The life cycle of Bolbophorus confusus (Krause, 1914) Dubois, 1935 (Tremotoda: Strigeoidea) and the effects of the metacercariae on fish hosts
by Alfred Carter Fox

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Zoology
Montana State University

Abstract:
A study was made to determine the life cycle of the strigeoid trematode Bolbophorus confusus and effects of the metacercariae on fish hosts. The adult trematode was found in the intestine of the white pelican (Pelicanus erythrorhynchos), sporocysts developed in Helisoma trivolvis, and metacercariae are known to infect 28 species of fish. Metacercariae produced a parasite cyst and also caused the formation of a host cyst. B. confusus is believed to be the only North American cyst-forming Diplostomulum. Temperature exerted a strong influence on the developmental stages of the parasite. Development of metacercariae in fish hosts produced hemorrhage, hyperemia, and muscle necrosis. A stamina tunnel was used to compare the swimming performance of rainbow trout infected by B. confusus and those free from the parasites. Heavily infected trout (8.4 - 12.0 metacercariae per cm of body length) swam at an average velocity of 1.5 feet per second, while parasite-free trout averaged 2.3 feet per second. Hematocrit values of 37 parasitized rainbow trout and 45 parasite-free trout were determined. The values of 51% of the parasitized trout were below the average of the parasite-free controls and only 5.4% of the parasitized fish had hematocrit values exceeding the average value of control-trout. The ability of 66 parasitized rainbow trout and 63 parasite-free trout to withstand high water temperatures was compared. It was observed that 88.9% of the parasite-free trout and 31.9% of the parasitized fish lived to a temperature of 82 F. Fish with the largest numbers of metacercariae per cm of body length died at lower temperatures than did fish with fewer parasites.

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Approved:

Head, Major Department

Chairman, Examining Committee

Dean, College of Graduate Studies

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ABSTRACT

A study was made to determine the life cycle of the strigeoid trematode Bolbophorus confusus and effects of the metacercariae on fish hosts. The adult trematode was found in the intestine of the white pelican (Pelicanus erythrorhynchos), sporocysts developed in Helisoma trivolvis, and metacercariae are known to infect 28 species of fish. Metacercariae produced a parasite cyst and also caused the formation of a host cyst. B. confusus is believed to be the only North American cyst-forming Diplostomulum. Temperature exerted a strong influence on the developmental stages of the parasite. Development of metacercariae in fish hosts produced hemorrhage, hyperemia, and muscle necrosis. A stamina tunnel was used to compare the swimming performance of rainbow trout infected by B. confusus and those free from the parasites. Heavily infected trout (8.4 - 12.0 metacercariae per cm of body length) swam at an average velocity of 1.5 feet per second, while parasite-free trout averaged 2.3 feet per second. Hematocrit values of 37 parasitized rainbow trout and 45 parasite-free trout were determined. The values of 51% of the parasitized trout were below the average of the parasite-free controls and only 5.4% of the parasitized fish had hematocrit values exceeding the average value of control-trout. The ability of 66 parasitized rainbow trout and 63 parasite-free trout to withstand high water temperatures was compared. It was observed that 88.9% of the parasite-free trout and 31.9% of the parasitized fish lived to a temperature of 82 F. Fish with the largest numbers of metacercariae per cm of body length died at lower temperatures than did fish with fewer parasites.

INTRODUCTION

Few investigations have been made concerning the quantitative effects of larval trematodes on the physical and physiological condition of fish. The extensive work of Smitherman (1964) evaluated some of the effects of Posthodiplostomum minimum (MacCallum) metacercariae on the "functional state" of the bluegill Lepomis macrochirus (Rafinesque). Experimentally infected fish showed "alteration of functional state" as exhibited by decreased growth rate, increased mortality, increased oxygen consumption of liver tissue, and depressed hematocrit levels. Krull (1934) reported that cercaria of Uvulifer ambloplitis may be responsible for the deaths of many small fish. His experiments with this parasite in centrarchids showed that larvae may produce hemorrhagic areas on the body and fins, popeye condition, impairment of muscle action, arrested development, and death. Hunter and Hunter (1938), working with the same parasite, observed that high infection rates produced significant weight loss in host fish, while Klak (1939) found that a severe infection of forage minnows by Neascus caused sterility and mortality. Experimental evidence produced by Hoffman (1956), (1957), (1958), Smitherman (1964), Olson (1964), et al. demonstrates that heavy infections of various species of metacercariae may kill fish, however, the effects of sublethal infections have not been well evaluated.

