DEVELOPMENT AND GROWTH OF *SCHISTOCEPHALUS SOLIDUS* MÜLLER *IN VIVO* AND *IN VITRO*

T H E S I S

for the

Degree of Doctor of Philosophy

in the

University of Glasgow

by

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C O N T E N T S

5. Adult

Section II

The occurrence of the plerocercoid stage of *Schistocephalus solidus* in two populations of the three-spined stickleback, *Gasterosteus aculeatus*

Section III

The in vitro cultivation of the plerocercoid of *Schistocephalus solidus*

Acknowledgements

I am grateful to Professor C.M. Yonge, C.B.E., F.R.S., for the provision of the facilities required for this work.

I would like to thank Dr. C.A. Hopkins for his invaluable advice, criticism and encouragement at all times during this investigation.

My thanks are also due to all my colleagues who helped in the collection of fish.

Introduction

The life cycle of *Schistocephalus solidus* had been known for over thirty years but there was little information in the literature on the maintenance of the life cycle in the laboratory. It was thus decided to obtain each stage of the life cycle under laboratory conditions and study their development. The data obtained is recorded in Section I.

The plerocercoid stage is the only stage in the cycle which can be obtained easily in nature. Hence numerous visits were made to two lochs to collect infected 3-spined sticklebacks. Records were kept of all the fish caught and the infection they harboured in order that information relating to the cycle under natural conditions could be obtained. This information is recorded in Section II.

The regular sampling of two lochs produced many small plerocercoids which it was found could not become established in a warm-blooded host. The cultivation *in vitro* of these small plerocercoids was thus commenced as well as the cultivation of any larger plerocercoids not required for feeding to potential definitive hosts. The results of this work are recorded in Section III.

Declaration

The work on the adult stage of the life cycle was published jointly with Dr. C.A. Hopkins [McCaig and Hopkins, 1963] but the majority of the results were obtained by McCaig. Another paper [Hopkins and McCaig, 1963] concerned the plerocercoid stage, the majority of this work being carried out by Dr. Hopkins. Two sections ("Infectivity of plerocercoids" and "Weight change during maturation") were carried out partly by McCaig and are reported in this thesis. In a third paper [McCaig and Hopkins, 1965] relating to the cultivation of the plerocercoid, many of the results were obtained by McCaig and are reported in this thesis.

Section I

The growth and development of the stages of the life cycle of *Schistocephalus solidus* under *in vivo* conditions in the laboratory.

INTRODUCTION

The plerocercoid and adult Schistocephalus solidus were described in the eighteenth century but it was Creplin (1824) who realised they were different stages in the life-cycle of one tapeworm and not two different worms as originally thought. Schauinsland (1886) developed the eggs of Schistocephalus and obtained coracidia but it was not until 1919, that the complete life-cycle was discovered when Nybelin showed that certain species of Cyclops were intermediate hosts.

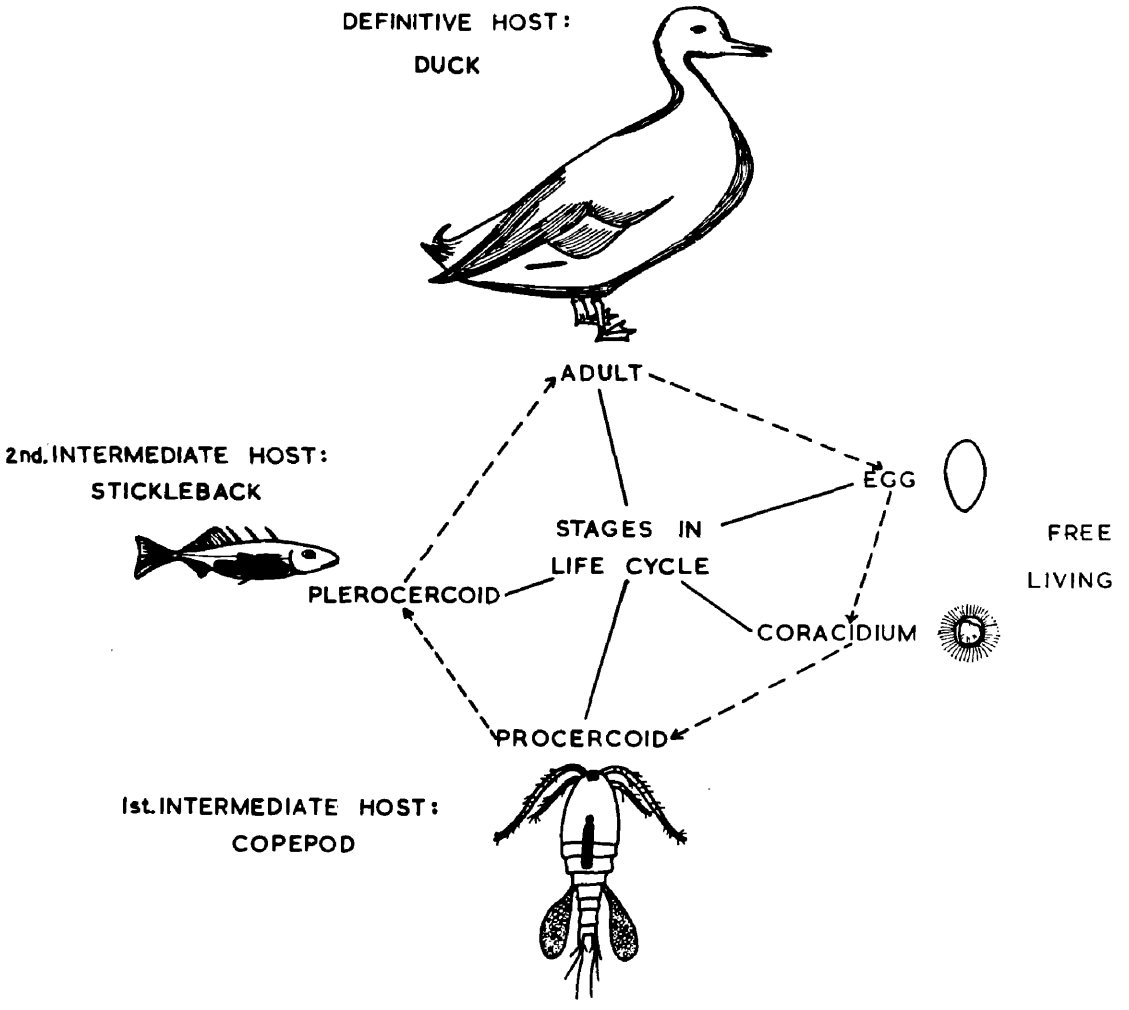
The life-cycle thus involves several phases of which two are free-living and three require to take place within hosts (Figure 1). The number of hosts required is one of the principle difficulties in establishing the life-cycle in the laboratory.

Although the outline of the life-cycle was known at the commencement of this work, little detail about the conditions which were limiting at each stage of the life history was known. The principle aim of the work recorded in this section was to investigate several stages in the life history about which more information was required if a large scale rearing of Schistocephalus solidus was to be attempted in the laboratory. As the object was primarily to establish methods for maintaining S. solidus in laboratory hosts, the time allocated to individual stages had to be restricted as it soon became/

Figure 1

The life cycle of *Schistocephalus solidus*

LIFE CYCLE OF SCHISTOCEPHALUS SOLIDUS.



/apparent many of the problems briefly investigated e.g. hatching stimulus of eggs, occurrence of specificity at certain stages in the life-cycle and retention response in the intestine, could have become full-time investigations.

The egg requires to be passed out in the faeces of the definitive host into water and there development occurs, the rate of development being directly affected by the temperature prevailing at that time. Development times are given by Hopkins and Smyth (1951) and Dubinina (1957) and it was to confirm these and extend the temperature scale that the investigation of the physical conditions affecting egg development was undertaken. The three physical conditions examined were temperature, light and pH but most of the work was concentrated on the effect of temperature. Various stages in the development of the eggs, which could be recognised by the examination of live eggs under a microscope, were determined in the course of the work and the time required to reach each stage used as a criterion of development. It was intended that hatching would be another criterion but it was often erratic even in control cultures and hence was not extensively used.

The actual stimulus causing hatching was reported to be light (Smyth, 1959) but this could not be unconditionally confirmed by the results obtained in the present work. The effect of various wavelengths of light was examined./

/ When fully developed, an egg hatches in water liberating a coracidium. The coracidium is a free living larva, moving through the water by means of its cilia. The coracidia were removed from egg cultures and used immediately to infect copepods.

The procercoid larval stage develops in the haemocoele of a copepod from the coracidium, the ciliated coat of which disappears at the time of infection of the copepod. This coat may be shed or reabsorbed by the tapeworm. The development of the procercoid under laboratory conditions was described by Clarke (1954) and its rate of development determined. Copepods were infected in the laboratory to confirm Clarke's results and establish methods for the infection of copepods on a larger scale.

When an infected copepod is eaten by a 3-spined stickleback (Gasterosteus aculeatus), the procercoid is liberated in the gut of the fish and burrows through into the body cavity of the fish; it develops there as the plerocercoid. After six months in a 3-spined stickleback, plerocercoids are infective to a warm-blooded host (Dubinina, 1957) but from the sizes of plerocercoids obtained during the survey of two lochs (SectionII), it became apparent that the worms could remain in sticklebacks for longer than the necessary six months. The effect of prolonging the plerocercoid phase was investigated. Some figures for the rate of growth of the/

/plerocercoid *in vivo* were given by Clarke (1954) but these only extended over the initial seven weeks in a fish and hence growth over longer periods was investigated. This growth rate became increasingly important with the commencement of the *in vitro* culture of the plerocercoid.

Potential definitive hosts must be warm-blooded or poikilothermic animals capable of surviving above the threshold temperature of 34°C which Smyth (1952) found necessary to stimulate maturation *in vitro*. *Digramma interrupta*, whose life history is similar to *Schistocephalus solidus*, has been induced to mature in the intermediate host, *Carassius auratus gibelio* by acclimatising the fish to 35-37°C (Dubinina, 1960) but for all practical purposes, the temperature bar limits the definitive host of *Schistocephalus* to a mammal or bird.

Various warm-blooded animals were fed plerocercoids to determine if they could serve as hosts, although only one of the animals used would, in nature, have reasonable access to infected sticklebacks. Another problem studied is the stimulus in the environment which triggers off the response in *Schistocephalus* to combat peristaltic pressure and how retention in the gut is achieved (unlike most cestodes, *Schistocephalus* has no hooks or suckers). Development to maturity occurs within approximately 36 hours but the time involved is still so long that an active response of the worm must occur. Hence, host specificity may arise because of a/

/failure to stimulate a retention response by the worm or the intestines of some animals are spatially unsuitable.

The adult stage was thus studied to determine what animals are reliable laboratory hosts and the effect the age of the hosts has on the establishment of the worms. The position of the worms in the intestine and the length of life of the adult in the various hosts was studied. Evidence of an immune response in the hosts was looked for.

Materials

Source

Three-spined sticklebacks (*Gasterosteus aculeatus*) infected with *Schistocephalus solidus* were obtained from two lochs, one a reservoir attached to Lennox Castle Hospital and the other a boating pond within the west end of Glasgow (see Section II). Uninfected three-spined sticklebacks were obtained from a third loch, at Craigton. The plerocercoid larvae were obtained from the fish by making a median ventral incision into the body cavity of the fish. The larvae were removed carefully, with forceps, and placed in Hanks's Balanced Salt Solution (BSS) without bicarbonate at a pH≈7. The larvae were fed, as soon as possible after removal from the fish, to a potential definitive host.

Potential definitive hosts were obtained commercially and maintained in the animal house until required. The animals used were:-

(i) *Anas boschas* - Aylesbury breed (Domestic duck)

(ii) *Columba livia* (Pigeon)

(iii) *Gallus domesticus* (Chicken)

(iv) *Rattus norvegicus* - albino Wistar strain (Rat)

(v) *Mesocricetus auratus* (Hamster)

Various species of *Cyclops* were obtained from Loch Lomond, off Balmaha pier, on the east side and off Rossdhu Peninsula/

/on the west side. Most of the plankton was collected by the use of a zooplankton net towed from a rowing boat but occasional hauls were taken in the region of the surface sand at Balmaha. Large numbers of Cyclops could be collected in a short space of time with both methods during the summer months.

Maintenance of Hosts

(i) Copepod Host - First Intermediate Host

The copepods were maintained initially in glass aquaria, 11" x 9" x 9" with moderate aeration but no running water. The temperature was 18°-20°C. Under these conditions, maximum survival time was six weeks and young copepods which did appear in the tanks survived for a very short time. A number of snails, Lymnaea peregra, were put in the tanks to remove algae which tended to grow on the sides of the tanks.

In later work, tanks 11" x 9" x 18" high were used after the method described by Mueller (1959). The tanks were kept on a bench in the laboratory, the temperature varying from 15° to 19°C. The tanks were exposed to normal daylight but were screened from direct sunlight. Fine aeration was maintained in each tank.

Phytoplankton collected from Loch Lomond was originally added as a source of food but various workers including Ewers (1930), Pine (1934), Clarke (1954) and Mueller (1959) maintained protozoa in the laboratory and added these as/

/food to their copepod cultures. After Mueller's method, newly cut hay was placed in polythene pails, water added and three to four days later a few hundred ml. of an existing protozoa culture added. Some of the pails were aerated. Initially, the protozoa cultures were not used until large ciliates were present but latterly the cultures were used earlier when only small forms were present. Cyclops agilis, a herbivorous copepod (Fryer, 1957) was one of the species maintained in the laboratory but it is not known what food it took.

(ii) Fish Host - Second Intermediate Host

The sticklebacks were maintained in both glass and polythene tanks with fresh running water and constant aeration. The tanks held water to a depth of ten to twelve inches. Sticklebacks will not eat Bemax or other dried foods and hence white worm (Enchytraeus spp.) and liver (Mouse, duck, etc.) were used. Uneaten food was removed to prevent fouling of the water.

Mortalities among the fish were occasionally high during the first day in the tanks. The cause of death was unknown but handling of the fish or change in water may have been contributory. The temperature in the tanks was not controlled, varying from 5° to 8°C during the winter months and up to 14°C in the summer.

Sticklebacks which had been exposed to infected copepods/

/were kept in tanks fitted with thermostatically controlled heating units.

(iii) Avian or Mammalian Host - Definitive Host

The definitive hosts were maintained in the animal house. The ducks and chickens were fed on chick mash (moistened in the case of the ducks), the pigeons on a mixture of grains. The rats and hamsters were fed diet 41 which was supplemented with vegetables and seeds for the hamsters. The animals had food ad. lib. except for the few hours before they were fed plerocercoids during which period they were starved.

/Stage in Life Cycle 1. Egg

A. Observations

The eggs are ellipsoidal, amber-coloured under aerobic conditions and show great variation in size.

Kiessling (1882)	49μ x 34μ
Fuhrmann (1896)	70μ x 29μ
Lühe (1899)	56-38μ x 38-22μ
Linton (1927)	71μ x 43μ
Callot and Desportes (1934)	70μ x 40μ
Thomas (1947)	87μ x 45μ
Hopkins and Smyth (1951)	77-58μ x 46-35μ
McCaig (1961, 1964)	75-55μ x 50-37μ

This variation in size may be due to inaccurate methods of measurement as the material obtained by Hopkins and Smyth and that obtained in the present work show an almost identical variation in the size of the eggs. However, several species of Schistocephalus have now been described, e.g. S. thomasi (Garoian, 1960), S. pungitius and S. nemachili (Dubinina, 1959) and the variation in egg size could be attributed to the different species. The eggs were obtained from worms matured in various hosts but their size does not appear to be influenced by the host in which the tapeworm matured.

There is no embryo in the egg when it is expelled from/

/the tapeworm but there is an operculum present at the more pointed end. The ovum itself is obscured by the dense mass of yolk present. Abnormally shaped eggs which were incapable of development were sometimes produced.

B. Experimental

Infection of the definitive host

This is dealt with in detail later in this section (see under "Adult").

The collection of eggs from the gut of a host

Procedure: With Spirometra mansonoides in cats, Mueller (1959) developed a technique for the collection of the cat faeces and the removal of the faecal material to leave the eggs.

Mueller's technique was slightly modified and used with Schistocephalus in ducks. The 24-hour output of faeces was collected from each host and suspended in water. The suspension was passed through a series of sieves (24, 60, 100, 150 and 200 meshes/inch), the resultant filtrate containing little faecal material. Cleaning of the eggs was completed by washing with water in a crystallising dish, the eggs being allowed to settle to the bottom before decantation of the excess fluid. This was repeated till the washing water remained clear.

Results: Very few eggs of Schistocephalus were obtained by this method, about 40-60 eggs per batch of faeces. Much loss of eggs must have occurred during the cleaning process as many hundreds of eggs were initially in the faeces (determined by rough egg counts on the newly suspended material).

/The collection of eggs directly from the worm

Procedure: Two methods were described by Smyth (1950, 1954) but a new method, developed partly from those of Smyth, has been worked out. The gut is removed from an infected animal, a median incision made and the adult worms so exposed, removed. The worms were placed singly in universal containers with 10-15 ml. of medium. Initially, this medium consisted of a balanced salt solution (BSS) with phenol red indicator; yeast extract and horse serum at final concentrations of 0.5% and 25% respectively were included in later work. The medium is prepared at approximately pH 7 but, as much acid material appears to be produced by the worm, changes in pH occur rapidly. The pH is controlled by the addition of $^{N}/_{5}$NaOH as required. This is necessary every hour for the first 4-5 hours in culture in BSS and then at longer intervals but the addition of serum provides partial buffering.

The containers were submitted to intermittent shaking in a water bath maintained at 40°C, this being approximately the body temperature of the host from which the worms have just been removed. After a suitable period (see Results), the worms were removed from the containers which were then filled up with water and left standing on the bench until the eggs had settled to the bottom (approx, 15 mins.). The water was then decanted or removed with a pipette attached to/

/a suction pump and the containers filled up with fresh water. This was repeated till all traces of the phenol red indicator had gone showing that the eggs were now in water. If serum had been added to the medium, the washing process took longer as additional washing had to be done to ensure that all the serum had been removed.

Various combinations of length of time in the host and time in culture were tried to determine the best method of collecting the eggs.

a) Time in host - Ducks were killed 2,4 and 6 days after feeding and the worms recovered. The worms were kept in containers for 24 hours, the medium used containing both serum and yeast extract. In another experiment, the worms were placed in fresh medium every 24 hours, the effect of this on the length of life of the worms and their egg production being recorded.

(b) Time in culture - Worms were kept in the containers for 24 hours, the medium being changed after $1\frac{1}{2}$, $4\frac{1}{2}$, and 7 hours then left overnight. Serum was included in the medium so that adjustment of the pH would not be required. After 24 hours, the medium was allowed to cool to room temperature with the result that the worms relaxed quite markedly. The worms were then dissected to obtain the eggs remaining in the uterus. I_n some experiments, the medium /

/was not changed until the worms had been in it for 7 hours, occasional adjustment of the pH being required.

Results; Worms in ducks for 2 and 4 days before being placed in culture produce mainly normal eggs for the first 24 hour period in culture but after that, the number of abnormal eggs increases with a decrease in percentage of cultures, with hatching eggs (Table 1). Abnormal eggs were slightly irregular in shape and their contents were not evenly distributed, as seen by transmitted light. After 6 days in a duck, the worm subsequently in culture produced less eggs than worms which had been in a duck for 2 and 4 days. Further, a greater percentage of the eggs were abnormal.

In the cultures recorded as having poor hatches, coracidia were seen occasionally over a period of two to three weeks whereas in the other cultures, if hatching occurred, few coracidia were seen some days but on others, at least 20-30 would be seen.

Eggs extruded in culture during the initial 7 hours develop readily and usually hatch well. However, eggs produced overnight have an increased percentage of abnormalities and hatching is less predictable. Occasionally, this it not the case and the majority of the eggs develop and hatch normally. Eggs dissected out of the worm after 24 hours usually develop readily but a number of the eggs are abnormal./

Table 1

Condition of eggs produced by worms after varying periods in a host and culture

Time in host (days)	Number of worms	Time in culture (days)	Total adult life (days)	Number of egg cultures	Number of cultures with eggs hatching	Comments
2	8	1	3	6	6 (100%)	Most eggs normal
		2	4	11	5 (45%)	>50% eggs abnormal
		3	5	5	1 (20%)	Most eggs abnormal
		4	6	2	0 -	Very few eggs
4	12	1	5	5	*5 (100%)	Most eggs normal
		2	6	4	*4 (100%)	>50% eggs abnormal
		3	7	4	0 -	Few eggs normal
6	9	1	7	6	2 (33%)	<50% eggs normal
		2	8	2	1 (50%)	Most eggs abnormal

* Poor hatching.

/The actual number of eggs produced by 14 worms in culture after 48 hours in a duck are shown in Table 2. The total number of eggs produced by each worm in 24 hours is shown in the right-hand column. Of the total eggs produced, 40% were produced in the first 7 hours.

(iv) The incubation and hatching of eggs

Procedure (i): Mueller (1959) developed a technique for the culturing of the eggs of Spirometra mansonoides in which clean eggs were placed in a flask and subjected to continual aeration and shaking.

A similar method was used with the eggs of Schistocephalus. The eggs were placed in about 150 ml. of tapwater in a stoppered 250 ml. flask. An air lead was passed through the stopper and a small airstone submerged completely in the water. Mechanical shaking was maintained twenty-four hours a day. When the effect of temperature was being investigated, the flasks were placed directly in an oven and shaken several times daily by hand. Water was added as required. To facilitate microscopic examination of the eggs, the cultures were poured into petri dishes after 10 days in the flasks.

Results: Several thousand eggs were kept in a single flask and hence, when hatching occurred, a fairly dense concentration of coracidia was obtained./

Table 2

Number of eggs produced by worm when placed in culture

Weight of Adult worms (mg)	Time in duck (days)	Eggs per period in culture					
		1 1/2 hrs	1 1/2-4 1/2 hrs	4 1/2-7 hrs	Overnight	Dissected	Total
55		700	420	510	540	980	3,150
58		1,260	0	360	390	900	2,910
73		660	260	610	730	240	2,500
85		570	960	470	1,410	1,440	4,850
97		690	220	560	480	1,370	3,320
102		360	360	380	600	670	2,370
105		510	350	610	600	2,270	4,340
110	2	900	1,680	990	3,870	1,100	8,540
144		1,260	170	990	1,250	1,680	5,350
159		600	430	430	1,920	4,350	7,730
181		2,400	270	720	1,560	3,080	8,030
214		2,640	360	180	1,350	1,060	5,590
275		4,890	360	450	2,760	2,900	11,360
390		900	90	60	5,010	2,360	8,420

40% of total eggs collected in first 7 hours.

/Comments: Since most of the egg cultures were to be examined regularly to follow the stages in development of the eggs, the flask technique was discarded. For large scale, routine maintenance of the life-cycle, this method would be ideal.

Procedure (ii): A technique using small containers was developed and used extensively. Approximately 500 eggs were placed in a Syracuse dish, 4 cms. in diameter, with a capacity of 3 cc. Originally, the water was changed daily but later it was found that a change of water twice a week was adequate if the eggs had been thoroughly clean initially. Water was always added to the cultures using a jet of water from a wash bottle to stir up the eggs.

Small petri dishes, $5\frac{1}{2}$ cm. in diameter, with a capacity of 10 cc. were occasionally used for culturing the eggs. One to two thousand eggs were placed in each dish.

Results: Hatches of up to 70% were recorded in cultures but the average was in the region of 50%. The percentage of eggs developing however was always noticeably greater than the hatch (Table 3). If the eggs had not been thoroughly clean, contaimination occasionally occurred, resulting in the eggs being bound together on to the glass. Hatching could, however, still occur under these conditions but many of the caracidia were trapped.

Table 3

Viability of eggs

Weight of Adult worms (mg)	Percentage of eggs viable	Percentage of eggs hatching	Mean	
			Viability %	Hatching %
< 60 mg	69	20	68%	22%
	68	4		
	56	17		
	80	45		
> 60 mg	70	63	74%	56%
	71	48		
	73	40		
	82	71		

Development of the Egg

Four stages in the development of the egg can be clearly recognised.

(a) When extruded from the adult worm, the egg is not embryonated. Little structure is visible, the egg being filled with yolk material. An operculum is present at the slightly more pointed end of the egg (Figure 2a). The operculum is more easily seen 2 to 3 days after extrusion from the worm.

(b) In the middle of the egg, a clearer area, visible by transmitted light, appears. This is the first indication of the development of the embryo. Most of the volume of the egg at this stage is still occupied by yolk material (Figure 2b).

(c) The clearer area has enlarged, now occupying at least half the volume of the egg. There has been a corresponding decrease in the amount of yolk material (Figure 2c).

(d) The embryo now appears fully formed, the six larval hooks being present and the various larval coats. The yolk material is represented by droplets surrounding the embryo which now occupies $^{2}/_{3}$ the volume of the egg. Contractions of the embryo occur frequently and the commencement of beating of the cilia causes movement of the yolk droplets. The hooks are found at the opposite end of the embryo from/

Figure 2

Stages in the development of the egg of S. solidus

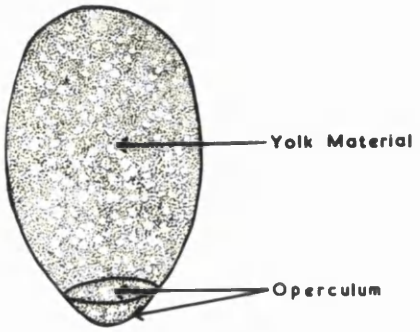
a. The egg immediately after extrusion from the tapeworm.

b. The developing embryo appears as a clearer area (as seen by transmitted light) in the middle of the egg.

c. The developing embryo occupies approximately half the volume of the egg. There has been a corresponding decrease in the quantity of yolk material.

d. The embryo is fully developed with the six larval hooks present. The cilia cannot be seen at present but their beating results in movement of the yolk droplets.

STAGE a.



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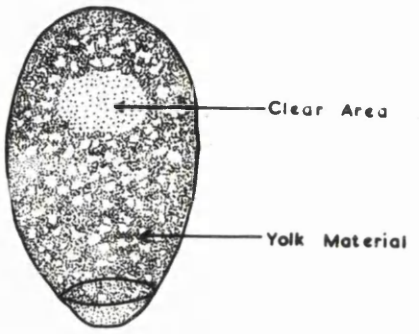

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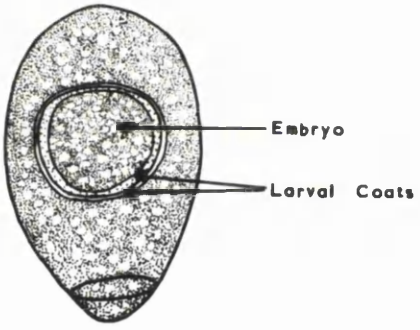
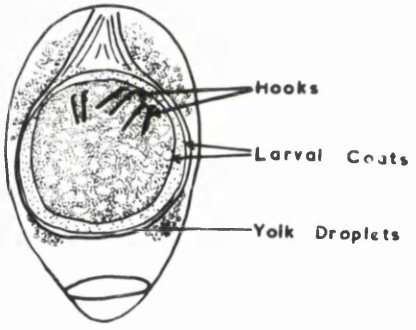


STAGE d.



/the operculum of the egg (Figure 2d). This embryo is known as the coracidium larval stage when it emerges from the egg. Hatching usually commences several days after the embryos appear fully formed but is often delayed for several weeks.

Hatching of the eggs

Procedure: The egg cultures were removed from the incubator, placed in daylight and some fresh water added. The move from the dark incubator necessitated approximately a 6°C drop in temperature. In later work, when the eggs appeared fully developed, stage (d), the cultures were placed in a refrigerator at 5°C. When coracidia were required, the cultures were removed from the refrigerator and placed in an incubator at 21°C.

Results: Good hatches were recorded from many of the cultures removed from the incubators into daylight. The change in temperature and the fresh water may also be contributory in causing hatching. More controlled hatching was obtained if the egg cultures had been placed in the refrigerator, then placed at a higher temperature. The eggs in a culture rarely hatched all together, hatching usually being spread over a period of several weeks.

Comments: Despite the various stimuli applied, many eggs/

/would not hatch although apparently fully developed embryos were present.

Physical Conditions affecting Egg Development

(a) Temperature

Procedure: The eggs from several worms were pooled then placed in covered Syracuse dishes, allowing approximately 500 eggs per dish. The dishes were placed in incubators at the required temperatures and left there, being removed only for daily examination of the eggs. The eggs were thus mainly in the dark. Water added to the cultures was placed in the incubator for half an hour before being used.

Results: The four temperatures used were 26°, 21°, 15° and 5°C. As all the eggs in a culture did not develop at an identical rate, times to reach a certain stage represent the time taken by at least 10% of the eggs in a culture. The time taken to reach each stage is shown in Table 4. At 21°C and 26°C. there is little difference in the rate of development of the eggs but at 26°C hatching precedes that at 21°C by several days. The rate of development at 15°C was markedly slower than at the higher temperatures (8-11 days to reach stage (c) but only 4-6 days at 21°C) with hatching occurring from the nineteenth day onwards as opposed to 10-14 days onwards at the higher temperature. At 5°C, the rate of development was very slow, 4 months for the clear area to appear (Figure 2c), but of 10 cultures left for 13-14 months/

Table 4

Effect of temperature on the development of the eggs of Schistocephalus

Number of Cultures	Temperature	Stages in development (days)			
		Stage (b)	Stage (c)	Stage (d)	Hatching
34	26°C	3-4	4-6	6-11	10-12
118	21°C	4-5	4-6	7-12	14-23
29	15°C	5-7	8-11	14-18	19-21
25	5°C	-	15-16 weeks	-	Completed in 56 weeks

Stage (b) clear area showing in middle of egg

(c) clear area occupies half the volume of the egg

(d) hooks are present in the fully developed embryo

/at 5°C., five contained eggs which may have hatched and five contained dead eggs (the contents of the eggs were still in situ). The other fifteen cultures originally put at 5°C were removed at intervals and kept at 21°C or 26°C to test the development times and viability of the eggs (Table 5). The development time at 21°C and 26°C is shortened by 1-2 days when the eggs have been at 5°C for 10-16 weeks. No accurate egg counts of the number which developed and hatched were made but it was estimated that 70-80% of the eggs developed after sixteen weeks at 5°C but the hatch was much less. The eggs that were left at 5°C had a higher hatch than those removed but as they came from different worms, it is possible that this accounts for the difference.

(b) Light

Procedure: The eggs from several worms were pooled and placed in Syracuse dishes. The external surfaces of the dishes had previously been painted black. The bases were not painted so that transmitted light could be used to examine the eggs at the end of the experiment. The lids of the dishes were either painted black or replaced by a series of light filters. Other dishes were not painted but placed in a box to exclude the light. All the dishes were placed at 21°C in an incubator and not examined for three weeks although the incubator door was opened to remove/

Table 5

Development of eggs after varying periods at 5°C

Time at 5°C (weeks)	Stage in development	Temperature	Time to be fully developed at higher temperature	Time at which hatching commenced
9-10	No external sign	21°C	9 days	12 days
16-17	Stage (c)	21°C	7 days	11 days
		26°C	6 days	7 days
56-60	Eggs either hatched or dead	-	-	-

/other egg cultures. After three weeks, the cultures were examined for degree of development and hatching of the eggs. Control cultures in untreated dishes were run concurrently, the water in these dishes not being changed during the three week period.

Results: Eggs developed and hatched when exposed only to light passing through yellow, blue and grey filters, good hatches i.e. more than 50% of the eggs hatching, being recorded (Table 6). Eggs kept in darkness for three weeks did develop but low hatches, approximately 10%, were obtained and two cultures did not develop, even when exposed to occasional daylight. One dish of eggs under a green filter did not develop until they were exposed to daylight but in the repeat of the experiment, no delay occurred. Later experiments with a green filter (Wheeldon, 1965) showed it had no effect on the development and hatching of the eggs. Only two cultures were treated with a red filter and in both no hatching occurred and in one, the eggs did not develop even after exposure to normal daylight. This filter had the most effect on the eggs. In the control cultures, hatching did not exceed 50-60% and hence it would appear that the yellow, blue and grey coloured filters had no effect on the eggs but complete darkness and red filters did have some effect especially on hatching./

Table 6

Effect of light on the development of the eggs of Schistocephalus

Colour of filter	Number of cultures	Number of cultures with eggs developed	Number of cultures with eggs hatching	Comments
Black	10	8	6	Two did not develop in dark, only when exposed to light. 10% hatch
Clear	3	3	3	Controls 50-60% hatch
Yellow	3	3	3	50-60% hatch
Red	2	1	0	-
Green	2	1	1	One did not develop initially, only when exposed to daylight. 40-50% hatch
Blue	8	8	8	50-60% hatch
Grey	1	1	1	50-60% hatch

/(c) pH.

Procedure: The eggs from several worms were pooled and placed in covered Syracuse dishes. Tap water was adjusted to pH 2, 4, 6, 10 and 12 with N/5 NaOH and N/5 H Cl and stored in firmly stoppered bottles. The eggs were washed twice with water at the pH at which they were to be cultured and then the dishes were filled up with the water and put at 21°C. Twice a week, the water was removed from the dishes, the pH recorded and fresh water at the correct pH added.

Results: As the pH was controlled by the addition of acid or alkali, water which was initially very alkaline (pH 10.0 and 12.0) dropped in pH during the course of the experiment (due to the formation of carbonates) but the acidified water remained at the initial pH. Eggs placed in water of pH 10 and 12 developed and hatched but it is not known whether the eggs were at a high pH for several days or only for a few hours. Eggs kept at pH 4 and 6 developed and hatched normally but those at pH 2 never developed (Table 7).

3. Discussion

It is much easier to mature Schistocephalus in a host than in culture but to obtain a large number of eggs, the worm must be maintained for several days. The difficulty that arises is the extraction of the eggs from the faeces of/

Table 7

Effect of pH on the development of the eggs of Schistocephalus

Initial pH	Final pH	Number of cultures	Hatching of eggs	Comments
2.0	<2.8	5	-	Eggs did not develop
4.0	4.0	2	good	-
6.0	5.5	2	good	-
10.0	7.0-8.0	2	good	Inadequate control of pH invalidates these results
12.0	8.2-9.0	5	good	

/the host. This was overcome by Mueller (1959) with his cats by placing them on a low bulk, high protein diet which was almost completely absorbed.

Several methods for obtaining the eggs of Schistocephalus have been described. Callot and Desportes (1934) took scrapings of the rectal mucosa after killing the host and Clarke (1954) washed out the gut contents, cleaning the eggs by a process of sieving and prolonged washing. Both these methods share the disadvantage that large masses of material are obtained and the complete cleaning of the eggs is time-consuming.

Smyth (1950) matured worms in a definitive host and on removal from the host relaxed them in tapwater before dissecting them to obtain eggs. In the present work, this method was improved by maintaining mature worms recovered from laboratory hosts, at 40°C in a medium for a few hours, in which time they produced large numbers of eggs (Tables 1 and 2). The worms were then dissected and more eggs obtained. With prolonged maintenance in a medium, many abnormal eggs were extruded by the worms (Table 1) indicating that either the culture conditions are unsatisfactory or that with increase in age, the worms' egg production becomes disturbed.

Worms had a shorter life span in culture with increase in time in a definitive host (Table 1). The number of eggs produced also decreased and after 6 days in a host, worms produced mostly abnormal eggs in culture. Whether/

/this is a result of ageing (see under "Adult") or deterioration due to culture conditions is not known.

Egg-production over a 24-hour period in culture varies considerably, initially many eggs being produced per hour with the rate of extrusion decreasing after the first hours (Table 2). The total eggs produced by a worm in 24 hours in culture varied from 2,000 to 11,000 (Table 2), the smaller worms tending to produce fewer eggs than the larger ones. Egg production *in vivo* has not been determined in sufficient detail to make comparisons possible.

Eggs commence development immediately after extrusion from the worm, water being a necessity. Vessels used for culturing eggs vary extensively from flasks of 250 ml. capacity as used by Mueller (1959) for *Spirometra* to Syracuse dishes with a capacity of 3 ml. Watch glasses enclosed within petri dishes were used by Hopkins and Smyth (1951) for *Schistocephalus* eggs while small containers is the only information given by Clarke (1954). The choice of containers thus depends on individual requirements.

In the present work, small Syracuse dishes were preferred as they fitted easily on a microscope, large numbers of cultures could be placed in a single incubator and thirdly, they were suitable for investigating the effects of various physical conditions on the eggs.

During the culturing of the eggs, the water was changed, originally daily, but in later work only about twice a/

/week. The water was changed daily to remove contaminating material adhering to the eggs but thorough washing of the eggs eliminated the need for this. Some eggs (those used in light experiment) never had the water changed for 3 weeks, no adverse effects being noted. Contamination had been controlled by the use of alcoholic iodine (Mueller, 1959), addition of Protozoa (Clarke, 1954) and antibiotics (Smyth, 1950). However, such additives were not used in the present work.

In most cases eggs develop if maintained in water at a suitable temperature (Table 4), but although eggs look fully developed, they may not hatch. Some egg cultures left undisturbed (those used in light experiment) hatched while others had to be stimulated. Stimuli which have been used include light (Clarke, 1954; Smyth, 1959 and Mueller, 1959) although Mueller also used temperature shock and the addition of fresh water. Good hatches were often recorded by these workers but during the present work, although good hatches did occur, some egg cultures would not hatch. In other cultures hatching continued for many weeks until the majority of the eggs had hatched.

Under natural conditions in a loch, sudden changes in conditions will not occur and hence, although light and temperature shock cause hatching in the laboratory, these cannot be the normal stimuli.

/ The oxygen tension in the water may be important, explaining the effect of adding fresh water to the cultures or certain light wavelengths may be sufficient to activate the enzyme which Smyth (1959) states attacks the cement on the operculum. The exact nature of the stimuli and the mechanism of hatching are not known.

Of the three physical conditions investigated, temperature was the most extensively studied. To determine the effect of altering the physical conditions, sound criteria are required. Those used were hatching and rate of evelopment. However, since hatching was sometimes variable, the rate of development proved the more useful.

Eggs could develop and hatch at both 5°C and 26°C but the death of some of the eggs at 5°C suggests that it is near the lower lethal temperature for the eggs. Under natural conditions, the surface temperature of a loch falls to 0°C (Table 23) but the temperature on the bottom may not remain at this level for long periods, the decomposition of material on the substrate raising the temperature locally. Hence, although some of the eggs will be killed, a proportion will survive. The upper lethal temperature was not determined, but it is certainly above 26°C.

