

DIPHYLLOBOTHRIMUM CORDICEPS:  
A TAPEWORM PROBLEM IN YELLOWSTONE LAKE FISHES  
NEW INVESTIGATIONS INTO THE LIFE CYCLE

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Objective

Diphyllbothrium cordiceps (Leidy, 1872) has been known from Yellowstone Lake fishes since 1872. Leidy described and named the species Dibothrium cordiceps from poorly preserved larval (plerocercoid) material collected from native trout, Salmo mykiss (= Salmo clarki) by members of the Hayden Expedition. The larval tapeworm has been reported only from cutthroat trout, Salmo clarki, brown trout, Salmo trutta, brook trout, Salvelinus fontinalis, and grayling, Thymallus arcticus, chiefly from Yellowstone Lake and adjacent waters in the northern Rocky Mountains. Simms and Shaw (1939), found D. cordiceps outside the Rocky Mountain area in brook trout from Elk Lake, western Deschutes County, Oregon.

Linton (1891 A & B) found and identified the adult stage of this tapeworm in the white pelican, Pelicanus erythrorhynchus. Also adults of Diphyllbothrium cordiceps have been found naturally infecting the California Gull, Larus californicus, the American merganser, Mergus americanus, and bears, both black, Ursus americanus, and grizzly, Ursus arctos, in Yellowstone Park (Scott, 1935). None of the other piscivorous birds or mammals from the area, e.g., the double-crested cormorant, Phalacrocorax auritus, the Caspian tern, Hydroprogne caspia, river otter, Lutra canadensis, is known to harbor this tapeworm.

The usual life cycle of a diphyllbothriid cestode involves three hosts: a piscivorous vertebrate (the primary, or definitive, host) in which the worm develops to sexual maturity, a copepod (1st intermediate host) which ingests the ciliated coracidium; the latter hatched from an egg passed out of the definitive host to develop externally in water. In the body of the copepod, the coracidium metamorphose into the proceroid. The infected copepod in turn is ingested by a fish (the 2nd intermediate host) where it develops into an elongate, vermiform parasite, the plerocercoid. If this infected fish is eaten by another fish, the plerocercoid may transfer to that host without further development. If the infected fish is eaten by a warm-blooded host of suitable species, the cycle is completed with the transformation of the plerocercoid into a sexually mature worm.

A) Most interest in this tapeworm has been directed towards the "wormy" fish first noted by Carrington in 1871 and described and identified in 1872 by Leidy. Hayden (1872) remarked that there was but one species of fish in the lake, trout weighing 2-4 lbs, with most of them infected with a peculiar intestinal (sic!) worm. These larval worms (plerocercoids) were among the intestines of trout and interlaced in the flesh, the largest being about 15 cm (6 inches) in length. Some of the worms were small and were in cysts clinging to the intestines. When pressed between the fingers they broke open and small worms emerged, in appearance similar to the larger worms. From 5-6 up to 40 or 50 parasites were found in a trout, the larger ones being in solid flesh through which they work causing the flesh to become almost putrid. Infected fish were detected by their appearance; the trout frequently were poor in flesh, dull colored, and they swam lazily though they fed voraciously.

B) Beginning in the 1920's, considerable thought and effort were directed towards the elucidation and completion of the life cycle with the aim of reducing the numbers of infected fish. Ward (1922) suggested reduction of pelican numbers but not the elimination of the birds from the park. He hoped this would indirectly reduce the numbers of infected fish by decreasing the number of infected copepods which would be exposed on a lesser basis to hatching tapeworm eggs. Ward apparently was unaware of the role of gulls and bears in the life cycle and their role in tapeworm egg production. Hall (1931) studied infections in pelicans and treated some of these birds with an anthelmintic. He considered that treatment of the definitive host would lessen the incidence of infection in trout. Hall also raised the question of determining the 1st intermediate host and suggested that copepod invertebrates may be involved. Woodbury (1931) thought a copepod species of the genus Diaptomus might be a likely 1st intermediate host owing to the large numbers of Diaptomus found in the stomachs of trout. Woodbury (1931) also treated a pelican with an anthelmintic and then reinfected the bird by feeding it heavily infected trout and grayling.

Scott (1935) and his associates, Honess and Simon, collected plerocercoids from Yellowstone fishes (cutthroat trout, grayling, and brook trout). Further plerocercoids were collected from Yellowstone Lake cutthroat trout by Crosby in 1970.

All previous workers have been unsuccessful in clarifying the development of the tapeworm embryo, the egg-hatching process and release of the coracidium and the identification of an intermediate host. In this study we have gathered and evaluated new information related to the foregoing topics and these data are now incorporated in this paper. In addition, natural infection in cutthroat trout and experimental infection of the dog, cat and hamster with adult worms are included in this study.

