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The life cycle of Cotylurus erraticus (Rudolphi, 1809) Szidat, 1928 (Trematoda: Strigeidae) and the effect of the metacercaria on rainbow trout (Salmo gairdneri)
by Robert Eldon Olson

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
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Abstract:
The life cycle of the strigeid trematode Cotylurus erraticus (Rud., 1809) Szidat, 1928 was completed experimentally in the laboratory. Eggs were obtained from laboratory infected immature Larus californicus. Most eggs hatched in 15-16 days at 24 C and the miracidium. was similar in morphology to other strigeoid miracidia.

Mother and daughter sporocysts developed in the operculate snail Valvata lewisi and the pharyngeate, furcocercous cercaria usually emerged 35 days after infection. The metacercaria is tetracotyliform and was originally described as Tetracotyle intermedia by Hughes (1928). It encysts in the pericardial cavity of salmonid fishes and was found in Salmo gairdneri, Salvelinus fontinalis, Oncorhynchus nerka, 0. kisutch and Thymallus arcticus collected from Georgetown Lake. When ingested by immature gulls, the metacercariae developed in the small intestine to patent adults in four days. This study is the first complete life cycle for a fish infecting Tetracotyle. The effects of C. erraticus metacercariae on S. gairdneri were studied by comparing the swimming performance, hematocrit value, high temperature tolerance and low oxygen tolerance of parasitized trout with parasite-free trout. No difference between parasitized and control fish was found in any of these tests.

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THE LIFE CYCLE OF <u>COTYLURUS</u> <u>ERRATICUS</u> (RUDOLPHI, 1809) SZIDAT, 1928 (TREMATODA: STRIGEIDAE) AND THE EFFECT OF THE METACERCARIA ON RAINBOW TROUT (<u>SALMO</u> <u>GAIRDNERI</u>)

by

ROBERT ELDON OLSON

A thesis submitted to the Graduate Faculty in partial
fulfillment of the requirements for the degree

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ABSTRACT

The life cycle of the strigeid trematode Cotylurus erraticus (Rud., 1809) Szidat, 1928 was completed experimentally in the laboratory. Eggs were obtained from laboratory infected immature Larus californicus. Most eggs hatched in 15-16 days at 24 C and the miracidium was similar in morphology to other strigeoid miracidia. Mother and daughter sporocysts developed in the operculate snail Valvata lewisi and the pharyngeate, furcocercous cercaria usually emerged 35 days after infection. The metacercaria is tetracotyliform and was originally described as Tetracotyle intermedia by Hughes (1928). It encysts in the pericardial cavity of salmonid fishes and was found in Salmo gairdneri, Salvelinus fontinalis, Oncorhynchus nerka, O. kisutch and Thymallus arcticus collected from Georgetown Lake. When ingested by immature gulls, the metacercariae developed in the small intestine to patent adults in four days. This study is the first complete life cycle for a fish infecting Tetracotyle. The effects of C. erraticus metacercariae on S. gairdneri were studied by comparing the swimming performance, hematocrit value, high temperature tolerance and low oxygen tolerance of parasitized trout with parasite-free trout. No difference between parasitized and control fish was found in any of these tests.

INTRODUCTION

During the fall of 1965, a number of salmonid fishes were collected from Georgetown Lake, near Anaconda, Montana. Examination of these fish revealed a high prevalence and intensity of a trematode metacercaria in their pericardial cavities.

This metacercaria proved to be Tetracotyle intermedia which was described by Hughes (1928) from the round whitefish (Prosopium cylindraceum) and the cisco (Coregonus artedii) collected in Lake Huron. Hoffman (1960) lists 15 species of Tetracotyle that infect fish, none of which has a completely described life cycle. Van Haitsma (1930a) reported the life cycle of Cotylurus platycephalus (syn. C. michiganensis and C. communis), but this was later shown to be in error by Olivier and Cort (1942). The present study is the first complete life cycle for a fish-infecting Tetracotyle.