Rapid development of the eggs occurs at 26°C but hatching was often irregular and hence, when other conditions were being studied, the eggs were maintained at 21°C. At this/

/temperature, experiments took a few days longer (Table 4) but more consistent results were obtained. Variation in the results from experiments performed at different times may be due to different worms producing the eggs. In an experiment, the eggs are obtained from several worms, pooled, then placed in the culture dishes but there is no way of standardizing the eggs collected at different times.

Development of eggs at 5°C is prolonged (Table 4) and takes many months to be completed. The rate of development during the initial 10 weeks is apparently very slow; eggs after 10 weeks at 5°C still require approximately 12 days at 21°C before hatching commences (Table 5) which is at the most 12 days earlier than if the eggs had been wholly at 21°C. However, after 16-17 weeks at 5°C, development has progressed to such an extent that the eggs complete their development and commence hatching several days earlier. The results of the experiments on rate of development at various temperatures compare favourably with the results obtained by Hopkins and Smyth (1951) and Dubinina (1957) although slightly different temperature ranges were used.

The effect of light on the development and hatching of eggs was ambiguous and did not confirm the statements of Dubinina (1957) and Smyth (1959) that light is essential for hatching. The red filter used in the experiments produced the greater effect, inhibiting development and hatching.

/Complete darkness partially inhibited hatching but in most cultures development occurred. Through water, certain wavelengths of light have poor powers of penetration viz. red and violet while others viz. blue, yellow, orange and green have greater powers of penetration. In daylight all these light wavelengths are present but when the eggs are submitted to red alone, development is disrupted (Table 6). Each of the other colours on their own, have no effect on the development and hatching of the eggs. The eggs used in this experiment had a 50-60% hatch in the controls, this figure being matched by eggs under the various filters. However, eggs kept in total darkness only had approximately a 10% hatch although the majority of the eggs were fully developed. Light would thus appear to be necessary for good hatches but a small percentage of the eggs can hatch in the total absence of light.

The third physical condition investigated was the pH of the water in which the eggs were developing. Under very acid conditions, pH 2.0, no development occurred but in nature a loch would not be so acid. Acid and alkaline conditions will not be important in culture as the pH does not fall below 5.6 but the length of time the eggs are above 39°C will be important. In vivo, eggs will be at 39°C for only a few hours but in vitro, the eggs will have to survive up to 24 hours at 39°C. In cultures, also, there is a lack of oxygen since oxygen disturbs egg formation and production (Smyth, 1959). The effect on the eggs of no oxygen is unknown but this and the effect of prolonged high temperature in saline must be/

/investigated before *in vitro* maturation can be used in the extensive rearing of *Schistocephalus*.

D. Summary

1) Eggs were obtained by feeding plerocercoids to definitive hosts, the eggs being collected either from the faeces of the host or directly from the worm. Collection of eggs from the faeces was time-consuming and produced few eggs.

2) Worms produce 2,000 to 11,000 eggs (depending on worm size) in 24 hours in culture after being in a duck for 48 hours. After the initial 24 hours *in vitro*, the number of abnormal eggs produced increases.

3) Eggs obtained from worms in culture require relatively little washing compared to those obtained from the faeces of a host.

4) Thorough washing eliminated the need for antibiotics in the egg cultures.

5) For large scale work, eggs may be kept in flasks but when regular microscopic examination was being carried out, solid Syracuse dishes of 3 ml. capacity proved ideal, with approximately 500 eggs per dish.

6) The criteria used for assessing the condition of the eggs were the time to reach each of four developmental stages and the number of eggs per culture which hatched.

7) The rate of development was dependent on the temperature the eggs were kept at, the rate being fastest at 26°C/

/(10-12 days to hatch); 14-23 days to hatch at 21°C; 19-21 days at 15°C and possibly 13 months to be fully hatched at 5°C. Eggs at 5°C for a period then put at a higher temperature required 15-16 weeks at 5°C before their development time at the higher temperature was noticeably reduced.

8) In darkness, most eggs develop but only approximately 10% hatch. Under yellow, blue, green and grey filters, development is normal with 50-60% eggs hatching. In control cultures, hatches of 50-60% of the eggs were recorded. Under a red filter no eggs hatched and only a few developed.

9) Under very acid conditions (pH 2.0), eggs do not develop but at pH 4.0 and 6.0, development is normal. The effect of very alkaline conditions (pH 10.0 and 12.0) is not certain.

Stage in Life Cycle 2. Coracidium

A. Observations

The sizes of coracidia recorded in the literature vary from 35-40μ (Clarke, 1954), 50μ (Smyth, 1950) to 113-147μ (Thomas, 1947). Coracidia hatched from eggs in this laboratory measured 40-50μ in diameter.

The coracidium is spherical and consists of an inner mass in which the six larval hooks are found and an outer ciliated coat. The cilia are 15-20μ in length (Figure 3). A rapid, flickering movement at two points in the inner mass of the coracidium indicates the position of the flame cells.

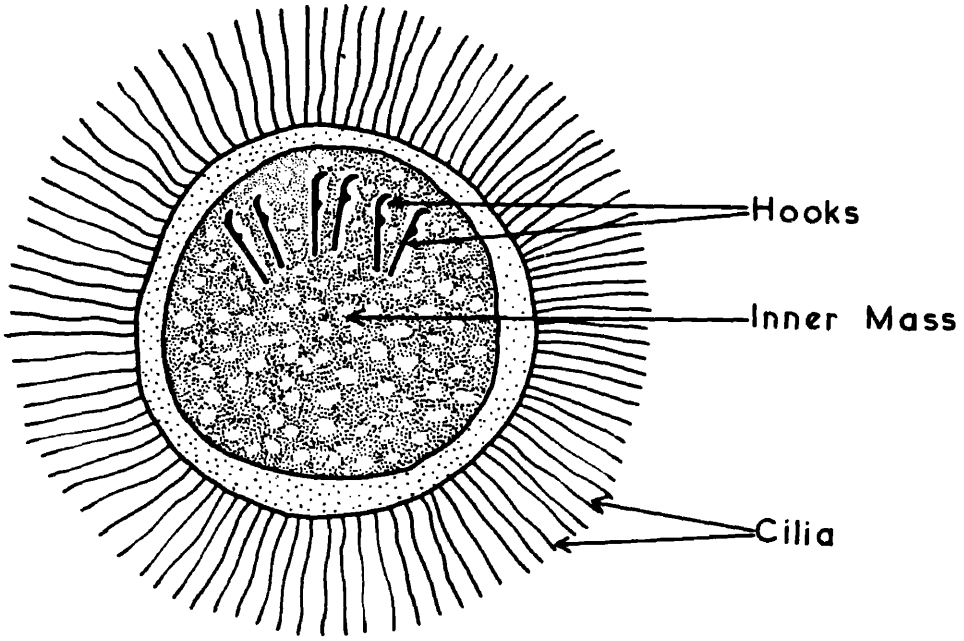
The coracidia revolved slowly immediately after hatching but a few hours later, they appeared as spherical bodies rotating rapidly across the field of the microscope. Thomas (1947) stated that coracidia were capable of moving vigorously for a maximum of 4-5 days but Smyth (1959) believes their life span is as short as 6-12 hours. It was noticed that coracidia in cultures at 26°C survived for a maximum of 24 hours whereas those at 21°C survived for 48 hours. This result is identical to that reported by Dubinina (1957).

Clarke (1954) suggested that the outer region of the coracidium contained mucilaginous material which took in water thus increasing the size of the outer layer. This/

Figure 3

The CORACIDIUM larval stage

CORACIDIUM.



/expansion of the outer layer was frequently observed and was always associated with a very slow rotating movement of the coracidium. It is possible the expansion of the outer layer eventually halts the movement of the cilia.

B. Experimental

Coracidia were obtained from eggs developed and hatched in the laboratory. Water was removed, with a fine pipette, from egg cultures in which hatching was occurring, care being taken to prevent the eggs from being sucked into the pipette as well as the coracidia. This was done daily. The dishes were refilled with tap-water and left for a further 24 hours before the next crop of coracidia were removed.

The coracidia so obtained were fed to copepods to obtain the next larval stage, the procercoid.

Stage in Life Cycle 3. Procercoid

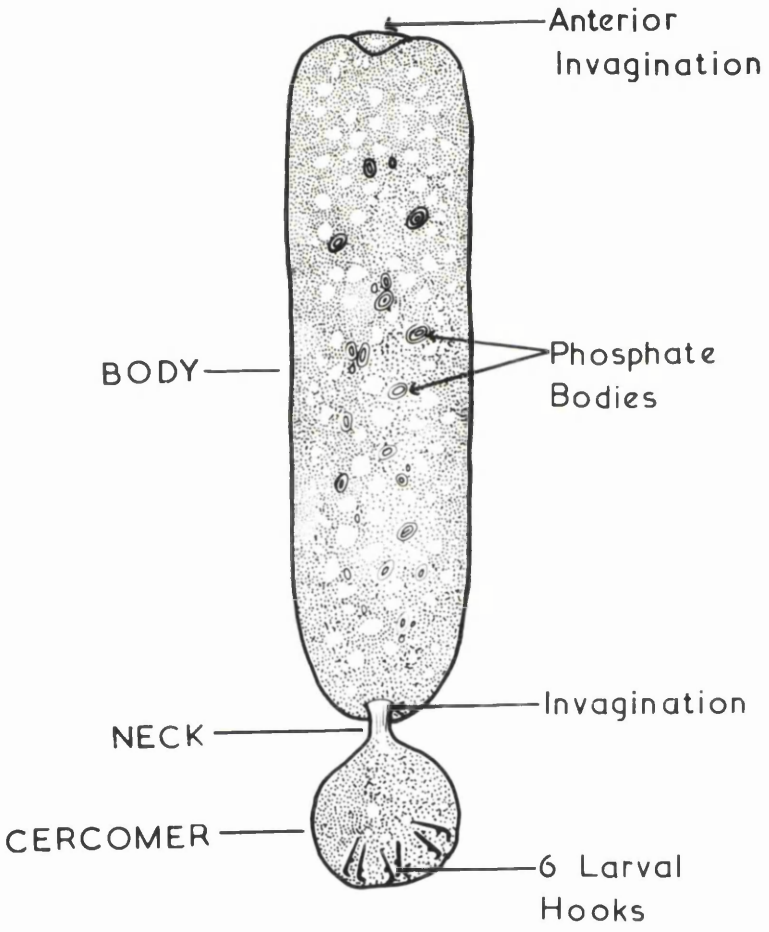
A. Observations

A fully developed procercoid is colourless but contains a number of dense bodies referred to as phosphate bodies; up to 60 have been observed in a procercoid. When fully developed, a procercoid is approximately cylindrical in shape with a length of up to 0.5 mm. and a diameter of 0.1 mm. At one end of the procercoid, there is a constriction, the neck, separating a small part of the body from the main body of the procercoid. In the small body, the cercomer, the six larval hooks are found. I_n a fully developed procercoid, the neck region, is slightly invaginated into the main part of the body (Figure 4). There is a thick cuticle covering the whole procercoid.

Figure 4

The PROCERCOID larval stage

PROCERCOID.


Anterior
Invagination
BODY
Phosphate
Bodies
Invagination
NECK
CERCOMER
6 Larval
Hooks

B. Experimental

Nine species of Cyclops and one species of Diaptomus are recorded as being intermediate hosts of Schistocephalus (Table 8). Of these copepods, four, Eucyclops agilis, Macrocyclops albidus, Acanthocyclops viridis and Cyclops strenuus abyssorum have been successfully infected in the course of the present work. No infections were obtained with Diaptomus gracilis.

(i) The Infection of the Copepods

Procedure: Twelve copepods were put in a 94 x 50 mm. crystallising dish containing water to a depth of approximately 1 cm. (80 ml.). About 50 coracidia from egg cultures were added to the water and left overnight. The following morning, the dish was filled 3/4 full with loch water and 50 ml. of a protozoa culture added. The contents of 4-5 crystallising dishes, after 2-3 days, were placed in a Brefitt, subjected to slight aeration through a fine pipette and maintained thus for 3-4 weeks.

Copepods were examined for infection by placing on a microscope slide in a drop of water. The water was gradually removed with a fine pipette until the copepods were held almost motionless by surface tension. The copepods were then examined on a stage microscope by transmitted light./

Table 8

Species of copepods reported as intermediate hosts of Schistocephalus

Copepod Hosts of Schistocephalus	Source
Macrocyclops albidus, Jurine	McCaig
Acanthocyclops bicuspidatus, Claus	Nybelin (1919); Clarke (1954)
Cyclops furcifer, Claus	Dubinina (1957)
Acanthocyclops gigas, Claus	Dubinina (1957)
Cyclops leuckarti, Claus	Thomas (1947)
(Cyclops serrulatus, Fischer) (Eucyclops agilis, Koch, Sars)	Nybelin (1919); Clarke (1954) McCaig
Cyclops strenuus, Fischer	Dubinina (1957)
Cyclops strenuus abyssorum, Sars	McCaig
Acanthocyclops viridis, Jurine	Callot, Desportes (1934); Clarke (1954); McCaig
Diaptomus gracilis	Dubinina (1957)

Results: This method resulted in at least 50% of the copepods acquiring the infection with, often, every copepod infected. Up to 6 procercoids have been recovered from a copepod but the average for laboratory infections was two. Of the four species of Cyclops used in laboratory infections, two, E. agilis amd A. viridis were more susceptible to the infection (Table 9) with over 80% of the copepods infected.

(ii) Mode of Infection

In the present work, no actual infection of a copepod was observed. However, the majority of the copepods used were adult and it is assumed that they ingested the coracidia which then penetrated through the gut wall.

(iii) Development of the Procercoid

Procedure: The procercoids were examined during their development without damaging the copepods but accurate measurements of their lengths were difficult due to contractions of the procercoid and the gut of the copepod. The size of the procercoids was thus determined by dissecting them from the copepods with two fine mounted needles. A micrometer eyepiece was used to measure the procercoids.

Results: On ingestion by the copepod, the ciliated outer layer of the coracidium disappears but it is not known whether it is shed or re-absorbed by the larva.. The/

Table 9

Rate of infection of copepods in the laboratory

Species of Copepod	Numer of copepods exposed to coracidia	Numer of copepods infected	Rate of infection %
Macrocyclops albidus	21	11	52
Eucyclops agilis	39	35	90
Cyclops strenuus abyssorum	23	15	65
Acanthocyclops viridis	37	30	81
Diaptomus gracilis	20	0	-

/procercoid, as it is now known, passes into the haemocoel of the copepod either in the thoracic or abdominal region but the former is more common with Schistocephalus. The procercoid rapidly loses its spherical shape and becomes slightly elongated.

During the first week of development at 16±2°C, the six hooks move to one end of the procercoid. At the end of the first week, the first calcium phosphate bodies appeared, only a few initially, but the number soon increased. At the same time, a small invagination was formed at the opposite end from the hooks and usually called the anterior end.

By the twelfth day, a slight constriction had developed just anterior to the hooks; this rapidly became more pronounced until only a narrow stalk connected the body of the procercoid and the small body, the cercomer, containing the hooks. Slight invagination of the stalk into the body of the procercoid then occurred.

The procercoid now appears fully formed and may be infective but no procercoids were administered to fish immediately the cercomer was formed.

From the measurements of the length and diameter of the procercoids, their volume was calculated, assuming them to be cylinders. A correction factor was determined for the cercomer and stalk region. In single infections, a maximum/

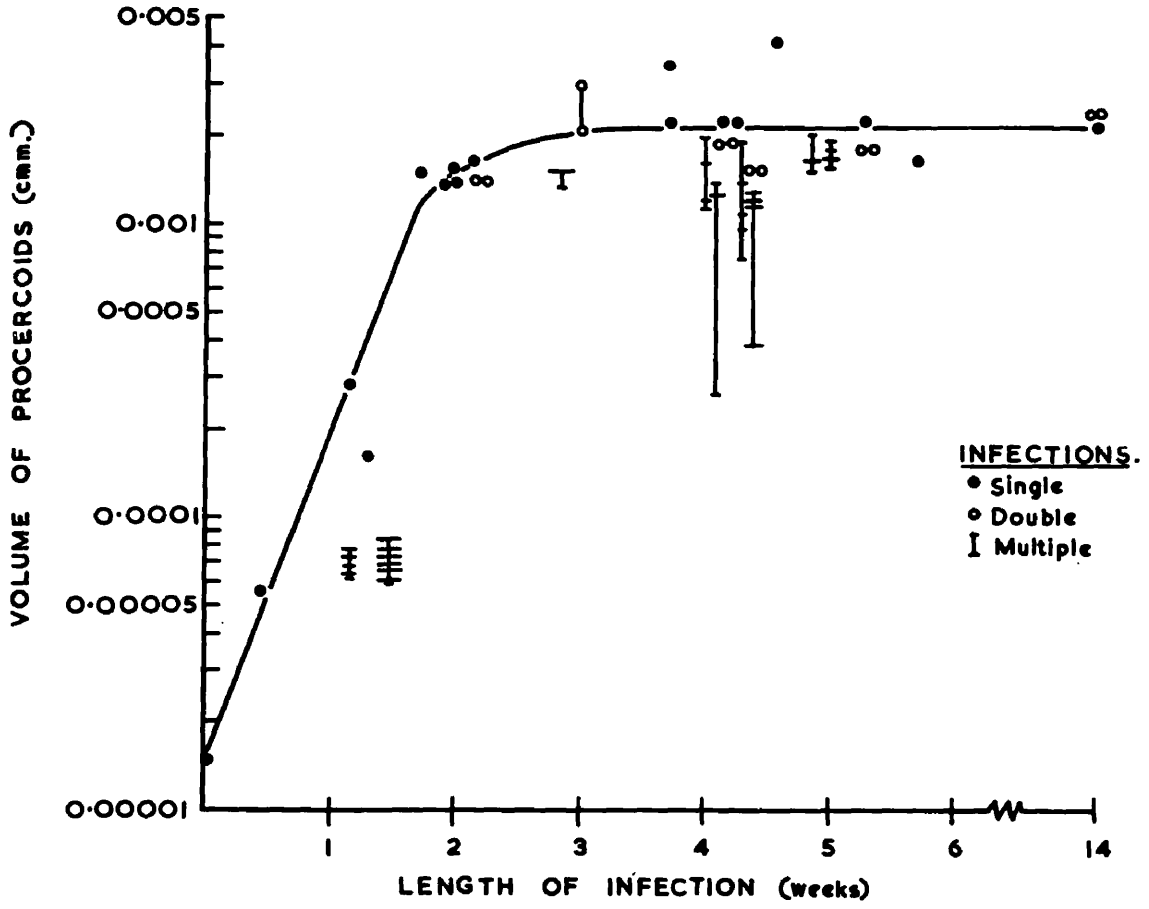
/volume of 0.0025 cmm. is attained in three weeks (Figure 5) at 16±2°C but in multiple infections, the maximum volume was 0.0020 cmm. but it took 5 weeks to attain this size. A procercoid with a volume of 0.0025 cmm. is approximately 0.48 mm. in length. No procercoid exceeded this size although a number of infected copepods were maintained for fourteen weeks in the laboratory.

The initial increase in size is very rapid but when cercomer formation commences at 12-14 days, the rate of growth drops sharply and eventually ceases a week later.

Figure 5

The rate of growth of the procercoid stage *in vivo*.

Procercoids were dissected from copepods which had been exposed to coracidia in the laboratory. The length and diameter of each procercoid was measured and the volume of the procercoid determined, assuming it to be a cylinder. The ordinate represents the volume of the procercoids and the abscissa the length of the infection in weeks.


0·005
0·001
0·0005
0·0001
0·00005
0·00001
VOLUME OF PROCERCOIDS (cmm.)
1
2
3
4
5
6
14
LENGTH OF INFECTION (weeks)
INFECTIONS.
Single
Double
Multiple

C. Discussion

The technique used for the infection of the copepods is essentially for the infection of small numbers and where the time of infection is required reasonably accurately. By the use of larger containers, Mueller (1959) achieved the infection of large numbers of copepods with Spirometra mansonoides. A similar method to his would be practical for the infection of copepods with Schistocephalus for the maintenance of the life-cycle in the laboratory.

The coracidia of Triaenophorus sp. were reported by Miller (1952) to be actively ingested by copepods while Smyth (1962) and Clarke (1954) made similar reports with the coracidia of Schistocephalus. Clarke also suggested that the integument of the copepod immediately after a moult could be penetrated by a coracidium. However, Freeman (1964) has described the actual penetration of the gut wall of a copepod by the coracidia of Proteocephalus parallacticus and it is assumed that penetration is similar for all coracidia.

It was suggested by Hunter (1929) that copepods were attracted to the eggs of Proteocephalus pinguis by the motion of the oncosphere within the egg. The rotating movements of the coracidia of Schistocephalus free in water may attract the copepods. Actual contact with potential prey/

/rather than the use of sight or smell was reported by Fryer (1957) to be the method used by copepods for the detection of prey. The carnivorous or omnivorous copepods, Acanthocyclops viridis, Mesocyclops albidus and Cyclops strenuus abyssorum (Fryer, 1957) may come in contact with the coracidia during swimming and ingest them but with the herbivorous copepod, Eucyclops agilis (Fryer, 1957), ingestion may occur during the browsing of algae on which coracidia have settled. From the good infections obtained with E. agilis (Table 9), browsing of coracidia is an efficient method of acquiring the infection or that species of copepod is the best copepod host. A high rate of infection was obtained with A. viridis which is a relatively large carnivorous copepod compared with the other two species used and may sieze food more voraciously or be a potentially better host than the other species.

The growth rate of the procercoid was first recorded by Clarke (1954) using copepods infected in the laboratory; a cessation of growth as well as an uneven growth rate during cercomer formation was found. This was found in the course of the present work (Figure 5). However, Clarke's decrease in volume during the cercomer formation was not found. The cessation of growth may be the result of a host-parasite relationship since, if the procercoid continued its/

/growth even at a decreased rate, the end result would be the death of the copepod due to pressure on its organs or even starvation.

With the plerocercoid stage, it is found that although the plerocercoids are infective at a certain size, growth beyond that size increases the possibility of becoming established in a host (see under "Adult"). This may also be the case with the procercoid stage. After cercomer formation, the procercoid is infective but the increase in size the following week may enhance its chances of penetrating the body cavity of a fish and becoming established there. In single infections the faster development to maximum size (Figure 5) is advantageous to the tapeworm whereas in multiple infections the slower growth rate means the procercoids must remain in the copepod for a longer period to have an equal chance of becoming established in a fish. The slower growth rate may be due to overcrowding resulting in less nutrients being available for each procercoid. If the slower rate were only due to pressure from overcrowding, the possible maximum size would never be reached.

D. Summary

1) In this work, 2 new species of Cyclops were infected with Schistocephalus as well as 2 other previously reported species. Thus, to date, 10 species of copepod have been recorded as hosts of Schistocephalus.

2) Eucyclops agilis and Acanthocyclops viridis were the copepods most susceptible to the infection (90% and 81% of the copepods infected respectively), with Macrocyclops albidus and Cyclops strenuus abyssorum slightly less so (52% and 65% respectively infected).

3) A method is described for the infection of small numbers of copepods.

4) The stages in the development of the procercoid from the coracidium are described.

5) The rate of growth of the procercoid was measured by the increase in volume, maximum size in a single infection being reached in 3 weeks at 16$\pm$ 20°C. With multiple infections, maximum size was attained in 5 weeks.

6) The effect of single and multiple infections in a copepod and the cessation of growth on attainment of a certain size is discussed.

Stage in Life Cycle 4. Plerocercoid

A. Observations

The plerocercoids of Schistocephalus solidus measured up to 40 mm. in length with a maximum breadth of 5 to 8 mm. Plerocercoids greater than 15 mm. in length were fully segmented and had from 65 to 95 proglottids. The first proglottid is triangular in shape with the most anterior point of the triangle invaginated. During movement of the plerocercoid, the invaginated portion was rhythmically evaginated. The plerocercoids are white in colour and firm to the touch.

In the larger plerocercoids, the genital primordia are visible to the naked eye as a denser white region in the middle of each segment. The primordia are found from the eleventh segment to the second last.

B. Experimental

(i) Infection of fish

Procedure: A three-spined stickleback was placed in a 120mm. crystallising dish half-filled with water and 4 to 6 infected and identified Cyclops added. Before an attempted infection, the fish were starved for 24 hours so that they would ingest the copepods more readily. After ingestion of the cyclops, the fish were returned to a tank and maintained for varying periods of time.

Results: Some fish ate the cyclops immediately, whereas others showed no interest for 2-3 hours. A total of 34 fish were exposed to infected copepods and of these, 20 (59% of the total) became infected. The actual number of procercoids fed was not determined since, in heavily infected copepods, accurate counts involved manipulation of the copepods and this often resulted in the death of the copepod. Sticklebacks would only eat moving copepods.

Comments: The fish were isolated during exposure to copepods in order that time of ingestion could be noted. Also, it ensured that each fish ate the copepods.

(ii) Development of the plerocercoid

Procedure: Originally, the temperature of the water in the fish tanks was carefully controlled but in later experiments, this was not possible. The fish were maintained for various/

/periods after ingesting infected copepods, then killed by pithing and the abdominal cavity opened laterally. This was done to avoid damaging the small plerocercoids which tended to lie against the ventral wall. Very small plerocercoids were washed out of the body cavity with a jet of warmed saline as forceps would have punctured them.

The fresh weight of the worms was measured by weighing in metal foil pouches to prevent moisture loss. Weighing errors in the region of $\pm$ 1 mg. occurred but this was insignificant in plerocercoids over 20 mg. but was important in very small plerocercoids. The plerocercoids were measured and examined for segmentation and genital primordia development.

Results: The following stages in development were observed:-

(a) The cercomer disappeared on penetration of the procercoid into the body cavity but it is not known if it broke off or was absorbed by the plerocercoid.

(b) The genital primordia were first detected as a cluster of densely nucleated cells in the mid-line of 2-3 mg. plerocercoids. The cells were arranged in segmented blocks although no external sign of segmentation was visible.

(c) Plerocercoids 3-5 mg. in weight (8mm. fully extended) showed the first signs of segmentation, the first segments appearing two-thirds of the distance down the body of the/

/plerocercoid from the anterior end. The development of the segments then continued anteriorly and posteriorly from the point at which the first segments appeared.

(d) Segmentation was complete in plerocercoids of 6 mg. upwards, the number of segments which had been formed by this time remaining constant for the rest of the life of the tapeworm.

(e) The next stage in development concerned the formation of the gential organs. As the number of nuclei in the middle of each segment increased, a compact mass of cells began to develop till the outline of the future genital organs became visible. By 20 mg. the gential primordia were easily recognisable in stained preparations.

(f) Plerocercoids > 20 mg. continued to grow fairly rapidly, increasing in size only. The size of the genital rudiments increased but there was no development of the genitalia.

Comments: Histological development was determined by examining serial sections of proglottids stained in Heidenhain's haematoxylin and by examining other regions of the body stained with catechol (Hopkins and McCaig, 1963).

(iii) Rate of growth in vivo

Procedure: Plerocercoids were removed from sticklebacks after varying times and measured fully extended. The plerocercoids, where possible, were then weighed in foil but/

/many of the very small plerocercoids were too small to be weighed.

Results: In one experiment, the fish were maintained constantly at 17± 1°C and the plerocercoids recovered from these fish had a markedly faster growth rate than plerocercoids in fish at lower temperatures (Figure 6, growth lines solid). These plerocercoids grew from 0.4 - 0.5 mm. initially to 1 mm. in 10 days and 5.6 mm. in 37 days.

The importance of temperature control is shown by the other results plotted in Figure 6. Plerocercoids recovered almost two months after infection of the fish had different rates of growth depending on the water temperature. With fish infected in July at a temperature of 14°C, the fastest growth was obtained, the late October infection at a temperature of 7°C being next and the slowest being obtained with fish infected in December at a temperature of 4°C. After the plerocercoids have attained 10 mm. in length, the rate of increase in their length appears to decrease but the weight of the plerocercoids continues to increase rapidly (Table 10).

Comments: With small plerocercoids, length is the only practicable method of measuring growth but once plerocercoids exceed 10 mm. it is a poor criterion and weight becomes a more accurate means of assessing growth.

Figure 6

The rate of growth of the plerocercoid stage *in vivo*.

Growth of the plerocercoids was measured in terms of increase in length.

Solid lines represent the growth of plerocercoids in fish maintained at 17 $\pm$ 1°C.

Broken lines represent the growth of plerocercoids in fish maintained in partially heated tanks. The water temperature in these tanks was 4°C during the winter and 14°C in mid-summer.

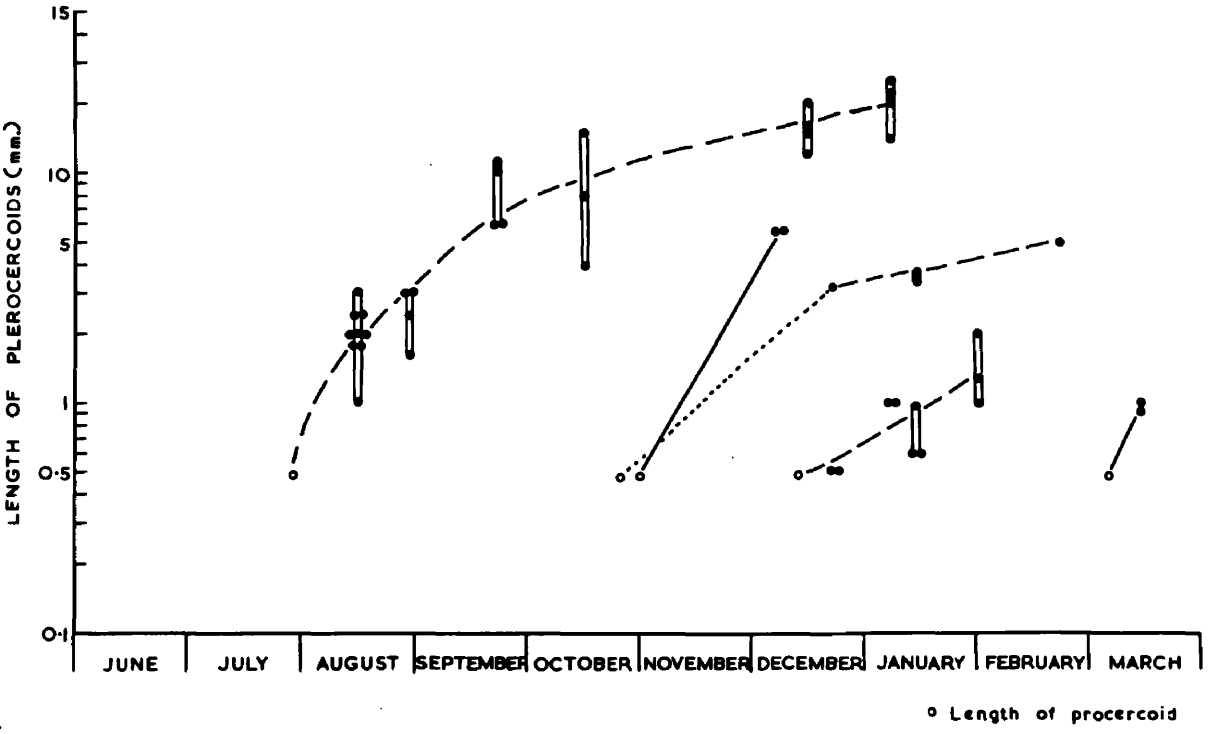

LENGTH OF PLEROCERCOIDS (mm.)
15
10
5
1
0·5
0·1
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
JANUARY
FEBRUARY
MARCH
o Length of procercoid

Table 10

Weights of plerocercoids recovered from fish-fed infected copepods

Temperature Range (°C)	Length of infection (weeks)	Weights of Plerocercoids from each fish
14 --10	9	0.7; 0.9; 1.3; 1.3mg
14 - 7	$12^{1}/2$	0.3; 0.7mg
14 - 7	$12^{1}/2$	10.5mg
14 - 7	19	0.8; 1.0; 1.2; 1.4; 1.5; 1.6; 1.9; 2.3; 2.8mg
14 - 4	21	5.9; 11.2; 13.0; 25.2mg
14 - 4	25	10.6; 23.1; 23.5; 36.6mg
7 - 4	$11^{1}/2$	0.1; 0.2mg
7 - 4	$12^{1}/2$	0.3mg

(iv) Infectivity of plerocercoids

To determine the effect of size of plerocercoids on their infectivity, plerocercoids were fed to pigeons and ducks and, after 48 hours, the hosts were killed and the weights of the worms recovered from the gut determined (Table 11). In the case of birds fed intact fish, the percentage recovery was estimated. One-third of the fish caught were dissected and their burden of plerocercoids determined. From this, the approximate number and weight of the plerocercoids fed in 72 fish to pigeons and in 120 fish to ducks, was calculated.

A minor correction was applied by expressing the number of adult worms recovered in the four weight categories <10, 10-19, 20-29 and $\geqslant$ 30 mg. as a percentage of the estimated number of plerocercoids in the <11, 11-21, 22-32 and $\geqslant$33 mg. weight categories. This was done to allow for the loss of approximately 10% in weight which occurs during 48 hour development in the duck (see under "Adult").

Table 11

The numbers of worms of different weight groups, recovered from definitive hosts two days after feeding, shown as a proportion of the number of plerocercoids fed

Host	Number of animals used	Infective dose	Number of worms recovered/ Number of plerocercoids fed			
			<10mg	10-19mg	20-29mg	>30mg
Pigeon	6	12 fish	0/40 0%	2/16 13%	5/23 22%	23/53 44%
Duck	10	12 fish	2/67 3%	12/27 44%	16/38 42%	74/88 84%
Duck	5	plerocercoids	0/7 0%	1/5 [20%]	2/6 33%	98/121 82%

C. Discussion

The plerocercoid stage was obtained from one species of fish, the three-spined stickleback, Gasterosteus aculeatus. In Lennox Castle Reservoir (see Section II), loach (Nemachilus barbatulus) were caught but were never found harbouring Schistocephalus plerocercoids. A number of teleost fish are reported in the literature as being hosts (Cooper, 1918 and Vik, 1954) but other workers (Hopkins and Smyth, 1951) suggest that Schistocephalus found in the intestines of these fish result from the fish eating infected sticklebacks or plerocercoids expelled from sticklebacks. The latter phenomenon was reported to occur on the death of a number of heavily infected fish (Vik, 1954). Schistocephalus solidus was also believed to occur in the ten-spined stickleback, Pygosteus pungitius but in 1959, Dubinina stated that the species was not Schistocephalus solidus but a new species, Schistocephalus pungitius. However, she makes two contradictory statements relating to the characters of the new species. The tapeworm used in this present work had 65-95 proglottids and was found in G. aculeatus. These two facts satisfy Dubinina's criteria for S. solidus which were based on the number of proglottids and the host from which the worm was obtained. Unfortunately, Pygosteus pungitius were not found locally and hence no examination of their tapeworms

/could be made.

The plerocercoid stage thus appears to be restricted in its hosts and may even be monospecific. The procercoid larva and the adult, however, can occur in many hosts. The factors causing the extreme specificity of the plerocercoid stage may be of a physical and/or chemical nature. Infected copepods must be eaten by other fish but there may not be a suitable stimulus to cause the procercoid to burrow through the gut wall or possibly G. aculeatus is the only fish which does not produce an immunological response against the procercoid of S. solidus. However, nothing is known of such conditions in sticklebacks.

Successful infections of sticklebacks were obtained in the laboratory with fish starved for 24 hours prior to exposure to infected copepods. Infections were attempted by Clarke (1954) with fish which had not been starved, but no infections occurred. After a period of starvation, enzymes will be at a minimum in the stomach and intestine and hence digestion will proceed slowly initially until secretion of more enzymatic material occurs. This would allow the procercoid to escape from the copepod and penetrate the wall of the gut of the host. Two hours was reported as adequate time for penetration of the gut wall (Clarke, 1954).

Apart from size increase, as measured by weight or length, the development of the plerocercoid can be described/

/in terms of proglottid formation and genital primordia development. The three principle variables to which development may be correlated are the weight, age and region of the strobila. The last point is important in very small plerocercoids as a development gradient extends both anteriorly and posteriorly from a central region in the strobila. Thus, in an adult of about 10 mg., only the middle proglottids produce eggs. The extent to which age, as distinct from size, affects the level of differentiation has not been determined as the plerocercoids were obtained from a natural population in which the age was not known. Plerocercoids reared in laboratory-infected fish would indicate the relationship but due to the use of different temperatures and variation in rate of growth, the relationship could not be determined.

The development of the genital primordia is segmental in distribution from its earliest stages, actual segmentation not appearing until the aggregations of nuclei in the midline of the strobila are well developed. In the development of the segments, thickening of the cuticle in restricted regions occurs (Clarke, 1954) to give the typical, craspedote structure of the plerocercoid. After the completion of segmentation, the genital primordia continue to develop, the genitalia being fully formed by the time the

/plerocercoid weighs approximately 19 mg. After this time, the genitalia increase in size by increasing the number of cells but no further development towards the adult condition occurs.

Two principal difficulties were encountered in investigating the capability of th plerocercoids to mature. Firstly, plerocercoids less than 20 mg. are unlikely to become established especially if removed from a fish before feeding to a duck or pigeon (Table 11). Secondly, plerocercoids less than 1 mg. were available in the lochs (this being the overwintering size) but worms of 5-20 mg. were scarce, as the majority of plerocercoids passed quickly through this size range in early summer (see Section II). Thus few results could be obtained since the percentage of small plerocercoids which can become established in a warm-blooded host is low (Table 11). It was found, however, that worms down to 10 mg. could mature in ducklings but only the most developed proglottids in the centre of the strobila actually matured within 48 hours.

The rate of growth of the plerocercoid in the stickleback was of interest for two reasons. Firstly, with respect to the time required by the plerocercoid to reach a size and stage in development capable of maturing in a warm-blooded host. Secondly, the rate of growth of plerocercoids <u>in vitro</u> may be compared with the <u>in vivo</u> results to ascertain the effect of the culture techniques on the plerocercoids.

From the growth curve commencing at the end of July, it can be seen that growth during the summer is rapid, the rate decreasing by September. This decrease is almost certainly due in part to the decrease in temperature, but may also result from an inherent slowing down after the initial rapid growth rate. At 17°C, the initial growth rate is more rapid than at 14°C, as indicated by the slope of the growth lines (Figure 6). The effect of temperature on the growth rate is also demonstrated by the growth curves obtained when fish were infected in October and December, the water temperatures being 7°C and 4°C respectively. Thus, plerocercoids occurring as natural infections in fish may grow rapidly initially during the summer months, with the rate decreasing at the onset of winter. This corresponds with the results obtained from the survey work carried out on two populations of naturally infected sticklebacks (see Section II).