#### Methods

D) Adult *Diphyllbothrium cordiceps* were recovered by necropsy from 2 of 3 pelicans found dead on Sandy Island of the Molly Islands in 1978-79. Terminal, gravid proglottids from eight live worms recovered from one pelican were removed from the worms and placed in lake water and refrigerated at 4°C. The remainder of the worms were relaxed and killed in hot (60°C) tapwater and fixed and preserved in formalin. Fragments of worms bearing eggs were recovered from a second dead pelican and this material was also stored in lake water and refrigerated until necropsied. Plerocercoids in the trout from the muscles, body cavity and those recovered by dissecting visceral cysts were counted and their position in the fish noted. The worms were then either relaxed, killed and fixed, or maintained alive in physiological saline for feeding experiments to putative, other intermediate or definitive hosts.

The dog and cat used in the experimental feedings were maintained on standard dog and cat chow or, in a later experiment, fed a pork-beef liver and/or heart diet, exclusively. Their feces were determined negative for tapeworm eggs by screening (final screen 53 µm), sedimentation and microscopic examination of the sediment prior to the experiment and checked at intervals after feeding of plerocercoids from naturally infected cutthroat trout. Prepatent periods and preliminary indication of the duration of infection in these hosts were established by these means. Adult tapeworms were recovered by necropsy from the intestines of the dog and cat following euthanasia. The adult worms were treated as noted previously. Hamsters were maintained on standard laboratory rat chow, in addition to some cracked grain. They were lightly anaesthetized and exposed per os by stomach tube to plerocercoids recovered from naturally infected cutthroat trout.

Feces were screened and eggs sedimented from this host, and the prepatent period and duration of infection determined. The hamsters were euthanatized and necropsied at various intervals after exposure for the recovery of adult tapeworms which were treated as previously described.

Plankton were collected in June, July and August from Yellowstone Lake using a hand-held Wisconsin Style Plankton sampler (18 x 76 cm) from the surface down to about 15 cm below the surface. The tow from a canoe usually lasted about 15 min. Plankton were flushed from the collecting bucket with lake water into 10 liter plastic bags containing about 2-3 liters of lake water. The bags were either breath-inflated or inflated with O<sub>2</sub> and the top of the bag was doubled over and secured with a rubber band. The bags were placed in an ice chest with ice until they were returned to the laboratory where the collections were transferred to aerated aquaria in a constant temperature chamber at about 10-12°C (45-55°F). Some of the copepods collected (*Eucyclops agilis*) were transferred to aerated aquaria at room temperatures about 21°C. Food in the form of strained hay infusion was provided as needed, indicated by the clearing of the aquarium as the food was consumed. Some of the copepod cultures

were fed Daphnia sp.

Tapeworm eggs from pelican feces and from gravid proglottids were used in incubation experiments. Three such experiments were conducted with the eggs from the pelican; the first, with eggs stored about 25 days; the second, with eggs stored about 150 days; and the third with eggs stored for about 250 days. In the first two experimental incubation trials, the eggs were exposed to two temperature regimes, 10C and 21C. In the final incubation experiment, eggs were incubated only at the higher temperature. When removed from refrigeration they were placed in 75 ml flasks containing about 50 ml of lake water and were agitated (180 strokes/min) and aerated. Development was followed by removing a sample of eggs at intervals and examining them microscopically. Photomicrographs of eggs and other stages were made using color transparency film in a 35 mm slr camera mounted on a Zeiss Research Microscope. Following development, and on hatching of the eggs, the contents of the flasks were centrifuged at 1000 RPM and the pelleted material was pipetted from the tubes into well slides. Copepods were collected from the aquaria using a 60 ml syringe to withdraw some of the bottom contents. This material was expressed into a petri dish in small amounts and examined under the stereomicroscope. Copepods were individually pipetted from the petri dish into the well of the slide containing coracidia. Usually, about 6-10 copepods were added to a single well containing 20-25 coracidia. Three such well slides, each with 2 well containing copepods, coracidia, and some unhatched eggs were placed in a moist chamber consisting of a folded piece of moistened Whatman filter paper in the lid of a 15 cm petri dish covering the slides in the bottom of the dish. These preparations were held at room temperature for as long as 24 hrs. At the end of this period, some of the copepods were examined microscopically for evidence of infection. The remaining copepods were transferred to lake water in 5 cm plastic petri dishes and returned to the moist chamber. Copepods were examined daily following exposure. Procercoïd stages, seen in situ or broken out of the copepod when it was slightly crushed, were photographed in the same manner used for the eggs.