My investigation, conducted from September, 1962 to December, 1964, was primarily an evaluation of effects of metacercariae on the stamina (swimming performance) and temperature tolerance of rainbow trout (Salmo gairdneri). The strigeoid trematode Bolbophorus confusus (Krause, 1914)

Dubois, 1935, was selected for the investigation because it is readily available. It was necessary to determine the life cycle of this parasite in order to carry out the primary objectives of the study.

LIFE CYCLE OF BOLBOPHORUS CONFUSUS (KRAUSE, 1914) DUBOIS, 1935

Dubois (1938) gave a historical account of B. confusus and reported that: Brandes first mentioned the parasite, from material collected by Kollar in 1858, and he called it "Hemistomum trilobum Dies."; Luhe, based on Brandes inadequate description, could not distinguish it from Distoma trilobum Rud. 1819, which Diesing had combined with the genus Hemistomum in 1850; Krause (1914) completely described the adult parasite and named it "Hemistomum confusum n. sp.". La Rue (1926a) and (1926b) placed B. confusus in a new genus, Proalaria, since he considered the two type species, Hemistomum spathaceum (Rud.) Dies. and Alaris alata (Goeze), to be non-congeneric. However, since LaRue's type species (Proalaria spathaceum) was described, in the larval form, as Diplostomum volvens by von Nordmann in 1832, it should have been Diplostomum spathaceum according to the law of priority (Hughes, 1929). This parasite was finally placed in the genus Bolbophorus by Dubois in 1935 who distinguished it from the genus Diplostomum because it had a muscular genital bulb in the copulatory bursa.

B. confusus has been reported from a number of places in Europe, but only a few records are known for the Western Hemisphere. Swanson found it in the intestine of the white pelican (Pelicanus erythrorhynchos) in Minnesota (Odlaug, 1954). McNeil (1949) in Washington and Hugghins (1956) in South Dakota also reported B. confusus from white pelicans. Specimens of B. confusus collected by M. C. Hall in 1930 from white pelicans on Yellowstone Lake, Wyoming were found deposited in the U. S. National Museum. Metacercariae of this parasite were reported from rainbow trout and brown

trout (Salmo trutta) from Meadow Lake, Montana (Fox, 1962).

Materials and Methods

Thirteen adult white pelicans were collected from Meadow Lake (Madison County) and Canyon Ferry Lake (Broadwater County) Montana. These contained numerous B. confusus. In addition, two fledglings were captured from a rookery in Yellowstone Lake, Wyoming. One died enroute to the laboratory and it contained a large number of adult B. confusus. The other was kept alive and served as a source of B. confusus eggs for two years. The captive bird was given fresh and frozen fish, principally suckers (Catostomus commersoni and C. catostomus), at a rate of four to seven pounds daily during early growth and two to four pounds every other day after the bird was five months of age. A vitamin supplement (Vita-King by Diamond Laboratories) was added to the diet (injected into fish) for about the first three months after capture.

Most trematode eggs were obtained from fresh fecal material of the captive bird, but some were secured from trematodes removed from autopsied pelicans. Living trematodes expressed eggs readily when put in distilled water which was replaced after a few minutes with 0.86% saline solution. In some instances this process had to be repeated several times before eggs were discharged. Fresh fecal material, from the captive pelican, was placed in pilsner glasses and mixed with dechlorinated tap water. After settling five minutes or more, the supernatant was decanted and the glasses refilled with water. This was repeated until the supernatant was clear and only eggs and a small amount of debris remained. Eggs were removed by

pipette, with the aid of a microscope, and placed in finger bowls containing dechlorinated tap water. Each bowl, containing 100 or more eggs, was partially covered and then floated in an aquarium at a constant temperature. Ciliates were added to the bowls to control bacteria (Hugghins, 1954a).