Commencing with the infection of the fish in July, plerocercoids reached a size which could be weighed (0.1 to 1.0 mg.) by late September and by December weighed more than 5.0 mg. The water temperature had just dropped to 4°C at this time. One plerocercoid, recovered in October, weighing 10.5 mg. was probably a previous infection in the fish, while the fish with 9 surprisingly small plerocercoids may have suffered overcrowding conditions within the body

/thus stunting the growth of the plerocercoids. By January, 25 weeks after infection, worms greater than 10 mg. were found. Thus, it would appear that under natural conditions, infective plerocercoids (see under "Adult" section) will develop by the spring if the fish have acquired the infection in the previous summer, allowing for the slightly lower water temperatures recorded in the lochs (Table 23). A time of 2-6 months to attain infectivity was reported by Dubinina (1960) but comparison of the results is not possible due to lack of information on the physical conditions.

No actual rate of growth was determined but from the results, it appears that small plerocercoids have a faster rate of growth than large ones. This was confirmed when plerocercoids of various sizes were maintained in a nutrient medium <u>in vitro</u> (see Section III).

D. Summary

1) Only one host, the 3-spined stickleback (Gasterosteus aculeatus) was used in this work but the position of other fish as hosts is discussed.

2) A method is described for the infection of small numbers of fish.

3) The stages in the development of the plerocercoid from the procercoid visible in living as well as stained whole mounts of the worms are described.

4) Some results were obtained for the rate of growth of the plerocercoid in vivo but due to temperature variations, the results can only be used to compare with the survey results and not used as a definite rate of growth.

5) The infectivity of various weights of plerocercoids was determined: 0-3% establishment of worms < 10 mg., 13-44% of worms 10-19 mg. in weight, 22-42% of worms 20-29mg. in weight and 44-84% established of worms > 30 mg. in weight. The effect of this in relation to the life-cycle is discussed.

Stage in Development 5. Adult

A. Observations

The adult, when freshly removed from a host, is very similar in appearance to the plerocercoid. However, the anterior end is more pointed, the plerocercoid anterior invagination having everted in the gut of the definitive host. The adult is longer than the plerocercoid, a maximum size of 50 to 60 mm. being reached but this would appear to be due to a relaxation of the body muscles, perhaps under the stimulus of peristaltic action, rather than to actual growth of the tissues occurring. The adults move actively at 40°C, the temperature of the host but almost cease to move at lower temperatures.

A pair of excretory canals extending the full length of the tapeworm, one near each lateral margin, can be easily seen. The genitalia have developed from the primordia seen in the plerocercoid and are found in segments eleven to the second last segment inclusive. Without staining, the uterus shows as a coiled tube packed with eggs about the midline of each proglottid. In the anterior third of each proglottid, the cirrus pouch can be seen but the uterine and vaginal openings to the posterior of it and to the right of it, in the case of the uterine openings, are more difficult to see. Occasionally the uterine opening is visible. The vitellaria are scattered throughout the/

/Cortical region of each proglottid and the testes are found in the medullary region, dorsally situated. The ovary is bilobed.

B. Experimental

(i) Infection of the definitive host

Procedure: To obtain adult worms, plerocercoids were fed by mouth to a variety of warm-blooded animals. Ducklings, up to 4 weeks of age were the most commonly used. The ducks, pigeons and chickens were fed by holding the beak or mouth open, the plerocercoids being placed at the back of the mouth with long blunt forceps, or with fingers. The mouth was then held shut and the animal made to swallow by stroking its throat. The rats and hamsters were lightly anaesthetised with nembutal or ether before introducing the plerocercoid, scolex first into their mouths. Plerocercoids were usually dissected from the fish before being fed to a definitive host but fish were fed on some occasions. (The gulls were allowed to eat the fish naturally as they regurgitated if under stress.) With pigeons, the animals had to be starved for a period to partially empty the crop of gritty material which would damage the plerocercoids. The other hosts were deprived of food for 1-4 hours before feeding so that the plerocercoids would be swallowed readily.

The animals were killed by breaking the neck. The intestines were removed at once as at 37-41°C, adult Schistocephalus can change their position rapidly. The position of each worm was expressed as a percentage of the/

/distance down the intestine, the length of the intestine being measured from gizzard (stomach) to anus. The worms were then used to obtain eggs as described previously. (See under "Egg").

Results: Five species of birds (chickens, ducks, pigeons, herring-gull and black-headed gull) and two species of mammals (rats and hamsters) were tested as possible definitive hosts (Table 12). The results are as follows:-

1. Schistocephalus can mature in all the species tested.
2. Ducks are good hosts up to four weeks of age after which the percentage of worms to become established decreases.
3. The feeding of plerocercoids still within the fish to pigeons probably leads to more of the plerocercoids becoming established than if free plerocercoids are fed.
4. With weighed plerocercoids, the percentage which became established is less than with plerocercoids which were not weighed prior to feeding. The removal of surface moisture may have damaged the plerocercoid. In more recent experiments, the plerocercoids were placed directly from the fish in pouches of aluminium foil. The pouch was closed and weighed. Small errors due to surface moisture were incurred but better results were obtained.
5. Both species of gulls are probably good hosts. The gulls and three 10-week-old ducks were fed two heavily infected fish each, but only the gulls were infected./

Table 12. The infectivity of laboratory animals to *Schistocephalus solidus*

Host			Number and method of infection		Number of animals infected		Number of worms recovered	
No. fed	Species	Age (wks)			Total	%	Total	%
15	*Anas boschas* (duck)	1	2-14	f	15	100	164	-
24		1	4-20	p	24	100	143	84
58		1	1-11	w.p	57	98	215	74
14		2-4	2-35	f	8	57	51	-
16		2-4	5-14	p	15	94	105	66
17		2-4	2-6	w.p	11	65	28	48
5		6-22	2-170	f	1	20	5	-
4		7-52	9-35	p	1	25	9	11
14	*Columba livia* (pigeon)	adult	5-18	f	13	93	88	-
15		adult	10-15	p	14	93	55	33
10		adult	15	w.p	3	30	3	2
4	*Gallus domesticus* (chicken)	1	3	p	4	100	12	100
6		2-3	3-6	f	3	50	64	-
23		4	5-10	p	16	70	22	32
15		2-5	2-7	w.p	10	67	3	38
3	*Larus argentatus* (herring gull)	8	2	f	3	100	24	-
2	*Larus ridibundus* (blackheaded gull)	52	2	f	2	100	14	-
9	*Rattus norvegicus* (rat)	8	10	p	9	100	18	29
11	*Mesocricetus auratus* (hamster)	8	6-10	p	10	91	-	-

f - fish
p - plerocercoids
w.p - weighed plerocercoids

/6. The percentage of worms to become established (Table 12) suggests that with ducklings, "takes" of over 50% may be expected; in chickens and pigeons the "take" is lower, about 30-40%, with one exception; and in rats, substantially lower, around 20%.

The distribution of the worms in the intestines of the various hosts was noted at autopsy and is summarised as follows:-

1. In pigeons (Figure 7).
   a) plerocercoids take about 2 hours to reach the intestine (the worms were found in the crop and gizzard in the three pigeons killed half, one, and one and a half hours after infecting);
   b) within 3-4 hours, the plerocercoids are found as far down the intestine as they normally go;
   c) between 3 - $13^{1}/2$ hours, most worms are in a region between 40-60% of the distance down the intestine, the mean position being 47%, S.D. 12.7;
   d) between 24-96 hours after infecting, over 90% of the worms occur in the anterior half of the intestine, the mean distance of the worms down the intestine after 1, 2, 3 and 4 days was, 31%, S.D. 11.2; 22%, S.D. 11.6; 37%, S.D. 15.0; 20%, S.D. 9.7 respectively.
2. In ducks, Schistocephalus is swept to the posterior half of the intestine in the first few hours and remains there,/

Figure 7

The distribution of Schistocephalus solidus in the intestine of pigeons at various time intervals after infecting.

• Position of individual worms in a multiple infection.

° Position of a worm in a single worm infection. Vertical line shows zone of intestine occupied by worms in each bird.

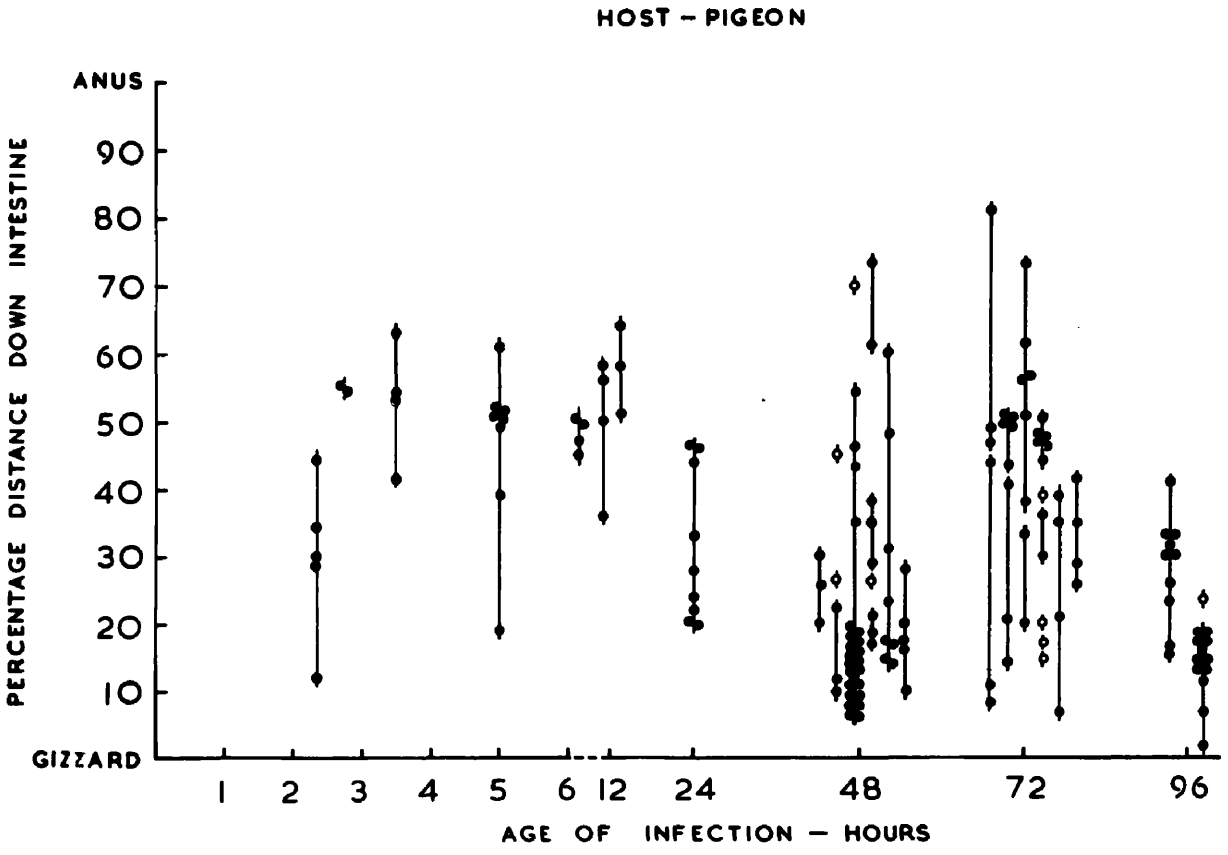

HOST – PIGEON
ANUS
90
80
70
60
50
40
30
20
10
GIZZARD
PERCENTAGE DISTANCE DOWN INTESTINE
1
2
3
4
5
6
12
24
48
72
96
AGE OF INFECTION – HOURS

Figure 8

The distribution of Schistocephalus solidus in the intestine of ducks at various time intervals after infecting.

X worm in a caecum; • position of individual worms in a multiple infection; ° position of a worm in a single worm infection. Vertical line shows zone of intestine occupied by worms in each bird.

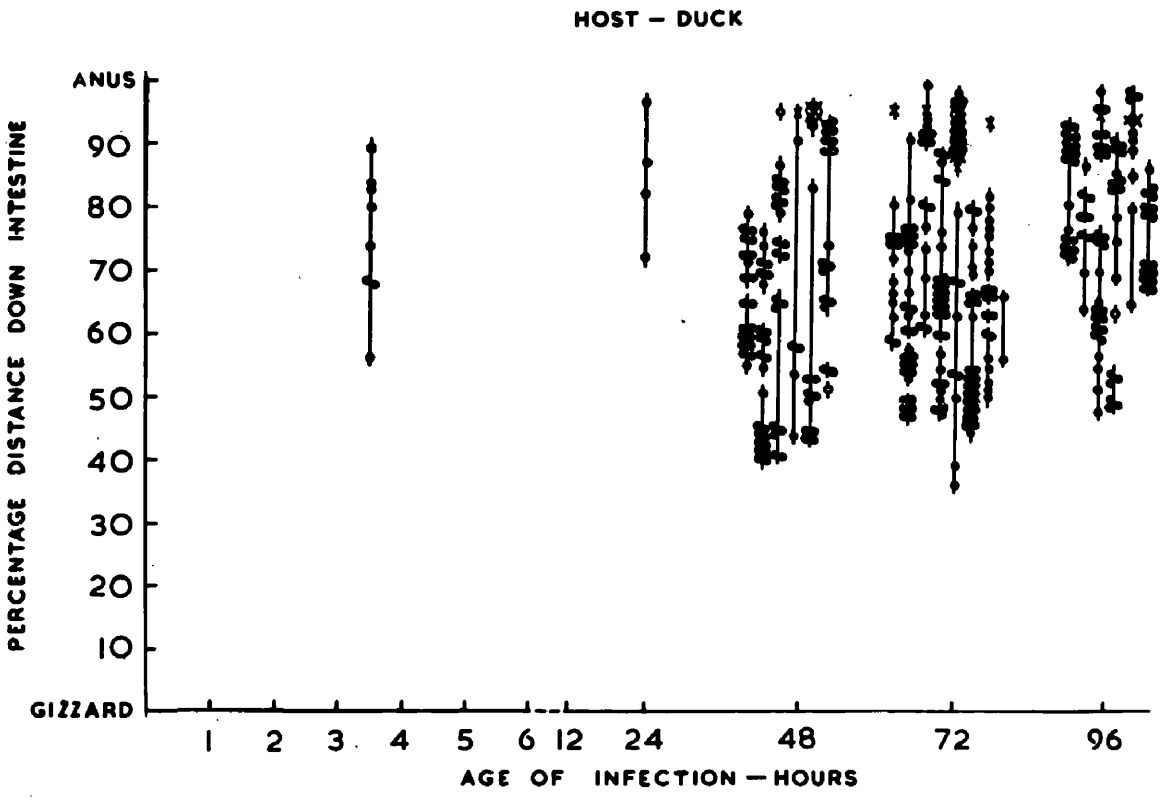

HOST – DUCK
ANUS
90
80
70
60
50
40
30
20
10
GIZZARD
PERCENTAGE DISTANCE DOWN INTESTINE
1
2
3
4
5
6
12
24
48
72
96
AGE OF INFECTION – HOURS

Figure 9

The distribution of Schistocephalus solidus in the intestine of chickens at various time intervals after infecting.

• Position of individual worms in a multiple infection.

° Position of a worm in a single worm infection. Vertical line shows zone of intestine occupied by worms in each bird.

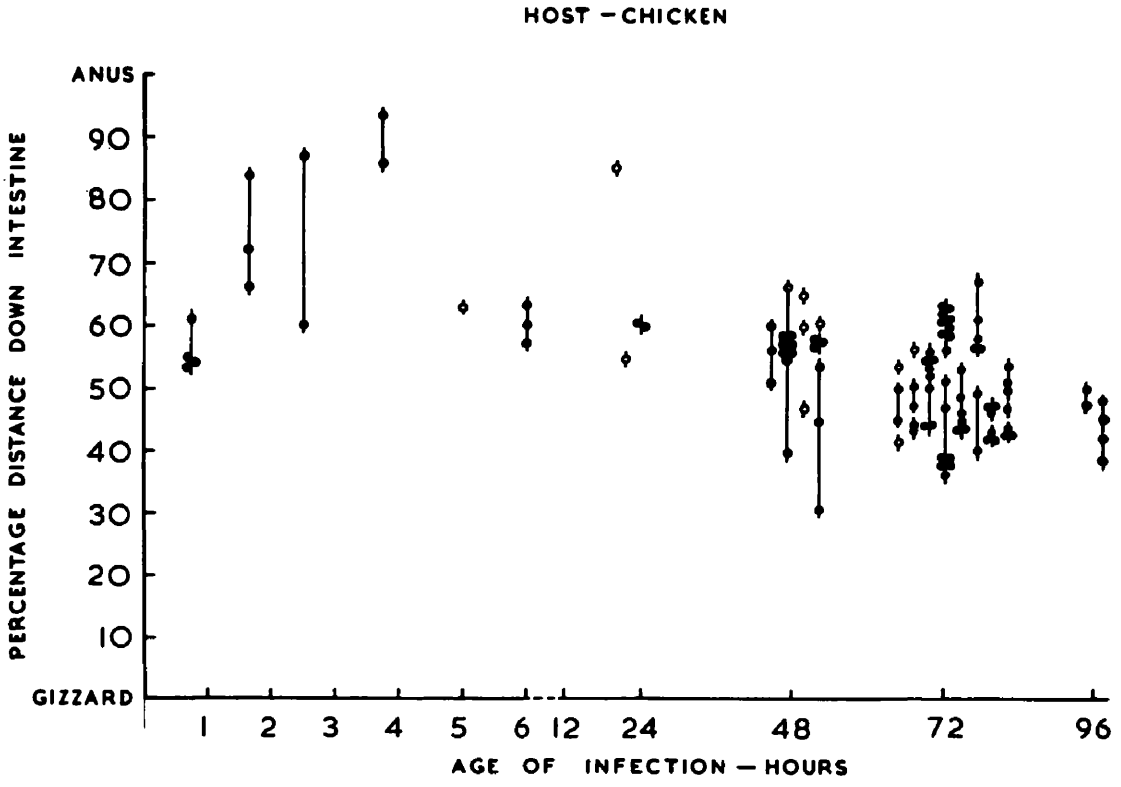
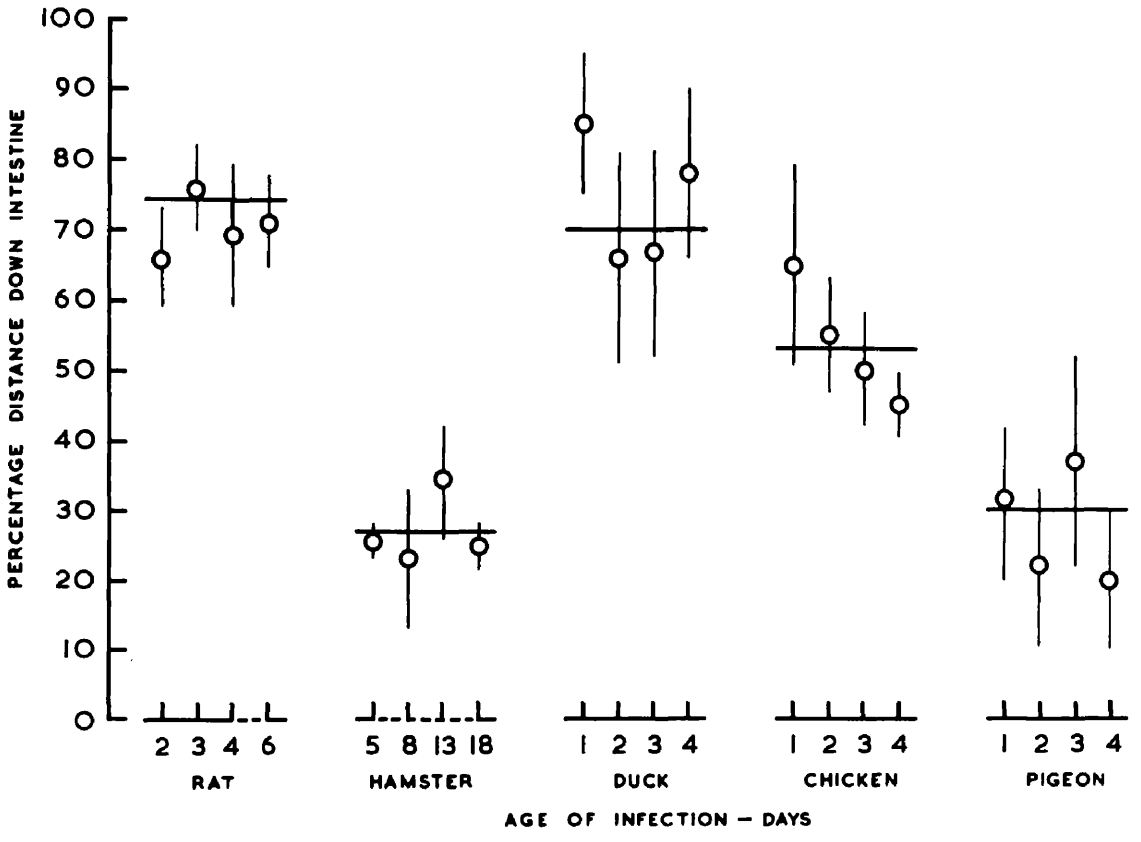

HOST – CHICKEN
ANUS
90
80
70
60
50
40
30
20
10
GIZZARD
PERCENTAGE DISTANCE DOWN INTESTINE
1
2
3
4
5
6
12
24
48
72
96
AGE OF INFECTION – HOURS

Figure 10

The position of Schistocephalus solidus in the intestine of various laboratory hosts.

The abscissa shows the age of the infection in days.

Open points indicate the mean position of worms after the specific period; vertical lines show the standard deviations. The horizontal line indicates the over-all mean position of worms in the host.


PERCENTAGE DISTANCE DOWN INTESTINE
100
90
80
70
60
50
40
30
20
10
0
2 3 4 6
RAT
5 8 13 18
HAMSTER
1 2 3 4
DUCK
1 2 3 4
CHICKEN
1 2 3 4
PIGEON
AGE OF INFECTION — DAYS

/sometimes entering the caeca (Figure 8).

3. In chickens, the plerocercoids usually become established within 5-6 hours in the mid region of the intestine, near the remains of the yolk sac attachment. Initial establishment may occur further back in the intestine and then the worms migrate forward to the central region (Figure9).

4. In herring-gulls, Larus argentatus, most worms occur in the third quarter of the intestine.

5. In rats, worms pass to the posterior part of the small intestine or even the caecum and anterior region of the colon (Figure 10).

6. In hamsters, the worms are usually found well forward in the anterior half of the small intestine (Figure 10).

(ii) Weight change during development in a definitive host

Table 13 shows the weight of plerocercoids fed to 7 ducklings, and the weight (column 4) of the worms recovered. The percentage change shown in column 5 is only an indication of the order of weight change to be expected. Where the adult worm recovered could not be identified with a specific plerocercoid but only with a weight group, the percentage change was calculated by comparing with the mean weight of the plerocercoids fed in that group, e.g. the 24 mg. worm in duck 1 was calculated as having lost 1.5 mg., and the 18 mg. worm in duck 2 as having lost 3.3 mg. The average loss in weight of the 13 intact worms recovered after 45

Table 13 The change in weight of Schistocephalus solidus after 45- and 96-hour development in a definitive host, the duck.

Bird no.	Hours in duck	Weight of plerocercoids fed	Weight of adult worms recovered	Change in weight (%)	
1	45	7 7 8	-	-	
		25 26	24	-6	
		34	32	-6	
		73 73 75	73 55[d]	-1	
2	45	3	-	-	
		21 21 22	18	-16	-9%
		63 63 64 65 68	53 53 55 60 61	-13	
		100 103	96 58[d]	-5	
3	45	12 13	-	-	
		44 45	40	-10	
		77 81 85	73 79	-5	
4	72	46	39[d]	-	
		79 139	71 131	-12	
		113 115	102 110	-6	-13%
5	72	64 64 87	53 54 79	-14	
		116 138	94 113	-19	
6	96	5 6 8	-	-	
		63 63 64 65	46 50 53 56	-19	
7	96	16	-	-	-20%
		25 26 29	21	-21	
		55	43	-22	

d damaged

/hours was 9%, of the 9 worms recovered after 72 hours, 13%, and of the 6 worms recovered after 96 hours, 20%.

(iii) Longevity

The longevity of the adult Schistocephalus in various laboratory hosts is shown in Table 14. The infecting dose consisted of 6 plerocercoids to the hamsters and 10 plerocercoids to the rats, ducks, chickens and pigeons. Thus, several worms may have become established in each animal but the results will only show the survival time of the longest lived worm in each host.

The number of eggs per unit area of faecal smear was counted every day after infecting the hosts and continued till the eggs disappeared from the faeces. Faecal checks were continued for two to three days after the eggs disappeared as a check that the infection had been terminated. In early work, some of the hosts were killed to determine if the worms disappeared immediately the eggs were no longer found in the faeces. The egg output from the various hosts is shown in Table 15.

From the results, it can be seen that maximum survival time varies extensively among the hosts, the egg output being at a maximum the second and third day after infection, decreasing with time after infection. The results obtained from the hamsters have been split into two - those in which the infection died out within 8-9 days and those in which the/

Table 14 The longevity of adult Schistocephalus solidus in laboratory hosts

Host	Number of animals used	Number of animals with Schistocephalus eggs in faeces on day:																		
		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Duck	6	6	6	6	6	6	6	6	4	3	0									
Pigeon	6	6	6	6	6	6	4	2	1	1	0									
Chicken	11	11	11	11	11	11	11	11	11	11	10	10	5	4	3	0				
Rat	5	5	5	5	5	5	3	0												
Hamster	7	7	7	7	7	7	7	7	6	6	6	6	6	6	5	5	3	2	1[a]	

[a] Killed on day 19 and a single Schistocephalus recovered

TABLE 15

Variation in Output of Eggs of Schistocephalus solidus during course of Infection in different Laboratory Hosts.

Host	Number of animals used	Age of host at infection		Egg Output from Various Hosts. Time in days. 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Anas boschas (Duck)	6	7 days	No. of Eggs[1].	-	-	61	55	38	-	-	19	10	5	3	0	-							
			No. of Hosts[2] infected	-	-	6	6	6	-	-	6	6	4	3	0	-							
			Average Eggs per host	-	-	10.2	9.2	6.3	-	-	3.2	1.7	1.3	1.0	0	-							
Columba livia (Pigeon)	6	Adult	No. of Eggs	-	-	33	25	-	18	11	5	2	1	1	0	-							
			No. of Hosts infected	-	-	5	6	-	6	6	4	2	1	1	0	-							
			Average Eggs per host	-	-	6.6	4.2	-	3.0	1.8	1.3	1.0	1.0	1.0	0	-							
Gallus domesticus (Chicken)	6	6 days	No. of Eggs	-	-	36	33	21	21	15	9	8	10	11	11	10	5	4	3	0	-		
			No. of Hosts infected	-	-	6	6	6	6	6	6	6	6	6	6	6	5	4	3	0	-		
			Average Eggs per host	-	-	6.0	5.5	3.5	3.5	2.5	1.5	1.3	1.7	1.8	1.8	1.7	1.0	1.0	1.0	0	-		
Gallus domesticus (Chicken)	5	6 days	No. of Eggs	-	-	18	16	12	12	13	6	5	4	6	5	2	1	0	-				
			No. of Hosts infected	-	-	5	5	5	5	5	5	4	4	5	4	2	1	0	-				
			Average Eggs per host	-	-	3.6	3.2	2.4	2.4	2.6	1.2	1.3	1.0	1.2	1.3	1.0	1.0	0	-				
Rattus norvegicus (Rat)	5	6 weeks	No. of Eggs	-	-	5	5	5	5	3	0	-											
			No. of Hosts infected	-	-	3	5	5	5	3	0	-											
			Average Eggs per host	-	-	1.7	1.0	1.0	1.0	1.0	0	-											
Mesocricetus auratus (Hamster)	4	3 months	No. of Eggs	-	-	6	7	7	8	6	5	2	0	-									
			No. of Hosts infected	-	-	3	4	4	4	4	4	2	0	-									
			Average Eggs per host	-	-	2.0	1.8	1.8	2.0	1.5	1.3	1.0	0	-									
Mesocricetus auratus (Hamster)	6	3 months	No. of Eggs	-	-	7	13	14	11	12	10	11	12	9	8	8	7	9	7	8	5	3	1
			No. of Hosts infected	-	-	6	6	6	6	6	6	6	6	6	5	6	6	6	4	5	4	2	1
			Average Eggs per host	-	-	1.2	2.2	2.3	1.8	2.0	1.7	1.8	2.0	1.5	1.6	1.3	1.2	1.5	1.8	1.6	1.3	1.5	1.

1. No. of Eggs per unit area of faecal smear.

2. No. of Hosts which are still infected each day.

Egg Output from Various Hosts.										Time in days.										
0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
-	-	61	55	38	-	-	19	10	5	3	0	-								
-	-	6	6	6	-	-	6	6	4	3	0	-								
-	-	10.2	9.2	6.3	-	-	3.2	1.7	1.3	1.0	0	-								
-	-	33	25	-	18	11	5	2	1	1	0	-								
-	-	5	6	-	6	6	4	2	1	1	0	-								
-	-	6.6	4.2	-	3.0	1.8	1.3	1.0	1.0	1.0	0	-								
-	-	36	33	21	21	15	9	8	10	11	11	10	5	4	3	0	-			
-	-	6	6	6	6	6	6	6	6	6	6	6	5	4	3	0	-			
-	-	6.0	5.5	3.5	3.5	2.5	1.5	1.3	1.7	1.8	1.8	1.7	1.0	1.0	1.0	0	-			
-	-	18	16	12	12	13	6	5	4	6	5	2	1	0	-					
-	-	5	5	5	5	5	5	4	4	5	4	2	1	0	-					
-	-	3.6	3.2	2.4	2.4	2.6	1.2	1.3	1.0	1.2	1.3	1.0	1.0	0	-					
-	-	5	5	5	5	3	0	-												
-	-	3	5	5	5	3	0	-												
-	-	1.7	1.0	1.0	1.0	1.0	0	-												
-	-	6	7	7	8	6	5	2	0	-										
-	-	3	4	4	4	4	4	2	0	-										
-	-	2.0	1.8	1.8	2.0	1.5	1.3	1.0	0	-										
-	-	7	13	14	11	12	10	11	12	9	8	8	7	9	7	8	5	3	1	0
-	-	6	6	6	6	6	6	6	6	6	5	6	6	6	4	5	4	2	1	0
-	-	1.2	2.2	2.3	1.8	2.0	1.7	1.8	2.0	1.5	1.6	1.3	1.2	1.5	1.8	1.6	1.3	1.5	1.0	0

/infection lasted considerably longer. This eliminates a dip in the average egg output due to the dying out of the infection in four of the hamsters.

(iv) Immunity

An immunity response was reported by Joyeux and Baer (1936) to occur in ducks following infection with the pseudophyllidean Ligula intestinalis. This immunity was reported to last for 20 days.

An immunity response was looked for in four species of hosts, ducks, pigeons, chickens and rats. The animals used in the longevity determinations were kept and given a challenge dose 10 days after the end of the first infection. Animals of an identical age were used as controls; they were maintained under the same conditions as the 'immunised' animals but only received the second dose of plerocercoids. Faecal checks were made on all the animals 48 hours after feeding the plerocercoids and at 72 hours the animals were killed and the number of worms established in the hosts determined. The percentage of worms which had become established was determined. The results obtained (Table 16) indicate the lack of an immunity response against Schistocephalus solidus in the hosts used.

Table 16

Effect of a previous infection on the number of Schistocephalus solidus to become established in a definitive host

Host		Host age at infection (days)	Number of animals used	Immunising dose (given day 0)	Length of infection (days)	Challenge Dose			% of worms established in host
						Day given	Number of plerocercoids	Day killed	
Anas boschas (duck)	A[a]	7	6	10 plerocercoids	8 - 10	20	10	23	70
	B[b]	7	5	-	-	20	10	23	76
Columba livia (pigeon)	A	adult	6	10 plerocercoids	6 - 10	20	10	23	27
	B	adult	6	-	-	20	10	23	33
Gallus domesticus (chicken)	A	6	5	10 plerocercoids	10 - 13	25	8	29	20
	B	6	5	-	-	25	8	29	15
Rattus norvegicus (rat)	A	6 weeks	4	10 plerocercoids	6 - 7	20	10	23	40
	B	6 weeks	4	-	-	20	10	23	40

[a] Animals given immunising and challenge dose

[b] Controls; given only one dose

C. Discussion

(a) Laboratory Hosts

The most striking fact about the development of the adult stage of Schistocephalus solidus is the lack of host specificity; all 7 species of birds and mammals tested were capable of being infected (Table 12).

Of the more easily handled and maintained laboratory birds, ducks appear to be the best hosts, provided they are used in the first few weeks of life. Older ducks can be infected but the "take" in them is much poorer (Table 12). This conclusion is not necessarily contradictory to several reports in the literature (summarised by Vik, 1954) of the use of ducks as laboratory hosts, as neither the percentage of plerocercoids to become established nor the size of plerocercoids used is stated by earlier workers. It was reported earlier (Hopkins and McCaig, 1962) that plerocercoids below 30 mg. showed a lower take than plerocercoids over that weight and in 1-4-week-old ducklings and chicks, plerocercoids of 30-60 mg. become established less frequently than larger plerocercoids. It was not possible to test whether the threshold weight at which establishment frequently occurs is much higher in older birds than in young ones, as few of the fish used as a source of Schistocephalus contained plerocercoids of over 150 mg. However, under more/

/favourable conditions of growth, plerocercoids of 200-350mg. are common, and these may be capable of establishing themselves in the intestine of full grown ducks.

The results obtained using pigeons as hosts were more variable than those using ducklings. This may merely reflect the fact that the pigeons used were a less homogeneous group. (The ducks were all obtained at 1-day-old and reared on a grit-free diet. The pigeons were purchased as adult birds). If fed fish, pigeons can act as good hosts but with unprotected plerocercoids, few infections were obtained (Clarke, 1952). A critical factor in infecting pigeons is the safe passage of the plerocercoid through the crop and gizzard. An investigation of the effect of diet, time of last feeding the pigeon, etc., might well indicate ways of reducing the variability in the infections, which at present limits the usefulness of this laboratory host. As long as ducklings were the only other easily maintained laboratory host, this investigation would have been profitable. However, the good results obtained with chickens (Table 14), a species not previously recorded as a possible host, suggests that this cheap and easily obtained bird is a suitable alternative to the duck.

Of the other animals tested, the gulls Larus argentatus and L. ridibundus appear to be good hosts. Vik (1954) has shown that this also applies to Larus canus. However, all/

/are expensive to maintain and difficult to feed with plerocercoids. Force feeding is unreliable because of their habit of regurgitating under stress.

Records concerning mammals are definitive hosts are very few compared with birds. Vik dismisses Schistocephalus as only "a pseudoparasite" of mammals, a conclusion possibly based in part on his failure to infect cats in the laboratory and the absence of Schistocephalus in 14 mammals shot in the field. Both hamsters and rats were capable of being infected (Table 12), an observation that became of particular interest when it was discovered that the longevity of the adult worms in these two species of host was completely different (see next heading).

(b) Weight change during development in a definitive host

The interpretation of the datum in Table 13 as indicating a cessation of somatic growth in all or nearly all of the worm is based on two reasons. Firstly, an increase of at least 10-20% in the dry weight of a plerocercoid in 48 hours at 22°C has been recorded; at 40°C, an even greater increase in body weight would be expected unless somatic growth is inhibited. Secondly, if it is assumed that no growth has taken place at 40°C and allowance is made for a glycogen loss similar to that reported for Schistocephalus in pigeons (Hopkins, 1950) and for the small loss in water which was found to occur, the calculated weight of the worm

/after 48 hours is similar to that found experimentally (Table 13).

Synthesis of both somatic and genital tissue occurs in the plerocercoid. In the case of the genitalia, as has been discussed, there are two phases; the qualitative, during which the various genital rudiments become differentiated, and the quantitative, during which the genital rudiments increase in size but show little further progress towards maturation. The final phases of maturation are inhibited at the lower temperatures and can only take place when stimulated by a high temperature (Smyth, 1952).

It is generally accepted, however, that with the onset of maturation, somatic growth ceases (Wardle & McLeod, 1952, p.563) and this appears to be the case with the worms developing in the ducks (Table 13). Further experiments may show whether the cessation of growth is due to an internal hormonal mechanism (a feed-back from the maturing genitalia), the absence of certain metabolites in the environment or some other cause.

(c) Longevity and position of worms in the intestine of definitive hosts

It was previously assumed that the length of life of the adult worm was around 2-3 days (Schauinsland, 1886), the worm being moved passively down the intestine to the anus. To account for the slower movement of the worm than of food,/

/it was suggested the worm, by some method, delayed its passage through the intestine. However, Vik (1954) found Schistocephalus still alive in gulls killed 7, 8, 11 and 13 days after infecting them.

The longevity results (Tables 14 and 15) and position of the worms in the intestine after various periods from time of infection (Figures 7-10) clearly show the concept, that the length of life of the adult is determined by the time it takes a worm to drift down the intestine, is wrong.

Likewise, the suggestion by Smyth (1962) that Schistocephalus should be able to mature in the intestine of any warm-blooded host is no longer valid since it is based on the assumption that a rise in temperature is the sole factor determining whether Schistocephalus will mature in an intestine, once ingested. In fact, the ability of a warm-blooded animal to act as a potential host depends primarily on it triggering off a retention response by the worm and offering a suitable environment spatially, for the worm to maintain itself against peristaltic pressure. As it happens, all the laboratory hosts tested were capable of being infected, but it should be borne in mind that the diameters of the intestines of the animals used were fairly similar, and that at present, there is no evidence that mammals and birds with intestines of considerably different sizes are potential hosts./

/The development of Ligula intestinalis, which like Schistocephalus matures in a wide range of warm-blooded hosts and has a short adult life, appears to be similar. In Ligula, the ability to mature depends upon the worm becoming established, and establishment depends on the presence of a scolex (Joyeux and Baer, 1936). That the function of the scolex is associated with retention in the intestine and does not affect maturation directly is shown by the fact that scolex-free fragments of Ligula (Smyth, 1948) and of Schistocephalus mature in vitro.

In general, one can conclude from the data in Table 13 and Figures 7-10, that the adult phase of the life-cycle is far more complicated than previously supposed, posing many interesting problems. The function of the scolex is not clear; it may be a sensory or a correlating centre. How the worm determines direction is unknown as well as how it counteracts the effects of peristalsis. The varied positions occupied by the worms in the intestines of the various hosts as well as the variation in life spans are interesting problems.

