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HOST TISSUE RESPONSE FOR TROUT INFECTED WITH DIPHYLLOBOTHRIUM CORDICEPS LARVAE

Terry N. Otto and Richard A. Heckmann

ABSTRACT.—Diphyllobothrium cordiceps (Leidy 1871) plerocercoids are present as enzootic parasites in the viscera and skeletal muscle of cutthroat trout (Salmo clarki) from Yellowstone National Park, Wyoming. Eight cutthroat trout from the Yellowstone River were examined by histological technique and scanning electron microscopy to determine the response of host tissue to the presence of diphyllobothriid larvae. All fish sampled contained encysted plerocercoids on the serosa of the pyloric caeca and intestine. In addition to infection of the exterior of the alimentary tract, muscle tissue was infected in two of the fish sampled, whereas one fish had infection of the liver, spleen, and testis. Intact plerocercoids were encapsulated with connective tissue that was infiltrated with lymphocytes and macrophages. Granulomatous tissue that was fibrotic was also present. Pancreatic tissue was displaced in infections associated with the alimentary tract. The liver showed general necrosis with edema, and the spleen demonstrated a reduction in cellularity and increased connective tissue. Testicular tissue compressed by an adjacent plerocercoid appeared to be in an otherwise normal stage of development. Necrotic myofibrils near encapsulated parasites were separated by edema and fatty infiltration. A brief morphologic description of D. cordiceps is presented. In general, D. cordiceps did not appear to produce a serious debilitation of cutthroat trout.

The plerocercoids in the life cycle of Diphyllobothrium cordiceps are enzootic in salmonids of Yellowstone National Park (YNP) and lakes of the Northern Rockies. This parasite, found primarily in cutthroat trout (Salmo clarki), has been reported in rainbow trout, brook trout, brown trout, and grayling (Post 1971). After the initial discovery by Hayden (1872), the diphyllobothriid plerocercoids of YNP began to receive a great amount of attention and study. The history of D. cordiceps epitomizes the taxonomic confusion that is prevalent for the plerocercoid (metacestode) stage of Diphyllobothrium.

Presence of diphyllobothriid larvae in trout of Yellowstone Lake was first recorded during Hayden's 1872 scientific exploration of Yellowstone Park (Hayden 1872). Plerocercoids were sent to Leidy, who referred the forms to Dibothrium cordiceps (=Diphyllobothrium cordiceps). Leidy's determination of Diphyllobothrium larvae present in Yellowstone trout was later verified by Linton (1891a, 1891b). Due to a high incidence of infection, public and scientific interest persists concerning D. cordiceps in trout of Yellowstone Park (Post 1971). The aesthetic appearance of infected fish has been of major concern to YNP biologists. Currently the number of plerocercoid infected trout that have been discarded by fishermen in YNP is estimated to be approximately 300 fish per month (Varley 1978).

Concerning the cestode from infected YNP fish, Skinker (1933) made a comparative study and reported that D. cordiceps and D. latum were synonomous. Since D. latum was known to be pathogenic for man (Davis 1953), more definitive research was necessary. To determine whether a public health danger was present, Woodbury (1935) ingested in two trials plerocercoids from Yellowstone trout. He concluded that humans were unsuitable hosts when no viable eggs of helminth parasites were found after administering an anthelmintic to himself. Meyer and Robinson (1963) evaluated Scott's (1955) redescription of D. cordiceps as inadequate for comparative studies, and then stated that D. sebago resembled D. cordiceps.

Confusion concerning the taxonomic status of D. cordiceps and other members of the genus still exists. The problem was appropriately summarized by Stunkard (1965), who quoted a colleague as saying, "The problem is one that might lead a respectable taxonomist to give up and to go into molecular biology."
The life cycle of *D. cordiceps* for YNP is similar to other *Diphyllobothrium* species. The definitive hosts were listed by Scott (1935) as White Pelican (*Pelecanus erythrorhynchos*), California Gull (*Larus argentatus*), and American Merganser (*Mergus merganser americanus*). Eggs are passed from the definitive host's feces into the water, where they hatch into coracidia. First intermediate host crustaceans ingest the coracidia. The crustacean host has not been identified and was once thought to be *Diaptomus* (Post 1971). Recent work implicates *Eucyclops agilis* (Kingston et al. 1980) as a first intermediate host, but the results are under scrutiny. The infected crustaceans are eaten by fish, the second intermediate host, which passes the developed plerocercoid to piscivorous birds.

Histopathogenesis has not been described for *D. cordiceps* larvae in cutthroat trout of YNP. However, there are pathological descriptions of other diphyllobothrid plerocercoids in trout. For example, *Diphyllobothrium sebago* does not encyst but penetrates the heart and liver in brook trout, causing extensive hemorrhage (Hoffman and Dunbar 1961). *Diphyllobothrium dendriticum* produces edematous granulation tissue in the body cavity with a severe anemia (Hickey and Harris 1947).