Miracidia were partially immobilized by the use of 0.4% chloretone or dilute methyl cellulose and stained with neutral red and/or Nile blue sulphate. Flame cells and portions of the excretory system could be observed as the miracidia slowly dehydrated under a coverslip (Hugghins, 1954a).

The snail host (Helisoma trivolvis Say) of this parasite was collected from Meadow Lake in Madison County and from Middle Three Forks Pond and Hebgen Lake in Gallatin County, Montana. Only those snails from Meadow Lake were naturally infected with B. confusus. Specimens of H. trivolvis were collected in large numbers during July, August, and September, but only a very few were found at other times of the year. Snails were kept in laboratory aquaria or finger bowls and fed on boiled lettuce. Infection was accomplished by either exposing snails directly to miracidia or by placing the parasite eggs in aquaria containing snails. Sporocysts were secured by carefully dissecting the snail host. These were studied alive and unstained in temporary water mounts sealed with vaseline.

To facilitate the collection of cercariae, host snails were isolated in small containers. Numbers of cercariae that emerged from snails were estimated by making actual counts of cercariae in subsamples totaling 5% of the volume of water into which they emerged. Cercariae were studied

in the same manner as miracidia. The dehydration method of Cort (1917) was best for studying the excretory system. Cercariae fixed in hot 5% formalin (Cort and Bracket, 1937), stained with Semichon's acid carmine, cleared in terpineol, were mounted in Permount or Customount.