/The longevity of a worm, as with any other animal, may be determined by either extrinsic or intrinsic factors. By intrinsic is meant factors which might be called senility changes. This is not a very satisfactory term as senility may involve very different processes in different animals. In Schistocephalus, senility might result from the exhaustion of a metabolite synthesised and stored in the plerocercoid but not available to the adult; the accumulation of waste, e.g. fat (Smyth, 1946), or it may be that around 40°C, the kinetics of reactions leading to the synthesis of genital products are so favourable that the somatic tissues are progressively starved. Reserves of glycogen are built up in the plerocercoid stage. When these worms are fed to a definitive host, the glycogen reserves are utilised as a source of energy. It has been shown that these reserves are depleted during maturation in a pigeon (Hopkins, 1950) 27% of the total glycogen being used in the initial 24 hours. Within 72 hours, approximately 40% of the glycogen reserves have been used. This rapid depletion must be an important factor in the longevity of the worms.

Extrinsic factors, as a cause of death, can be considered under the headings of environmental risks and immunity responses. As most worm infections died out at specific times (Tables 14, 15), despite the variable number of worms/

/established, it seems probable that the loss of worms is not a cumulative effect of environmental risks. This conclusion was verified by studying the survival of single worm infections in rats and hamsters (Hopkins and Thomas, private communication).

If it is accepted that the risk of death increases sharply after a certain length of adult life, one is left to decide whether this rise in risk results from intrinsic (senility) or extrinsic(immunity) causes. At first the latter was rejected as being highly improbable, but the claims of Joyeux and Baer (1936) that ducks could not be infected with Ligula for 20 days after a previous infection but then could be, and that this had been repeated seven times, could not be ignored. Table 16 shows the results of attempts to immunise ducks, chickens, pigeons and rats. The experiment was designed merely as a test of the worm doses used in the longevity experiments. A period of 10 days before challenging was selected to give time for an immunity response to develop and to lie within Joyeux and Baer's 20-day immunity period. The results failed to demonstrate the development of an immunity response.

It therefore appears, by elimination, that death may be due to senility and hence that the rate at which Schistocephalus becomes senile depends on, and is characteristic of, the host. That survival should be longer/

/in a mammal (hamster) than in a bird seemed reasonable since the rate of metabolism in the worm would be much slower at 37.5°C than at 41.5°C; unfortunately, survival in the rat is shorter than in birds. This, it was thought, might be due to Schistocephalus having access to food in the anterior part of the small intestine and caecum of a rat (Figure 10). However, this assumption does not correlate with the greater survival time of Schistocephalus in the duck, where it occurs in the posterior half of the gut (Figure 8), or in the pigeon, where it occurs in the anterior half of the intestine (Figure 7). It is possible, therefore, that the longevity of Schistocephalus in the definitive host is intrinsically determined, resulting from senility changes, but that the nature of the senility changes may be different in different hosts.

D. Summary

1. Schistocephalus matures in ducks (Anas boschas), pigeons (Columba livia), chickens (Gallus domesticus), herring-gull (Larus argentatus), black-headed gull (L. ridibundus), rats (Rattus norvegicus), and hamsters (Mesocricetus auratus).

2. The age of the host was important in determining the percentage of worms to become established. Best results were obtained with 1-4-weeks old ducks (Over 50% established) and 2-5-weeks old chickens (nearly 40% established).

3. Worms reach the intestine of the host in 2-3 hours and occupy a specific region of the intestine within the first 6 hours. This specific region varies in the different hosts.

4. In hosts, in which the worms occupy an anterior position in the intestine, there is evidence of an initial overshoot followed by an anterior migration.

5. The length of life appears specific in each host species but varies greatly among the host species; approximately 18 days in hamsters and 6 in rats, 14 days in chickens, 11 in ducks and 10 in pigeons.

6. The advantages and disadvantages of the various hosts

/are discussed as well as how the adult worm retains its position in the intestine.

7. The factors determining the longevity of the worms viz. senility changes, immunity reactions and environmental risks are discussed. Present evidence suggests longevity of Schistocephalus is limited by senility changes.

Section II

The occurrence of the plerocercoid stage of *Schistocephalus solidus* in two populations of the three-spined stickleback, *Gasterosteus aculeatus*.

A. Introduction 1.

The existence of Schistocephalus solidus in its plerocercoid stage has been recorded in many populations of the 3-spined stickleback (Gasterosteus aculeatus). Most reports of the infection are the result of single examinations of a number of fish from canals and lakes. Such reports include those of Abildgaard (1790) who reported "that every fifth or sixth stickleback was infected" and Hickey and Harris (1948) who found 20.6% incidence of infection at Poulaphouca, Eire. Fluctuations in the infection during one and two years were reported by Clarke (1954) and Walkey (1963) respectively. Hopkins (1959) recorded the variations in a stickleback population while investigating another tapeworm Proteocephalus filicollis.

The procercoid stage in the life-cycle occurs in the haemocoele of cyclopoid copepods but no work on a natural infection has been published. Copepods of the genus Cyclops were infected with the tapeworm by Clarke (1954), Dubinina (1957), and in the course of the present work. The first larval stage in the life-cycle, the coracidium, is probably ingested by the copepods of which ten species are now known as intermediate hosts. Development in this host takes two to three weeks under laboratory conditions but the time required under natural conditions has not been ascertained./

/Fish acquire the infection by ingestion of infected copepods. Cooper (1918) and Vik (1954) reported the plerocercoids from various hosts but Dubinina (1957) suggested that total specificity of the tapeworm in the plerocercoid phase occurred, although in her paper, two contradictory statements are made (see under "Plerocercoid", Section I). She also reported that plerocercoids grew to infectivity in 2 - 6 months under laboratory conditions but gave no indication of their size. However, it is known that plerocercoids are infective over 10 mg. in weight (see Section I). Eggs are produced within 40 hours in a definitive host (Smyth, 1950) the adult phase being relatively short (see Section I).

The main aims of the investigations were firstly to obtain information on variation in incidence and intensity of the infection in the fish populations; secondly, to ascertain the length of time taken for the plerocercoids to attain infectivity in a natural population and thirdly, to record variations in the composition of the fish populations. The latter would be invaluable in the elucidation of some of the fluctuations in the tapeworm population.

During the surveys, information relating to the physical nature of the lochs and their flora and fauna was accrued. It is appended below. The main investigation was carried out at Lennox Castle, that at Bingham's being for comparison.

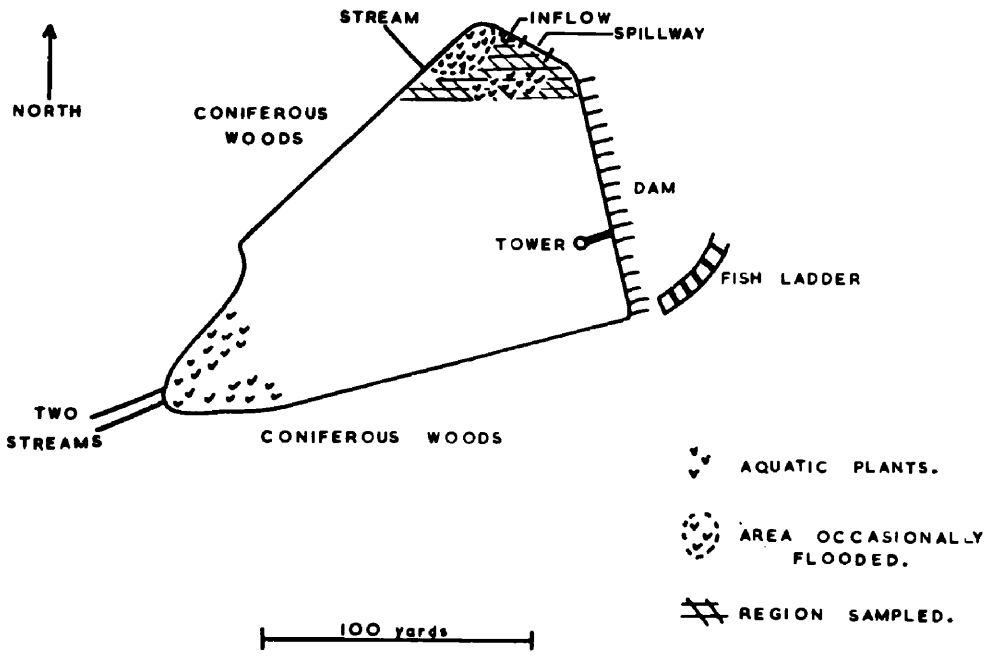
Introduction 2.

The loch at Lennox Castle is at an altitude of approximately 600 feet and lies 10 miles to the North of Glasgow, just to the South of the Campsie Hills from which it is separated by a valley. The loch was formed by the building of a dam across a V-shaped valley, the slopes of which are planted with coniferous trees. The maximum depth of the loch is 16 feet. The loch covers an area of approximately 2.5 acres.

There are three inflows associated with the loch although two of them carry a small volume of water in comparison with the third. The main inflow is a pipe, 6" in diameter, carrying water from the Campsie Hills. This water is screened before entering the loch. Two systems of drainage ditches feed the loch, the water passing through silt traps before entering the loch. The ditches drain the wooded slopes and a neighbouring moor to the West and North.

The main outflow is the withdrawal of approximately 10,000 gallons of water per day for use in Lennox Castle Hospital, the water being withdrawn at a tower built out from the dam. There is a spillway but it functions only after prolonged rain. At the opposite end of the loch from the dam, the fish ladder opens but no fish have been observed in the open part of the ladder beside the dam. A small sluice ensures a flow of water from the loch down the ladder/

LENNOX CASTLE RESERVOIR.



/to a stream.

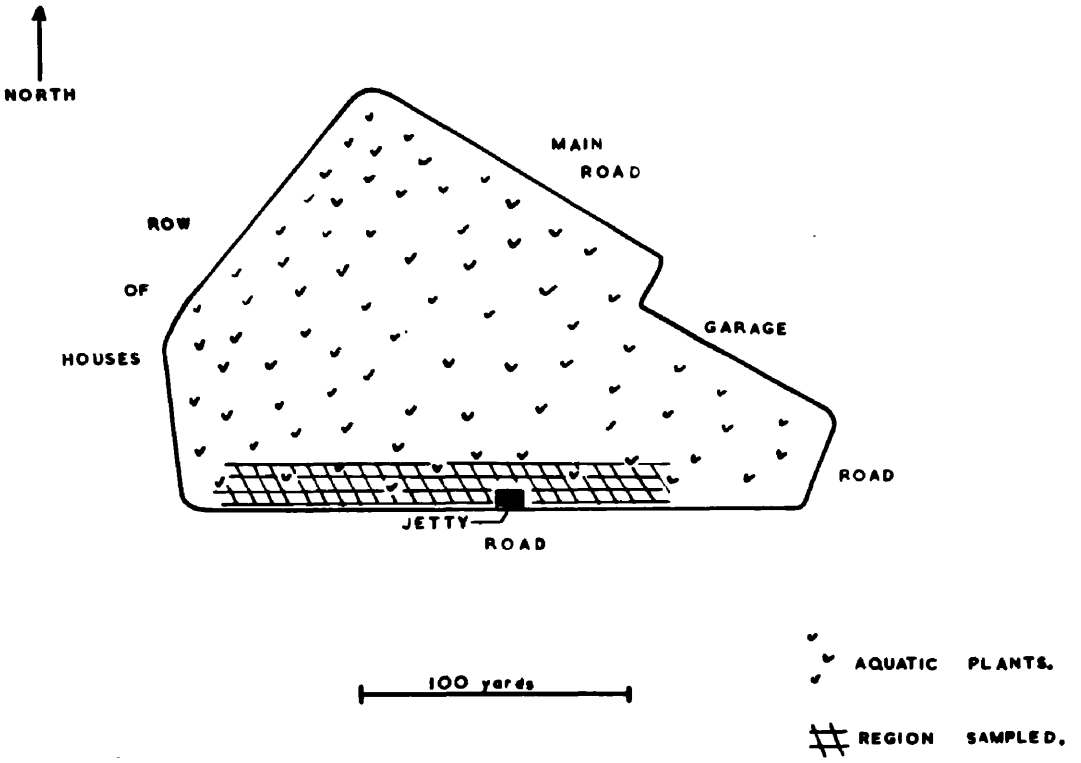
The bottom of the loch appears to be silt on a firm base. There are several banks of dense weed in the loch, their position being marked in the sketch map. These banks of weed appear to be mainly Elodea canadensis.

The loch beside Great Western Road within the west-end of Glasgow is referred to as Bingham's (after its former owner). It is at an altitude of approximately 50 feet. The loch appears to be hollowed out of the ground, the banks of the loch rising 6-10 feet above the level of the water. It is believed the water cannot be more than 4-5 feet in depth but the presence of a thick layer of mud on the bottom makes depth determination difficult. The loch covers an area of approximately 4.5 acres. This loch due to its shallowness freezes rapidly in the winter.

On two sides of the loch there are roads, a row of houses and a large garage complete the surrounds. There is always a sooty deposit in the surface of the loch but in the last three months of the survey, sewage fungus was found adjacent to the row of houses. This was the first sign of serious contamination.

There are no obvious inlets and outlets associated with the loch so it is possible that drainage may be down through the soil, rain being the source of water; during a prolonged dry-spell the level drops considerably./

BINGHAM'S LOCH.



/There is a dense growth of vegetation throughout the loch, consisting of various forms of algae as well as Elodea canadensis.

B. Material and Methods

The tapeworm *S. solidus* occurs as a natural infection in both lochs examined. The 3-spined stickleback *G. aculeatus* was the sole fish host of this parasite in the lochs although loach (*Nemachilus barbatulus*) occurs at Lennox Castle.

The stickleback population was sampled from February 1961 to December 1963 at Lennox Castle and from March 1962 to January 1964 at Bingham's. Initially, samples of at least 200 fish were taken from both lochs but during 1963, difficulties in catching the fish caused the sample size at Bingham's to be reduced to approximately 100 fish.

The fish were caught by hauling a light-weight beam trawl, 4 feet wide, 2 feet deep and 8 feet long about 6 feet from the shore or across a corner. The net was allowed to sink almost to the bottom before being slowly hauled across the loch. At Bingham's, the net was towed 3-4 feet from the shore. During the summer months 1-4 hauls produced the required number of fish but during the winter, many more hauls were required.

The same regions of the lochs were sampled each time but it is uncertain how random the samples were and it is certainly not known if the same group of fish were sampled each time. Migrations of fish to and from deeper water may have occurred, particularly at times of breeding and/

/with variations in the level of the loch.

During the winter months, ice often had to be broken before the commencement of fishing but as far as possible, even under these conditions, the net was hauled across the same region of the loch, if necessary under ice. The temperature of the lochs was recorded at most visits.

Fish were brought back alive to the laboratory and maintained in aquaria equipped with fresh-running water and aeraters. The fish were killed by pithing, the length from the mouth to the tip of the tail measured and a median ventral incision extending from the origin of the pectoral fins to just anterior to the anus made in the body wall. Any plerocercoids present in the body cavity were removed, touched on filter paper to remove excess moisture and placed on a piece of aluminium foil. The foil and plerocercoid was weighed, the plerocercoid removed and the foil reweighed. Examination of the fish was always completed within 3 days of the visit to the loch.

The plerocercoids removed from the survey fish were used for the life-cycle and culture work reported elsewhere in this thesis.

C. Results

(i) Length frequency distribution of Gasterosteus aculeatus Lennox Castle

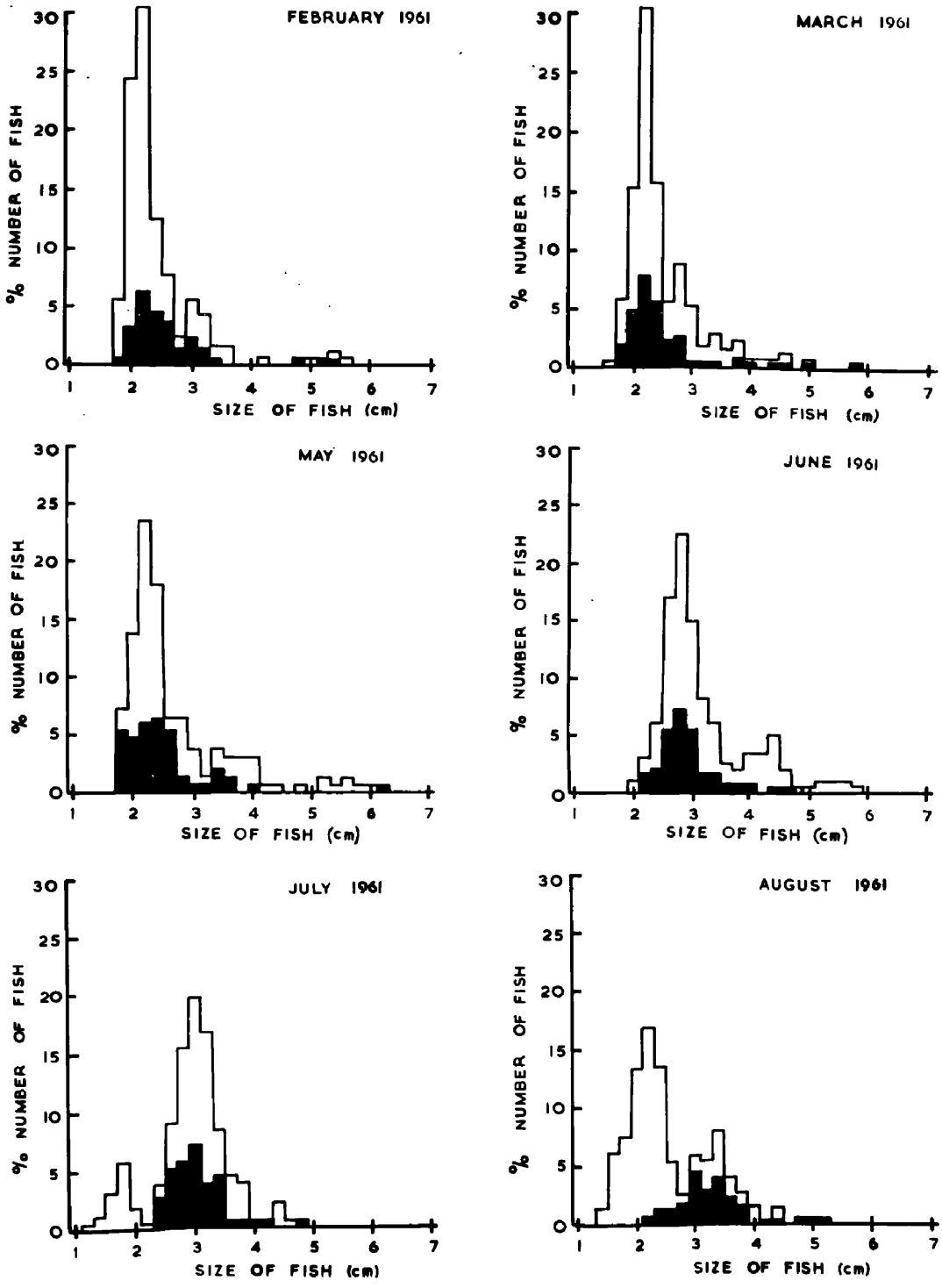
The monthly length frequency distribution of the three-spined stickleback is shown in Figure 11. The ordinate represents the number of fish of each length frequency caught, expressed as a percentage of that total sample with the abscissa representing the length frequency. Each length group is 2 mm. The black area represents the distribution of the plerocercoid stage of S. solidus in the fish population.

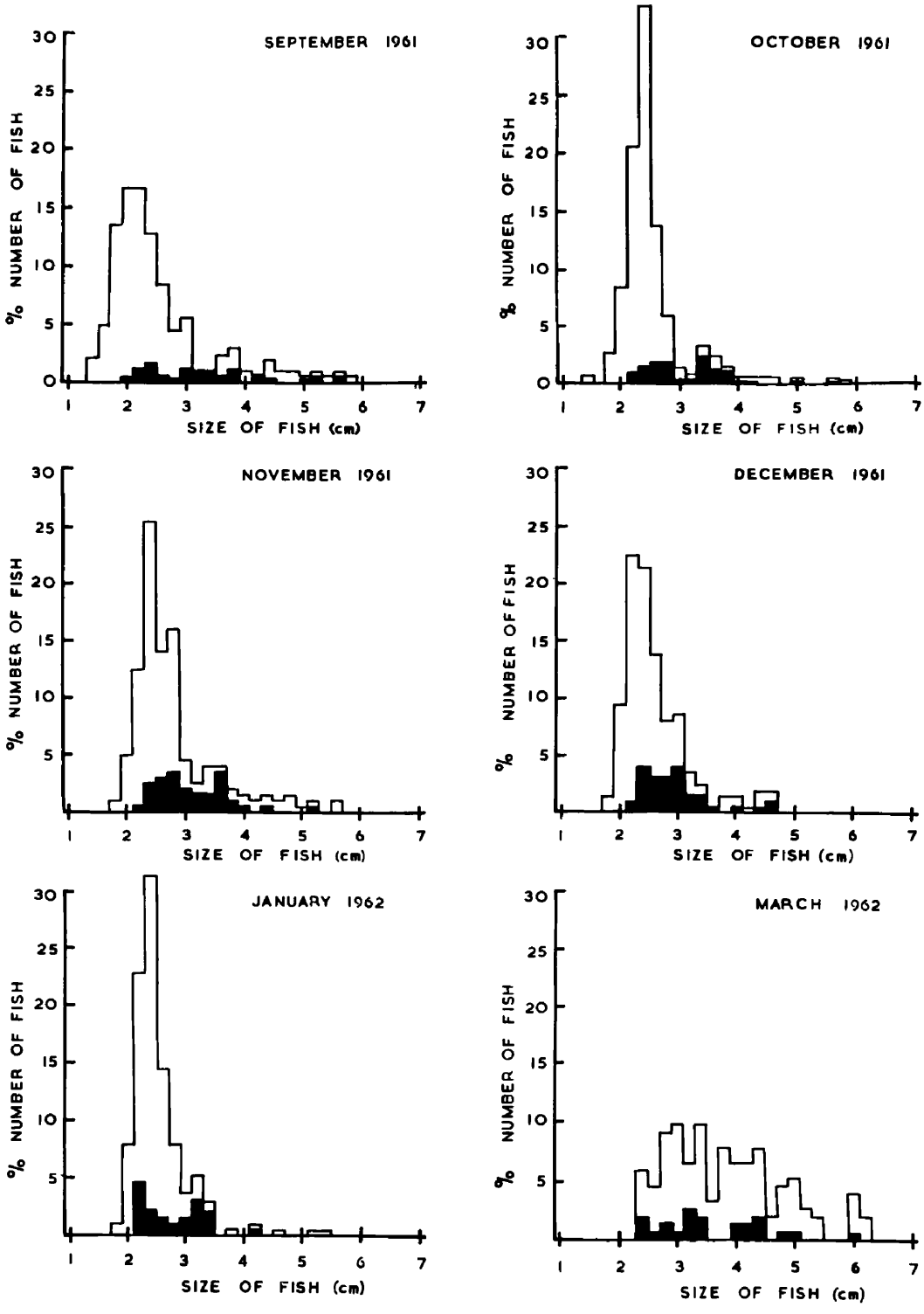
In the July samples of 1961 and 1962, newly-hatched fish first appeared, initially few in number, but increasing rapidly until, in 1961, they constituted the majority of the sample by September. In 1962, the newly-hatched fish were not recognisable as a distinct frequency range after the first month. In 1963, newly-hatched fish were first recorded in the August sample, their number increasing rapidly in the following months. The newly-hatched fish formed recognisable groups until September in 1961 and November in 1963.

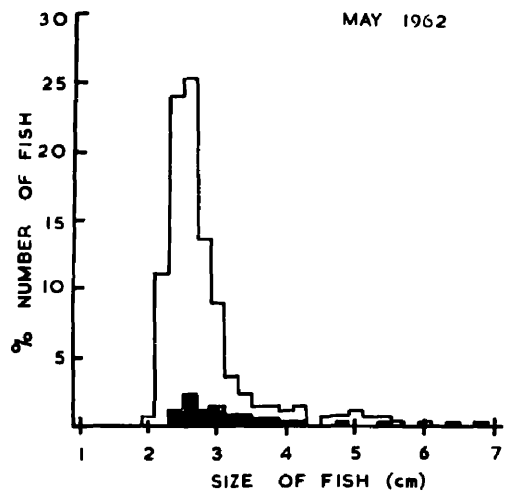
The newly-hatched fish had a mean length of 1.7 cm., with a range of 1.0 to 2.0 cm. in July 1961, increasing to 2.1 cm. in September with a range of probably 1.4 to 2.8 cm. A similar estimation was not possible for the 1962 survey/

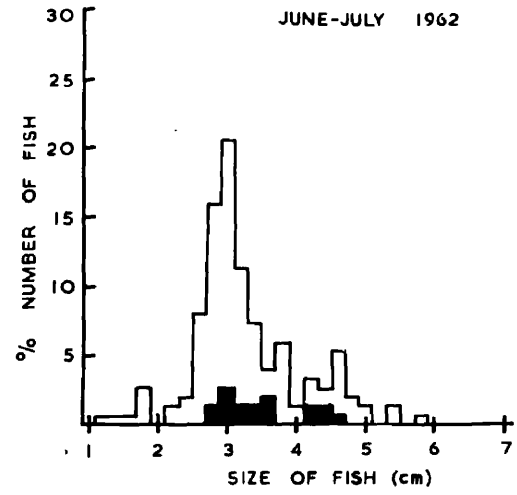
Figure 11

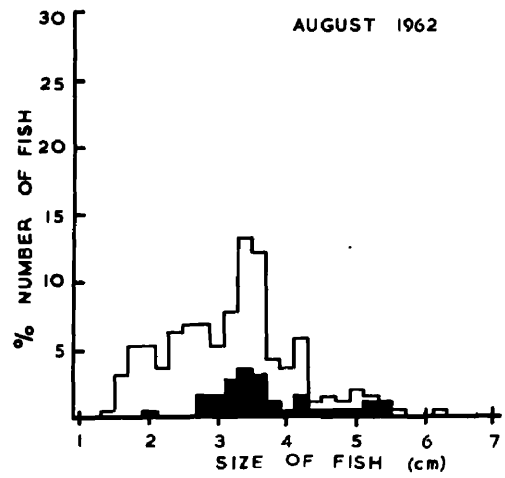
The length frequency distribution of the three-spined stickleback in Lennox Castle Reservoir. The ordinate represents the number of fish of each length caught expressed as a percentage of the total sample. The abscissa is the length frequency. The distribution of the plerocercoid stage in the fish population is denoted by the black area.

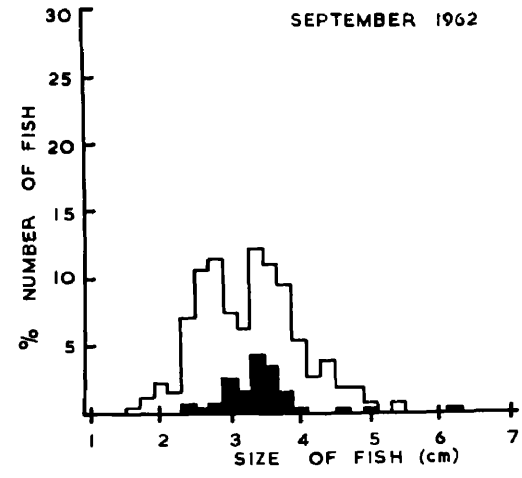

FEBRUARY 1961
MARCH 1961
MAY 1961
JUNE 1961
JULY 1961
AUGUST 1961
% NUMBER OF FISH
SIZE OF FISH (cm)
0
5
10
15
20
25
30
1
2
3
4
5
6
7

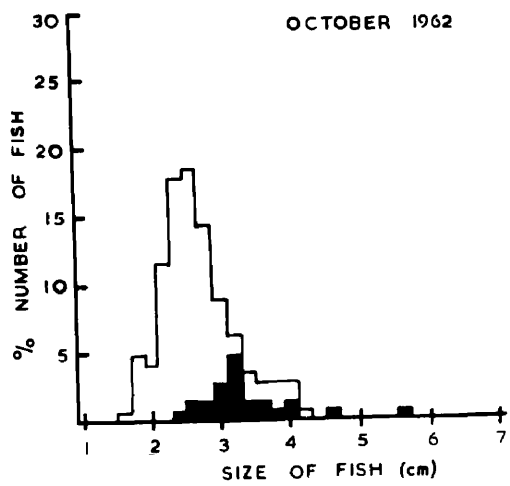

SEPTEMBER 1961
OCTOBER 1961
NOVEMBER 1961
DECEMBER 1961
JANUARY 1962
MARCH 1962
% NUMBER OF FISH
SIZE OF FISH (cm)
0
5
10
15
20
25
30
1
2
3
4
5
6
7

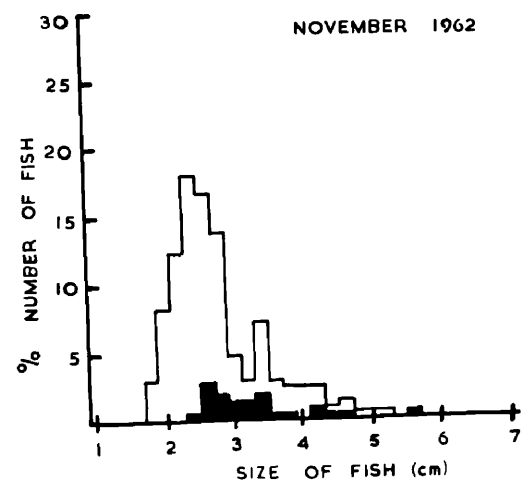

MAY 1962
% NUMBER OF FISH
SIZE OF FISH (cm)

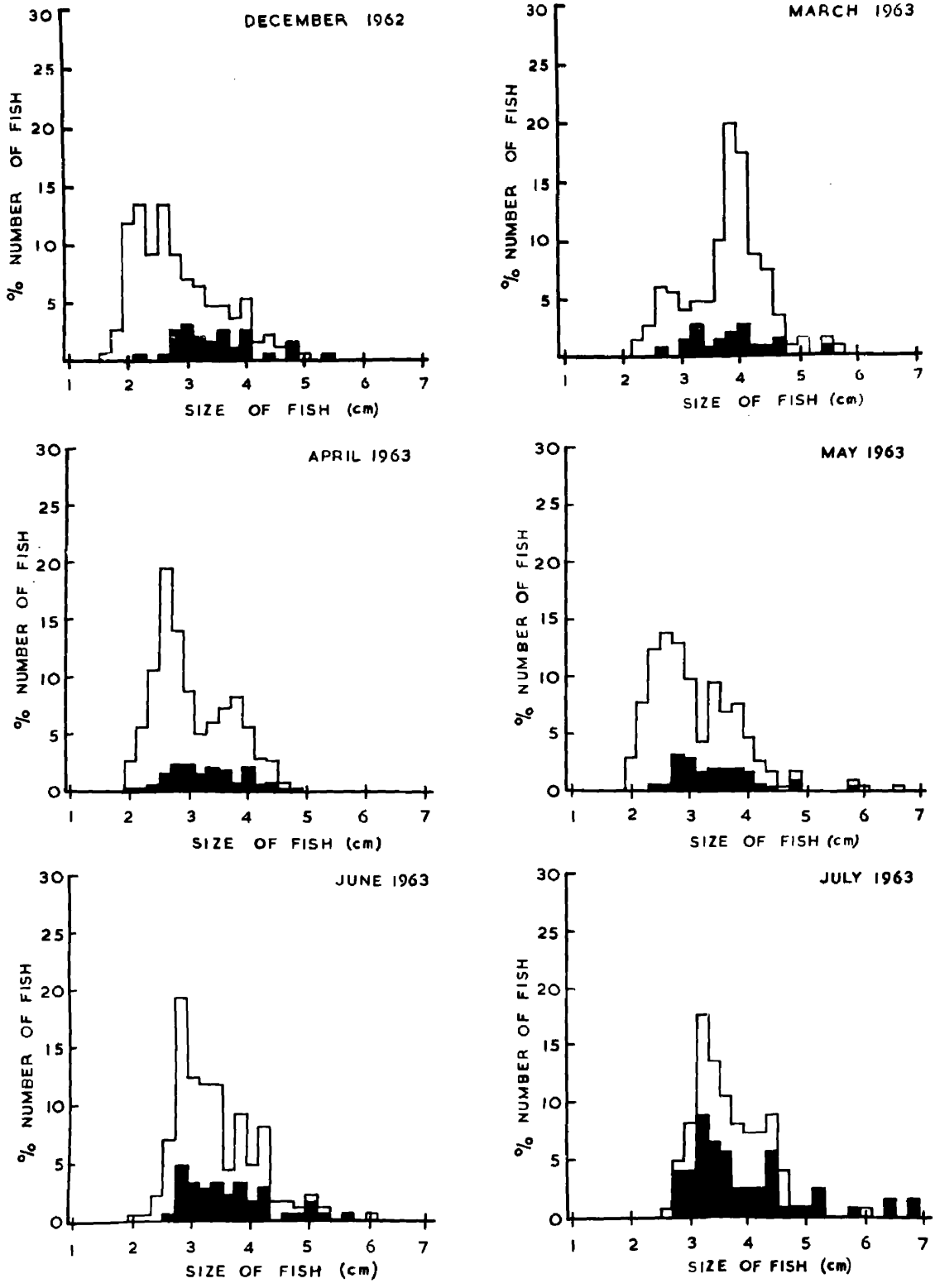

JUNE-JULY 1962
% NUMBER OF FISH
SIZE OF FISH (cm)

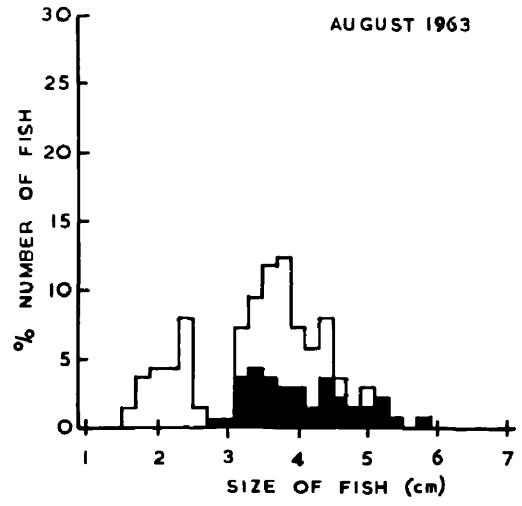

AUGUST 1962
% NUMBER OF FISH
SIZE OF FISH (cm)

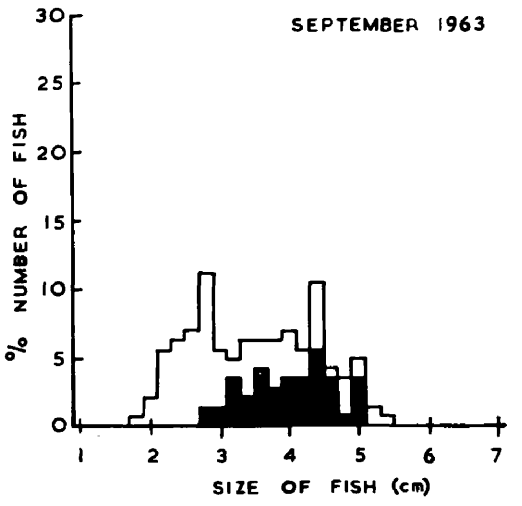

SEPTEMBER 1962
% NUMBER OF FISH
SIZE OF FISH (cm)

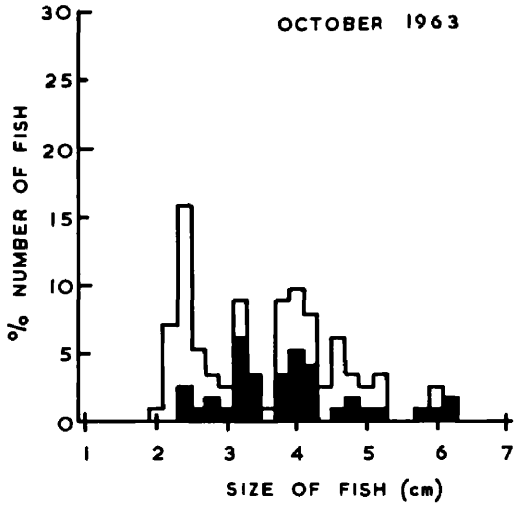

OCTOBER 1962
% NUMBER OF FISH
SIZE OF FISH (cm)

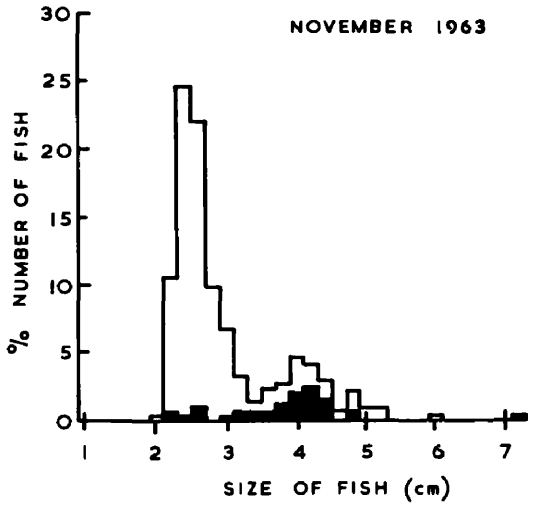

NOVEMBER 1962
% NUMBER OF FISH
SIZE OF FISH (cm)


DECEMBER 1962
MARCH 1963
APRIL 1963
MAY 1963
JUNE 1963
JULY 1963
% NUMBER OF FISH
SIZE OF FISH (cm)
0
5
10
15
20
25
30
1
2
3
4
5
6
7


AUGUST 1963
% NUMBER OF FISH
SIZE OF FISH (cm)


SEPTEMBER 1963
% NUMBER OF FISH
SIZE OF FISH (cm)


OCTOBER 1963
% NUMBER OF FISH
SIZE OF FISH (cm)


NOVEMBER 1963
% NUMBER OF FISH
SIZE OF FISH (cm)

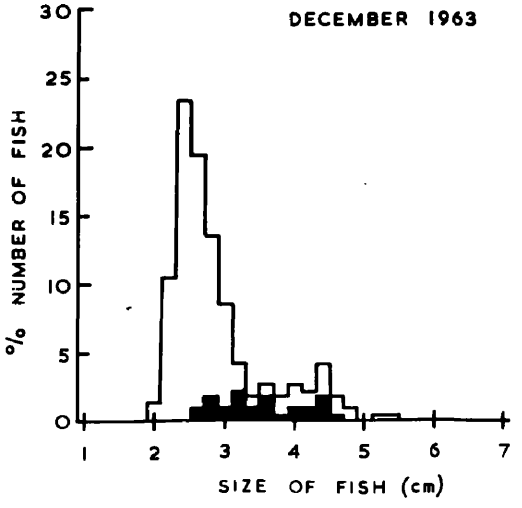

DECEMBER 1963
% NUMBER OF FISH
SIZE OF FISH (cm)

Table 17

The occurrence of three size groups of fish expressed as a percentage of the total fish caught in Lennox Castle Reservoir

Length of fish (cm)	Length frequency distribution of fish		
	1961	1962	1963
< 3.0	73.6%	56.4%	44.6%
3.0- <4.0	19.9%	29.7%	33.5%
⩾ 4.0	7.2%	14.1%	21.9%

/In 1963, the new fish had a mean length of 2.2 cm., ranging from 1.6 to 2.6 cm. in August increasing to 2.5 cm. in November with a range of probably 2.0 to 3.0 cm. Figure 11 indicates that Schistocephalus infections exist in the stickleback host all the year round.

