Studies on the Life-Cycle of *Ornithodiplostomum ptychocheilus* (Faust) (Trematoda: Strigeoieda) and the "Self Cure" of Infected Fish*

Glenn L. Hoffman
US Fish and Wildlife Services

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STUDIES ON THE LIFE-CYCLE OF *ORNITHODIPLOSTOMUM PTYCHOCHEILUS* (FAUST) (TREMATODA: STRIGEOIDEA) AND THE "SELF CURE" OF INFECTED FISH*

Glenn L. Hoffman

Department of Bacteriology, University of North Dakota**

During the summer of 1956 the following fish of Turtle River (Arvilla), North Dakota were found infected with the *Neascus* metacercaria of *Ornithodiplostomum ptychocheilus* (Faust): Northern common shiner (*Notropis cornutus frontalis*), bigmouth shiner (*Notropis d. dorsalis*), creek chub (*Semotilus a. atromaculatus*), fathead (*Pimephales p. promelas*), and johnny darter (*Boleosoma n. nigrum*). The cysts were usually very numerous (up to 400 per fish) in the mesenteries of the chub and common shiner; they were also very numerous in the cranial cavity and in the fat bodies behind the eyes of the common shiner and fathead, but were never seen in these locations in the chub. These unusual locations were briefly reported previously (Hoffman, 1954). Only 1 specimen of *O. ptychocheilus* was found in the mesenteries of the johnny darters examined.

MATERIALS AND METHODS

Thirteen newly hatched chicks were force-fed 100–450 metacercariae which had been removed mechanically, or by 10-minute pepsin-HCl digestion from the viscera or cranial cavities of the above infected fish; An average of 23 (0–89) ovigerous adults was recovered per chick at 2–5 days. These adults were identical with adults described from mergansers (*Mergus americanus, M. serrator, Lophodytes cucullatus*) and the old squaw duck (*Harelda hyemalis*). There was no morphological difference in the adults recovered from chicks which were fed metacercariae from different fish species, or from different locations in the fish. As is characteristic of this species, there were very few eggs *in utero,* usually 1 or 2, and never more than 6.

Small numbers of eggs for life cycle studies were collected in 3 ways: (1) collection of infected chick feces in dechlorinated water, and subsequent straining, washing and sedimenting; (2) collecting worms containing eggs from autopsy; and (3) mechanically removing the eggs from the worms. Those obtained by method (1) were incubated with forced aeration; the latter 2 groups were incubated in 5-cm petri dishes. Free-swimming miracidia were observed in group (3) at 9 days; the eggs in the worms of group (2) developed to the miracidium stage but none hatched. Both groups (1) and (3), however, were successful methods for collecting and incubating eggs for subsequent infection of snails. Snails were placed in half-pint milk bottles after several days' exposure to the egg cultures. Cercariae were allowed to accumulate for 6–12 hours in the bottles and then added to about a liter of aerated dechlorinated water in a gallon jar with 1 or more fish which were maintained in the same jar at an average temperature of 24° C throughout the course of the experiment.

RESULTS AND OBSERVATIONS

No source of trematode-free fish was available but 11 control fatheads were infected with only 10 (0–40) *O. ptychocheilus* from natural sources, whereas the experimental fish contained 832 (387–1441). Three bigmouth shiners (*Notropis d. dorsalis*) contained 70 (38–131) *O. ptychocheilus,* whereas the experimental fish contained 722 (387–1368). In addition, the immature experimental metacercariae could be easily distinguished from the mature natural metacercariae because

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** Present address: Eastern Fish Disease Laboratory, Leetown (P. O. Kearneysville) W. Va.

the experiments were done in late fall (October 30–November 2) and the natural metacercariae were mature by the time the earliest experimental fish were examined.

All measurements given below are in microns unless otherwise stated.

The Miracidium

Because of a scanty supply of eggs only 3 miracidia were studied. These moved very rapidly in the usual spiral fashion of strigeoid miracidia except that sometimes they swam intermittently in very small circles. The following description is based on 2 specimens only: Size 127–171 by 46–49; eye spots 8–10 by 5 dorsally, and very close together with concave sides facing each other and containing what appear to be 2 lenses; cilia *ca* 10 long; a rather large oval structure, 30 in diameter, containing cells with nuclei 5 in diameter, just posterior to eye spots.

