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Authors: Rosen, R., and Dick, T. A.

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EXPERIMENTAL INFECTIONS OF RAINBOW TROUT, *SALMO GAIRDNERI* RICHARDSON, WITH PLEROCERCOIDS OF *TRIAENOPHORUS CRASSUS* FOREL

R. Rosen and T. A. Dick
Department of Zoology, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada

ABSTRACT: This study concerned the suitability of rainbow trout as an experimental host for plerocercoids of *Triaenophorus crassus*. Twenty-five trout were exposed to *Cyclops bicuspidatus thomasi* infected with procercoids of *T. crassus*. Plerocercoids were recovered from 11 of these fish. Hemorrhaging in the muscle was the first evidence of infection in live fish between Days 22–58 postinfection (PI). Migration of worms created extensive lesions in the muscle by Day 30 PI followed by formation of granulomas between Days 45–75 PI. One to three plerocercoids were wound throughout the muscle after Day 30 PI, and penetration into the body cavity and through the integument was common. Mortality of infected trout was first observed at Day 44 PI, and by Day 56 PI, 45% of the trout died. The swimming behavior of infected trout was marked by decreased activity and loss of equilibrium.

INTRODUCTION

Triaenophorus crassus occurs as an adult in the small intestine of the northern pike (*Esox lucius*) and as a plerocercoid in the flesh of a variety of salmonid fish (Kuperman, 1973). There is little information on mortality of fish naturally infected with this parasite (Michajlow, 1962), but recent studies showed that plerocercoids of *T. crassus* killed experimentally infected whitefish (*Coregonus clupeaformis*) fry (Dick and Rosen, 1982; Rosen and Dick, 1983a). Interestingly, *T. crassus* has not been found in rainbow trout in Canada, but is reported as a parasite of *S. irideus* in the USSR by Kuperman (1973). The ability of *T. crassus* to infect several species of trout in the USSR (Kuperman, 1973) and lake trout (*Salvelinus namaycush*) in North America (Margolis and Arthur, 1979) suggested that rainbow trout might be susceptible to infection with *T. crassus*. Furthermore, stocking of rainbow trout may be into waters containing both pike and coregoniids. The objectives of this study were to determine the suitability of rainbow trout as a host for *T. crassus* and to assess the pathology and mortality of this experimental host.

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MATERIALS AND METHODS

Gravid adults of *T. crassus* were obtained from spawning northern pike at Falcon Lake, Manitoba (49°40'N, 95°20'W) during the spring of 1982. Specimens of *Cyclops bicuspidatus thomasi* were collected from ponds in the vicinity of Winnipeg, Manitoba, and 6-mo-old rainbow trout (group-domestic Nisqually) about 4 cm long were donated by the Rockwood Experimental Fish Hatchery, Manitoba. The methods for processing adult tapeworms, culturing coracidia, infecting *C. b. thomasi* and maintenance of fish have been described by Rosen and Dick (1983b). Trout were divided into two groups of 25 fish each and acclimated to a water temperature of 15–17 C for 5 days. One group of fish was exposed to large numbers of infected cyclopids following the methods of Rosen and Dick (1983b), while the other group was exposed to *Artemia* (brine shrimp) only. Fish were held at 15–17 C following exposure to infected cyclopids or brine shrimp and fed equal volumes of brine shrimp daily which were adjusted upon death or removal of infected fish.

Infected fish were killed at selected times (i.e., 30, 45, 49, 58, 59 and 75 days postinfection [PI]), fixed whole in Bouin's, embedded in paraffin and serially sectioned at 10 μm with a rotary microtome. Slides were stained with hematoxylin and eosin, PAS-hematoxylin (Humason, 1979) and picro-sirius red (Puchtler et al., 1973). Infected fish which died during the experiment

were necropsied and plerocercoids recovered, fixed, stained, mounted and their lengths determined.