The number of sticklebacks caught per year within each of three length frequencies was determined and expressed as a percentage of the total number of fish caught. The three length groups used were <3.0 cm., 3.0-<4.0 cm. and ≥4.0 cm. (Table 17).

In the three years of the survey, the majority of the fish caught were less than 3.0 cm. in length although in 1963 the proportion of small fish was markedly lower than in the previous two years. Large fish viz. those greater than 4.0 cm. in length always formed the smallest group. During the years of the survey there was a tendency for a decrease in the proportion of small fish caught with a corresponding increase in the proportion of the other two groups.

Bingham's

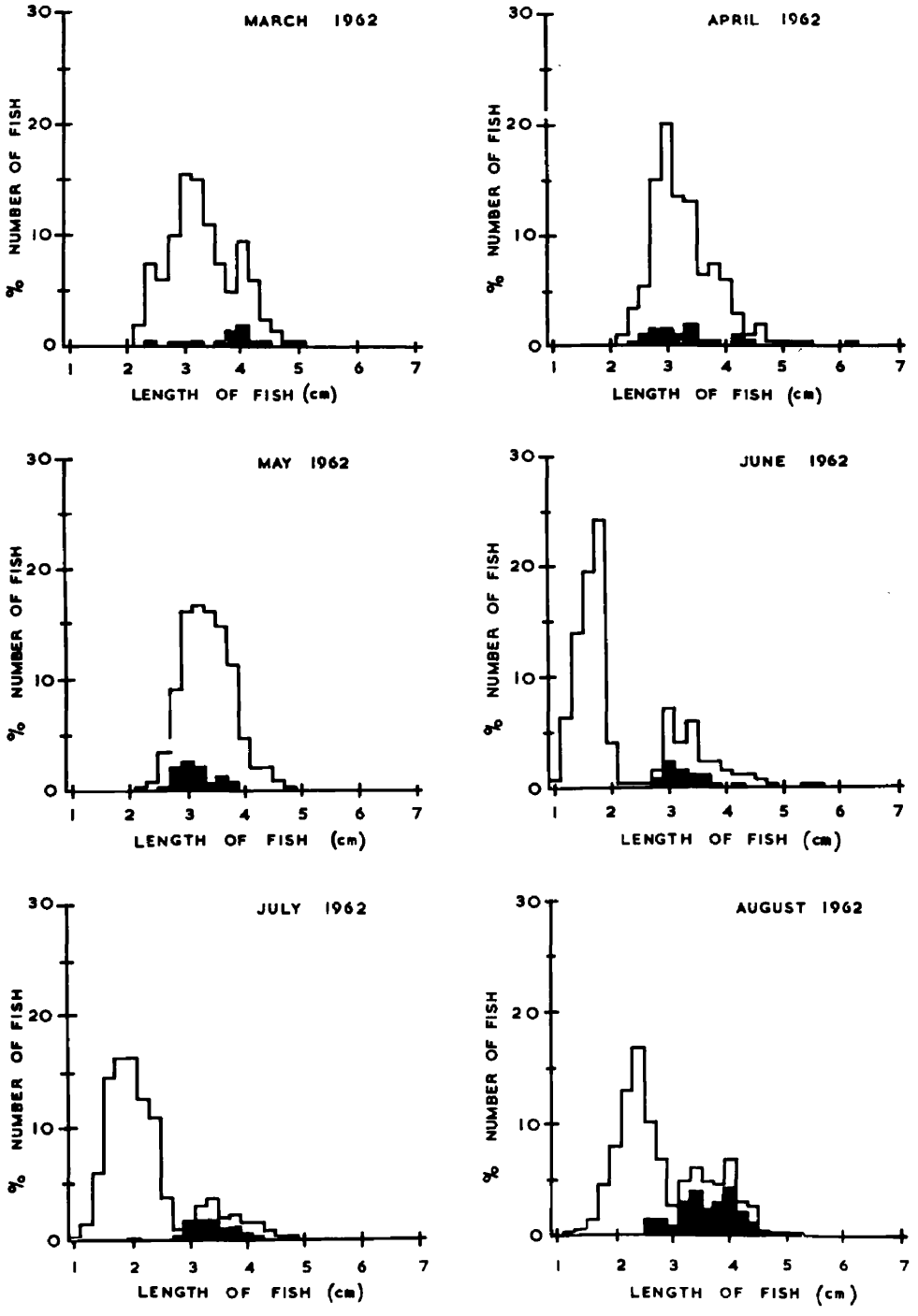
The monthly length frequency distribution of the three-spined sticklebacks and the tapeworm infection are shown in Figure 12.

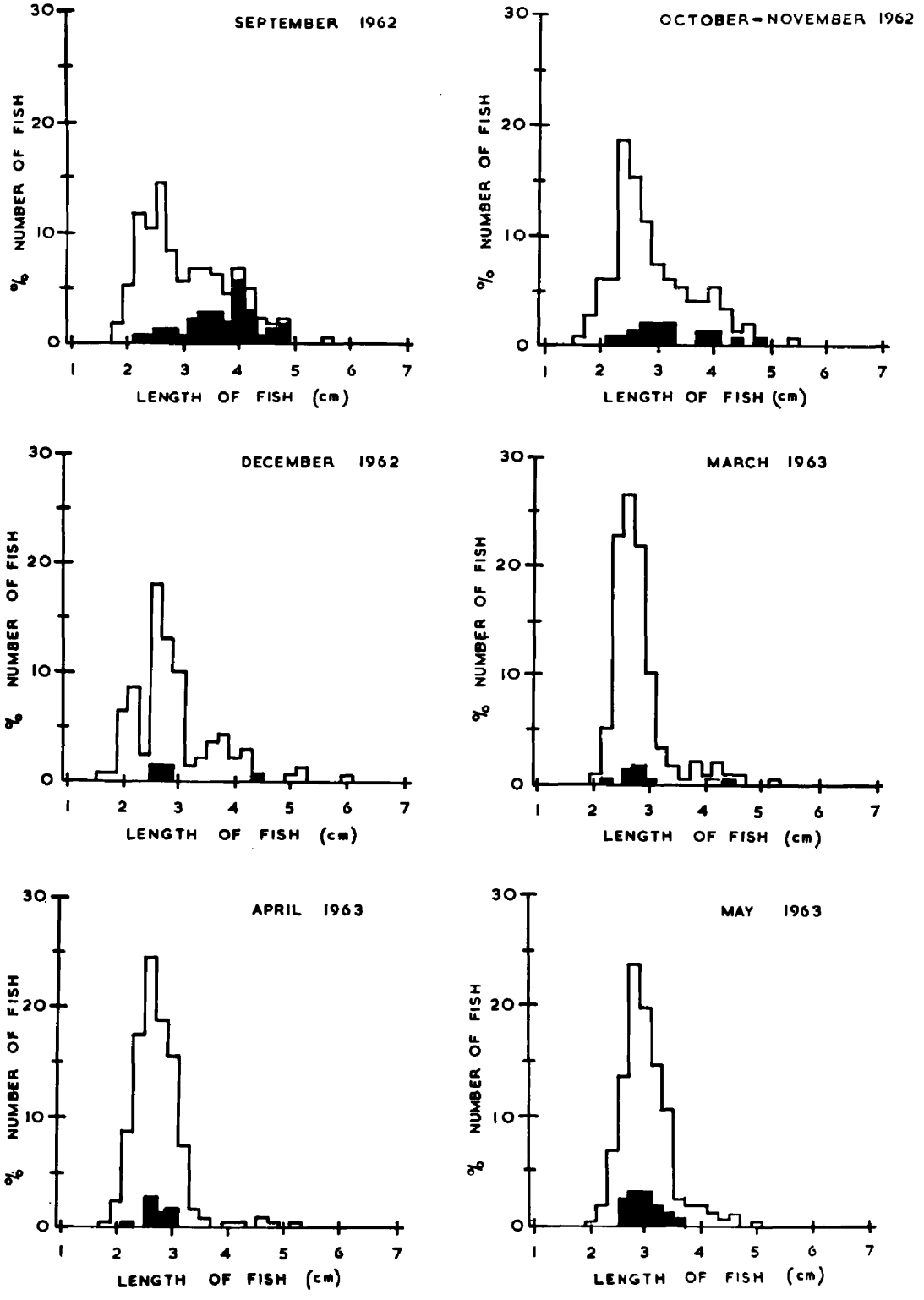
Newly-hatched fish appeared initially in June of both years of the survey. In 1962, the new fish immediately/

Figure 12

The length frequency distribution of the three-spined stickleback in Bingham's Loch.

The ordinate represents the number of fish of each length frequency caught expressed as a percentage of the total sample. The abscissa is the length frequency The distribution of the plerocercoid stage in the fish population is denoted by the black area.


MARCH 1962
APRIL 1962
MAY 1962
JUNE 1962
JULY 1962
AUGUST 1962
% NUMBER OF FISH
LENGTH OF FISH (cm)
0
10
20
30
1
2
3
4
5
6
7


SEPTEMBER 1962
OCTOBER-NOVEMBER 1962
DECEMBER 1962
MARCH 1963
APRIL 1963
MAY 1963
% NUMBER OF FISH
LENGTH OF FISH (cm)
0
10
20
30
1
2
3
4
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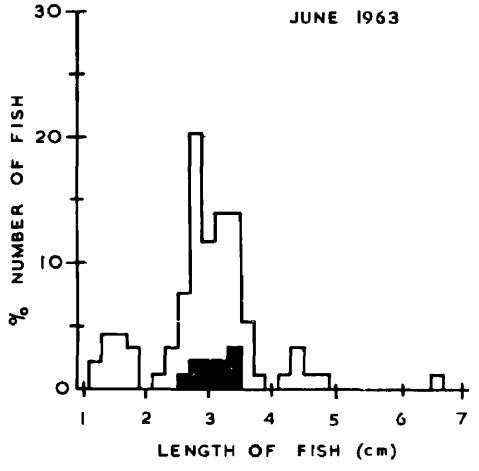
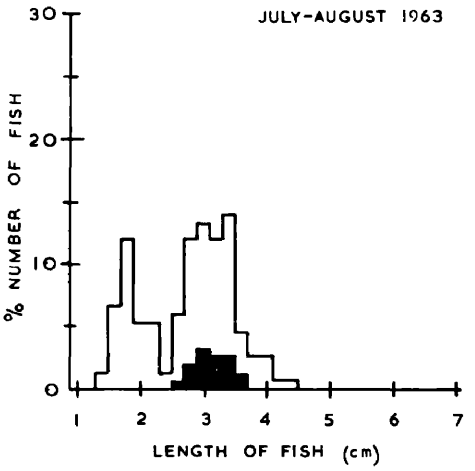
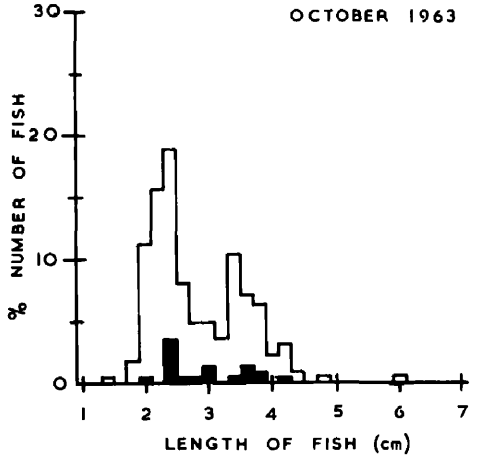
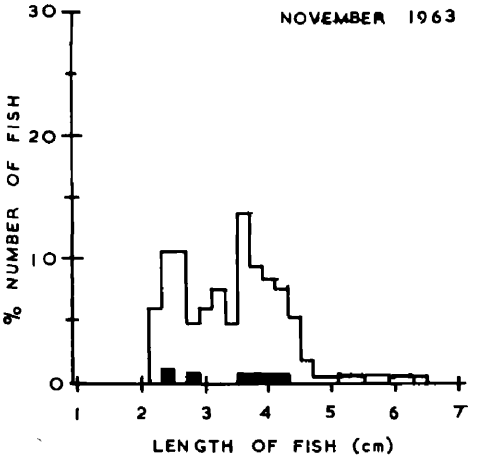
6
7


JUNE 1963
% NUMBER OF FISH
LENGTH OF FISH (cm)


JULY-AUGUST 1963
% NUMBER OF FISH
LENGTH OF FISH (cm)


OCTOBER 1963
% NUMBER OF FISH
LENGTH OF FISH (cm)


NOVEMBER 1963
% NUMBER OF FISH
LENGTH OF FISH (cm)

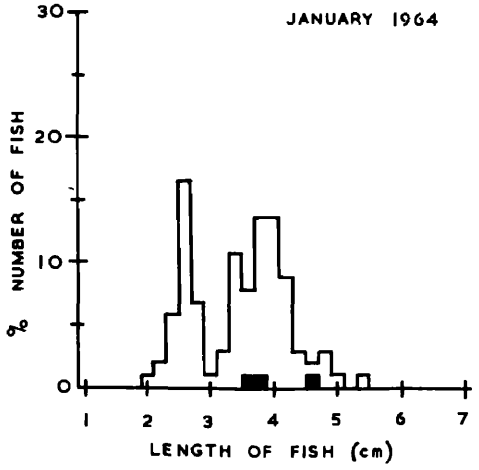

JANUARY 1964
% NUMBER OF FISH
LENGTH OF FISH (cm)

Table 18

The occurrence of three size groups of fish expressed as a percentage of the total fish caught in Bingham's Loch

Length of fish (cm)	Length frequency distribution of fish	
	1962	1963
< 3.0	55.5%	55.0%
3.0 - < 4.0	33.2%	35.9%
≥ 4.0	11.3%	9.2%

/constituted the majority of the sample whereas in 1963, their numbers were initially small, increasing till October when possibly they constituted the majority of the sample. A mean length of 1.6 cm. with a range of 1.0 to 2.0 cm. was recorded for the new fish in June 1962 increasing to 2.3 cm. with a range of probably 1.2 to 3.0 cm. in August. In 1963, a similar increase in length was recorded despite the difference in numbers of newly-hatched fish caught.

The number of sticklebacks in each of the three length frequencies was expressed as a percentage of total catch of fish as described for Lennox Castle. The results so obtained are shown in Table 18.

As at Lennox Castle, the majority of the fish were less than 3.0 cm. in length, accounting for 55% of the fish caught both years. Large fish, however, only accounted for 9-11% of the fish caught. In the two years of the survey, the population of the fish in each size group remained remarkably constant.

(ii) Incidence of Schistocephalus solidus in G. aculeatus Lennox Castle

The number of infected fish in each of the three length groups, <3.0 cm., 3.0 - <4.0 cm. and ⩾ 4.0 cm. was expressed as a percentage of the total fish caught in each group (Table 19).

Fish less than 3.0 cm. in length showed the lowest/

Table 19

The incidence of infection at Lennox Castle Reservoir, calculated by expressing the number of infected fish in each size group as a percentage of the total fish of that size caught

Length of fish (cm)	Incidence of infection		
	1961	1962	1963
< 3.0	17.1%	7.2%	7.4%
3.0 - < 4.0	40.3%	27.8%	31.4%
≥ 4.0	23.7%	22.2%	38.1%

/incidence of infection of the three groups although in 1961, a slightly higher incidence (17%) than in the other two years (7%) was recorded. In 1961 and 1962, the highest incidence was found in fish 3.0 - <4.0 cm. in length but in 1963, the highest incidence occurred in fish ≥ 4.0 cm. in length.

For each month of the survey, the number of infected fish was expressed as a percentage of the total fish caught in that month. The results are plotted in Figure 13b, the ordinate representing the percentage of infected fish and the abscissa the months of the years. In Figure 13a, the ordinate represents the approximate number of newly-hatched fish expressed as a percentage of the total monthly catch.

In 1961, the monthly incidence increases from 24% in February to 40% in May then decreasing to 31-32% in June, July and 11% in September. In November and December, the incidence rose to 21%. A gradual decrease in incidence for the period January to May was recorded in 1962. The incidence from August to December fluctuated in the range 13-20%. From January to May 1963, the incidence fluctuated in the range 15-20% then rose to 50% in July. The incidence then dropped to 30% for the following three months and then dropped further to 11% in November.

In 1961 and 1963, the newly-hatched fish were initially few in number in July and August respectively. Their/

Figure 13a

The incidence of the infection in Lennox Castle Reservoir.

The ordinate represents the approximate number of newly-hatched fish expressed as a percentage of the total fish and the abscissa is the time scale in months.

Figure 13b

The occurrence of infected fish in Lennox Castle Reservoir.

The ordinate represents the number of infected fish expressed as a percentage of the total fish and the abscissa is the time scale in months.

FIGURE a.

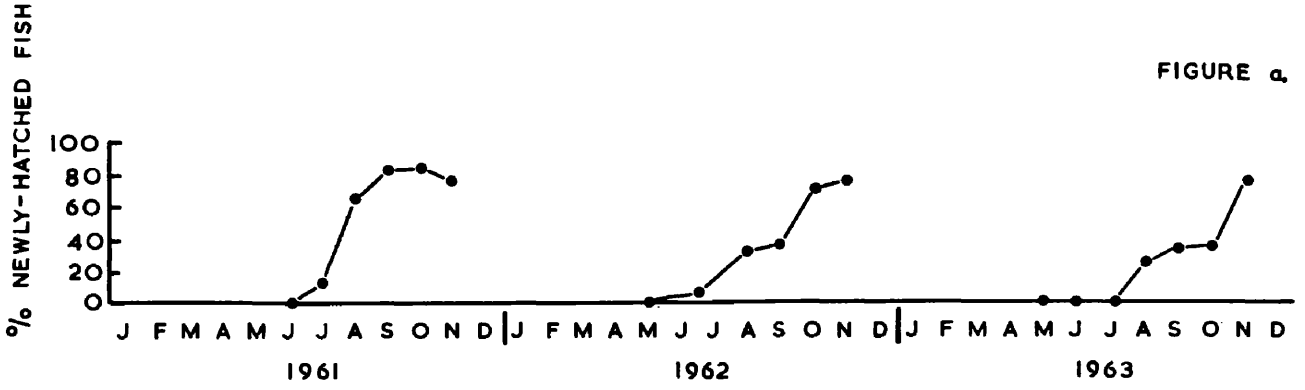
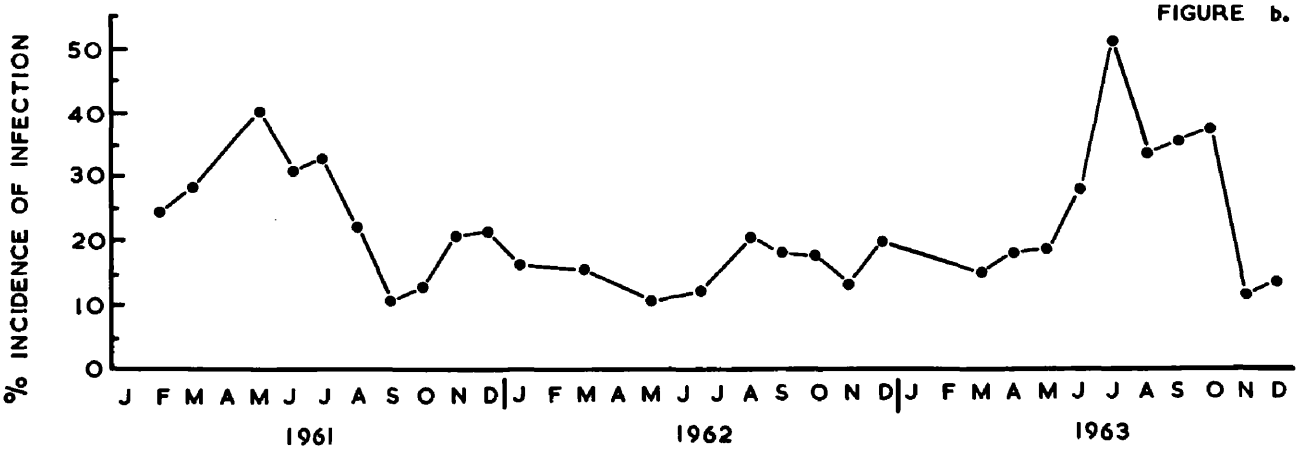


FIGURE b.



/numbers increased rapidly in 1961 reaching 60-80% of the total fish in August and September. This rise corresponded with a decrease in the incidence of the infection. In 1963, the new fish constituted 20-30% of the fish in August to October, increasing to 70% in November. The initial appearance of the new fish corresponded with the first drop in incidence and in November a further increase in numbers of new fish and a decrease in incidence occurred.

Bingham's

The incidence of the infection relative to the size of the fish was determined as for Lennox Castle, and the results obtained are shown in Table 20.

In 1962, as at Lennox Castle, the smallest fish had the lowest incidence of infection. With increase in size of fish to the largest fish, there was an increase in the incidence. The incidence in 1963 was lower than in 1962 and hence variations in the distribution of the infection relative to fish size were less marked. However, there was a definite decrease in the incidence of the infection in fish greater than 4.0 cm. in length.

The percentage of newly-hatched fish caught each month and the monthly incidence of infection were determined and are shown in Figures 14a and 14b respectively.

The incidence of infection was approximately 10% from/

Table 20

The incidence of infection at Bingham's Loch, calculated by expressing the number of infected fish in each size group as a percentage of the total fish of that size caught

Length of fish (cm)	Incidence of infection	
	1962	1963
< 3.0	3.8%	6.7%
3.0 -<4.0	22.0%	11.2%
⩾ 4.0	29.2%	4.3%

Figure 14a

The incidence of the infection in Bingham's Loch.

The ordinate represents the approximate number of newly-hatched fish expressed as a percentage of the total fish and the abscissa is the time scale in months.

Figure 14b

The occurrence of infected fish in Bingham's Loch.

The ordinate represents the number of infected fish expressed as a percentage of the total fish and the abscissa is the time scale in months.

FIGURE a.

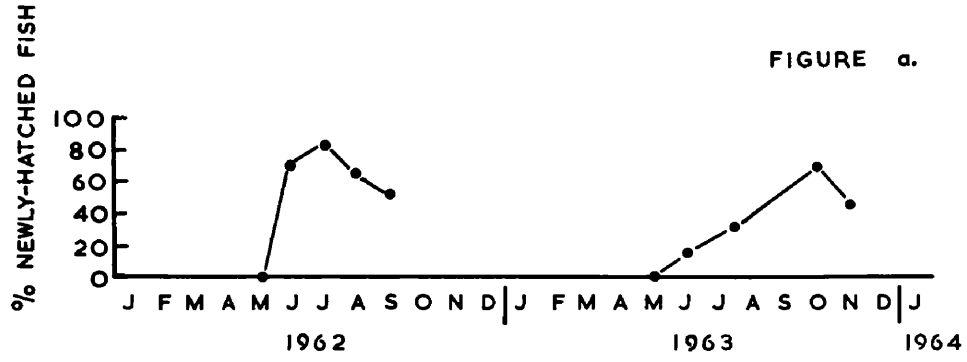
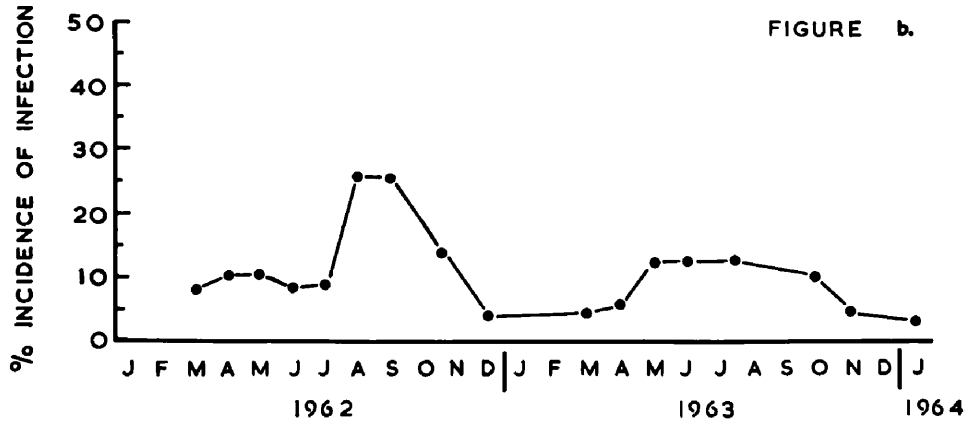


FIGURE b.



/March to July 1962, increasing to 25% in August and September before dropping to 4% in December. During 1963, the incidence increased to 12% in May then dropping to 3% in the following January.

Newly-hatched fish appeared in June of both years constituting 70% of the monthly catches in June and July 1963 and then appearing to drop to 50% of the fish. In 1963, however, the new fish increased to 30% of the fish in August and 70% in October.

(iii) Intensity of the infection

Lennox Castle

For each month of the survey the average worm burden i.e. $\frac{\text{total number of worms}}{\text{number of infected fish}}$ was calculated. Mean intensities of 1.8, 1.4 and 1.6 were obtained for 1961, 1962 and 1963 respectively. Table 21 is a record of the monthly intensities of the infection.

Bingham's

The average worm burden or intensity of infection was calculated for each month. Mean intensities of 1.4 and 1.1 for 1962 and 1963 respecitvely were obtained. The monthly intensities are recorded in Table 22.

(iv) Weight distribution of the plerocercoids

Lennox Castle

The weights of all plerocercoids removed from live survey/

Table 21

The average number of plerocercoids found in G. aculeatus in Lennox Castle Reservoir

	Average worm burden		
	1961	1962	1963
January	-	1.1	-
February	2.2	-	-
March	2.0	1.5	1.3
April	-	-	1.5
May	2.1	1.2	1.4
June	2.2	1.4	1.6
July	1.8		1.8
August		1.5	1.6
September	1.6	1.2	2.4
October	1.3	1.4	1.5
November	1.8	1.5	1.8
December	1.5	1.4	1.4

Table 22

The average number of plerocercoids found in G. aculeatus in Bingham's Loch

	Average worm burden		
	1962	1963	1964
January	-	-	1.0
February	-	-	-
March	1.9	1.0	-
April	1.4	1.1	-
May	1.5	1.1	-
June	1.2	1.2	-
July	1.8	1.1	-
August	1.3		-
September	1.2	-	-
October	1.0	1.2	-
November		1.4	-
December	1.0	-	-

/fish were recorded and the plerocercoids divided into four groups based on their weights, the groups being as follows:-

(i) those < 5 mg. in weight i.e. unsegmented plerocercoids

(ii) those 5-10 mg. in weight i.e. plerocercoids undergoing segmentation

(iii) those 10-30 mg. in weight i.e. infective plerocercoids with a low % "take" in a definitive host

(iv) those > 30 mg. in weight i.e. infective plerocercoids with a high % "take" in a definitive host (see Section I)

The number of plerocercoids of each group caught in a month was expressed as a percentage of the total worms found in that month. The yearly distribution of each size group of plerocercoids was then calculated and expressed as a percentage. In Figure 15 the ordinate represents the proportion of plerocercoids of each group found per month and the abscissa the months of the year. Figure 15 a) b) c) and d) records the results of the < 5mg., 5-10 mg., 10-30mg., and > 30 mg. plerocercoids respectively.

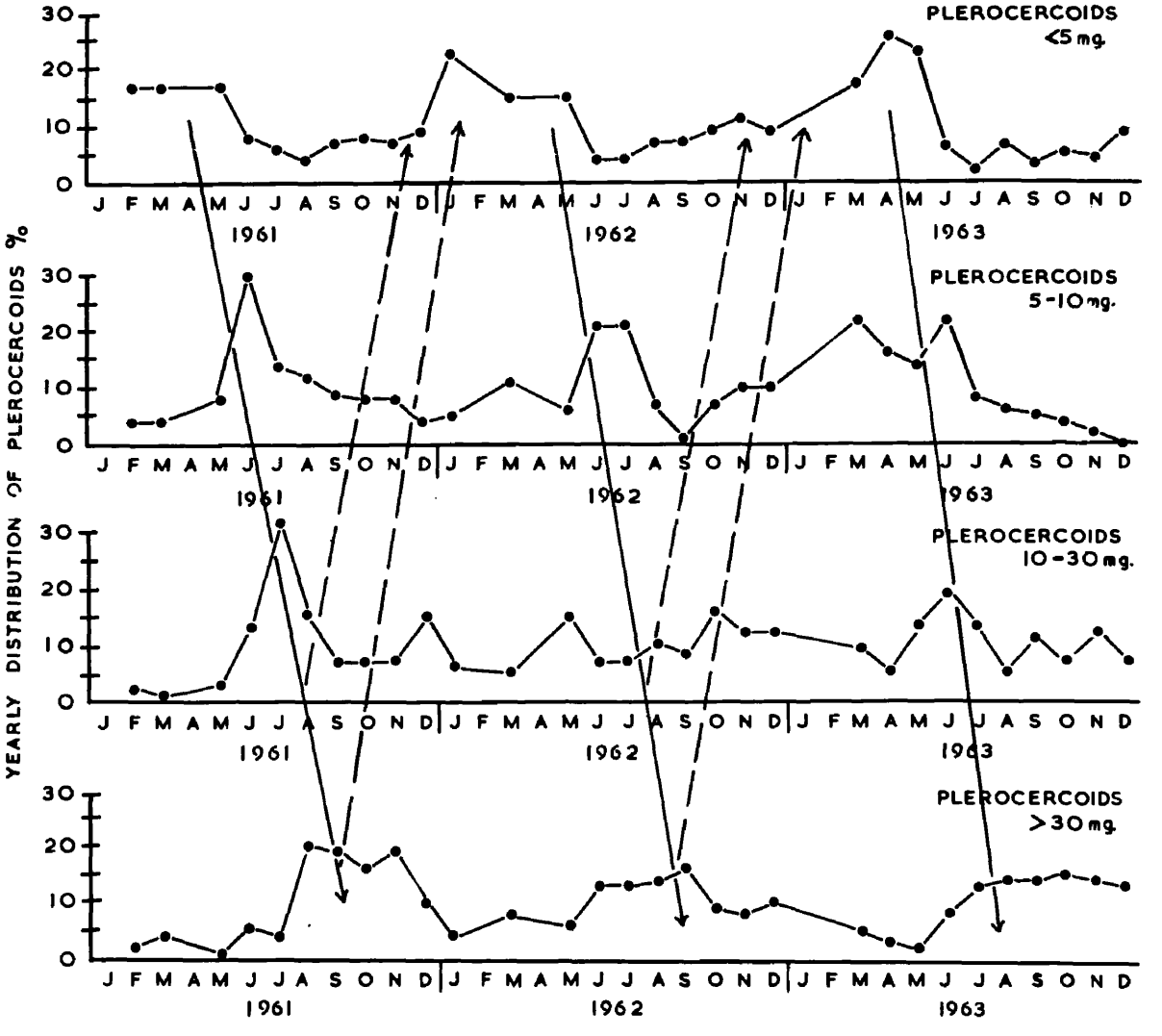
The peak in the yearly distribution of the unsegmented plerocercoids occurred during the period January to May in all three survey years. By June, their numbers were decreasing and from June to December, the proportion per month never exceeded 10% of the total yearly catch. However, unsegmented plerocercoids were found in the fish throughout the year.

Plerocercoids undergoing segmentation were also found

Figure 15

The occurrence of plerocercoids in the fish at Lennox Castle Reservoir.

The plerocercoids were divided by weight into four groups and the number of plerocercoids in each group was expressed as a percentage of the total worms caught in that month. The ordinate represents the occurrence of the four size groups of plerocercoids in the fish and the abscissa is the time scale in months.


YEARLY DISTRIBUTION OF PLEROCERCOIDS %
PLEROCERCOIDS <5 mg.
PLEROCERCOIDS 5-10 mg.
PLEROCERCOIDS 10-30 mg.
PLEROCERCOIDS >30 mg.
0
10
20
30
J F M A M J J A S O N D
1961
1962
1963

/throughout the year with a peak in the yearly distribution occurring in May to August in 1961 and 1962 but from January to July in 1963. At other periods of the year, fewer plerocercoids of this size were found each month, the decrease in numbers being very marked from July to December in 1963.

In the third size group, a definite peak was recorded in July 1961 but otherwise considerable fluctuations in distribution occurred. A peak was recorded in June-July in 1963, although not so clear as that in 1961 but in 1962 no definite peak appeared in the distribution graph.

Large plerocercoids were present in the fish throughout the year, being more abundant from August onwards in 1961 and from June-July in both 1962 and 1963.

The solid lines indicate the possible passage of plerocercoids from one size group to the next. Plerocercoids could attain a size of 10-30 mg. by July-August, after over-wintering in the fish, at which size a few would survive in a warm-blooded host to produce eggs. These eggs could hatch by August and September, produce infective copepods by September and October, and so give rise to the small unsegmented plerocercoids found in fish during the winter. Growth could be continued to > 30mg. when the possibility of survival in the definitive host would be greater. The dotted lines indicate the possibility of the/

/plerocercoids giving rise to the next generation of plerocercoids.

(b) Bingham's

During the survey of this pond, only small numbers of plerocercoids were found and hence a division of the worms identical to that used with the Lennox Castle plerocercoids was not done.

D. Discussion

Examination of the length frequency range of the newly-hatched fish indicates a gradual increase in the length range occupied by the fish in each month until overlapping with the length range of the older fish occurs. Initially the new fish form a separate group during which time at least 90% of the fish in that group are newly-hatched. As overlapping commences, the accuracy with which fish can be separated into their year groups solely by length diminishes and other methods, such as ageing by the number of rings in the otoliths (Jones and Hynes, 1950) are necessary.

The years' small fish first appear in June at Bingham's, with a mean size of 1.6 cm. At Lennox Castle, they are not found until July but it is probable from the size of the fish (mean 1.7 cm.) and the fact that the temperature increased (Table 23) in April [which Craig-Bennett (1931) states stimulates spawning some 6-8 weeks later] that spawning occurs during May. By August, the fish in both lochs have reached 2.0 - 2.1 cm. mean length. From September onwards, it is difficult to separate the year groups at Bingham's but at Lennox Castle, this year's fish appear from the shape of the histogram (Figure 11) to form a separate size group until at least November (1961 and 1962) and December (1963), though even in this population some fish have grown faster and been excluded./

Table 23

Water temperature recorded in two feet of water at Lennox Castle Reservoir

	Water remperatures (°C)		
	1961	1962	1963
January	-	0	-
February	-	-	-
March	-	-	-
April	-	-	6
May	13	-	12
June	13	-	17
July	16	-	16
August	16	-	15
September	17.7	-	14
October	17	-	9.5
November	13	-	9
December	0	-	5

/The difference in numbers of young fish caught in Bingham's loch in the two years of the survey (Figure 12) may be attributable to yearly variation in the population or the physical conditions prevailing in the loch at the time of sampling. In the second year of the survey, the loch level was lower than in the previous year and may have had an effect on the distribution of the fish. In March 1962 and 1963 at Lennox Castle, large fish were present in appreciable numbers although few in number in January and May of these years. The presence of these fish may be indicative of some spawning migration.

The disappearance of the largest fish i.e. those greater than 4.0 cm. in length, from the survey area may be due to several causes: Death from senility (sticklebacks can live for up to $3^{1}/2$ years [Jones & Hynes, 1950]), predation by birds or migration. The last two possible causes could explain the decrease in numbers of 3.0 - 3.8 cm. fish found. Small fish will be less obvious to terrestrial predators in shallow water than larger ones and hence small fish can remain in the shallow water which protects them, to some extent, from the cannibalistic attacks of larger fish reported by Walkey (1963).

Consideration of Tables 17-20 indicates a possible relationship between the size of the fish and the incidence/

/of the infection. Small fish, viz. those less than 3.0 cm. in length had the lowest infection rate in all years of both surveys. The incidence of infection in the next size group of fish showed a marked increase which was maintained to the largest fish (those 4.0 cm. and longer) in two years but in the other three years a decrease occurred.

A low rate of infection in the smallest fish could be due to several factors: the incidence of infection in the copepod intermediate host at a low level; few copepods in the lochs relative to other food organisms; or the relatively short time the fish have been eating infected copepods. Since the incidence of infection never exceeded 40% in any group of fish, it could be assumed that infection in the copepods is not 100% and is possibly much less. Prolonged exposure to copepods could thus result in a gradual increase in the incidence.

It was reported by Hynes (1950) that with increase in fish size, there was an increase in food size although small forms such as cyclops would still be taken if available. The alteration in the diet of the fish may limit the chance of eating infected copepods and thus be contributory in the limiting of the incidence of the infection. Selective elimination of heavily infected, and hence distended, fish by predators such as birds could also limit the incidence of infection in the larger fish. Clarke (1954) and Arme and Owen (1964)/

/reported that heavily parasitised fish were slow-moving. Arme also stated that these fish often swam at the surface of the water, behaviour which was also seen in both Lennox Castle and Bingham's fish. Such behaviour makes the fish more liable to predation.

Few fish were caught which harboured more than three large plerocercoids although a number containing up to six plerocercoids of less than 10 mg. were caught in both lochs. It has been suggested that the growth of several small plerocercoids in a fish may result in its death due to pressure or actual penetration of the organs but no evidence of this has been found. Competition for nutrients in a heavy infection could result in the death of some of the worms. However, plerocercoids in a moribund condition have never been found and Hopkins and Smyth (1951) and Vik (1954) record much heavier infections in fish. The presence of a very large and a very small plerocercoid in a number of fish caught in both lochs indicates that the acquisition of the infection by a fish does not prevent further worms entering and surviving in the fish. Competition for nutrients could not account for the size difference often found in a multiple infection of a fish. Hence, low worm burdens are probably due to a generally low level of infection in the lochs./

Consideration of Figures 13 and 14 shows that the infection is present in the fish throughout the year but there are fluctuations in the incidence of the infection. In a true seasonal cycle, there would be phases of infection, growth and maturation followed by release of eggs and subsequent loss of worms from the definitive host, as is found in tapeworms living in the intestine of fish [Miller (1943), Hopkins (1959) and Chubb (1963)]. A cycle of this nature could not be expected with Schistocephalus as the plerocercoids once established in the host, can only be lost by host reaction, since escape by rupture of the fish abdominal wall would result in the death of the fish (Vik, 1954). However, Walkey (1963) described a cycle of infection at Newcastle where the fish acquired the Schistocephalus infection during the summer. In the following spring, the plerocercoids grew to an infective size and heavy predation by ducks ensured a supply of eggs and hence infected copepods by late summer.