The Sporocyst

Thirty-six *Physa anatina, 27 Stagnicola palustris,* and 20 *Helisoma anceps,* all laboratory-reared, were exposed to cultures prepared as described above; of these 5 *Physa anatina* became infected and cercariae first emerged at 45 days.

Mother sporocyst:

One mother sporocyst 2.8×0.1 mm was removed from a snail liver; 1 end was slightly invaginable and may have contained the birth pore. No distinguishing morphological features were noted.

Daughter sporocyst:

These were also in the liver. Three were 232–490 by 40–54. No ear-like structures were seen and the birth pore is apparently at the anterior end, which is slightly invaginable; germ balls were *ca* 24 in diameter.

The Cercaria

The cercaria of *O. ptychocheilus* is of the longifurcate monostome type. No acetabulum, pharynx, esophagus or ceca can be ascertained. The penetration organ (oral sucker) is oval, elongate and *ca* 50 by 17 alive. The anterior tip is usually inverted. Small scattered spines, *ca* 1.5 long, cover the anterior surface of the cercaria for *ca* ½ the length of the penetration organ; those of the area corresponding to the anterior end of the penetration organ are shorter. Posterior to this crown of spines are 8 single transverse rows of small spines *ca* 1 long which can be seen with phase only; the terminal row is at the level of the first pair of penetration glands. One pair of laterally projecting flagellets, 10 in length, emerges from the body between the posterior pair of penetration glands and the posterior end of the body.

There are 6 pairs of flame cells in the body of the cercaria plus 2 in the tail-stem. Two pairs are located postero-laterally to the penetration organ, 2 centrally, and 2 posterior. Only the 2 main lateral excretory ducts of the body and the main tail-stem tubules could be discerned. The excretory bladder is bipartite with a small "island of Cort".

A pair of simple eye spots, *ca* 3 to 4 in diameter, is located just posterior to the middle of the body, but cannot always be demonstrated. Three pairs of penetration glands, *ca* 10 in diameter, lie posterior to the eye spots but do not reach

the posterior end of the body; ducts lead anteriorly from them through the penetration organ, in which they are enlarged, and open in small pores lateral to the oral opening. Between the eye spots is a body of cells which are *ca* 2 in diameter. Between the penetration glands are 2 tandem bodies composed of cells *ca* 4 in diameter. Posterior to the last pair of glands is a larger body of cells which is probably the genital Anlage.

The body is 86/100 as long as the tail-stem which is slightly narrower. Most of the cercariae have 5 pairs of caudal bodies, but in some newly emerged cercariae there are 6 pairs which extend almost to the furcae; these bodies disappear (become used up?) at about 12 hours after the emergence of the cercaria. There are 2 pairs of flame cells in the tail-stem, 1 anterior and 1 near the posterior end. The excretory duct from the excretory bladder passes through the center of the tail-stem and bifurcates at the origin of the furcae; each duct proceeds to the proximal anterior surface of the furca where it empties through a minute pore about 2/3 the distance from the furcal base. The surface of the tail-stem bears about 60 annulations which correspond to underlying cells. There are 8 to 10 flagellets *ca* 10 in length on each side of the tail stem. Very small flagellets, *ca* 1 long, are present on the anterior edge of the furcae. A very small fin-fold extends from about midway on the anterior edge of the furca and around the end to about midway of the posterior edge.

Measurements of 10 specimens fixed in hot formalin: Body 169 (144–196) by 31.8 (26–39), tail stem 196 (162–220) by 27 (24–34), furcae 177 (159–196) by 13 (12–14) dorsal aspect, greatest lateral width of furcae 19, penetration organ 66 by 19.

This cercaria is almost identical with the cercaria of *Posthodiplostomum minimum centrarchi* as described by Miller (1954). However, the following differences are noted: *Cercaria O. ptychocheilus:* (1) is smaller—the body is 12% smaller, tail-stem 21% smaller, furcae 19% smaller; (2) has 8 rows of body spines instead of 10; (3) has 6 pairs of body flame cells instead of 8; (4) has a slightly longer tail-stem in proportion to the body-length; (5) has an anterior area of very short spines instead of all anterior spines of equal size; (6) sometimes has 6 pairs of caudal bodies instead of 5. The writer realizes that some of these features may be variable and are not generic differences. It seems apparent that the 2 cercariae are very closely related; perhaps the 2 worms should be placed in the same genus.

