OCCURRENCE OF CERCARIAE IN A PERMANENT POND AND STREAM

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ABSTRACT

This investigation deals with the occurrence of cercariae in a permanent pond in contrast to those occurred in a stream. The snails were hand collected and microscopically observed for the emergence of cercariae in the laboratory. Temperature and the dissolved oxygen content of the water were recorded for the observation of their effect on the occurrence of cercariae. Current of the stream and the chemical content of the water were also recorded for the same purpose. In general five types of cercaria were found. Three belonged to the group Furcocercus and two belonged to the group Xiphidiocercus. Only one cercaria, ornatae cercaria, was common in both sites and was the only one present in the stream. It appears that the host specificity of the cercariae and the current of the stream are the two main factors contributing to the occurrence of the cercariae in the two environments.

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INTRODUCTION

The purpose of this study was to compare two completely different environments where cercariae occurred. This enabled the investigator to account for the cercariae found in one environment and to contrast them to those found in the second environment and to consider the ecological factors that might have been responsible for their occurrence.

Cercariae are the juvenile forms of the adult digenetic trematodes (Schmidt & Roberts, 1977). Digenetic trematodes are a group of trematodes that require at least two hosts in their life cycle, an intermediate host and a definitive host (Schmidt & Roberts, 1977). The intermediate host is almost always a mollusk and the definitive host a vertebrate.

At one stage of the life cycle, cercariae emerge from their snail host before infecting the vertebrate host. This phenomenon has generated an interest among the scientists to study the larval trematodes.

The two environments examined in this study were a pond and a stream. The pond was located in Esophea park in Vernon County Wisconsin (Fig. 1), and the stream was located in the lower portion of the Gundersen Arboretum in La Crosse County Wisconsin (Fig. 2).

This investigation was conducted from May 1978 to October 1979. The collections were suspended during the period of November to April when the pond and the stream were frozen. Water samples were collected and examined for the nutrient content. Temperature and the dissolved oxygen content of the water were also recorded to account for their influence on the snails and/or cercariae.

LITERATURE REVIEW

Digenetic Trematodes cause numerous diseases in animals and man. For this reason, scientists have been greatly interested in the life history of these parasites since the beginning of this century. Surveys of cercarial occurrence have been conducted by many investigators to account for the presence of these parasites, for they supply the information as to where these parasites occur that cause diseases.

Cort (1914) conducted the first survey of the cercariae in the North American Continent. He cataloged the larval trematodes in the fresh water snails of North America. He examined six species of fresh water snails and reported cercariae belonging to the groups monostome, amphistome and distome. He also described 14 new species of cercaria in his report and stated that except for one monostome and one amphistome, the rest belonged to the distome group. Faust (1919) confirms some of Cort's findings. He reported that the general bulk of cercariae are distome. He also reported that three groups; the Stylet cercariae, echinostome, and forked dominate most of these cercariae. He reported that the furcocercus cercariae are not known to encyst.

Other investigators have also conducted surveys in different localities in the United States. Faust (1918), Brooks (1943 a,b) and Hall (1960), Acholonu (1964) and Erlandson (1972) reported cercarial occurrence from Illinois, Iowa, Indiana, Michigan, Colorado and Wisconsin, respectively.

Yet, other investigators were interested in the life cycle of these parasites. Park (1936), McMullen (1937), Oliver (1940), Hunter, et al (1939), Chandler (1942a), Krull (1934 and 1935), Huggins (1954), Macy (1956), Macy (1960), and Howell (1968) investigated the life histories of some digenetic trematodes and in some cases reported new parasites which were of great importance to the scientists for these types of information supplied the first road to overcoming the problems of parasites.

McCoy (1928) investigated the seasonal fluctuations in the infection of Planorbis trivolvis with larval trematodes in St. Louis, Missouri. He showed no clear cut seasonal fluctuations in the infected snails. However, he noted that the degree of infection varies widely from time to time regardless of the time of the year. A work done by Cort et al (1939) confirms McCoy's report. They collected larger snails only and reported that the number of incidence of infection was by chance and not due to seasonal fluctuations. However, Najarian (1952) working in Michigan reported that snail infection was greatest in April and least in September. Smith (1967) also reported that in the case of Ferrisia fragilis the infection peaks were in Spring and Autumn, but in Helisoma trivolvis the infection was constant. Gambiano (1959) studied the seasonal incidence of infection of Nassarius obsoletus with larval trematodes in Greenwhich Bay, Rhode Island. He reported that the incidence was low in October to early March and it rose sharply from March 21 and it tapered off in April and May. He reported that by June the infection disappeared. He based his finding on the migration of Avian hosts of these parasites and not the time of the year.

Brackett (1940, 1942) reported seven new species of schistosome cercariæ from Wisconsin and Michigan. He reported that 10% of schistosomal dermatitis occured in Wisconsin and that the snail Gyraulus pravus could be involved in its incidence.

Cort et al (1941) studied the larval trematode infection in juveniles and adults of Physa parkeri. They reported that the juveniles of P. parkeri were infected with larval trematodes at all stages of their development and the infections were carried over winter. They also reported that the adults can also acquire the infection. They suggested that the miracidia (the stage of the trematode life cycle after hatching from egg) penetrate the snail after the host hatches from the egg.

Bedinger et al (1967) worked on the biology of Posthodiplostomum minimum from Madison County, Texas. They reported that there were different strains of cercariae for P. minimum based on the host specificity of cercariae.

Krull (1930) described the life history of two North American frog lung flukes, Pneumonoeces medioplexus and P. parviplexus. He demonstrated that the dragon fly nymphs are the second intermediate hosts in which the metacercaria (a stage in the trematode life cycle after cercariae) develop.

Harper (1934) studied the larval trematodes of Britania. He found four new xiphidiocercus cercariae and in total described six cercariae in detail. He reported that the liver and the gonads of the snail host were destroyed by the larval trematodes. He also reported of no double infection on the snail host.

McMullen (1934) studied the life history of the turtle trematode Cerchis medius. He reported that in the cercarial stage there was a correlation between the type of the second intermediate host and the strength of the stylet of the cercariae.

Miller (1935) studied the North American cercariae. He described eight new species and found that cercariae emerged from the snail by artificial raise in the temperature, but died before encystment due to the fact that the process may have been too fast. He also concluded that the diversity in the cercarial shape depends upon their cosmopolitan adjustment to various hosts in different localities.

Herber (1939) studied the biology of the frog lung fluke Diplodiscus temperatus. He noted that upon metamorphosis of the frog, the flukes were dispersed throughout the digestive system apparently because of increased peristalsis.

Leigh (1946) studied the cercariae of Glypthelminus quieta. He noted that the cercariae did not respond to light and lived up to 65 h. He also showed that a period of high productivity of cercariae from the snail was followed by a period of low productivity. He noted that the cercariae swam randomly and came into contact with the second intermediate host by chance. He also noted that during molting, the frog ingested its own skin, therefore, the metacercariae were transferred to the gut and developed into adult parasites.

Hoffman (1955) investigated the life history of Fibricola crutera. He found that cercariae would not penetrate frog embryos prior to hatching, but did penetrate one-day old tadpoles. He also noted that cercariae were unable to penetrate the adult frog.

Mathis and Cort (1956) studied the larval trematode infection in snails of different sizes. They reported that larger snails harbored greater number of cercariae. They noted that in Physa parkeri the size of the snail was related to the number of rediae (a stage of trematode life cycle in the snail).

Hoffman (1958) studied the cercariae and metacercariae of Posthodiplostomum minimum. He reported that the cercariae lived for 32.5 h. and were infective up to 24 h. He also noted that the snail shed cercariae at room temperature but not at 15°C. The cercariae were not infective at this temperature, but were infective at 18°C and above.

Goodchild and Kirk (1960) investigated the life history of Spirorchis elegans. They reported that this organism was different from other spirorchids in that there was no host-organ specificity. They also noted that the time required for the maturation of the cercariae in the snail host was characteristic of the species and that this time was related to the temperature.

Hunter and Birkenholz (1961) reported the larval trematodes of Gunnison County, Colorado. They reported that in this region echinostomes were more predominant and their prevalence in the snail increased in the season. They also found that the abundance of the larvae varied according to the time of collection, species of snail collected and the location of the collection.

Hall and Groves (1963) studied the virgulate xiphidiocercariae from Nitocoris dilatatus Conrad. They found that cercariae are very specific with regard to second intermediate host due to ecological or mechanical factors.

Lang (1963) compared the xiphidiocercariae of Plagiorchis muris and P. proximus. He based the separation of these two cercariae on the size and the shape of the stylet. He concluded that the length of the stylet used with the body shape was a unique characteristic for the identification of cercariae.

Holliman and Fisher (1968) studied the life cycle and pathology of Spirorchis scripta in Chrysemys picta picta. He found that the host showed reaction to the penetration of the cercariae and miracidia by rubbing tentacles in the case of the snail; and by rubbing of claws on the body in the case of the turtle. They noted that the time between the penetration of miracidia and the emergence of the cercariae was related to temperature. Watertor's work (1968) confirms this point. He reported that temperatures of 4°C and 10°C inhibited cercarial development. After transfer to 30°C, he noted, cercarial development followed by emergence. He found that the temperature of 30°C seemed to be the optimum temperature for development of cercariae.

Acholonu (1964) surveyed the larval trematodes of Colorado. He reported four major groups of cercariae in that region: furcocercus, monostome, echinostome and xiphidiocercus. He also noted that the snails heavily infected with cercariae died sooner than snails with no infection (presumably due to the mechanical injury to the snail's internal systems.)

Erlandson (1972) studied the ecological factors affecting the occurence of of larval trematodes in a semi-permanent pond in Madison, Wisconsin. He reported great seasonal fluctuations in the annual and periodic emergence of cercariae due to temperature, drying of the pond, snail populations and the presence of the definitive hosts in the area.

All of the investigators mentioned in this review have contributed to the taxanomy and biology of the larval trematodes while helping others to better understand these larval trematodes. However, there was no comparative study between two or more environments to establish possible factors having adverse effect on the cercariae; which, was the prime objective of this investigation.

MATERIALS AND METHODS

Snails were hand collected from Esophea Park pond and from the lower Gundersen Arboretum stream. They were placed inside a plastic container with water from the collection sites and brought to the laboratory. In the laboratory the snails were put in small glass jars half filled with specially prepared water. The prepared water contained the following ingredients:

Calcium Sulfate	0.45 g
Magnesium Sulfate	0.45 g
Sodium Bicarbonate	0.72 g
Potassium Chloride	0.06 g
Distilled Water	14 l

Jars were identified as to the collection stie, each jar usually containing four snails. Glass jars containing the snails were kept at room temperature under a 60-watt desk lamp connected to a timer, which established the natural diurnal environment for the snails. After 24 h., the snails were examined in the glass jars with a dissecting scope for the emergence of cercariae. If cercariae were present in a particular jar, that jar was set aside and each of the snails removed and placed in separate marked jars. Twenty-four hours later these jars were examined again with the same dissecting scope. If cercariae were present, a drop of water containing cercariae was placed on a slide prestained with methylen blue or neutral red. A cover slip was placed on the drop of water and the slide was examined with phase contrast microscopy. This procedure was followed for each type of cercariae.

In order to determine the measurements of each cercarial type, ten cercariae were fixed by placing them in boiling hot water for a few seconds. Fixed cercariae were then placed on a slide in a drop of water. A cover slip was placed on the drop of water and the slide was examined with a phase contrast microscope. The cercariae were measured with an occular micrometer, values were recorded, and averaged.

In order to determine the size of the snails, they were placed on the back of a wet petri dish. The diameter of the shell and the length of the shell, depending on the snail type, was measured with a ruler.

Water temperature of both sites was measured in degrees of celcius with a standard laboratory thermometer. The dissolved oxygen content of water at each site was measured with a dissolved oxygen meter (YSI model 54 A). Measurements for both sites were taken within 2 hs. of each other.

The current of the stream was measured by placing an empty shotgun shell in the water and recording the amount of time it took to travel 1 meter. This procedure was repeated ten times in order to establish an average velocity for the stream.

Samples for the water chemistry tests were taken to the Limnology Laboratory of the Department of Biology of the University of Wisconsin-La Crosse. These tests included the tests for nitrite, nitrate, total phosphates, orthophosphates, ammonia, pH, conductivity and turbidity. The Single Reagent Method (EPA 1979) test was used to measure phosphate. The tests for nitrate were done according to the Mullen & Riley Reduction Method and the nitrite tests by the Coloro-metric, Hydrozene Reduction Method (EPA 1979). Tests for ammonia were done according to the

Phenolhypochlorite Method (APHA 1971). The pH was measured with an O'Brien ® pH meter model 701 (APHA 1971). Conductivity of the water samples were measured with a Marxon Conductivity Meter. Water turbidity was measured with a Hach ® turbidometer.