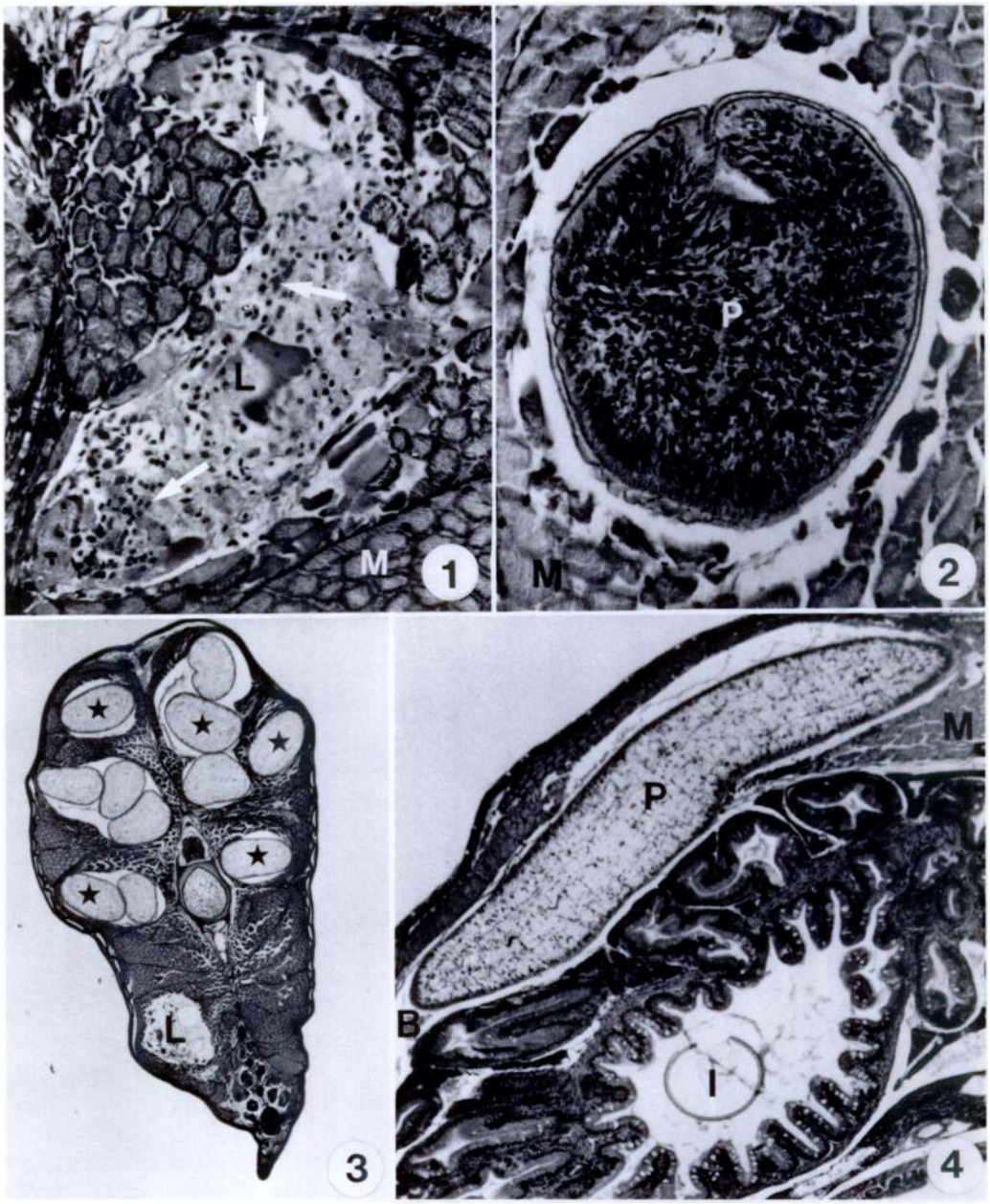
RESULTS

Positive infections were obtained in 11/25 of the exposed trout and the mean intensity of infection was 1.7 (range 1–6). Hemorrhaging (Fig. 1) created by migrating plerocercoids in the muscle was the first evidence of parasitism between Days 22 and 58 PI ($\bar{x}$ = 37 days PI). Lesions were considered to be any traumatic discontinuity in the muscle. Plerocercoids were observed in the muscle of sectioned trout by Day 30 PI (Fig. 2) creating extensive cavitation. These extensive lesions indicated that worms entered this tissue much earlier. Worms caused muscle necrosis (Fig. 1), and replaced considerable areas of muscle by Day 56 PI, having attained an average length of 45.8 ± 28.6 mm (n = 13) between Days 44 and 56 PI. Plerocercoids were wound throughout the myotomes after Day 44 PI (Fig. 3) and often penetrated into the body cavity (Fig. 4). A loss of pigmentation on one side of a trout was noted in which the plerocercoid had penetrated through the integument and into the vertebral cavity (Fig. 5), brain (Fig. 6) and gill chamber (Fig. 7). Infiltration of macrophages and polymorphonuclear leucocytes into lesions (Fig. 8) led to formation of granulomas between Days 45 and 75 PI (Fig. 9). Granulomas did not enclose the plerocercoid, and were comprised of a necrotic central core surrounded by fibroblasts. Plerocercoids were still wound through the musculature and were not enclosed in a host capsule by Day 75 PI (Fig. 3). The initiation of hook development was observed in the scolex region by Day 75 PI (Fig. 10).

