalog)

Station II

Station III (Caegwydd)

Temperature.

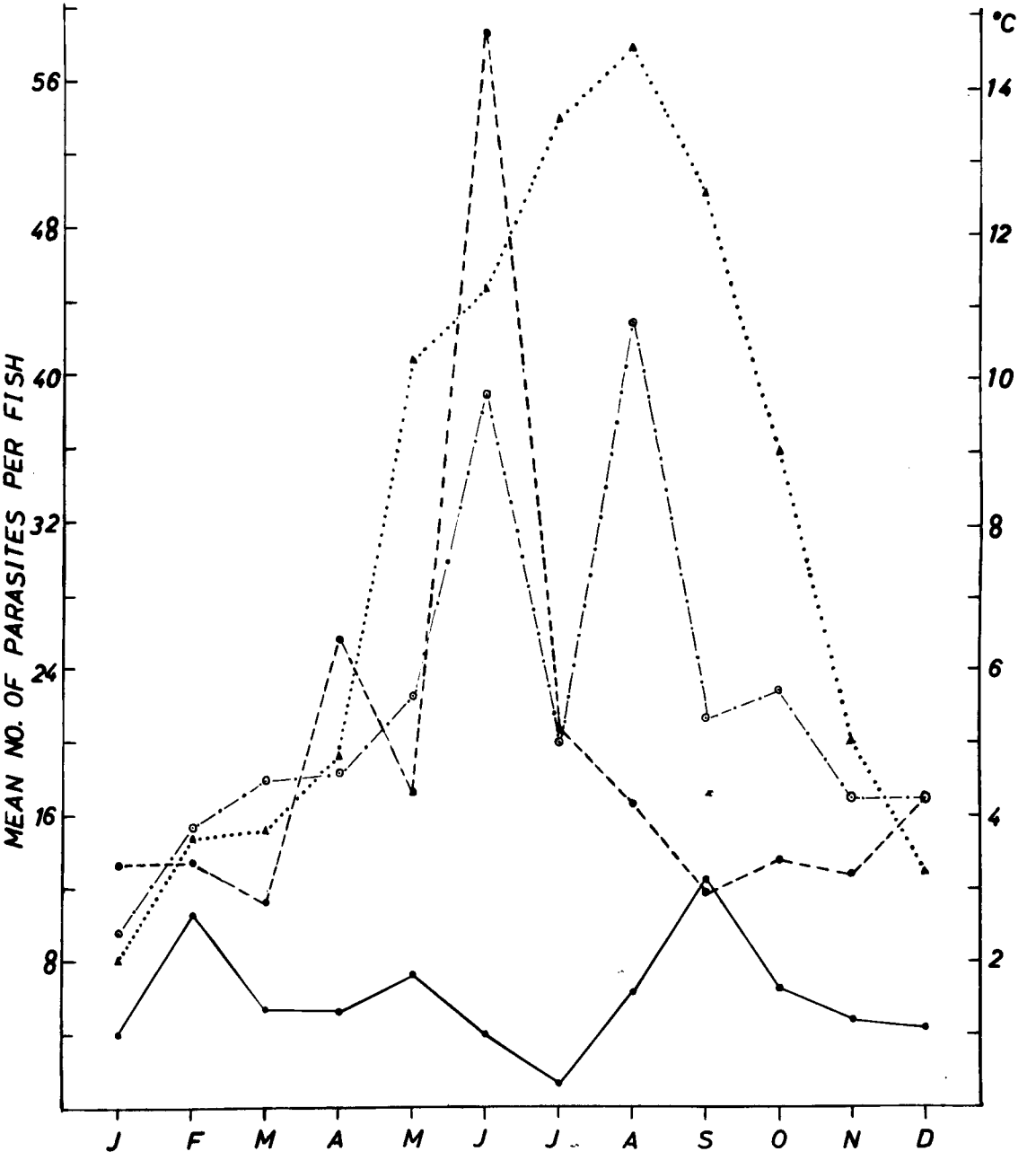

MEAN NO. OF PARASITES PER FISH
°C
56
48
40
32
24
16
8
14
12
10
8
6
4
2
J
F
M
A
M
J
J
A
S
O
N
D

Fig. 7. 4 The Relationship between the Size of the Trout of Afon Terrig and Parasitisation by E.truttae.

Note: Fish < 100 mm. in length taken in connection with other investigations, were used in the preparation of this diagram.

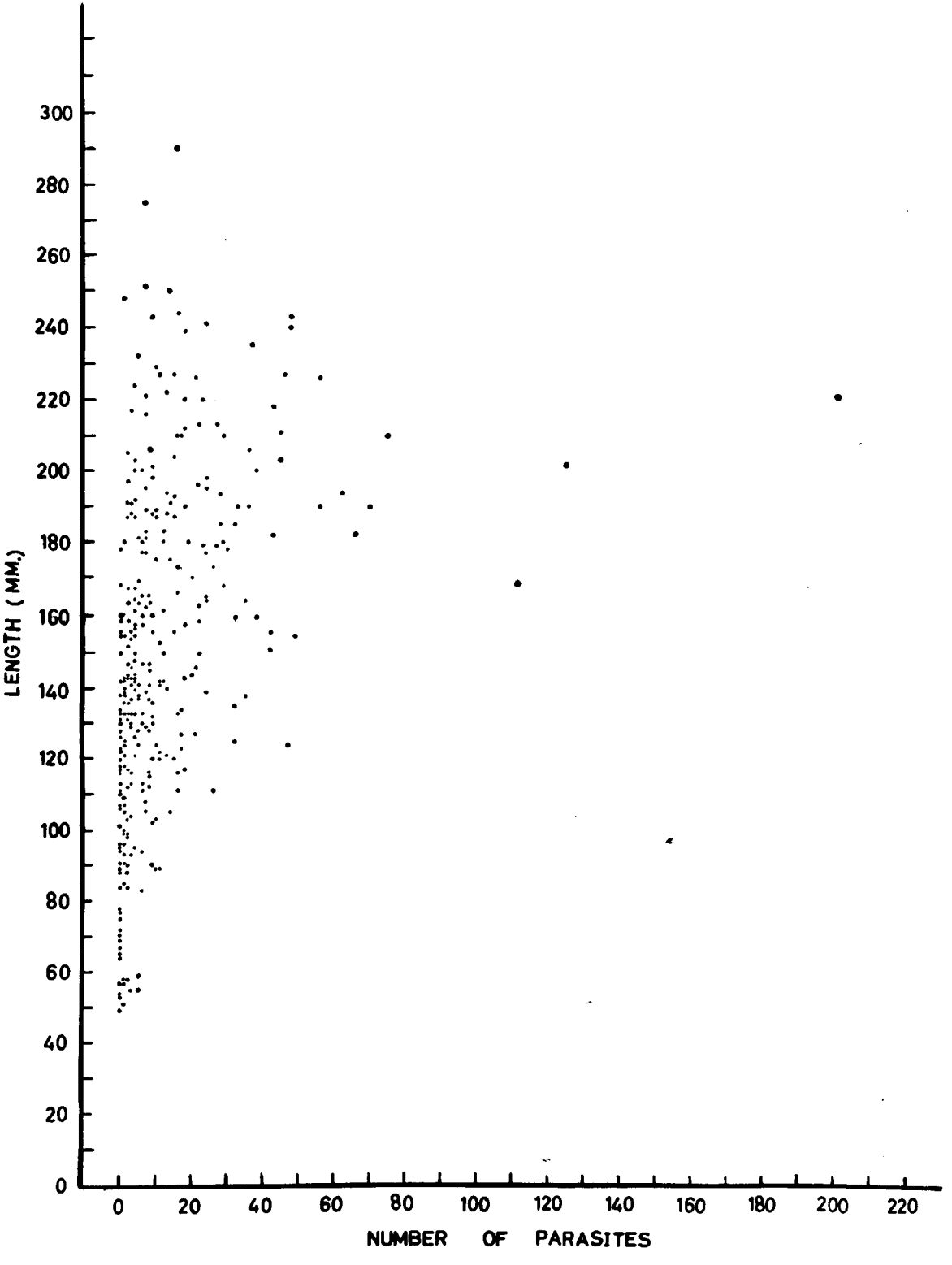
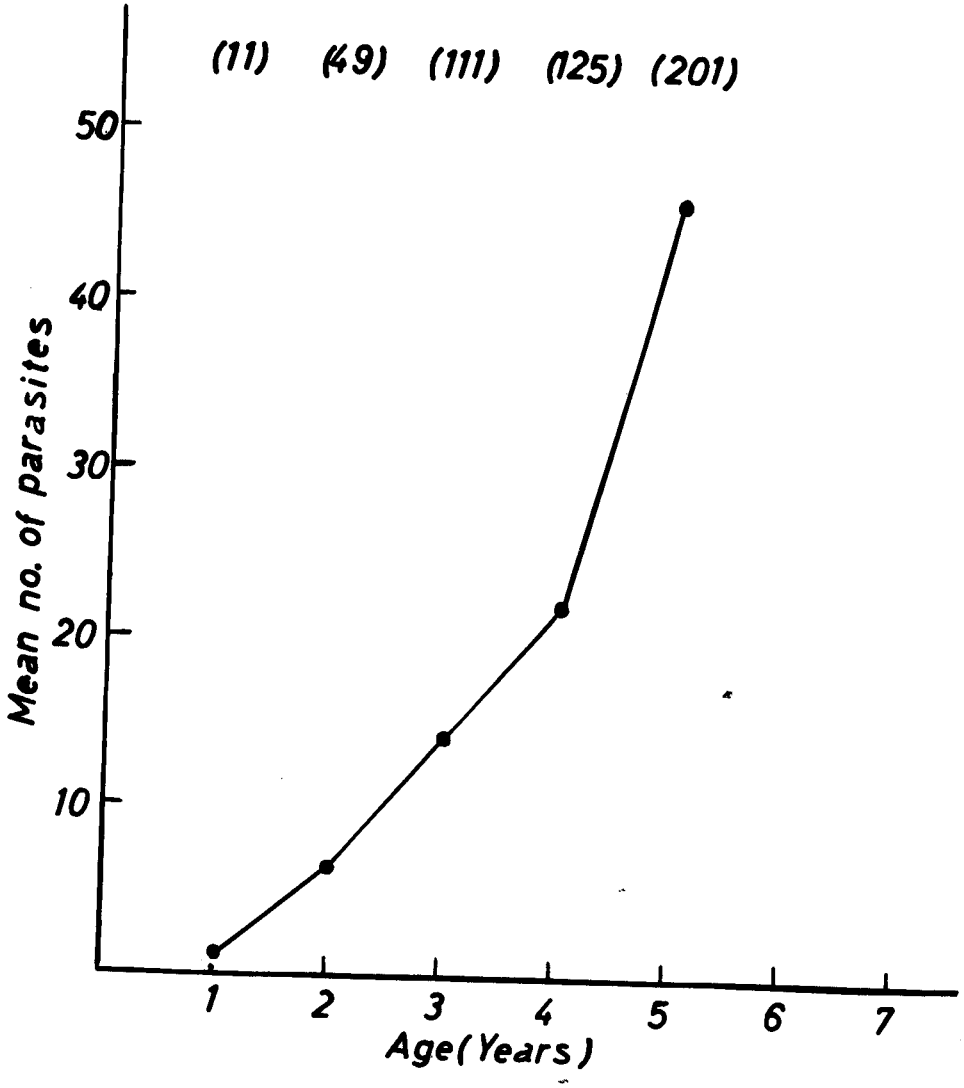

LENGTH (MM.)
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
0
20
40
60
80
100
120
140
160
180
200
220
NUMBER OF PARASITES

Fig. 7. 5. The Relationship between the Age of Trout of Afon Terrig and Intensity of Infection by *E. truttae*.

Note: 1. Numbers in brackets show the highest number of parasites recorded for a fish in each age group.

2. Fish <100 mm. in length taken in the course of other investigations in the stream, used in the preparation of this diagram.


(11) (49) (111) (125) (201)
50
40
30
20
10
Mean no. of parasites
1
2
3
4
5
6
7
Age(Years)

It seems relevant to add here that in the single trout in its seventh year of life mentioned above, the stomach contained a frog.

(e) The Sex of Trout and the Occurrence of the Parasite.

It seems, from Table 7. 7, that there is no correlation between the sex of trout and the incidence and degree of parasitisation. The monthly figures show that the percentage infection was high and fluctuated irregularly in both sexes (76.9 - 100% in males and 62.5 - 100% in females) throughout the year. As might be expected from earlier observations, the highest mean number of parasites was found during the summer for both sexes - 73 in June for female fish and 46.2 in August for the male. Attention may be called to the fact that there appears to be no appreciable difference in the occurrence of the worm during the spawning season (September - December) and the other comparable parts of the year. From the amount of food in the stomach (see below), it was obvious that trout fed less during the spawning period. The probable relationship between the amount of food eaten and the occurrence of this parasite will be considered.

(f) The Food and Feeding of the Brown Trout and the Occurrenee of the Parasite.

The food and feeding of trout in relation to its environment has received a good deal of attention and for such information reference may be made to the works of Phillips (1929), Pentelow (1932), Slack (1934), Allon (1938, 1951), Neil (1938), Frost (1939, 1945, 1950), Swynnerton and Worthington (1940), Wingfield (1940), Butcher (1945), Frost and Smyly (1952) Nilsson (1955), Swift (1955), Ball (1957, 1961), Stube (1958), Holmes (1960),

Table 7. 7

Showing the relation between the sex of trout, the incidence and intensity of infection by E. truttae.

Month	Male trout				Female trout			
	No. examined	No. infected	Percentage infected	Mean No. per infected fish	No. Examined	No. Infected	Percentage infected	Mean No. per infected fish
January	7	6	85.7	9.0	14	14	100	10.1
February	7	7	100	6.7	15	15	100	15.4
March	12	12	100	8.6	11	11	100	13.1
April	7	6	85.7	13.7	15	15	100	17.7
May	13	13	100	10.7	9	6	66.7	24.5
June	14	11	78.6	10,7	8	5	62.5	73.0
July	13	10	76.9	17.4	9	8	88.9	14.1
August	7	6	85.7	46.2	15	12	80.0	10.4
Sept.	8	7	87.5	8.3	13	12	92.3	19.6
October	10	8	80	17.9	11	8	72.7	12.9
Nov.	18	15	83.3	9.9	11	9	81.8	9.6
Dec.	29	28	96.6	12.8	21	17	81.0	10.9
TOTAL	145	129	89.0	14.3	152	132	86.8	19.3

Graham and Jones (1962) McCormack (1962) "inter alia".

Stomach content examination has revealed that trout in Afon Terrig fed on the following organisma:

List of Food Organisms.

a) Bottom Fauna

Nematomorpha
Gordius sp.

Annelida
Oligochaeta (including Eiseniella sp.)

Mollusca
Limnaea pereger Müller
Hydrobia (Potamopyrgus) jenkinsi Smith
Ancylastrum fluviatile Müller
Pisidium casertanum Poli.

Crustacea
Gammarus pulex pulex L.

Insecta
Ephemeroptera - nymphs
Baetis rhodani Pictet
Rithrogena semicolorata Curtis
Ecdyonurus torrentis Kimmins
Ephemerella ignita Poda
Ephemera danica Müller
Paraleptophlebia submarginata Stephens

Plecoptera - nymphs
Leuctra hippopus Kempny
L. fusca L.
L. inermis Kempny
L. geniculata Stephens
L. nigra Olivier
Perla bipunctate Pictet
Perlodes microcephala Pictet
Amphinemura standfusi Ris.
A. sulcicollis Stephens
Nemoura cambrica "
N. erratica Classen
Protonemura praecox Morton
Brachyptera risi "
Isoperla grammatica Poda
Chloroperla torrentium Pictet
Capnia bifrons Newman

Trichoptera larvae
- Agapetus fuscipes Curtis
- Odontocerum albicorne Scopoli
- Stenophylax sp.
- Halesus sp.
- Plectrocnemia sp.
- Philopotamus sp.
- Silo sp.
- Rhyacophila sp.
- Hydropsyche sp.
- Polycentropidae

Coleoptera larvae
- Helmis maugetii Bedel
- Helophorus sp.
- Agabus sp.
- Platambus sp.
- Helodidae

Megaloptera larvae
- Sialis lutaria L.

Diptera larvae and pupae
- Simulium ornatus Meigen
- S. latipes "
- S. aureum Fries
- Tipula larvae (large)
- Orthocladinae
- Tanytarsinae
- Tanypodinae
- ? Empididae

b) Surface and Aerial organisms

Mollusca
- 'Slugs'

Crustacea
- 'Woodlouse'

Insecta
- Collembola
  - Proisotoma sp.
- Ephemeroptera
  - Ecdyonurus torrentis Kimmins - subimago and adults
  - Bactis rhodani Pictet - subimago
  - Paraleptophlebia submarginata Stephens-subimagos
  - Rithrogena semicolorata Curtis-subimago and adults
  - Ephemera danica Muller - subimagos
  - Ephemerella ignita Poda "

Plecoptera

Leuctra sp. - adults
Nemoura sp. - adults

Orthoptera

'Grasshoppers'

Dermaptera

Forficula sp.

Hemiptera

Psylla sp. - nymphs and adults
Velia caprai " " "
Jassidae " " "
Cercopidae " " "
Anthocoridae " " "

Coleoptera

Carabidae - adults
Cantharidae "
Scarabacidae - larvae
Agabus sp. - adults
Platambus sp. "
Other Dytiscidae "
Helophorus sp. "
Esolus sp. "
Staphylinidae "
Clavicornia "
Curculionidae "

Megaloptera

Sialis lutaria L. adult

Trichoptera

Agapetus fuscipes -adults

Diptera

Simulium sp. adults
Tipuloidea "
Chironomidae "
Cecidomyidae larvae and adults
Empididae ? " "
Dolichopodidae adults
Leptidae "
Asilidae larvae and adults
Syrphidae larvae
Cyclorrhapha adults

Lepidoptera

'Caterpillars'
Adult moths

Hymenoptera - adults
Ichneumonidae
Proctotrupoidea
Formicoidea
Vespula sp.
Apidae

Aranea - probably all terrestrial

Acarina including aquatic forms

Myriapoda
Chilopoda
Iulidae

Pisces
Teleostei
Salmo trutta L. - eggs and whole fish

Amphibia
Anura
Frog

Vegetable matter - including algae, mosses etc.....

From the monthly analysis of stomach contents, the relevant data given in Tables 7. 8, 7. 9 and Figs. 7. 6 and 7. 7. have been worked out. It was observed (Chapter I) that G.pulex , and the young stages of Ephemeroptera, Plecoptera, Trichoptera and Diptera, were the dominant components of the fauna of Afon Terrig. This may be seen to be reflected in the percentage occurrence of the above food types in the stomach of trout viz: 33%, 36.2%, 29.5%, 52.7% and 38% respectively (cf. Table 7. 8).

From Fig. 7. 6, a seasonal cycle in the feeding activity of fish may be observed. The amount of food taken (fullness index) tended to be higher with increasing daylength and temperature. The reverse was also true. (Allen 1940, 1941b, Wingfield 1940, Brown 1946a, b, c., Swift 1955). A summer drop in feeding activity is indicated. A similar drop has been reported by Hewitt (1943), Ball (1961).

Fig. 7. 6 Showing the Relationship between Daylength, Temperature, and Seasonal Variation of Food Intake.

⊙ - - - - - ⊙ Stomach fullness index

●————● Temperature

● . . . . . ● Daylength

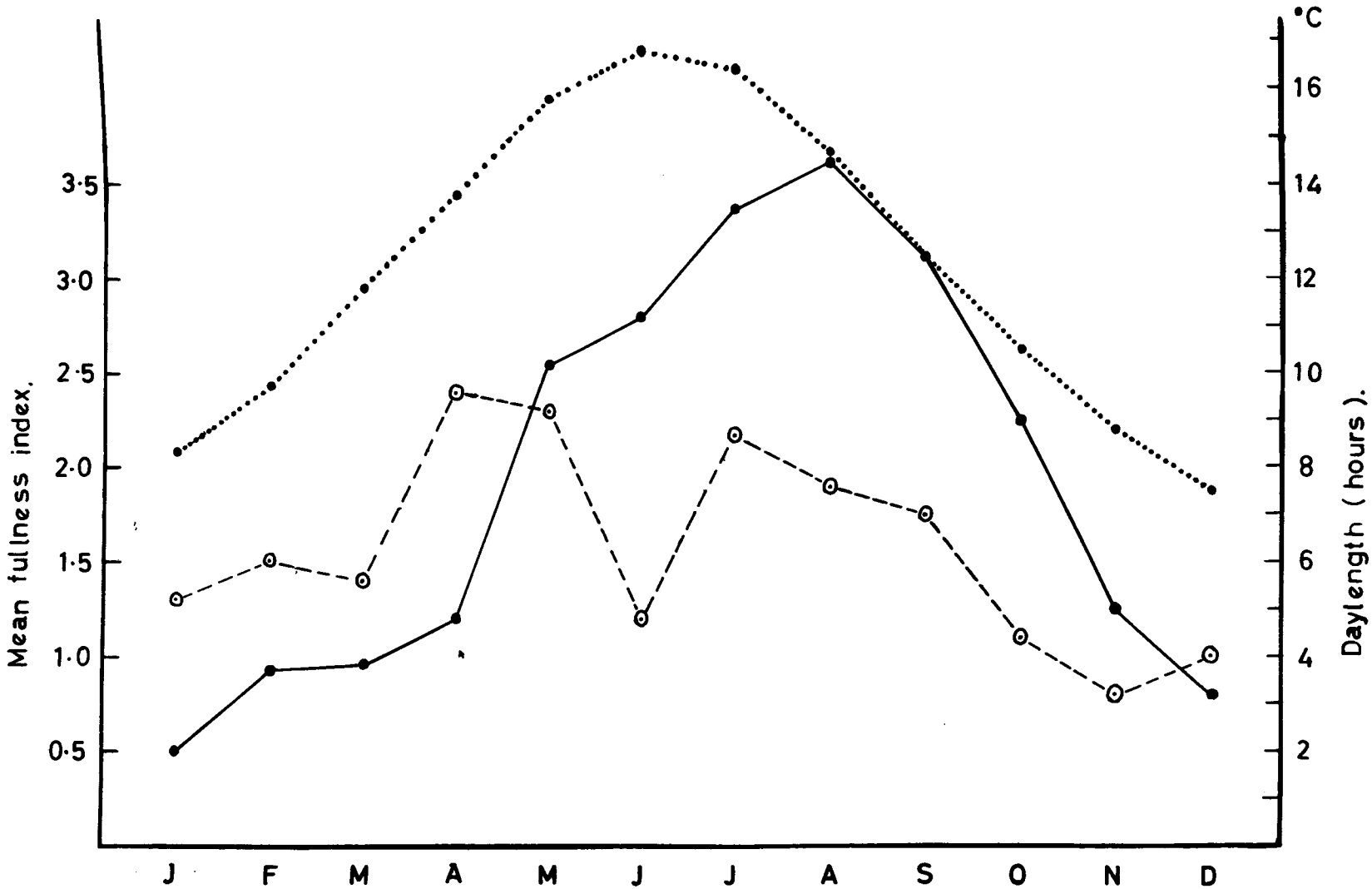


Fig 7·6 SEASONAL VARIATION IN FOOD INTAKE.

Table 7. 8

Composition of the diet of brown trout in Afon Terrig. Percentage representation of each of the main food types as assessed by the occurrence method, Based on 316 stomachs.

Food type	Percentage by occurrence
(i) Bottom fauna	
Annelida	
Oligochaeta	4.1
Mollusca	
Limnaea pereger	2.9
Potamopyrgus jenkinsi	3.5
Ancylastrum fluviatile	7.9
Crustacea	
Gammarus pulex	33.0
Ephemeroptera nymphs	
Ecdyonurus torrentis	14.3
Baetis rhodani	6.0
Unidentified	15.9
Plecoptera nymphs	
Leuctra spp.	2.2
Unidentified	27.3
Trichoptera larvae	
Encased	39.4
Unencased	13.3
Diptera larvae	
Tipula sp.	3.8
Simulium sp.	7.3
Chironomidae	19.0
Unidentified	7.9
Megaloptera	
Sialis lutaria larva	0.9
Coleoptera larvae	2.2
Invertebrate eggs	6.7

(ii) Surface and Aerial food	Percentage by occurrence
Mollusca	
Slugs	3.8
Crustacea	
Woodlouse	1.3
Ephimeroptera - sub-imagos and adults	6.7
Plecoptera " " "	10.8
Dermaptera	
Forficula sp.	0.6
Trichoptera - adults	10.5
Hemiptera - hymphs and adults	6.3
Coleoptera - adults	18.4
Diptera - pupae and adults	23.8
Hymenoptera - adults	3.8
Lepidoptera - larvae and adults	1.3
Aranea (terrestrial)	2.9
Acarina (aquatic)	1.6
Pisces - Trout(whole fish)	1.3
Trout eggs	2.2

Table 7. 9 Percentage composition of the monthly food intake, by number.

	Jan	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.
No.of stomachs examined	22	22	27	23	23	22	22	21	21	21	33	58
Bottom Fauna												
Limnaea pereger	2.2	4.7	0.7	0.2	-	0.6	2.1	4.8	0.6	-	-	-
Potamopyrgus jenkinsi	-	0.5	-	-	-	-	0.3	1.1	0.4	0.9	-	-
Ancylastrum fluviatile	1.1	0.5	-	2.1	-	15.1	-	1.4	0.4	3.5	-	-
Gammarus pulex	10.7	14.0	14.2	10.9	7.6	26.4	10.0	18.3	6.1	46.1	8.7	21.5
Ephemeroptera nymphs	12.9	4.7	5.0	1.2	7.8	3.1	20.7	23.1	9.6	6.1	20.7	4.0
Plecoptera "	11.8	12.1	24.1	11.2	21.2	1.3	1.6	5.7	3.6	0.9	-	2.3
Trichoptera larvae	47.1	52.3	41.2	11.8	21.4	21.4	1.8	4.8	0.4	7.0	31.0	20.9
Simuliid larvae	2.2	7.5	-	1.8	-	-	0.3	1.4	2.3	-	1.7	-
Chironomid larvae	-	1.3	-	25.3	3.6	4.4	3.9	10.8	15.4	1.7	13.9	38.4
Other Diptera larvae	2.2	0.5	-	1.3	0.6	-	1.3	0.3	-	0.9	1.7	-
Sialis sp. larvae	-	-	-	0.3	-	-	-	0.3	-	-	-	1.1
Coleoptera larvae	1.1	-	2.1	0.5	1.3	-	1.0	-	-	-	-	-
Invertebrate eggs	-	-	-	-	-	-	1.6	+numerous	+num-erous	+num-erous	-	-

+ not counted

Surface and mid-water food	Jan.	Feb.	Mar.	April	May	June	July	August	September	October	November	December
Ephemeroptera subimagines and adults	-	-	-	-	-	3.1	-	3.7	8.7	-	-	-
Plecoptera " "	-	-	-	-	-	1.9	-	0.3	7.0	7.0	3.4	1.1
Dermaptera	-	-	0.7	0.2	0.3	0.6	-	-	-	-	-	-
Trichoptera-emergent forms and adults	-	-	-	-	4.9	-	5.5	3.7	6.4	3.5	5.2	-
Hemiptera - nymphs and adults	-	-	-	-	0.6	0.6	2.9	1.4	0.2	4.3	1.7	0.6
Diptera - pupae and adults	1.1	-	7.8	31.4	20.4	14.5	23.6	12.9	34.5	-	5.2	1.7
Coleoptera adults	-	0.5	2.1	0.6	1.9	5.7	21.5	3.7	3.8	4.3	-	1.1
Hymenoptera adults	-	-	-	-	5.8	-	0.3	0.6	0.2	3.5	-	-
Lepidoptera larvae and adults	-	-	-	0.2	0.9	-	-	-	-	-	1.7	-
Aquatic mites	-	-	-	-	-	-	-	1.7	-	10.4	-	0.6
Aquatic and aerial spiders	1.1	-	0.7	0.3	0.8	1.3	0.8	-	-	-	1.7	-
Miscellaneous												
Oligochaetes - aquatic and aerial	1.1	0.5	-	-	0.3	-	0.8	-	0.8	-	3.4	5.6
Pisces - Trout	-	-	-	0.2	0.6	-	-	-	-	-	-	1.1
Trout eggs	5.4	0.9	0.7	0.5	-	-	-	-	-	-	-	-
Amphibia - Frog	-	-	0.7	-	-	-	-	-	-	-	-	-
Summary												
Bottom Food	91.3	98.1	87.3	66.6	63.5	72.3	44.6	72.0	38.4	77.0	77.7	88.2
Surface and mid-water food	8.7	1.9	12.7	33.4	36.5	27.7	55.4	28.0	61.6	33.0	22.3	11.8

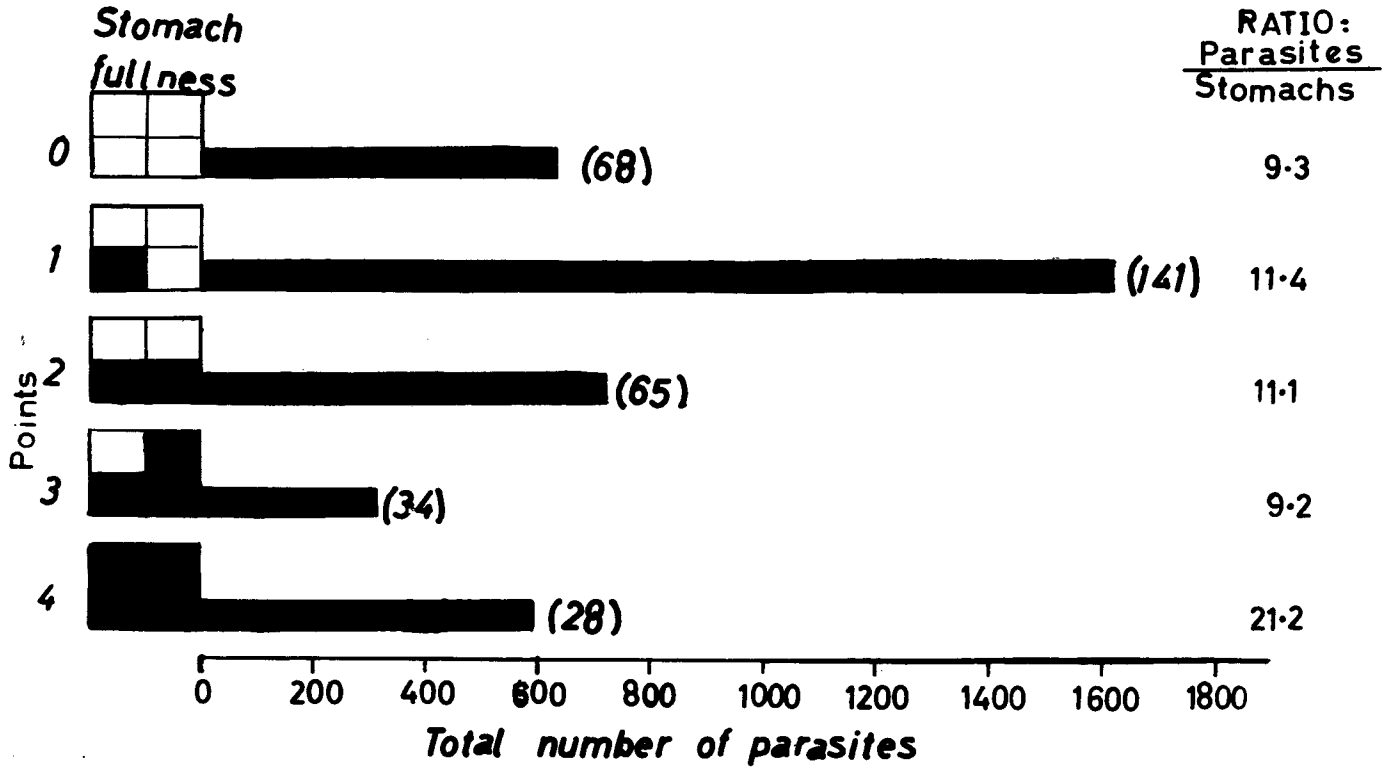
For the details of the seasonal fluctuation in the numerical representation of each of the main food organisms, see Table 7. 9. In summary, it may be said that the changes in the number of the dietary elements shown in the table are reflected in the availability of food animals which, in turn, was seasonal to varying extents. Thus, while G.pulex constituted an important food item throughout the year, Trichoptera larvae had a higher representation and may even be considered dominant during the winter (November to March). Bottom fauna were important in the food of trout throughout the year, though a definite trend in increasing reliance of trout on animals captured at the surface and midwater may be noted during the summer months (April to October). Organisms from the latter source exceeded bottom fauna in June (55.4%) and August (61.6%). In this connection, it should be pointed out that the highest temperature of the stream (15°C) was recorded in August 1962,. Brown (1946c) has shown that food intake in trout increased with temperature up to 19°C. It may be added that the tendency to feed on aerial fauna was more marked in larger trout. Also within the size range investigated (100 - 375 mm), larger fish were more carnivorous. Thus younger trout were recovered from the stomach of fish above 170 mm. in length and frog from trout 375 mm. long. It seems justifiable to say in summary that trout in Afon Terrig is a generalised carnivore capturing its food in moving or drifting condition.