In order to develop metacercariae, tadpoles of Bufo americanus were collected from the pond in Esophea Park. They were placed in the water containing cercariae. If metacercariae were established, the tadpoles were sacrificed and observed microscopically. Tadpoles of the African Clawed frog (Xenopus leavis) were purchased from the Carolina Biological Supply Company for exposure to cercariae. Once the metacercariae were established, they were stained according to the procedure for staining the flatworms in Animal Micrology (Guyer, 1953).

A female Map Turtle (Graptemys geographica) was collected from the pond. This animal was sacrificed and dissected for the recovery of adult trematodes. A Green Frog, (Rana clamitans melanata) was recovered from the same pond to recover adult trematodes. These animals were collected in July, 1978. In September, 1979, a Leopard Frog, (Rana pipiens) and a species of Rana catesbiana were collected from the pond and stream respectively. These animals were doubly pithed and were dissected for the recovery of mature parasites.

Three laboratory mice were used for the infection experiments. These animals were anesthesized with diethyl ether and infected with the metacercariae by stomach tubing. In this method, a narrow plastic tube was placed on the tip of a hypodermic needle attached to a small syringe. Metacercariae were sucked into the syringe with water. The

tube of the syringe was forced into the stomach of a mouse via the mouth cavity and emptied into the stomach. Two of these mice were killed and dissected two weeks after being infected. The third mouse was sacrificed and dissected one month after being infected.

One dozen chickens were purchased from the Poultry Science Research Laboratory of the University of Wisconsin-Madison during August, 1978 for infection experiments. The stomach tubing procedure was not followed in this case due to the desire of the animals to readily eat the infected tadpoles. Three chickens were sacrificed and dissected two weeks after infection. The rest of the chickens were sacrificed and dissected at two week intervals. During the course of these experiments the chickens were kept in special cages prepared for them.

All animals involved in these experiments were observed for parasites in the body cavity. Different organs were dissected with teasing needles and probes in a glass dish filled with saline solution. If any parasites were recovered, they were fixed and stained according to the staining procedure for the flatworms in Animal Micrology (Guyer, 1953), and examined with phase contrast microscopy.

A special device was set up to observe the flame cell pattern of cercariae. Flame cells are part of the excretory system of cercariae and act as kidneys. Their arrangement in the cercariae (their pattern) is an important factor in identification of cercariae. The cercariae were placed on a slide in a drop of water. A piece of wire was attached to a metal bar which was attached to two ring stands (Fig. 23). The

wire was placed on the cover slip of the slide containing the cercariae. Movement of the bar was established by moving the metal bar along the ring stands (Fig. 24). Pressure on the cercariae pressed different organs out of the way making it possible to observe the excretory system.

A Minolta camera was used to take photomicrographs of the materials under the microscope. Kodak film (ASA 160, Tungsten) was used to make color slides. The same camera with Kodak film, Ektachrome ASA 200, was used to take pictures of the collection sites, the snails, and the apparatus for observation of the flame cells.

RESULTS

Snails from the pond were identified as Helisoma trivolvis (Fig. 3) and Physa parkeri (Fig. 4) (Hyman, 1967). Only one species of snail was collected from the stream, P. parkeri.

A total of 1,390 H. trivolvis and 51 P. parkeri were collected from the Esophea Park pond. A total of 682 P. parkeri were collected from the Lower Gundersen Arboretum stream. Thirty-six of H. trivolvis (2.6%) and three P. parkeri (5.9%) from the pond were found to be infected. Eight P. parkeri (1.2%) recovered from the stream were also infected. These values are shown graphically (Figs. 5,6,7,8,9,10) and are tabulated in Table 1.

Results of the water chemistry tests are recorded in Tables 2 and 3. Table 4 shows the temperature and dissolved oxygen contents of both collection sites. These measurements were taken within 2 hs. of each other.

Five types of cercariae were found, two xiphidiocercus and three furcocercus cercariae (Erasmus 1972, La Rue 1957, Olsen 1967, Schell 1970, Skrjabin 1964).

STRIGEA CERCAIRA: This organism was a member of the furcocercus cercariae. It had a prominent oral sucker. No acetabulum was seen in this organism (Figs. 11 & 12). A "Y" shaped excretory bladder was observed. No pharynx in this organism was observed, however, an esophagus was noted. The body length ranged from 290 µm to 470 µm with a width of 70 µm to 160 µm. The diameter of the oral sucker was 90 µm to 120 µm. The tail ranged from 450 µm to 550 µm in length with a width of 50 µm to 100 µm.

The tail divided into two branches at the bottom making the appearance of a forked tail, hence, the name furcocercus. The length of the furcae ranged from 450 μm to 500 μm, almost equivalent to the length of the main stem of the tail. One excretory pore opened to the outside on the upper surface of the furcae. Spines were observed to project from the main stem of the tail and the body. This cercariae was the larval form of either family Diplostomatidae or Strigeidae. Due to the lack of information on the flame cell pattern of this organism, identification to the species level was not possible. The snail host of this organism was H. trivolvis. Detailed measurements of this cercaria are listed in Table 5.

BREVIFURCATE-PHARYNGEATE CERCARIA: This organism was also a furcocercus cercariae (Figs. 13,14,15). The oral sucker was prominent and active. No acetabulum was observed in this organism. Two dark colored spherical bodies called eyespots were visible in the midventral portion of the body. A cellular mass staining red with neutral red dye, the germinal mass, was observed. The excretory bladder was "Y" shaped. A dorsomedian finfold was observed with phase contrast microscopy. Excretory pores were present on the tip of the furcae. The body of this cercariae was short compared to other cercariae ranging from 200 μm to 360 μm in length and 100 μm to 150 μm in width. The tail measured from 260 μm to 600 μm in length and 50 μm to 70 μm in width. The pharynx of this pharyngeate organism was visible under high magnification. The furcae ranged from 150 μm to 250 μm in length, and the oral sucker was 50 μm to 70 μm in diameter. This organism belonged to the family Clinostomatidae.

Comparison of the photographs of this organism and the drawings made by Krull (1934) revealed that this organism belonged to the genus Clinostomum and possibly the species marginatum, however, the latter cannot be established with certainty due to the lack of information on the flame cell pattern. More detailed measurements of this organism can be found in Table 9.

BREVIFURCATE-APHARYNGEATE CERCARIA: This organism was also a furcocercus cercaria (Figs. 16,17). It had a prominent oral sucker and acetabulum was present and very active. Eyespots were present in the upper half of the body and a "U" shpaed excretory bladder was observed. The furcae were short and each bore a finfold. Execretory pores could be seen on the tip of the furcae. The body length measured 350 μm to 810 μm, with a width of 130 μm to 320 μm. The stem of the tail ranged from 1,000 μm to 1,500 μm in length and 100 μm to 180 μm in width. Furcae ranged from 250 μm to 450 μm in length. The oral sucker ranged from 50 μm to 150 μm in diameter. No pharynx was observed in this organism which coincided with its name. This organism was observed to be the most active cercariae collected. It was identified to be the juvenile form of the members of the family Spirorchiidae. Since the flame cell pattern was not observed, further identification was not possible. This organism was found only in H. trivolvis. More detailed information on the measurements of this cercariae were outlined in Table 6.

ARMATAE CERCARIA: This organism belonged to the general class of xiphidiocercus cercariae (Figs. 18,19). This type of cercariae was different from the Furcocercus cercaria in that they had the tail with no furcae. Usually the body length in these organisms was longer than

the length of the tail. They normally had a stylet in the region of the mouth sucker; this stylet, a very large spine, was important in the identification between the members of this type of cercaria. The body also contained a large number of granular bodies. In the Armatae cercaria the oral sucker was present and prominent, and the acetabulum was present in the lower half of the body. A "Y" shaped excretory bladder was present. The penetration glands were present and stained red with neutral red dye. The exact number of these structures could not be counted. One tubule from the penetration glands on each side of the body ran towards the oral sucker. The openings of these tubules opened at the base of the stylet. A pharynx was also noted as well as the prepharynx. The esophagus led to the branched intestinal ceca located on each side of the body. Excretory pores of this organism were not observed. The body was long compared to the other cercariae measuring from 640 μm to 800 μm in length and 250 μm to 350 μm in width. The oral sucker measured from 100 μm to 150 μm in diameter. The stylet was short and ranged from 20 μm to 40 μm. The length of the tail ranged from 300 μm to 500 μm. More detailed information on the measurements of this cercaria are listed in Table 7. This organism was identified as the larval form of the families Plagiorchiidae and Telorchiidae. It was found only in H. trivolvis as other cercariae previously mentioned.

ORNATAE CERCARIA: This organism was also a xiphidiocercus cercaria. It had a prominent oral sucker and the acetabulum was also present. The penetration glands were present and stained red with neutral red dye. The exact number of these glands could not be determined. The

body ranged from 600 μm to 850 μm in length with a width of 240 μm to 300 μm. The stylet was large and measured from 65 μm to 80 μm in length, much larger than that of armatae cercaria. The length of the tail was 220 μm to 440 μm. The oral sucker ranged from 100 μm to 150 μm in diameter. A finfold was present on the tail. Spines have also been observed along the body length. This organism belonged to the families Macroderoididae and Haplometridae. It is demonstrated in Figs. 20 and 21. Comparison of the photographs of this organism and the drawings by Schell (1965) revealed that this organism was from the genus Haematoloechus and probably the species breviplexus. However, since the flame cell pattern of this organism was not known, the specific identification could not be made for certain. The excretory tubules of this organism were observed (Fig. 21). More detailed information on the measurements of this cercaria is tabulated in Table 8. Attempts to establish the location of the excretory pores of this organism failed. This organism was found in both H. trivolvis and P. parkeri, thus from both sites of the collection (Table 10).

Bufo americanus tadpoles taken from the pond were successfully infected with Strigea cercariae. Metacercariae were established in the tadpoles (Fig. 22). Chickens did not become infected with the metacercariae of Strigea cercaria established in Xenopus tadpoles. In similar experiments, mice did not become infected. These experiments were not carried on with metacercariae of other cercarial types, because there were no metacercariae available.

The Map Turtle (G. geographica) from the pond was not infected with digenetic trematodes. Dissection of the green frog (R. clamitans melanata) recovered four flukes. Unfortunately, the specimen were destroyed in the process of preparation for examination. Thus, there was no identification. No trematodes were recovered from the dissection of R. catesbiana and R. pipiens.

Attempts to observe the flame cells of the cercariae were unsuccessful, and flame cell patterns were not established. However, some of the excretory tubules of the Ornatae cercaria were observed (Fig. 21).

Measurements on the current of the stream were recorded for ten trials. The average speed of the stream was calculated to be 10 cm/sec. The pond had no measurable current.

Fig. 1. The Pond Environment in Esophea Park.

Fig. 2. The Stream Environment in Gundersen Arboretum

Fig. 3. The Snail Host, *Helisoma trivolvis*.

Fig. 4. The Snail Host, *Physa parkeri*.

Fig. 5: Distribution of non-infected *Helisoma trivolvis* from the pond according to their size.

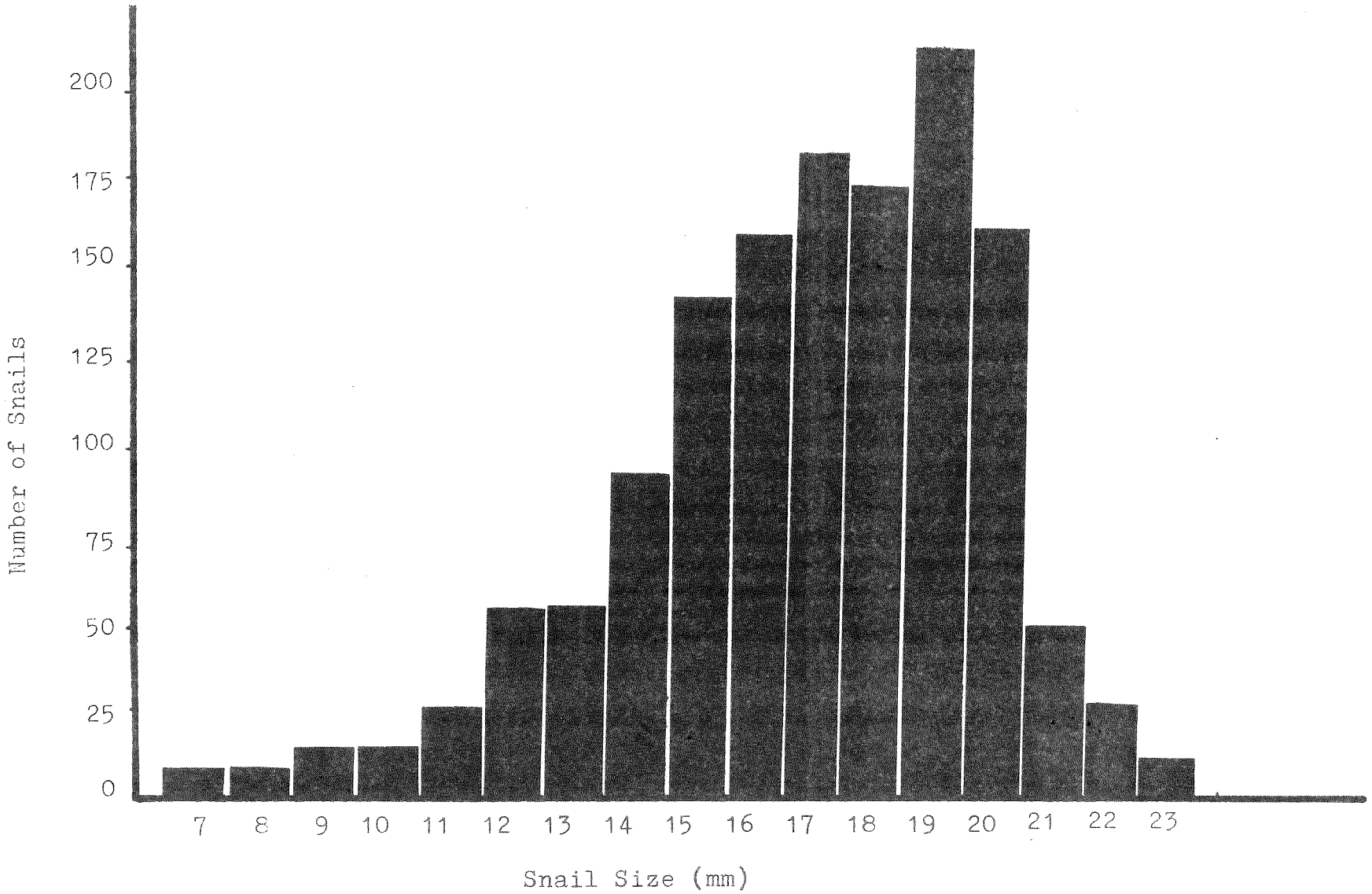

Number of Snails
200
175
150
125
100
75
50
25
0
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
Snail Size (mm)

Fig. 6: Distribution of infected Helisoma trivolvis from the pond according to their size.