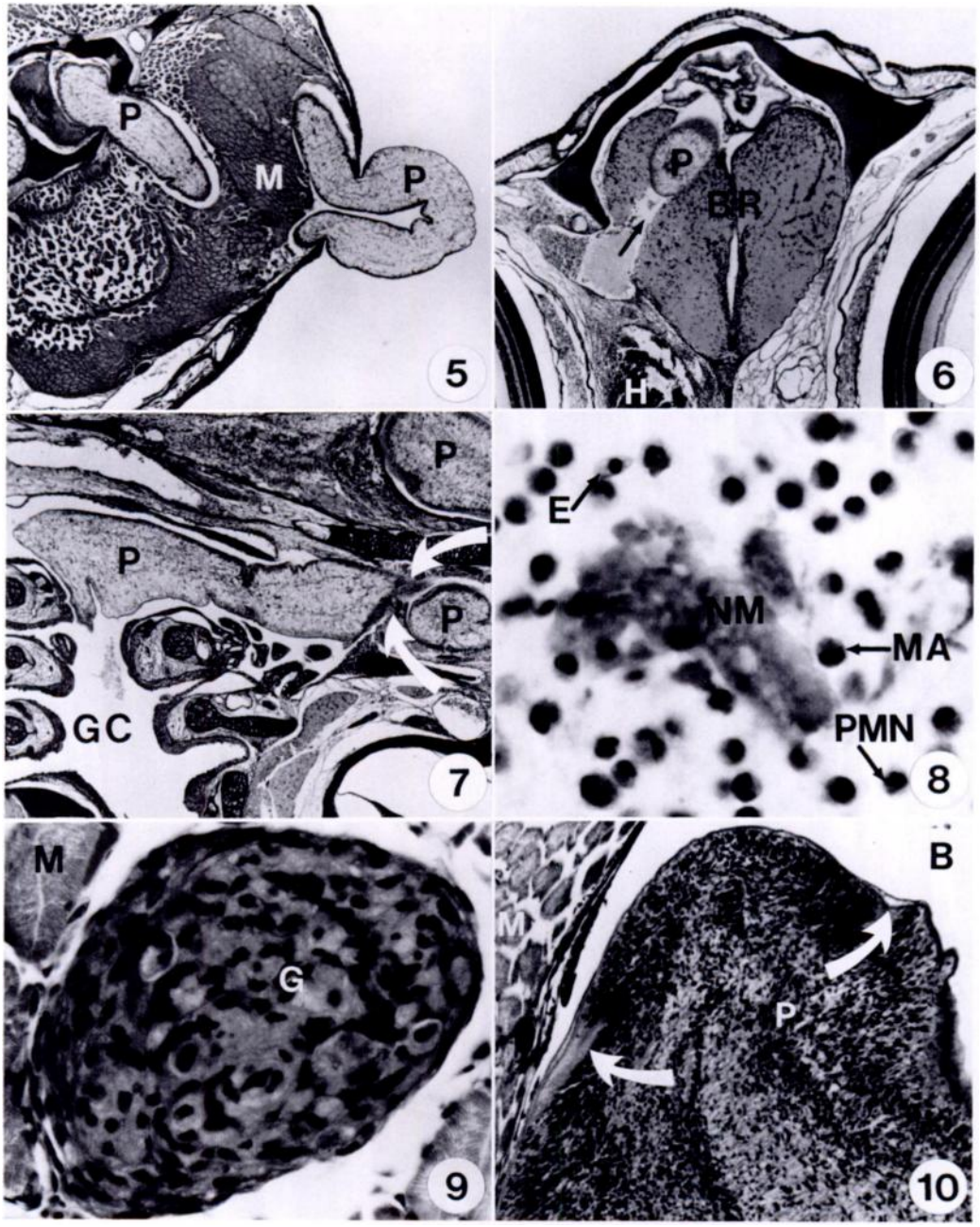
Swimming activity was reduced and a loss of equilibrium was evident for fish after Day 40 PI. Mortality of infected fish was first noted at Day 44 PI, and by Day 56 PI, 45% of infected trout died. The majority of dead trout had plerocercoids penetrating their integument. There was no evidence of secondary bacterial or fungal infections. Furthermore, there was no mortality of control fish during the experiment, and infected fish did not appear to be smaller than control fish.