The above findings are in agreement with the conclusions reached by various investigators named above and reviewed by Ball (1961). The salient points of the review are "that the extent to which trout feed on any particular food organism depends mainly on its accessibility and representation in the fauna, these factors alone accounting for the com-

position without involving discrimination by the fish". No evidence for some degree of selective feeding put forward by Allen (1938), Frost (1939, 1945) was found by Allen (1951). "A further point of some importance is that as trout grows it takes bigger food organisms, the larger fish tending to be exclusively piscivorous. Seasonal dietary changes appear to be determined by changes in the availability and size of the food organisms".

Fig. 7. 7 summarises the relationship between the quantity or volume of food taken and the occurrence of the worm. It would seem that the quantity of food taken is neither reflected in the incidence nor in the intensity of infection. Thus the ratio of the total number of stomachs in each category of 'stomach fullness' to the corresponding total number of parasites is about 1:10. The higher ratio of about 1:20 recorded for the full stomachs may be due to the smaller number of stomachs in this category. The above findings lend support to an earlier postulation, that from a parasitologist's point of view, the volume of food eaten is relatively unimportant. It also not only shows that the conclusion arrived at on experimental grounds viz: that temporary starvation does not dislodge established E.truttae, is probably correct, but also indicates that such periods of starvation exist in nature and may be frequent or even diurnal. Empty stomachs were found throughout the year, but were common during the colder months (October to February) when trout was spawning and feeding less actively.

Fig. 7. 7. The Relationship between Stomach Fullness and the Number of E.truttae present in the intestine of brown trout at the time of capture. (An indication of the effect of temporary starvation on the acanthocephalan fauna of S.trutta).


Stomach fullness
RATIO: Parasites / Stomachs
0
1
2
3
4
Points
(68)
(141)
(65)
(34)
(28)
9·3
11·4
11·1
9·2
21·2
0
200
400
600
800
1000
1200
1400
1600
1800
Total number of parasites

5. OBSERVATIONS ON THE OCCURRENCE OF E.TRUTTAE IN THE BULLHEAD, COTTUS GOBIO L.

A total of 44 bullheads were examined from November 1961 to January 1963. They were within the size range of 40 - 107 mm. with most of them above 64 mm. in length. The absence of samples in some months (February, June, August, September and October 1962) was due to the fact that the fish was limited in its distribution in the stream and not very common even within its range. Added to this is the fact that the fish is camouflaged in its habitat - the bottom of the stream, and, in the above months, fish stunned by electric fishing, disappeared in the debris.

It may be seen from Table 7. 10 that the incidence and intensity of 'infection' (one per fish) was very low. Since there appears to be no previous record of this parasite in the bullhead, all the worms found were carefully examined for their condition and mode of attachment to the intestine.

It was found that, except in the case of one worm taken in March, the parasites were normally attached to the intestine. None of the females had copulatory caps but in one occurring alone in one intestine, although there was no copulatory cap, a few immature acanthors (Stage I of Chapter III) were present. Several cases of younger trout harbouring females with developing acanthors in the absence of male worms were found. In these cases it may be safely assumed that male worms responsible for insemination had dropped out since it has been pointed out above that males disappear sooner than females. As far as is known, there has been no evidence of parthenogenesis among the Acanthocephala.

Table 7. 10

The occurrence of *Echinorhynchus truttae* in the bullhead, *Cottus gobio*, from Afon Terrig

Month	No. of fish examined	No. of fish invaded	Mean No. per infected
Jan.	5	1	1
Feb.	0	-	-
March	4	1	1
April	3	2	1
May	1	1	1
June	0	-	-
July	4	2	1
Aug.	0	-	-
Sept.	0	-	-
Oct.	0	-	-
Nov.	10	0	-
Dec.	17	0	-

An examination of the stomach contents showed that the bullhead, like trout, fed mainly on available bottom fauna viz: G.pulex, Ephemeroptera and Plecoptera numphs, Trochoptera and Diptera larvae, as well as Oligochaetes and molluscs. The low incidence and intensity of 'infection' of this fish by the worm may reflect the smaller size of this species of fish and the consequent smaller number of food organisms the gut can accommodate. For instance, in a 77 mm. fish, one large G.pulex and three Ecdyonurus torrentis nymphs caused the distention of the stomach. It may also be recalled that brown trout in the same size group had the lowest incidence and intensity of infection. (cf.Fig.7.4).

6. A NOTE ON SIZE VARIATION WITH REFERENCE TO TAXONOMIC DESCRIPTIONS OF THIS SPECIES.

Detailed systematic data on E.truttae have been given by Shrank (1788), Von Linstow (1895), Lühe (1911), Meyer (1928, 1931), Hoffmann (1954) and Petrochenko (1956). During the present investigation measurements were made of the various parts of the body of some worms recovered from the field.

Attention is called here to a point in which these field, as well as some experimental specimens, showed variation from the data given by Lühe and Petrochenko. Both authors gave the length of female worms as varying from 15 - 20 mm. While, however, Lühe noted that males were 8 - 11 mm. in length, Petrochenko recorded a range of 8 - 10 mm. It is noted here that the range of body length in nature varied widely for both sexes in both shrimps (cf. Chapter II) and fish. Several cases in which

adult worms fell outside the above given ranges were found. Thus male worms as small as 6 mm. as well as up to 13 mm. in length were taken. Several females were above 20 mm. with the maximum length recorded being 22 mm. It may be added in this connection that during the course of experimental investigations of the worm in trout (Chapters II and V), male worms 6 - 12 mm. long were recovered after similar periods in fish. In none of the above cases was there evidence that the variation may have been due to crowding. It has also been shown (Chapter V) that within the range of infection experimentally investigated (30 worm infectinns) and met in nature (up to 201 parasites) in fish of various size groups and age, there was no apparent crowding effect on this species of Acanthocephala. That the length of 6 mm. in male worms taken from the field may be due to a more recent establishment in fish, should be viewed in the light of experimental results from Chapters II and VI, viz: (i) the cystacanths of the species are sexually mature in shrimps and copulate as soon as they are primed in the pyloric region, (ii) worms continue to grow in fish, most males attaining the size of 8 - 10 mm. after 4 weeks and 8 - 11.5 mm. after the 5th week. In females no further increase in range of length was observed after 6 weeks when this was 14 - 16 mm.

As, however, only worms that seemed strikingly short or long were measured, the statistical significance of the observed variations in the length of the body in the natural population could not be determined.

DISCUSSION

Chubb (in press) has reviewed the literature on the periodicity of occurrence of fish Acanthocephala. The survey showed that there was

some relation between the geographical position of the body of water in which a host lives and the presence or absence of a definite seasonal distribution. Thus while Shulman and Shulman-Albova (1953) found a seasonal cycle in the development of E.gadi in the fish of the White Sea, Polyanski (1955) observed no such cycle for the same parasite species in the fish of Barents sea which does not freeze over in the winter. Chubb also found no cyclical fluctuations in the occurrence of E.clavula in Llyn Tegid which is not iced in normal winters. He thus postulated that temperature may play a major part in determining the presence or absence of a well-defined seasonal periodicity of development of some of the Acanthocephala.

The present study shows no seasonal cycle in the incidence of E.truttae in the trout of Afon Terrig. The stream is geographically in the same region as Llyn Tegid (North Wales). Unlike the latter, however, it is relatively small and shallow with open stretches of it being at least snowed-in in winter. It was iced in stretches in 1961/62 and during the very cold winter of 1962/63, the entire stream was frozen-over for two months. Since the period when the stream may be frozen-over is usually short, it would seem that the absence of long cold spells in North Wales may be the underlying factor which leads to the non-existence of a periodicity in the percentage infection of trout by E.truttae. As has been noted above, the latter is directly due to the fact that the two main broods in the life cycle of the intermediate host G.pulex, are overlapping and mature acanthors and cystacanths were found throughout the year. It seems pertinent to add also that while Steinstrasser (1936) found

evidence for seasonal periodicity in N.rutili, no similar results in the same environment were obtained for E.truttae in which the intermediate host was G.pulex. In the River Terrig, shrimps were shown to constitute an important part of the food of trout. This would point to the existence of a dynamic equilibrium in the number of worms gained and lost. Hence the more or less uniformly high incidence in trout. Chubb (in press) postulated that the population of E.clavula in fish at any given time would be the result of the interaction of two variables - a variable but continuous level of infection of fish by cystacanths, and an equally variable and continuous loss of matured worms.

Hoffmann(1954) for the same parasite and final host as currently studied, found that, of a total of 2816 parasites from River Syre, Luxenbourg, only 21.7% were males. Chubb (1963) recorded that the percentage of male E.clavula in grayling, pike, roach and eel was 44.4%, 26.3%, 41.0% and 47.1% respectively. In Afon Terrig there were 1447 males and 2096 female worms in the regular samples. This, as well as the fluctuations in the proportion of males to females (cf. Fig. 7.2, Tables 7. 4 - 7. 6) agrees more with the findings of Chubb for E.clavula. It has been pointed out above that except in June, when there were 259 males and 234 females, females outnumbered males with a tendency for more female worms to be found in the colder months. Considering the latitude of Luxembourg, it seems extremely unlikely that the ratio of almost 4 females to 1 male recorded by Hoffmann, may have been due to temperature effects aided, as noted above, by the earlier disappearance of males. It can be suggested that his datum may not represent the usual relationship in the

proportion of males to females in a population.

The percentage infection of G.pulex by the parasite shows a different picture. Fluctuations were, on the whole, seasonal in character. The lower incidence in summer (June to August) is attributable to (i) the birth of young shrimps and the death of the older generation in which infection would be greater, (ii) the observed fact that a higher proportion of female worms in June - August had immature acanthors and earlier stages (cf. Fig. 7 2b). The higher incidence and intensity of infection in shrimps upstream, as compared with downstream, may be accounted for as follows: (a) The level of parasitisation of trout was found to be generally higher upstream than downstream; (b) The shrimp population is much higher upstream; (c) The stream is markedly narrower in this region. These three factors operating together would lead to a greater incidence of E.truttae upstream than downstream.

The movements of trout, spawning and otherwise, do not appear to affect, to any appreciable extent, the regional differences in the occurrence of this worm in its hosts. This is not surprising, as the stream is small, fast and stony (Chapter I) and most parts of it appear to have suitable nesting sites. In this connection it may be noted that running-ripe females were taken from all sections of the stream from September to January. The indications are that in Afon Terrig, trout movements may be rather limited. Allen (1951) has shown that the brown trout occupy definite home areas in streams. The 'home area' phenomenon has also been found in non-salmonid fish of American streams (Gerking 1953, 1959). Frost (1963) has been able to demonstrate a homing

behaviour, restricted to that associated with breeding habits, in the charr Salvelinus willughbii Günther. However, the possibility that local spawning movements may have been contributory in the establishment and maintenance of the usually higher incidence and intensity of infection in shrimps upstream around Rhydtalog, as well as in the peak infection and intensity found in this region in December and January (cf. Table 7.1), is not ruled out.

The intensity of infection in both hosts was found to be seasonal and to bear an inverse relationship to each other. Thus, while it was high in summer (May to September) and low from October to April in the brown trout, the reverse was the case in shrimps (cf. Fig. 7. 8). This rhythm in the fluctuations of the intensity of infection in both hosts may be correlated with each other by considering the following factors: (i) the food of the brown trout, (ii) the trends in the composition of the parasite population in both hosts, (iii) seasonal variation in the food intake of trout, (iv) seasonal cycles in the life history of G.pulex. It has been noted above that G.pulex appeared to be the only intermediate host in Afon Terrig and that this animal formed an important component of the food of trout at all seasons. Although, therefore, the incidence and intensity of infection in shrimps were found to be relatively lower during the warmer months, yet over 50% of the larval worms in June to August (cf. Fig. 7. 1b) were infective (cystacanths). This higher availability of cystacanths seems to be reflected in the higher proportion of worms without mature acanthors present in fish from May to August. Thus the summer lower incidence and intensity of infection in shrimps

Fig. 7. 8. Comparison of the Seasonal Variation of the Intensity of Infection of the Intermediate and Definitive Host by Echinorhynchus truttae.

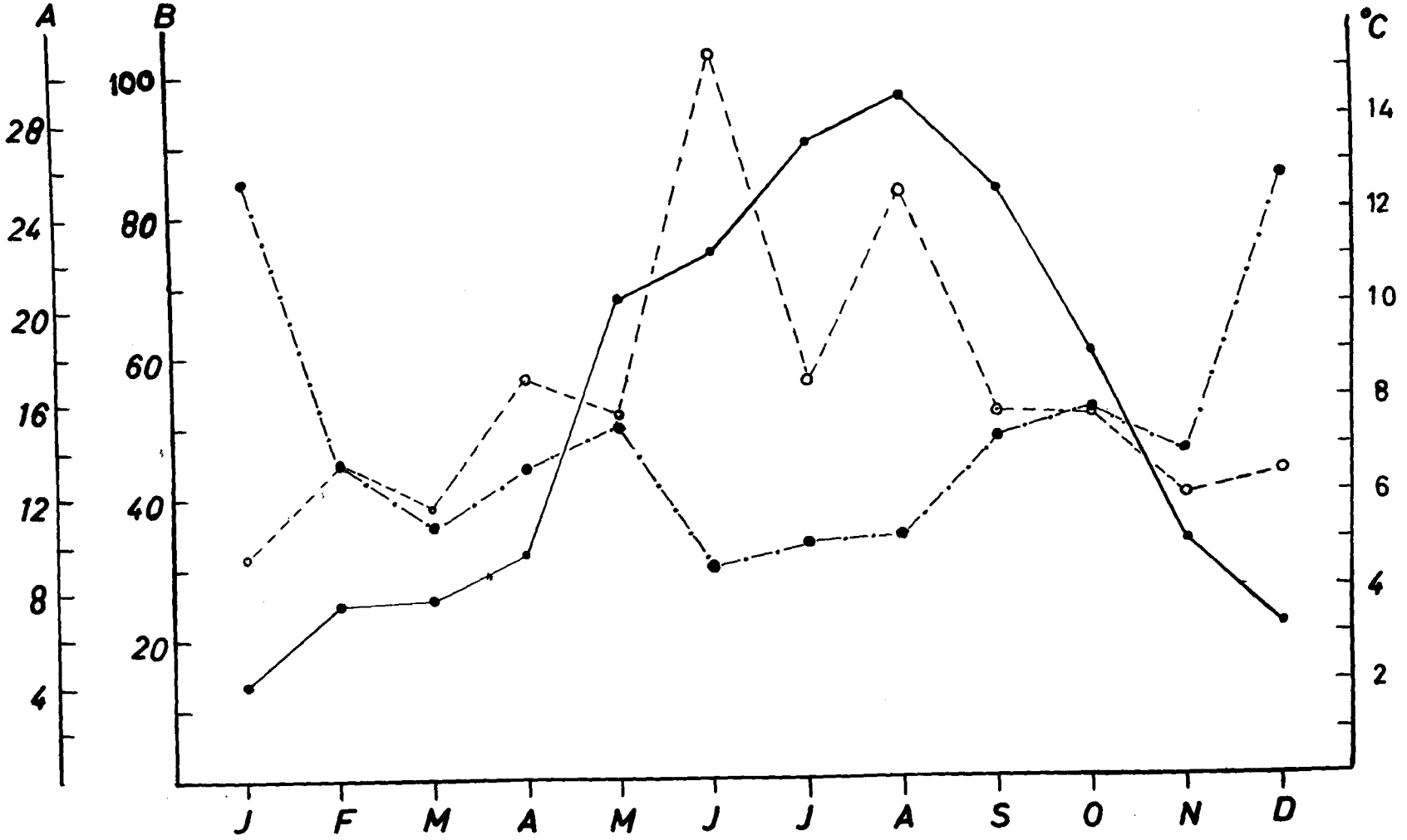
A. - Ordinate showing Mean number of parasites/ fish.

B. - Ordinate showing No.of parasites/1200 shrimps.

•—·—·—• Intensity of infection in shrimps

o— — — —o Intensity of infection in trout

•———— Temperature


A
B
°C
28
24
20
16
12
8
4
100
80
60
40
20
14
12
10
8
6
4
2
J
F
M
A
M
J
J
A
S
O
N
D

were not only offset by the greater feeding activity of trout with rising temperature and increase in day length, but the latter led to a definite increase in the intensity of infection of trout. The studies of Brown (1946b), Swift (1955, 1962), Ball and Jones (1962) have shown that the activity cycle of trout rises with temperature to a maximum of about 10 - 12°C. In this connection, it is interesting to note that the highest mean number of parasites in fish (30.8), as well as the highest percentage composition (84.6%) of worms without mature acanthors, representing recently acquired infections, was recorded in June when the stream temperature was 11.2°C and after two months of intensive feeding (cf. Fig.7. 6). It may also be noted that although in June the food consumption fell, nonetheless G.pulex formed the largest single item (26.4% by number) in the total food intake. The sharp drop in the mean number of parasites in July may, therefore, be a reflection of the above general drop in the amount of food taken in June. It may also be partly due to the predominance of aerial fauna (55.4%) which substantially weights the volume of uninfective meal taken during this month.

In spite of the fact that the temperature rose to a peak in August, a more gradual drop of food intake was recorded from July to September. A similar observation has been reported by Hewitt (1943) Ball (1961). Hewitt explained that the drop may be partly due to increased digestive activity in trout at the higher summer temperature. As it has been experimentally shown (Brown 1946c) that food consumption in trout increased up to 19°C, Ball (1961) contended that the almost entire

reliance by trout on surface food from July to September in Llyn Tegid, coupled with the fact that these (surface food) were smaller and would be more sporadically encountered than bottom forms, could lead to an effective decrease in food supply. The latter would, in turn, account for the lowered food intake. A condideration of the data on the food of trout in Afon Terrig would indicate that the latter explanatinn is broadly applicable. Perhaps the uncertainty and other features of aerial fauna outlined above may have been partly responsible for the unexpected high representation of bottom organisms in June (72.3%) and August (72.0%) It should also be noted that the winter peak (December and January) in both the intensity of infection of shrimps and the percentage of cystacanths available to fish (cf. Fig. 7. 1a,b) did not result in a corresponding higher intensity of infection in trout, probably because trout was feeding less actively with decreasing temperature (October to January) and day length (October to December) (cf. Fig. 7. 6).

The main trends in the composition of the developmental stages of the parasite in the final host appear to be tied to the life cycle of the intermediate host. Hynes (1955) has pointed out as follows: (i) that G.pulex born in March mature in July (3 - 4 months) and breed through August and September. (ii) the late summer brood of G.pulex overwinter as juveniles, mature about March (7 months) and breed from April to June. As is mentioned above, female worms with mainly mature acanthors reached a peak in April and December. It would seem, therefore, that the abundance of young shrimps in March to April and October to January, is correlated with the liberation of a large number of acanthors in the stream. This

would ensure the attainment of the cystacanth stage in the largest possible number of shrimps in each brodd and hence reinfection of the final host and the continuation of the parasite species. As might be expected from the above relationships, it may be noted that appreciable changes in the composition of the developmental stages of the worm in one host were reflected in the other, e.g. the relatively higher occurrence of mature acanthors in the colder months led to the recovery from shrimps, of a higher proportion of larvae at the spherical and earlier stages of development.

It has been shown experimentally that starvation causes the expulsion of M.dubius (Burlingame and Chandler 1941), P,minutus (Nicholas and Hynes 1958) but not of E.truttae (Chapter V). In the present study, evidence has been adduced to show that in nature, trout is subjected to temporary starvation which has no effect on the number of worms harboured (cf. Fig. 7. 7). It is suggested that the different reactions of these acanthocephalan species may be associated with feeding habits of their final hosts. Trout is poikilothermous with experimentally proved diurnal and seasonal variation of activities (Brown 1946c, Swift 1962). E.truttae would thus be expected to be adapted to conditions of partial or complete starvation for varying periods. Rats and ducks, the definitive hosts of M.dubius and P.minutus respectively on the other hand, are homothermous and their feeding activities are independent of environmental temperature. Their intestinal parasites may thus be expected to be pronouncedly affected by the absence of food in the digestive tract.

It was demonstrated experimentally (Chapter V) that there is no size limit of trout that can be infected by E.truttae. Results from the present study not only show that there is no age resistance but that there is a marked increase in the incidence and intensity of infection with the age of fish. Ekbaum (1938) noted that Echinorhynchus lageniformis Ekbaum was more frequent in male than in female starry flounder Platichthys stellatus Pallas caught in the shore waters of Departure Bay, Vancouver Is. No relation between the sex of trout and the incidence and intensity of infection by E.truttae was found in Afon Terrig.

Sandground (1929) has stressed the importance of restricting the term "infection" or "infestation" to cover those cases in which the parasite regularly develops to functional maturity in the particular host species. He went further to point out that "in nature, the introduction into the body of a host of the infectious egg or larva is usually a matter of chance, and, not infrequently, the larva can undergo considerable development before the absence of special conditions in its environment causes the inhibition of further development. Consequently the finding of larvae which have not yet attained functional maturity cannot be taken as constituting infection....." Baer (1952) while discussing reported cases of different bird orders harbouring identical Acanthocephala, within the context of the general problem of host specificity, has also emphasised the importance of specimens being normally attached and fully mature in determining the "proper host". Petrochenko (1956) listed the following as fish species in which E.truttae has been found: Salmo fario, S.irideus, S.erythreus, S.trutta, Thymallus thymallus, Coregonus

laveratus, Esox lucius and two dubious cases in Australian fishes Pomadasys hastata and Sparus berda. Apart from the latter, there was no indication as to whether all the above cases represented actual infections judged by the criteria outlined above. In the present investigation, E.truttae has been recovered from the bullhead Cottus gobio, which, as far as is known, has not been recorded as a definitive host for this parasite. That all the parasites were normally attached and that in one female there were free immature acanthors, would point to the probability of the bullhead being a potential final host. As mature acanthors were not recovered, however, a definite conclusion on the 'infection' of the bullhead by this acanthocephalan species has to await further observational and experimental evidence.

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PART III

STUDIES ON *CREPIDOSTOMUM* SPECIES

(TREMATODA : DIGENEA)

C H A P T E R VIII

THE LIFE CYCLE OF *CREPIDOSTOMUM SPP.*

1. INTRODUCTION

Observations by Chubb (1961), Hynes (pers. comm.) have shown that the fish of Afon Terrig are parasitised by *Crepidostomum farionis* O.F.Müller 1784 (Lühe 1909) and *C.metoecus* Braun 1900 (Braun 1900 b). Other reports of both species in the British Isles have been made by Corbett (1955) in Ireland and Thomas (1957, 1958) in mid-Wales. *C.farionis* has also been recorded from various parts of Britain (Nicoll 1909, 1924; Southern 1912, Brown 1927, Baylis 1928, 1939, Rawson 1952, and Robertson 1953).

A survey of the literature shows that both *Crepidostomum spp.* are widely distributed in the Northern Hemisphere. While *C.farionis* has always been regarded as a common parasite of fish especially the salmonids, *C.metoecus* was first thought to be a parasite of bats. The latter situation arose from the fact that the first description of this species by Braun (1900a) was made on specimens taken from bats. Later Braun (1900b) revised and supplemented the description and established the genus *Crepidostomum*, with *C.metoecus* as type. Odhner (1905, 1910), using the same specimens, amended and improved on Braun's work. Nybelin (1932)

set up a new species Crepidostomum suecicum from specimens taken from five Swedish freshwater fish species.

After a detailed comparative study involving original specimens as well as personal collections, Hopkins (1934) considered C.suecicum Nybelin 1932 to be synonymous with C.metoecus. The recently mounting evidence of the abundance of C.metoecus in fish, as well as the presence of a fewer number of eggs in the original Central European specimens from bats, is now taken to show that fish are the 'natural hosts' and bats only occasional or 'accidental hosts' (Hopkins 1934). However, it remains intriguing that, as Corbett (1955) pointed out, a species "normally parasitic in a cold-blooded animal adapts itself to a very different environment including a temperature of 98°F. "

While the life cycle of C.farionis has been worked out in some detail (Brown 1927), there appears to be little information on that of C.metoecus beyond reports of its occurrence in its final host. Nöller (1928) suggested that Cercaria arhopalocerca Nöller 1925, taken from a sphaeriid mollusc and encysting in chironomid larvae, may belong to C.metoecus. Because of the scanty state of knowledge of this species, it was thought desirable to study and compare the life cycles of both Crepidostomum species in Afon Terrig. The present study also deals with their distribution in the stream. The details of seasonal periodicity will be dealt with in a later work (Chapter IX).

2. FIELD STUDIES

(a) Materials and Methods.

The procedure adopted in sampling and examining fish and shrimps for parasites has been given in Chapter VII. In examining each fish for trematodes, however, the following additional routine procedure was used. After observations on Acanthocephala, the intestine was divided into two halves, each of which was placed in a separate shallow glass dish. The position of the digeneans in the intestine, as well as opened pyloric caeca, was then noted. The intestinal contents were then flushed out and allowed to settle before counts of worms were made. The gall bladder of each fish was also opened and examined. Apart from the regular samples, observations for the parasites were also made in over 100 brown trout taken from the stream in connection with other investigations in November 1962, January, February, April and June 1963.