Hatchery reared cutthroat trout and laboratory reared grayling were fed experimentally infected copepods following various periods of incubation. The fish were maintained for various periods of time and then necropsied for evidence of infection.

## Results

### Eggs

Unembryonated eggs of D. cordiceps are small, ovoidal, operculate and possess a knob or boss on the abopercular end. They contain a number of cells and vitelline granules. Measurements of fecal eggs and eggs from tapeworm proglottids in the pelican are given in Table 1.

The embryo (coracidium), as it develops within the egg, first appears as a spherical mass with an outer limiting boundary, the ciliated embryophore, though no ciliary action was seen in the unhatched embryo. Various other features of the developing embryo were apparent by day 14 of incubation, the three pairs of embryonic hooks being the most notable. No flame cell activity was noted in unhatched or hatched embryos. Eggs hatched following 15-22 days of incubation, depending on temperature. Results of the three incubation experiments are summarized in Table 2. Some incubated, fully-developed, unhatched eggs were held at 10C without further agitation for up to 35 days before being exposed to light and allowed to hatch.

### Hatching

After short to long exposures to bright light, eggs appeared to hatch spontaneously. There was no indication when a particular egg was about to hatch, except, in some instances a slightly loosened operculum was noted just before hatching. The first event in hatching was the sudden release of the opercular cap which opened and extended perpendicularly. The coracidium, still retained within the vitelline membrane, emerged through the opercular opening. Almost immediately the coracidium escaped, rapidly swam away, turning and spinning. Vitelline granules and other material streamed from the hatched egg coincident with the vitelline membrane collapse, following escape of the coracidium. A species of Stylonichia, a ciliated protozoan much larger than the coracidium, was observed feeding on the egg contents following the escape of the coracidium. Coracidia were never observed to attract or to be bothered by such organisms. The presence of these ciliated protozoans in the lake water cultures may have been beneficial helping to keep down populations of bacteria.

### Coracidia

Free-swimming coracidia are spherical, ciliated organisms. The ciliated embryophore surrounds the pyriform hexacanth embryo. Typically the coracidium swims with the embryonic hooks located posteriorly. Swimming activity was continuous and generally at the water surface, although coracidia were seen at all levels within the water column. Coracidia were concentrated by centrifugation (1000-1500 RPM/10 min) for use in feeding experiments. Detailed studies of the structure of coracidia are still in progress.

### Infection of Copepods

Several species of Yellowstone Lake plankton organisms were returned to the laboratory. The copepod, Eucyclops agilia, grew and reproduced readily at low (10C) and ambient ca. 21C) temperatures. About 6-10 specimens, usually gravid females, of this species were exposed to

TABLE 1  
 MEASUREMENTS ( $\mu\text{m}$ ) OF EGGS OF *DIPHYLLOBOTHRIUM CORDICEPS*  
 RECOVERED FROM VARIOUS HOSTS BY VARIOUS INVESTIGATORS.

| Host          | Author                        | n  | $\bar{x}$ |       | Range       |             |
|---------------|-------------------------------|----|-----------|-------|-------------|-------------|
|               |                               |    | Length    | Width | Length      | Width       |
| White Pelican | This Study<br>Crosby<br>Scott | 24 | 68.82     | 44.76 | 57-80.75    | 41.25-52.48 |
|               |                               |    | 63.1      | 42.97 | 55.6-66.3   | 38.52-48.81 |
| Dog           | This Study<br>Crosby<br>Scott | 20 | 61.20     | 42.06 | 57.5-66.5   | 39.1-45.08  |
|               |                               |    | 61.86     | 41.61 | 55.67-67.88 | 38.52-56.26 |
| Cat           | This Study<br>Scott           | 54 | 56.74     | 37.58 | 53.2-61.6   | 36-40.0     |
|               |                               |    | 61.0      | 45.0  |             |             |
| Hamster       | This Study                    | 40 | 58.31     | 39.1  | 48.8-66.7   |             |
| Bear          | Scott                         |    | 62.0      | 45.0  |             |             |

TABLE 2.  
Development and Hatching of *D. cordiceps* eggs  
Under Variable Periods of  
Dormancy and Incubation Temperatures

| Incubation | No. Days Eggs<br>In Storage | Day Development<br>First Noted |     | Day eggs<br>.Hatched |       |
|------------|-----------------------------|--------------------------------|-----|----------------------|-------|
|            |                             | 10C                            | 21C | 10C                  | 21C   |
| 1          | 25                          | 14**                           | 8*  | 21                   | 19    |
| 2          | 150                         | 13                             | 13  | 20-22                | 16-17 |
| 3          | 250                         | -                              | -   | -                    | 15-19 |