In the two Scottish populations, partial seasonal variation was observed in the incidence of the infection. Increase in water temperature in spring and the appearance of the newly-hatched fish will be two contributory factors. The monthly incidence of infection at Lennox Castle, but not at Bingham's, shows a definite relationship to the occurrence/

/of the newly-hatched fish. The incidence rises in spring and early summer in 1961 and 1963 but when the newly-hatched fish, and as yet uninfected fish, appear, there is sharp decrease in the incidence. In the same two years, there is a suggestion of an increase in incidence by September which could be due to the new fish acquiring the infection.

At Bingham's, the incidence remains at a low level until the number of new fish begins to decrease in August 1962 when an increase commences. It is suggested the earlier appearance of the new fish, about a month earlier than at Lennox Castle, masks a gradual increase in incidence in the fish more than a year old. The decrease in numbers of new fish or the acquisition of the infection by them allows the increase to be observed. The generally low infection rate in 1963 at Bingham's makes the interpretation of fluctuations in incidence for that year unsatisfactory.

Further evidence of a seasonal cycle is found in the monthly distribution of the four size groups of plerocercoids (Figure 15). Although unsegmented pleroceroids are present in fish throughout the year, they are predominantly found in the period December to May. It is suggested the acquisition of the infection is continuous throughout the winter period when, due to low temperatures, development has ceased (Dubinina, 1957) and viability times are at a maximum. This is true of pseudophyllidean eggs, procercoids/

/and plerocercoids. Examination of the gut contents of sticklebacks (Hynes, 1950) indicated that copepods are eaten by the fish throughout the year. Hence, if unsegmented plerocercoids are at a maximum during the winter, maximum infection in the copepods would occur in late summer and autumn to account for the unsegmented plerocercoids over winter. When the water temperature is 14-16°C, procercoid development could be completed within 4-6 weeks in a copepod (see Section I) and egg development would be complete within another 4-6 weeks (see Section I). Thus, under summer conditions, when the loch temperature is in the range 14-16°C, infective plerocercoids could give rise to the next generation of plerocercoids in 3-4 months.

The factors affecting a seasonal cycle will be complex, involving the two intermediate hosts and the definitive host of the parasite. The effect of temperature on some stages of the life cycle has been studied in the laboratory (see section on Life Cycle) but other factors such as length of light, level of the loch and most important, food chains, have not been investigated.

The rate of growth of plerocercoids in a natural infection cannot be determined accurately when the life cycle is not completely seasonal. Under laboratory conditions, Clarke (1954) obtained a 12-13 mm (≡10-15 mg.) plerocercoid from a/

/fish exposed to infected copepods seven weeks previously while Dubinina (1957) reported that plerocercoids were infective after 6 months, segmentation having commened at 5 weeks. The rate of growth of the plerocercoids at Lennox Castle has been studied in relation to its importance in the completion of the life cycle and not in absolute terms.

It is known that sticklebacks live for up to three and a half years (Jones and Hynes, 1951) and as Schistocephalus plerocercoids cannot be lost from the host once established, the larvae may thus live for up to three and a half years. However, large plerocercoids may affect the swimming of the fish and thus render them more liable to attack by predators. The presence of plerocercoids >30 mg. throughout the year indicates that the worms can live in fish for more than twelve months but no actual life span can be determined under natural conditions. The seasonal influx of worms from the group 10-30 mg. into the group > 30 mg. would account for the increase in numbers in that group in the latter half of the year.

In Figure 15, the possible growth of the plerocercoids through each of the size groups is indicated. From this figure it would seem probable that fish may acquire the infection during the autumn but little growth will occur during the winter months. With the onset of spring and the rise in water temperature, the growth of the plerocercoids/

/will be resumed. This is indicated by the increase in plerocercoids 5-10 mg. in weight during May to August and the July peak in plerocercoids 10-30 mg. in weight. The life cycle may thus make one complete turn in 12-14 months under natural conditions in central Scotland but the constant presence of large infective plerocercoids indicates that the cycle may take up to two to three years depending on the time the tapeworm spends as a plerocercoid. The winter cessation of growth under natural conditions will account for the longer time to attain infectivity from that reported by Dubinina.

E. Summary

1) Newly-hatched fish formed a distinct length group from July to November at Lennox Castle and from June to September at Bingham's. After this time, ageing of fish by length alone was unsatisfactory.

2) The disappearance of fish >4.0 cm. in length from the survey regions may be due to death from senility, attacks by predators or migration of these fish to deeper water.

3) Newly-hatched fish show the infection by September of the year in which they hatched.

4) Fish may acquire the infection during their second summer as indicated by the increase in the incidence of the infection before the appearance of the new fish.

5) Fish may be infected several times during their life span as is indicated by the co-existence of very large and small plerocercoids in the same fish; no immunity against the plerocercoids appears to be developed.

6) The infection is present in the fish population throughout the year.

7) Seasonal variation occurs in the incidence of the infection when expressed as a percentage infection rate in all the fish caught, the appearance of the newly-hatched, and as yet uninfected, fish being the main cause./

8) The incidence probably increases throughout the life span of a cohort of fish as demonstrated by the positive correlation between length of fish and percentage incidence.

9) Seasonal variation occurs in the distribution of the various sizes of plerocercoids, unsegmented plerocercoids being at a maximum over winter with the maxima of the 5-10mg. and 10-30mg. plerocercoids occurring in May to August and July onwards respectively. Plerocercoids greater than 30mg. are found throughout the year, an increase in their numbers in the second half of the year being due to the seasonal influx of worms growing from the 10-30mg. weight group.

10) Infective plerocercoids can be obtained in 6 months under laboratory conditions (Dubinina, 1957 and 1960) but in Scotland under natural conditions, infectivity is not attained for nearly twelve months.

11) The life cycle can be completed in 12-14 months under natural conditions but this may be extended to two to three years depending on the time the tapeworms spends in the plerocercoid stage.

Section III

The *in vitro* cultivation of the plerocercoid of *Schistocephalus solidus*.

A. Introduction

The conditions necessary for the maturation of *Schistocephalus solidus* in culture were investigated by Smyth (summarized 1959). As a result of his work, it became apparent that adult development was a matter of differentiation of tissue and the utilisation of endogenous reserves built during the plerocercoid stage; no growth occurs. (see under "Adult", Section I). Hence the growth requirements of this tapeworm must be investigated using the plerocercoid or earlier larval stages. In the work reported here, the plerocercoid larval stage was used.

In the initial work, a medium was assessed on its ability to induce a plerocercoid to increase its fresh weight but there were several disadvantages to this. As few figures for the rate of growth of *Schistocephalus* plerocercoids in fish existed, comparison of *in vivo* and *in vitro* results was impossible. Also, small increases in weight could be interpreted as growth when, in reality, they were only due to swelling of the tissues, a degenerative change. Hence, change in dry weight was used in the assessment of growth. The dry weight was known to be a constant proportion of the fresh weight of plerocercoids over 100 mg (Hopkins, 1950). The range of this earlier work was extended to determine the water content of worms down to 1 mg in weight./

However, an increase in dry weight need not indicate normal growth and, in the course of the work, other criteria have been introduced, including the appearance of the worm by eye, water and glycogen contents, ability to mature at 40°C and the ability to continue growth in culture for varying periods of time.

All these criteria involved the investigation of plerocercoids taken direct from fish. The results of these studies are reported and comparisons made with plerocercoids which have been cultured.

In culture work, it is necessary to establish the physical conditions under which a worm can survive before proceeding to investigate the nutrient requirements of the worm. A basal medium consisting of saline, glucose, yeast extract and horse serum was used, during the investigation of these physical conditions.

A carbohydrate, usually glucose, was included in most media but it was not known how necessary this was since two constituents of the basal medium, yeast extract and horse serum, have a carbohydrate fraction. In yeast extract, this fraction accounts for 13% of the yeast (Sykes, 1956) but its nature is unknown. Horse blood contains approximately 0.07% glucose (Spector, 1956). Thus to determine the effect of including a sugar in the medium, yeast extract and horse serum were omitted, the medium consisting solely of saline/

and the sugar.

Initially, glucose was the sugar used but later the effect of pentose and disaccharide sugars was measured as well as that of another hexose sugar.

A basal medium (Hanks's saline, glucose, yeast extract and horse serum) induced a certain level of growth in plerocercoids. It was not possible to compare this rate of growth with the few figures for *in vivo* growth. However, extra amino acids and vitamins were added to the basal medium to determine if, in the presence of extra nutrients, growth could be increased.

The medium used in the investigation of the physical conditions of culture contained two non-chemically defined components, horse serum and yeast extract. To determine the nutrients required by the plerocercoid and hence possibly move towards the understanding of the metabolism of the larva, breakdown of the yeast component was attempted. Two approaches were used; firstly, the yeast was fractionated by hydrolysis and secondly, attempts were made to replace the yeast by a defined amino acid and vitamin supplement.

B. Material and Methods

(i) Preparation of glassware and instruments

All glassware used in the course of culturing the plerocercoids was cleaned and sterilised according to the methods described by Wilson (1960). This included vessels which contained the various components of the media as well as the roller tubes used for culturing the worms and the pipettes required for transference and changing of media.

Syringes and stainless steel instruments were sterilised in boiling water. Aluminium foil seals and pouches were placed in petri dishes before being autoclaved for 15 minutes at 15 lbs pressure.

(ii) Culture vessels

Cultivation was carried out in 125 x 20 mm roller tubes containing 5 ml of medium. The culture tubes were closed by placing a 7 cm diameter disc of sterile aluminium foil on top of the tube and twisting tight below the rim, followed by a slightly larger, square piece of parafilm. This method of closure has proved excellent - quick to handle, aseptic and permits CO_2 equilibration but only a negligible water loss. The roller drum was fitted inside a gas tight container, the whole being rotated at 30 revs/hour. If required, the container could be flushed with a gas mixture. The entire roller drum apparatus was set up inside an incubator for the maintenance of the cultures at a required/

/temperature.

(iii) Aseptic procedure for setting up cultures

Fish (see Section II) were killed by pithing, dipped in alcoholic iodine, allowed to dry and the abdominal cavity opened by a ventral incision. Plerocercoids were removed with fine forceps and placed in small sterile pouches made of aluminium foil. The pouches were closed to reduce evaporation and prevent contamination of the plerocercoid, and weighed. The plerocercoids were placed in culture and the pouches re-weighed. This method worked satisfactorily down to 2 mg wet weight (W.W.). Wet weight is not quite the same as fresh weight (F.W.) which is the weight of a plerocercoid after light blotting with filter paper. The difference, however, is small in plerocercoids recovered from fish, the F.W. ranging from approximately 90% of the W.W. in a 3 mg plerocercoid to 96% of the W.W. in a 100 mg worm. Since the difference is small, no correction was made for it.

In most cases, cultures were run for 8 days at 21$\pm$ 1.5°C. At the end of this period, the worms were removed from the culture tubes, weighed then dried for 18-24 hours at 100°C on aluminium foil. The worms were then weighed (D.W.). Dried worms were stored over silica until their carbohydrate content could be determined using anthrone (Trevelyan and Harrison, 1952).

/The fresh weight of plerocercoids taken direct from fish was determined and the worms dried at 100°C to obtain their dry weight. These worms were stored over silica gel for carbohydrate determination using anthrone.

(iv) Changing of cultures

The medium was usually changed every 48 hours but was never left for longer than 72 hours. The medium was withdrawn from the roller tube with a fine pipette and fresh medium added. A new foil seal and piece of parafilm were used to re-seal the cultures. If pH readings were required, the medium from the roller tube was placed in a small dish, 2 cm in diameter with a capacity of 6-8 ml. The pH was determined using a glass electrode and a Pye meter. Readings were taken within five minutes of the removal of the medium from the roller tube.

(v) Basal medium

The basal medium used in this work consisted of:-

(a) Balanced salt solution (BSS), Hanks's formula (supplied in a packet by OxO, Ltd.)

Magnesium sulphate	0.1g
Potassium dihydrogen phosphate	0.06g
Sodium phosphate	0.06g
Dextrose	1.0g
Sodium chloride	8.0g
Potassium chloride	0.4g
Calcium chloride	0.14g
Magnesium chloride	0.1g

per litre of deionised water.

/25 ml of 1.4% W/V sodium bicarbonate was added to each litre after autoclaving.

(b) Glucose (G) made up as an isotonic (6.5%) solution and added to the medium to give a final concentration of 0.65%.

(c) Horse serum (HS) supplied commercially by Burroughs Wellcome. (This is collected in potassium oxalate, recalcified and pasteurised for 2 hours at 58°C). It was added to the medium to give a final concentration of 25%.

(d) 'Difco' dehydrated yeast extract (YE) used in a final concentration of 0.5%.

(e) The sodium salt of penicillin (50 units per ml), streptomycin (100 units per ml) and phenol red as a pH indicator (2 mg per 100 ml of medium). Phenol red was normally incorporated in the BSS. Components (a), (b) and (d) were sterilised by autoclaving for 15 minutes at 10-15 lbs pressure and components (c) and (e) were supplied sterile.

(vi) Sugar solutions

Three sugars, apart from glucose were used in the medium. These were ribose (a pentose sugar), galactose (a hexose sugar) and sucrose (a disaccharide). The sugars were made up as isotonic solutions, 5.5%, 6.5% and 13.2% respectively and/

/added to the media to give final concentrations of 0.55%, 0.65% amd 1.32% respectively.

(vii) Amino acid and vitamin supplement

Initially, Eagle's medium (taken from Paul, 1959) was used as a supplement with the basal medium of horse serum, yeast, glucose and saline. In later experiments, an amino acid and vitamin supplement based on Medium 703 (Healy, Fisher and Parker, 1954) was used. The constituents were:-

Solution 1	mg		mg
Arginine monohydrochloride	70.0	Isoleucine	40.0
Histidine monohydrochloride	20.0	Valine	50.0
Lysine monohydrochloride	70.0	Glutamic acid	150.0
Tryptophane	20.0	Aspartic acid	60.0
Phenyl alamine	50.0	α-Alanine	50.0
Methionine	30.0	Proline	40.0
Serine	50.0	Hydroxyproline	10.0
Threonine	60.0	Glycine	50.0
Leucine	120.0	Sodium acetate	94.0

Dissolved in 100 ml Hanks's BSS by heating to 80°C. The solution was allowed to cool then the following were added:-

	mg		
Glutamine	100.0	Ascorbic acid	50.0
Cysteine hydrochloride	260.0	Glutathione	10.0

/Solution 2

	mg
Tyrosine	40.0
Cystine	20.0

Dissolved in 50 ml 0.075N HCl.

Solution 3

	mg		mg
Niacin	12.5	Riboflavin	5.0
Niacinamide	12.5	Calcium pantothenate	5.0
Pyridoxine hydrochloride	12.5	p-amino benzoic acid	25.0
Pyridoxal hydrochloride	12.5	Inositol	25.0
Thiamine hydrochloride	5.0	choline chloride	500.0

Dissolved in 50 ml distilled water. To make the stock solution, 1 ml of the 50 was made up to 50 ml with more distilled water.

Solution 4

Crystalline biotin 5.0 mg

Dissolved in 50 ml distilled water and 1 ml N.HCl. To make the stock solution, 1 ml of the 50 was made up to 50 ml with distilled water containing 1 ml N.HCl per 50 ml of water.

Solution 5

Folic acid 5.0 mg

Dissolved in 50 ml Hanks's BSS. To make up the stock solution 1 ml of the 50 was made up to 50 ml with more BSS.

/Solution 6

Vitamin B_{12} 100 µg ampoule (1 ml)

Obtained commercially as cyanocobalamin BP.

Solutions 1 - 5 were sterilised separately by negative pressure filtration through sintered glass filters.

Preparation of 100 ml of Medium

Solutions	1	10 ml	BSS	33 ml
	2	5	Glucose	0.65g in 10ml H_2O
	3	0.5	Horse serum	25 ml
	4	0.5	Yeast extract	0.5g in 10ml H_2O
	5	0.5	Double strength saline	5 ml
	6	0.5		
		17.0 ml		83 ml

Double strength balanced salt solution is included in the medium to compensate for the low osmotic pressure of the yeast extract.

(viii) Yeast component of the medium

a) The basal medium contained yeast extract at a concentration of 0.5 %. The level of yeast was altered in the medium to give concentrations of 0.11%, 0.34%, 0.79% and 1.1%.

b) The yeast extract was fractionated by acid or alkali hydrolysis.

/Alkali hydrolysis: 3g of yeast extract was dissolved in 30 ml N.NaOH and autoclaved for 15 minutes at 15 lbs pressure. After colling, the solution was neutralised with 30 ml N.HCl.

Acid hydrolysis: 3g of yeast extract was dissolved in 30 ml N.HCl and placed in a 250 ml flask fitted with a condenser. Gentle boiling of the solution was maintained for one hour. After cooling, the solution was neutralised with 30 ml N.NaOH.

In 100 ml of basal medium, there was normally 40 ml of Hanks's saline

which contains 40 x 8 mg NaCl = 320 mg NaCl

On neutralising 1 litre of N.NaOH with N.HCl, 58.5g (58,500mg) of NaCl is produced

$$[HCl + NaOH \rightleftharpoons NaCl + H_2O]$$

∴ To obtain 320 mg of NaCl, one needs to neutralise

$$\frac{320}{58,500} \times 1000 \text{ ml of N.NaOH}$$

$$= 5.5 \text{ ml of N.NaOH.}$$

On completion of hydrolysis and neutralisation, the 3g of yeast extract was contained in 60 ml of H_2O+NaCl. To make up 100 ml of medium, 11 ml of this solution was used (11 ml contains 320mg NaCl and 0.55g yeast).

/Horse serum and glucose were added to the hydrolysed yeast extract as required but the saline solution used contained the normal concentration of salts (Hanks's formula) except sodium chloride as this was already present in the yeast solution (see above).

C. Results

(i) Water content of plerocercoids in vivo

The ratio of the dry weight of a plerocercoid to its fresh weight was determined and expressed as a percentage. This dry/fresh weight ratio was plotted against the fresh weight of the plerocercoid (Figure 16).

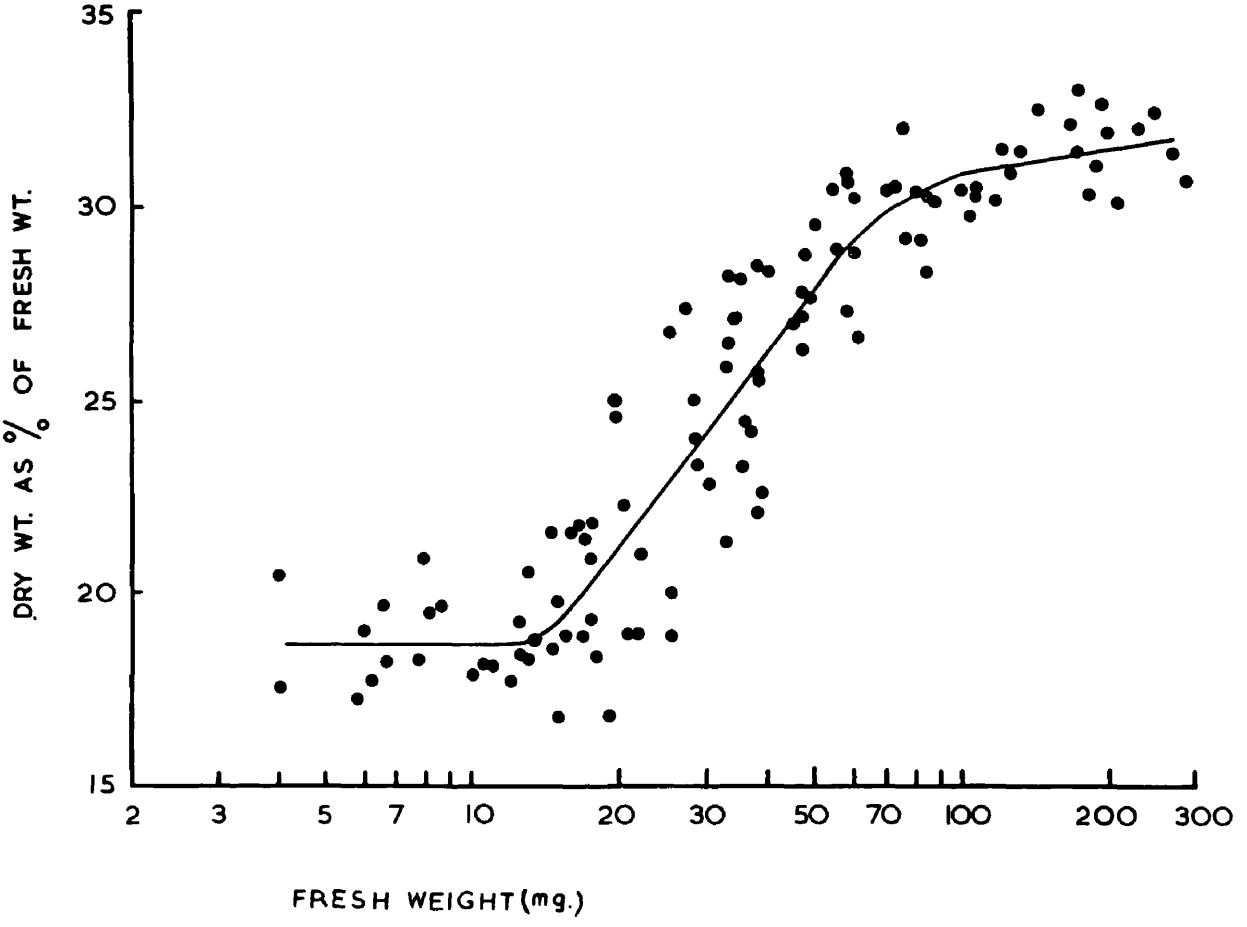
Under normal conditions, the amount of water present in a particular weight of plerocercoid is constant and changes with the weight of the plerocercoid. The plot of the dry/fresh weight ratio can be divided into three sections.

(i) Between 5 and 15 mg F.W. (1-3mg D.W.) the percentage water content is almost constant.

(ii) Between 15 mg and 100 mg F.W. (3-30 mg D.W.) the plerocercoid becomes progressively drier until the water content has fallen from an initial level of just over 80% to 68-69%.

(iii) Over 100 mg F.W. When the water content of the plerocercoids either remains as a fixed percentage of the total weight or continues to decrease at a very slow rate.

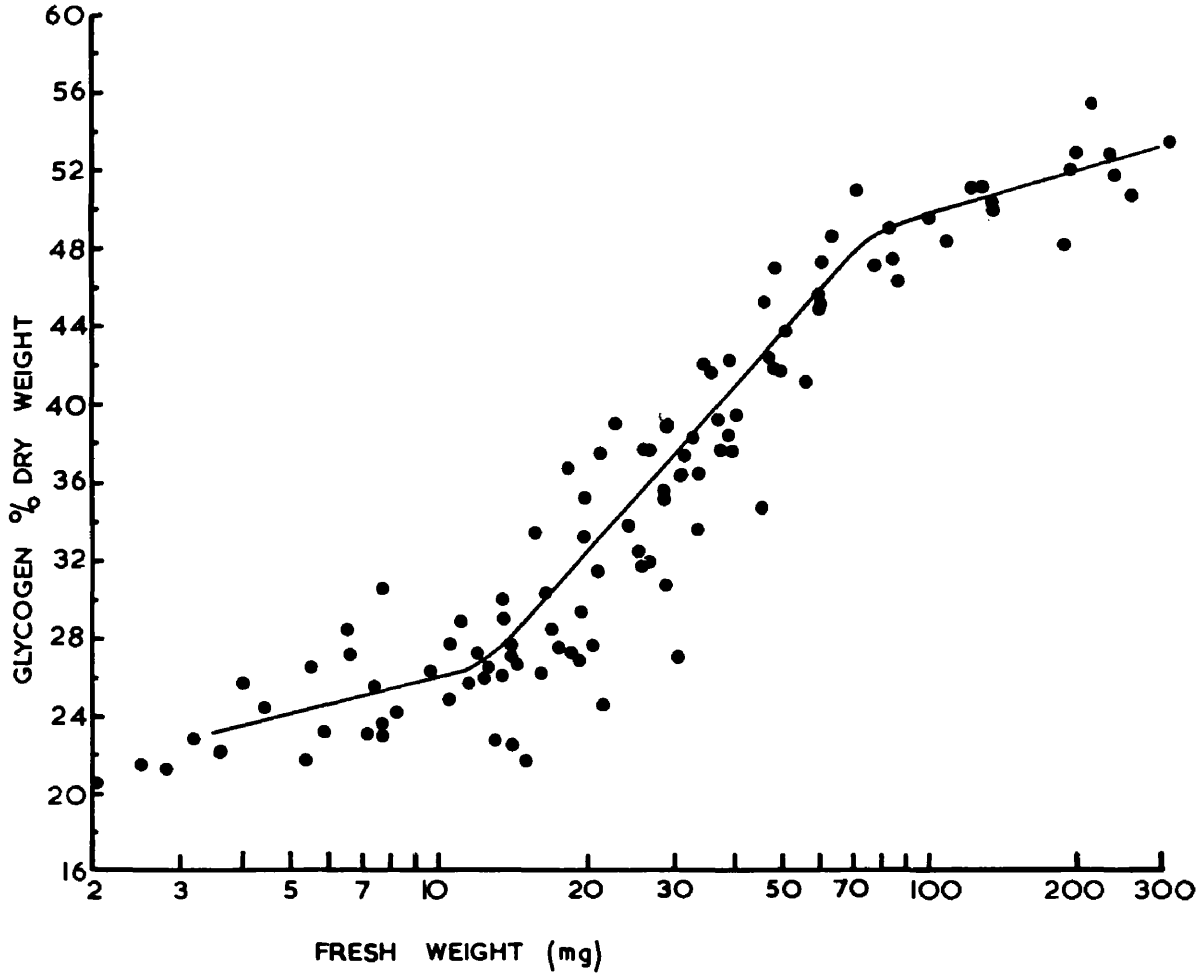
Figure 16. The change in percentage dry weight content of Schistocephalus solidus plerocercoids with increase in fresh weight.


35
30
25
20
15
DRY WT. AS % OF FRESH WT.
2
3
5
7
10
20
30
50
70
100
200
300
FRESH WEIGHT (mg.)

/(ii) Glycogen content of plerocercoids in vivo

The glycogen content of plerocercoids, when plotted against fresh weight (Figure 17) shows a strong similarity to the water content of the plerocercoids (Figure 16). The glycogen content changes little in plerocercoids 2-15 mg F.W. and increases rapidly between the fresh weights of 15 and 100 mg. In the third section, containing plerocercoids greater than 100 mg in F.W., there is little increase in the glycogen. Few plerocercoids in the last weight range were analysed due to their scarcity in the fish population. This weight range had been examined previously (Hopkins, 1950) and the present results agree with the earlier work that plerocercoids over 100 mg F.W. contain 50-55% glycogen (measured relative to their D.W.).

Figure 17. The change in percentage glycogen content during growth of Schistocephalus solidus plerocercoids in the body cavity of Gasterosteus aculeatus.


60
56
52
48
44
40
36
32
28
24
20
16
GLYCOGEN % DRY WEIGHT
2
3
5
7
10
20
30
50
70
100
200
300
FRESH WEIGHT (mg)

/(iii) Glycogen and water content in vitro

Since dry weight can be more accurately determined than fresh weight, water and glycogen content have been plotted against dry weight (Figure 18) for comparison with the results obtained with worms developing in vitro (Figure 19). The curve drawn on Figure 18 which fits both the water and glycogen content of the worms in vivo, is reproduced in Figure 19. The data on Figure 19, however, is from cultured worms (medium used is 0.65% glucose, Hanks's saline, 0.5% yeast extract and 25% horse serum). The values for glycogen content (symbol-open points) are nearly all higher and those for dry-matter content (closed points) all lower than would be expected in plerocercoids of the same weight removed directly from the perivisceral cavity of the fish.

Figure 18. The change in percentage glycogen content (open points) and dry weight (closed points) of S. solidus plerocercoids with increase in dry weight.
The solid line is seen to fit both sets of data.

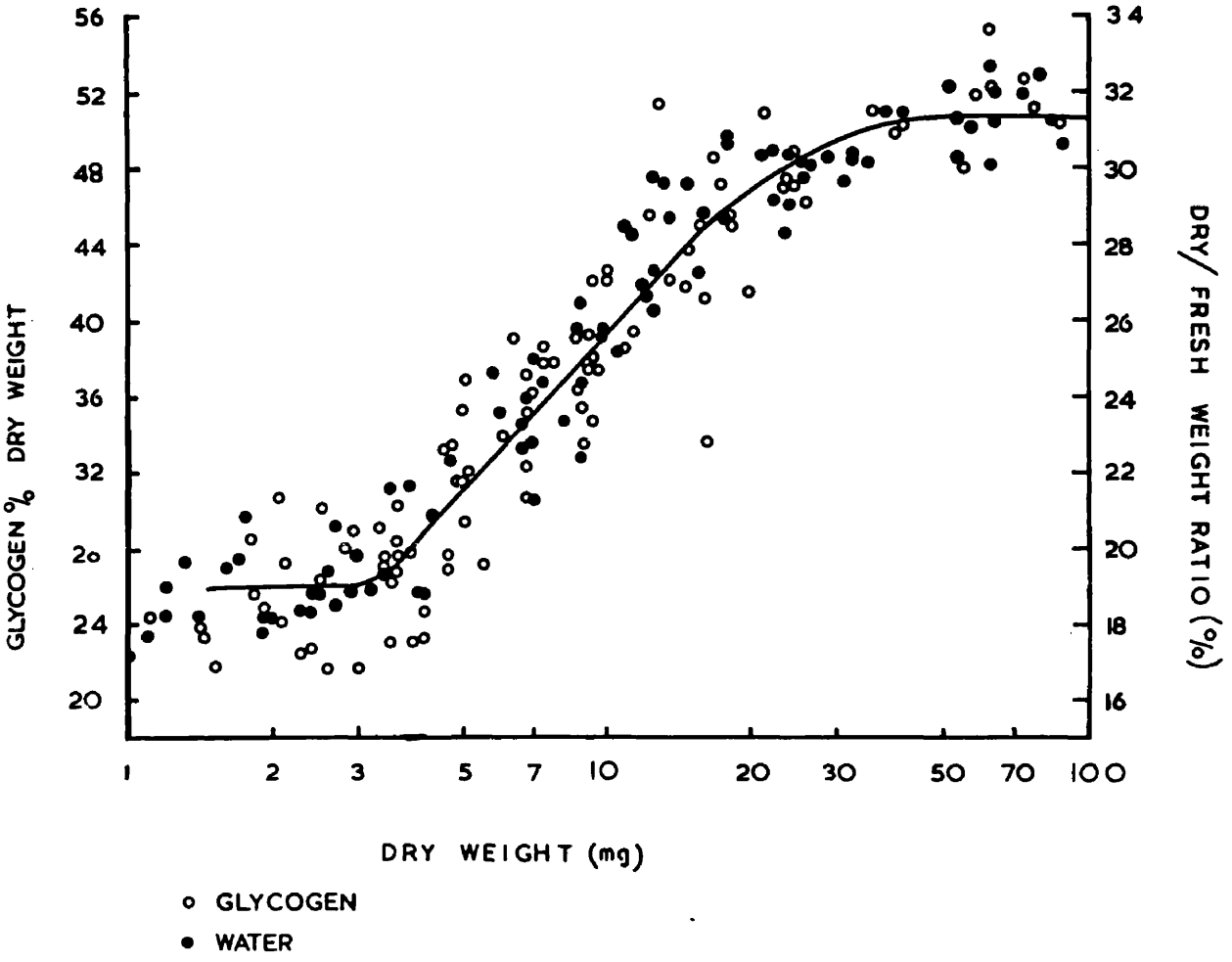
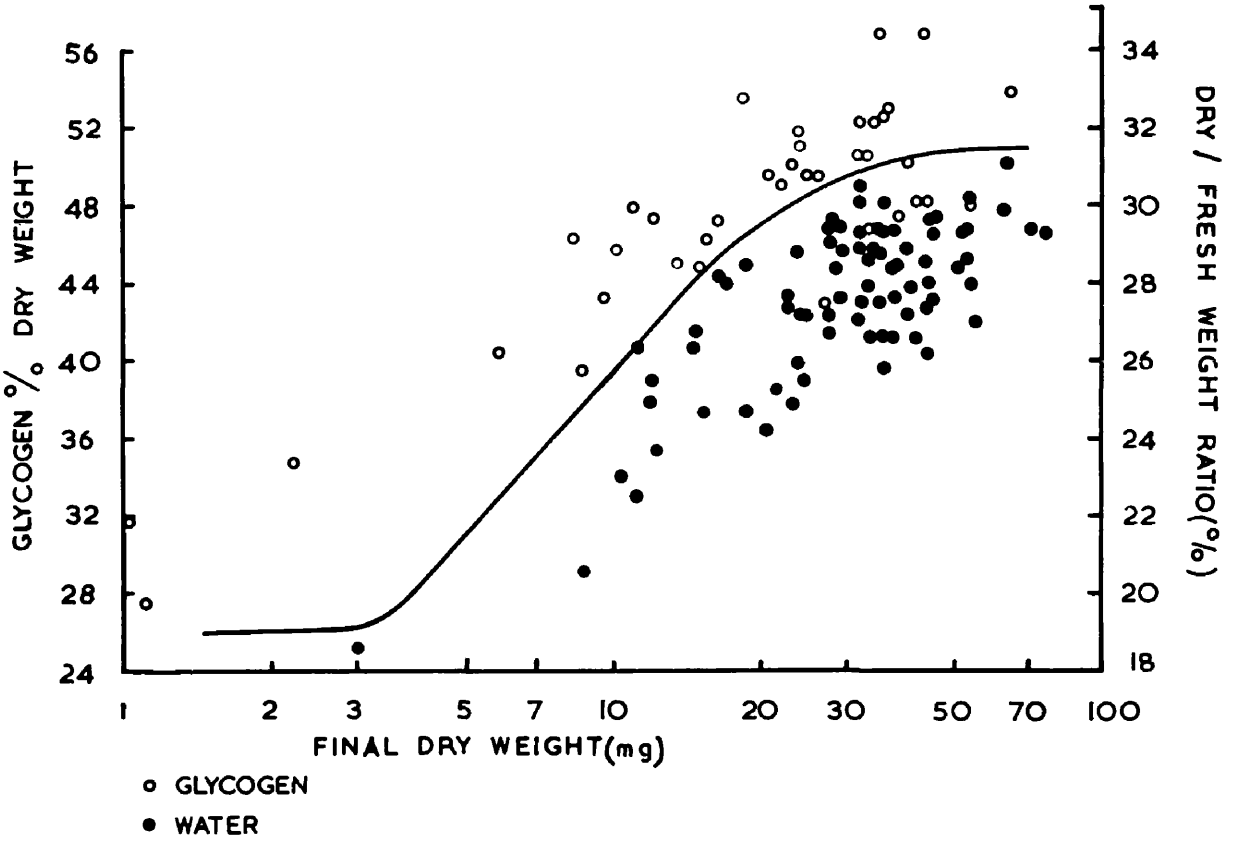

56
52
48
44
40
36
32
20
24
GLYCOGEN % DRY WEIGHT
34
32
30
28
26
24
22
20
18
16
DRY/ FRESH WEIGHT RATIO (%)
1
2
3
5
7
10
20
30
50
70
100
DRY WEIGHT (mg)
GLYCOGEN
WATER

Figure 19. The percentage glycogen content (open points) and percentage dry weight (closed points) of plerocercoids after 8 days growth *in vitro*.

The solid line is the normal amount found *in vivo* (Figure 18).

SCHISTOCEPHALUS AFTER 8 DAYS IN BSSG+HS+YE



(iv) Cultivation of plerocercoids in the basal medium

The percentage changes in dry weight of plerocercoids cultured for 8 days in Hanks's saline + 0.65% glucose [BSSG], 75% BSSG + 25% horse serum (v/v) [BSSG+HS], BSSG + 0.5% yeast extract [BSSG+YE] and BSSG + 25% HS + 0.5% YE are shown in Figure 20. The initial dry weight was calculated from the initial fresh weight using Figure 16. Three main points of interest occur in Figure 20: the smaller the plerocercoid, the greater the growth rate; the rate of decrease of the growth rate approaches an exponential (Figure 20d); serum and yeast extract together promote good growth of the plerocercoid.

Figure 20. The percentage increase in dry weight shown by plerocercoids after 8 days cultivation at 21°C in various media.