All the molluscs, as well as the commoner larval insects occurring in the stream (Chapters I, VII), were also examined regularly for the young stages of the trematodes. Unlike the arthorpods, the molluscs were not frozen. They were separated according to species and kept in petri dishes, specimen tubes and occasionally in large aerated glass tanks depending on the number available. They were maintained at both room temperature and the prevailing stream temperature. The stream water in which they were kept was examined at convenient intervals for cercariae. Where, as in Ancylastrum fluviatile Müller, molluscs and stones on which they were attached were kept in large aerated tanks or enamel dishes, uninfected shrimps were introduced in the hope that they would be infected by cercariae. In all cases, as soon as possible after

the examination of fish, some of the molluscs were dissected for larval worms. Others were preserved in formalin for future reference.

When necessary, measurements of both adult and larval worms were made on fresh specimens relaxed in cold or hot water, 60°C, (mainly used for cercariae). Then followed a rapid visual assessment of the stage of development of the parasites. The presence or absence, number and colour of intrauterine eggs, as well as the condition of the eye spots and hence the distribution of pigment spots arising therefrom, were observed. Specimens required for critical study were stained with Acetic Horens Trichrome Stain and Acetic Haematoxylin (Chubb 1962).

(b) Observations.

Only one digenean genus, Crepidostomum was found in the fish of Afon Terrig. It soon became obvious that while C.metoecus (see Fig.8.1) was found in brown trout from all parts of the stream, C.farionis (see Fig. 8. 2) was observed in small numbers and only in fish from Station III. The two parasite species occur in different parts of the intestine. C.metoecus inhabits the pyloric caeca and the upper intestine, C.farionis the lower intestine.

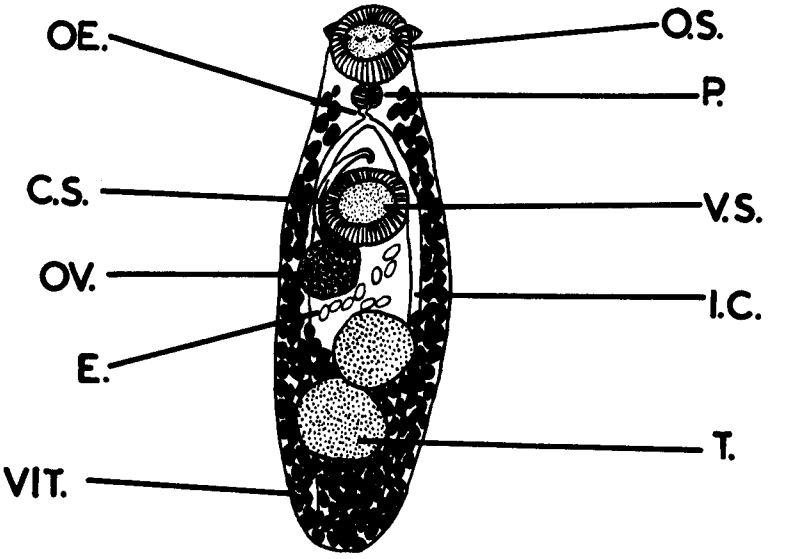
Living metacercariae were found attached mainly to the digestive caeca and often to the intestine of G.pulex. They were abundant at Station II, common at Station III and very rare in shrimps from Station I. Dead and dying spherical metacercariae attached to the muscles, fat body and often Malpighian tubules were recovered from the nymphs of Ecdyonurus torrentis Kimmins, Baetis rhodani Pictet, Paraleptophlebia submarginata Stephens, larval Sialis lutaria L. and once in a Leuctra

Fig. 8.1. Crepidostomum metoecus
(From Chubb 1961)

Note: Figs. 8.1 and 8.2. are drawn to the same scale.

Legends.

O.S. - Oral Sucker
V.S. - Ventral Sucker
P. - Pharynx
OE. - Oesophagus
IC. - Intestinal caecum
OV. - Ovary
VIT. - Vitellaria
E. - Eggs
T. - Testis
C.S. - Cirrus sac



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nymph. Infected specimens of other Plecoptera nymphs, Ephemera danica Müller and Chironomid larvae, were not taken. Although at first an attempt was made to characterise the metacercaria of both species by examining worms from shrimps taken from Station III, this was suspended in favour of experimental establishment of worms in fish, after the examination of hundreds of metacercariae gave no clues. Cheng (1957a, b) has commented on the extreme similarity of metacercariae within the genus Crepidostomum. Harper (1929) has observed that the form of metacercariae depends on the host. It seems likely that the pear-shaped metacercariae of C.farionis described by Brown (1927) may have a different form in G.pulex.

In the light of the above observations, an intensive collection and examination of the molluscan fauna at Stations II and III were made in an effort to complete the life cycle of the two species, with the following results:

(i) Crepidostomum metoecus.

Limnaea pereger Müller serves as the first intermediate host. The developmental cycle in this snail is similar to that described by Brown (1927) for C.farionis. There are two generations of rediae. These are oval to irregular in outline, light brown in colour, and measure from 198 x 165 microns to 649 x 308 microns. They were found all over the visceral mass, particularly the digestive glands or liver, and the mantle. From August to October, the liver in some snails was literally riddled with rediae bearing emerging cercariae. Similar extensive damage

in molluscs often leading to castration, has been reported by various workers (Giard 1888, Lebour 1912, Dubois 1929, F. G. Rees 1931, W. J. Rees, 1936, inter alia).

The cercaria is an elongated, slender, transparent ophthalmoxiphidiocercaria, similar to Cercaria arhopalocerca described by Nöller (1925). It is interesting to note that Nöller (1928) assigned his cercaria, on the basis of resemblance to Brown's (1927) cercariae, to C.metoecus although he found no bats or fish in the vicinity investigated (Thüringia). Cercariae taken from Afon Terrig had the maximum body dimensions of 0.3 x 0.12 mm. In the present investigation, metacercariae have been obtained by exposing uninfected G.pulex to cercariae from L.pereger.

No larval trematodes were observed in A.fluviatile, Potamopyrgus jenkinsi Smith, and slugs from the stream. It may be added, however, that during the swarming season, a few opthalmoxiphidiocercaria were found in the petri dish in which P.jenkinsi were kept at room temperature. It is thought that this may be due to contamination.

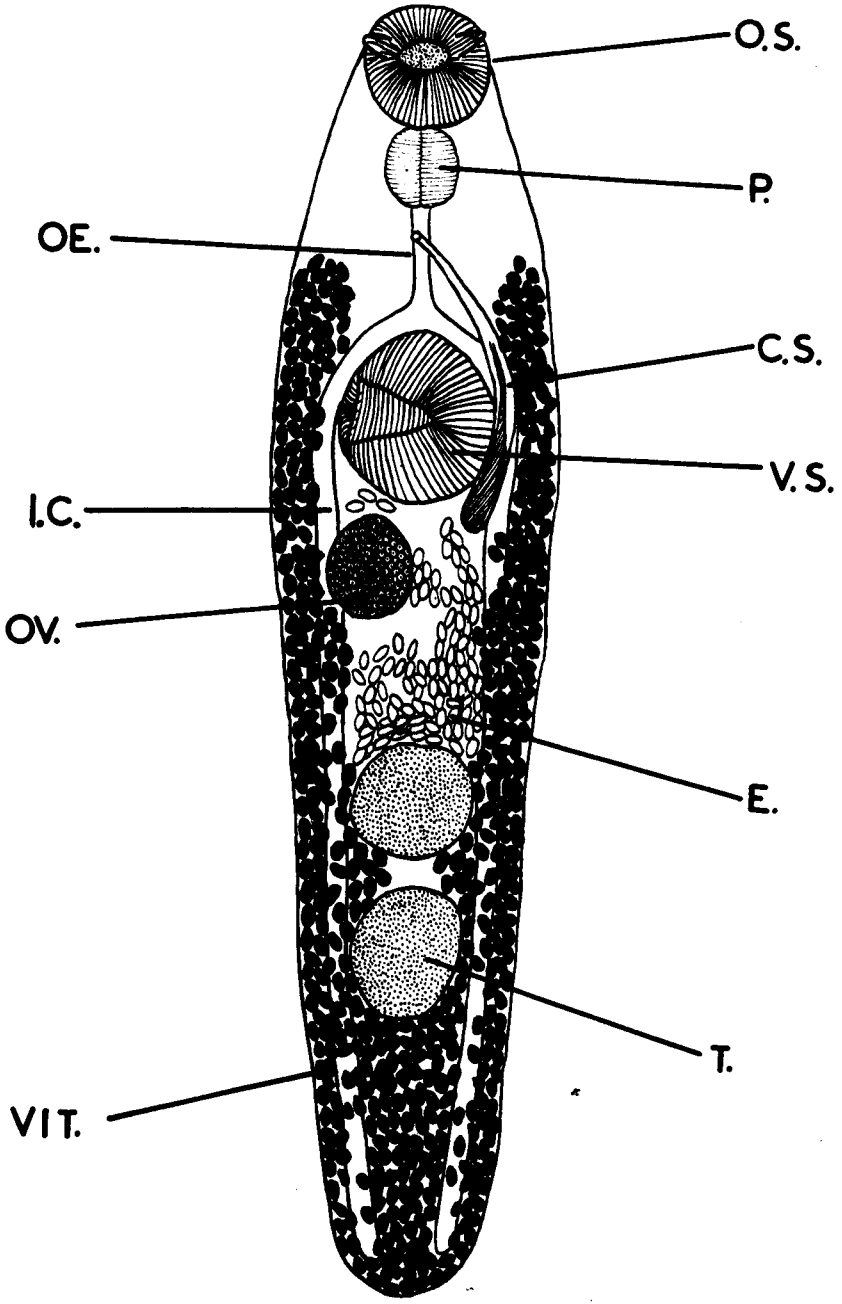
(ii) C.farionis.

The first intermediate host was found to be a sphaeriid mollusc, Pisidium casertanum. This is in agreement with the findings of other investigators on this species. The rediae are attached to the gills of the bivalves. Carcariae encyst in G.pulex (Baylis 1931). G.pulex introduced into petri dishes with bivalves, were infected with metacercariae. As pointed out above, ingested metacercariae liberate young worms which establish in the lower intestine of S.trutta. For the details of the larval stages and life history of this species, reference may be made to Brown (1927).

Fig. 8.2. Crepidostomum farionis
(From Chubb (1961)

Note: Figs. 8.1 and 8.2. are drawn to the same scale

Legends: As for Fig. 8.1.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3. LABORATORY INVESTIGATIONS.

Unless otherwise stated, most of the investigations were done on the larval stages of C.metoecus.

(a) The Cercariae.

(i) Emergence from molluscan host.

During the swarming season, Lpereger were introduced into petri dishes containing stream water and kept under constant observation under the binocular microscope. Some snails were dissected in physiological saline and prolonged observations made on the mode of escape of the cercariae.

It was found that emergence is by active penetration. Cercaria, aided by its stylet, bores its way out through the thin wall of the second generation rediae. Depending on the location of the latter, cercariae tunnel through the body until they reach the outside world. Most emerge through the mantle, the ventral surface of the tentacles and foot. Very few were observed wriggling free of the snail from the dorsal surface of the head and tentacles. In October, scores were seen leaving the snail within minutes and a total of 25 emerged through the rim of the right tentacle in 30 minutes. It may be added that on the ventral surface of the same tentacle, sat two rather elongated rotifers. During the period mentioned above, one of them captured and swallwed 5 cercariae as they were freeing their tails from the rim of the tentacle by side-to-side movement of their body. It is likely that many parasites are lost this way during the swarming season, a feature that may be of some importance in maintaining a balance in the economy of nature.

By leaving snails overnight at both room and stream temperature, it was found that more cercariae were shed during the day and at a higher temperature (Cort 1922, Harper 1929, Smyth 1962). This is in contrast with the observations made on C.farionis by Brown (1927). Brown found no cercariae during the day. In the present investigation a few cercariae of C.farionis were shed from Pisidium when the latter was kept in stream water at 18 - 25°C, during the swarming season.

(ii) Activity and span of free life.

On wriggling clear of the snail host, the cercaria swims rapidly, the body assuming a U shape with the actively lashing tail sticking out from one end. This 'swimming form' contrasts with that of C.farionis. The latter assumes an oval figure of OO with the bent tail arising, as it were, from the ventral side. Tailed and decaudated larvae of C.metoecus were found crawling at the bottom of petri dishes. They are phototropic and the direction on them of a strong beam of artificial light, caused all larvae to swim actively to the surface. By leaving 10, 20 .....100 cercariae repeatedly in stream water in open petri dishes at 8°C, the prevailing temperature of the stream, it was found that cercariae live for a maximum of 5 days. In most of the experiments 50% of the worms were dead after 4 days.

(iii) Mode of invasion of G.pulex.

Uninfected G.pulex, reared in the laboratory, were used to study the method of entrance and encystment of larvae in shrimps. Ten shrimps with light cuticles, were selected and exposed to infection in a petri dish containing many cercariae as well as several snails known to be liberating cercaria.

After swimming for a short time, the shrimps settled on the snails and crawled round them for periods of up to 30 minutes at a time. This behaviour of G.pulex enabled detailed observations to be made in the more natural condition. However, in order to follow the movement of worms within the haemocoel, suitable specimens were slowed down by depressing their heads between a pair of forceps. The results are as follows:

Cercariae come to rest and attach themselves on any part of the shrimp. They crawl over the body, tail raised, until they find a weak spot at the intersegmental areas. The worms then bore through the cuticle and any tissues of the shrimp on their path till they enter the haemocoel. The actual mechanism of penetration takes 15 - 20 minutes and may be less. Many cercariae find their way into the haemocoel through the ventral surface of the body. Thoracic limb joints were also favourite spots of entry. It seems unlikely that, in living specimens, the swimmerets are invaded to any appreciable extent, as even in partially arrested shrimps, these limbs served more to bring cercariae in contact with shrimps.

On successful penetration of the cuticle, the tail is lost and the hind body squeezed into the haemocoel by alternative contraction and expansion of the worm. Some worms lose their tails after unsuccessful attempts at the 'wrong' points but the loss of this organ does not prevent worms already on shrimps from getting into the haemocoel. Whatever their point of entry, all worms crawl about in the haemocoel until they find the digestive caeca. Here they encyst. Cercariae entering the

shrimp through the ventral surface of the abdomen were observed to complete the entire process of penetration to encystment in 15 minutes. Those invading shrimps through the appendages took a longer time, and one larva was observed to encyst after migrating up and down along a limb segment for over an hour.

At encystment, a very thin transparent membrane is formed round the worm, the recently encysted worm thus remaining still erect. Further movements of the worm result in the 'doubling' or folding of the latter which from then on revolves at irregular intervals within the cyst wall. The cyst wall thickens, gradually assumes oval or spherical shape but remains transparent. No encystment was observed outside the body cavity of shrimps.

(b) The Metacercariae .

As indicated above, these are oval or spherical bodies with transparent cyst walls. The diameter or length ranges from 120 - 276 microns.

(i) Artificial Hatching.

In a preliminary attempt to establish the identity of metacercariae from shrimps taken downstream, where both species of Crepidostomum occurred, some metacercariae were hatched with interesting side results. The pepsin/trypsin method, described by Erasmus (1962) and outlined in Chapter III, was employed.

It was observed as follows: (i) all metacercariae which in fresh saline mounts were opaque, with many body folds, well developed, larger and readily visible suckers especially the oral, were hatched successfully.

On wriggling free of their cysts, they moved actively and when transferred to 0.6% saline, they remained active for 6 days, when the specimens were used up in infection experiments; (2) metacercaria which were transparent in fresh mounts, did not hatch successfully. On dissolution of the cyst walls, the contents were discharged into the medium; (3) Metacercaria between the above two categories, were immobile and freed only by complete dissolution of the cyst wall. The worms so recovered were transparent with small rudimentary gonads.

It would appear, therefore, that the metacercarial stage of C.metoecus and probably of C.farionis may not just be a transient phase in the life cycle but one in which considerable development and morphological differentiation occur. It is noteworthy that in September (peak swarming period), less than 30% of hundreds of metacercaria passed through the hatching solution, emerged alive.

Experiments designed to confirm the above observations, did not lead to conclusive results, as shown hereunder:

(ii) Development of metacercaria in G.pulex, and the establishment of the identity of metacercariae.

Experiment I.

G.pulex were infected as before, by leaving them in a petri dish containing cercariae and snails. After 2 - 7 days they were isolated and kept at both room and stream temperature. After 7, 14 and 77 days infected shrimps were naturally administered to hatchery trout as described in Chapter II. Samples dissected before each infection showed that the shrimps were heavily infected, e.g. in those kept for 77 days at room temperature before use, the 12th shrimp contained 39 apparently mature

metacercariae, on dissection. The fish were autopsied 16, 2, and 64 days respectively after infection.

No worms were recovered after a through search of the gall bladder, pyloric caeca and the intestine.

Experiment II.

In this investigation, hatchery brown and rainbow trout were fed with infected shrimps taken from the field, each fish receiving a total of 80 - 500 shrimps. At autopsy, after intervals varying from 7 - 50 days, no trematodes were taken though Echinorhynchus truttae Schrank 1788, present in two shrimps, were established. In all, a total of 14 Salmo trutta L. and 1 S.gairdneri Richardson were used in this experiment.

Experiment III.

In a final effort to establish metacercariae experimentally in trout, a large number of experimentally and naturally established metacercariae were hatched. Again, it was noticed that, while worms 120 days in shrimps at stream temperature were hatched, those only 28 days failed to do so. Excysted worms, in 0.6% saline, were delivered directly into the gut by way of a slender polythene tube passed down the oesophagus with a rubber catheter at the other end. 30 and 50 worms were administered to the two fish fed by this method. The fish were left in a small tank for observation, before they were transferred to the larger and strongly aerated aquarium tanks.

It seemed from the movements of the fish that some parasites may have been regurgitated. After 8 and 9 days respectively, the entire digestive tract and gall bladder were carefully examined for infection. No trematodes were found.

(iii) Development of metaceraariae in some other crustacean species.

Although it was desirable, because of the inconclusive nature of the results of the above experiments, to work out the details of the development of metacercariae in G.pulex, uninfected shrimps were not available. However, as some specimens of other Gammarus spp. and Asellus aquaticus L. were left, it was decided to carry out a preliminary investigation on the establishment and development of larval C.metoecus in these unusual hosts. G.lacustris Sars, G.duebeni Lilljeborgi and A.aquaticus L.were exposed to infection as described for G.pulex. G.lacustris.

All the shrimps used were full grown adults. Infected specimens were kept at room (18 - 20°C) and stream (3 - 4°C) temperatures. Two shrimps were dissected after 21 hours, 2 days and subsequently at weekly intervals.

At stream temperature, most worms taken up to the 35th day after infection were alive, transparent and poorly differentiated. Some shrimps, on the latter date, bore dead metacercariae. The two shrimps left on the 49th day after infection harboured pear-shaped metacercariae 168 - 180 microns in length. These were still transparent with large granules, suckers not readily visible and thus apparently had not attained the relevant final stage.

At room temperature, development appeared to have progressed more rapidly. Specimens taken 35 days after infection, varied from small spherical and poorly differentiated forms 120 microns in diameter, to pear-shaped individuals 0.276 mm. in length, with opaque bodies and easily observable oval and ventral suckers. In the five shrimps remaining after

94 days, only a living metacercaria was taken and even this was ensheathed by host cells. All the shrimps had darkened, dead and disintegrating larvae, also covered with host cells.

It seems clear that more investigations are called for to arrive at a final conclusion on the development and life span of metacercariae in G.lacustris. The above results would suggest that the establishment of C.metoecus is possible.

G.duebeni.

The procedure was as in G.lacustris. Only full-grown shrimps were used.

At 3 - 4°C, 'balls' of host cells were found associated with the digestive caeca. On teasing these, disintegrating larvae were observed. All five of eight shrimps left after 12 days contained dead worms between the digestive caeca.

At 18 - 20°C, 1 of 21 shrimps examined 7 days after infection, contained one revolving metacercaria ensheathed by a mass of host cells. Nine contained dead larvae and 11 were uninfected. On repetition, the experiment was allowed to run for 46 days. Of the 15 shrimps remaining, only 5 contained dead brown parasites.

The above results seem to indicate that while the trematode was destroyed soon after entering the haemocoel at 3 - 4oC, it was able to encyst before being killed, probably by host reaction, at the higher room temperature.

Asellus aquaticus L.

The observations made in this species were as follows. After

49 days at 3 - 4°C, the 2 water hoglouse left, contained dead and dying metacercariae with the remains of the worm shrunken to the centre of some cysts. On the other hand, all 14 Asellus examined 14 days after infection at 14 - 20°C, had living and actively rotating metacercariae.

Summary.

In summary, attention may be called to the interesting similarity of the reaction of the above crustacean species to larval C.metoecus and the acanthocephalan E.truttae. The probable explanation for this has been discussed (cf. Chapter II).

4. DISCUSSION.

The present study has revealed that C.metoecus is more abundant and widespread in Afon Terrig than C.farionis. Perhaps a parasitological investigation of the neighbouring Alun-Dee system would show that this species is even more widely distributed. The above finding is in full accord with the more recent observations made by Thomas (1958) in mid-Wales, and Slusarski (1958a, b, c) in various Polish waters. Commenting on the recent advances in the knowledge of the incidence and abundance of C.metoecus, Slusarski (1958c) wrote: "This species has been considered to be extremely rare in the European continent before. It follows from the analysis of literature and the survey of some original materials that much seems to suggest that many writers have erroneously denoted the fluke they have found and have mistaken C.metoecus for another species, namely C.farionis, which, according to a generally accepted yet probably mistaken opinion, is far more common in Europe". He concluded by saying that "..... C.metoecus should be regarded as a species of considerable

geographical and ecological distribution. Chubb (1961) has suggested that a re-examination of the records may show that C.farionis in Europe is found only in oligotrophic waters.

Hopkins (1934) postulated that C.farionis "must be able to adapt itself to other invertebrate hosts in different parts of its range, though probably always dependent on Sphaeriidae for its molluscan host". The life cycle of this species in Afon Terrig, lends support to this view. G.pulex is shown to be the arthropod host. Dead metacercariae were found lodged in the fat body of Sialis lutaria, and no worms, dead or alive, were observed in Ephemera danica. Both species are the commonly reported arthropod hosts (Brown 1927, Robertson 1953, et al). The current observations would indicate that while the absence of metacercariae in Ephemera danica may be due to its scarcity in the stream, Sialis lutaria appears to react adversely to the establishment of infection in Afon Terrig. In this connection it is significant to note that many dead metacercariae were recovered from Eedyonurus torrentis, a species which is abundant in all stretches of the stream. At this juncture, Harper's (1929) remarks on the occurrence of metacercaria in G.pulex and insect larvae seems pertinent and I quote:"In the literature, there are numerous records of larval distomes in G.pulex and in insect larvae but instances in both appear to be rare".

A.fluviatile was found not to serve as first intermediate host for Crepidostomum in spite of the fact that it is the most abundant mollusc all over the stream. It is suggested that behavioural and habitat isolation from the eggs of the digeneans may be partly contributory to this.

These freshwater 'limpets' are attached to stones and are rarely found at the bottom. This location, coupled with their extremely slow movement in comparison to that of Limnaea, would minimise the chances of this pulmonate ever getting infected.

Another feature of the life cycle of the two Crepidostomum species which has been emphasised by the current study is the importance of the metacercarial stage. Hatching experiments and preliminary observations on the development of C.metoecus under experimental conditions in Gammarus spp. have shown that the metacercaria undergoes considerable organogeny before attaining the opaque and apparently infective form. Unfortunately it was not possible to determine exactly the time these larvae become infective to fish, as all attempts to establish worms in trout failed (compare Robertson 1953). The above observations on morphological differentiation of metacercaria are not without precedent among the Digenea. Harper (1929) has shown that the encysted form of Cercaria X.1. shows many structural advances over the cercaria. Smyth (1962) has noted that the time required for metacercaria to become infective after encystment, varies from a few hours to several months and that the degree of morphological development achieved varies accordingly e.g. from Fasciola hepatica L. where metacercariae become infective soon after encystment, to Coitocaecum anaspidos Hickman 1934 (MacFarlane 1939) which shows progenesis. Buttner (1955), Dawes (1956) have reviewed cases of progenesis among digenetic trematodes.

It has been shown experimentally above that the shape of metacercariae is variable and may depend on the stage of development and on the

host. Thus, while apparently mature metacercariae were oval in G.pulex, they were distinctly pear-shaped in G.lacustris, Harper (1929) made a similar observation on Cercaria X.1. in various arthropods.

Finally, it may be added, that although the chemical processes aiding the drilling action of the stylet during the penetration of shrimp cuticle by Cercariae were not studied, it seems reasonable to assume that the secretions from stylet glands (Wunder 1923c) and probably the cystogenous glands (Harper 1929) were involved.

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C H A P T E R IX

SEASONAL PERIODICITY OF OCCURRENCE OF CREPIDOSTOMUM SPP.

1. INTRODUCTION.

The preceding study (Chapter VIII) dealt with the life cycle as well as some aspects of the distribution of Crepidostomum metoecus Braun 1900 and C.farionis O.F.Müller 1784 in Afon Terrig. In the present chapter the seasonal cycles in the occurrence of these species, in their hosts, are considered.

2. MATERIALS AND METHODS

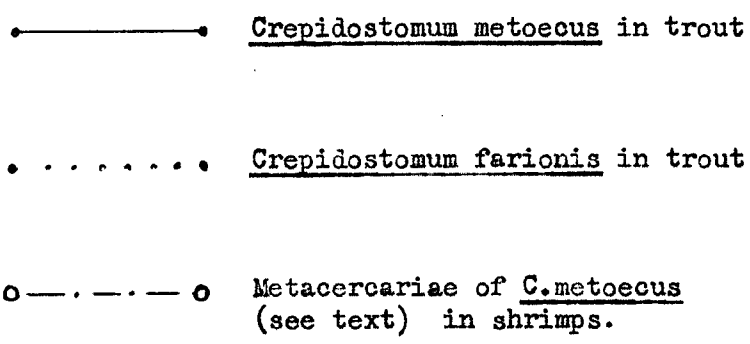
As in Chapters VII and VIII.

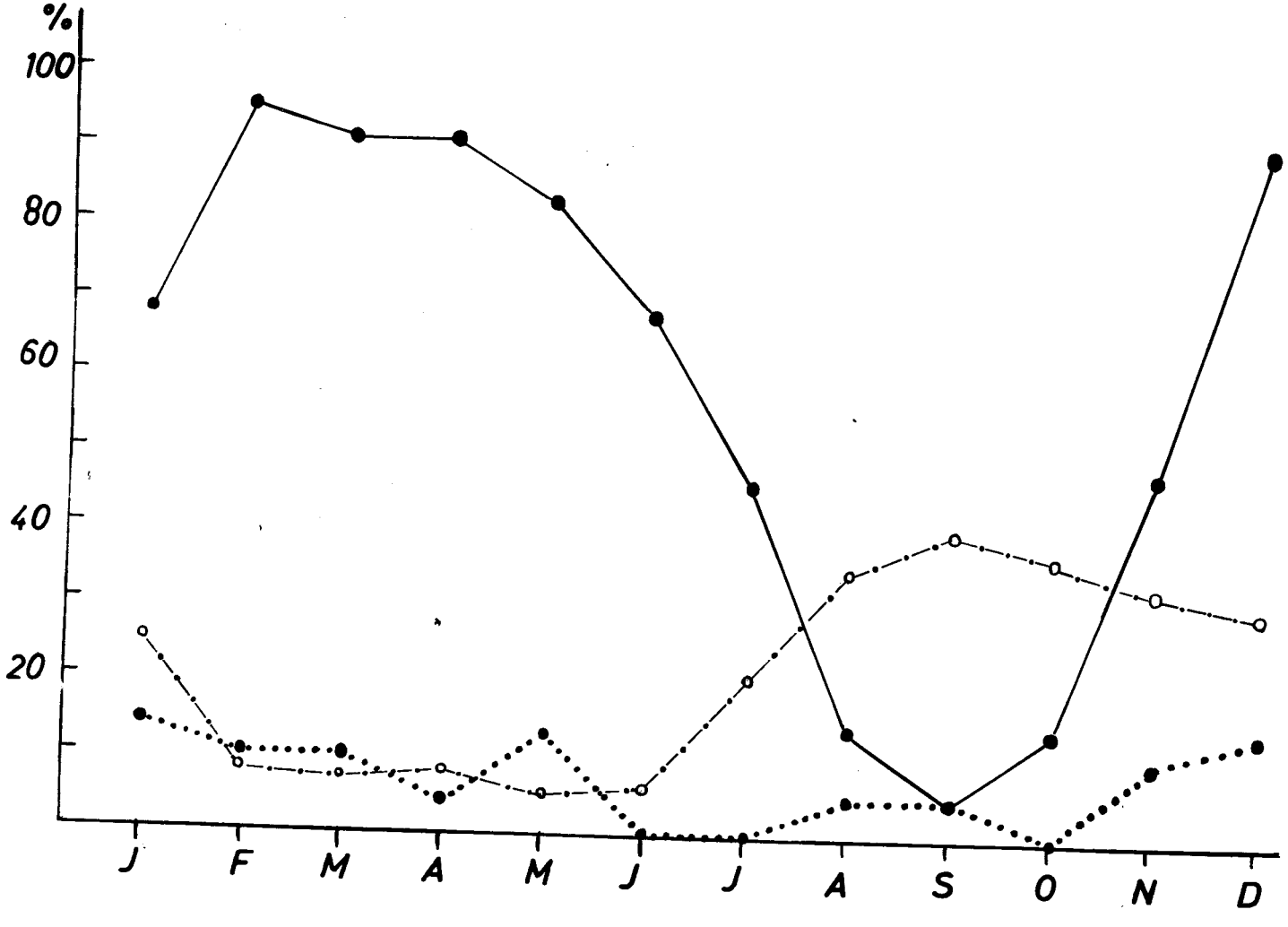
3. THE OCCURRENCE IN THE BROWN TROUT

(a) The Incidence and Intensity of Infection.