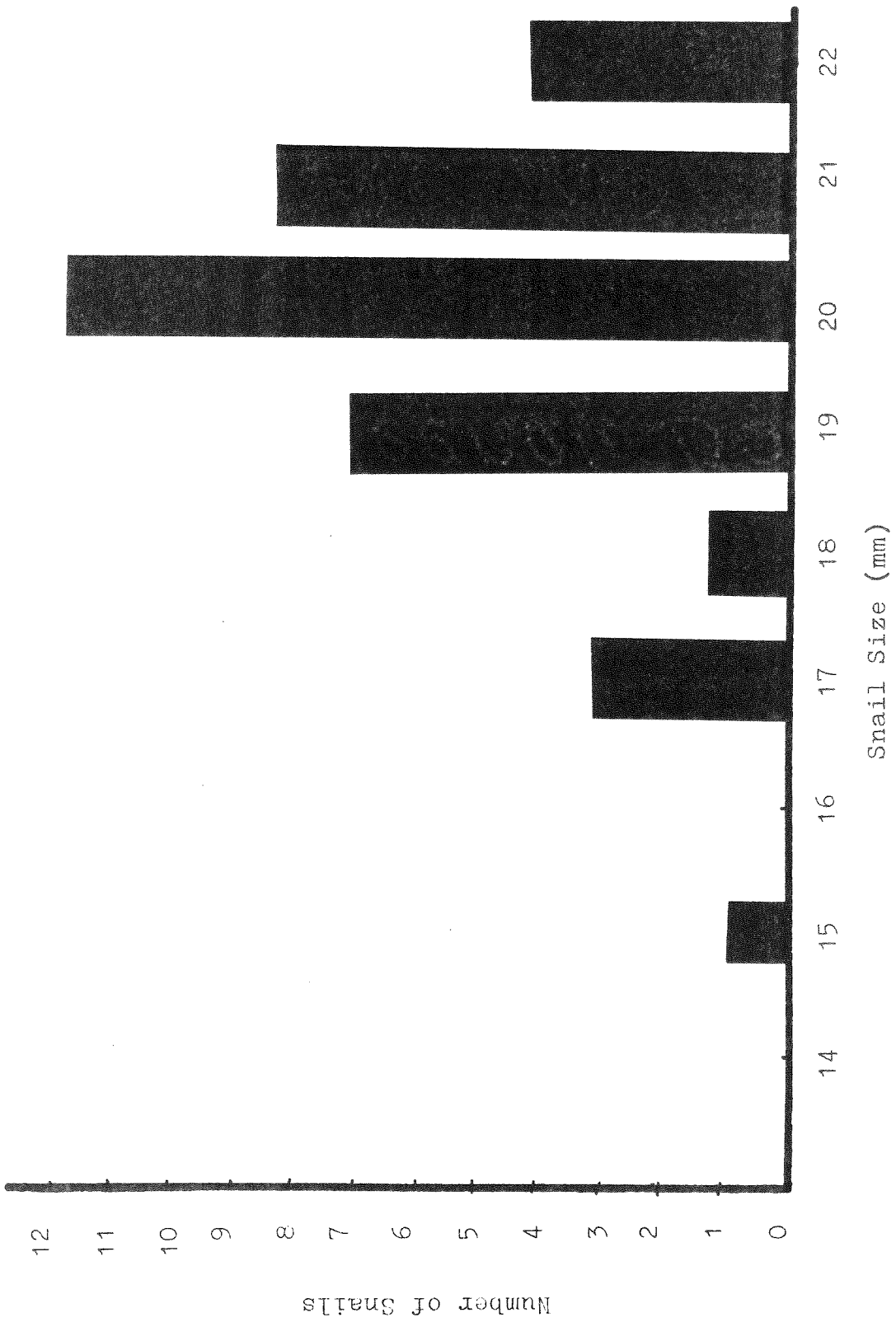

Number of Snails
0
1
2
3
4
5
6
7
8
9
10
11
12
14
15
16
17
18
19
20
21
22
Snail Size (mm)

Fig. 7: Distribution of non-infected *Physa parkeri* from the pond according to their size

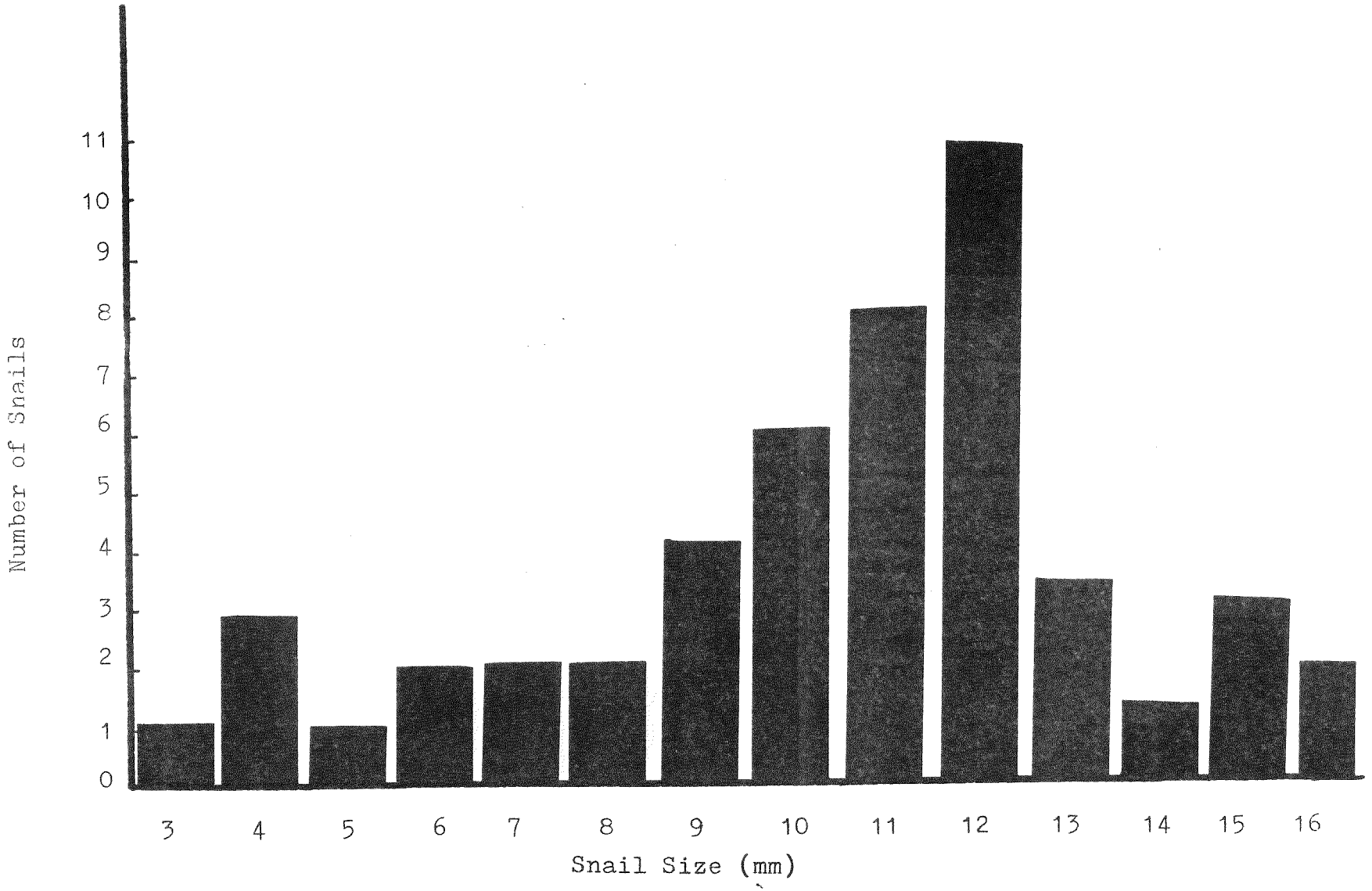

Number of Snails
0
1
2
3
4
5
6
7
8
9
10
11
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Snail Size (mm)

Fig. 8: Distribution of infected Physa parkeri from the pond according to their size.

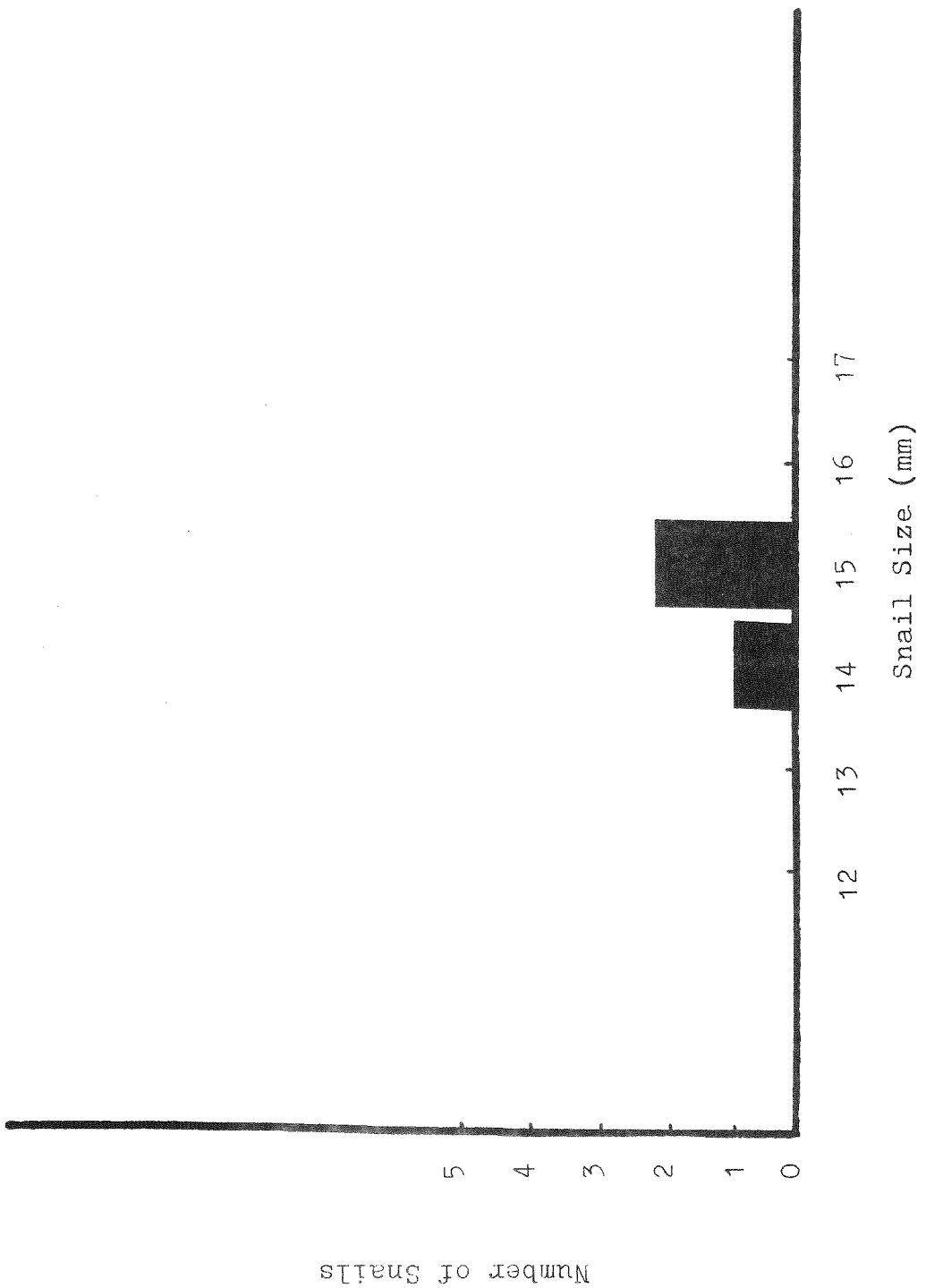

Number of Snails
0
1
2
3
4
5
12
13
14
15
16
17
Snail Size (mm)

Fig. 9: Distribution of non-infected Physa parkeri from the stream according to their size.