Georgetown Lake is an impoundment formed by a dam athwart Flint Creek constructed in 1894. According to Fred Beal (unpublished, undated manuscript) the dam was raised 5 feet in 1919 and another 3 feet in the 1940's, resulting in a lake of 1120 hectares and an average depth of 5.5 meters. This lake is situated at an elevation of 1942 meters and is usually ice covered from mid-November to mid-May. It is highly eutrophic and supports a large variety of plant and animal life. In recent years, rainbow trout (Salmo gairdneri) have been regularly planted in the lake. Arctic grayling (Thymallus arcticus) were stocked in 1966 and 1967 and coho salmon (Oncorhynchus kisutch) in 1967.

Other fish which are numerous are: brook trout (<u>Salvelinus</u> <u>fontinalis</u>),
kokanee salmon (<u>O.</u> <u>nerka</u>), longnose sucker (<u>Catostomus</u> <u>catostomus</u>) and
redside shiner (<u>Richardsonius</u> <u>balteatus</u>). Large areas of mucky bottom
and decaying vegetation in Georgetown Lake make conditions favorable for
the eight species of snails found during the study period.

The purpose of this investigation was to determine the adult stage
and life cycle of <u>T.</u> <u>intermedia</u> and to study the effects of sublethal
infections of the parasite on swimming performance, high temperature
and low oxygen tolerance of young rainbow trout. Study on this project
extended from September, 1965 to June, 1968.

MATERIALS AND METHODS

Life Cycle

A number of aquatic birds were collected at Georgetown Lake. One of these, an immature California gull (<u>Larus</u> <u>californicus</u>) was infected with <u>Cotylurus</u> <u>erraticus</u> in the intestine. In addition, four young California gulls were collected from breeding islands at Freezeout Lake, Teton Co., Montana. These birds were checked for natural trematode infections and although negative, were given 1 cc. doses of CCl_4 to ensure that they were free of parasites. One of these birds died after receiving a laboratory infection. The remaining three gulls served as a source of parasite eggs throughout the study. Gulls were fed fish from Georgetown Lake, principally longnose suckers and salmonids that had been frozen.

All <u>C</u>. <u>erraticus</u> eggs used were obtained from laboratory-infected California gulls. Fresh fecal material from infected birds was placed in quart jars and mixed with distilled water. After settling for about 10 minutes, the supernatant was decanted and more distilled water added. This process was repeated until the sample consisted only of eggs and larger particles of fecal material. Distilled water was again added but since the remaining fecal particles settled faster than the eggs, a settling period of less than one minute allowed the still-suspended eggs to be decanted into finger bowls along with a small amount of fecal material. Eggs were then removed with a pipette, using a dissecting microscope, and placed in a finger bowl with clean water where they were incubated at room temperature, approximately 21 C.

Some eggs were incubated in finger bowls floated in a constant temperature aquarium. Eggs were agitated and the water was changed daily during the incubation period. Hatching of eggs was delayed when desired by placing them in a refrigerator at 5 C when fully embryonated (Harris, Harkema and Miller, 1967). Miracidia were studied alive, both unstained and supravitally stained with neutral red. Methyl cellulose was used to inactivate living individuals. The excretory system was most easily studied by allowing miracidia to dehydrate slowly under a coverslip. Permanent mounts were also made after fixation in hot AFA and staining in Semichon's carmine. The epidermal plate pattern was determined using the silver nitrate method described by Lynch (1933).

Snails were collected at Georgetown Lake by hand-picking them in shallow water and with an Ekman dredge in deeper water. Representatives of all species found in the lake were maintained in laboratory aquaria on a diet of boiled lettuce. Attempts to infect various species of snails were accomplished by placing miracidia and snails together in small Stender dishes for a period of about 12 hours.

Sporocysts were carefully dissected out of infected snails and studied alive unstained. Permanent mounts were also made, but were not as good as living specimens for the study of internal structure.

Cercariae were collected from finger bowls containing infected snails. Methods used to study cercariae were the same as for miracidia.