The two objectives of this work are to assess the tissue damage in YNP cutthroat trout by *D. cordiceps* plerocercoids and to provide a brief morphological description of *D. cordiceps* larvae at the light and electron microscope levels.

Materials and Methods

In May 1979, four cutthroat (*Salmo clarki*) were taken by line from each of two locations, Fishing Bridge and Le Hardy Rapids, on Yellowstone River, which drains from Yellowstone Lake, Yellowstone National Park, Wyoming. Plerocercoids (metacestodes) were excised from muscle and viscera and fixed in 10% neutral buffered formalin for light microscopy. Glutaraldehyde was used to fix plerocercoids for scanning electron microscopic (SEM) studies of the larvae.

Approximately 50 tissue samples were processed according to standard methods (Humason 1972) for light microscopy (LM). The samples were embedded in Paraplast, sectioned at 6 μm, and stained using Harris hematoxylin (H & E), Giemsa (G), and Mallory's trichrome (MT). Periodic-acid Schiff reaction (PAS) was used to demonstrate mucopolysaccharides (Galigher and Kozloff 1971, Humanson 1972). Cytological and histological structures of trout tissue, as outlined by Anderson (1974), were observed and pathological changes recorded. The morphometric data for *D. cordiceps* were recorded from sections of plerocercoids in five observations of 10 different sections.

Sections of host tissue containing cestode larvae were fixed with 3% buffered glutaraldehyde followed by standard dehydration methods. The samples were mounted on a specimen holder, coated with gold in a CS minicoater sputter apparatus, and viewed with an AM Ray 1000 A high resolution SEM operating at 10 kv. Micrographs were taken at varying magnifications. Permanent data on the micrographs are registered with a digital data keyboard entry system attached to the AM Ray SEM. On each micrograph the KV, magnification, micron bar, plate number, laboratory location, and specimen code (PLER CES) are printed at the bottom.

Results and Discussion

Cysts were found in all cutthroat trout sampled. The majority of the cysts were attached to the alimentary tract (Fig. 1). However, 3 fish had infections of other organs.
Both liver and spleen in one fish were infected with diphyllobothrid plerocercoids, and another individual trout had a plerocercoid infection of the testis and muscle adjacent to the kidney. In a third instance a single plerocercoid was observed encysted in the hypaxial muscle. Cysts varied from white to light brown and were approximately 2 mm in diameter and 1–2 mm thick. Cysts were either in intimate contact or were attached to the serosa of the alimentary tract by a pedunculated string of connective tissue (CT). The plerocercoid infection of the testis was a peritoneal attachment, and those of the muscle, liver, and spleen were next to the parenchyma. Examination of cysts using histologic technique showed some cysts to contain larvae, whereas other cysts appeared to have degenerating parasites, presumably *D. cordiceps*, with granulomatous tissue present.

The reaction of cutthroat trout to infection with *D. cordiceps* was a multicellular connective tissue chronic inflammatory response. Plerocercoids were encapsulated by dense CT (Figs. 2 and 3) and infiltrated predominantly by lymphoid cells with occasional macrophages. For some plerocercoid infections a short acute phase characterized by congestion, edema, and hemorrhage is usually followed by the longer lasting chronic phase typified by CT encapsulation (Cosgrove 1975).

A characteristic granulomatous inflammatory response was present around apparent degenerative parasites with lymphocytes, macrophages, and epitheloid cells as demonstrated by G and MT stains. PAS positive cells were usually found in the interior milieu and may be cells of the mononuclear phagocyte system.

Chronic inflammation leading to fibrosis is characteristic in many diseases of fish and results in the encystment of a parasite as a method of isolating an irritating agent (Cosgrove 1975, Hauck 1977, Heckmann and Jensen 1978). Many of the sections that were prepared had a pronounced affinity for aniline blue of MT, which indicates an active fibrosis in capsules surrounding *D. cordiceps* (Arne and Owen 1967, 1969).

Encapsulation of a parasite removes it from many of the host defense mechanisms, thus reducing parasitic stress to some degree. Attempts by the host to remove or resolve the parasitic infection may continue within the capsule.

In long-term infections fibrous capsules can become calcified, resulting in death of the plerocercoid (Sweeting 1977). No evidence of calcification was observed in this sample or in previous research on *D. cordiceps* by other authors.

Intestine and pyloric cecum adjacent to the cyst had an edematous muscularis externa, and degenerative adipose, displacement of pancreatic tissue, and occasional monocellular infiltration of the muscularis mucosa and externa (Fig. 2). Fibrosis of adipose and pancreatic tissue appeared to be more extensive near cysts with degenerating parasites than those with intact larvae.