\*Formed Body Seen

\*\*Embryonic Hooks Seen

C

concentrated coracidia in well slides for up to 24 hrs. Some of these copepods were examined microscopically at the end of this period for evidence of infection by (1) observing procercooids or embryonic hooks in situ in live copepods lightly restrained under coverslip pressure, or (2) observing procercooids expelled from ruptured copepods. The remaining copepods were examined for evidence of infection daily for the next 7 days and then on days 10, 13, 16, 18 and 20 after exposure. Infections with procercooid stages were seen in copepods examined 1-13 days after exposure. No procercooid bodies could positively be identified in copepods examined after 13 days of exposure. However, embryonic hooks were found as late as 20 days after exposure in copepods cleared in Hoyer's fluid. Almost all copepods examined during days 1-6 post exposure were infected. Subsequently, increasing numbers of negative copepods were encountered. This result likely represents a dilution effect owing to the relatively short egg-adult life cycle of this species of copepod.

Copepods which had been exposed to coracidia 17-18 days and 28-33 days previously were fed to hatchery reared cutthroat trout and laboratory reared grayling by stomach tube. The fishes were negative for infection when examined approximately 2 months following exposure.

#### Natural Infections of Fish with Plerocercoids.

A total of 66 cutthroat trout were collected from various locations in Yellowstone Lake in 1978-79. Plerocercoids recovered from these fish were preserved for study or were used in feeding experiments (vide infra) to other potential intermediate or definitive hosts. Twenty-five (64%) of 39 fish examined in 1978 were infected with a minimum of 74 plerocercoids (numerous small cystic worms were noted in some of these fish but not all were counted). In 1979, of 27 fish examined, 25 (92%) were infected with 184 plerocercoids, a mean of 7.4 plerocercoids (Table 3).

Visceral cystic worms typically were small; these cysts were primarily associated with the pyloric caeca, though some larger cysts were found in other parts of the viscera. Visceral cysts usually contained but a single plerocercoid, however, on one occasion 2 worms were recovered from a cyst and 4 small worms from another cyst. In addition to the plerocercoid, the cysts contained a tan homogeneous material similar to that seen in the normal caeca. Occasionally, a visceral cyst was empty except for this material or sometimes the cysts held, not a plerocercoid but, a nematode of the genus Bulbodacnitis.

Plerocercoids free in the body cavity were larger than cystic worms but did not reach the length of plerocercoids found in the flesh of the fish. Plerocercoids in the muscle tissue were found free (i.e., not enclosed in a cyst membrane) though they were intertwined and folded in the flesh. Careful dissection of the surrounding muscular tissue was required to free the worms.

TABLE 3.  
Incidence of Infection and Distribution of  
D. cordiceps in Yellowstone Lake Cutthroat Trout, 1978-79.

| Year | Sex | No. Fish Examined/<br>No. Fish Infected (%) | Number (%) of Plerocercoids Found in: |             |             |
|------|-----|---------------------------------------------|---------------------------------------|-------------|-------------|
|      |     |                                             | Visceral<br>Cysts                     | Body Cavity | Musculature |
| 1978 | M   | 12/9 (75)                                   | 2 ♀ (17)                              | 1 (08)      | 9 (75)      |
|      | F   | 7/5 (71)                                    | 6 (100)                               | 0 (0)       | 0 (0)       |
|      |     | 20*/11 (55)                                 | 38 (68)                               | 9 (16)      | 9 (16)      |
| 1979 | M   | 17/16 (94)                                  | 76 (68)                               | 20 (18)     | 16 (14)     |
|      | F   | 10/9 (90)                                   | 59 (82)                               | 7 (10)      | 6 (08)      |

\*Sex Not Recorded.

All of the plerocercoids remained alive, active, and infective in the refrigerated fish host for up to 8 days. Plerocercoids were collected, washed in 0.85% saline and briefly refrigerated before being fed to other hosts. Both small and large worms became very active rapidly extending and contracting the body when placed in saline.