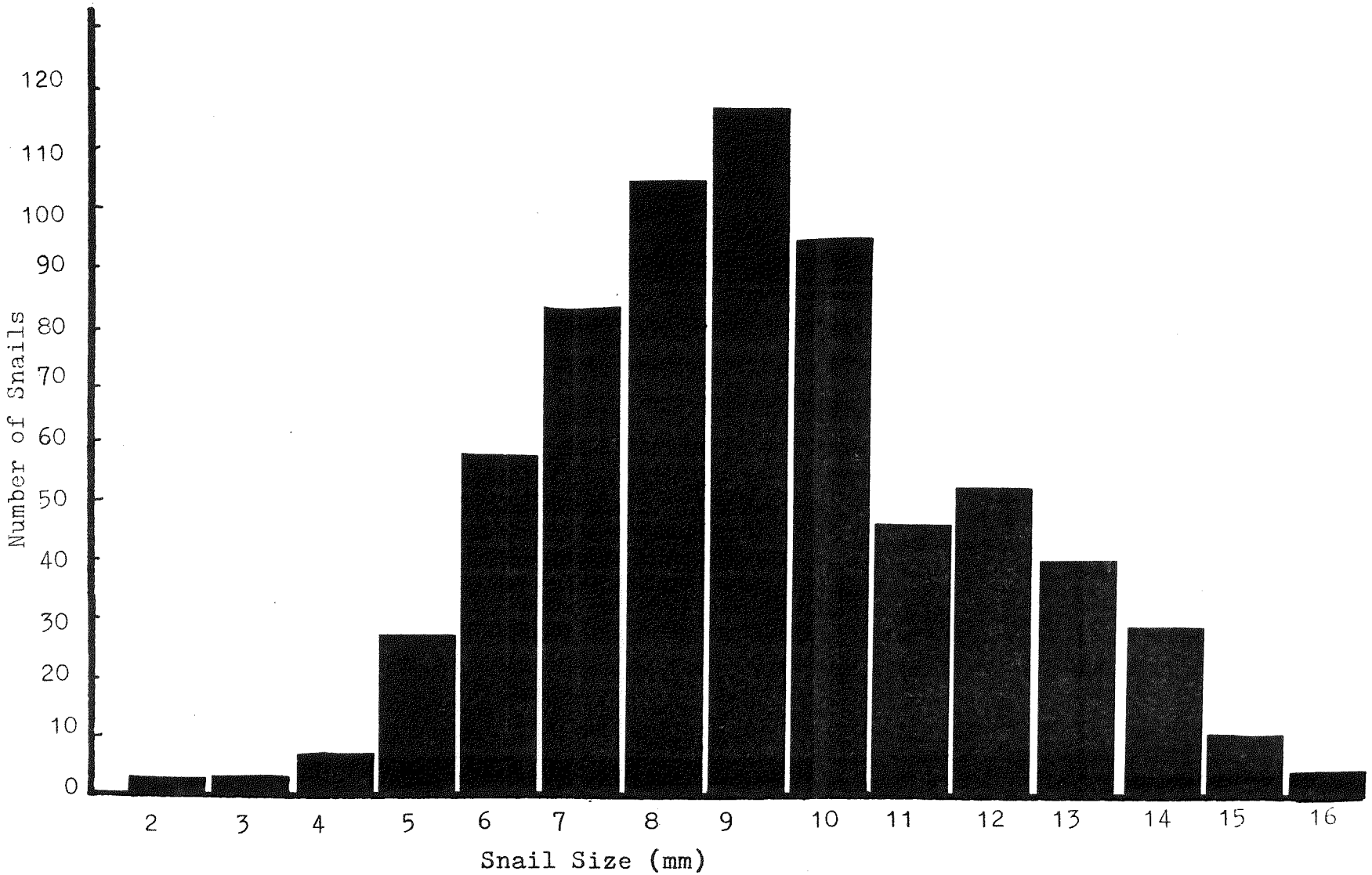

Number of Snails
120
110
100
90
80
70
60
50
40
30
20
10
0
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Snail Size (mm)

Fig. 10: Distribution of infected Physa parkeri from the stream according to their size.

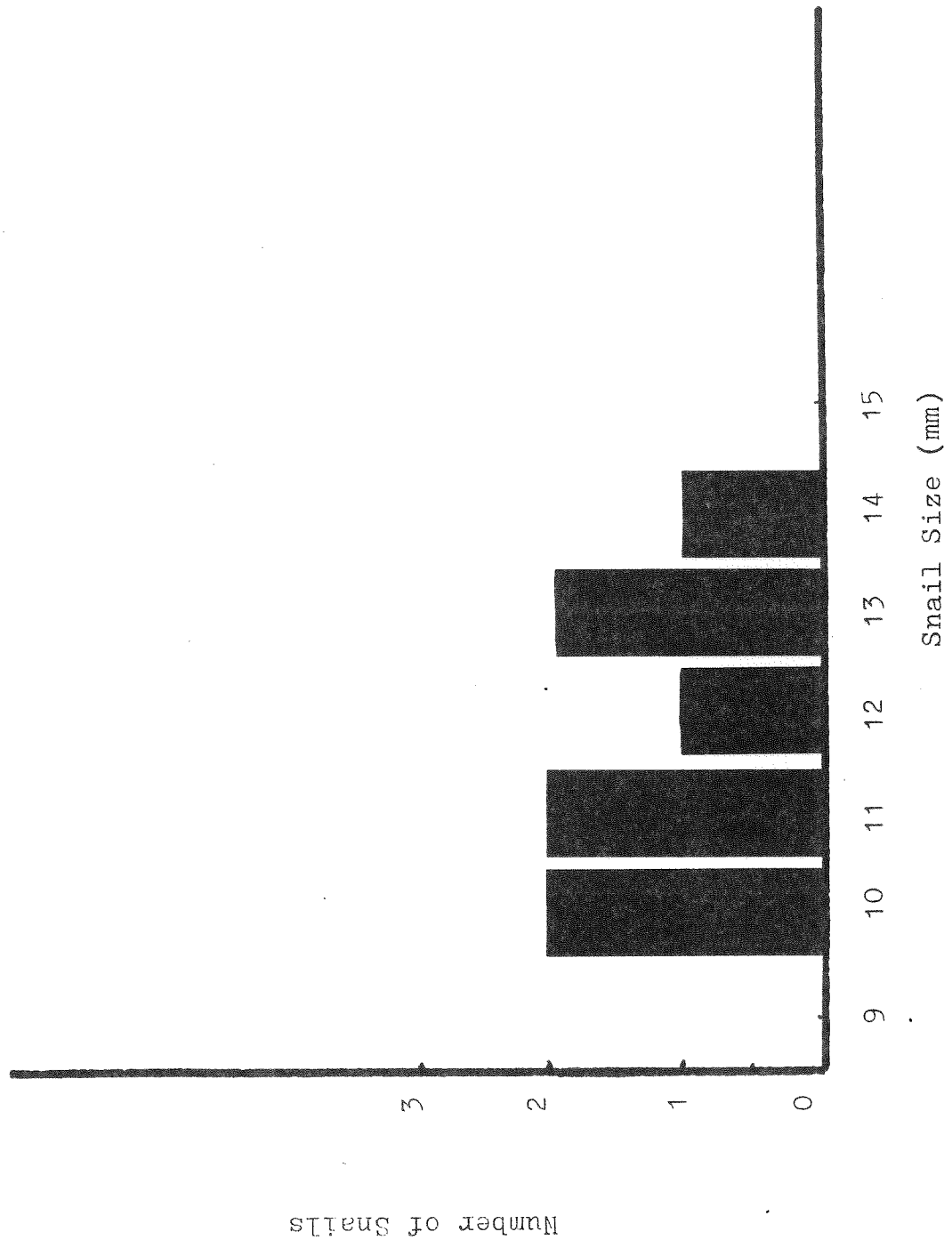

Number of Snails
0
1
2
3
9
10
11
12
13
14
15
Snail Size (mm)

Fig. 11: Strigea Cercaria
OS = Oral Sucker
EB = Excretory Bladder
TS = Tail Stem
F = Furcae
Host: H. trivolvis

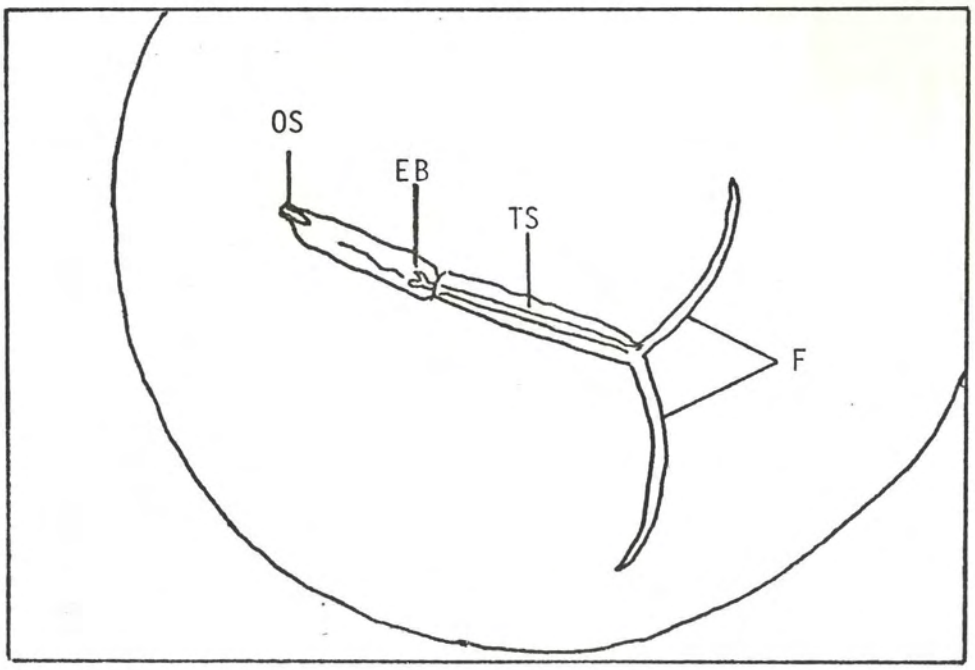
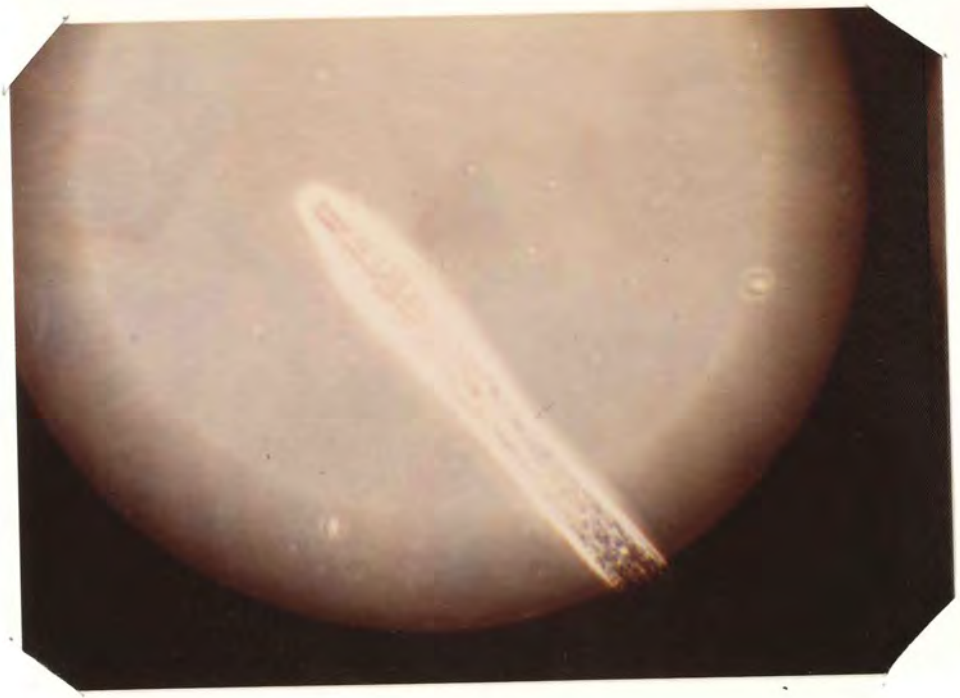