There seemed to be no reaction in the intestinal mucosa related to the presence of cestode larvae on the serosal surface, which might interfere with digestion. In one instance there was hyperplastic CT around a submucosal cyst that extended into the intestinal lumen. The cause of the cyst was not apparent. The cyst was also accompanied by a disruption of the stratum compactum and inner circular muscle layer of the muscularis externa. The injury to the mucosa was localized and minor.

Only one instance of gonadal infection was observed in a male cutthroat trout. The CT of the tunica albuginea was hyperplastic in close proximity to the cestode larva. The capsule was typically infiltrated with lymphocytes and macrophages. A major effect

![Fig. 2. Fibrosis of adipose and pancreatic tissue (A) with edema of pyloric ceca (PC) near *Diphyllobothrium cordiceps* larva (L) in connective tissue capsule (arrow). H & E X 25.](image-url)
was the compression of the seminiferous tubules (Fig. 4). In spite of the compression, germinal tissue in the seminiferous tubules appeared to be in a normal stage of development as compared to Henderson's (1962) description of brook trout testis. Gonadal retardation in fish has been caused by other pseudophyllidean larva (*Ligula intestinalis*), which produced an atrophic condition where it was difficult to separate reproductive tissue from mesenteries (Arme 1968). Since Woodbury (1935) stated that sterility of trout could result from a *D. cordiceps* infection, a more thorough investigation of gonadal infection rate and possible effects on germinal tissue and gonadotropic hormones should be pursued.

Skeletal muscle was not extensively infected in this sample, as only two of eight fish had noticeable encysted larvae. Effects of plerocercoid larvae in muscle produced degeneration and necrosis of nearby myofibrils, fatty infiltration, and edema. Mononuclear infiltration of the adipose and CT was concomitant. In one fish, larvae encysted near kidney tissue produced an edematous condition, interruption of the epithelial limiting membrane, and hypertrophy of the renal organ. Cells of the kidney appeared to be in a normal condition. The necrosis present in muscle sections studied would probably not produce a serious debilitation of the trout.

A single infection of liver by a *Diphyllolbothrium* plerocercoid induced necrosis of hepatocytes (Fig. 5). Melanomacrophages, lymphocytes, and tissue macrophages were present, indicating active phagocytosis of dead host tissue and parasite by-products. Connective tissue infiltration, localized fibrosis, into the sinusoids was also prevalent. The plerocercoid was intact and did not appear degenerative.

Hepatocyte necrosis is not common in many parasite infections (Cosgrove 1945), but in severe infections general necrosis may occur (Hauck 1977) with extensive damage (Stromberg and Crites 1974). As long as the infection persists, fibrous scarring and cirrhosis might result in reduced liver function. Obstruction and portal hypertension may occur when sinusoids are compressed or occluded (Stromberg and Crites 1974).

Fig. 3. Plerocercoid of *Diphyllolbothrium cordiceps* (L) partially removed from connective tissue capsule (C).
One instance of spleen infection by plerocercoid larvae produced a compression of the spleen but no necrosis. The spleen appeared edematous, with a decrease in germinal center size and cellularity and an increase of CT in the stroma. There was an abundance of melano-macrophages distributed fairly evenly throughout the spleen. Erythropoiesis is an important function of the spleen (Lane 1979). Therefore, damage to the spleen, whether partial or total, could lead to anemia and accompanying reduced vigor. Depending upon the incidence of spleen infection in the cutthroat trout of YNP, damage to the erythropoietic centers in the spleen may be an important factor contributing to any observed ill-health of infected fish (Ellis 1979).

The histopathogenesis of *D. cordiceps* larvae in YNP cutthroat trout did not appear to be gravely debilitating. The chronic inflammation associated with *D. cordiceps* plerocercoids is typical of such a parasitic infection. The amount of damage to infected viscera was minimal except perhaps in the case of spleen and liver, both of which had structural changes that could be detrimental to the health of the trout. The infection appears to be long term, and a decrease in vigor of infected fish may result from the presence of large numbers of *D. cordiceps* larvae.

Little attention has been given to numbers of *D. cordiceps* larvae in cutthroat trout. Scott (1935) recorded approximate plerocercoid populations ranging from 50 or more in each fish. Heckmann (1971) observed over 400 plerocercoid larvae in one cutthroat trout. Extensive studies have not been recorded elucidating effects of sublethal infections of *D. cordiceps* larvae on the health of cutthroat trout, either. Histologic examination of *D. cordiceps* in the viscera and muscle did not appear to cause lesions that would be damaging to the point of impairment, except in the case of liver and spleen infection.

Woodburry (1935) stated that the physiological well-being of cutthroat trout infected with *D. cordiceps* larvae could be compromised by reduced vitality or sterility. Parasitism by other pseudophyllidean larvae produced poor condition, reduction in size, and anemia in host fish (Arne and Owen 1969, Hickey and Harris 1949, Mahon 1976, Pitt and Grundman 1957). Although not seen in our sampling period, Linton (1891) described heavily infected fish of Yellowstone Lake as being emaciated. He also observed erratic swimming of infected fish, which has also been seen in recent years (Post 1971) in YNP.