Figs. 9. 1 and 9. 2 summarize the incidence and intensity of infection of Crepidostomum species in G.pulex and S.trutta. It may be readily observed from Fig 9. 1, that C.metoecus shows a clear seasonal cycle in its distribution in the brown trout of Afon Terrig. The rate of infection was higher during the colder than the warmer months of the year. Thus, over 80% of the sampled fish population were infected from December

Fig. 9. 1. Seasonal Variation in the Incidence of *Crepidostomum spp.* in their Hosts in Afon Terrig.




%
100
80
60
40
20
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F
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A
M
J
J
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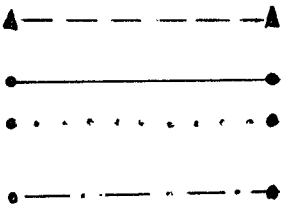
D

to May with a peak of 95.2% in February. The drop in January appears to be due to sampling deficiency especially as there was no corresponding fall in the incidence of metacercariae. The incidence then fell through June, July, August to September when only 1 of 21 fish harboured two dying or moribund specimens of C.metoecus. The rate of infection would seem to rise more gradually from September to October than thereafter. This is probably due, as will be discussed later, to the fact that most metacercariae were unable to establish in trout (Chapter VIII).

The intensity of infection (See Fig. 9. 2) shows a definite and similar trend, the lowest degrees of infection being recorded from June to October. A sharp rise occurred in November, and the peak intensity (42.5 parasites/fish) was recorded in March.

A closer examination of the worms taken showed that November specimens were very young, small and almost transparent, with the remains of the eye spots scattered dorsally in the antero-lateral areas. In December only 3 of 583 specimens taken had a few pale brown eggs within the uterus. The others were immature as in November. By February most parasites bore eggs. In June, the eggs were observed to be fewer and the disintegration of the internal organs had begun. In August, September and October, the worms were either not found or where present, appeared dead and almost empty. From the above observations, the developmental cycle of C.metoecus in fish seems clear. It is annual. Young worms are established about November. Depending on the time of infection, eggs begin to appear in the uterus about January and February. By March practically all worms are functionally mature. Since parasites

Fig. 9. 2. Seasonal Variation in the Intensity of Infection of the Brown Trout and Shrimps by Crepidostomum spp. in Afon Terrig

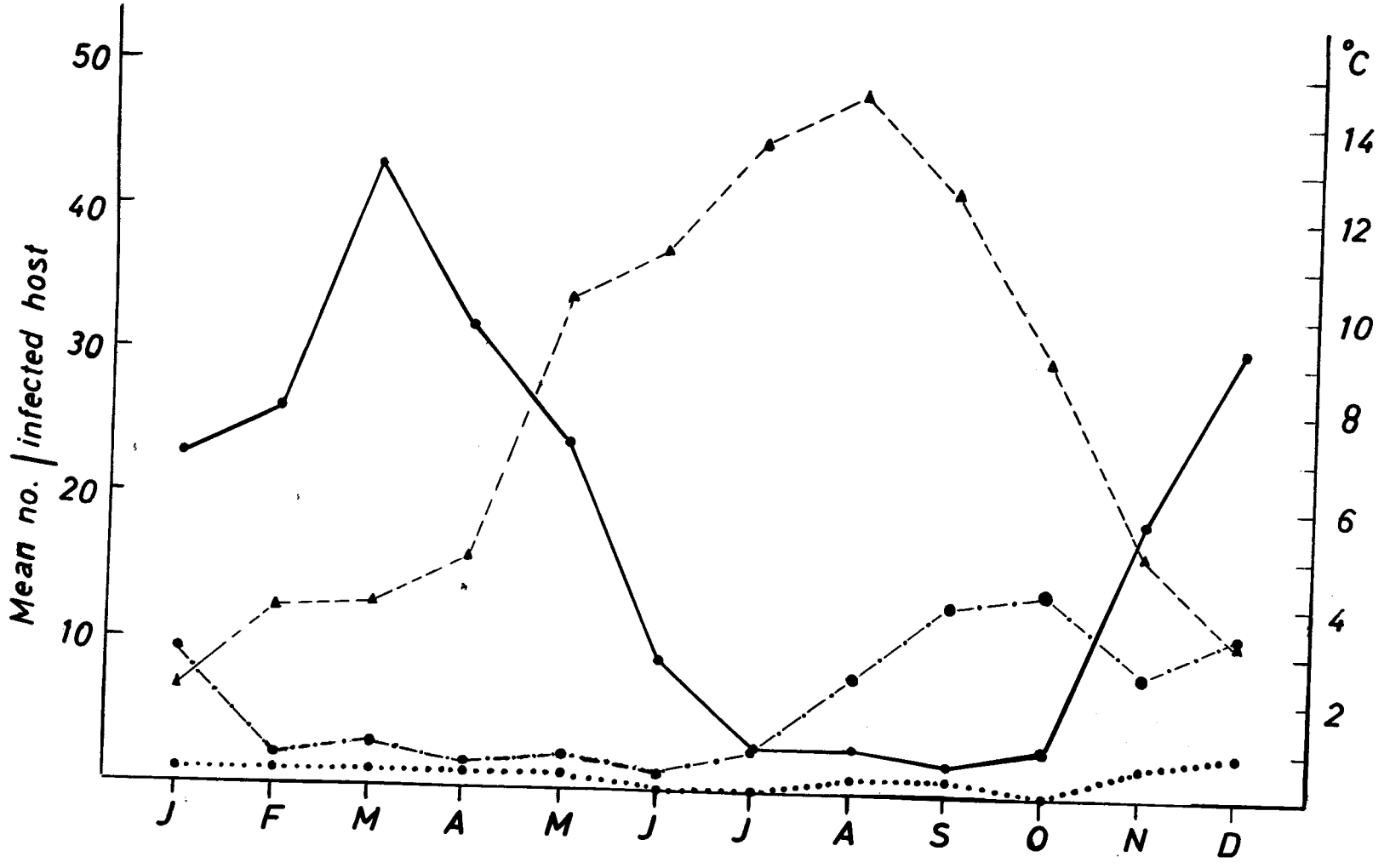


Temperature

C.metoecus in trout

C. farionis in trout

Metacercaria of C.metoecus (see text) in shrimps.


Mean no. / infected host
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40
30
20
10
°C
14
12
10
8
6
4
2
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F
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taken from July to October had only a few eggs, it may be assumed that eggs are gradually shed into the stream from April. By the end of August, this species disappears from the intestine. It may be added that the occasional specimens taken in September and October were moribund, with the vitellaria, ovary and the gut, except the pharynx having disappeared.

Despite the relative paucity of C.farionis population in the stream, available data on its incidence and intensity in trout, would suggest the same seasonal trends as shown by C.metoecus (see Figs. 9.1 and 9.2). In this connection, it seems significant to note that more worms were taken from November to May with the highest recorded rate of infection in December (13.6%) and January (14.3%). No parasites were taken in June, July and October. As in C.metoecus, specimens recovered in November were transparent with pigment spots distributed antero-dorsally (Brown 1927) and no intra-uterine eggs. One of three specimens taken in December had a few light brown eggs. This trend in functional maturity continued as described for C.metoecus. Despite the scanty data on C.farionis, therefore, its life cycle appears to be the same as outlined for C.metoecus. From the observations recorded above, it seems patent that there is a close relation between the temperature of the stream and the life cycle of the two trematodes. Worms are established in late autumn when the temperature has fallen below 10°C. In May/june the rapid rise to 11.2°C coincides with a steep fall in both incidence and intensity of infection. These observations seem to indicate that Crepidostomum may not be able to establish in trout at temperatures above 10°C. If so,

this would account for the failure to establish Crepidostomum spp. in trout in the laboratory with a temperature considerably greater than 10°C (cf. Chapter VIII).

The details of the occurrence of these two species at each of the three Stations sampled, are given in Tables 9. 1 and 9. 2. It may be noted that while the seasonal trends of occurrence of C.metoecus were the same at all three Stations, the intensity of infectinn was markedly higher at Stations II and III. This is most probably a reflection of the distribution of the parasite in its intermediate hosts. The correlations will be discussed. The absence of infection in fish in August and September (Station III) September and October (Station II), where the parasite occurred in greater numbers, is noteworthy.

No Crepidostomum farionis was taken from fish upstream (Stations II and III) throughout the period of investigation (cf. Table 9.2). Even downstream where the species occurred, both the incidence and intensity of infection were low. The restriction of this species downstream is probably due to the rarity of Pisidium sp. upstream. An intensive search for this bivalve has shown that it has a low and patchy distribution downstream and it has not been taken above Station III(cf. Chapters I and VIII).

(b) The Relationship between the Size of Trout, the Incidence and Intensity of Infection by C.metoecus.

Figure 9.5 summarises the relationship existing between the size of the final host and parasitisation by this trematode. It should be pointed out that in preparing the diagram, the information obtained from fish below 100mm. in length, taken from the stream in connection with

Table 9. 1.

The Occurrence of Crepidostomum metoecus in brown trout.

Station I (Rhydtalog)

Month	Jan.	Feb.	Mar.	April	May	June	July	August	September	October	November	December
No.fish examined	7	7	7	7	7	7	7	7	7	7	7	7
No.(%) infected	5(71.4)	7(100)	5(71.4)	5(71.4)	4(57.1)	4(57.1)	4(57.1)	2(28.6)	1(14.3)	2(28.6)	4(57.1)	5(71.4)
Total No.parasites	25	92	23	62	8	43	16	6	2	6	122	18
Mean no./infected fish	5.0	13.1	4.6	12.4	2	4.8	4.0	3.0	2.0	3.0	30.5	3.6
Station II												
No.fish examined	7	7	7	9	7	7	7	7	7	7	7	7
No.(%) infected	7(100)	7(100)	7(100)	9(100)	6(85.7)	5(71.4)	4(57.1)	1(14.3)	0	0	3(42.9)	7(100)
Total no.parasites	336	232	394	356	164	49	11	2	-	-	185	373
Mean no./infected fish	48.0	33.1	56.3	39.5	27.3	9.8	2.8	2.0	-	-	61.7	53.3
Station III (Caegwydd)												
No.fish examined	8	7	7	7	9	8	8	7	7	7	7	7
No.(%) infected	5(62.5)	6(85.7)	7(100)	7(100)	9(100)	6(75.0)	2(25.0)	0	0	1(14.3)	3(42.9)	7(100)
Total no.parasites	93	191	391	247	286	41	3	-	-	2	93	192
Mean no./infected fish	18.6	31.8	55.9	35.2	31.8	6.8	1.3	-	-	2.0	31.0	27.4

Table 9. 2

The Occurrence of Crepidostomum farionis in the brown trout

Station I (Rhydtalog)

Month	Jan.	Feb.	Mar.	April	May	June	July	August	September	October	November	December
No.fish examined	7	7	7	7	7	7	7	7	7	7	7	7
No.(%) infected	0	0	0	0	0	0	0	0	0	0	0	0
Total no.parasites	-	-	-	-	-	-	-	-	-	-	-	-

Station II

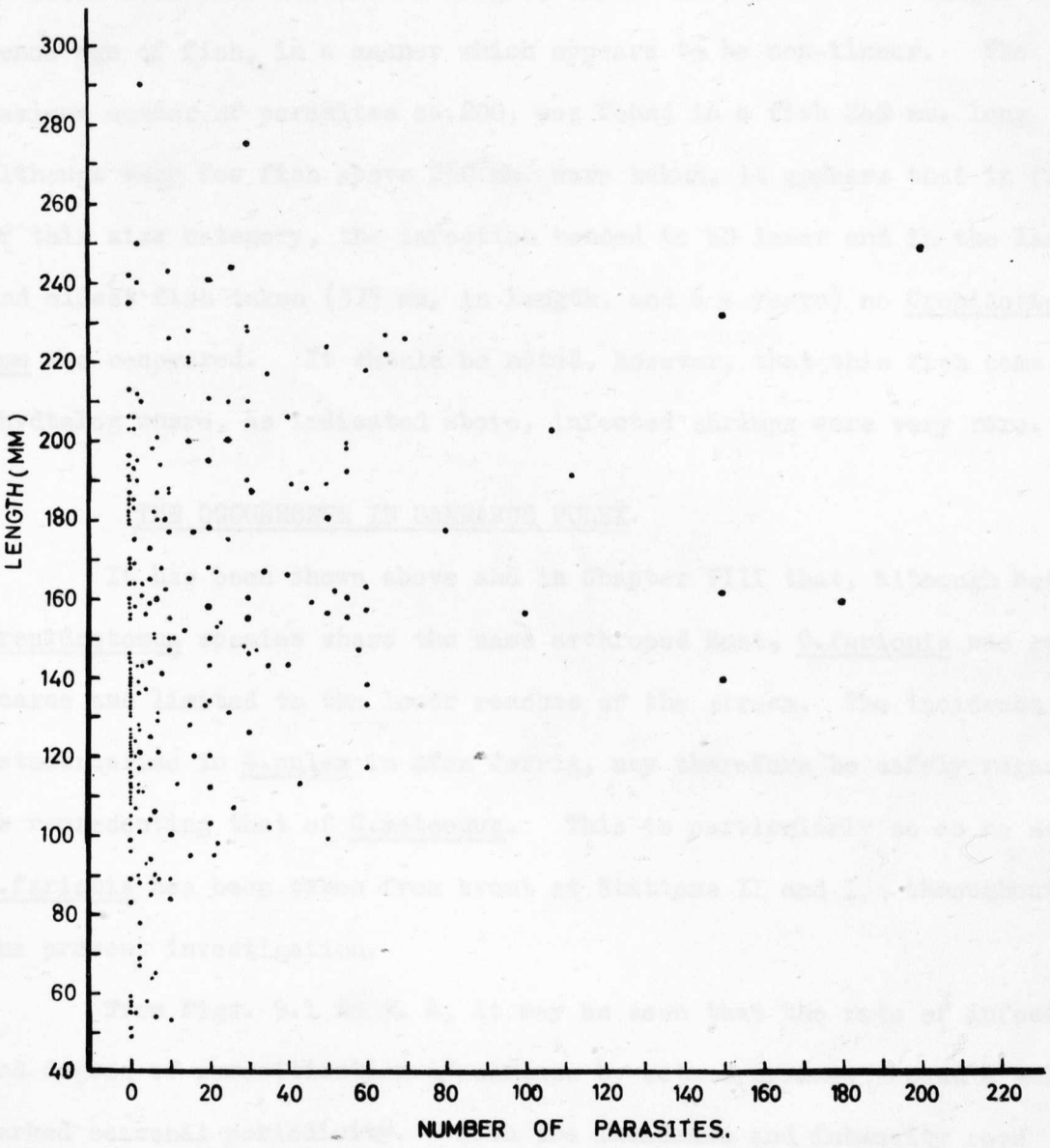
No.fish examined	7	7	7	9	7	7	7	7	7	7	7	7
No.(%) infected	0	0	0	0	0	0	0	0	0	0	0	0
Total no.parasites	-	-	-	-	-	-	-	-	-	-	-	-

Station III (Caegwydd)

No.fish examined	8	7	7	7	9	8	8	7	7	7	7	7
No.(%) infected	3(37.5)	2(28.6)	2(28.6)	1(14.3)	3(33.3)	0	0	1(14.3)	1(14.3)	0	2(28.6)	3(42.9)
Total no.parasites	2	2	2	1	3	-	-	1	1	-	2	3
Mean no./infected fish	1.0	1.0	1.0	1.0	1.0	-	-	1.0	1.0	-	1.0	1.0

Fig. 9.5. The Relationship between the Size of the Brown Trout of Afon Terrig and Parasitisation by Crepidostomum metoecus.

Note: Fish < 100 mm. in length taken during the course of other investigations, used in the preparation of this diagram.


LENGTH (MM)
300
280
260
240
220
200
180
160
140
120
100
80
60
40
0
20
40
60
80
100
120
140
160
180
200
220
NUMBER OF PARASITES.

other investigations, was used.

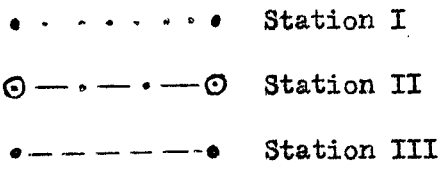
It may be seen from the diagram that the smallest fish infected was 53 mm. in length. The incidence and intensity of infection were lower in trout less than 100 mm. in length, and increased with the length and hence age of fish, in a manner which appears to be non-linear. The maximum number of parasites ca.200, was found in a fish 249 mm. long Although very few fish above 250 mm. were taken, it appears that in fish of this size category, the infection tended to be lower and in the largest and oldest fish taken (375 mm. in length, and 6 + years) no Crepidostomum was recovered. It should be noted, however, that this fish came from Rhydtalog where, as indicated above, infected shrimps were very rare.

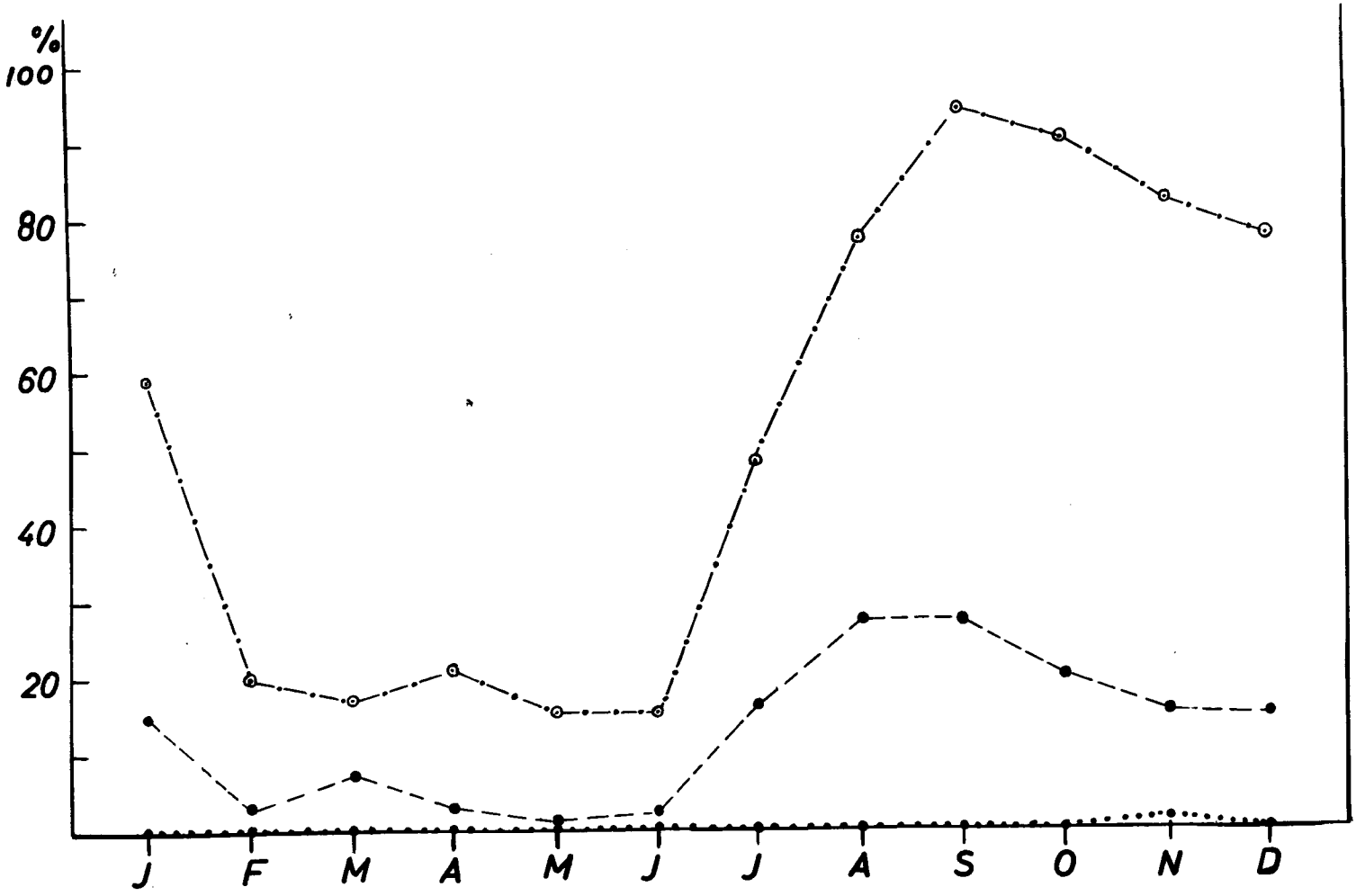
4. THE OCCURRENCE IN GAMMARUS PULEX.

It has been shown above and in Chapter VIII that, although both Crepidostomum species share the same arthropod host, C.farionis was rather scarce and limited to the lower reaches of the stream. The incidence of metacercariae in G.pulex in Afon Terrig, may therefore be safely regarded as representing that of C.metoecus. This is particularly so as no adult C.farionis has been taken from trout at Stations II and I throughout the present investigation.

From Figs. 9.1 and 9. 4, it may be seen that the rate of infection and degree of parasitisation of shrimps by metacercariae, showed a well-marked seasonal periodicity. Both the incidence and intensity rose markedly from July and remained high till January, with peaks in September and October respectively. It is interesting to note from Figs. 9. 1 and 9. 2 that the peak incidence and intensity in shrimps (August to October),

Fig. 9.3. Comparison of Seasonal Incidence of the Metacercariae of Crepidostomum in various stretches of Afon Terrig.



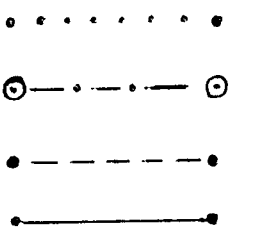

%
100
80
60
40
20
J
F
M
A
M
J
J
A
S
O
N
D

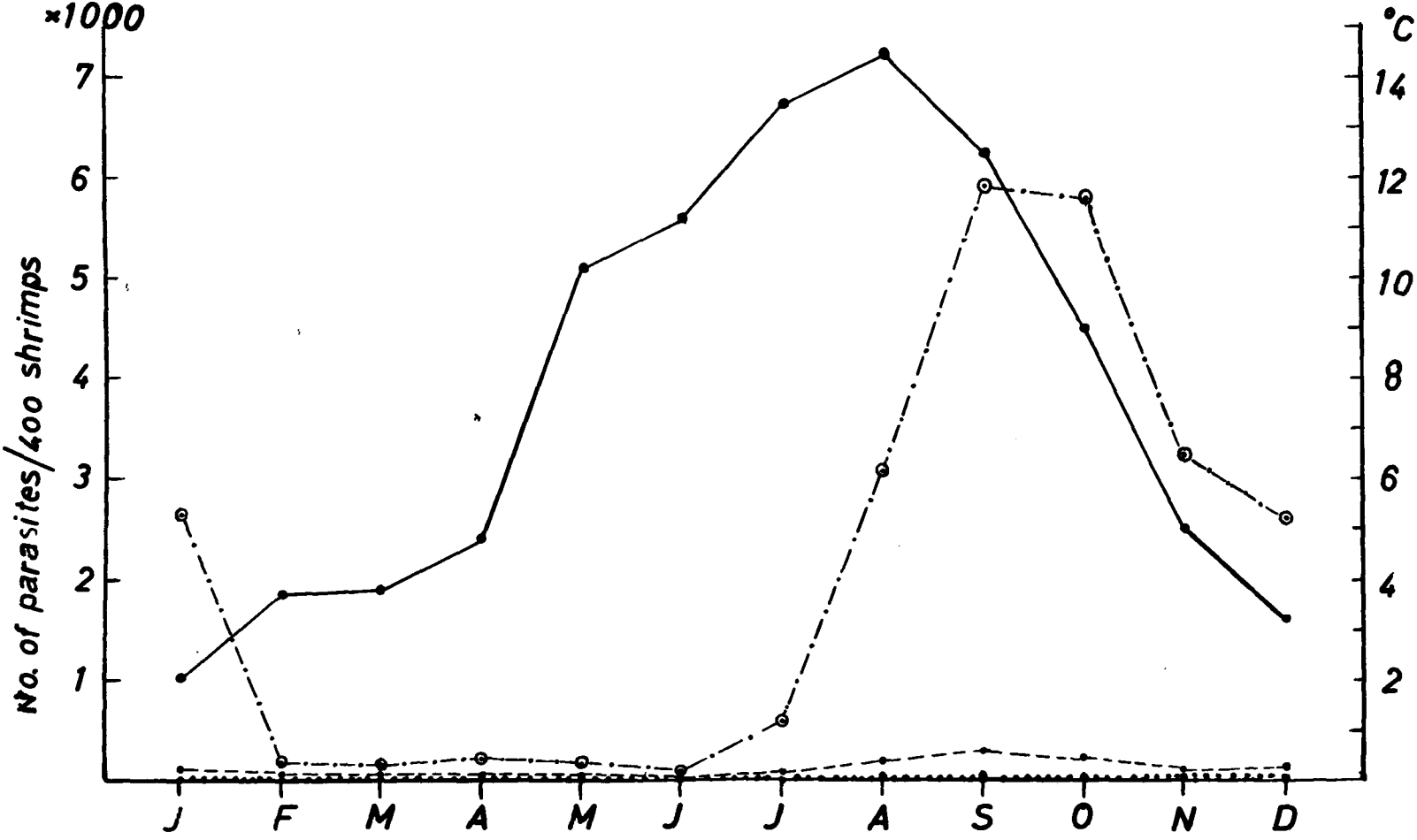
corresponded with the lowest occurrence in the final host. It seems obvious that this is not due to trophic factors as shrimps have been shown to form an important component of the food of the brown trout all the year round (Chapter VII). A probable explanation may be found by considering certain features intrinsic to the developmental cycle of the parasite. Experimental observations (Chapter VIII) have shown that metacercariae undergo considerable differentiation before attaining the infective stage. Although the exact period required by larvae to become infective was not confirmed experimentally, it seems from experimental data (Chapter VIII) and the current field observations, that a period of about 2 - 3 months may be necessary. Thus, although metacercariae were abundant in July to September, they were not able to establish till November.

It may also be noted that the incidence of metacercariae was a true reflection of the swarming of cercariae from L.pereger, The May sample of this snail harboured rediae with cercariae at all stages of development. In June only a few certariae were being shed while in July to October, snails taken and kept at stream temperature in the laboratory liberated hundreds of ophthalmoxiphidiocercariae (Nöller 1925, Brown 1927, Thomas 1957, 1958).During the rest of the year, only very few cercariae were released after long periods in the laboratory.

As might be expected from observations made in Chapter VIII, the incidence and intensity of infection were markedly higher at Station II than at Station III (cf. Figs. 9.3, 9.4.) Shrimps were practically uninfected upstream at Station I (Rhydtalog). Only in November were infected shrimps taken - 3 of 400 examined. There seems to be little

Fig. 9.4. Seasonal Variation in the Intensity of Infection of G.pulex by Crepidostomum metacercaria at the three Stations sampled.




×1000
No. of parasites/400 shrimps
7
6
5
4
3
2
1
°C
14
12
10
8
6
4
2
J
F
M
A
M
J
J
A
S
O
N
D

doubt that these features of the incidence and intensity of infection are related to the distribution of the molluscan host, L.pereger, in the stream (Chapters I and VIII). The population of this snail was found to be concentrated around Station II. Further downstream, it was common but not abundant, while further upstream, the population fell sharply and around Rhydtalog this species has not so far been taken. It is also interesting to note that an analysis of the developmental stages of metacercaria taken from Station II showed very interesting trends in the invasion and encystment of cercaria in G.pulex, outlined hereunder.

Month	No. shrimps examined	No. with encysting or recently encysted worms	Total encysting or recently encysted worms
June	400	31	40
July	400	84	183
August	400	283	922
September	400	231	974 +
October	400	80	335
November	400	56	146
December	400	8	9

Table 9. 3. Showing the trends in the population of establishing and recently established metacercaria in G.pulex at Station II.

\+ Maximum of 60 unencysted and recently encysted metacerceria per shrimp recorded in September.

It seems clear from the above table (Table 9.3) that swarming is almost over by December. This was found by examining snails in Chapter VIII.

As might be expected, the incidence and intensity of infection were found to be higher in adult shrimps than in young ones. In September, at Station II where shrimps were naturally exposed to heavy infection, it was found that 146 of 374 infected shrimps (400 examined), belonged to the smaller size group ($<$ 6 mm.) and accounted for only 477 of a total of 5937 metacercaria recovered. It may be added that in September also, the digestive caeca of a shrimp 10 mm. long were found to be literally riddled with 150 metacercariae - the maximum intensity recorded during the present investigation. It is suggested that the observed habit of G.pulex, of crawling round stationary and slowly-moving objects for long periods (Chapter VIII), may be contributory to this high incidence and intensity of infection. Observations on both naturally and experimentally infected specimens indicate that except in cases of gross over-infection, above 50 - 70 cercariae in adult shrimps, metacercariae have little effect on life span of shrimps.