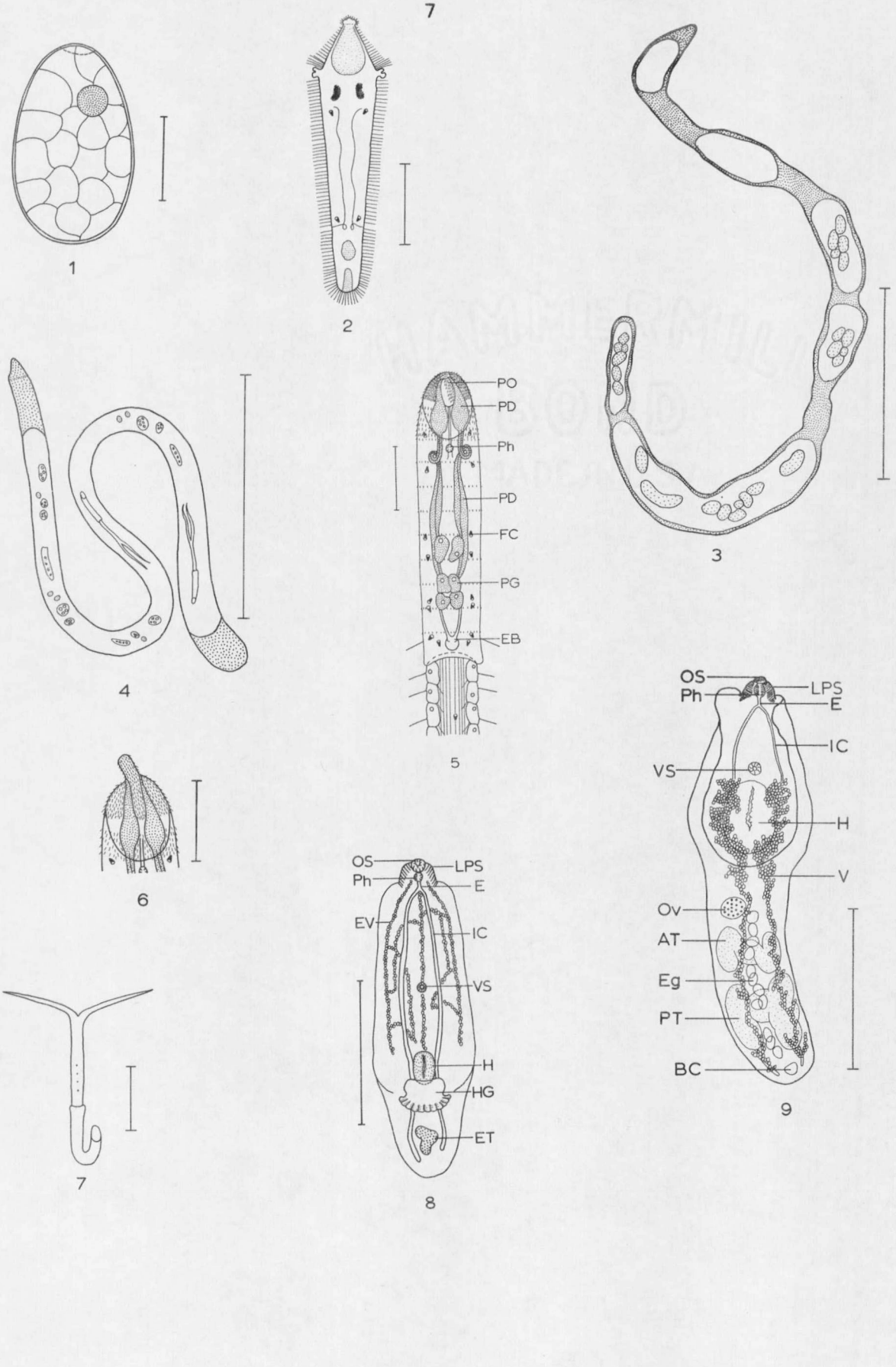
Metacercarial cysts were dissected from infected fish. The metacercariae were removed from cysts by teasing the cyst walls apart. The artificial digest recovery method of Hoffman (1955) was attempted without success, since metacercariae as well as cysts were destroyed by pepsin digestion. Permanent mounts of metacercariae were prepared in a similar manner to cercariae, but hot Bouin's fluid was used instead of 5% formalin.

Living adult B. confusus were removed from the intestines of autopsied pelicans. The entire intestine was slit open and its content washed out. This was mixed with fresh water and repeatedly decanted until the supernatant was clear. Trematodes were then recovered from the material remaining in the container. Adults were fixed in hot Bouin's fluid. Some were dropped directly into the fixative while others were first flattened under a coverslip.

Description of Stages in the Life Cycle of B. confusus

(measurements in microns unless otherwise designated)

Egg (Fig. 1). Eggs are generally ovate in shape, however a few were rounded or elongated. All are amber colored and operculate. Fifteen eggs, obtained from fresh fecal material, ranged in size from 115 x 67 to 125 x 82 and averaged 119 x 72. Ciurea (1930) reported a range of 94 x 64 to 101 x 66, but his measurements were made on eggs in utero. Dubois gave a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Explanation of Figures

Fig. 1 Unembryonated egg. Scale = 0.05 mm.

Fig. 2. Miracidium. Scale - 0.05 mm.

Fig. 3 Mother sporocyst containing germ masses and developing daughter sporocysts. Scale = 1.0 mm.

Fig. 4 Daugher sporocyst containing germ masses and developing cercariae. Scale = 1.0 mm.

Fig. 5 Body of cercariae with protrusible portion of penetration organ invaginated. Scale = 0.05 mm.

Fig. 6 Head of cercaria with protrusible portion of penetration organ extended. Scale = 0.05 mm.

Fig. 7 Cercaria in resting position. Location of flame cells in tail stem indicated. Scale = 0.20 mm.

Fig. 8 Metacercaria in ventral aspect. Calcareous corpuscles indicate extent of reserve excretory system in forebody. Scale = 1.0 mm.