Cercariae used to infect fish were obtained by placing infected snails in finger bowls containing clean water for a period of 18-24 hours

before infections were attempted. The number of cercariae used in experiments was estimated by the method of Olson (1966). All fish were kept in water slowly tempered to 21 C and then exposed to cercariae for 30 minutes in a small (5.6 liter) aquarium. This aquarium allowed the cercariae and fish to be concentrated and increased the chances of cercarial contact. After exposure, fish were held at 21 C to facilitate rapid metacercarial development.

Metacercariae were collected both from naturally and laboratory infected fish and the parasite cysts were mechanically removed. They were fixed in hot AFA or 10% formalin, stained in Semichon's carmine and mounted in Permount.

Adult worms were obtained from the infected immature California gull from Georgetown Lake and from a laboratory infected California gull that died during experiments. The intestines were slit open and the contents washed into a quart jar. This material was decanted several times until the supernatant was clear. Worms were then recovered and whole mount slides prepared in the manner described for metacercariae.

All measurements of parasite stages are given in microns. When length and width measurements are given, length is given first. Ranges are given in parentheses.

Experimental

Fish were procured from a single stock of 50-85 millimeter rainbow trout at the National Fish Hatchery, Ennis, Montana, on March 8, 1968.

The 500 fish obtained were held in hatchery troughs containing running water (5-11 C) until used in experiments.

A stamina tunnel made by the Bureau of Commercial Fisheries, Seattle, Washington, was used to test the swimming performance of experimental rainbow trout. This apparatus was modified and described by Fox (1965). Briefly, the tunnel was constructed of plexiglass, had a length of 132 centimeters and an inside diameter of 7.6 centimeters. Water was supplied by a centrifugal pump powered by an electric motor. Fish were stimulated to remain in the tunnel by an electrical field produced by three electrodes near the outlet. A sliding gate with three openings of different sizes was located at the outlet of the tunnel. The small opening was used for velocities of 0.12-0.30 m/sec., the intermediate for 0.30-0.76 m/sec., and the large for removing test fish from the chamber.

Fish to be used in tests were transferred from the troughs into 84 liter aquaria in which the water temperature could be controlled at desired levels (Fox, 1965).

Experimental fish were exposed to cercariae in the manner described above. Control fish were treated the same as experimental fish, but were not exposed to cercariae. Control and experimental fish were placed in the same aquaria so that they were indistinguishable until post-test necropsy.

After a period of at least two weeks at 21 C to allow rapid metacercarial growth, the water temperature was lowered to 11 C. Fish were transported about 8 kilometers to the Fish Hatchery Development

Center and were allowed at least two days to acclimate before tests were
started.

The stamina tunnel was situated over a hatchery trough which had a
continuous flow of water. Water was pumped from the upper end of the trough,
through the tunnel and emptied into the lower end. Some reuse of water may
have occurred, especially at high velocities when a larger volume was used,
but no problems with metabolic waste, increased temperature, lowered oxygen
etc. were encountered.

Test fish were held in a trough adjacent to the one on which the
stamina tunnel was situated. One fish at a time was netted and placed in
the experimental chamber. The water velocity was then raised to 0.30 m/sec.
over a period of 5 minutes. At this time, the sliding gate was moved to the
high velocity position and the electrical field activated. The water
velocity was then slowly increased 0.03 m/sec. every 5 minutes until the
test terminated. Fish were allowed two passes through the electrical
field. After the first pass, fish were allowed 5 minutes to return to the
velocity at which they failed and the test continued. The test terminated
when one of the following occurred: 1. The fish passed through the electri-
cal field and failed to return to the experimental chamber after the water
velocity was reduced to zero and the electrical field inactivated. 2.
The fish returned to the experimental chamber but did not reach the
velocity at which it failed within the allowed 5 minute period. 3. The
fish passed through the electrical field a second time.

At the completion of each test, the highest velocity attained and the total swimming time were recorded. Each test fish was measured to the nearest millimeter and a blood sample taken. Blood samples for use in hematocrit determinations were collected from the caudal artery after severing the caudal peduncle. Heparinized capillary tubes containing the blood sample were centrifuged at 12,500 r.p.m. for 5 minutes and hematocrit values obtained with a microhematocrit reader. All test fish were labeled and taken to the laboratory, frozen and later examined for parasites.