____ . _____ . Growth in BSSG (Hanks's balanced salts + glucose);

---------- growth in BSSG + HS (horse serum);

.... growth in BSSG + YE (yeast extract);

_____ growth in BSSG + HS + YE.

FIGURE a

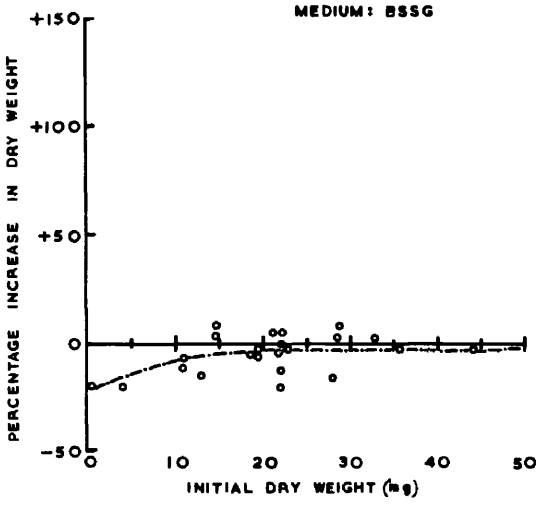


FIGURE b

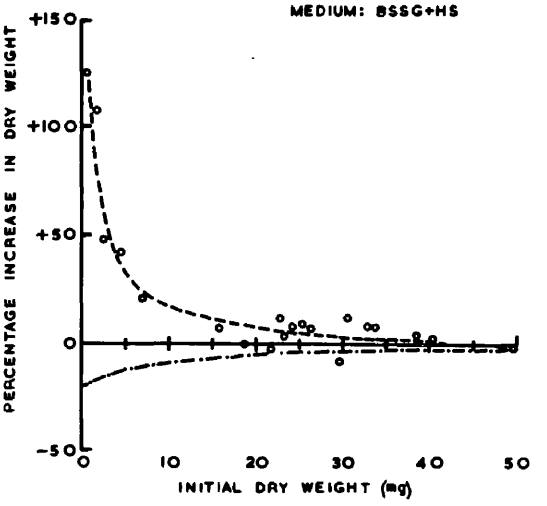


FIGURE c

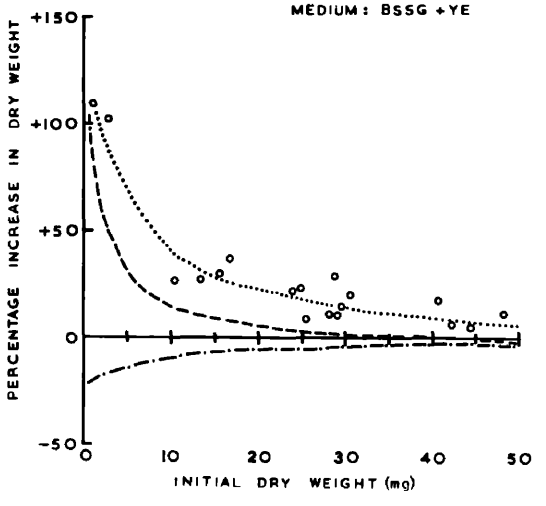
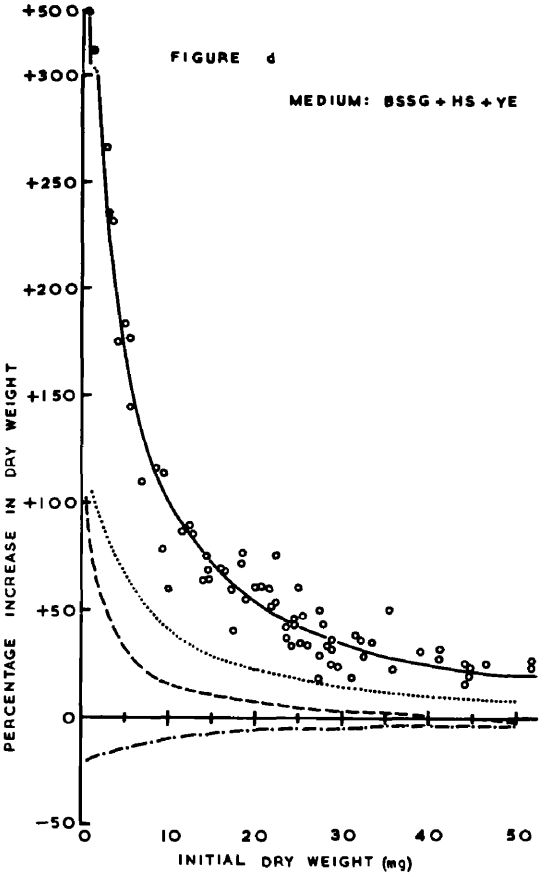


FIGURE d



(v) Physical conditions of culture

In addition to the 69 results shown in Figure 20d, 180 plerocercoids have been cultured in nearly the same medium but with one modification:-

i.e. a) CO_2 - 10% or 0.04% (air) instead of 5%

b) pH - 8 $\pm$ 0.2 or 6.3 $\pm$ 0.2 instead of 7.1

c) serum - 10, 20 or 30% instead of 25% of the medium

d) medium changed 24-hourly instead of 48-hourly.

In all cases, the results were similar to the controls (Figure 20d) though in some cases, both the controls and the modified cultures showed a slightly lower growth rate at all weights.

(vi) Effect of length of culture period on the growth rate

The growth rate was determined over a period of 8 days with a number of culture maintained for 16 days. Growth during the second period of 8 days was more variable, in most cases varying between 50 and 70% of the expected amount (based on 18 worms). By the end of a third period of 8 days, (Hopkins, private communication) growth had stopped or nearly so (based on seven worms weighed every second day).

In another series of experiments, plerocercoids were maintained in culture for 2, 4, 6 and 8 days and the growth rate for each period of 2 days determined. (Figure 21). From this, the actual growth of three weights of plerocercoids (10, 20 and 30 mg D.W. initially) was determined for 2, 4, 6 and 8 days. From the plot of the results of worms cultured only for 2 days (symbol, a plus), the expected growth of the same three weights of plerocercoids was calculated. The actual growth recorded was 85-89% of the expected amount (Table 24).

Figure 21. The percentage increase in dry weight shown by plerocercoids after 2, 4, 6 and 8 days cultivation at 21°C in BSSG + HS + YE.

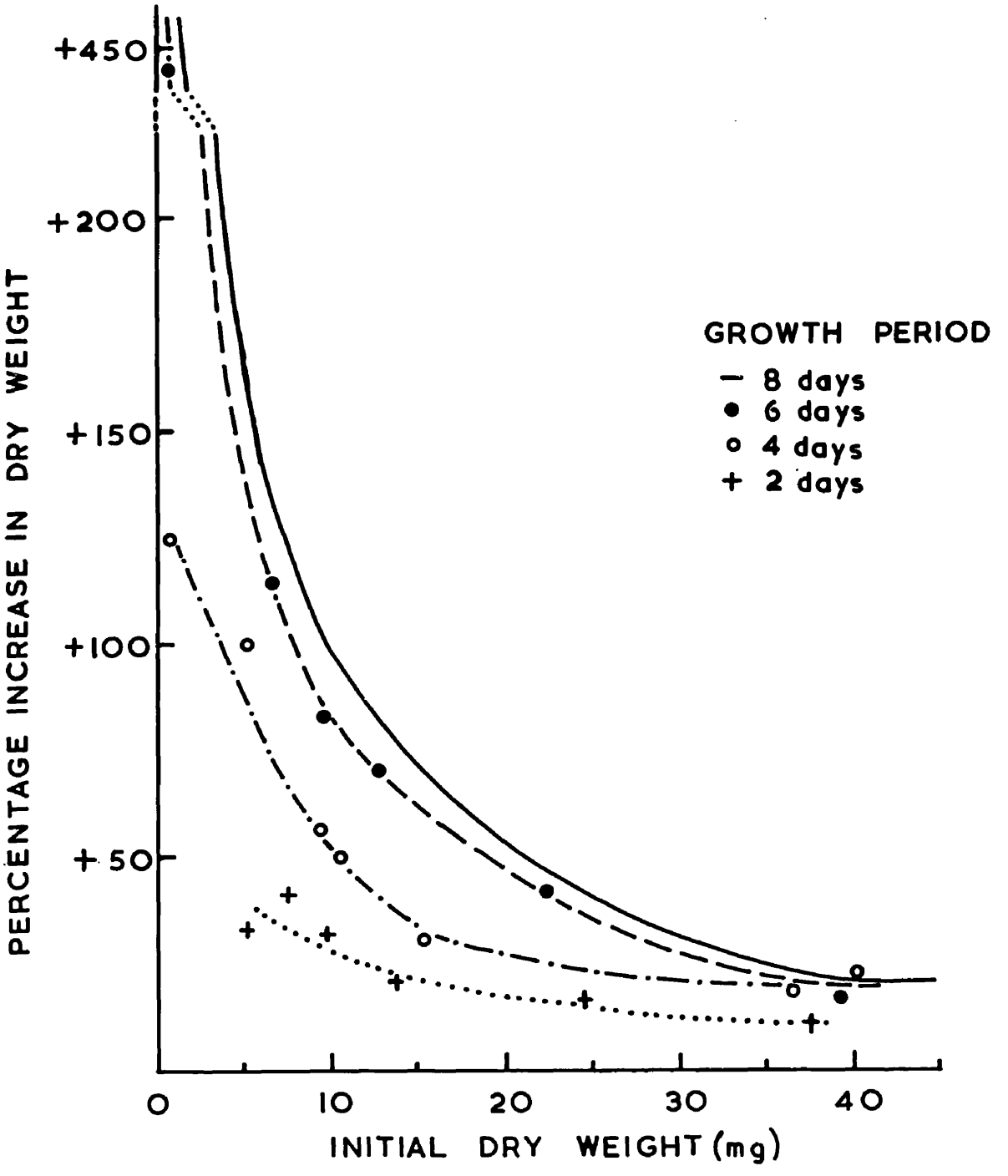

+450
+200
+150
+100
+50
PERCENTAGE INCREASE IN DRY WEIGHT
GROWTH PERIOD
8 days
6 days
4 days
2 days
0
10
20
30
40
INITIAL DRY WEIGHT (mg)

Table 24

Comparison of the actual increase in weight of plerocercoids in the basal medium with the increase which would be expected if the initial rate of growth were maintained

Initial dry weight	Growth	Weight of plerocercoids (mg) at				Actual/Expected Growth in 8 days (%)
		2 days	4 days	6 days	8 days	
10 mg	Actual	12.8	15.2	18.2	20.0	89
	Expected	12.8	15.9	19.1	22.5	
20 mg	Actual	23.4	25.4	29.4	30.8	89
	Expected	23.4	27.1	30.9	34.6	
30 mg	Actual	33.6	36.3	38.1	39.3	85
	Expected	33.6	37.5	41.6	46.2	

(vii) Effect of including sugars in the medium

In a medium consisting of Hanks's BSS with a sugar, there is a decrease in the dry weight of the plerocercoid, a 0 to 20% loss in weight. This loss in weight occurred in saline alone and in saline with glucose, ribose or sucrose (Figure 22). The water content of the plerocercoids decreased slightly in 8 days from the *in vivo* level (solid line, Figure 23) and the *in vitro* level (Figure 19), the small plerocercoids (<10 mg D.W.) having the largest decrease. This alteration in the dry to fresh weight ratio occurred with all the sugars and the saline alone. There was an appreciable increase in the glycogen of the small plerocercoids with a small increase in the large plerocercoids in saline + sugar. This increase is similar to that recorded in the basal medium (Figure 19). Small plerocercoids with no external source of sugar decreased in glycogen content with the larger plerocercoids remaining at the *in vivo* glycogen content level (Figure 23).

In a medium consisting of saline and yeast, the rate of growth of the worms was independent of the presence or absence of glucose (Table 25). The dry/fresh weight ratio of the worms were normal in both media. However, the glycogen content of the small worms increased in both media above the *in vivo* level with the content of the large/

Figure 22. The percentage change in dry weight shown by plerocercoids after 8 days cultivation in various media.

- o Growth in BSS (Hanks's balanced salts);
- • growth in BSS + glucose;
- x growth in BSS + sucrose;
- \+ growth in BSS + ribose.

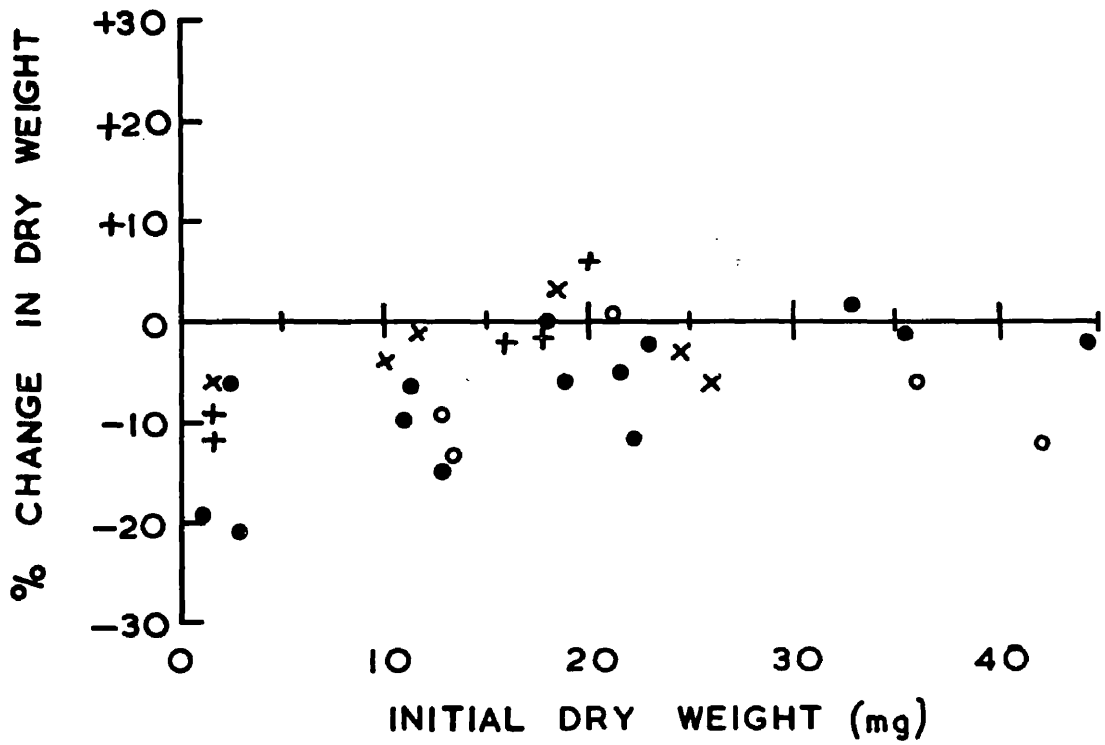

+30
+20
+10
0
-10
-20
-30
% CHANGE IN DRY WEIGHT
0
10
20
30
40
INITIAL DRY WEIGHT (mg)

Figure 23. The percentage glycogen content and percentage dry weight of plerocercoids after 8 days growth in various media.

The solid line is the normal amount found *in vivo* (Figure 18).

- x Percentage dry weight in BSS;
- o percentage dry weight in BSS + sucrose, ribose, or glucose;
- \+ percentage glycogen content in BSS;
- • percentage glycogen content in BSS + sucrose, ribose or glucose.

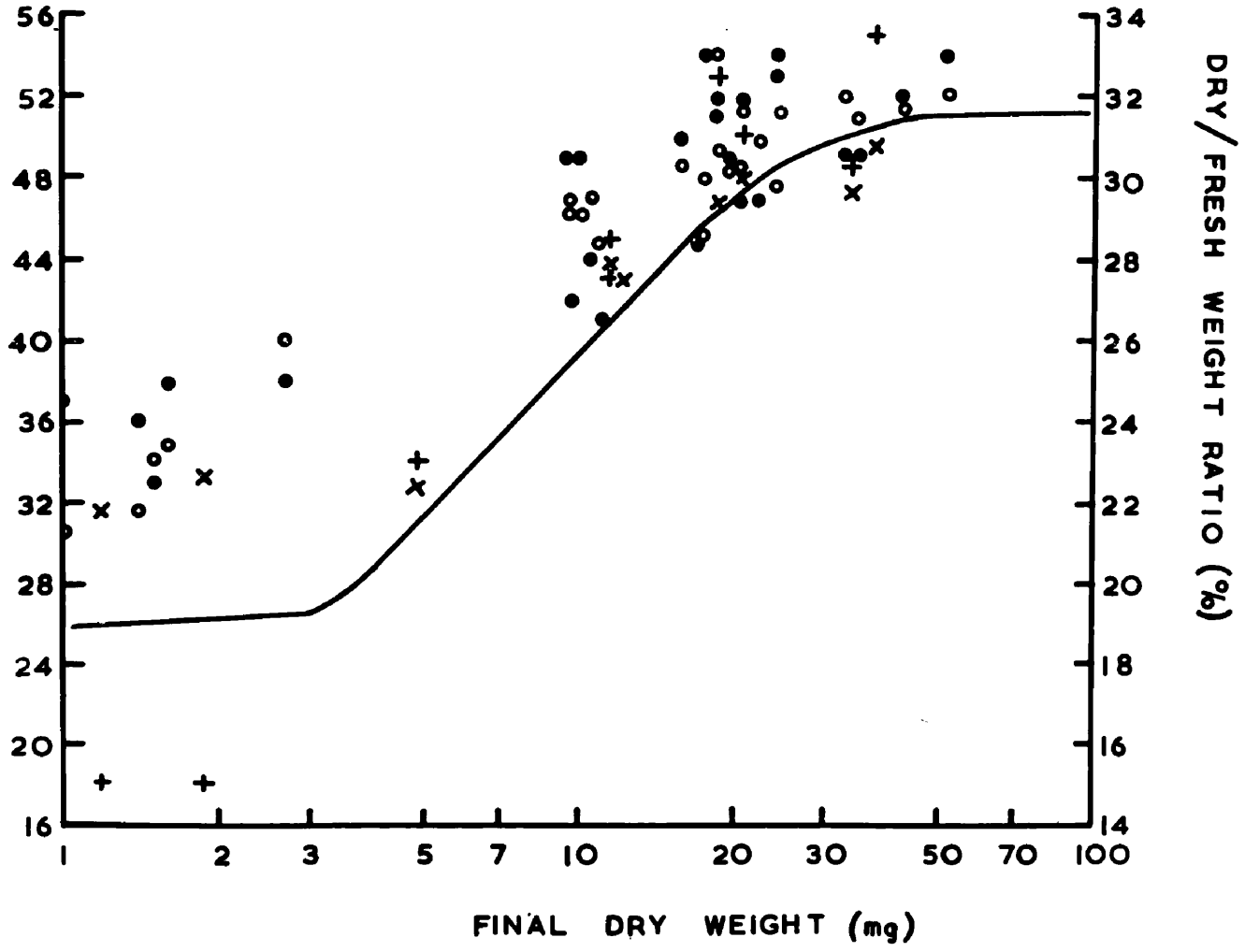

56
52
48
44
40
36
32
28
24
20
16
34
32
30
28
26
24
22
20
18
16
14
1
2
3
5
7
10
20
30
50
70
100
FINAL DRY WEIGHT (mg)
DRY/FRESH WEIGHT RATIO (%)

Table 25

The effect of adding glucose to a medium consisting of Hanks's saline and yeast extract

Initial dry weight (mg)	Final dry weight (mg)	Change in dry weight (%)	Dry/Fresh weight ratio (%)	Glycogen as % of dry weight (%)
BSS + YE				
0.8	1.3	+63	25.0	56
5.2	6.5	+25	26.1	46
28.3	31.6	+12	30.2	55
29.0	32.2	+11	29.8	50
36.8	39.5	+7	30.7	52
48.3	54.4	+12	29.6	54
69.0	64.0	-7	25.0	59
72.3	75.6	+5	30.5	56
85.6	91.6	+7	28.3	55
BSS + YE + G				
1.2	2.1	+75	23.6	-
6.4	8.4	+31	27.2	-
7.7	10.2	+33	27.5	-
10.2	13.0	+27	28.2	51
25.7	28.0	+9	29.4	-
29.7	33.7	+15	31.2	52
30.8	37.0	+20	30.5	-
41.4	42.8	+3	30.6	50
44.5	47.9	+8	30.9	52

/plerocercoids unaltered.

Growth is erratic in a medium of saline and serum; the presence of glucose appeared to reduce the variations in growth (Table 26). The dry/fresh weight ratio was also erratic in the absence of glucose. There was no change in the glycogen content of even the smallest plerocercoid in either medium.

The presence or absence of glucose in a medium of saline, serum and yeast does not affect the rate of growth, the water content or the glycogen content of the plerocercoids (Table 27, Figure 20). A similar result was obtained replacing glucose with galactose (Table 27).

Table 26

The effect of adding glucose to a medium consisting of Hanks's saline and horse serum

Initial dry weight (mg)	Final dry weight (mg)	Change in dry weight (%)	Dry/Fresh weight ratio (%)	Glycogen as % of dry weight (%)
BSS + HS				
0.4	1.0	+150	33.3	26
1.2	2.5	+108	31.0	34
18.6	18.6	0	29.8	53
22.0	21.1	-4	26.7	57
26.0	24.6	-5	24.6	44
29.6	27.0	-9	28.6	51
38.4	39.6	+3	31.3	56
40.2	41.4	+3	30.3	53
84.0	82.4	-2	29.6	48
BSS + HS + G				
0.4	0.9	+125	18.8	-
2.4	2.9	+21	23.0	31
4.2	5.0	+19	26.3	43
7.2	10.0	+39	27.6	-
15.7	17.1	+9	28.1	-
24.2	26.2	+8	29.6	50
27.8	25.7	-8	29.4	-
33.7	36.6	+9	30.3	52
59.2	59.1	0	31.1	50

Table 27

The effect of adding galactose to a medium consisting of Hanks's saline, horse serum and yeast extract

Initial dry weight (mg)	Final dry weight (mg)	Change in dry weight (%)	Dry/Fresh weight ratio (%)	Glycogen as % of dry weight (%)
BSS + HS + YE				
0.3	1.8	+500	31.6	30
0.4	1.8	+350	26.4	30
1.1	3.4	+209	23.3	45
1.9	5.5	+190	25.4	56
7.8	13.8	+77	27.3	56
16.5	24.5	+49	27.8	53
17.8	23.6	+33	28.3	57
24.8	31.6	+27	29.1	-
69.9	75.9	+9	28.3	48
BSS + HS + YE + Galactose				
0.3	1.1	+267	20.0	32
0.4	1.2	+200	18.5	38
2.0	4.2	+110	23.4	40
3.6	7.6	+111	24.1	42
12.2	17.5	+43	25.9	48
16.1	22.7	+41	26.8	47
25.0	29.1	+16	27.5	45
47.8	53.8	+13	28.9	51
57.6	62.2	+8	28.4	46

(viii) Effect of adding an amino acid and vitamin supplement to the medium

The amino acid and vitamin supplement was used with the basal medium, the yeast component of which was different from that used in the previous section. The nature of the difference is unknown but the yeast used in this series of experiments stimulated a slightly lower growth rate in all sizes of plerocercoids. This growth rate is plotted in Figure 24 (broken line). With the addition of amino acids and vitamins, the growth rate of the smaller plerocercoids was increased but there was no effect on the growth of plerocercoids larger than 25 mg D.W. (80 mg F.W.) [Figure 24, solid line].

To determine if the amino acids and vitamins were sufficient to replace either the yeast or the serum constituents of the basal medium, the supplement was used with BSSG + HS and BSSG + YE. In Figure 25, the solid line represents the growth rate in a medium consisting of BSSG + HS + amino acid + vitamin supplement, and the broken line, the control medium of BSSG + HS. The solid line in Figure 26 represents growth in a medium consisting of BSSG + YE + amino acid and vitamins and the broken line the control medium of BSSG + YE.

With both media, growth was improved in the presence of/

Figure 24. The percentage increase in dry weight shown by plerocercoids after 8 days cultivation in various media.

+ (---) Growth in BSSG + HS + YE;

• (___) growth in BSSG + HS + YE + amino acids + vitamins.

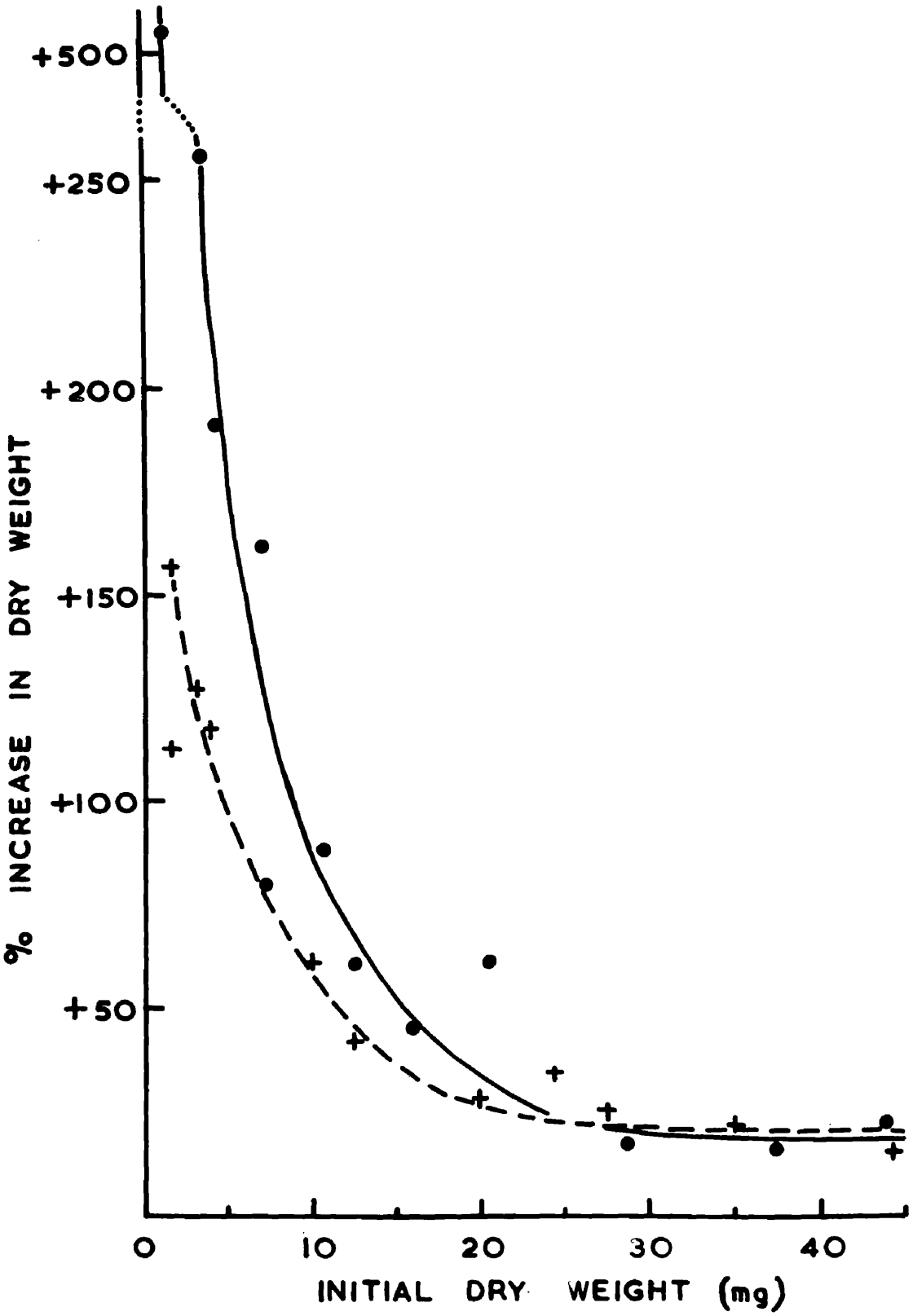

+500
+250
+200
+150
+100
+50
% INCREASE IN DRY WEIGHT
0
10
20
30
40
INITIAL DRY WEIGHT (mg)

Figure 25. The percentage increase in dry weight shown by plerocercoids after 8 days cultivation in various media.

- o (----) Growth in BSSG + HS;
- ● (____) growth in BSSG + HS + amino acids + vitamins.

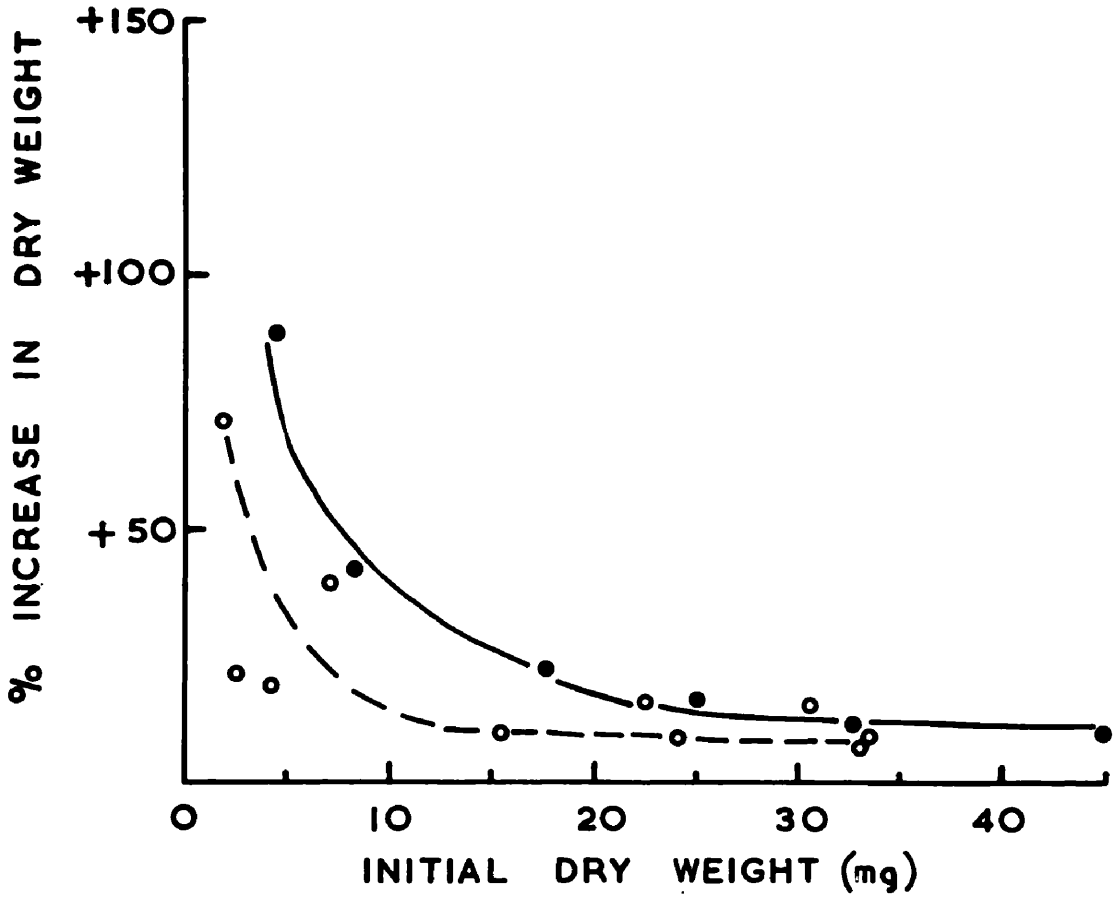
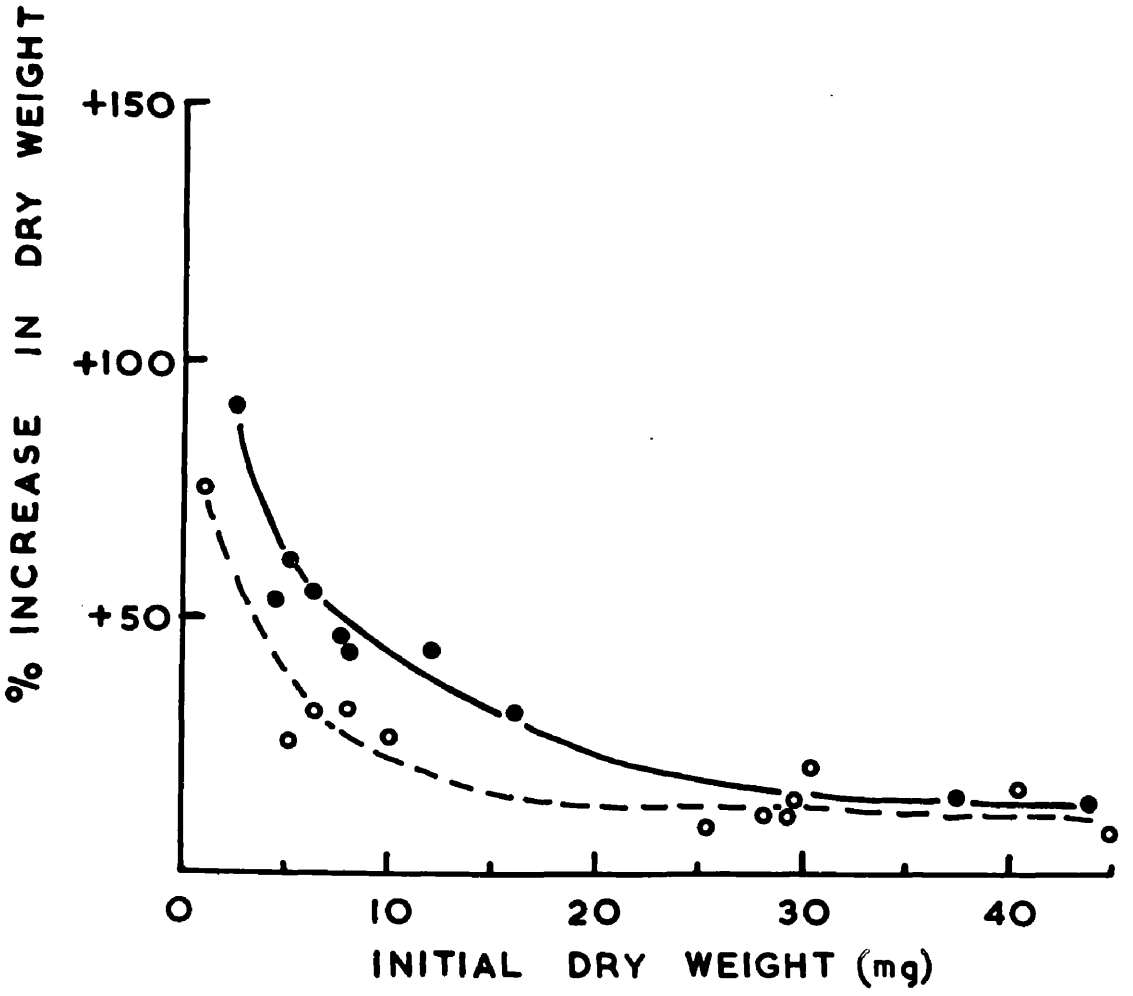

+150
+100
+50
% INCREASE IN DRY WEIGHT
0
10
20
30
40
INITIAL DRY WEIGHT (mg)

Figure 26. The percentage increase in dry weight shown by plerocercoids after 8 days cultivation at 21°C in various media.

o (----) Growth in BSSG + YE;

• (——) growth in BSSG + YE + Amino acids + vitamins.


% INCREASE IN DRY WEIGHT
+150
+100
+50
0
10
20
30
40
INITIAL DRY WEIGHT (mg)

/the amino acids and vitamins, the increased growth rate being very marked in small plerocercoids. However, growth did not increase to the rate obtained with the basal medium (Figure 24).

(ix) Effect of altering the yeast concentration in the medium

Plerocercoids were maintained in media consisting of Hanks's saline, and various concentrations of yeast extract for 16 days. The concentrations of yeast in the media were 0.11%, 0.34%, 0.79% and 1.1% with a control group of worms maintained in 0.56% yeast. Consistent growth rates were obtained for each concentration with no different in the rates among the five media (Figure 27).

Figure 27. The percentage change in dry weight shown by plerocercoids after 16 days cultivation in various media.

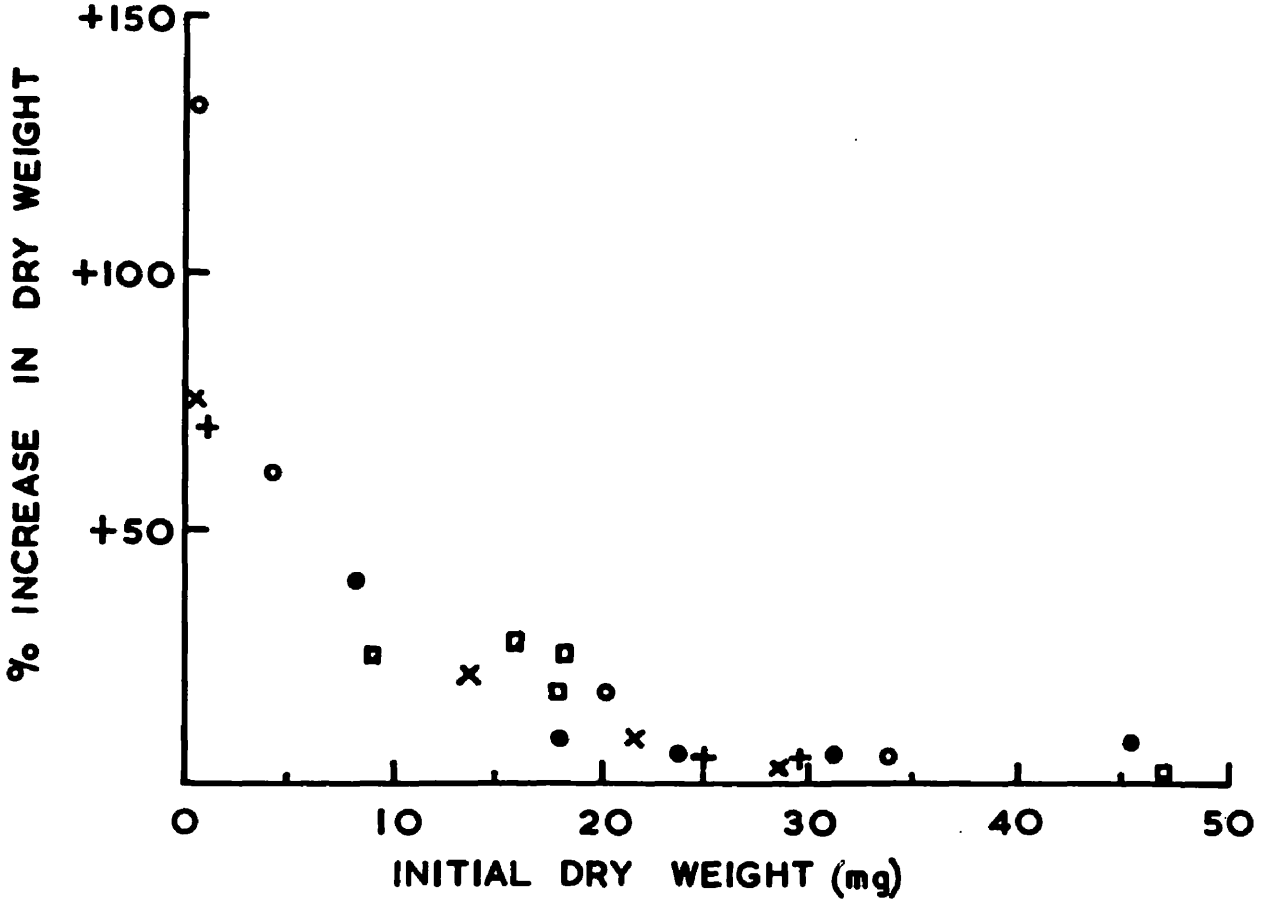
o Growth in BSS + 0.11% YE;

□ growth in BSS + 0.34% YE;

● growth in BSS + 0.56% YE;

x growth in BSS + 0.79% YE;

\+ growth in BSS + 1.1% YE.