Fig. 9 Adult trematode in ventral aspect. Scale = 1.0 mm.

Abbreviations: AT, anterior testis; BC, bursa copulatrix; E, esophagus; EB, excretory bladder; Eg, egg; ET, embryonic testes; EV, excretory vessel; FC, flame cell; H, holdfast; HG, holdfast gland; IC, intestinal caecum; LPS, lateral pseudosucker; OS, oral sucker; Ov, ovary; PD, penetration duct; PG, pentration gland; Ph, pharynx; PO, penetration organ; PT, posterior testis; V, vitellaria; VS, ventral sucker.

size range of 90 x 55 to 102 x 72. He credited Krause with measurements of 95.4 x 58 (Dubois, 1938). These latter measurements were probably made on eggs in utero since they are similar to Ciurea's. The egg shell appeared smooth and was less than 1 thick. The opercular cap, located at the narrow end of the egg, averaged 22 wide by 5 deep.

The development of the embryo was influenced by temperature. Time prior to hatching and the hatching period was shorter as temperature increased. Of several thousand eggs kept at 60 - 68 F, a few hatched in 65 days and hatching continued sporadically for several weeks; however, only a very small percentage hatched. Miracidia from these eggs were inactive and were probably not invasive. Many of them were malformed. Eggs generally hatched as follows: 16 - 21 days at 70 - 75 F; 14 - 18 days at 75 - 80 F; 12 - 15 days at 80 - 85 F; and 12 - 13 days at 90 F. Several hundred eggs kept at 35 - 40 F for 30 days showed no embryonic development, but these started to hatch in 12 days when the temperature was raised to 90 F. A considerable number of eggs subjected to freezing were no longer viable when thawed and held at 90 F. Eggs kept at comparable termperatures hatched at the same time and rate whether illuminated or in darkness, which suggests that heat rather than light may be a stimulus (Hugghins, 1954a).

Miracidium (Fig. 2). Development, within the egg, was similar to that of the related species Hysteromorpha triloba (Hugghins, 1954a). The developmental characteristics and measurements made on embryonic miracidia (based upon several individuals for each stage) kept at 88 - 90 F were as

follows:

First day - egg unembryonated when laid, ovum with average diameter of 18.5 and eccentrically located ca. 19 from the operculated anterior end.

Second day - egg with many large vitelline cells, ovum irregularly round, nearly twice original size.

Fourth day - egg with numerous large clear globules, few vitelline cells, embryo elongate ca. 40 x 30.

Sixth day - embryo with flame cells visible (anterior pair and posterior pair), eye spots faintly discernible as diffuse circular patches of pigment granules, cilia not visible on embryo within egg but seen beating when egg shell was ruptured, embryo ca. 70 x 35.

Eighth day - embryo extending almost length of egg, eye spots more compact and approaching reniform shape, faintly discernible goblet-shaped apical gland present ca. 33 x 23, embryo ca. 101 x 38.

Tenth day - embryo longer than egg, with posterior one-third flexed, eye spots reniform shaped measuring ca. 10 x 4 and ca. 8 apart.

Twelfth day - embryo active in egg with increased activity when exposed to strong light, embryo probing with terebratorium, most eggs hatched, miracidia generally escaped via opercular opening but some through split in shell without disturbing opercular cap.

Miracidia were generally cylindrical in shape. They were difficult to measure, due to their contractility. Measurements ranged from 150 - 190 by 30 - 40. The anterior end of the body (ca. one-fourth) is cone-shaped, with a terebratorium located at the apex. The latter is ca. 4