#### Feeding of Plerocercoids to Potential Fish Hosts

A 15 cm hatchery-reared cutthroat trout was fed 10 plerocercoids per os by stomach tube. The experimental fish died 67 days later with no external signs of infection. Upon necropsy, this fish was found to be infected with 5 plerocercoids free in the body cavity, 2 other plerocercoids located retroperitoneally (1 near the kidney; 1 on the inner body wall surface near the anus), and 3 visceral cysts, attached to a pyloric caecum and 2 imbedded in the stomach wall). Four of the free plerocercoids from the body cavity and outside the peritoneum were fed per os by stomach tube to another hatchery-reared cutthroat trout. The remaining worms were fixed. No plerocercoids were recovered when the second experimental trout was examined 130 days post exposure. In another experiment, 6 small cystic worms from a naturally infected cutthroat trout were fed per os by stomach tube to a laboratory-reared grayling and 6 larger worms to another grayling. These grayling were negative for infection on necropsy 120 days post exposure.

Six other grayling have been exposed per os to small to medium sized cystic worms, to scolices only (removed from large worms) and to the bodies of plerocercoids lacking scolices. These fish have yet to be examined for evidence of infection.

#### Feeding of Plerocercoids to Mammalian Hosts

In 1978, a 6-year old, mongrel male dog and a human volunteer considered negative for tapeworm infection by fecal examination (screening) were fed a mixture of 17 small and large plerocercoids and 14 plerocercoids, respectively. Subsequent fecal examinations on days 15, 20, 25, and 30 post exposure revealed no tapeworm eggs.

In 1979, the same dog and human volunteer were fed 10 and 11 plerocercoids, respectively. Also, 2 hamsters were fed 2 plerocercoids each per os by stomach tube. The hamsters passed tapeworm eggs in their stools (collected over water) on day 9 following exposure. The human volunteer was negative on day 9 and on all subsequent examinations. Tapeworm eggs were detected in the stool of the dog on day 10 following exposure. The dog continued to pass eggs, though in decreasing numbers, for 3 days, then the stool was negative thereafter. The hamsters continued to pass eggs for 12 days following patency. At necropsy on day 21 post exposure one hamster was positive and one negative by stool examination, but no worms or worm fragments were recovered. The worms apparently had been digested and the eggs found in the feces of one hamster were considered residual eggs passed after the worm had been digested.

The same dog 32 days following the previous exposure was exposed for the 3rd time to 6 large plerocercoids in hamburger. A kitten was similarly exposed to a mixture of 8 small and large worms. Six hamsters (2 males, 2 females, and a male-female pair) were exposed per os by stomach tube; the 2 males and the 2 females received 1 long and 1 short worm each, the male-female pair received 2 small, cystic worms each. The stool of the dog was negative on days 4 and 5 following exposure; eggs were first seen in the stool of the dog on day 6 by direct microscopic examination. The dog was necropsied on day 7. Six mature intact tapeworms measured between 15-45 cm in length by approximately 1 cm distal to the pylorus. Eggs were detected in the stool of the cat on day 10 by direct microscopic examination following administration of an enema. The cat was necropsied and two mature worms, one 30 cm and the other 40 cm in length, were recovered. A male hamster necropsied on day 10 following exposure contained a tapeworm measuring 15 x 1 cm. Comparatively, a female hamster was negative. On day 11, necropsy of a female hamster produced a 7 x 1 cm mature worm. Comparatively, necropsy of a male hamster produced negative results. The remaining male hamster continued to pass eggs and was necropsied 16 days following exposure. Two worms were recovered, one 8 x 1 cm and the other 16 x 1 cm. The remaining female hamster escaped and was not recaptured. All three groups of hamsters passed eggs in their stools, beginning on day 6. The male-female pair, however, did not pass eggs until 9 days post exposure and then only a few eggs were recovered.

#### References Cited

- Hall, M.C. 1931. Kamala as an anthelmintic for removing bothriocephalids from pelicans. Proc. 141st Meeting Helminthol. Soc. Washington, In J. Parasitol. 18: 304.
- Hayden, F.V. 1872. Preliminary Report of the United States Geological Survey of Montana and Portions of Adjacent Territories. Washington: Government Printing Office. pp. 97-98.
- Leidy, J. 1872. Notice of some worms collected during Professor Hayden's Expedition to the Yellowstone River in the summer of 1871. pp. 381-382. In Hayden, 1872.
- Linton, E. 1891a. On two species of larval Dibothria from the Yellowstone National Park. Bull. U.S. Fish comm. 9: 65-79.
- Scott, J.W. 1935. On the Diphyllobothrium of Yellowstone Park. J. Parasitol. 21: 443.
- Ward, H.B. 1922. The pelicans of Yellowstone Lake. Unpub. Mss. in files of Dr. J.W. Scott.
- Woodbury, L. 1931. The development of Diphyllobothrium cordiceps (= Dibothrium cordiceps) in Pelecanus erythrorhynchus. Proc. 141st meeting Helminthol. Soc. Washington. In J. Parasitol. 18: 304-305.