5. A NOTE ON THE OCCURRENCE OF CREPIDOSTOMUM SPP. IN COTTUS GOBIO L.

Although the bullhead occurred in the region of the stream where the two species of Crepidostomum were found, none of a total of 44 specimens of this fish examined during the present study, was found to be infected by these digeneans. Nybelin (1932) found C.metoecus (= C.suecicum Nybelin 1932 (Hopkins 1934)) in various freshwater fish of Sweden including Cottus gobio. As it is not known whether the eggs he recovered and measured were viable, the status of the bullhead as a "proper host" (Sandground 1929, Baer 1952) would bear further investigation. Hynes

(pers.comm) has observed three specimens of Crepidostomum in Cottus from Afon Terrig but did not examine them critically. The above observations by Nybelin and Hynes would, however, indicate that the present failure to take Crepidostomum, especially C.metoecus, from the intestine of the bullhead, may be due to a combination of the following factors, 1. the relatively small number of fish examined; 2, occurrence of the bullhead around Station III where the population of G.pulex and L.pereger is comparatively low,and 3. the smaller capacity and lower feeding activity of this fish in comparison with trout of similar size (Chapter VII). The records of other allocreadiid species from the bullhead by Nicoll (1924), Dawes (1947, 1956) would seem to favour the latter contention.

6. DISCUSSION.

A well-defined seasonal periodicity has been found by various workers for Crepidostomum metoecus and C.farionis in their predominantly salmonid hosts, viz: Dyk (1954, 1956, 1957), Dyk et al (1954) in Czechoslovakia, Robertson (1953) in Scotland, Thomas (1957, 1958) in Mid-Wales, Slusarski (1958a, b, c) in the basin of Vistula, South Baltic, and the Polish part of the High Tatra. The observations of Olsson (1876) Nicoll (1909), Brown (1927) and Rawson (1952) on C.farionis in trout, though limited to short periods and a few fish, were suggestive. The present investigation not only shows clear cyclic changes in the final host, but also marked and correlated seasonal rhythms of occurrence in all the three hosts involved in the life cycle of Crepidostomum spp.

In the more abundant species C.metoceus (Corbett 1955, Thomas 1958, Slusarski 1958 a, b, c, Chapter VIII), well-developed cercariae were found inthe rediae in April and May. The cercariae were being liberated from L.pereger in appreciable numbers by the end of May. Swarming reached a peak in September and was practically over by December. The above picture is reflected in the incidence and intensity of infection in G.pulex. As might be expected, although swarming dropped sharply in October (cf. Table 9. 3 and Fig. 9.4), the occurrence in shrimps fell gradually through the winter till February, when a sharp drop was recorded. From the latter month till June, the occurrence remained low but remarkably uniform in shrimps and metacercariae recovered had attained the fullest possible morphological differentiation. The latter observation in addition to what is known about the life cycle of G.pulex (Hynes 1955) would indicate that infected shrimps found from February to May, largely represent a carry-over from the previous autumn infection of the late summer brood of G.pulex. Infected shrimps have been shown experimentally to live for up to 240 days under imitated stream conditions. It is postulated that environmental temperature may be the main factor which underlies the above correlated seasonal dynamics of Crepidostomum spp. in their hosts. By determining the time of establishment of worms in fish, it sets the clock, as it were, for the development of the parasite in the other two hosts.

An interesting and perhaps ecologically significant feature found in the life cycle rhythms of C.metoecus in its hosts, is that there is a time lag of about 3 - 4 months between the beginning of swarming of

carceria and hence a high incidence in shrimps, and the appearance of newly established worms in the intestine and pyloric caeca of trout. It has been pointed out that this may be due to the time required for metacercariae to attain the infective stage. Recent studies by Cheng (1957 a,b), Cheng and James (1960), DeGiusti (1962), on other allocreadiid metacercaria in crustacean hosts, have shown that remarkable development of the encysted worm culminating in progenetic forms in Crepidostomum cornutum Osborne 1903 and Allocreadium lobatum Walton 1909, occurs in this family. It seems patent that in C.metoecus, an immense loss of worms is inevitable due to: 1) the failure of a large number of uninfective larvae to establish in trout from June to September when the latter is still feeding actively; 2) death of adult and the more heavily infected shrimps belonging to the spring brood; 3) expected failure of some cercariae to find suitable hosts. This appears to be relatively unimportant in this species,. However, the large numbers of cercariae released tips the balance in favour of the parasite's survival.

Another feature of considerable interest and importance as far as the two parasite species are concerned, is the correspondence of the swarming of their cercariae with the presence in the stream, of the largest possible population of young G.pulex (Hynes 1955). De Giusti (1962) reported a similar correlation between the life cycle of A.lobatum and that of the amphipod Gammarus pseudolimnaeus in a small Michigan stream (U.S.A). As above, he found that maturation to cercaria in Pisidium sp. occurred in late summer, coinciding with the height of juvenile Gammarus population.

The distribution of Crepidostomum species in their final host in Afon Terrig, calls for some comment. No C.farionis was taken at Stations I and II. C.metoecus, on the other hand, was abundant at Stations II and III but showed a markedly lower intensity at Station I (Rhydtalog). It is explained that the extent of the ecological barriers imposed by the distribution of the molluscan first intermediate host and the limited movements of the brown trout in the stream (Allen 1951, Chapter VII) may be responsible for this. Pisidium sp.has been shown not only to be scarce but to be limited to patches of shallow stream, with sandy or gritty bottom, around and below Station III. With regard to L.pereger, it may be noted that this species has been taken from the confluence of Afon Terrig with River Alun to a point about one-third way upstream between Stations II and I. Thus, while the local movements or migrations of trout (breeding and otherwise) led to infection of the final host taken from Rhydtalog, by the more abundant and widely distributed C.metoecus, they were unable to produce the same effect in C.farionis with very limited ecological distribution.

It would appear from the literature that experimentally proven cases of host immunity to adult Trematoda, even among the better known and studied species, are few. Smyth (1962) observed that "although complement-fixation antibodies have been detected in the serum of sheep and other hosts of F.hepatica, there is no evidence that effective immunity to this trematode is ever developed". Urquhart et al (1954) showed that immunisation of rabbits prior to infection by this parasite inhibited development without reducing their numbers significantly.

Among schistosomes, however, host resistance and acquired immunity have been demonstrated in man and other animals (Fisher 1934, Kagan 1958). The present study shows no evidence of age resistance or natural resistance (Sandground 1929, Taliaferro 1929, 1940) in the parasitisation of the intestine of trout by Crepidostomum spp. A definite increase in the numbers of Crepidostomum with age and length of fish, was found in Afon Terrig. The trout with largest numbers of C.metoecus recorded during the current study (ca. 200 worms), was 249 mm. long and 4 years old. Fish less than 100 mm., mainly in the 0 + 1 year group, had markedly lower infection rate. Essex and Hunter (1926), Bangham (1944), Bangham and Venard (1946), Robertson (1953), Frankland (1955) and Thomas (1958) have all found increased infection rate of intestinal helminths with advance in age of fish. These observations would indicate that age resistance may not exist in helminth parasitisation of the intestine of fish. In this connection, it may be noted that the apparent lower intensity of infection in the few trout above 250 mm. in length from Afon Terrig, is probably a reflection of the general tendency of larger fish to feed on larger organisms. In the 375 mm. fish, in which no parasites were found, the stomach contained a frog.

Finally it may be added that it seems patent from the results of the present study, that premunition to Crepidostomum infection occurs neither in the intermediate hosts nor in the brown trout.

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PART IV

C H A P T E R X

OTHER HELMINTHS

CESTODA

1. THE OCCURRENCE OF CYATHOCEPHALUS TRUNCATUS PALLAS 1781

(a) Introduction.

Cyathocephalus truncatus Pallas 1781 belongs to the family Cyathocephalidae Nybelin 1922 in the order Spathebothriidea Wardle McLeod 1952. As far as is known, this worm is the only known member of the single genus Cyathocephalus Kessler 1868 within the family.

The details of the life history of this worm have been experimentally investaged by Wiśniewski (1932, 1933). Since then the species has received little attention. Vik (1954, 1958) recorded some observations on the distribution and pathogenicity of this parasite (cf.Chapter XI) in the Ånöya water system, Norway. Bauer (1959) has remarked that ecological information on this species is very scanty. The present study was thus undertaken in the hope that some addition may be made to the existing state of knowledge of this parasite.

(b) Materials and Methods.

The proeedure followed was the same as for the Trematoda (cf.Chapters

VII and VIII) except that all cestodes, larval or adult, were relaxed in cold water only before fixation in Alcohol-formol-acetic (Van Cleave 1953).

(c) Observations.

Only one species of cestode, C.truncatus, occurs in the brown trout of Afon Terrig. The parasite was not taken from 44 bullhead (Cottus gobio L) examined. First described from specimens taken from pike (Esox lucius L.) from St.Petersburg by Pallas (1781), the tapeworm has also been commonly reported from Coregonus sp.(Wiśniewski 1932, 1933, Bauer and Nikolskaya 1957 inter alia). Other species of fish from which the worm has been taken include Perca fluviatilis L., Lucioperca lucioperca L., Lota lota L., Salmo fario, S.umbla, S.gairdneri Richardson, Salvelinus sp., Thymallus, Stenodus, Cristovoner, Leucichtys and Myoxocephalus quadricornis labradoricus (Wiśniewski 1933a, Yamaguti 1959, Bauer 1959).

The intermediate host in Afon Terrig was Gammarus pulex L. No other arthropod in the stream, (cf. Chapter 1) was found to be infected by this worm. This is in consonance with the findings of other workers (Wolf 1906, Nybelin 1922, Schäferna 1922, Wiśniewski 1932, Beckman 1954 etc.) who found this species only in amphipods, viz: Rivulogammarus pulex, R.spinicaudatus Schäferna, Fontogammarus bosniacus Schäferna, Pontoporeia hoyi Stimpson, Pontoporeia affinis,and Pallasea quadrispinosa.

The parasite lives in the body cavity of the shrimp, while in fish it inhabits principally the pyloric caeca and is only occasionally taken in the upper intestine. It is doubtful if the worm lives for any length of time in the upper intestinal lumen. The occasional young individual

in the pyloric region of the intestine may be on its way to a caecum. Two adult specimens taken in this region were moribund and probably on their way out of the intestine.

(i) Occurrence in the final host.

The incidence of this parasite in its hosts is summarised in Fig.10.1. It may be seen from this figure that the pattern of the rate of infection in trout, is indicative of seasonal periodicity. The rate was high during the winter and spring months (November to April) with a peak (52.4%) in February, dropped in May, and by September no parasites were taken. It may also be observed from Table 10.1 that the dynamics of the total number of parasites recovered as well as the mean per infected fish, were in accordance with the general pattern of the incidence.

An examination of the worms taken throughout the year shows that specimens taken in October/November were very young, recently established and essentially similar to progenetic procercoids found in shrimps. Worms taken from March to July, were predominatly adult and functionally mature, as shown by the presence of intrauterine eggs. It seems clear from these observations that the species has a definite annual cycle in the trout of Afon Terrig. As in the trematodes species (Chapter IX), C.truncatus establishes in fish from late autumn. Depending on the time of infection, they mature in late winter and spring and disappear by late summer and early autumn., (August/September). Wolf (1906), Bauer and Nikolskaya (1957) did not find a seasonal cycle in the incidence of this parasite in the fish host. It may be pointed out that both series of observations were limited to certain parts of the year. Bauer and Nikolskaya's data are reproduced as follows, for comparison:

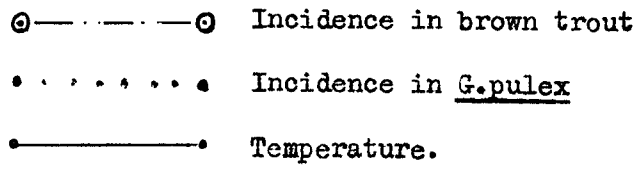
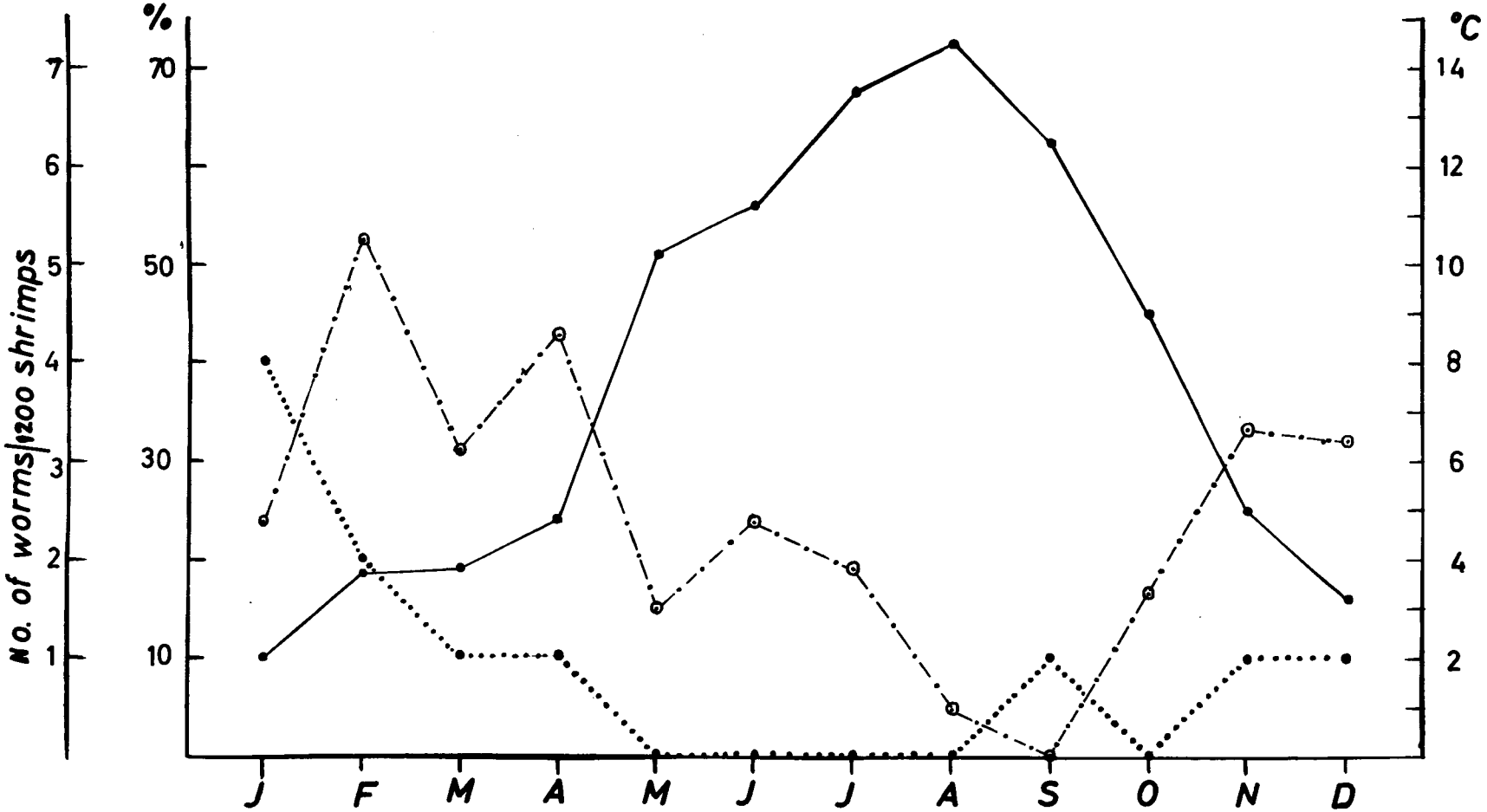


Fig. 10. 1. Seasonal Changes in the Occurrence of Cyathocephalus truncatus in the Intermediate and Final Hosts in Afon Terrig.


No. of worms/1200 shrimps
%
°C
7
6
5
4
3
2
1
70
50
30
10
14
12
10
8
6
4
2
J
F
M
A
M
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J
A
S
O
N
D

Table 10. 1. The occurrence of *Cyathocephalus truncatus* in the brown trout of Afon Terrig.

Month	No. fish examined	No. (%) infected	Total no. parasites	Mean no./ infected fish
January	21	5(23.8)	9	1.8
February	21	11(52.4)	15	1.4
March	21	8(32.1)	17	2.1
April	21	9(42.9)	13	1.4
May	21	3(14.4)	5	1.7
June	21	5(23.8)	7	1.4
July	21	4(19.0)	11	2.8
August	21	1(4.8)	2	2.0
September	21	0	-	-
October	21	4(19.0)	10	2.5
November	21	7(33.3)	19	2.7
December	21	8(32.1)	25	3.1
Total	252	65(25.8)	133	2.0

Species of parasite: C.truncatus.

Parasitisation in different months.

July		August		September		October		November	
%	Mean intensity	%	Mean intensity	%	Mean intensity	%	Mean intensity	%	Mean intensity
27	0.9	13	0.8	40	0.6	13	0.13	60	1.6

Table 10. 2. Seasonal changes in the parasite fauna of Coregonus laveratus baeri n. ladogensis. (After Bauer and Nikolskaya 1957)

Wolf's observations were less systematic and scattered between July - September 1903 and March and July 1904. A re-examination of both sets of data, in the light of the results of the present study, shows that they are suggestive of seasonal periodicity. Thus, in March, Wolf found only 5 small worms in 3 trout, whereas in July of the same year, one of 12 C.truncatus recovered was 4 cm. in length - the maximum so far recorded for this species. Bauer and Nikolskaya's figures also show an appreciable upward trend in infection rate in November (cf. Table 10.2). There seems to be little doubt, therefore, that the failure to observe cyclic fluctuations of occurrence by the above investigators, was due to the limited periods over which studies were made.

It may also be noted that in Afon Terrig, C.truncatus was more common in fish taken upstream than downstream.

(ii) Occurrence in the intermediate host.

The occurrence of the parasite in G.pulex (cf. Fig.10.1) was found to be generally low throughout the year. In spite of this, it may be seen from the above figure that there seems to be a seasonal trend which is tied

up with the incidence in the final host. Thus, worms were taken mainly during the colder months, November - April, when the temperature was 2 - 5°C. In May - August, with a temperature range of 10.2 - 14.5°C, no infected shrimps were found. All the worms recovered belonged to Wiśniewski's groups - 'Mittelprocercoidstadien' and 'Reifeprocercoidstadien' - and had the caudal vesicle or cercomer. No earlier stages, 'Fruhprocercoid stadien' were observed. It is possible that these earlier forms may have been present in summer but were missed due to the rarity of the worm in the stream. However, it would appear from available data that temperature may play a very important part in determining the seasonal rhythm of occurrence by controlling the establishment of worms in the final host. Young worms were taken mainly in October - December (9 - 3°C). The rapid rise of temperature from May to August (from 4.8°C towards the end of April to the recorded maximum of 14.5°C in August), corresponded with the decrease in the occurrence of worms in fish.

It was also found that the incidence in shrimps was more sparse downstream. A similar observation is recorded above for the final host. As pointed out for the acanthocephalan Echinorhynchus truttae Schrank 1788 (Chapter VII), it is highly probable that this regional differential in incidence reflects the higher population density of G.pulex upstream. The finding also emphasises the limited nature of the movement of the brown trout in Afon Terrig.

(d) Discussion.

As indicated above, C.truncatus is essentially a Salmonid parasite of Northern Hemisphere. Reports of the parasite in this region include

those of Pallas (1781), Wolf (1906), Nybelin (1922), Wiśniewski (1932) for the European continent; Baylis (1928) for Great Britain; Layman (1933) Barysheva and Bauer (1948, 1957) for the U.S.S.R., and Cooper (1918) for NlAmerica. S. S. Shulman (in Dogiel et al 1958) includes this parasite among other species considered typically circumpolar in distribution, though also found in high mountain streams in lower latitudes within the range. The position of the Afon Terrig falls in with the above view.

Wiśniewski (1933) followed the development of the worm. Eggs are fertilised, accumulate within the uterus and are then shed into the intestinal cavity of fish. Here they are covered by a thin layer of intestinal mucus to which particles of excrement are stuck. He emphasised the ecological importance of the often thick envelope so formed in protecting eggs from unfavourable temperature and osmotic pressure effects. He further showed experimentally that normal embryonic development occurs only in the faeces of fish. Embryonic development takes 35 - 40 days. Eggs are ingested directly by gammarids. Onchospheres with no apparent hooks, liberated in the intestine of shrimps, penetrate into the body cavity where worms develop to the progenetic procercoid stage, with the full complement of genitalia, but no eggs. On ingestion by the fish host, fertilisation and functional maturation ensue. It is unfortunate that Wiśniewski's data give no indication of the duration of the postembryonic developmental stages or the life span of experimental adults. Bauer (1959) has suggested that the worm may live for approximately one year. It seems reasonably clear from the current study that the worm has an annual cycle

and a periodicity of occurrence. As has been pointed out earlier, the failure of Wolf (1906) Bauer and Nikolskaya (1957) to detect definite cyclical fluctuations in Tübingen and Lake Ladoga respectively, is probably due to the limited nature of their investigations. Perhaps a critical re-examination of the latter's specimens for size and stage of development may reveal that the November sample contained mainly newly established worms.

It has been noted that temperature may be the underlying factor controlling the life cycle and hence the incidence of the parasite in its hosts. A similar relation was also found for the trematodes Crepidostomum metoecus and C.farionis (cf.Chapter IX). It is apparent that worms begin to establish in fish in October when the temperature has fallen to 9°C. It also seems significant to note that a rise above 10°C in May was accompanied by a sharp drop in both the rate and intensity of infection. A threshhold of about 10°C above which the worm does not establish in the final host may be suggested. This apparent effect of temperature on the life cycle of this worm may be correlated with Shulman's (1958) observation that C.truncatus (as well as Crepidostomum farionis and Echinorhynchus truttae) is of northern origin and occurs in Arctic fish species of the U.S.S.R.

Bauer and Nikolskaya (1957) have shown that there is a relation between the rate and intensity of infection by the parasite and the age of the Ladoga white fish Coregonus laveratus baeri natio ladogensis. Infection was recorded only in 4+ fish and above and increased with age. They,

however, explained that the changes in parasite fauna are not due to the acquisition of age immunity but mainly to ecological factors: change of diet and migrations of fish within the lake. Thus, while young fish live mainly near the shores and feed mainly on Cladocera, Copepoda and Chironomid larvae, adult fish spend a large part of their lives in deeper water, where the diet includes a considerable proportion of the amphipods Pontoporeia and Pallasea. A very similar observation has been made on the brown trout of Afon Terrig. No age resistance is apparent in the incidence of C.truncatus nor is there any indication of a lower limit of size of fish that may be infected. The smallest infected fish taken during the present investigation was 94 mm. in length, 1+ years old, and harboured two worms, Although 4+ fish were infected, the highest number of worms (6 per fish) was taken from two and three year-old fish 159 and 190 mm. in length. From what has been noted above about the life cycle of the worm in fish, it seems significant that the above maxima in both young and older fish were recorded in November and December. It is also likely that the non-occurrence of the highest intensity in 4+ fish (there was no 5+,and only one 6+ was taken, cf. Chapter VII), may be connected with the generally low incidence of this parasite coupled with the observed tendency of larger and older fish to feed on larger organisms in Afon Terrig (cf. Chapter VII). The parasitisation of 1 - 3 year-old fish in Afon Terrig but not in Lake Ladoga, may be associated with the differential effects of the physical features of the environment in determining the distribution of fauna. Unlike the situation in the Ladoga outlined above, the Afon Terrig is a narrow, shallow, and stony stream, in which both young and

older fish as well as the intermediate host G.pulex, are ecologically in close proximity.

Wiśnewski (1932) examined 3850 specimens of F.bosniacus and R.spinicaudatus from the sources of Rivem Bosna, Sarajevo, and found approximately 7% infection asopposed to 100% in trout from the same area. Senk (1952) has recorded an infection rate of 0 - 16.69% in shrimps from various parts of the main river. Vik (1958) in the Anoya water system, observed that while the incidence in shrimps was up to 63.1% only about 23% of trout was infected. Still more recently, DeGiusti and Budd (1959) in a three-year survey of the combined infection rate of Echinorhynchus coregoni Van Cleave 1919 (= E.salmonis Muller 1780 (Petrochenko 1956)) and C.truncatus in their intermediate host Pontoporeia affinis from South Bay Mouth, Ontario, reported a marked decrease over the period. A total of 8115 P.affinis were examined over the period and the overall rate of infection was 7% in 1956, 4% in 1957 and 1.5% in 1958. They suggested that the decrease in overall rate of infection may reflect the decrease in Coregonid population in the area. In the Afon Terrig, the incidence of C.truncatus in shrimps is shown to be very low. Only about 0.1% of a total of 14260 G.pulex examined was infected, with a mean number per infected shrimp of one. It may be pointed out, however, that in shrimps dissected in connection with other studies, two progenetic and apparently infective worms have been recovered from a male shrimp 12 mm. in length. Wisniewski has noted that only in 2% of infections were procercoids at the early and late stages of development found. He thus suggested that shrimps develop immunity to secondary infection. The current observations

would seem to support his hypothesis. The nature of shrimp resistance to this worm, if any, is unknown, and it seems that experimental investigation of the above problems would be interesting.

The possibility that interspecies competition with other helminths utilising the shrimp as intermediate host may be responsible for the rarity of the worm in shrimps, is ruled out by the observation that procercoids of the species have been found in the same shrimp, but on different occasions, with larval E.truttae, Crepidostomum sp. and Cystidicola farionis Fischer 1798. A low incidence of the worm in shrimps has also been observed by Hynes (pers.comm.). Since Hynes's observations were made before the current study began, the scarcity may not be connected with any drop in the population of fish. The comparative rarity of C.truncatus in shrimps but not in fish, as found in Afon Terrig and vice versa (cf. Vik 1958) remains problematical. It can be suggested, however, that this may be due to a hitherto undiscovered aspect of host-parasite relationship in the water systems.

Finally, it may be added that the low infection rate in shrimps and hence the failure to take an appreciable number of infected specimens during the breeding seasons, did not allow any objective observations to be made on the effect of this relatively large worm, on the reproductive potentialities of shrimps. Beckman (1954) has reported that even one plerocercoid could destroy the gonads of the female amphipod and sterilise it.

2. NEMATODA

(a) Notes on the Occurrence of Intestinal Roundworms.

Roundworm infestation of the intestine of the brown trout of Afon Terrig was found to be comparatively low. Only 17 specimens of *Capillaria sp.* (Nematoda: Trichuridea[+]) were recovered during the present investigation. These were all taken downstream (Stations II and III) and were distributed as follows: January, 2 worms from one fish; March, 1; April, 1; June, 12 from four fish, one of these harbouring 9 worms; and August, 1 worm.

Capillaria is world-wide in its distribution in fish having been recorded from various fishes from the Arctic circle (White Sea, Shulman-Albova 1953) down to the Antarctic (Johnston and Mawson 1945). The intermediate host for *Capillaria* in Afon Terrig is not known. Markevich (1951) found that *Gammarus sp.* served as intermediate host for *C.tuberculata* Linstow and *C.brevispicula* Linstow in the acipenserids of Ukraine. Unless there are other ecological factors limiting the distribution of the worm to the lower stretches of Afon Terrig, it seems likely from what is known of the distribution of *G.pulex* population (cf. Chapters I and VII) that shrimps may not be the intermediate host in this stream. No capillarid larvae have been taken from *G.pulex.*

Smyth (1962) has stated that the bird worms *C.longicollis* Rudolphi 1819 and *C.annulata* Molin 1858 make use of earthworms as intermediate hosts. Considering the population and distribution of shrimps and earthworms in conjunction with the incidence and degree of parasitisation of the fish of

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+ After Yamaguti (1961)

Afon Terrig by Capillaria, it seems more likely that earthworms may be the invertebrate host. Earthworms, including the aquatic form Eisiniella sp. were shown (Chapter VII) to have a low occurrence by number (4.1%) and constituted 0.3% - 5.6% of the food of the brown trout during the year. The possibility that another arthropod, relatively common downstream but rare around Station I, may be the intermediate host, is not ruled out. It may also be recorded here that this parasite was more frequently taken from the intestine of experimental trout from Chirk Fishery than that from the stream investigated.

(b) Observations on the Occurrence of Non-intestinal Roundworms.

During the current study of the intestinal helminth parasites of the brown trout, records were also kept of the occurrence of two nematodes Metabronema truttae Baylis 1935 and Cystidicola farionis Fischer v. Waldheim 1798, found to be common in the stomach and swim bladder respectively of this fish in Afon Terrig.

(i) Metabronema truttae * Baylis 1935 (Nematoda: Spiruridea +)

This parasite is closely associated with the mucosa of the intestine and was especially common in the hollows formed by the longitudinal pleating of the undistended stomach.

The main features of the occurrence of M. truttae in the Afon Terrig trout are summarised in Figs. 10. 2 and 10.3. The graph for the whole stream shows that the rate of infection was generally high, and more or less

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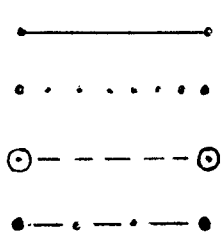
* Yamaguti (1961) has indicated that this species is synonymous Metabronema salvelini Fujita 1922 .