long and 7.5 wide and has a number of pores in the proximal region. The widest part of the body is the base of the anterior cone, which bears two pair of lateral papillae. The anterior papillae are short rod-like structures ca. 5 x 2.5, while the posterior ones, located immediately behind the anterior pair, are knob-shaped and measure ca. 7 x 5. The body of a miracidium is tapered from the level of the lateral papillae to the blunt posterior end which has a width of ca. 12. The entire body is ciliated, with the exception of the lateral papillae. The cilia are ca. 2 in length at the anterior end, but increase in length on the anterior cone until they are ca. 7 at the level of the lateral papillae. The cilia, in a tuft which is situated immediately anterior to the lateral papillae, are ca. 10. Cilia posterior to the papillae are ca. 7, except for those on the distal end which are ca. 8. An obscure goblet-shaped apical gland nearly fills the anterior cone. Directly posterior to this are two reniform-shaped eye spots of ca. 10 x 5. These are 8 - 12 apart. Their concavities face laterally and they appeared without lenses. The eye spots were observed to move considerably within the body, but always maintained their relative position to one another. Just posterior and lateral to the eye spots are the anterior flame cells. The posterior flame cells are also in a lateral position 70 - 90 posterior to the anterior flame cells. Both pairs of flame cells usually face anteriorly. The two main excretory tubules originate near the anterior flame cells and extend directly to the level of the posterior flame cells where they become convolute. Each opens into a lateral excretory pore. An irregular ovoid

structure ca. 12 x 8 is centrally located between the posterior flame cells and the posterior end. It contained granules which may be germinal material. A sac-like structure ca. 15 x 5 at the posterior end of the body also contained similar material.

Most miracidia swam in a path approximating a straight line, sometimes rotating on their longitudinal axes. They were usually extended while swimming, but some were slightly contracted and a few were very much contracted and appeared as small spheres. Miracidia, believed to be nearly spent, were observed to swim slowly in circles without rotating on their axes. Miracidia were not observed to be phototrophic and could not be concentrated with light as were miracidia of Hysteromorpha triloba (Hugghins, 1954a). Most miracidia swam for 12 hours (range 3 - 24 hours) in water kept at 75 - 80 F. The presence of host snails did not appear to stimulate miracidia and contact with snails was probably by chance. Miracidia were not seen to penetrate host snails, but some were observed to crawl over the shells and others disappeared into the shell apertures.

Sporocyst (Fig. 3 and 4). Both mother and daughter sporocysts were found in H. trivolvis. None were found in Physa sp., Gyrulus sp., and Lymnea sp., although miracidia were seen to enter their shell apertures. Only one mother sporocyst was found per snail and it was always located in the mantle. The elongate sporocysts are 4 - 5 mm in length and are composed of 6 - 8 sac-like nodules ranging in size from 0.4 - 1.6 mm long by 0.13 - 0.21 mm wide. Most nodules contained germ masses and/or daughter sporocysts in various stages of development. The anterior end of

a mother sporocyst is attenuated, while the posterior end is rounded and blunt. Tissue plugs were present at either end and between each of the nodules. Birth pores were not observed. Mother sporocysts exhibited limited mobility.

Daughter sporocysts appeared as a tangled mass of thin worm-like organisms (Fig. 4), completely obscuring the digestive gland of the snail. The number present in a representative snail exceeded 1000. These were somewhat smaller than mother sporocysts and most were without nodules. The few nodulated ones observed were probably old specimens which had produced most of their cercariae. Size was variable depending upon development, but those containing recognizable cercariae ranged from 3 - 4 mm long by 0.08 - 0.15 mm wide. The anterior and posterior ends of a daughter sporocyst are similar in shape to those of a mother sporocyst, but the daughter has a distinct anterior cone. A birth pore is present at the base of the anterior cone; ca. 100 posterior to the apex. The body is slightly tapered with the posterior end ca. 5 wider than the base of the anterior cone. Both ends of the body contain tissue plugs, while the portion between is a continuous chamber. Germ masses and cercariae in various stages of development were observed in this chamber. The maximum number of fully developed cercariae in a daughter sporocyst was seven. Cercariae were active in the body chamber and some were seen to move its entire length. Cercariae were observed leaving the birth pore, one at a time and always went head first, appearing to use the tail stem for propulsion. Daughter sporocysts were very active and a number were