Fish Infection and Metacercarial Development

All of the following fish became infected: 11 fatheads (*Pimephales p. promelas*), 9 central bigmouth shiners (*Notropis d. dorsalis*), and 1 creek chub (*Semotilus d. dorsalis*). However, neither of 2 johnny darters (*Boleosoma n. nigrum*) or 1 stickleback (*Eucalia inconstans*) became infected. Surprisingly, 1 johnny darter from Turtle River was found with 1 natural *O. ptychocheilus* metacercaria. The experimental fish became infected with large numbers and the "crowding effect" probably retarded the development of the metacercariae as well as the development of the cyst of parasite origin in some instances. The development was very similar to that of *Posthodiplostomum minimum centrarchi* as reported by Hoffman (1958) and Miller (1954).

Development of the Metacercaria and Parasitic Cyst

7 days—Many metacercariae are found in the mesenteries, cranium, fat bodies behind the eyes, and in the vitreous chambers. They are very actitve and plastic, and measure 313 to 732 long by 188 to 260 wide. The penetration ducts are still present, the holdfast can be seen as a slight invagination, but the ventral sucker can not yet be ascertained. At this stage the metacercariae can not be differentiated from larvae of *Posthodiplostomum minimum* of similar age.

10 days—No evidence of cyst of parasite origin yet, but the metacercaria appears nearly mature.

17 days—The secreting of the cyst of parasitic origin is in progress. Some were left overnight in saline and the parasite cyst separated from some of them as reported for *Posthodiplostomum* by Hoffman (1958).

20 days—Many parasites are enclosed in "swollen" recently secreted parasite cysts. The metacercaria itself has a swollen appearance and cannot be distinguished from *Posthodiplostomum* at this time; in fact, the writer could not identify the parasite certainly until after this stage of development.

24 days—In fish with a moderate number of parasites the cysts and the parasites were fully formed. However, many of the metacercariae in very heavily parasitized fish were retarded in development and appeared like the 10–17-day stage above.

55 days—Of 2 heavily infected fish 1 had a few immature metacercariae, the rest being mature, and in the other, 20% were immature, presumably because of the "crowding effect." A few dead metacercariae were observed.

107 days—In 1 extremely heavily infected fish all of the 1368 metacercariae appeared mature and normal except for a few dead ones, and a few which were about 2/3 as large as the rest.

The Host Cyst

The cysts can be easily removed from adjacent tissue, and the larvae easily liberated by tearing the cyst with dissecting needles. Experimental cysts, 20 to 68 days old, were studied in histologic sections stained with hematoxylin and phloxine, and Mallory's triple connective tissue stain. The cyst of parasite origin stains blue with Mallory's and is *ca* 1.6 microns thick. At 20 days the host cyst is *ca* 3 microns thick, and is composed of closely packed connective tissue which stains red with Mallory's stain at this time; the nuclei are oval and *ca* 16×13 microns in diameter.

At 68 days the nuclei can no longer be seen and the host cyst stains purplish with Mallory's stain. The cysts from the eye orbit, viscera, brain, and muscle are essentially the same.

Effect on the Fish and the "Self-Cure"

It is not uncommon to find large numbers of metacercariae in fish and often there is no evidence of deleterious effect on them. Fischthal (1953) has summarized the effects of parasitism on the fish host. Sillman (1957) observed no deleterious effect of *Posthodiplostomum minimum centrarchi* on a sunfish in an aquarium although the fish's kidney and liver were riddled with parasites. It is difficult to understand how fish can withstand extremely heavy infections and remain

apparently healthy. Hoffman (1958) reported a peculiar means by which excessive numbers of larval *Posthodiplostomum m. minimum* are eliminated by minnows—a bursting of the body wall, allowing some of the parasites to fall out followed by closure of the wound. In the present study this rupture was repeated with *O. ptychocheilus*. The 19 fish (*P. p. promelas, N. d. dorsalis, S. a. atromaculatus*) were exposed to relatively large numbers of uncounted cercariae and of these 19, 12 were examined carefully for evidence of lacerations at 31 to 107 days post-infection; 8 showed evidence of having burst, releasing parasites, followed by complete healing (Fig. 1). Histopathologic examination of the affected area showed simple scars completely through the body wall. The fish were not examined for an acumulation of tissue fluid (ascites) in the body cavity and there is a possibility that it might have contributed to this phenomenon.