OS
EB
TS
F

Fig. 12: Strigea Cerceria (Magnified)
OS = Oral Sucker
E = Esophagus
EB = Excretory Bladder
ET = Excretory Tubule



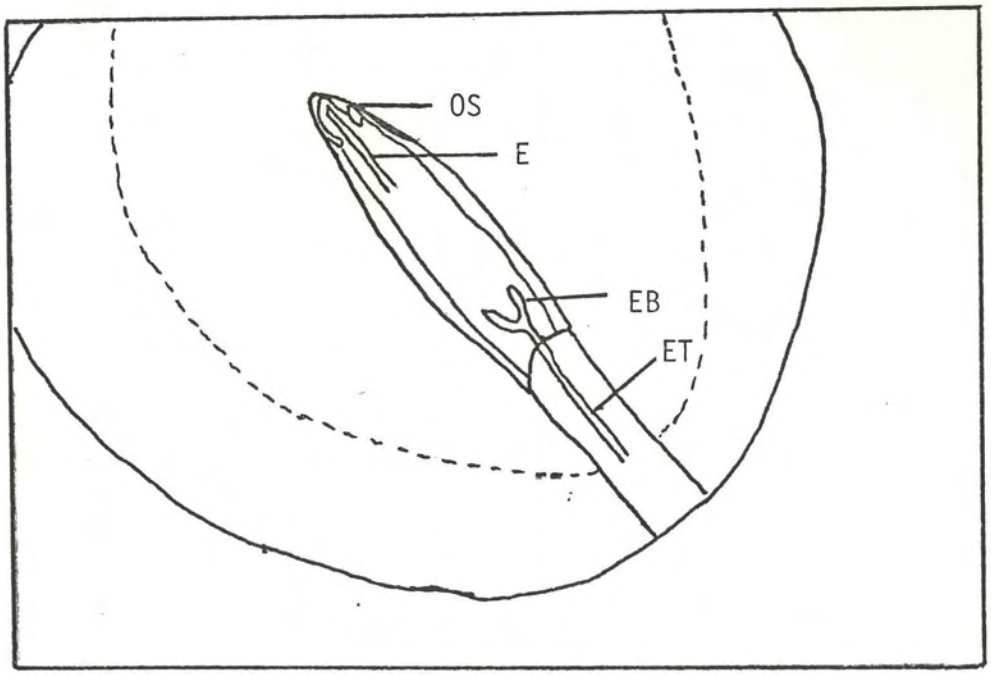

OS
E
EB
ET

Fig. 13: Brevifurcate-Pharyngeate Cercaria
OS = Oral Sucker
ES = Eyespots
TS = Tail Stem
F = Furcae
Host: H. trivolvis

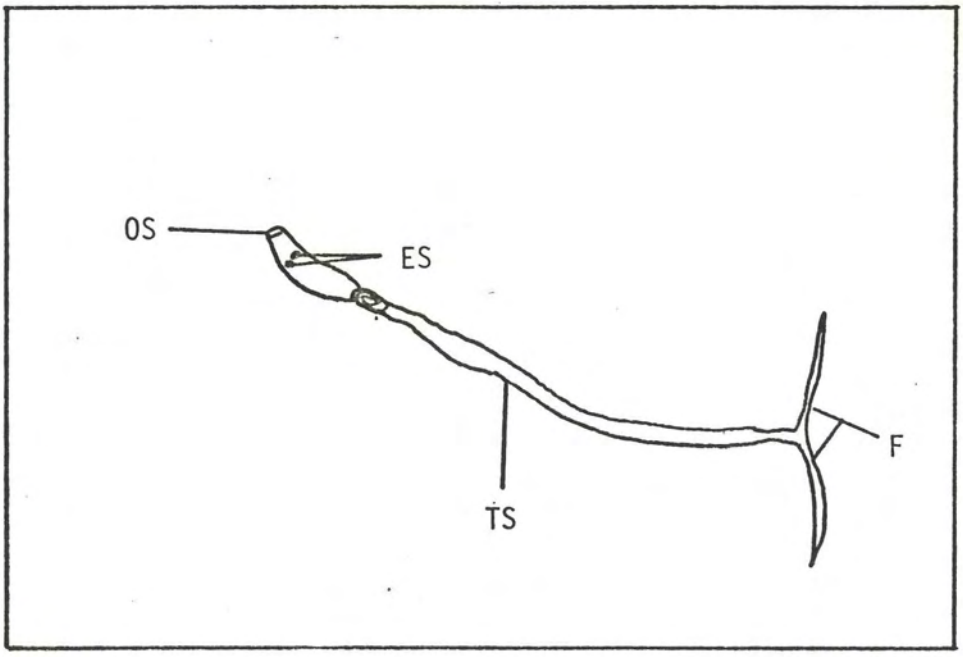

OS
ES
F
TS

Fig. 14: Brevifurcate-pharyngeate Cercaria(Magnified)
ES= Eyespots
EB= Excretory Blader
GM= Germinal Mass
DMF= Dorsomedian Finfold
TS= Tail Stem

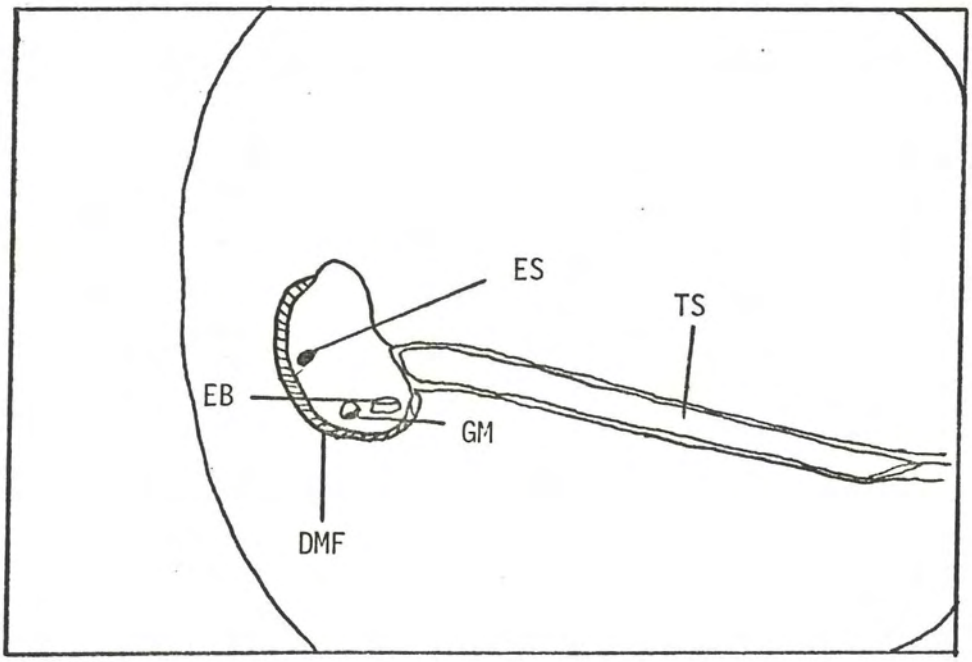
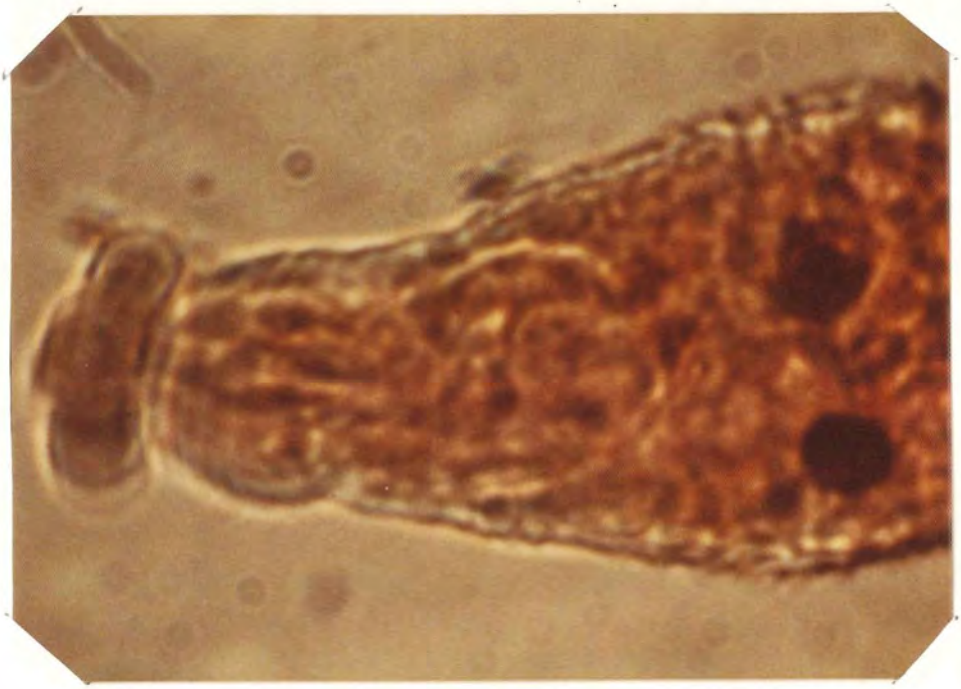

ES
TS
EB
GM
DMF

Fig. 15: Brevifurcate-pharyngeate Cercaria (Further magnification)
OS= Oral Sucker
E = Esophagus
P = Pharynx
ES= Eyespots



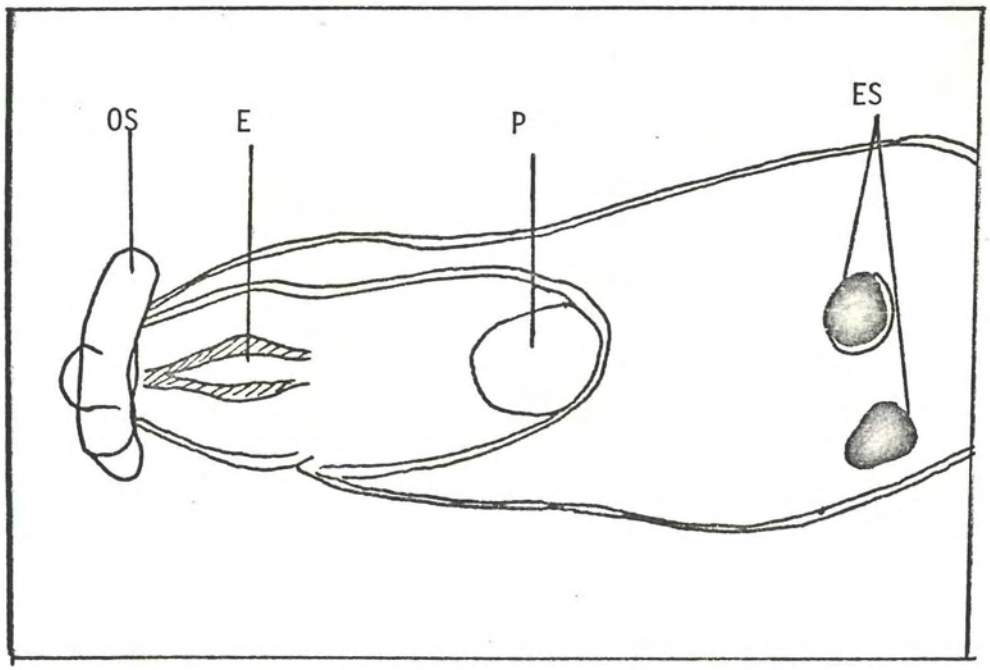

OS
E
P
ES

Fig. 16: Brevifurcate-apharyngeate Cercaria.