+ After Yamaguti (1961)

Fig. 10.2. The Incidence of Metabronema truttae in the Brown Trout of Afon Terrig

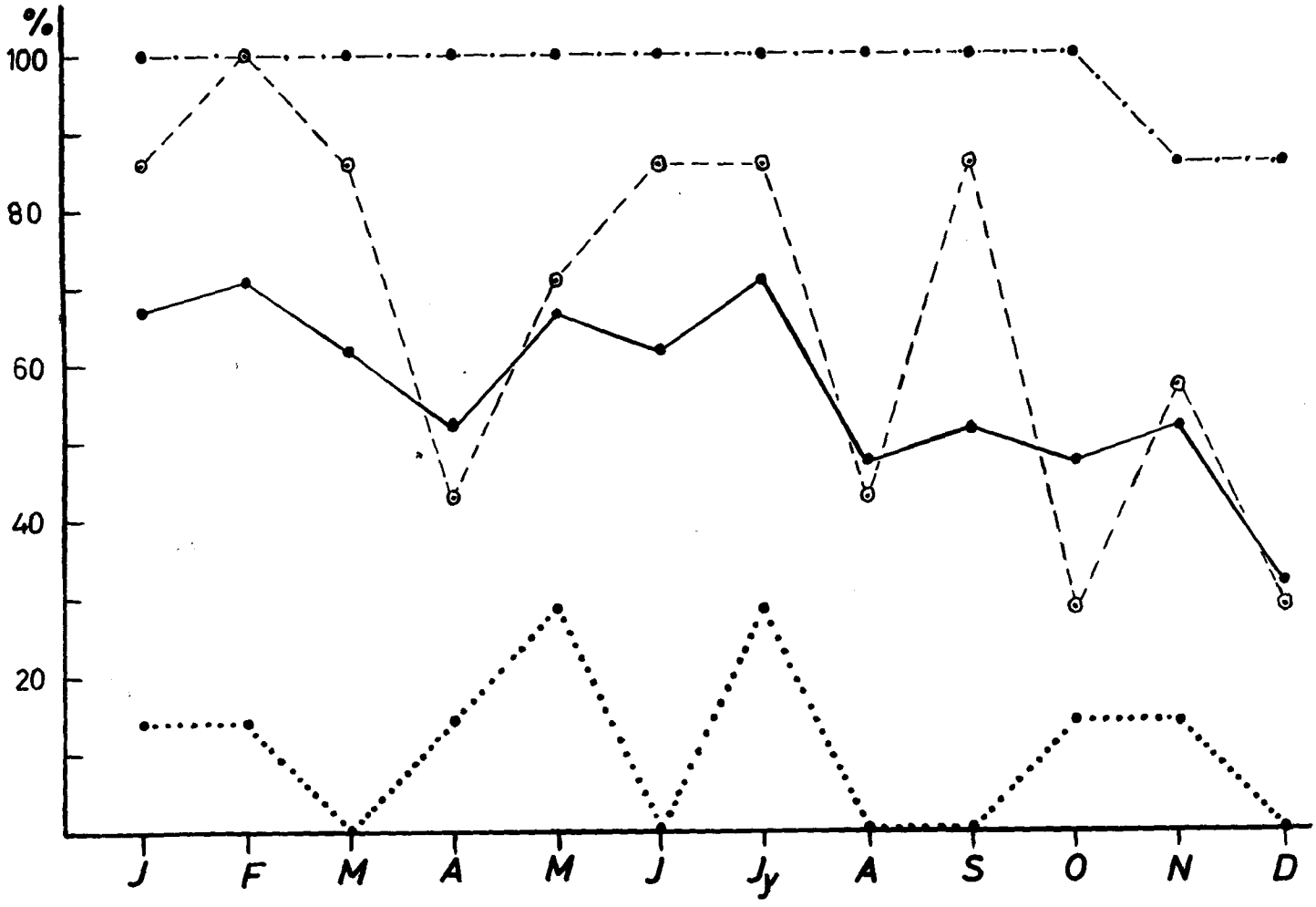


Percentage infection for the Whole Stream

" " at Station I

" " " Station II

" " " Station III


%
100
80
60
40
20
J
F
M
A
M
J
Jy
A
S
O
N
D

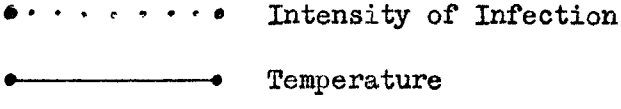
even throughout the year. A markedly higher infection was found downstream (Station III) than upstream (Station I). Thus, at Station III the percentage infection remained remarkably uniform at 100% for the first 10 months of the year and only fell slightly to 85.7% in November and December. Trout from Station I (Rhydtalog) on the other hand, had a very low incidence - only a maximum of 2 out of 7 fish being infected with 3 and 4 worms in May and July. It is interesting to note that the result from Station II is somewhat intermediate between those of Stations I and III and closely approximates that of the whole stream.

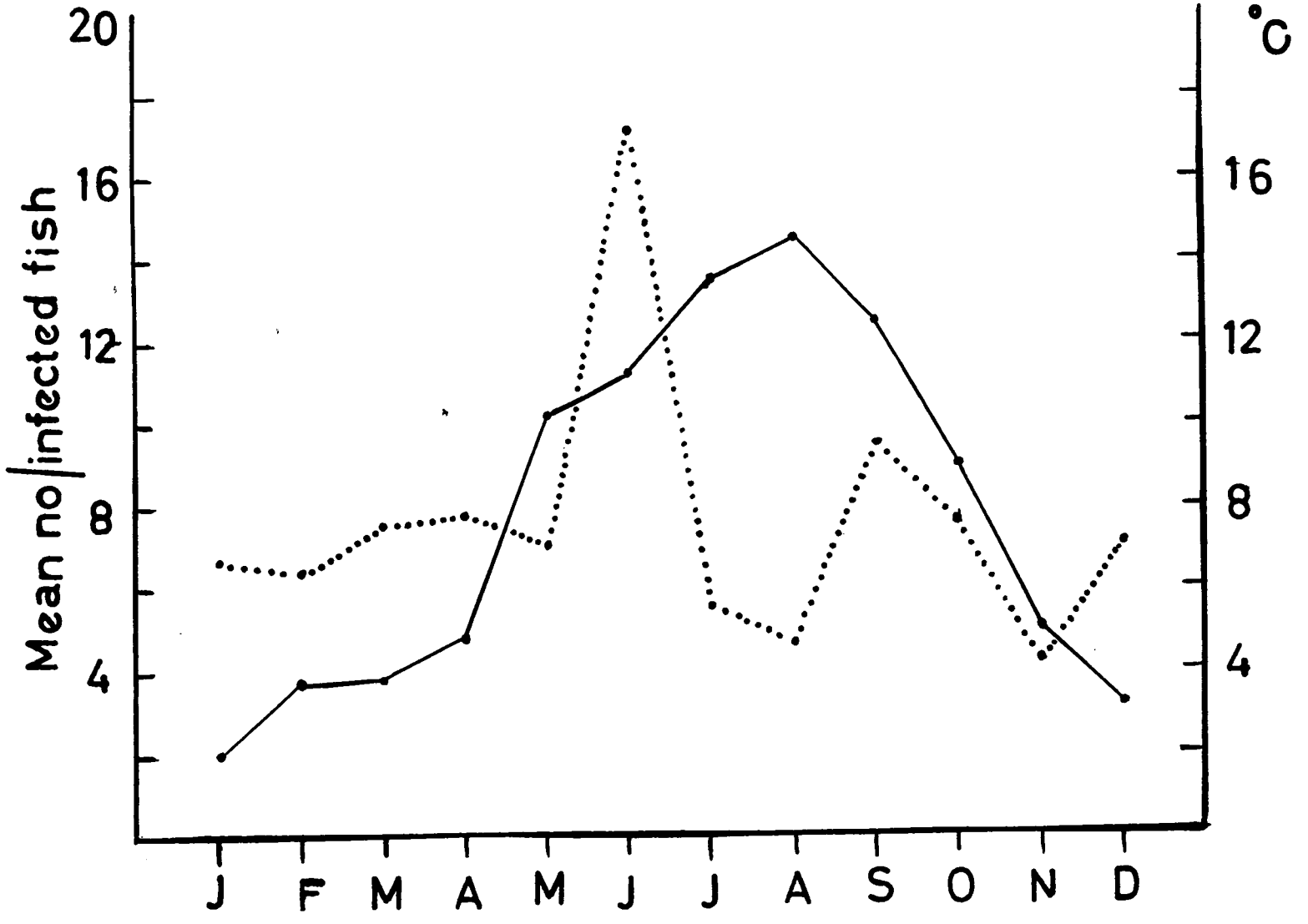
From Fig.10.3 it may be observed that there was a tendency for the intensity of infection to be higher during the warmer months of the year. This may well be a reflection of the seasonal dynamics in the feeding intensity of trout discussed in Chapter VII. In connection with the drop in the degree of parasitisation in July and August, it may be recalled that a sharp fall in both amount and number of food organisms was recorded in June (cf.Chapter VII).

Brown trout 1 - 7 years of age were infected and it is probable that at Station III where the infection was greatest, actively feeding 0+ fish may have been infected. Fish in the latter age group were not taken at Station III during the current study. As also the number of worms increased with age of fish, it may be safely concluded that there is no age resistance of trout to infection by this worm.

The intermediate host of this worm in Afon Terrig is not known. However, the pattern of the regional incidence indicates that the intermediate host is very common downstream and sparse upstream. This is

Fig. 10.3. Seasonal Variation in the Intensity of Infection of the Brown Trout of Afon Terrig by Metabronema truttae.




Mean no/infected fish
20
16
12
8
4
°C
16
12
8
4
J
F
M
A
M
J
J
A
S
O
N
D

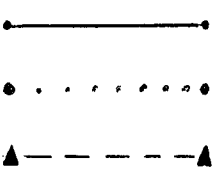
certainly the reverse of what is known about the population of shrimps in the stream. That G.pulex may not be such a host is further supported by the following viz: 1. nematode larvae were very rare in shrimps taken downstream especially at Station III; 2. larvae of roundworms recovered from G.pulex have been identified as various instars of Cystidicola farionis Fisher. Choquette (1953) recorded the Ephemeropterans Hexagenia recurvata and Polymitarcys as vectors of M.truttae (= M.salvelini Fujita 1922) and demonstrated experimentally that adult worms developed in fish 60 - 70 days after being fed on H.recurvata. During the current investigation, roundworm larvae were observed frequently in the body cavity of Ephemera danica Müller and various encased Trichoptera but the speciemens were not preserved.

(ii) Cystidicola farionis Fischer v Waldheim 1798 (Nematoda: Spiruridea)

As indicated above, this parasite inhabits the swim bladder of brown trout in Afon Terrig. The larval stages are passed in the haemocoel of G.pulex. The latter observation is in agreement with the finding of Baylis (1931). Bauer and Nikolskaya (1952) have also reported another amphipod Pontoporeia affinis as the intermediate host for this worm in Lake Ladoga (U.S.S.R.) The parasite has been recorded in Europe and the Soviet Union in various fish including Salmo trutta L., Coregonus Laveratus, Osmerus, Thymallus, Salvelinus and Gasterosteus. It is apparent that Cystidicola farionis is predominantly a salmonid parasite.

The fluctuations in the distribution of the worm in the brown trout of Afon Terrig are shown in Fig.10. 4. No obvious seasonal cycle seems apparent. Thus, at Station I where the parasite was abundant, the

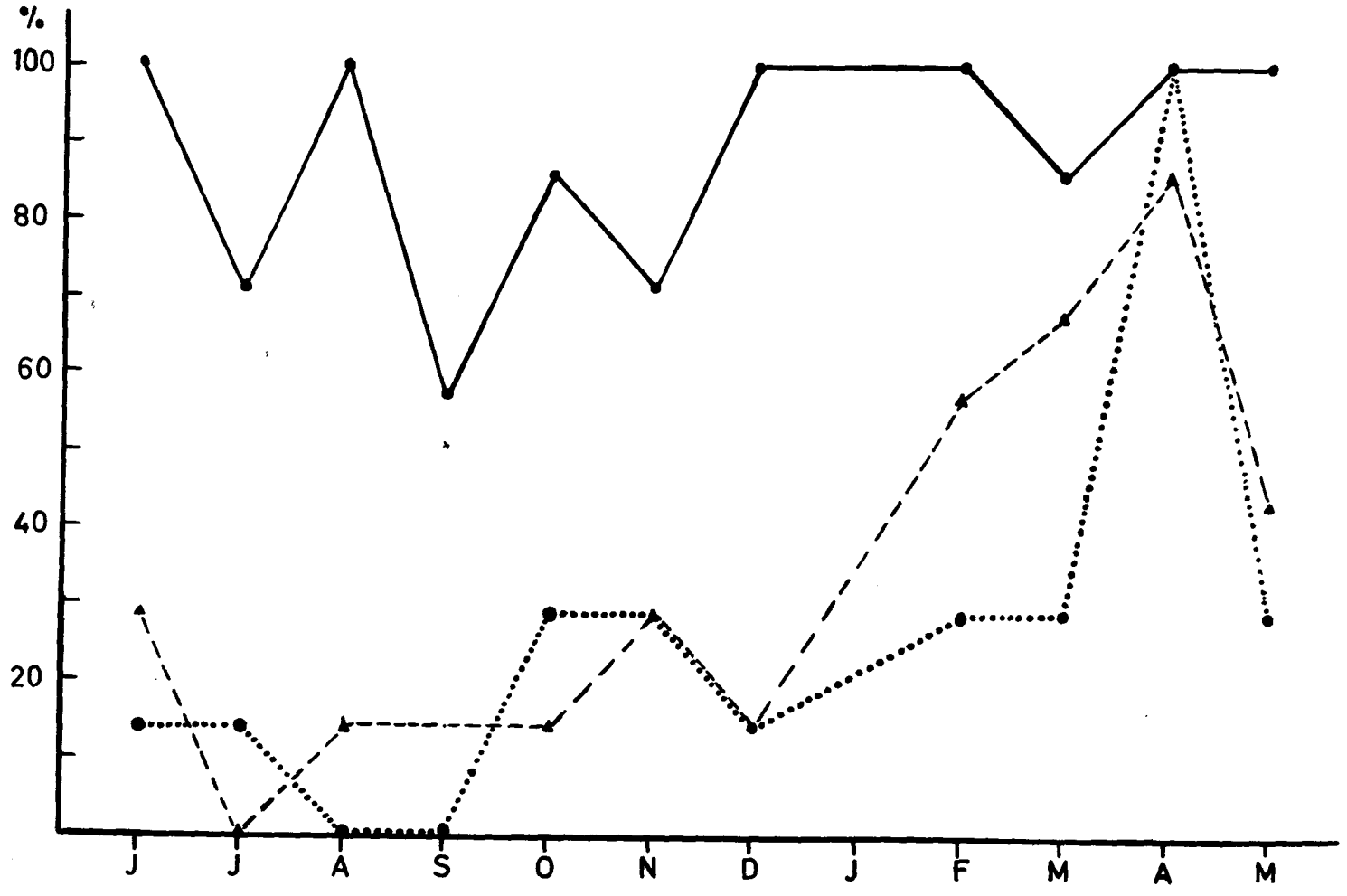
Fig. 10.4. The Incidence of *Cystidicola farionis* in the Brown Trout of Afon Terrig.



Infection rate at Station I

Infection rate at Station II

Infection rate at Station III


%
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M

percentage infection varied within a range throughout the year. Downstream (Stations II and III) the incidence was generally lower. This, as will be shown below, is due to the distribution of the worm in its intermediate host. The absence of a definite seasonal cycle of incidence in fish is in accord with the conclusion of Bauer and Nikolskaya (1957) arrived at after a five-month survey of the parasite fauna of the fish of Lake Ladoga. It may be added here that in two cases at Station I, the swim bladders were so heavily parasitised that they were badly damaged.

The rate of infection and degree of parasitisation of shrimps (see Table 10.2) were found to be high upstream (Station I) and remarkably low downstream (Stations II and III). There seems little doubt that this is a reflection of the abundance and distribution of G.pulex in these parts of the stream (cf. Chapters I and VII). Since C.farionis is virtually concentrated around Rhydtalog (Station I), the features of occurrence of the worm in this region may be taken as indicating the pattern for the stream. A close examination of Table 10.2 and Fig.10.5 shows that the dynamics of larval population at the latter Station does not appear to coincide with the incidence of the worm in its final host. A seasonal trend in the intensity of infection is indicated with greater numbers of worms during the colder months. The peak rate and intensity of infection were recorded in December. It is suggested that this may be due to one or both of the following: 1) a factor intrinsic to the developmental cycle of the parasite in shrimps. The developmental history was not investigated but it is possible that, like Crepidostomum spp. (cf.

Table 10.2. The occurrence of Cystidocola farionis in Gammarus pulex.

Station I. (Rhydtalog)

Month	Jan.	Feb.	March	April	May	June	July	August	September	October	November	December
No. shrimps examined	454	380	400	400	400	400	400	400	400	400	400	400
No. (%) infected	53(11.5)	34(8.9)	30(7.5)	22(5.5)	19(4.8)	15(3.8)	13(3.3)	15(3.8	38(9.5)	43(10.8)	40(10.0)	74(18.5)
Total no. parasites	56	35	35	26	19	15	13	17	42	45	47	83
Mean no./infected shrimps	1.1	1.0	1.2	1.2	1.0	1.0	1.0	1.1	1.1	1.0	1.2	1.1

Station II.

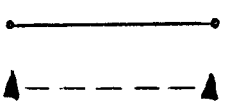
No. shrimps examined	314	420	360	400	400	400	400	400	400	400	400	400
No. (%) infected	0	1(0.3)	0	0	0	0	1(0.3)	1(0.3)	0	0	0	0
Total no. parasites	-	1	-	-	-	-	1	1	-	-	-	-
Mean No./infected shrimps	-	1.0	-	-	-	-	1.0	1.0	-	-	-	-

Station III (Caegwydd)

No. shrimps examined	468	380	320	400	400	400	400	400	400	400	400	400
No. (%) infected	0	1(0.3)	1(0.3)	2(0.5)	0	0	0	4(1.0)	1(0.3)	1(0.3)	2(0.5)	6(1.5)
Total no. parasites	-	1	1	2	-	-	-	4	1	1	2	6
Mean no./infected shrimps	-	1.0	1.0	1.0	-	-	-	1.0	1.0	1.0	1.0	1.0

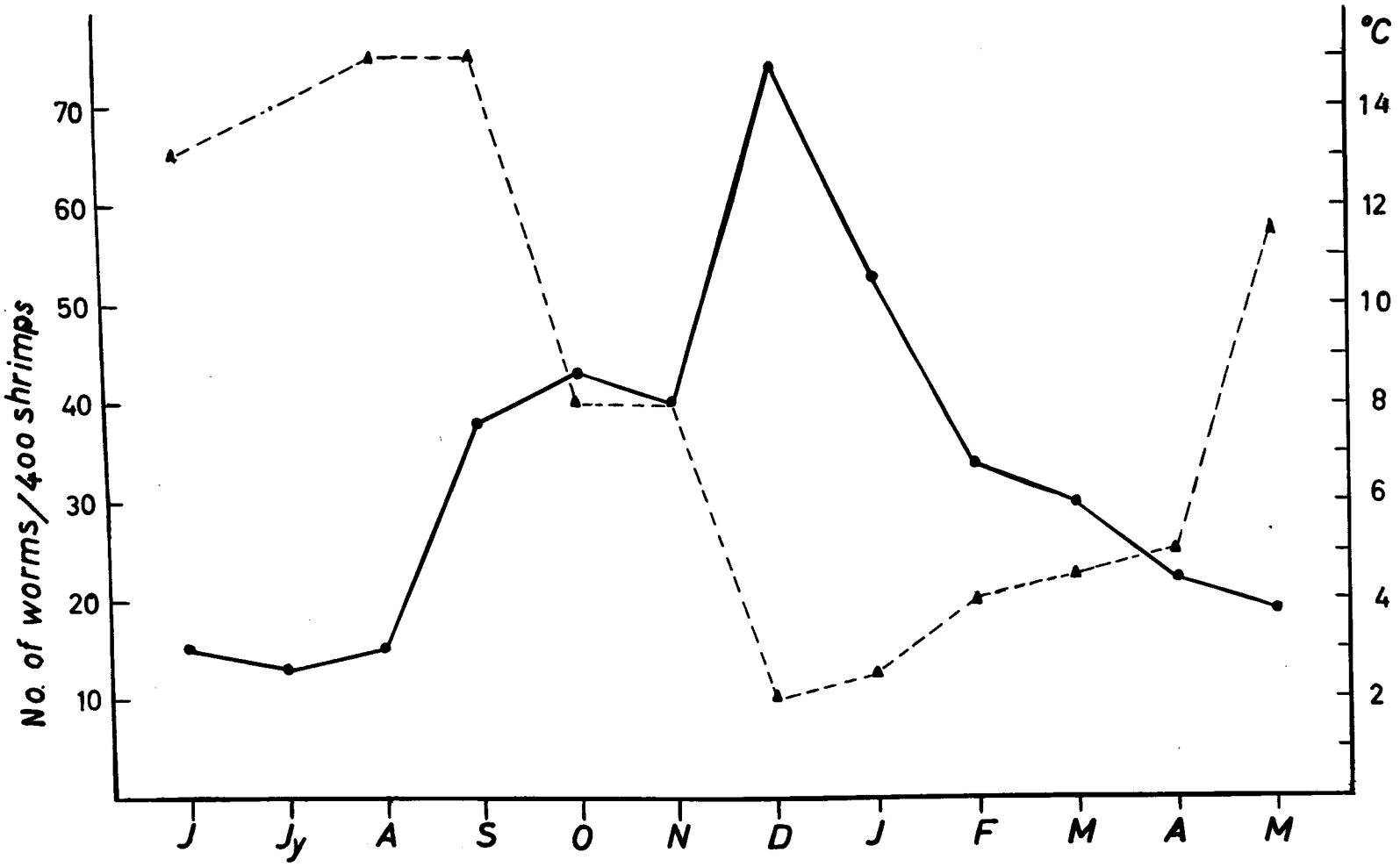
10.17

Fig. 10.5. Seasonal Variation in the Occurrence of *Cystidicola farionis* in the Intermediate Host *G.pulex*, at Rhydtalog (Afon Terrig)



Total number of larvae/400 Shrimps

Temperature.


No. of worms/400 shrimps
70
60
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30
20
10
°C
14
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10
8
6
4
2
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A
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Chapter IX), Cystidicola farionis has a limited period during which most worms invade the intermediate host. On the strength of the available evidence, it seems that the parasite establishes in late fall and winter when the temperature is lowest. Over-wintering juvenile shrimps carry the infection over to the summer. In this connection it is interesting to note that Tornquist (1931) has reported that all the specimens of another Spiruroid Camallanus lacustris Zöega 1776 taken from fish in the Swedish winter, were sexually immature. Sexual maturity was attained from May to June.

2) a favourable balance of worms in shrimps arising from the difference between the available infected specimens and the number ingested by trout during the colder months when the feeding activity of the fish is reduced (cf. Chapter VII).

It may also be pointed out that the above marked regional differences in the incidence and abundance of C.farionis and M.Truttae are confirmatory of the earlier established fact that the movements of the brown trout in Afon Terrig is limited. As it appears from the literature that these two species have received little ecological study, it is hoped that the observations made on their seasonal dynamics in Afon Terrig would be useful to future investigators.

3. MISCELLANEOUS PARASITES FROM GAMMARUS SPP.

The following parasites were recovered from the body cavity of shrimps during the present investigation.

Trematoda: Orchipedum (probably O.tracheicola Braun 1901) - metacercariae

Host: G.lacustris

Locality: Llyn Llywenan (Anglesey)
(A parasite of anseriform birds)

Cestoda : 1.Hymenolepis bifurcatus Hamann - cysticercoids

Host : G.pulex, G.lacustris

Locality: Afon Terrig (Flintshire/Denbighshire)
Shotwick stream (Cheshire)
Llyn Llywenan (Anglesey)
(A parasite of watershrew)

2.Davainea minuta? Cohn 1901 - cysticercoids

Host : G.pulex

Locality: Afon Terrig (Flintshire/Denbighshire)
Shotwick stream (Cheshire)
Raby ponds "
(A parasite of charadriiform birds.

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C H A P T E R XI

PATHOGENICITY AND HISTOPATHOLOGICAL OBSERVATIONS

1. INTRODUCTION

Intestinal helminths of trout can be of considerable pathological significance. Thus Davis (1937) has reported that inflammation of the intestine may result from parasitisation by the trematode Crepidostomum farionis Müller 1784. Steinstrasser (1936) recorded that while the thorny-headed worm Echinorhynchus truttae Schrank 1788 caused laceration of the intestinal mucosa, retardation of growth and noticeable loss of weight, a much smaller species Neoechinorhynchus rutili Müller 1787 inflicted a more extensive damage with the consequent death of trout. Other investigators who have observed varying degrees of and occasionally serious pathogenicity in infections involving E.truttae include Marochino (1926) Lestage (1933), Wurmbach (1937), Reichenbach-Klinke (1934) and Hoffmann (1954). Deterioration in the condition (Wolf 1906, Wiśniewski 1932, 1933, Vik (1954) and often mass mortality of trout (Huitfeldt-Kaas 1927, Bauer et al 1954 (in Bauer 1959), have been associated with infections involving the cestode Cyathocephalus truncatus Pallas 1781. It can be seen from the above brief survey that even for the same parasite species in the same

fish host, the pathogenic effects on the latter may vary.

It was thus thought desirable to ascertain the extent of the damage, if any, done by the helminths found in the intestinal tract of the brown trout in Afon Terrig.

2. MATERIALS AND METHODS

Both naturally infected brown trout, and hatchery brown and rainbow trout experimentally infected in the laboratory, were employed in the study. On the whole, just over 480 fish from the field and about 50 fish with acanthocephalan infection of known age were examined for the effect of the parasites (E.truttae, Crepidostomum metoecus, C.farionis and Cyathocephalus truncatus). Several uninfected fish from the stream, as well as hatchery fish used as controls for various experiments, were also examined and compared with infected specimens. The general procedure adopted for each worm species has been described in Chapters VII, VIII and X. After a rapid survey of the intestine and attached worms, the former was flushed out and carefully examined for gross damage under the binocular microscope. Worms were gently moved around the point of attachment without dislodging them and signs of host reaction as well as the degree of association between the organ of attachment and host mucosa noted.

For histological study, suitable parts of the intestine and caeca were fixed in cold Alcohol-formal-acetic described by Van Cleave (1953) and Bouin's fluid. Sections were made at 10 µ and stained in Ehrlichs haematoxylin and eosin.

3. GENERAL OBSERVATIONS ON GROSS PATHOLOGY

No adverse effects on the intestine, pyloric caeca or the general condition of trout was evident in infections of fish involving the digeneans Crepidostomum metoecus Braun 1900 and Crepidostomum farionis. Thus, a male fish harbouring about 200 C.metoecus was 249 mm. in length, weighed 142 gm. in spite of the fact that the gonads were nearly spent.

A much similar observation was made on trout parasitised by the Acanthocephalan E.truttae under both field and laboratory conditions. Signs of host reaction at the points of attachment of the probosces were not noticed under the binocular microscope. Nor was it possible to locate the positions of former attachment despite the fact that it has been established from both field and experimental studies (cf.Chapters II, V and VII) that this worm changes its place in the intestine and migrates down the latter with age. It is interesting to note here that Bullock (1963) has just published a similar observation on the movement of another echinorhynchid Acanthocephalus jacksoni Bullock 1962 in trout. Even mechanically dislodged worms left no obvious trace of their position under the binocular microscope. These observations would suggest a very light or loose association between the worm and the intestine in spite of what may be presumed from the formidable-looking proboscis armature of E.truttae.

At Rhydtalog where the degree of parasitisation of fish by this worm was high, with a worm population of 40 - 125 per fish being common, there was no epizootic of acanthocephalosis in trout. It may be added, however, that in experimental infections in which 30 infected shrimps were

administered simultaneously to fish which were autopsied 2 weeks later (for details see Chapter V), a copious secretion of mucus was observed in all parts.of the intestine especially the middle region. It is thought that this may be correlated with the sudden appearance of a comparatively large number of worms (up to 47/fish) in the intestinal environment.

A very different picture is presented by incidental observations made on two other acanthocephalan species met with in the course of the current series of studies on fish helminths. Hatchery fish were found to be occasionally infected by a few specimens of N.rutili. Examination of control fish from various experiments showed small red swellings or nodules and hence hyperaemia around the probosces of the parasite species. Several other such reddened patches were also readily noticeable in the mid- and hind-intestine of parasitised fish including those where the worms had disappeared from the intestine. There is little doubt that these red areas indicate places of former attachment by the worm. This finding of an apparently greater pathological importance of a small species with markedly smaller attachment organ, is in full agreement with Steinstrasser's (1936) observations.

In another investigation in which ducks experimentally infected with Polymorphus minutus Goeze 1782 were autopsied after 37 and 47 days, extensive damage to the intestinal wall was apparent. The locations of worms in the intestine were easily recognisable on opening the abdominal cavity. These were seen as large discoloured nodules along the lower intestine. On slitting open the intestinal lumen, P.minutus was observed

to be deeply embedded in the intestinal wall and attempts to dislodge live parasites mechanically, as was accomplished without difficulty with E.truttae led to the disruption of the worm. From the extent of damage done by about 7 - 30 worms taken during the investigation referred to above, it is not surprising that Polymorphus has been reported as an important pathogen to the final host (Nicholas and Hynes 1958, Petrochenko 1958, Logachev and Bruskin 1959, and Hynes and Nicholas 1963).