The ability of parasitized fish to withstand high water temperatures was compared with that of parasite-free fish using the constant temperature aquaria mentioned earlier. Experimental fish to be used in temperature stress tests were handled and exposed to cercariae as described above. Control fish were treated similarily but were not exposed to cercariae. A number of infected and control fish were then placed in each of two aquaria and held at 21 C. Oxygen was maintained at high levels throughout the experiment by bubbling air through an air stone. After allowing two weeks for metacercarial development, the temperature in each tank was raised 1 C every 24 hours. When a fish died, the temperature was recorded and the fish was frozen and later examined for parasites.

Fish used in experiments testing the ability to withstand oxygen depletion were handled and exposed as described above. After the period allowed for metacercarial development, 50 experimental fish (half infected, half control) were placed in a 84 liter aquarium and the air and inflow water turned off. The water temperature was 20.5 C. The oxygen level was

checked about once an hour with a Precision Galvanic Cell Oxygen Analyser. When a fish died, the oxygen level was recorded and the fish frozen and later examined for parasites.

HISTORICAL ACCOUNT OF C. ERRATICUS

A historical account of Cotylurus erraticus (Rud., 1809) Szidat, 1928
is given by Dubois (1938). Some confusion arose among the early workers,
due in part to the number of different hosts in which the parasite was
found. All of the early work on C. erraticus was done in Europe and can
be summarized as follows: The parasite was first described by Rudolphi in
1809 as Amphistoma erraticum from specimens that he found in the intestine
of the red-throated loon, Larus septentrionales (now Gavia stellata).
Later, he observed specimens of the same parasite from the arctic loon
(G. arcticus). In 1844, Bellingham described trematodes from the intestine
of the common loon (G. immer) as A. gracile; these were actually C.
erraticus. In 1845, Dujardin changed A. erraticum of Rudolphi to
Holostomum erraticum. The name was changed again in 1909 to Strigea
erratica by Lühe who reviewed the work reported by Brandes in 1888.
Finally, in 1928, Szidat placed the parasite in the new genus Cotylurus
characterized by vitellaria confined to the hindbody and a muscular bursa
copulatrix.

Other European host records were found by Dubois (1938) who studied
specimens in the Koningsberg collection. The trematodes found in the
common gull (Larus canus) and the lesser black-backed gull (L. fuscus)
were identified as C. erraticus.

Guberlet (1922), working in Oklahoma, described Strigea aquavis as a
new species from the common loon (G. immer) and from the ring-billed gull
(L. delawarensis). Szidat (1928) placed this parasite in the genus

Cotylurus. Dubois and Rausch (1950) found the parasite in G. immer in

Wisconsin and suggested that C. aquavis may be the same as C. erraticus.

Dubois (1953) placed the two species in synonomy.

DESCRIPTION AND LIFE CYCLE OF C. ERRATICUS

Egg

The ovoid, operculate egg is amber in color. Ten eggs taken at random averaged 116 X 62 (105-122 X 60-65). These measurements are very similar to those reported by Szidat (1929) and Dubois (1938). Measurements of eggs given by Guberlet (1922) and Dubois and Rausch (1950) were slightly smaller. It may be that the latter were measured in utero since my measurements are slightly less for eggs in utero than for free eggs. An operculum in the open position measured 20 X 7 although the aperture of the hatched egg was 30 across. The aperture may increase in size after the operculum opens.