OS= Oral Sucker
VS= Ventral Sucker
ES= Eyespots
EB= Excretory Blader
ET= Excretory Tubule
TS= Tail Stem
F =Furcae
FF= Furcal Finfold
EP= Excretory Pores
Host: H. trivolvis

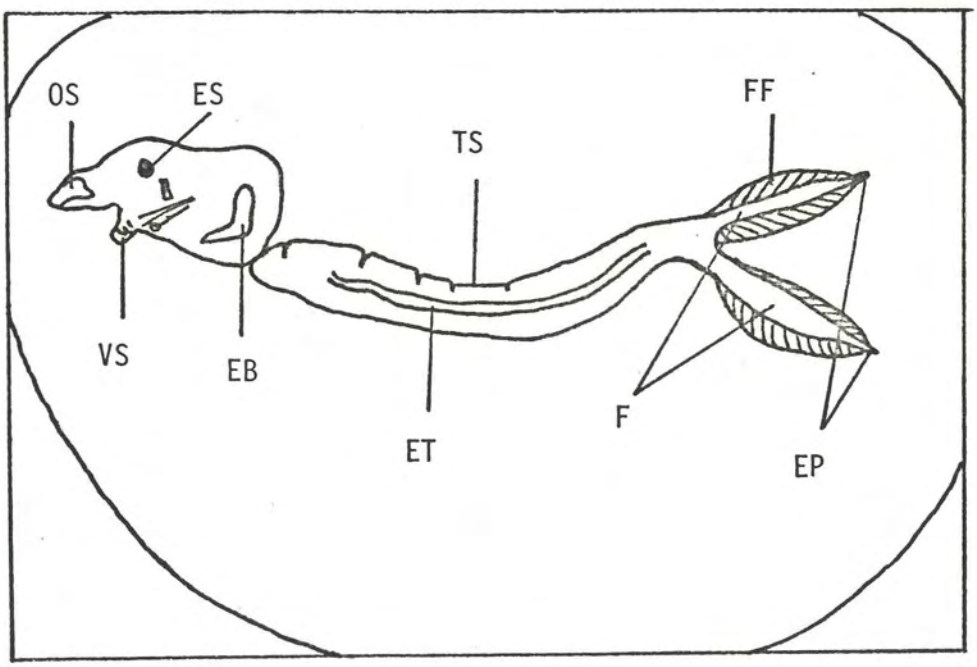
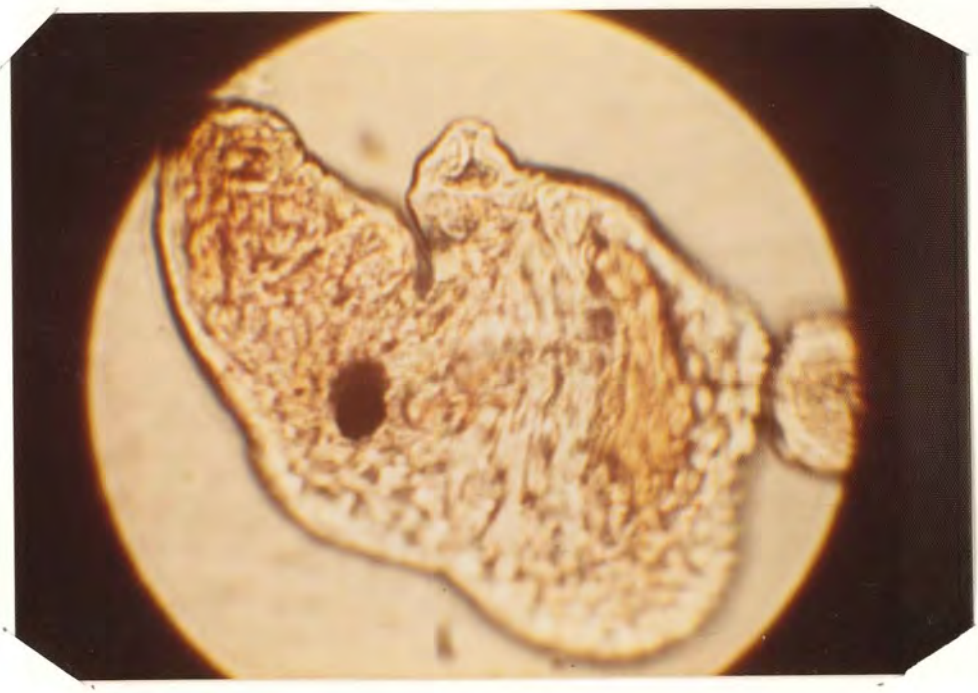

OS
ES
TS
FF
VS
EB
ET
F
EP

Fig. 17: Brevifurcate-apharyngeate Cercaria (Magnified).
OS= Oral Sucker
VS= Ventral Sucker
ES= Eyespots
EB= Excretory Blader



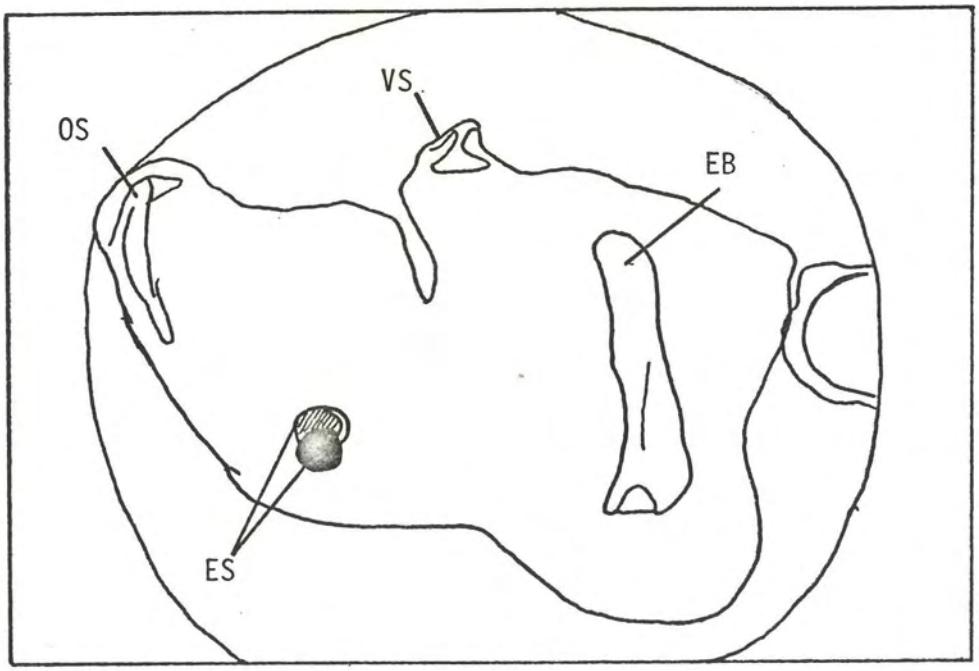

OS
VS
EB
ES

Fig. 18: Armatae Cercaria.
OS= Oral Sucker
S = Stylet
VS= Ventral Sucker
T = Tail
Host: H. trivolvis

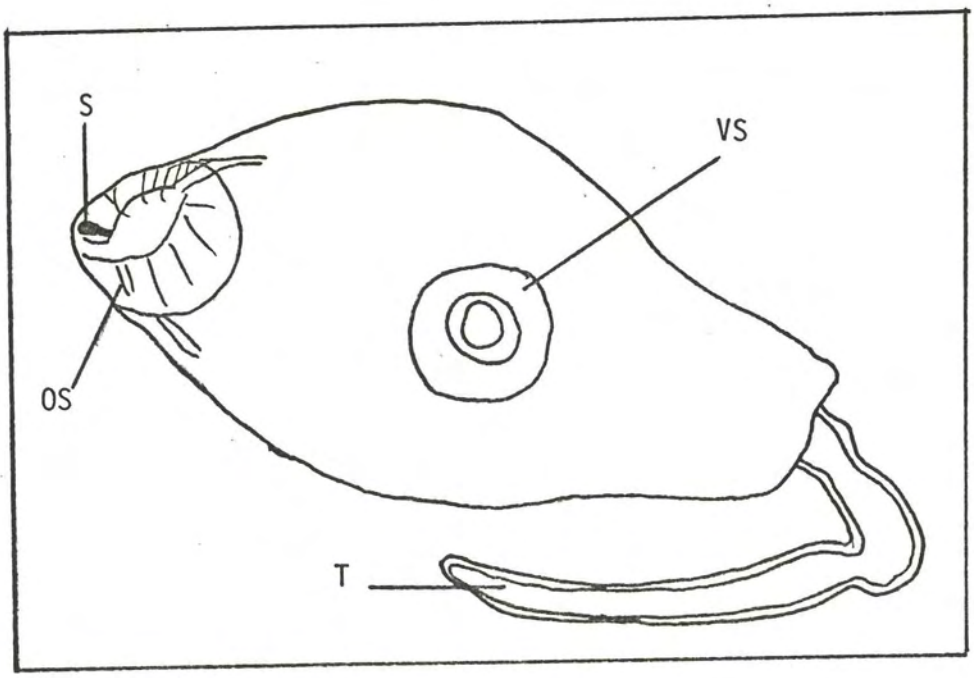
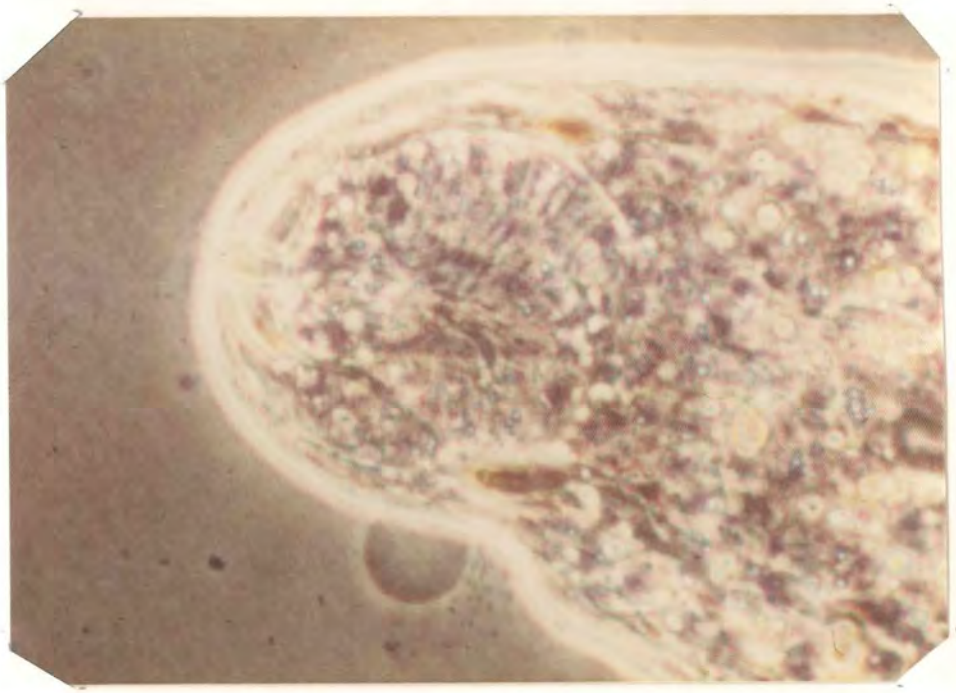

S
VS
OS
T

Fig. 19: Armatae Cercaria (Magnified).
OS= Oral Sucker
ST = Stylet
C.T.O.P.G.= Collecting Tubule
Of Penetration Glands



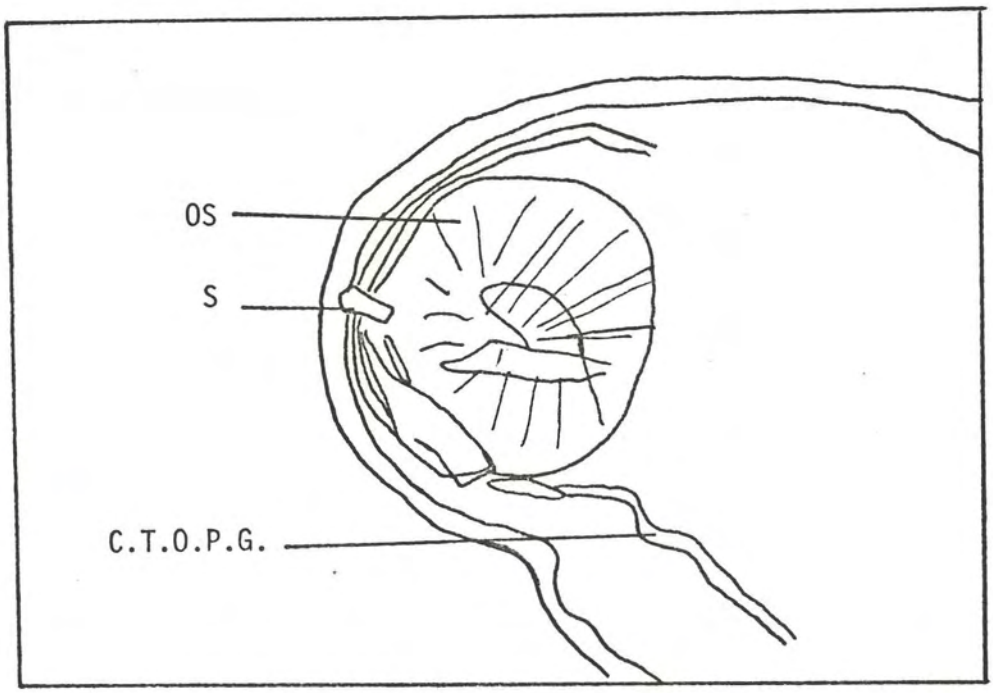

OS
S
C.T.O.P.G.