DISCUSSION

This study has clearly shown that plerocercoids of *T. crassus* become established and develop in rainbow trout. Complete differentiation of plerocercoids (i.e., fully-formed hooks) was not observed in trout, but this may be due to the age of infections (maximum 75 days) since fully-developed plerocercoids in whitefish fry were occasionally found as late as Day 100 PI at similar temperatures (Rosen and Dick, 1983a). The host response of trout to *T. crassus* during the early migration of the worm in muscle was similar to that in whitefish (Rosen and Dick, 1983a), but hemorrhaging was later in trout ($\bar{x}$ = 37 days PI) than in whitefish ($\bar{x}$ = 25 days PI). Extra-muscular positions were more frequently occupied by plerocercoids in trout than in whitefish where epaxial and hypaxial muscles were the usual site (Miller, 1952; Rosen and Dick, 1983a). Plerocercoids in trout did not coil upon themselves and were not surrounded by a host capsule at Day 75 PI, although this was the usual situation in whitefish (Rosen and Dick, 1983a). Similarly, Kuperman (1973) found unencapsulated plerocercoids of *T. crassus* in salmon and *T. orientalis* in the Amur sleeper (*Perccottus glehni*). These findings corroborate other experimental studies in which the host response to a particular species of helminth varied with the species of fish (Bylund, 1972; Sommerville, 1981). The high mortality of infected trout was similar to whitefish fry mortality (Dick and Rosen, 1982; Rosen and Dick, 1983a). Mortality occurred during the same period of time in trout and whitefish (i.e., 1.5–2 mo PI), and coincid-



FIGURES 1–4. 1. Lesion (L) in muscle (M) at Day 45 PI. Note extensive hemorrhaging (arrows) into lesion; hematoxylin and eosin (H&E), ×220. 2. Plerocercoid (P) in muscle (M) at Day 30 PI; H&E, ×214. 3. Single plerocercoid (stars) wound throughout muscle in post-anal region of trout at Day 75 PI. Note lesion (L) in hypaxial muscle; H&E, ×14. 4. Plerocercoid (P) reentering body cavity (B) from muscle (M) at Day 45 PI: I = host intestine; H&E, ×55.



FIGURES 5–10. 5. Sections through a single plerocercoid (P) at Day 58 PI. Note worm (P on left of photograph) in vertebral cavity, muscle (M) and breaking through integument; H&E, ×26. 6. Same plerocercoid (P) as in Figure 5 penetrating into brain (BR). Note lesion in brain (arrow) and extensive hemorrhaging (H) adjacent to left eye; PAS-hematoxylin, ×32. 7. Same plerocercoid (P) as in Figures 5 and 6 penetrating into gill chamber (GC) from muscle (arrows). Note hemorrhaging associated with worm in muscle at top of figure; PAS-hematoxylin, ×35. 8. Macrophages (MA), polymorphonuclear leucocytes (PMN) and erythrocytes (E) in muscle lesion shown in Figure 3. Note necrotic muscle cells (NM); H&E, ×648. 9. Granuloma (G) in muscle (M) at Day 45 PI: H&E, ×478. 10. Area of developing hooks (arrows) in scolex region of plerocercoid (P) at Day 75 PI. Note plerocercoid is in body cavity (B) rather than in muscle (M); H&E, ×134.

ed with the large increase in plerocercoid size at this time. The mortality observed during the muscle phase of *T. crassus* in experimentally infected trout differed from Kuperman's (1973) interpretation that plerocercoids of *T. crassus* were not harmful to the host.

The extra-muscular sites of plerocercoids, lack of capsule formation and high mortality of rainbow trout infected with *T. crassus* are probably indicative of an abnormal host that plays little or no role in natural transmission of this parasite. But, the severe lesions and death attributed to even very low levels of infection (one to three plerocercoids) in small rainbow trout clearly established the importance of *T. crassus* as a potential pathogen. It is not known whether or not larger rainbow trout (50–100 g and greater) would be susceptible to *T. crassus*. Nevertheless, in light of this study, stocking of rainbow trout in aquatic systems known to have *T. crassus* should be carefully considered.

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