Of the parasites of the brown trout in Afon Terrig, the cestode C.truncatus appears to be the most important from the point of view of harmful effects induced on the host. This species inhabits the pyloric caeca and does serious damage to the latter. Thus,it was found that the distal blind tips and often distal quarter, depending on the age of the infection of parasitised caeca were swollen and solid. Vik (1958) reported that in experimental infections "cartilagisation of the pyloric coeca was observed after 43 days". Such caeca, without worms, were common in summer -a fact that ties up with observations on the life cycle of this species in fish (cf. Chapter X). Worms were difficult to detach even after their death and relaxation in tap water. In 2 of ca.480 fish from Afon Terrig, the caeca were in a very poor condition and were firmly glued on to the ventral abdominal wall by connective tissue. In yet another fish, a rotting caecum with disintegrating but attached worm was recovered from the abdominal cavity. That no external indications of the effect of this parasite was obvious on fish, may be due to the low occurrence of the worm in the stream. Even in the latter case, only four other caeca of the fish were infected.

4. HISTOLOGICAL OBSERVATIONS ON PATHOLOGY.

Very recently, Bullock (January 1963) has reviewed the work done on intestinal histology of salmonid fishes. Bullock (1961, 1963) has also studied and compared the histology of four species viz: the brook trout (Salvelinus fontinalis), Atlantic salmon (Salmo salar) the rainbow trout (S.gairdneri), and the brown trout (S.trutta). For further details on the latter, reference may be made to his papers. Suffice it to outline here the various regions of the intestine of the brown trout. These are: 1. the mucosa, 2. lamina propria, 3. Stratum compactum, 4. Stratum granulosum, 5. circular muscle layer, 6. longitudinal muscle layer, and 7. the serosa (see Plate 11 Fig.1). The hind intestine or rectum is characterised by the presence of 'annulo-spiral septa', 8, (Burnstock 1959) which involves all the intestinal layers except the longitudinal muscles and the serosa.

(a) Echinorhynchus truttae Schrank 1788.

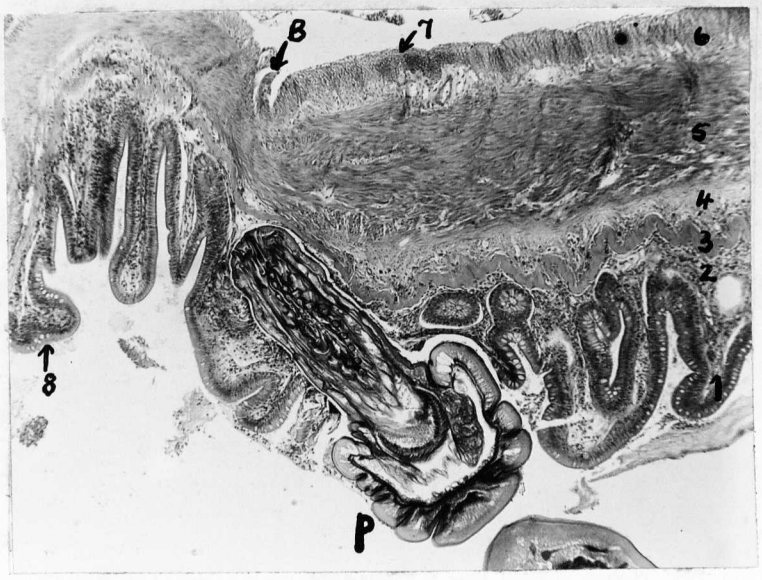
Histological examination of the infected intestine (see Plate 11 figs.1 and 2) showed that E.truttae does comparatively little damage. The latter appears to be mainly mechanical. The proboscis penetrates the epithelium and is embedded in the lamina propria and even in infections of 10 weeks' standing (cf. Plate 11. fig.1) the proboscis did not penetrate the Stratum compactum. Apart from the destruction of the epithelial layer at the point of attachment, no other damage was apparent. The inflammatory reaction was very limited. In areas where a gap (g) had been left in the epithelium by former attachment of a worm (see Plate 11 fig.2) there was no marked aggregation of leucocytes. As might be expected

PLATE 11

PHOTOMICROGRAPHS SHOWING DAMAGE DONE BY E.TRUTTAE
TO THE INTESTINE OF BROWN TROUT

Fig. 1 - Transverse section of posterior small intestine adjacent to the 'rectal' region with worm attached. Note that proboscis does not penetrate the stratum compactum (X60).
B. — boundary between lower intestine and 'rectum'

Fig. 2 - Transverse section of small intestine showing a temporary gap (g) in the mucosa caused by attachment of proboscis of the acanthocephalan. Note that there is no marked inflammation of the area.



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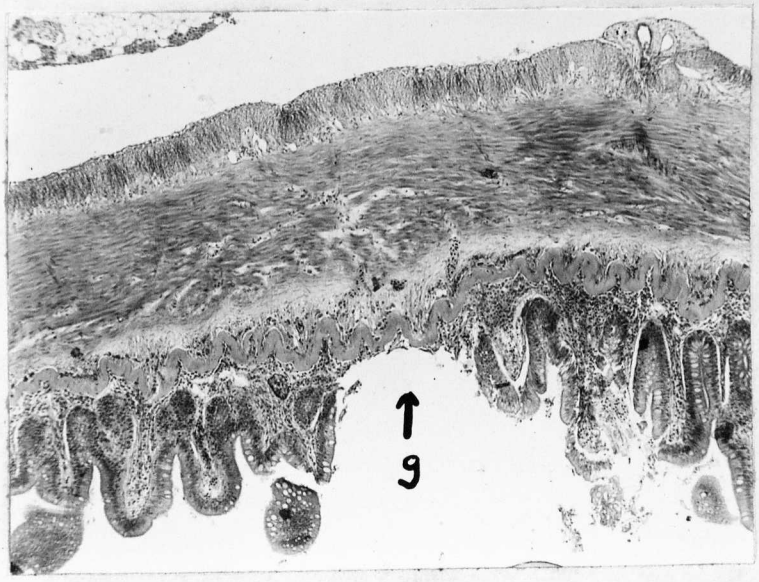

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PLATE II

however, pycnosis and karyorrhexis were evident in the degenerating epithelial as well as other displaced cells in the area immediately adjacent to the point of proboscis attachment. No thickening of the lamina propria reported in infections of Acanthocephalus jacksoni Bullock 1962 by Bullock (1963) was observed.

In sharp contradistinction to E.truttae, P.minutus, a smaller worm sharing the same intermediate host with E.truttae in Afon Terrig, was found to engender enormous histological decay in the intestine of its final bird host. Thus, after 47 days in the latter (see Plate 12) the praesoma of the worm was completely buried in the intestinal wall. The entire very thick band of circular muscles in the neighbourhood of the worm was swollen, degenerate and their place taken by a mass of fibrous tissue encircling the praesoma. In one case, where the proboscis had gone through the intestine, all the layers of the intestinal wall, from the epithelium to the serosa, were transformed into a collagenous capsule. It is interesting to note here that while the larger larvae of E.truttae had no effect on the reproductive phenomena in their common intermediate host G.pulex, P.minutus caused parasitic castration in female shrimps (cf. Chapter IV for details)

(b) Cyathocephalus truncatus Pallas 1781

The histological effects of C.truncatus on the pyloric caeca were similar to those described for P.minutus. The structure of the caecum is essentially similar to that of the intestine except that the muscularis is thinner (cf. Plate 13. fig.1).

PLATE 12

PHOTOMICROGRAPH SHOWING THE EXTENT OF DAMAGE DONE BY POLYMORPHUS MINUTUS TO THE INTESTINE OF AYLESBURY DUCK 47 DAYS AFTER INFECTION (X25). Compare E.truttae, 126 days in brown trout (cf. Plate 11. Fig.1)

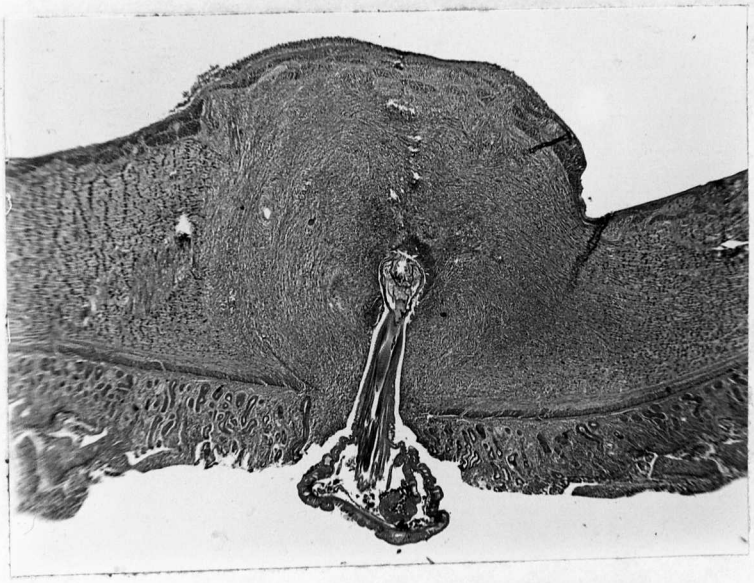


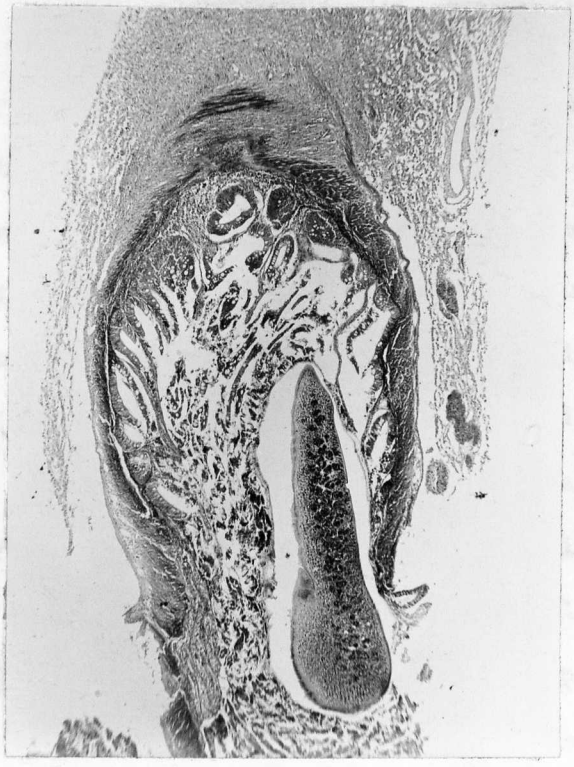
PLATE 12

PLATE 13

DAMAGE DONE BY CYATHOCEPHALUS TRUNCATUS TO THE PYLORIC CAECA OF BROWN TROUT.

Fig. 1 - Vertical section of caecum yet undamaged by parasite. Note the position of the worm (X30)

Fig. 2 - Vertical section of caecum showing the badly damaged blind tip. Note thickening and complete replacement of the layers of the wall by fibrous connective tissue (X30).



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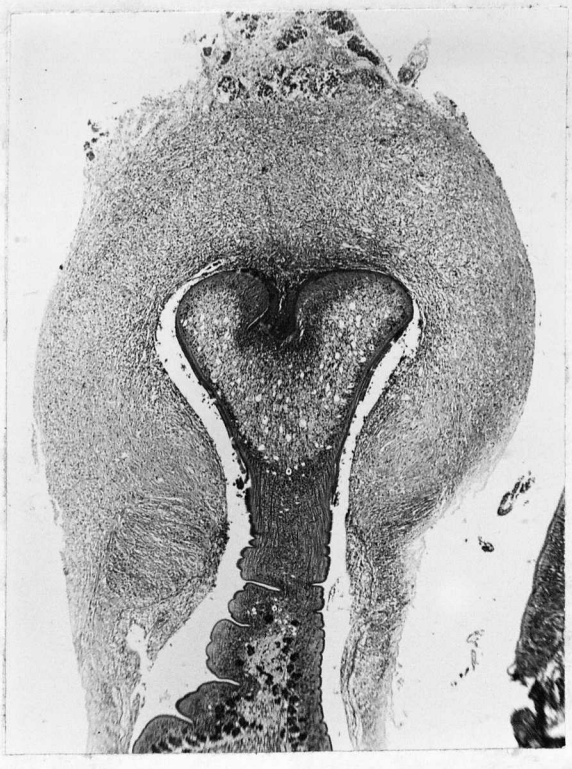

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PLATE 13

In a recently invaded caecum the epithelium was compressed and at the blunt end where the worm is attached, the epithelium was erroded. There was a marked influx of lymphocytes and polymorphonuclear leucocytes. As infection aged, vigorous metaplasia set in. The muscularis degenerated and the area was invaded by fibroblasts (see Plate 14, fig.1). In long-standing infections (see Plate 13. fig.2 and Plate 14, fig.2) not only was the blind end of the caecum transformed into a fibrous mass part of which was sucked into the funnel-shaped end of the worm, but also a considerable stretch of the organ was thickened and changed into a collagenous mass (cf. Plate 13. fig.2). It has been noted that caeca impaired to the extent described above, were found in nature without worms. Such caeca appear, therefore, to be permanently incapacitated.

5. DISCUSSION

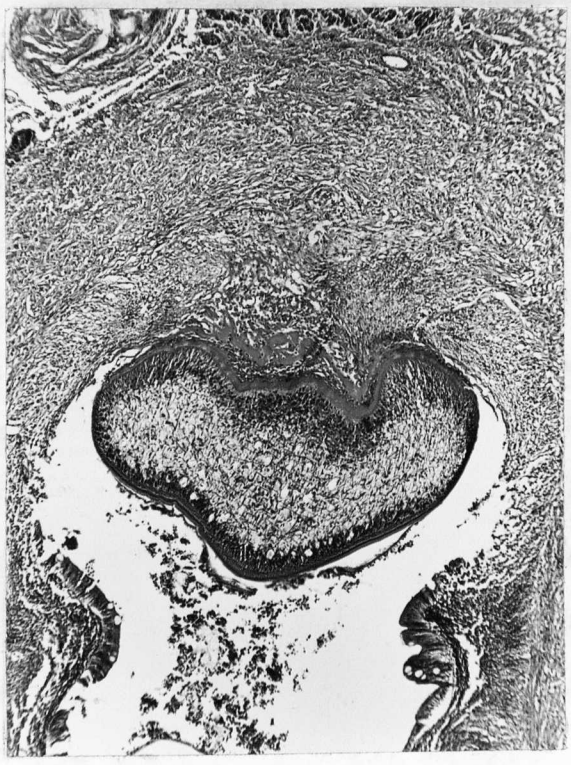
From the results of the present study, there seems to be little question of the relative pathologic importance of the various worms parasitising brown trout in Afon Terrig. The trematode, C.metoecus, which infests fish in large numbers in the stream, could not be associated with any obvious adverse effects on the infected part or the general condition of the host. Although a good deal of mucus was often found in the infected caeca, it was not possible to be sure that this was not due to the state of digestive activity. This is especially so as no appreciable increase in the number of goblet cells was found. Crepidostomum farionis is also shown to have no detectable effect. However, Davis (1937) has reported that mass infection of trout by this parasite, caused a general inflammation of the intestine. Wales (1958) made a similar observation in

PLATE 14

DETAILS OF TWO PHASES IN THE REACTION OF CAECA TO PARATISATION BY C.TRUNCATUS

Fig. 1 - An earlier stage. Note complete erosion of mucosa, inflammation, and metaplasia in attached area and immediate neighbourhood. Behind the latter, caecal wall is swollen, inflammation is evident but mucosa and other layers are still distinguishable. Necrotic displaced cells entangled in mucus lie in the lumen (X60)

Fig. 2 - A later stage (magnified tip of Fig.2 Plate 13) showing the fibrous nature of the now solid blind end of caecum (X80).



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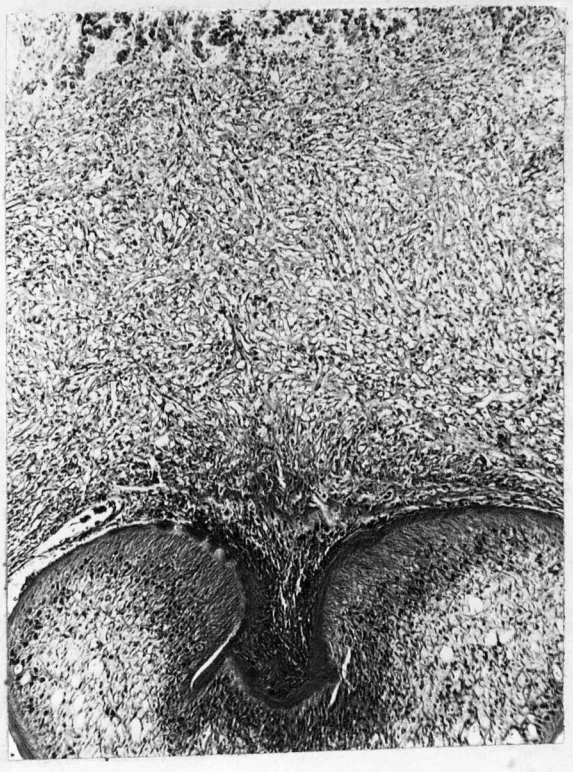

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Plate 14.

two cases of trout mortality in California where up to 446 trematodes were taken from dead fish. He went further to suggest that this fluke may be the cause of epizootics often ascribed to poisoning, "winterkill", and various other causes. It has been shown (cf. Chapters VIII and IX) that C.farionis is scarce in Afon Terrig, and that the mean number per infected fish is one. The absence of a detectable effect on fish may thus be due to the low occurrence of the worm in the environment investigated.

Although as indicated earlier, extensive damage and pathogenicity are often assumed in acanthocephalan infections on the grounds of the spiny nature of the proboscis and the numbers of worms often present in sick and dying animals, a survey of the literature and the results of the current investigation, show that this may not be applicable to some Acanthocephala. Thus, while the bird parasite P.minutus is shown to be capable of penetrating through the intestinal wall with consequent metaplasia and polypoid extrusion of the infected area into the abdominal cavity as reported by Pflugfelder (1956) for P.baschadis Schrank, Logachev and Bruskin (1959) for P.magnus Skrjabin 1913, and Prakash and Adams (1960) for the echinorhynchid Echinorhynchus lageniformis Ekbaum 1938 in starry flounders, E.truttae is very lightly attached to the intestinal mucosa and does no more histologically apparent damage, beyond the laceration and perhaps temporary ulceration (Bullock 1963) of the columnar epithelial layer. This finding closely approximates those reported for this worm (E.truttae) by Marochino (1926), Steinstrasser (1936), Wurmbach 1937; for Leptorhynchoides thecatus Linton in the large mouth bass by Venard and Warfel (1953) and for A. Jacksoni in four salmonid species by Bullock (1961, 1963).

Sternstrasser and Bullock were, however, able to associate the incidence of E.truttae and A.jacksoni with emaciation and retardation of growth in the fish of the Mosel and State Fish Hatchery of New Hampshire respectively. No such adverse effects were found in both naturally infected fish and experimental fish maintained in the laboratory for up to 18 weeks after infection. The most heavily parasitised fish in the stream with just over 200 parasites, had a well-formed body, had attained a length and weight (221 mm. and 110 g.m) that compared favourably with uninfected fish of the same age (4+). Two rainbow trout which were fed 15 and 20 infected shrimps, and from which 11 and 7 E.truttae were recovered 96 and 126 days respectively after infection, were in very good condition and fed well throughout the experimental period. At autopsy, masses of adipose tissue lined the gut and the dorsal wall of the abdominal cavity, almost obliterating the swim bladder from view. It seems also pertinent to add that although G.K.Petrushevski and S.S.Shulman(in Bogiel et al (1958)) have suggested that E,truttae and E.gadi O.F.Müller 1776 were "undeniably important" as a source of epizootics among the Salmonidae and Gadidae respectively, Bauer (1953, 1959) while accepting the economic importance of these species and E.salmonis (Barysheva 1949), has stated that so far deaths of trout due to infestation by the worms have not been recorded in the U.S.S.R.

Of the intestinal helminths of the brown trout of the Terrig, the tapeworm C.truncatus is shown to be of great pathologic potentiality. Although no obvious loss of condition in fish was apparent, the effects of the worm on the infected parts were such that it appears that but for -

1. the low rate and intensity of infection in Terrig trout (cf. Chapter X), 2. the abundance of food organisms, especially G.pulex, upstream where the infection was common, there would probably have been external manifestations of the damage done by this species to the fish host. Large portions of pyloric caeca were permanently defunct and deformed. Since this organ is important in the digestive mechanism of fish, the incapacitation of a good number would lead to malnutrition and attendant disturbances in growth and development.

It is not surprising, therefore, that Huitfeldt-Kaas (1927) has described epizootics caused by the incidence of this parasite in trout and char in two Norwegian lakes. Death occurred when fish were infected by 200 worms or more. Dying fish were exhausted, their muscles discoloured, pyloric caeca inflamed, and a general anaemia observed. Cyathocephalosis has also been reported in salmonids and coregonids which, in the Arctic province of the U.S.S.R., are infested upto 80 - 90% with intensity of up to 80 (G.K.Petrushevski and S.S.Shulman in Dogiel et al (1958)). Vik (1958) has followed the pathgenic effects of C.truncatus in trout experimentally and his findings are in agreement with those recorded above.

The present investigation emphasises an interesting but general parallel existing between the closeness of association, mobility and hence relative permanence of attachment of an intestinal helminth, and the host reactions vis-a-vis the damage done to the latter. Experimental evidence shows that E.truttae changes its position in the intestine and gradually migrates down the latter with age. On the other hand, observations on

C.truncatus recorded above (cf. also Chapter X) indicate a close and lifelong (worm's) association between a parasite and a caecum. Thus, while E.truttae causes only temporary gaps in the epithelium of the intestine as it moves down the latter (Morachino 1927, Bullock 1963), C.truncatus causes necrosis of infected parts which may lead to the death of the host as reported elsewhere. An examination of the behaviour of a parasite species and its incidence in conjunction with a consideration of host physiology as well as some ecological factors such as the abundance and dietary value of available food organisms in a habitat, would thus help to explain at least some of the varying and often conflicting reports of the pathogenicity of Acanthocephala and may-be other fish parasites . Finally, it is noted that mobile and apparently less harmful species, could 'unwittingly' act as vector for other pathogens (and thus become epizootologically important) by serving as inoculating needle through which disease bacteria and other microbes invade fish.

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C H A P T E R XII

INTER-RELATIONSHIPS OF THE FISH, THE PARASITES AND THE INVERTEBRATE FAUNA OF AFON TERRIG

From the foregoing chapters, it is clear that the Afon Terrig is typically a trout stream. It is well-aerated (fast flowing) with stony bottom, maximum temperature about 15°C and more important still, the predominant vertebrate fauna is Salmo trutta L. (Ricker 1934, Van Deusen 1954). Because of the features exhibited by the occurrence of intestinal helminth parasites in Cottus gobio L., the only other fish resident in the stream (cf. Chapters I, VII - X), the current investigation has been essentially one of the parasites of the brown trout.

Of the main invertebrate elements of the fauna - G.pulex, Ephemeroptera and Plecoptera nymphs, Trichoptera and Diptera larvae, only the freshwater shrimp is shown to be important from the parasitological standpoint, harbouring the larval stages of the parasites whose life - cycles in the environment are known. Thus, G.pulex serves as intermediate host of 4 out of 5 intestinal helminths of the brown trout. It also houses the larval stages of Cystidicola farionis a swim bladder parasite of trout, the cysticercoids of two cestodes Hymenolepis bifurcatus and a Davained

(D.minuta?) parasitising homothermous animals, and the larvae of the bird acanthocephalan Polymorphus minutus.

The details of the development and life history of the acanthocephalan Echinorhynchus (Metechinorhynchus) truttae in both its hosts have been worked out in both experimental and natural infections (cf.Chapters II, VII), while the life cycles of the digeneans Crepidostomum metoecus and C.farionis, and the cestode Cyathocephalus truncatus in the stream have been followed (Chapters VIII - X). Clearly defined and similar seasonal cycles are shown by the two trematodes and the cestode. In all three cases the worms are shown to begin to establish in the trout of Afon Terrig in late autumn when the temperature is 9.5°C. The incidence rises sharply as temperature falls further with the highest percentage infection in February C.metoecus and C.truncatus, December/January for Crepidostomum farionis. As the temperature of the stream rises in spring, the incidence begins to fall, and by May, when the temperature is above 10°C, a sharp decline in the rate of infection is recorded. The decrease in both incidence and intensity continued till late summer, when worms disappear from most fish. Where present, the parasites are either gravid as in C.truncatus, or moribund with evanescent internal organs, as in the digeneans. In Crepidostomum metoecus experimental and field observations emphasise the importance of the metacercarial stage in the life cycle. A developmental period of about 2 - 3 months in shrimps in nature, which is essential for the attainment of the infective form, is indicated.

In the light of the above observations, it seems patent that temperature may have a considerable influence on the determination of seasonal

periodicity by controlling the period during which worms are able to establish in the final hosts - the brown trout. A threshold temperature of about 10°C above which conditions appear unfavourable for the establishment of worms, is suggested.

The situation in the acanthocephalan E.truttae is shown to be slightly different owing to the intervention of certain ecological factors. No seasonal periodicity is shown in the incidence of the parasite in trout in the stream. This is associated with the comparatively high level of infection of the intermediate host (ca. 3 - 7°C) and more important still, the cystacanths are available in good numbers throughout the year. The occurrence of the parasite in G.pulex has, however, a seasonal character which is tied up with the seasonal cycle in the life of shrimps. The cyclic nature of the intensity of infection of trout by E.truttae is probably a reflection of the seasonal dynamics of the feeding intensity of the fish.

Despite the absence of cyclic fluctuations in the rate of infection of trout, an analysis of the population of larval and adult E.truttae shows definite trends in the composition of the population by the different developmental stages. Thus, although in trout, female worms with acanthors at all stages of development are found during the year, cyclic changes in the peak proportion and hence attainment of each of the four main stages of development, are detectable, e.g. an upswing in the number of worms reaching functional maturity can be noticed as the temperature falls in the autumn and by December (3.2°C) and again in April (4.8°C), peak percentage composition of worms with mainly mature acanthors is recorded. Correlated

dynamics in the percentage composition of larval developmental stages in shrimps are also found. It is explained that the underlying factor responsible for these changes in the proportion of the developmental stages of E.truttae may be temperature. Thus, the occurrence, during the colder months, of two peaks of worms with mainly mature acanthors, is probably due to the experimentally established differential in the effect of temperature on the development of parasites acquired in summer and the fall - the former worms being largely responsible for the December peak, and the latter, developing during the coldest part of the year, are probably accountable for the peak in April.

It seems clear, therefore, that in the four main intestinal helminths of S.trutta in Afon Terrig viz: E.truttae, Crepidostomum metoecus C.farionis and Cyathocephalus truncatus, temperature appears to be the underlying factor which controls the seasonal dynamics of development and life cycle. This effect of temperature may be correlated with the geographical distribution and probable origin of these parasites. Attention is drawn to the fact that the above worms are holarctic and parasitise a definitive host, which is essentially a fish of the higher latitudes. S. S. Shulman (in Dogiel et al 1958) includes these parasites (except C.metoecus) among 20 species which are of northern origin and circumpolar. The non-inclusion of C.metoecus is no doubt due to a hitherto and, as shown earlier, mistakenly held view about the distribution and abundance of C.metoecus (cf. Chapters VIII and IX). It is understandable, therefore, that having apparently originated from very cold climes, temperature has a profound effect on the life of these worms, even when found in the more

temperate areas within their range.

On the general problem of host-parasite relations, some interesting observations have been made on the parasite fauna of fish in Afon Terrig. Apart from the nematode Capillaria, which is shown (Chapter X) to be world-wide and to parasitise various fish groups, the other four intestinal parasites named above are predominantly parasites of the Salmonidae. Dogiel (1948a) has stated that the "formation of the intestinal fauna is a result of action of two forces: phylogenetic (relationship between the host species) and ecological (similarity of diet etc.)". Thus the acanthocephalan E.truttae, of S.trutta origin, has been successfully passed through S.gairdneri experimentally even though the latter fish species is only of comparatively recent introduction to Britain. On the other hand, Cottus gobio, which is ecologically similar to S.trutta e.g. occurs in the same stream, has similar diet etc., is found to harbour only E.truttae during the present investigation. Even so, the acanthocephalan has comparatively very low occurrence in the bullhead. The worms found were normally attached, but no functionally mature female was recovered. One, however, contained a few immature acanthors in the body cavity. The indications are that the worm may develop normally in Cottus but until further observational and experimental data are available, no definite conclusion can be reached on the status of the 'infection' (cf. Sandground 1929, Baer 1952) recorded here in the bullhead.

Of the other intestinal parasites, only Crepidostomum metoecus has been reported from Cottus gobio (Nybelin 1922, Hynes pers.comm.) Since Nybelin recovered eggs from his specimens, though it is not known if

these eggs were viable, it is thought that the failure to take this species from Cottus during the present investigation may be due to a lower exposure of this fish to infection in comparisonwith trout, due mainly to ecological factors. The bullhead is limited to the area around Station III where the incidence of C.metoecus in shrimps is low. Added to this, Dottus gobio is a bottom type, poor swimmer, and not only has a low capacity for food but feeds less actively than trout (cf.Chapter VII) and hence stands a lower chance of acquiring an infection. It may also be noted here that the cystacanths of the bird acanthocephalan Polymorphus minutus, in both natural and experimental infections, passes through the digestive tract of trout apparently unstimulated and unharmed.