The major impact of YNP cutthroat trout infected with *D. cordiceps* seems to be aesthetic. Response of fishermen has been to discard infected trout. In July 1959, 7500 uncleared trout were found in garbage cans of YNP. This same survey was duplicated in 1978, and a monthly estimate of discarded trout for the month of July was approximately 300 (Varley 1978). A decrease in discarded trout was probably due to a change of legal size limit in which only younger fish were kept. Presumably the younger fish would have a lower worm burden and would be more acceptable to sportmen (Varley 1980).

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**Fig. 4.** Compression of testis by encysted *Diphyllobothrium cordiceps* larva with fatty infiltration and edema. H & E X 25.

**Fig. 5.** Necrotic hepatocytes with fibrosis adjacent to capsule (C) of cestode larva. H & E X 25.
The value of histologic features of *Diphyllolothrium* plerocercoids for taxonomic purposes was cited by Halvorsen (1970), Kuhlow (1953), and Meyer (1963). Some morphological characteristics often used in the study of diphyllobothriid larvae are length of microtrichia, thickness of the integument, and longitudinal parenchymal musculature. These and other histologic features are shown in Figure 6.

Length and morphology of microtrichia have been used for taxonomic purposes both at the LM (Halvorsen 1970) and SEM (Anderson 1975) levels. The length of microtriches (Table 1) was shorter than some other species of *Diphyllolothrium* (Halvorsen 1970). The measurement of microtriches was hampered since they do not lie flat, nor was the base properly seen in histological sections.

In the SEM microphotographs of the surface topography for *D. cordiceps* larvae, microtrichia appear slender (Fig. 7) and somewhat densely arranged. The scolex region is contracted with bothridia visible as a shallow groove with a decreased number of microtrichia. The integument of *D. cordiceps* larvae had shallow folds. *Diphyllolothrium* integument has active metabolic properties (Arme 1966, Von Bonsdorff et al. 1971), and PAS staining showed presence of mucopolysaccharides in the integument. No PAS reaction was seen in the plerocercoid environment precluding possible alkaline phosphatase activity by *D. cordiceps* larvae (Moog and Wenger 1952). The integument was approximately 8.6 μm thick (Table 1). The longitudinal parenchymal musculature (PM) was approximately 88.1 μm. The longitudinal parenchymal musculature may vary in thickness in various parts of the body of diphyllobothriid plerocercoids (Halvorsen 1970). Therefore, due to the fact that the plerocercoids were sectioned in situ, the morphometric data for the longitudinal parenchymal muscle may require further study on relaxed whole larvae.

Earlier in the history of *D. cordiceps*, the larvae were believed to be similar to *D. lataum* (Skinker 1933). It is evident from the morphometric data supplied by this report that *D. cordiceps* does not resemble *D. lataum* since the latter has considerable differences in morphology (Halvorsen 1970). Comparisons of histologic structures of *D. cordiceps* larvae can be made with other *Diphyllolothrium* plerocercoids to assist in establishing the identity of *D. cordiceps* in cutthroat trout of YNP.

**Acknowledgments**

We thank the National Park Service at Yellowstone National Park for their help on this project and James Allen of the Brigham Young University Electron Optics Laboratory for his help with the scanning electron microscopy.

**Literature Cited**


**Table 1.** Measurements of anatomical features of *Diphyllolothrium cordiceps* larvae. Mean ± S.E. and range are given in μm.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Mean ± S.E.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of microtrichia</td>
<td>8.2 ± 0.6</td>
<td>(4.0 – 20.0)</td>
</tr>
<tr>
<td>Thickness of integument</td>
<td>8.6 ± 0.3</td>
<td>(4.0 – 14.0)</td>
</tr>
<tr>
<td>Thickness of longitudinal parenchymal muscle</td>
<td>88.0 ± 11.8</td>
<td>(24.0 – 247.0)</td>
</tr>
<tr>
<td>Width of sections</td>
<td>606.9 ± 21.5</td>
<td>(400.0 – 1030.0)</td>
</tr>
</tbody>
</table>

Fig. 6. Longitudinal section of *Diphyllolothrium cordiceps* larva showing some morphological features: microtrichia (M), integument (I), outer parenchyma (OP), longitudinal parenchymal muscle (PM), and inner parenchyma (IP). H & E X 100.
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Fig. 7. Microtrichia appear slender and densely arranged.


Lane, H. C. 1979. Some haematological responses of normal and spleenectomized rainbow trout (Olmio gokdneri) to a 12% blood loss. J. Fish Biol. 14:159-164.