Fig. 20: Ornatae Cercaria.
S == Stylet
OS= Oral Sucker
PG= Penetration Glands
VS= Ventral Sucker
EB= Excretory Blader
CF= Caudal Finfold
T = Tail
Hosts: H. trivolvis & P. parkeri

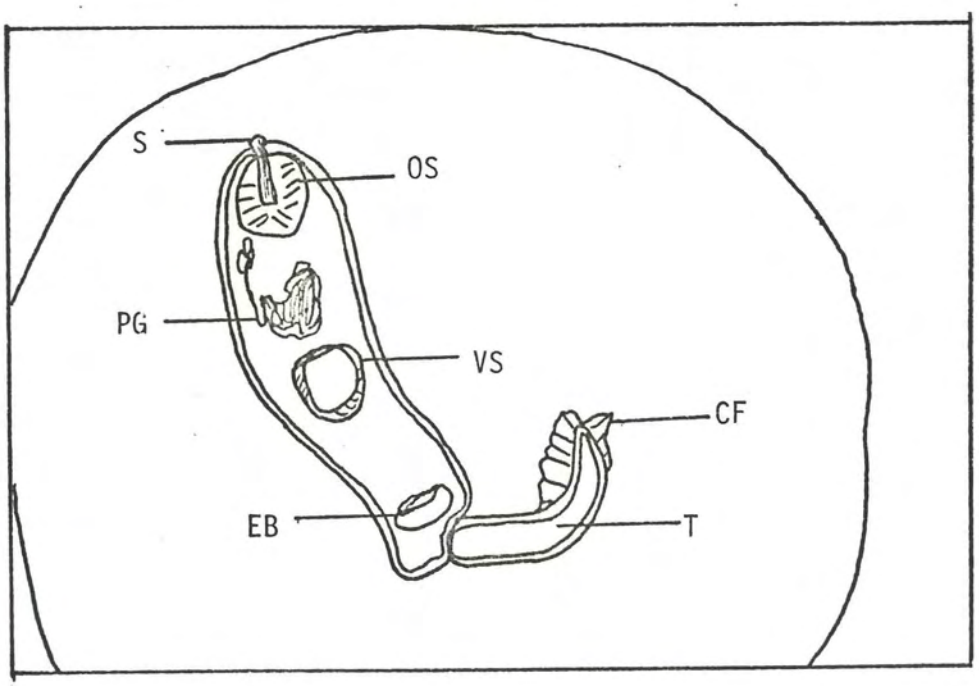
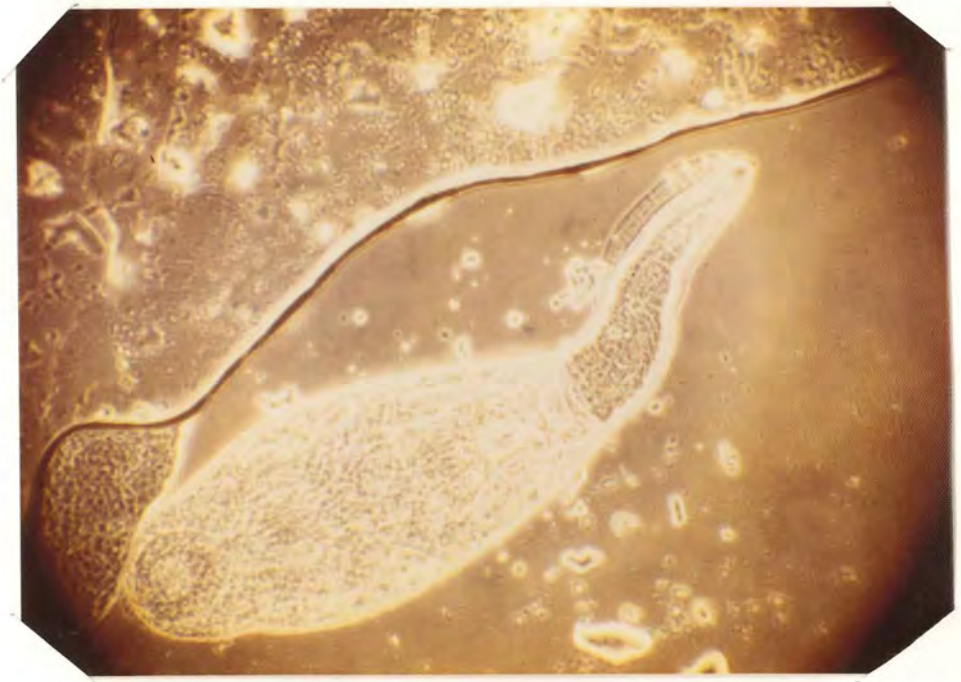

S
OS
PG
VS
CF
EB
T

Fig. 21: Ornatae Cercaria (Magnified).
S = Stylet
OS= Oral Sucker
VS= Ventral Sucker
ET= Excretory Tubule
C = Cecum
EB= Excretory Blader
CF= Caudal Finfold
T = Tail



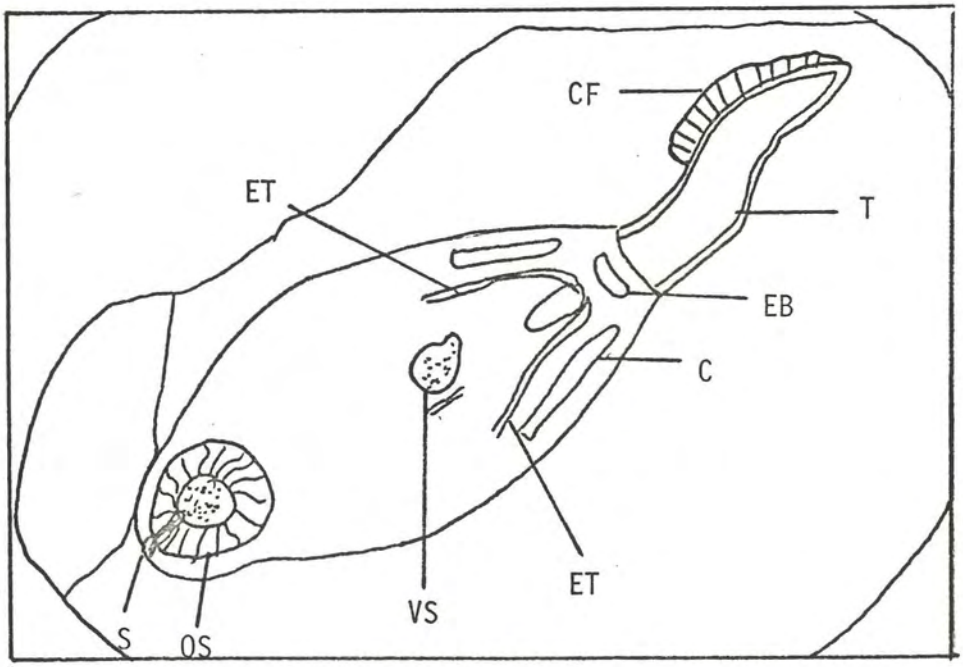
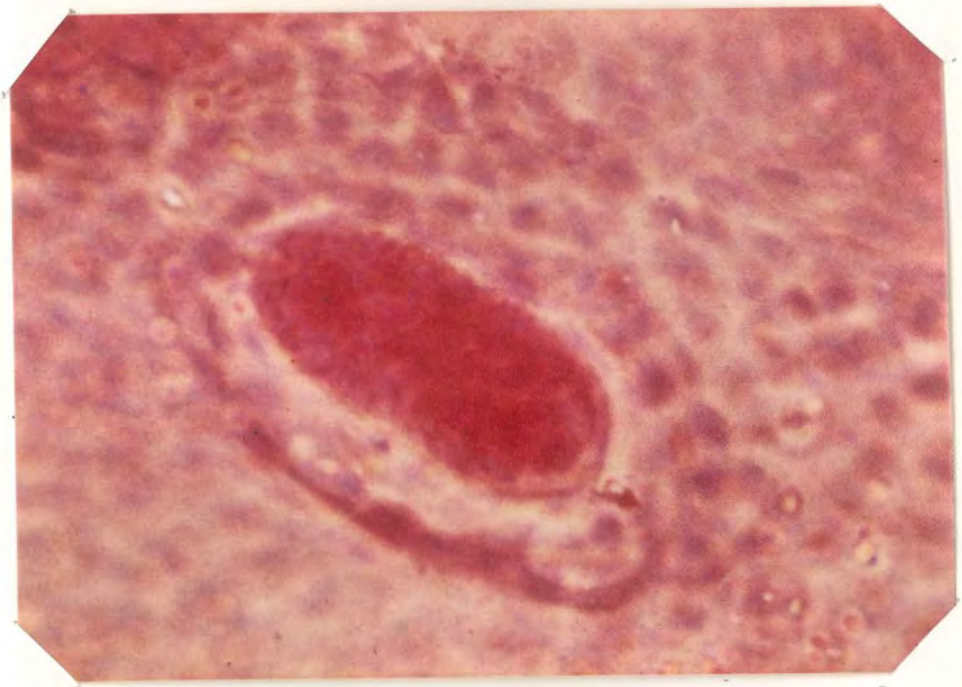

CF
ET
T
EB
C
ET
VS
S
OS

Fig. 22: Strigea Metacercaria in Tadpole tissue.
OS= Oral Sucker



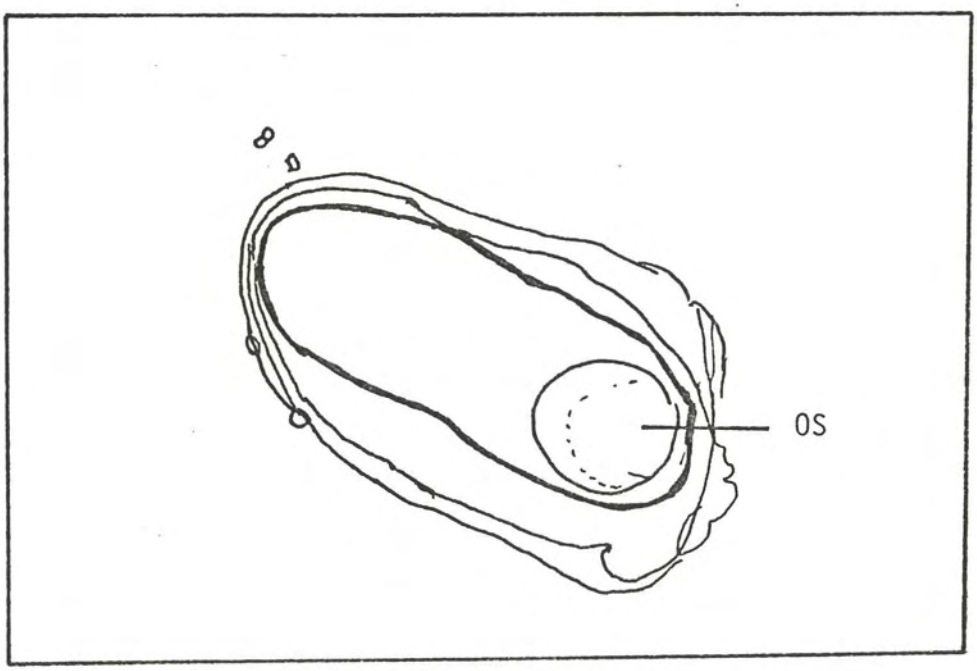

OS

Fig. 23. The Experimental Apparatus used in search for the Flame Cells.

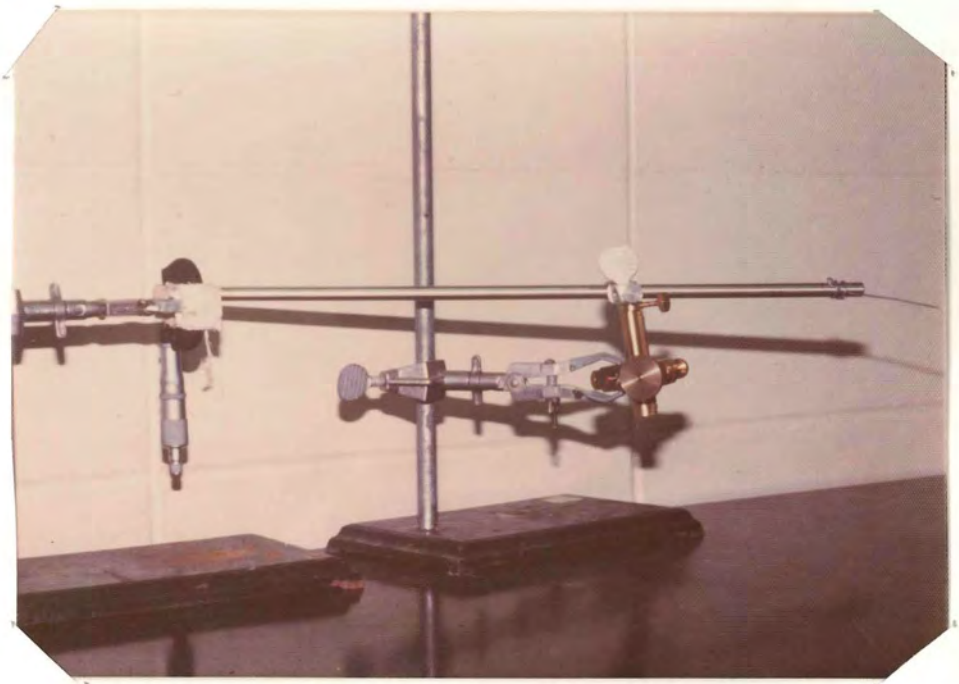


Fig. 24. The Experimental set up used in search for the Flame Cells.