Bauer (in Dogiel et al 1958) in dealing with the effects of fish parasite on the host has noted as follows: "The body of the fish can react to the parasite by hypertrophy of individual tissues, by metaplasia, by inflammatory process and, finally, by the development of immunity. Here again, in relation to fishes, these phenomena have not been studied sufficiently". An examination of the reactions of the trout of Afon Terrig to its parasites has emphasised the relation between host-parasite intimacy of association and the reactions of the fish host. Thus, while the trematodes Crepidostomum farionis and C.metoecus which are lightly attached and as observed under the binocular microscope are likely to shift their points of attachment continually in nature, are shown to have no easily demonstrable effects on the Terrig trout, the cestode has a close and apparently life-long association with the pyloric caeca. It thus elicits a profound reaction and does extensive damage culminating in the

permanent defunctioning of parasitised parts of the caeca. As the latter are very important in the nutrition of fish (especially in processing of food for absorption), it is apparent that the failure to detect external symptons of cyathocephalosis in parasitised fish is probably due to the low occurrence of the worm in the Afon Terrig. In Sweden, Huitfeldt-Kass (1927) has reported cases of mass mortality of trout due to this worm. The intennity of infection in one case was up to 400 and dying fish were extremely emaciated. In 'Ropsha' farm (U.S.S.R.) 1954, Bauer (in Dogiel et al 1958) has observed that "when present in great numbers, this parasite rapidly causes the fish to lose weight and retards the maturation of the ovaries. Sometimes, particularly during the spawning season, it can become the cause of mortality".

As far as the current consideration is concerned, the acanthocephalan E.truttae appears to be somewhat intermediate between Crepidostomum spp. and Cyathocephalus truncatus. Contrary to expectations, E.truttae is shown to be lightly attached to the intestinal wall (cf. Chapter XI), and migrates down the intestinal tract with age, causing superficial damage as it moves along. Van Cleave (1952) dealing with some host-parasite relationships of the Acanthocephala with special reference to the organs of attachment, has described the mechanics of attachment and withdrawal of the proboscis. From his description, it would appear that damage of the type caused by E.truttae is done during the process of attachment. This superficial ulceration of the intestine is temporary, unlike the damage caused by C.truncatus. Bullock (1963) has described the process involved in the healing of a similar breach in the epithelial layer of the intestine

of trout caused by another echinorhynchid with a similar behaviour, Acanthocephalus jacksoni.

From the standpoint of survival and perpetuation of the parasite species, the above feature of the association between E.truttae and the intestine of trout puts the worm in a bad stead. It has been shown from experimental infections, that this loose attachment of the proboscis and the continual changes of position along the intestinal tract leads to appreciable loss of worms with faecal matter (cf. Chapters II and V). It also appears that a similar loss does occur in nature for in most infected fish taken from the stream, otherwise perfectly normal parasites free in the intestinal lumen or with their probosces embedded in intestinal contents are found (Chapter VII). It is also noted (Chapters II and VII) that the diet can be related to the frequency of occurrence and hence loss of such worms in both experimental and natural infections. Thus, when the diet is fleshy (and probably 'simulates' the gut wall) e.g. earthworms and young trout in nature and minced meat in laboratory-maintained fish, more worms are found attached to intestinal contents. However, from both experimental and field studies of E.truttae in S.trutta (cf.Chapters IV, V and VII), it would appear that any disadvantages emanting from 'accidental' loss of immature and unspent mature worms referred to above, is probably offset in favour of the parasite by 1. non-existence of premunition in infections of E.truttae in both the intermediate and final host. Concurrent infections are experimentally possible and occur widely in nature; 2. the established fact that starvation does not expel established worms;

and 3. the observation (experimental and natural) that fish of all ages are infected by E.truttae.

The present study also shows that there is no age resistance or natural immunity as expounded by Taliaferro (1929, 1940) and various other authors (cf. Table 6. 1). Sandground (1929) has questioned the occurrence and extent of immunity in helminth infections while the results of various workers on fish helminths (Essex and Hunter 1926, McCoy1930, Bangham 1944, Bangham and Venard 1946, Robertson 1953, Frankland 1955 etc.) are in agreement with the present finding. It can be concluded, therefore, even if tentatively, that available evidence shows that host immunity does not occur in intestinal helminth infections of fish.

The studies on resistance and other aspects of host-parasite relations in G.pulex as well as parallel observations on other relevant invertebrates, show a substantially different, but variable picture. A host-parasite specificity that is entirely independent of ecological surroundings is evident. Although, as indicated above, other arthropods are abundant in the stream, G.pulex only serves as the arthropod host for the four main intestinal helminths - E.truttae, Crepidostomum farionis, C.metoecus and Cyathocephalus truncatus. Live larvae of these worms have not been found in any other arthropod in Afon Terrig. For instance, even at Station II, where insect nymphs and larvae are exposed (and have been for years exposed) to heavy infection by C.metoecus due to their close ecological proximity with Limnaea pereger the first intermediate host, only dead cysts are recovered. The same thing goes for insects and the acanthors of E.truttae at Rhydtalog. Similarly, although the prosobranch Potamopyrgus

12.9a

Table 12. 1. Changes in the gut helminth fauna of the brown trout with age in Afon Terrig.

Species of parasite	Age of trout (years)													
	1		2		3		4		5		6+		7*	
	% infected	Mean	%Infected	Mean	%Infected	Mean	%Infected	Mean	%Infected	Mean	%Infected	Mean	%Infected	Mean
Echinorhynchus truttae	50.0	1.0	79.4	6.3	94.0	14.1	100	22.1	100	45.9	-	-	100	41.0
Crepidostomum metoecus	53.3	10.1	45.9	22.5	63.1	24.0	71.4	28.8	91.7	29.1	-	-	0	-
Crepidostomum farionis	0	-	7.5	1.0	10.7	1.0	2.0	1.0	0	-	-	-	0	-
Cyathocephalus truncatus	10.0	1.0	21.9	2.0	22.6	2.6	51.0	1.8	33.3	1.8	-	-	0	-
Capillaria sp.	0	-	2.1	1.0	7.1	2.5	2.0	1.0	0	-	-	-	0	-
Metabronama truttae	26.7	4.6	50.7	5.2	60.7	12.4	42.9	7.1	33.3	3.3	-	-	0	-

\+ No fish in this age group taken

* Only one specimen taken.

jenkinsi, and the pulmonate L.pereger are common downstream, nevertheless the mulluscan host for Crepidostomum farionis is the sphaeriid bivalve Pisidium which is scarce with very patchy and limited distribution downstream. It is also noted that although the freswater 'limpet' Ancylastrum fluviatile is abundant in all parts of the stream, it does not function as first intermediate host for the digeneans. Physiological considerations apart, it is shown that the snail is ecologically and behaviourally isolated from the eggs of the parasites.

From experimental evidence (cf.Chapters II and IV), it is clear that not all species of Gammarus nor the ecologically similar freshwater hoglouse Asellus, can be infected with equal success by the acanthors of E.truttae. All species investigated, including the natural intermediate host G.pulex, possess natural resistance which is humoral in character and increases at low temperatures. It is also shown that the strength of resistance to the establishment of E.truttae is correlated principally with the phylogenetic relationships of the species. Thus, while viable cystacanths have successfully developed under experimental conditions in G.lacustris a close relation of G.pulex (Hynes and Nicholas 1958), all worms were destroyed after varying periods in G.duebeni and G.tigrinus. Both shrimp species are distantly related to G.pulex in comparison with G.lacustris. Also, in spite of their ecological similarity with G.pulex the isopods are found to be completely resistant to E.truttae. An identical pattern of reaction is observed in a more limited experimental investigation of the development of the trematode Cœtoecus in G.lacustris and G.duebeni. Unfortunately, because of limitation of material, it was not possible to

conduct a similar investigation on Cyathocephalus truncatus. It may be noted, however, that a similar differential in the success of invasion of copepods by the coracidia of other tapeworms has been found by Michajlow (1938) and Baer (unpubl. in Baer 1952).

An aspect of the relationship between these parasites in their common intermediate host revealed by the current series of studies is that their larvae are not mutually exclusive in the haemocoel of G.pulex. Concurrent infections involving metacercariae, procercoids and acanthellae (including cystacanths not only of E.truttae but also P.minutus) in various combinations, are found in nature. Intra-species co-invasion of shrimps by the four parasites are also found in the field. For C. truncatus, however, there is observational evidence (Wisniewski 1933 and Chapter X) which seems to indicate that the presence of an infective progenetic procercoid in G.pulex may prevent the establishment of a new infection. This is in contrast with Crepidostomum, E.truttae and the bird parasite P.minutus in which it is found that intra-species challenges involving the relevant final larval form in G.pulex and the invasive larva are naturally and experimentally possible.

Unlike P.minutus, E.truttae has no effect on adult G.pulex but retards the development of those shrimps acquiring infection at a tender age in both the field and the laboratory (cf. Chapter IV). Although it was not possible to make objective observation on the pathogenic effects of C.truncatus in shrimps because of the very low occurrence in the latter, M. Yu Beckman's (1954)observation indicates that this worm castrates female G.pulex - an effect paralleled by that of P.minutus in Afon Terrig

(cf. Chapter IV), and as serious as the damage done by the adult worm to the final host. The trematodes are not associated with any obvious pathological effect on shrimps. C.metoecus is, however, shown to do extensive damage to its molluscan host L.pereger. In infected specimens taken from August - November, practically all parts of this snail except the central nervous system, were parasitised. The most severely infected areas are the digestive gland and the ovo-testis and in very bad cases, the testis is found to be very small. Here again, there is little doubt that the degree of association between the worm and its snail host coupled with the entire dependence on the latter for the supply of the material necessary to meet the requirements of the enormous tissue synthesis involved in the multiplicative asexual reproduction in the snail, has resulted in the extensive damage inflicted on L.pereger. Although it was not possible to carry out a similar assessmemt of the damage done by Crepidostomum farionis in Pisidium because of the low population of both parasite and host in Afon Terrig, it is noteworthy that Cheng and James (1960) have recorded that the hepato-pancreas of another sphaeriid, Sphaerium striatinum, is completely filled with second generation rediae of Crepidostomum cornutum at various stages of maturity. They also observed ingested liver cells in the blind sac of several living daughter rediae under phase-contrast and thus suggested that C.cornutum may utilise the liver cells of Sphaerium as nutrient.

Comparative study of the distribution and abundance of the parasites of fish in relation to the invertebrate fauna in Afon Terrig, illustrates a general ecological point, i.e. that a stream is an environment with varying habitats or regional differences, which may be, as in this case,

comparable to those found in the more localised lake or pond. For instance, the lamellibranch Pisidium is limited to discontinuous gravelly and sandy patches of the wider stretches of the stream around and below Caegwydd (Station III). The population of L.pereger is highest around Station II, decreases sharply upstream, and the snail has not been taken at Rhydtalog. Further downstream from Station II, the population falls more gradually and the snail is found all over the lower stretches of the stream. Even the dominant invertebrate species in Afon Terrig, G.pulex, shows a regional pattern of distribution. Shrimps are abundant at Rhydtalog where the stream is narrow with plenty of grass drooping into it (see Wolf 1906, Senk 1952). In the wider lower stretches G.pulex is common with the population decreasing as confluence with Afon Alun is approached.

The above features of the invertebrate fauna are truly reflected in the abundance and distribution of the parasites of trout. The population of E.truttae, Cyathocephalus truncatus and Cystidicola farionis utilising G.pulex as the only intermediate host is highest upstream. Depending on the relative abundance of these worms in Afon Terrig as a whole, (see Table 12.2) they are sparsely distributed downstream or scarce (cf. also Chapters VII - XI). In the digenean species, the molluscan host controls the distribution and relative abundance of the worms in Afon Terrig. Thus C.metoecus has a dense population around Station II whereas Crepidostomum farionis is scarce and limited to areas around and below Station III.

12.14

Table 12.2 Summary of the occurrence of gut helminth parasites of the brown trout of Afon Terrig in their intermediate and definitive hosts.

	TROUT			SHRIMPS		
Species of Parasite	Incidence (%)	Intensity (Mean)	Total no.parasites	Incidence (%)	Intensity (Mean)	Total no.parasites
Echinorhynchus truttae	88.5	15.9	3543	3.6	1.2	588
Crepidostomum metoecus	59.8	22.5	3954	21.4	8.9	27228
Crepidostomum farionis	6.9	1.0	18	? +	-	-
Cyathocephalus truncatus	25.8	2.0	133	0.1	1.0	11
Capillaria sp.	3.1	2.1	17	- *	-	-
Metabronema truttae.	57.5	7.6	1104	- *	-	-

\+ See Chapter IX

* See Chapter X

The existence of these clearly marked regional differences in the incidence and numbers of these parasites in both the intermediate and the final host shows conclusively that the movements of the brown trout, spawning or otherwise, are very limited in Afon Terrig. It may be recalled that a similar result has been obtained by Allen (1951) for the brown trout of Horokiwi Stream, New Zealand, by marking fish with small oxidised silver discs attached to the dorsal fin. The present finding in Afon Terrig, therefore, stresses the value of parasitological studies as a means of gaining knowledge about the host. In the Afon Terrig studies, the gut parasites Echinorhynchus truttae, Crepidostomum farionis C.metoecus, Cyathocephalus truncatus, Metabronema truttae and the swim bladder nematode Cystidicola farionis, have served as very useful tags or indicators in determining the movements of the brown trout (Salmo trutta) in the stream.

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APPENDICES.

Appendix I

Table 1. The Effect of E.truttae on the Life Span of G.pulex. Room Temperature

Expt.No. and Date	Initial No. of infected G.pulex	No. of G.pulex remaining after (DAYS) 7	14	21	28	35	42	49	56	63	70	77	84	%age remaining after 35 days	%age remaining after 56 days	%age infected after 56 days	%age remaining after 84 days	%age infected after 84 days	Range of Temp. °C.
No.1 24.3.63																			
Light Infection	40	37	33	32	32	32	32	32	31	-	-	-	-	80	77.5	100	-	-	10-15
Heavy Infection	40	36	34	31	31	26	23	23	23	-	-	-	-	65	57.5	100	-	-	
Control	40	40	38	37	37	32	30	30	29	-	-	-	-	80	72.5	-	-	-	
No.2 22.8.62																			
Light Infection	40	40	-	31	31	30	30	25	20	13	10	6	2	75	50	100	5	100	10-15
Heavy Infection	40	40	-	32	31	28	28	21	16	13	8	5	3	70	40	100	7.5	100	
Control	40	40	-	36	36	33	33	32	32	26	23	9	5	82.5	80	-	12.5	-	
No.3 11.4.63																			
Light Infection	40	33	30	29	26	24	24	19	5	1	0	-	-	60	12.5	100	0	-	10-20
Heavy Infection	40	38	34	34	32	31	29	27	21	13	8	7	4	77.5	52.5	100	10	100	
Control	40	39	36	36	36	30	22	17	17	17	16	16	15	75	42.5	-	37.5	-	
No.4 11.4.63																			
Light Infection	40	36	33	32	32	29	29	29	24	22	13	10	5	72.5	60	100	12.5	100	10-20
Heavy Infection	40	38	35	35	35	34	32	31	24	20	14	5	1	85	60	100	2.5	100	
Control	40	40	37	36	35	31	26	23	19	16	10	7	7	77.5	47.5	-	17.5	-	

Appendix I

Table 2. The Effect of E.truttae on the Life Span of G.pulex. Cold Room

Expt.No. and Date	Initial No. of G.pulex infected		No. of G.pulex remaining after (DAYS) 7	14	21	28	35	42	49	56	63	70	77	84	112	%age remaining after 35 days	%age remaining after 56 days	%age infected after 56 days	% remaining after 84 days	%age infected after 84 days	Range of Temp. °C
No.1 24.3.63																					
Light Infection	40	H & N	40	40	39	37	36	34	34	34	-	-	-	-	-	90	85	100	-	-	4-10
Heavy Infection	40	"	39	38	35	35	33	29	29	28	-	-	-	-	-	82.5	70	100	-	-	
Control	40		40	40	40	40	38	38	38	36	-	-	-	-	-	95	90	-	-	-	
No.2 22.8.62																					
Light Infection	40	H & N	40	40	31	28	24	24	21	17	14	13	8	7	-	60	42.5	?	17.5	100	14-15
Heavy Infection	40	"	28	27	27	27	27	26	24	22	20	17	16	12	-	67.5	55	?	30	100	
Control	40		40	40	33	31	17	7	4	2	0	-	-	-	-	42.5	5	-	0	-	
No.3 31.10.62																					
Light Infection	40		40	36	32	31	29	29	29	28	26	25	25	25	-	72.5	70	?	62.5	100	2-8
Heavy Infection	40	Deg.	19	17	12	9	7	7	7	6	6	6	5	5	-	17.5	15	?	12.5	100	
Control	40		40	40	37	30	29	27	27	27	26	26	26	26	-	72.5	67.5	-	65	-	
No.4 24.11.62																					
Light Infection	40		40	39	38	37	37	37	35	35	35	34	34	34	33	92.5	87.5	?	85	-	2-4
Heavy Infection	39	Deg.	39	39	38	38	38	38	36	36	36	36	35	34	30	97.4	92.3	?	87.2	-	
Control	40		40	40	40	38	38	37	36	35	33	32	32	32	30	95	87.5	-	80	-	

H. & N. - Infection method after Hynes & Nicholas (1957)
Deg. - " " " DeGiusti (1949)

Appendix 2. Table 1.

The occurrence and developmental stages of E.truttae in G.pulex from Afon Terrig. Summary for the stream.

Month	Jan	Feb.	March	April	May	June	July	August	September	October	November	December
No.of shrimps >6 mm. examined	840	840	780	810	840	840	840	840	840	840	840	840
No.of shrimps< 6 mm. examined	360	360	240	390	360	360	360	360	360	360	360	360
No.(%) shrimps > 6 mm. infected	53(6.3)	31(3.8)	30(3.9)	32(4.0)	40(4.8)	25(3.0)	31(3.7)	30(3.6)	41(4.9)	47(5.6)	34(4.1)	59(7.0)
No.(%) shrimps< 6 mm. infected	10(2.8)	8(2.2)	3(1.3)	5(1.3)	5(1.4)	2(0.6)	0	0	0	2(0.6)	6(1.7)	11(3.6)
Total no.of worms found	85	45	36	44	50	30	33	34	48	52	46	85
No.(%) acanthella in spherical and earlier stages	8(9.4)	10(22.2)	11(30.6)	14(31.8)	8(16.0)	2(6.7)	3(9.1)	2(5.8)	9(18.8)	6(11.5)	5(10.9)	8(9.4)
No.(%) acanthella in oval to positioning of cortical nuclei stages	13(15.3)	10(22.2)	4(11.1)	3(6.8)	12(24.0)	8(26.7)	6(18.2)	4(11.8)	9(18.8)	8(15.4)	5(10.9)	4(4.7)
No.(%) acanthella in elongation to erect proboscis differentiation stages	19(22.4)	6(13.3)	7(19.4)	9(20.5)	12(24.0)	4(13.3)	5(15.2)	9(26.5)	14(29.1)	17(32.7)	17(36.9)	22(25.9)
No.(%) acanthella in invaginated proboscis and later stages.	45(52.9)	19(42.3)	14(38.9)	18(40.9)	18(36.0)	16(53.3)	19(57.5)	19(55.9)	16(33.3)	21(40.4)	19(41.3)	51(60.0)

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Appendix 2.

Table 2. The occurrence of E.truttae and developmental stages of the female parasite in the brown trout of Afon Terrig. Summary for the Stream.

Month	Jan.	Feb.	March	April	May	June	July	August	September	October	November	December
+ Total no. of trout examined	21	21	21	21	21	21	21	21	21	21	21	21
Total no. of trout infected	21	21	21	20	18	16	17	17	19	16	18	19
% trout infected	100	100	100	95.2	85.7	76.2	80.9	80.9	90.5	76.2	85.7	90.5
Total no. of parasites found	197	278	242	340	283	493	288	423	294	246	214	245
Mean no.parasites/infected fish	9.4	13.2	11.5	17.0	15.7	30.8	16.9	24.9	15.5	15.4	11.9	12.9
No. of male parasites	59	108	83	137	129	259	109	199	116	92	81	75
No. of female parasites	138	170	159	203	154	234	179	224	178	154	133	170
No.(%) with ovarian balls only	15(10.9)	13(7.6)	17(10.7)	36(17.7)	39(25.3)	49(20.9)	45(25.1)	51(22.8)	20(11.2)	16(10.4)	14(10.5)	15(8.8)
No.(%) with ovarian balls and immature acanthors	45(32.6)	62(36.5)	51(32.1)	90(44.3)	83(53.9)	149(63.7)	94(52.5)	112(50.0)	75(42.2)	49(31.8)	57(42.9)	51(30.8)
No.(%) with immature and mature acanthors	78(56.5)	85(50.0)	78(49.1)	50(24.6)	32(20.8)	33(14.1)	39(21.8)	55(24.6)	77(43.3)	76(49.4)	47(35.3)	80(47.1)
No.(%) with mature acanthors mainly	0	10(5.9)	13(8.1)	27(13.3)	0	3(1.3)	1(0.6)	6(2.6)	6(3.4)	13(8.4)	15(11.3)	24(14.1)

+ All the fish examained were 100 - 375 mm. in length

Appendix. 3.

Table 1. The Occurrence of Crepidostomum metoecus in the brown trout of Afon Terrig.

Month	No. fish examined	No. (%) infected	Total no. of parasites	Mean no./ infected fish
January	22	15(68.2)	344	22.9
February	21	20(95.2)	515	25.8
March	21	19(90.5)	808	42.5
April	23	21(91.3)	665	31.7
May	23	19(82.6)	458	24.2
June	22	15(68.2)	133	8.9
July	22	10(45.5)	30	3.0
August	21	3(14.4)	8	2.7
September	21	1(4.8)	2	2.0
October	21	3(14.4)	8	2.7
November	21	10(47.6)	400	19.1
December	21	19(90.5)	583	30.7
Total	259	155(59.8)	3954	25.5

Appendix 3

Table 2. The Occurrence of Crepidostomum farionis in the brown trout of Afon Terrig.

Month	No. fish examined	No.(%) infected	Total no.of parasites	Mean no./ infected fish
January	22	3(13.6)	3	1.0
February	21	2(9.5)	2	1.0
March	21	2(9.5)	2	1.0
April	23	1(4.3)	1	1.0
May	23	3(13.0)	3	1.0
June	22	0	-	-
July	22	0	-	-
August	21	1(4.8)	1	1.0
September	21	1(4.8)	1	1.0
October	21	0	-	-
November	21	2(9.5)	2	1.0
December	21	3(14.3)	3	1.0
Total	259	18(6.9)	18	1.0

Appendix 3.

Table 3. The Occurrence of Metacercaria of Crepidostomum* in Gammarus pulex. Summary for Stream.

Month	No. shrimps examined	No (%) infected	Total no. of parasites	Mean no./ infected shrimps
January	1200	298(24.8)	2752	9.2
February	1180	94(8.0)	200	2.1
March	1080	72(6.7)	192	2.7
April	1200	95(7.9)	207	2.2
May	1200	65(5.4)	153	2.4
June	1200	69(5.8)	97	1.4
July	1200	255(21.3)	680	2.7
August	1200	414(34.5)	3258	7.9
September	1200	481(40.1)	6209	12.9
October	1200	439(36.6)	6025	13.7
November	1200	398(33.2)	3350	8.4
December	1200	365(30.4)	4105	11.2
Total	14260	3045(21.4)	27228	8.9

* Crepidostomum metoecus. See text.

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Appendix 3

Table 4. Showing details of the Occurrence of Metacercaria of + Crepidostomum in Gammarus pulex.at the 3 Stations.

Station I (Rhydtalog)

Month	Jan.	Feb.	Mar.	April	May	June	July	August	September	October	November	December
No.shrimps examined	400	380	400	400	400	400	400	400	400	400	400	400
No.(%) infected	0	0	0	0	0	0	0	0	0	0	3(0.8)	0
Total no.parasites	-	-	-	-	-	-	-	-	-	-	3	-
Mean no./infected shrimps	-	-	-	-	-	-	-	-	-	-	1	-

Station II

No.shrimps examined	400	420	360	400	400	400	400	400	400	400	400	400
No.(%) infected	237(59.2)	83(19.8)	60(16.7)	85(21.3)	61(15.3)	61(15.3)	190(47.5)	307(76.8)	374(93.5)	360(90.0)	334(81.8)	308(77.0)
Total no.parasites	2623	170	146	195	148	89	598	3061	5937	5800	3248	3966
Mean no./infected shrimps	11.1	2.0	2.4	2.3	2.4	1.5	3.1	10.0	15.9	16.1	9.7	12.9

Station III (Caegwydd)

No.shrimps examined	400	380	320	400	400	400	400	400	400	400	400	400
No.(%) infected	61(15.3)	11(2.9)	12(3.8)	10(2.5)	2(1.0)	8(2.0)	65(16.3)	107(26.8)	107(26.8)	79(19.8)	61(15.3)	59(14.8)
Total no.parasites	129	30	46	12	5	8	82	197	272	225	99	139
Mean no./infected shrimps	2.1	2.7	3.8	1.2	1.3	1.0	1.3	1.8	2.5	2.8	1.6	2.4

+ Crepidostomum metoecus. See text

Appendix 4

Table 1. The Occurrence of Cyathocephalus truncatus in the brown trout.

Station I (Rhydtalog)

Month	Jan.	Feb.	March	April	May	June	July	August	September	October	November	December
No.fish examined	7	7	7	7	7	7	7	7	7	7	7	7
No.(%) infected	2(28.6)	4(57.1)	4(57.1)	3(42.9)	2(28.6)	4(57.1)	4(57.1)	0	0	1(14.3)	3(42.9)	2(28.6)
Total no.parasites	2	12	9	4	4	5	11	-	-	3	8	4
Mean no./infected fish	1.0	3.0	2.3	1.3	2.0	1.3	2.8	-	-	3.0	2.7	2.0

Station II

No.fish examined	7	7	7	7	7	7	7	7	7	7	7	7
No.(%) infected	3(42.9)	2(28.6)	3(42.9)	4(57.1)	1(14.3)	1(14.3)	0	1(14.3)	0	3(42.9)	3(42.9)	5(71.4)
Total no.parasites	7	8	6	7	1	2	-	2	-	7	10	20
Mean no./infected fish	2.3	4.0	2.0	1.8	1.0	2.0	-	2.0	-	2.3	3.3	4.0

Station III (Caegwydd)

No.fish examined	7	7	7	7	7	7	7	7	7	7	7	7
No.(%) infected	0	(5(71.4)	1(14.3)	2(28.6)	0	0	0	0	0	0	1(14.3)	1(14.3)
Total no.parasites	-	5	2	2	-	-	-	-	-	-	1	1
Mean no./infected fish	-	1.0	2.0	1.0	-	-	-	-	-	-	1.0	1.0

Appendix 4

Table 2. The occurrence of *Metabronema truttae* in the brown trout of Afon Terrig.

Station I (Rhydtalog)

Month	Jan.	Feb.	March	April	May	June	July	August	September	October	November	December
No.fish examined	7	7	7	7	7	7	7	7	7	7	7	7
No.(%) infected	1(14.3)	1(14.3)	0	1(14.3)	2(28.6)	0	2(28.6)	0	0	1(14.3)	1(14.3)	0
Total no.parasites	1	1	-	1	3	-	4	-	-	1	1	-
Mean no./infected fish	1.0	1.0	-	1.0	1.5	-	2.0	-	-	1.0	1.0	-

Station II

No.fish examined	7	7	7	7	7	7	7	7	7	7	7	7
No.(%) infected	6(85.7)	7(100)	6(85.7)	3(42.9)	5(71.4)	6(85.7)	6(85.7)	3(42.9)	6(85.7)	2(28.6)	4(57.2)	2(28.6)
Total no.parasites	25	27	29	7	15	16	16	11	29	6	11	2
Mean no./infected fish	4.2	3.9	4.8	2.3	3.0	2.7	2.7	3.7	4.8	3.0	2.8	1.0

Station III (Ceegwydd)

No.fish examined	7	7	7	7	7	7	7	7	7	7	7	7
No.(%) infected	7(100)	7(100)	7(100)	7(100)	7(100)	7(100)	7(100)	7(100)	7(100)	7(100)	6(85.7)	6(85.7)
Total no.parasites	68	68	69	77	80	206	62	35	75	69	34	55
Mean no./infected fish	9.7	9.7	9.9	11.0	11.4	29.4	8.9	5.0	15.0	9.9	5.7	9.2

Appendix 4

Table 3. The occurrence of *Metabronema truttae* in the brown trout. Summary for the stream.

Month	No.fish examined	No.(%) infected	Total no. parasites	Mean no./ infected fish
January	21	14(66.7)	94	6.7
February	21	15(71.4)	96	6.4
March	21	13(61.9)	98	7.5
April	21	11(52.4)	85	7.7
May	21	14(66.7)	98	7.0
June	21	13(61.9)	222	17.1
July	21	15(71.4)	82	5.5
August	21	10(47.6)	46	4.6
September	21	11(52.4)	104	9.5
October	21	10(47.6)	76	7.6
November	21	11(52.4)	46	4.2
December	21	8(32.1)	57	7.1
Total	252	145(57.5)	1104	7.6