+150
+100
+50
% INCREASE IN DRY WEIGHT
0
10
20
30
40
50
INITIAL DRY WEIGHT (mg)

(x) Replacement of yeast by vitamins

a) To a medium consisting of Hanks's saline, glucose and horse serum was added vitamin B_6, pyridoxine hydrochloride. The concentrations used in the medium were 0.03% and 0.1%. The broken line in Figure 28 indicates the rate of growth obtained with BSSG + HS with the symbols representing the two concentrations of vitamin B_6. The symbols all lie above the line representing growth in BSSG + HS but such a small increase could be attributed to an osmotic effect and not to growth.

b) A complete vitamin B supplement was added to BSSG + HS to give a concentration of 0.005%. The growth obtained with this medium is shown in Figure 29, the broken line representing the control medium, BSSG + HS. The complete B vitamin supplement appeared to induce a slight increase in growth as compared to growth in the control medium, but as with the vitamin B_6 supplement, this slight increase could be due to an osmotic effect.

Figure 28. The percentage change in dry weight shown by plerocercoids after 8 days cultivation in various media.

———— growth in BSSG+HS+YE;

-------- growth in BSSG+HS;

• growth in BSSG+HS+0.03% pyridoxine hydrochloride;

o growth in BSSG+HS+0.1% Pyridoxine hydrochloride.

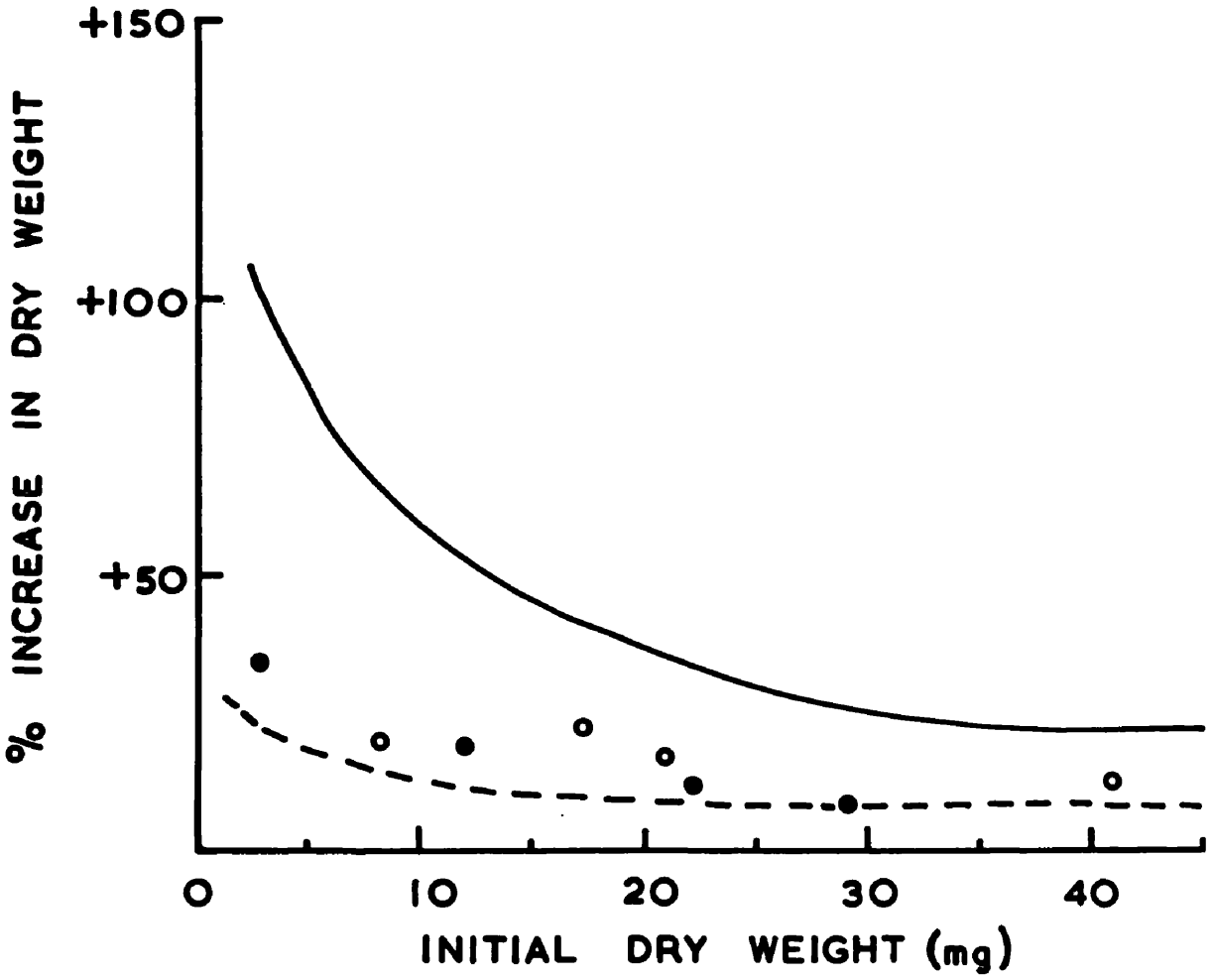
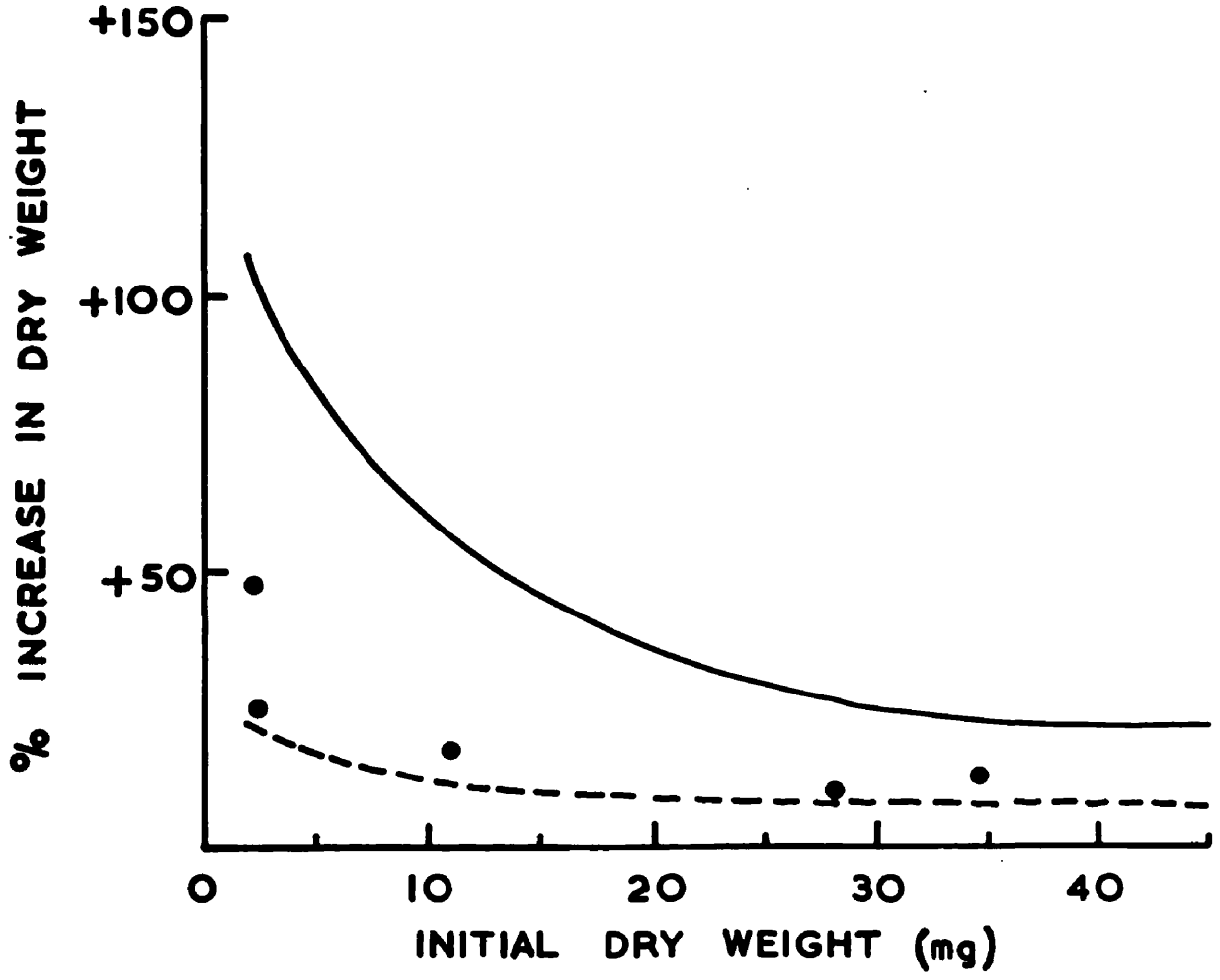

+150
+100
+50
% INCREASE IN DRY WEIGHT
0
10
20
30
40
INITIAL DRY WEIGHT (mg)

Figure 29. The percentage change in dry weight shown by plerocercoids after 8 days cultivation in various media.

———— Growth in BSSG+HS+YE;

---------- growth in BSSG+HS;

• growth in BSSG+HS+0.005% vitamin B.


+150
+100
+50
% INCREASE IN DRY WEIGHT
0
10
20
30
40
INITIAL DRY WEIGHT (mg)

(xi) Effect of using hydrolysed yeast in the medium

Yeast extract, hydrolysed with acid or alkali, was included in a medium with saline, serum and glucose. The effect of hydrolysis of the yeast is shown in Figure 30. Acid hydrolysis (open points) had little effect but alkali hydrolysis (closed points) had destroyed some essential substances (as indicated by the decrease in growth of the plerocercoids from that obtained with non-hydrolysed yeast - symbol +). However, the horse serum could have been contributing some of the substances destroyed in the yeast.

A medium consisting of saline, glucose and yeast was thus used, the results of which are shown in Figure 31. The rate of growth in both media was less than that in the control medium (BSSG+normal YE), with the alkali hydrolysed yeast possibly inducing slightly less growth than the acid hydrolysed yeast.

Figure 30. The percentage change in dry weight shown by plerocercoids after 8 days cultivation in various media.

———— Growth in BSSG + HS + YE;

o growth in BSSG + HS + acid hydrolysed yeast;

• growth in BSSG + HS + alkali hydrolysed yeast.

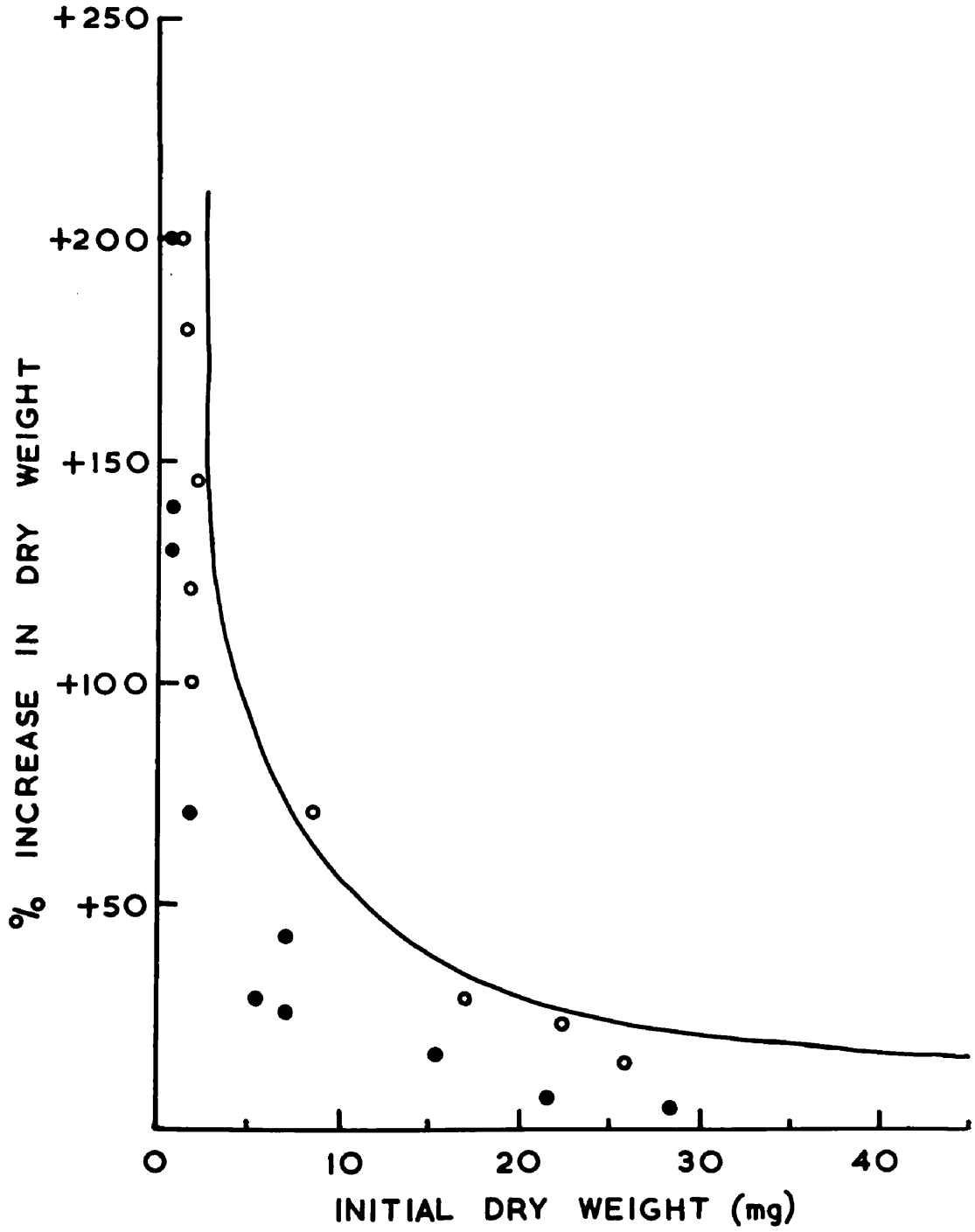
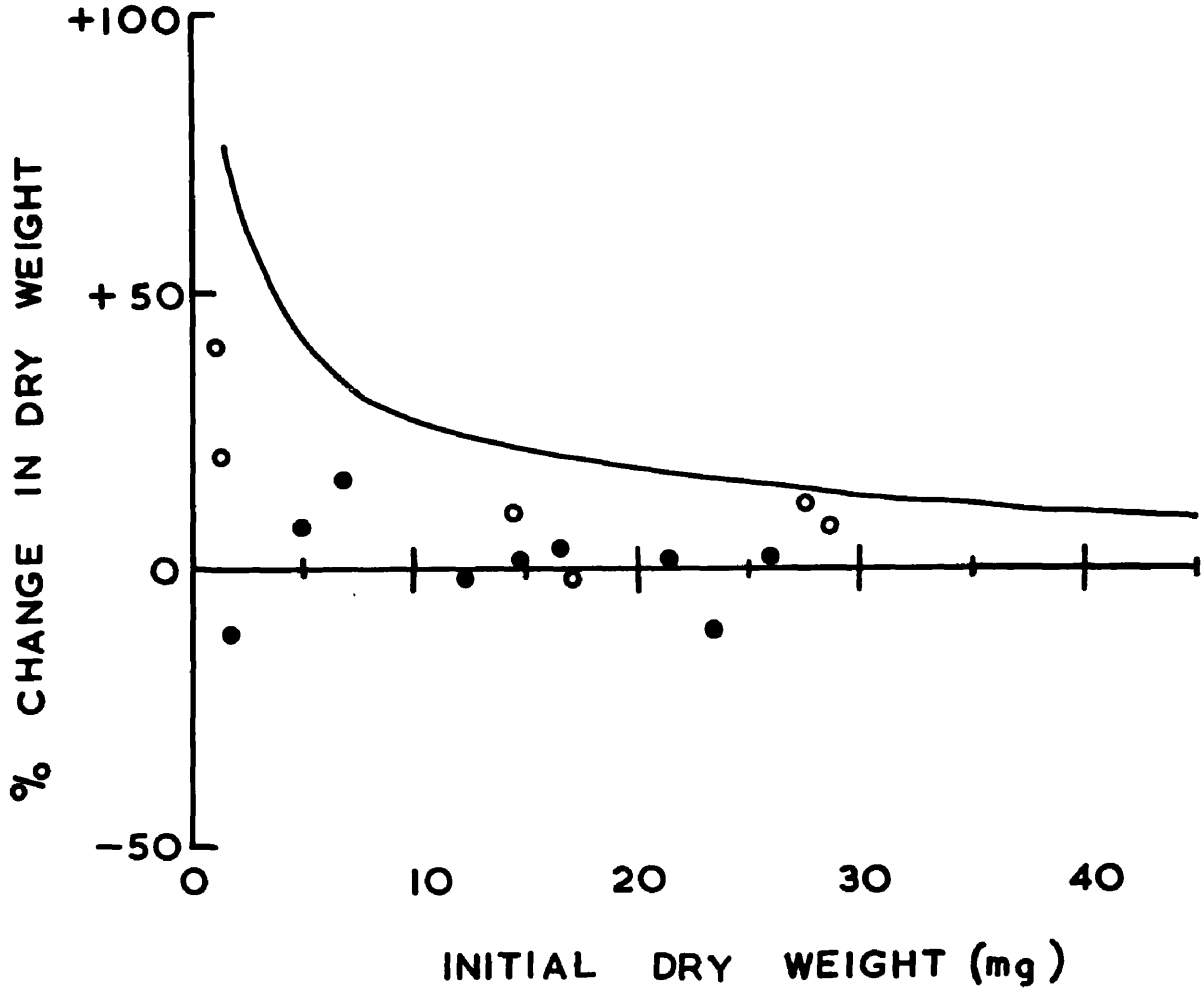

+250
+200
+150
+100
+50
% INCREASE IN DRY WEIGHT
0
10
20
30
40
INITIAL DRY WEIGHT (mg)

Figure 31. The percentage change in dry weight shown by plerocercoids after 8 days cultivation in various media.

———— Growth in BSSG + YE;

o growth in BSSG + acid hydrolysed yeast;

● growth in BSSG + alkali hydrolysed yeast.


+100
+50
0
-50
% CHANGE IN DRY WEIGHT
0
10
20
30
40
INITIAL DRY WEIGHT (mg)

D. Discussion

Growth of plerocercoids in the basal medium

Plerocercoids of Schistocephalus had been kept alive and normal in appearance in salines for 114 S.D. 9 days by Smyth (1946) and hence it was expected that in 8 days, little loss of weight would occur in Hanks's saline plus glucose (Figure 20a). This result, however, provides a baseline with which to compare the results obtained from more complex media. The addition of serum or yeast extract prevented weight loss, with the latter inducing more growth than the former (Figure 20b, c). On combining yeast and serum, a synergistic effect was observed (Figure 20d) which was similar to that observed with Diplostomum phoxini by Wyllie, Williams and Hopkins (1960).

At the end of the cultivation period, the worms appeared healthy, i.e. no 'blistering', sloughing of cuticle (integument) or loss of muscular contractility had occurred. There had been an increase in the water content of the plerocercoids (Figure 19) during the 8 days in culture but when this increase occurred is not known. If it occurred soon after the worms were put in culture it would probably be an osmotic change correctable by increasing the osmotic pressure of the medium, rather than a degeneration change. However, the fact that plerocercoids over 50 mg F.W. at the end of/

/cultivation were capable of producing eggs (McCaig & Hopkins, 1965) suggests that no major pathological change occurred. Also, the plerocercoids grew to 85-89% of their expected weight (Table 24), the difference from expected to actual growth being in the range which might be expected if the osmotic pressure of the medium differed slightly from that of the medium. However, cultured worms failed to grow during a successive period of 8 days as rapidly as plerocercoids of the same weight taken fresh from fish. Various explanations have been suggested such as exhaustion of certain metabolites in the worm and lack of their precursors in the medium but electron microscopy has demonstrated the presence of a thin layer of material either deposited or secreted on the outside of the microvilli of the integument. A possible correlation between this masking of the villi and decrease in ability to grow in culture is being investigated by other workers.

Small plerocercoids were metabolically much more active than old plerocercoids, the rate of growth of a 4 mg D.W. worm being 8-10 times that of a 40 mg D.W. worm (Figure 20). With the worms available in the supply lochs, the growth curve did not go to zero, the largest plerocercoids always increasing in weight in 8 days and from the shape of the growth curve, it seems probable that the line never reaches zero.

/Glycogen and water content of plerocercoids

During growth in the perivisceral cavity, a specific level of glycogen exists in a plerocercoid at any given weight, the amount ranging from 24± 4% D.W. in 2-15 mg F.W. worms to around 52± 3% D.W. in worms over 70 mg F.W. (Figure 17). This implies that the plerocercoids have a regulatory mechanism. The fact that in culture, the level of glycogen in small plerocercoids rises above the *in vivo* level (Figure 19) means either, there is less glucose available to the plerocercoid *in vivo*, or the regulatory mechanism is being overwhelmed *in vitro*.

Under *in vivo* conditions, the rate of accumulation of glycogen is similar to the increase in dry weight (Figure 18) but *in vitro*, the two rates differ. The change commences at approximately 3 mg D.W. (15 mg F.W.) which is the size at which distinct genital primordia can be observed and the plerocercoids are capable of maturing if subjected to a rise in temperature (see under "Plerocercoid," Section I). This parallels the report of Hopkins and Hutchison (1958) that the relative rate of synthesis of protein to dry weight alters at the time the larva reaches infectivity.

The decrease in water content which occurs during growth from 3 -30 mg D.W. results from the increase in glycogen (Figure 19). The dry weight increases to a plateau about 31% of the fresh weight which is much higher than in/

/Cyclophyllidean cestodes (Hopkins and Hutchison, 1960). In that group, the glycogen content is much lower, e.g. 32% D.W. (range 19 - 43%) in adult Raillietina cesticillus (Reid, 1942), 40-44% larval Hydatigera taeniaeformis (Hopkins, 1960), and a maximum of about 40% in adult Hymenolepis diminuta (Roberts, 1961).

Physical conditions of culture

The plerocercoids show a wide pH tolerance despite the fact that the pH of the body cavity of G. aculeatus is precisely maintained (7.2 to 7.4 as recorded with a glass electrode). However, the growth rate within the range pH 6.3 - 8.1 showed no significant difference. This lack of sensitivity to pH is similar to that reported for Hymenolepis nana by Bernzten (1962). Carbon dioxide, in the concentration range, 0.04 (air) - 5% had no effect on the worms but our method of sealing cultures in a drum filled with a gas mixture does not expose the worms to the gas mixture as efficiently as Bernzten's method of bubbling the gas actually through the mixture. The plerocercoids exhibited tolerance to a high oxygen tension which is the opposite to the situation with the adult where bubbling air through the medium causes the worm to go brown and egg production to become abnormal (Hopkins, 1952: Smyth, 1956).

/An environmental factor which was not considered was the temperature at which the plerocercoids were before being placed in culture. Walkey & Davies (1964) while measuring the Q_{10} of Schistocephalus found a marked acclimation effect. Plerocercoids recovered from fish in summer and heated to 20°C had a lower rate of respiration (and hence growth rate?) than had plerocercoids recovered from fish in winter and heated to 20°C.

Schistocephalus plerocercoids can thus grow in a wide range of physical conditions in a medium not identical with the coelomic fluid of a stickleback.

Effect of including sugars in the medium

Three criteria were used in determining the effect of sugars on the plerocercoids. Firstly, the effect on the rate of growth of the worms and the reproducibility of that rate. Secondly, it was known that in the basal medium, the dry/fresh weight ratio of the worms was decreased after cultivation for 8 days (Figure 19); variation from the in vivo and in vitro ratio was used as a criterion. The third criterion used was the glycogen content of the worms compared with the initial content of the worms [the in vivo level (Figure 17)].

Growth did not occur in saline or saline and sugar media, but the worms remained healthy in appearance. The rate of growth of plerocercoids in a medium consisting of/

/horse serum and saline was arratic but on the additon of glucose to the medium, the growth did not increase but became more regular. It is not known how the presence of glucose in the medium effected this change in the growth rate; it is possible that the level of glucose in the serum is low for the plerocercoid and the extra glucose allows the worm to maintain slow growth. In medium containing yeast, the rate of growth is independent of the presence of glucose no matter whether serum is present or not (Tables 25, 27). This could be attributed to the relatively high carbohydrate content of yeast itself.

The dry to fresh weight ratio of the worms in saline + a sugar was slightly raised above that obtained in the basal medium (Figure 19) and, since the sugars were added as isotonic solutions, it is suggested the saline may have been slightly hypertonic to the worms. Variations in the dry to fresh weight ratios of small worms (<10 mg D.W.) occurred when only serum and saline were used (Table 26) but on the inclusion of glucose in the medium, the ratio became normal. The osmotic pressure of the medium was similar to that of other media and the glucose was added as an isotonic solution. Hence a medium of serum and saline disturbs the development and growth of the plerocercoids in culture but the presence of glucose offsets this effect./

/A general increase in the glycogen content of small plerocercoids less than 5 mg D.W. occurred in culture except in a medium consisting of saline alone. As discussed previously in relation to the basal medium this increase in glycogen may mean that *in vivo* there is less glucose available to the worm or a regulatory mechanism is being overwhelmed. When no glucose was available (Figure 23), the glycogen content of the small worms decreased below the *in vivo* level but no decrease occurred in the large plerocercoids.

Small plerocercoids potentially have a very rapid growth rate (Figure 20) and hence their uptake of nutrients and turnover thereof must be equally rapid. When nutrients are present, rapid growth occurs but when nutrients are limited either in quantity or by total lack of certain substances, growth is limited. In the medium of serum and saline, growth is erratic but the addition of glucose steadies the growth rate suggesting that low carbohydrate can limit or disturb growth. In saline alone or with sugars, growth does not occur due to a general lack of nutrients with the glycogen level dropping in small plerocercoids as they utilise their stored glycogen to maintain themselves, the larger plerocercoids possibly having a slower metabolism and hence utilising less of their stored glycogen. Yeast extract, with its high carbohydrate/

/content requires no extra sugar for growth to occur (Table 25).

Effect of adding an amino acid and vitamin supplement to the medium

The increase in dry weight of very small plerocercoids (< 2.5 mg D.W.) was in the region of 150-200% in 8 days in the basal medium containing the first type of yeast extract. With the addition of amino acids and vitamins, the increase in dry weight for the same size of plerocercoids was about 500% (Figure 24). These small plerocercoids indicate the effect of a medium on growth. With increase in initial size of plerocercoids, the difference in the growth rate induced by the control medium and the medium being tested becomes less until plerocercoids greater than 20 mg D.W. initially have similar growth rates.

Since small plerocercoids grow more rapidly than larger ones (Figure 20), and are concurrently undergoing segmentation and development of genital primordia, the increased supply of amino acids and vitamins in the medium allows the whole process to proceed at a greater rate than in the basal medium alone. The greater rate of growth may be achieved by the increase in quantity of nutrients available to the plerocercoid or they may be in a balance more suitable to the plerocercoids' requirements. The basal medium may thus be limiting growth by lack generally of nutrients or the scarcity of one or a few of the essential/

/nutrients. This would explain the effect of the amino acids and vitamins. The actual state the amino acids and vitamins are supplied in will be similar to that in yeast and serum.

The manner in which the amino acids and vitamin supplement affects the increased growth rate was first investigated by adding the supplement to media lacking either yeast extract or horse serum. Both yeast (Sykes, 1956) and horse serum (Spector, 1956) contain amino acids and vitamins although in the latter, the exact composition is not known. Growth in the two media (Figures 25 & 26) was increased over that in the control media but did not attain the rate in the complete basal medium. The supplement thus enhances the effect of both yeast and serum but cannot completely replace them in the medium.

Apart from the different proportions of amino acids and vitamins, the supplement, used to replace the yeast and serum, did not contain any of the trace elements found in yeast or any of the globulins and sterols present in serum. Their addition might improve the supplement. [Williams, 1961, showed that the globulin fraction of serum was important for the growth and development of the strigeid, Diplostomum phoxini.] From later experiments, it became apparent that the amino acid part of the supplement was the effective part during short-term cultures (see next paragraph)./

/However, the maintenance of plerocercoids for many weeks *in vitro* might show the necessity for vitamins in the medium (Bernzten, 1961 ... includes them in his media for long-term cultures of the *Hymenolepids* and *Trichinella*.)

Effect of adding a vitamin supplement to the medium

The use of various concentrations of vitamin B_6 resulted from the work of Williams, Hopkins and Wyllie (1961) who reported that concentrations of pyridoxine exceeding that in yeast, enhanced the development of *Diplostomum phoxini*. With *Schistocephalus*, a slight increase in the growth rate occurred with various concentrations of pyridoxine (Figure 28), greater than that in yeast with a B vitamin supplement having the same effect (Figure 29). Williams *et al* (1961) also found that B_6 gave as good results as whole B vitamin supplement. Since the increase in growth was so small, there is some doubt as to its cause - it may represent growth or be an osmotic change. The dry/fresh weight ratio of the plerocercoids was normal for cultured worms and hence it is assumed that the vitamins have induced a slight increase in growth. It is not comparable to that induced by the amino acids and vitamins.

Concentration of yeast in the medium

The basal medium contained yeast at a concentration of 0.5%. It was not known whether this concentration limited the growth of the plerocercoids or if the nutrients were being provided in excess. The results, using a range of yeast concentrations, indicate that in a medium consisting of saline and yeast, a 0.5% concentration supplied the nutrients in excess (Figure 27). Further, 0.1 g of yeast per 100 ml of medium provided sufficient nutrient as indicated by the identical growth rates obtained with 0.11%, 0.5% and 1.1% yeast.

However, the growth obtained with the various concentrations of yeast in 16 days was identical to that in 8 days with 0.5% yeast (Table 25e Figure 20). Thus the inability of the plerocercoids to maintain growth for a second period of 8 days occurs both in the basal medium (BSSG+HS+YE) and in a medium lacking horse serum and glucose (BSS+YE).

Effect of hydrolysed yeast in the medium

To determine the components of yeast extract which are important for the growth of the plerocercoids, some splitting of the yeast is necessary. This splitting was achieved by hydrolysing yeast extract with either normal hydrochloric acid or normal sodium hydroxide./

/The replacement of untreated yeast by alkali hydrolysed yeast in the basal medium (BSSG+HS+YE) resulted in a marked decrease in the growth rate (Figure 30). Acid hydrolysed yeast in the same medium induced similar growth to that achieved with untreated yeast (Figure 30). When horse serum was omitted from the medium, acid hydrolysed yeast did not induce similar growth to untreated yeast (Figure 31). It is suggested thus that acid hydrolysis destroys some proteins and amino acids which are also present in horse serum. The substances destroyed by the alkali are either not present in horse serum or are not present in sufficient quantity to overcome the effect of destroying those in yeast.

Three amino acids, serine, threonine and tryptophane are destroyed by acid treatment and five, cystine, cysteine, arginine, serine and threonine are destroyed by alkali treatment (Williams, Hopkins & Wyllie, 1961). From the results shown in Figures 30 and 31, it would appear that some or all of these amino acids are required by the plerocercoids for normal growth and untreated yeast in the medium provides the total requirements of these substances for the plerocercoids.

Yeast extract is a source of vitamins as well as amino acids but the addition of vitamins alone to the medium had/

/a negligible effect on the growth of the plerocercoids (Figure 29). Hence, the reduction in growth of the plerocercoids in the presence of hyrdolysed yeast suggests that some of the amino acids in yeast are essential for growth.

E. Summary

1) Schistocephalus plerocercoids, 2-200 mg F.W., were recovered from the perivisceral cavity of G. aculeatus and cultured in a basal medium.

2) In the basal medium which consisted of 25% horse serum, 0.5% yeast extract, 0.65% glucose and Hanks's saline at pH 7.1, 21°C, 95% air + 5% CO_2, dry weight increases of up to 500% were recorded in eight days. The growth rate of large plerocercoids was only one tenth the rate observed in small plerocercoid.

3) After 8 days cultivation, plerocercoids had a slightly higher than normal glycogen and water content.

4) Growth during a second period of 8 days was variable, the worms growing only 50-70% of the expected amount.

5) The in vivo glycogen and water content of plerocercoids from 3-300 mg F.W. was determined. Glycogen rose from 24% in plerocercoids of 10 mg F.W. to 50-55% in plerocercoids over 80 mg F.W. The water content was found to mimic precisely this change, falling from 82% to a plateau of 67-69%.

6) The glycogen content of plerocercoids less than 5 mg D.W. after 8 days in saline had dropped below the in vivo level but the presence of ribose, sucrose or glucose in the saline/

/caused a large increase in the glycogen content.

7) The presence of glucose in a medium consisting of serum and saline stabilises the rate of growth and the dry/fresh weight ratio of the plerocercoids.

8) The addition of glucose to a medium containing yeast produced no effect on worms.

9) The use of galactose instead of glucose in a medium has no effect on the plerocercoids.

10) Growth in the basal medium is increased, especially the small plerocercoids, with the addition of an amino acid and vitamin supplement.

11) In media lacking either yeast extract or horse serum, growth is increased with the addition of amino acids and vitamins. The rate induced was lower than that obtained with the complete basal medium.

12) It is uncertain if the vitamins affect the growth rate in the absence of amino acids during an 8-day period but the possibility that they are necessary for long-term maintenance in culture is discussed.

13) Yeast at a concentration of 0.11% to 1.1% in a medium induces identical growth of the plerocercoids.

14) Certain constituents of the yeast were destroyed by treatment with normal HCl or normal NaOH.

/15) Horse serum masks the effect of acid hydrolysed yeast on the growth of the plerocercoids. This did not occur with alkali hydrolysed yeast.

16) In the absence of serum, plerocercoids showed very little increase in size in the presence of either acid or alkali hydrolysed yeast.

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TABLE 15

Variation in Output of Eggs of Schistocephalus solidus during course of Infection in different Laboratory Hosts.

Host	Number of animals used	Age of host at infection		Egg Output from Various Hosts. Time in days. 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Anas boschas (Duck)	6	7 days	No. of Eggs[1].	-	-	61	55	38	-	-	19	10	5	3	0	-							
			No. of Hosts[2] infected	-	-	6	6	6	-	-	6	6	4	3	0	-							
			Average Eggs per host	-	-	10.2	9.2	6.3	-	-	3.2	1.7	1.3	1.0	0	-							
Columba livia (Pigeon)	6	Adult	No. of Eggs	-	-	33	25	-	18	11	5	2	1	1	0	-							
			No. of Hosts infected	-	-	5	6	-	6	6	4	2	1	1	0	-							
			Average Eggs per host	-	-	6.6	4.2	-	3.0	1.8	1.3	1.0	1.0	1.0	0	-							
Gallus domesticus (Chicken)	6	6 days	No. of Eggs	-	-	36	33	21	21	15	9	8	10	11	11	10	5	4	3	0	-		
			No. of Hosts infected	-	-	6	6	6	6	6	6	6	6	6	6	6	5	4	3	0	-		
			Average Eggs per host	-	-	6.0	5.5	3.5	3.5	2.5	1.5	1.3	1.7	1.8	1.8	1.7	1.0	1.0	1.0	0	-		
Gallus domesticus (Chicken)	5	6 days	No. of Eggs	-	-	18	16	12	12	13	6	5	4	6	5	2	1	0	-				
			No. of Hosts infected	-	-	5	5	5	5	5	5	4	4	5	4	2	1	0	-				
			Average Eggs per host	-	-	3.6	3.2	2.4	2.4	2.6	1.2	1.3	1.0	1.2	1.3	1.0	1.0	0	-				
Rattus norvegicus (Rat)	5	6 weeks	No. of Eggs	-	-	5	5	5	5	3	0	-											
			No. of Hosts infected	-	-	3	5	5	5	3	0	-											
			Average Eggs per host	-	-	1.7	1.0	1.0	1.0	1.0	0	-											
Mesocricetus auratus (Hamster)	4	3 months	No. of Eggs	-	-	6	7	7	8	6	5	2	0	-									
			No. of Hosts infected	-	-	3	4	4	4	4	4	2	0	-									
			Average Eggs per host	-	-	2.0	1.8	1.8	2.0	1.5	1.3	1.0	0	-									
Mesocricetus auratus (Hamster)	6	3 months	No. of Eggs	-	-	7	13	14	11	12	10	11	12	9	8	8	7	9	7	8	5	3	1
			No. of Hosts infected	-	-	6	6	6	6	6	6	6	6	6	5	6	6	6	4	5	4	2	1
			Average Eggs per host	-	-	1.2	2.2	2.3	1.8	2.0	1.7	1.8	2.0	1.5	1.6	1.3	1.2	1.5	1.8	1.6	1.3	1.5	1.

1. No. of Eggs per unit area of faecal smear.

2. No. of Hosts which are still infected each day.

Egg Output from Various Hosts.										Time in days.										
0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
-	-	61	55	38	-	-	19	10	5	3	0	-								
-	-	6	6	6	-	-	6	6	4	3	0	-								
-	-	10.2	9.2	6.3	-	-	3.2	1.7	1.3	1.0	0	-								
-	-	33	25	-	18	11	5	2	1	1	0	-								
-	-	5	6	-	6	6	4	2	1	1	0	-								
-	-	6.6	4.2	-	3.0	1.8	1.3	1.0	1.0	1.0	0	-								
-	-	36	33	21	21	15	9	8	10	11	11	10	5	4	3	0	-			
-	-	6	6	6	6	6	6	6	6	6	6	6	5	4	3	0	-			
-	-	6.0	5.5	3.5	3.5	2.5	1.5	1.3	1.7	1.8	1.8	1.7	1.0	1.0	1.0	0	-			
-	-	18	16	12	12	13	6	5	4	6	5	2	1	0	-					
-	-	5	5	5	5	5	5	4	4	5	4	2	1	0	-					
-	-	3.6	3.2	2.4	2.4	2.6	1.2	1.3	1.0	1.2	1.3	1.0	1.0	0	-					
-	-	5	5	5	5	3	0	-												
-	-	3	5	5	5	3	0	-												
-	-	1.7	1.0	1.0	1.0	1.0	0	-												
-	-	6	7	7	8	6	5	2	0	-										
-	-	3	4	4	4	4	4	2	0	-										
-	-	2.0	1.8	1.8	2.0	1.5	1.3	1.0	0	-										
-	-	7	13	14	11	12	10	11	12	9	8	8	7	9	7	8	5	3	1	0
-	-	6	6	6	6	6	6	6	6	6	5	6	6	6	4	5	4	2	1	0
-	-	1.2	2.2	2.3	1.8	2.0	1.7	1.8	2.0	1.5	1.6	1.3	1.2	1.5	1.8	1.6	1.3	1.5	1.0	0