The egg is unembryonated when laid, with the ovum located in the anterior one-third. The rate of egg development depends on temperature as reported for many other strigeid trematodes. Eggs incubated at 24 C began hatching in 13 days, but most hatched in 15-16 days. Hatching was complete in 21 days. Lower temperatures resulted in a corresponding lengthening of incubation time. Eggs allowed to develop almost to hatching and then kept at 5 C for a month before returning to room temperature, showed good survival. Some of the eggs placed at 5 C immediately after collection and held for 1 to 2 months, resumed development upon return to warmer temperatures but survival was noticeably reduced. No eggs survived 12 months at 5 C. Survival was very low when eggs were incubated at 32 C.
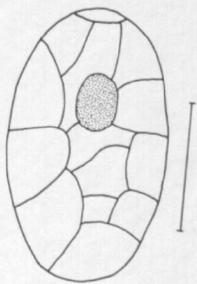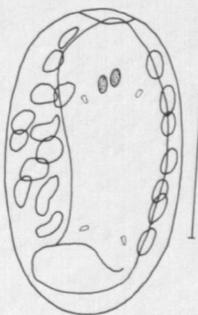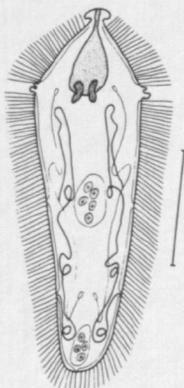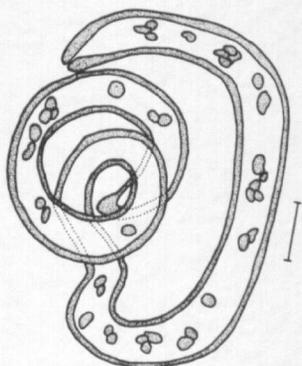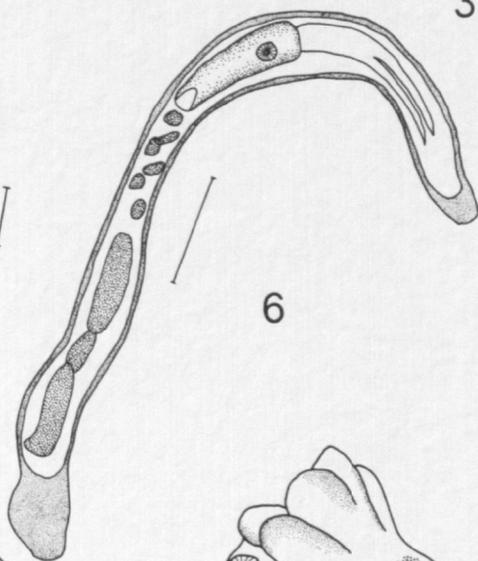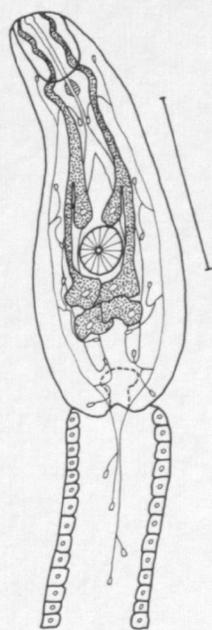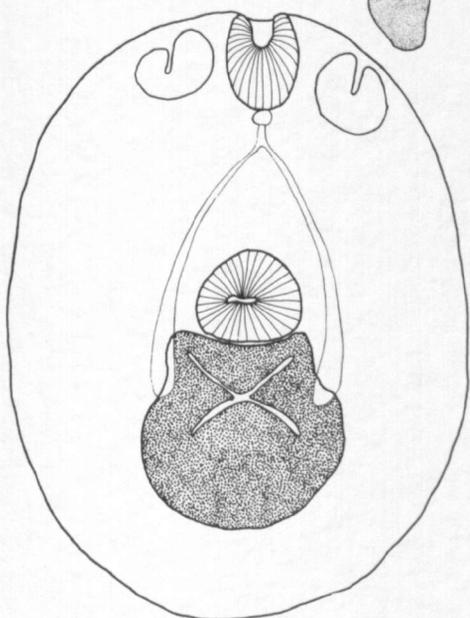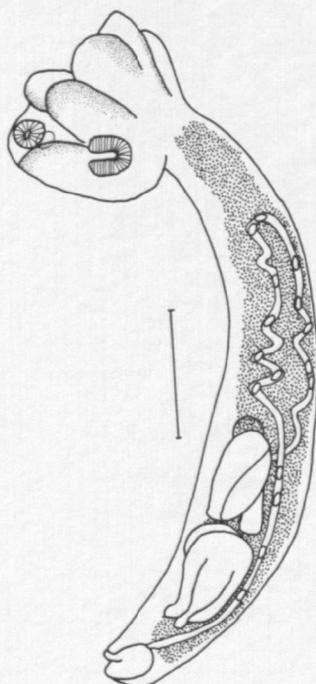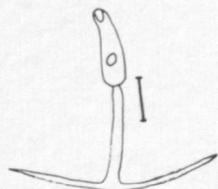
Explanation of Figures