Table 1. Quantitative Data on Snail Collection

	POND		STREAM	
	H. trivolvis	*P. parkeri*	*H. trivolvis*	*P. parkeri*
Total Number	1390	51	0	682
No. Infected	36	3	0	8
% Infected	2.6%	5.9%	0%	1.2%

Table 2. Water Chemistry Results From The Pond

Date	NO_2^- (mg/l)	NO_3^- (mg/l)	NH_3 (mg/l)	TP[1] (mg/l)	OP[2] (mg/l)	pH	Cond.[3] (m.mohs/cm)	Turb.[4] (JTU)
9/11/79	0.006	0.32	0.09	0.05	0.03	7.9	360	5
9/25/79	0.005	0.28	0.005	0.03	0.015	8.1	390	1

[1]Total Phosphates
[2]Ortho Phosphates
[3]Conductivity
[4]Turbidity

Table 3. Water Chemistry Results From The Stream.

Date	NO_2^- (mg/l)	NO_3^- (mg/l)	NH_3 (mg/l)	TP[1] (mg/l)	OP[2] (mg/l)	pH	Cond.[3] (m/mohs/cm)	Turb.[4] (JTU)
9/11/79	0.015	0.315	0.145	0.025	0.005	7.79	420	6
9/25/79	0.015	0.255	0.005	0.02	0.005	8.1	410	2

[1]Total Phosphates
[2]Ortho Phosphates
[3]Conductivity
[4]Turbidity

Table 4. Temperature & Dissolved Oxygen Data of the Pond and the Stream.

Date	Pond		Stream	
	Temp.* (C^o)	D.O.** (ppm)	Temp. (C^o)	D.O. (ppm)
6/1/79	18	7.52	12	6.0
6/7/79	19	12	14	9.8
6/24/79	21	9	18	9.5
7/14/79	24		15	
7/21/79	19	9.5	14	10
8/6/79	23	9	14	10
8/24/79	17	8.8	13	9.1
9/11/79	20	8.9	15	9.8
9/25/79	15	9.5	13	7.5
Average	19.4	10.4	14.2	10.1

* Temperature
** Dissolved Oxygen

Table 5. Measurments of Strigea Cercaria

No.	Body		Tail		Furcae	Oral Sucker
	Length (μm)	Width (μm)	Length (μm)	Width (μm)	Length (μm)	Diameter (μm)
1	290	70	460	50	450	100
2	390	120	550	70	450	100
3	380	130	450	80	500	90
4	450	150	520	70	450	110
5	320	100	500	70	450	100
6	460	140	550	80	450	120
7	350	100	450	90	460	90
8	350	160	500	100	500	100
9	460	140	500	80	410	120
10	470	150	550	100	450	120
Average	357	126	473	79	457	105

Table 6. Measurments of Brevifurcate-apharyngeate Cercaria

No.	Body		Tail		Furcae	Oral Sucker
	Length (µm)	Width (µm)	Length (µm)	Width (µm)	Length (µm)	Diameter (µm)
1	490	130	1150	130	250	110
2	570	230	1350	100	300	100
3	690	320	1400	150	310	120
4	600	240	1450	150	350	100
5	600	250	1500	180	400	100
6	810	310	1450	150	450	140
7	350	200	1500	150	400	70
8	790	250	1500	150	400	120
9	720	250	1000	140	300	130
10	350	160	1150	100	380	50
Average	597	234	1345	140	354	104

Table 7. Measurments of Armatae Cercaria

No.	Body		Tail	Oral Sucker	Stylet
	Length (μm)	Width (μm)	Length (μm)	Diameter (μm)	Length (μm)
1	720	250	300	100	20
2	750	250	400	120	40
3	750	280	380	100	30
4	790	310	420	120	40
5	740	320	400	120	30
6	700	350	500	120	20
7	800	350	400	120	30
8	800	350	500	100	30
9	690	350	400	150	30
10	700	310	380	150	30
Average	744	312	408	120	30

Table 8. Measurments of Ornatae Cercaria

No.	Body		Tail	Oral Sucker	Stylet
	Length (μm)	Width (μm)	Length (μm)	Diameter (μm)	Length (μm)
1	640	240	400	100	70
2	640	260	400	120	70
3	670	250	350	120	80
4	850	280	300	100	65
5	600	280	310	100	80
6	610	300	220	150	60
7	650	300	260	100	70
8	680	300	400	120	70
9	750	280	350	150	65
10	650	240	300	100	65
Average	674	273	329	116	69.5

Table 9. Measurments of Brevifurcate-pharyngeate Cercaria

No.	Body		Tail		Furcae	Oral Sucker
	Length (µm)	Width (µm)	Length (µm)	Width (µm)	Length (µm)	Diameter (µm)
1	200	100	350	60	220	50
2	200	100	280	50	200	70
3	250	100	560	50	250	60
4	320	100	550	50	200	50
5	360	100	600	70	250	50
6	300	150	600	70	150	60
7	350	110	600	70	200	60
8	350	110	500	70	200	50
9	350	100	550	70	200	60
10	360	100	600	60	200	50
Average	298	107	517	62	207	51

Table 10. Occurance of Cercariae in the Snail Host

Cercaria	Snail Host	Location
Ornatae	H. trivolvis & P. parketi	Pond & Stream
Armatae	H. trivolvis	Pond
Strigea	H. trivolvis	Pond
Brevifurcate-apharyngeate	H. trivolvis	Pond
Brevifurcate-pharyngeate	H. trivolvis	Pond

DISCUSSION AND CONCLUSIONS

The objective of this investigation was to consider two distinctly separate and different environments for the occurrence of larval trematodes and account for the factors that might be influencing their presence in one environment in contrast to the other.

Data listed in Table 1 clearly indicate that, although snails of Physa parkeri were present in both sites of the study, the snails of Helisoma trivolvis were present only in the pond. This indicates that the conditions in the stream may not be suitable for the survival of H. trivolvis. During the course of this investigation it was noted that the average temperature of the pond was 19.4°C and that of the stream was only 14.2°C (Table 4). This suggested that temperature may be one factor in explaining why Helisoma trivolvis was not present in the stream. When the snails were brought to the laboratory, they were kept at room temperature, approximately 20°C. P. parkeri could survive only up to one week at this temperature but H. trivolvis survived throughout investigation. This also suggested the strong possibility that temperature was a factor in the survival of the snails. Since the fate of H. trivolvis was not determined in the lower temperature range, it was not possible to draw any substantial conclusion from the temperature role on the survival of H. trivolvis in the stream. The findings of this investigation with regard to P. Parkeri is in agreement with the work of Cort et al (1941). They reported that P. parkeri was a very delicate snail and that in order to prolong its existence in the laboratory it had to be maintained under running water and at temperatures below room temperature. These conditions are in accordance with the natural environment of the stream investigated in this study.

The amount of vegetation available to the snails at these two sites could also have bearing on the population of snails. It was observed that the amount of vegetation in the pond was greater than vegetation in the stream. Although vegetation was abundant on the sides of the stream, the bed of the stream was almost empty of vegetation. In the pond, however, vegetation was abundant both in and on the sides of the pond.

The dissolved oxygen content of the water at the two environments are listed in Table 4. There was no significant difference in values for either site; therefore, dissolved oxygen probably did not have any effect.

Tables 2 and 3 demonstrate the results of water chemistry tests. These results indicate similarity in water chemistry tests in both environments, and for this reason the factors considered in this investigation probably had little effect on cercarial occurrence.

The average current velocity of the stream could also affect snails. P. parkeri are more aerodynamically shaped than H. trivolvis. This enabled P. parkeri to better withstand the current velocity of about 10 cm/h than could H. trivolvis. This could be another reason why H. trivolvis was not found in the stream.

Figures 5-10 graphically demonstrate the distribution of snails, both infected and noninfected, from both sites. Figure 5 and 6 show the distribution of H. trivolvis from the pond. Figures 7 and 8 demonstrate the distribution of P. parkeri from the pond. Figures 9 and 10 show the distribution of P. parkeri from the stream. Comparison of these graphs from each snail type and population indicate that unless the snail was of

a particular size, the emergence of cercariae was not observed. This does not mean that the snails referred to as noninfected, do not harbor any cercariae. The term infected in this investigation was used to mean cercarial release from a snail and not the actual infection of the snail with the parasite. The results obtained from these graphs indicate that, there may be a time required for the maturation of cercariae in the snail host. Also physiological factors may be required to stimulate the emergence of cercaria. If the latter was true, then it is reasonable to assume that perhaps the snail must be a certain age before that factor is manifested by the snail. It has been noted that the actual infection of the snail takes place while the snail is quite young (Cort et al 1941, Mathis et al 1956). This could probably be due to immunological defense mechanism of the young snail permitting the parasite to establish itself more successfully. Cort et al (1941) noted that the adults of Physa parkeri could be infected if they were encountered with meracidia; however, they did not find this to be true for other snails examined.

Table 10 demonstrates the occurrence of cercariae in the snail hosts. The Ornatae Cercaria was the only cercaria found in both snails. This was probably related to the host specificity of miracidium and sporocyst generations. The snail secretes a mucus that attracts the miracidia (Ulmer, 1971). It is realistic to assume different snails secrete mucosa with different chemical contents. If this is true, then it may account for the observation that certain cercariae were only found in certain snail hosts. Even if all miracidia penetrated all snails, it could be

assumed that some cercariae could not survive the internal environment of the snail body. Another possibility was that the definitive host of the adult trematode may not have been present in the vacinity of the stream.

Table 1 demonstrates frequency of the P. parkeri infection in both environments. It shows that although the number of P. parkeri collected from the stream was much greater than the number collected from the pond; only 1.2% of P. parkeri were infected from the stream compared to 5.9% infection of this organism from the pond. The reason for this difference can be explained in two ways. One could be linked to the frequencies that the definitive host visits the stream. The second reason could be attributed to the rapid current of the stream. The miracidia after hatching swim randomly until they come into contact with the snail or its mucosa (Ulmer, 1971). The current of the stream would wash away the miracidia and would wash away the mucosa. This would minimize the chance of the miracidia contacting the snail host. This factor could be the most reasonable explanation of the difference between the cercarial occurrence in these two environments. These factors did not apply to Ornatae cercaria since it was present in both the pond and the stream. However, since the number of infections was small, the validity of these data could be questioned. Other investigators are encouraged to undertake similar experiments to substantiate these findings.

The objective in the infection experiments was to develop a life cycle pattern for the parasites. Also specific identification of adult parasites would be possible. Unfortunately, these experiments were not successful.

In the case of mice, the failure can be explained in two ways. One would be the host specificity of the parasites. In this case, metacercariae entered the stomach of the host, passed through to the intestine, and been chemically stimulated by the host to excyst. The excysted worms would then migrate to the appropriate sites in the body of the host and establish themselves. If the host was not the proper host, the excystment would not occur and the cysts would pass through the system. Even if they did excyst they could be destroyed by the defense mechanism of the host.

Another explanation for the failure of the infection experiments in the case of the mice would be due to the experimental procedure. The cysts may have been destroyed in the process of stomach tubing, resulting in no infection. However, since the process of stomach tubing is a common means of infecting the experimental animals, the first explanation may be the more accurate one. In the case of the chickens, stomach tubing was not necessary. The animals ate the infected tadpoles readily. The explanation of the experiments' failure could only be the host specificity of the parasites.

The objective of collecting and dissecting different wild animals throughout this investigation was to recover the adult trematodes so that they could be linked to the cercariae collected. This was important for the specific identification of the cercarial species. Unfortunately, no adult trematodes were found in the animals collected. This does not mean there were no trematodes in these environments. Juvenile forms were present, therefore, adults must have been.

The environments sampled were frozen from November to mid March; therefore, snails could not be collected during this period. Cercariae were available from late March until middle to late September. The most probable time for the recovery of cercariae would be from mid-June to early August. Table 4 shows that the temperature was normally higher during this time than the rest of the year.

The research areas for this project were two very different sites. Current and the temperature in the stream differed from the pond substantially. Along with this, the species of snails and cercariae were quite different. Only one cercaria, Ornatae cercaria, was found in both sites, and it was the only species found in the stream. The physical differences in the two sites were reflected in the taxonomic distributions of the cercariae.

Unfortunately, no other investigation similar to this study had been conducted and therefore, results of this study could not be compared or contrasted to other studies. Other investigators are encouraged to undertake similar investigations of this nature so that reasons for occurrence of some cercariae in a particular environment, in contrast to cercariae in other environments, can be better understood. These studies can have great impact in the fields of medicine and veterinary medicine, for they supply information regarding the nature of many parasitological diseases of man and animals and can help the scientists to develop better methods in control of these disturbances.

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