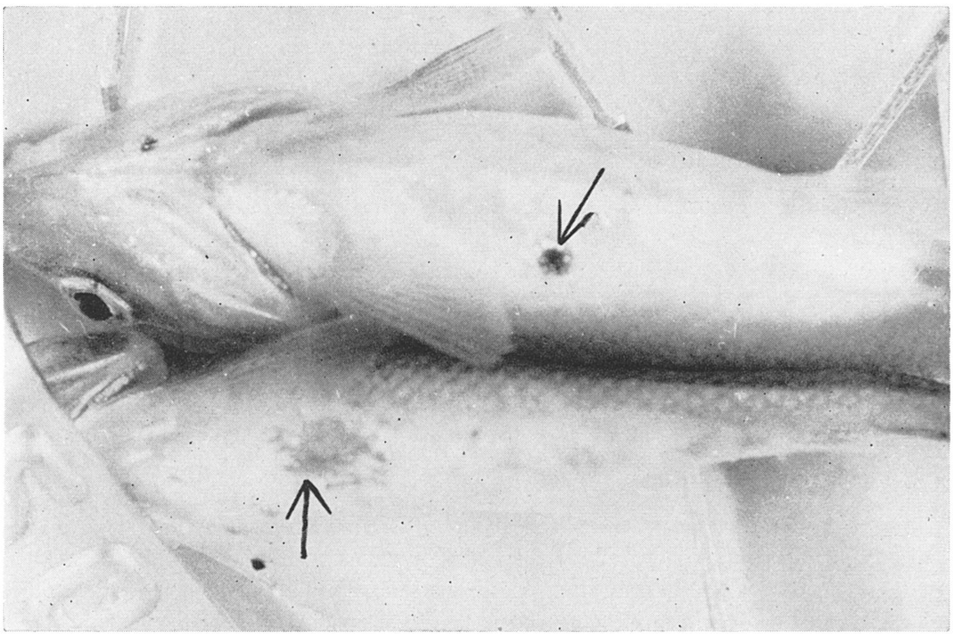

FIGURE 1. Two *Pimephales p. promelas* 40 days post-infection with a large number of cercariae of *Ornithodiplostomum ptychocheilus*. Note the large dark scars where the body wall had burst and healed over.

There was considerable hyperemia at the base of the fins of several of the infected fish at 1 to 24 days post-infection; in 1 instance several spots of hyperemia on the body were also seen.

In moderate natural infections the metacercariae were almost always in 2 locations, the body cavity and on the brain. In the heavy experimental infections, however, the metacercariae were found in 3 additional locations: the eye orbit, the vitreous chamber of the eye (occasionally subcorneal), and in the musculature. The larvae from 5 experimentally infected fish were counted with the following results: (1) total, average 832 (387–1441) (2) body cavity, 89.2% of total average (3) eye orbits, 4.5% (4) eyes, 4% (5) brain, 1.8%, (6) musculature, 0.5%. During the examination of histologic sections of the brain several larvae were noted in the brain tissue itself; most, however, were on the surface of the brain.

Except for the afore-mentioned hyperemia and the thin connective tissue host cyst, there appears to be little host reaction; when numerous, some of the cysts on the surface of the brain are sunken into the brain tissue, presumably because of resorption, and the meninges are intact, although thinner, beneath the cyst. In very heavy infections the meninges become considerably thicker and very vascular adjacent to the cysts. The cysts in the brain tissue itself are essentially identical with those on the surface. No unusual physical symptoms were noticed except that 1 experimental fish became greatly emaciated.

SUMMARY

The life cycle of *Ornithodiplostomum ptychocheilus* is described. Adults were obtained by force-feeding baby chicks with metacercariae from the mesenteries and brain of the shiner (*Notropis cornutus frontalis*) and fathead (*Pimephales p. promelas*) and mesenteries of the creek chub (*Semotilus a. atromaculatus*). Snails (*Physa anatina*) were infected with the resulting miracidia, and the sporocysts and cercariae are described. Fish (*Pimephales p. promelas, Notropis d. dorsalis, Semotilus a. atromaculatus*) were experimentally infected and the development of the metacercaria and cyst is described. A bursting of the body wall of the fish, resulting in release of metacercariae ("self-cure") is described. The histopathology of the infected fish is briefly described.

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