All drawings made with the aid of a camera lucida.

Fig. 1. Unembryonated egg. Scale = 50 μ.

Fig. 2. Egg after twelve days of incubation at 24 C.

Miracidium about ready to hatch. Scale = 50 μ.

Fig. 3. Miracidium showing internal structure. Scale = 50 μ.

Fig. 4. Miracidium showing epidermal plates. Scale = 50 μ.

Fig. 5. Mother sporocyst, 44 days old and contains no developing daughter

sporocysts. Scale = 100 μ.

Fig. 6. Daughter sporocyst containing embryonic cercariae and one nearly

mature cercaria. Scale = 100 μ.

Fig. 7. Body of cercaria showing internal structure. Scale = 100 μ.

Fig. 8. Entire cercaria showing relative size of furcae to body.

Scale = 100 μ.

Fig. 9. Metacercaria. Scale = 100 μ.

Fig. 10. Adult. Scale = 500 μ.

Miracidium

The development of the miracidium was studied within the egg and was similar to that observed for the miracidium of other strigeids (Hugghins, 1954a, Fox, 1965). The developmental characteristics and measurements of representative individuals incubated at 24 C are described below.

First day: Ovum unembryonated (Fig. 1), 15 in diameter and located in the anterior one-third of the egg.

Second day: Ovum 30 in diameter and vitelline cells scattered throughout egg.

Third day: No apparent change.

Fourth day: Most ova still about 30 in diameter, but more rounded. Vitelline cells decreased in number.

Fifth day: Ovum 35 in diameter, vitelline cells absent, large clear cells surround ovum.

Sixth day: Ovum 45 in diameter and more centrally located within the egg.

Eighth day: Embryo showed form of miracidium. A representative individual measured 58 X 40, the eyespots and flame cells were barely discernible.

Tenth day: A representative embryo measured 88 X 45. Movement was observed for the first time. Eyespots were prominent and flame cells were more easily seen.

Twelfth day: Miracidium fully developed within the egg (Fig. 2). It was longer than the egg and the body was flexed at the posterior end so

that only approximate measurements were possible. One miracidium was 130 X 40. Eyespots had reached maximum size (12 X 5). Movement of the miracidium exerted pressure on the operculum.

Hatching occurred almost exclusively during the early evening hours. This is contrary to the observations of Hugghins (1954a) for Hysteromorpha triloba and Van Haitsma (1930b) for Diplostomum flexicaudum. They reported that most miracidia hatched in the early morning hours and suggested that light and heat may play a role in hatching. .I did not observe any relationship between either light or heat and hatching time. Temperature was held constant and hatching occurred at approximately the same time whether the room was illuminated or not. After hatching, miracidia appeared to be positively phototactic in that they consistently congregated near the surface and at the edge of the container nearest the light source. Freshly hatched miracidia rapidly swam in a straight line with a rotating motion. After about 6 hours, they swam more slowly and erratically. Most miracidia remained active for 12 hours after hatching and a few were observed to swim as long as 17 hours. Miracidia held at 5 C for 12 hours after hatching were immobilized, but regained activity upon return to room temperature. Most of these swam for as long as 12 hours following refrigeration.

The body of the miracidium is cylindrical and is widest at the level of the lateral papillae (Fig. 3). The anterior end is cone-shaped and contains a protrusible terebratorium. When extended, the terebratorium measures 16 X 10. The body tapers posterior to the lateral papillae and is quite narrow at the posterior end. Living miracidia were difficult to