5-7-1997

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DIPLOSTOMIASIS IN NATIVE AND INTRODUCED FISHES FROM YELLOWSTONE LAKE, WYOMING

Victor H. Inchaustyl, Michael Foutz l , Richard A. Heckmann l,2 , Claudete Ruas 3 , and Paulo Ruas 3

ABSTRACT.—Totals of 101 native Yellowstone cutthroat (Oncorhynchus clarki bouvieri), 27 introduced lake trout (Salvelinus namaycush), and 40 introduced longnose sucker (Catostomus catostomus) from Yellowstone Lake, Wyoming, USA, were examined for eye flukes. Metacercariae of the trematode fluke Diplostomum were in vitreous humor and/or lens of 94% of Yellowstone cutthroat trout, 92% of lake trout, and 78% of longnose sucker. Longnose sucker had 7% prevalence of infection in both lens and vitreous humor of metacercariae, while Yellowstone cutthroat trout had 3% and lake trout 8%. Diplostomum spathaceum was in lens tissue of 5% of infected Yellowstone cutthroat trout and 93% of longnose sucker; Diplostomum baeri was in vitreous humor of 92% each of infected Yellowstone cutthroat trout and lake trout. Morphological characteristics indicate that a single species infected the lens of Yellowstone cutthroat trout and longnose sucker, while another species infected lake trout. Impacts of the parasite interchange between native and introduced fishes of Yellowstone Lake, Wyoming, are unknown but should be monitored each year.

Key words: Diplostomum, metacercariae, Oncorhynchus clarki, Salvelinus namaycush, introduced fish, Wyoming.

Eye fluke disease or diplostomiasis is a parasitic fish disease caused by strigeoid trematodes (Trematoda: Diplostomatidae), primarily of the genus Diplostomum. The parasite was first reported in the New Jersey State Hatchery at Hackettstown during 1937 and 1938, where it caused considerable damage to several fish species (Palmer 1939). Thousands of rainbow trout (Oncorhynchus mykiss) blinded by Diplostomum spathaceum were destroyed at the state fish hatchery in New Jersey (Ferguson and Hayford 1941). Metacercariae of strigeoid trematodes caused similar problems in Europe (Bauer et al. 1969, Schiiperclaus 1991).

Brassard et al. (1982a) reported that the penetration of D. spathaceum cercariae into the host was directly proportional to exposure and involved chance contacts between host and parasite. There is a significant decrease in the proportion of penetrating cercariae that become established in the fish lens at a high exposure density, suggesting that many cercariae die before reaching this destination. Diplostomum spathaceum appears to reduce host survival rates. The host death rate increases exponentially rather than linearly with parasite burden under natural conditions (Anderson 1978).

Rau et al. (1979) and Pennycuick (1971) examined frequency distributions of D. spathaceum in natural fish populations and found a high parasite frequency within these populations. This may ensure that relatively few fish succumb to direct effects of the infection. The direct effect of D. spathaceum is only 1 component of rate of parasite-induced host mortality. Another component is the degree to which a parasite reduces the host’s vigor and hence increases susceptibility to predation (Anderson 1978). It has been suggested that heavily parasitized fish are preferentially taken by predators (Pennycuick 1971, Crowden and Broom 1980). Brassard et al. (1982b) showed experimentally that even light infections with D. spathaceum cercariae predispose fish to predation.

Earlier studies of the parasites of cutthroat trout (Oncorhynchus clarki bouvieri) from Yellowstone Lake reported no infections by D. spathaceum (Heckmann 1971). Subsequently, Heckmann and Ching (1987) found metacercariae of D. spathaceum in longnose sucker (Catostomus catostomus) and D. baeri in Yellowstone cutthroat trout of Yellowstone Lake. Furthermore, Dwyer and Smith (1989) found D. spathaceum in the lens of longnose sucker and Diplostomum sp. in the vitreous humor.
and retina of Yellowstone cutthroat trout. Heckmann (1994) reported *D. spatiaeum* and *D. baeri bacculentum* from Yellowstone cutthroat trout from Yellowstone Lake.

Lake trout (*Salvelinus namaycush*), first caught in Yellowstone Lake during 1993 and 1994, has the potential to upset the ecological balance of Yellowstone Lake and the surrounding area (Kaeding et al. 1996). It is a major problem for the future of the lake’s native cutthroat trout, the only native fish of the lake (Kaeding et al. 1996). Introduction of lake trout may have affected the densities of cutthroat trout, and it is probable that parasites were introduced along with the lake trout. Effects of any such parasite are unknown, as are effects of the native parasitofauna on the introduced lake trout. Dynamics of the parasitism may be important in the ultimate outcome of the invasion of lake trout into Yellowstone Lake. A recent example of the effect of parasites introduced with fish is that of the Asian fish tape-worm (*Bothriocephalus acheilognathi*) introduced to the Virgin River with infected fish from Lake Mead; these parasites had devastating effects on new fish hosts (Heckmann et al. 1986, 1987).

The purpose of our research is 2-fold. First, we discriminate the different *Diplostomum* spp. infecting fishes in Yellowstone Lake, and second, we evaluate possible interactions of parasites infecting native and introduced species.

**MATERIALS AND METHODS**

During 1994 and 1995 we gill-netted 168 specimens of 3 fish species in Yellowstone Lake, Yellowstone National Park, through the cooperation of park fisheries biologists. Fish heads were removed, fixed in buffered 10% formalin, and dissected in the laboratory. Metacercariae found in the lens and vitreous humor were

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**Fig. 1.** Metacercariae of *Diplostomum spatiaeum* found in the lens of *Oncorhynchus clarki howeri* (cutthroat trout) and *Catostomus catostomus* (longnose sucker); line drawing and light optics micrograph. H = holdfast organ, N = intestine, O = oral sucker, P = pseudosuckers, V = ventral sucker. Bar = 100μm. Magnification 100X for photo.
isolated, stained with Semichon's carmine, decolored with acid-alcohol, and whole-mounted in Permount (Sheehan and Hrapchak 1973, Lillie 1991). Drawings of metacercariae were made using camera lucida and an American Optical compound light microscope.

We recorded the following measurements from 20 metacercariae of each trematode species: body length and width, oral sucker length and width, pharynx length and width, ventral sucker length and width, holdfast organ length and width, distance from oral sucker to ventral sucker, and distance from oral sucker to holdfast organ. Data were then statistically analyzed using Number Cruncher Statistical Software.

Eyes of infected fish were fixed in buffered 10% formalin and processed by standard histological techniques (Sheehan and Hrapchak 1973, Lillie 1991). Paraffin-embedded tissue was sectioned at 4–6 μm and stained with hematoxylin and eosin, Mallory's trichrome, and toluidine blue. Slides were examined with a compound light microscope to help determine species of metacercariae present. Metacercariae were identified with available keys and descriptions (Hoffman 1967, Bauer 1985, Shigin 1986).

To further evaluate species of metacercariae, we processed samples for scanning electron microscopy (SEM) following standard techniques (Dawes 1994). Gold-coated samples were viewed with a JOEL 8400 Scanning Electron Microscope.

**RESULTS**

We collected longnose sucker, Yellowstone cutthroat trout, and lake trout at Yellowstone Lake. *Diplostomum* spp. metacercariae were found in the vitreous humor and/or lens of all 3 species. Prevalence of metacercariae was 78% in 40 longnose sucker, 94% in 101 Yellowstone cutthroat trout, and 93% in 27 lake trout. When both lens and vitreous humor were infected, we considered the result a mixed infection. Among infected fish, longnose sucker had 7% prevalence of mixed infections; 93% were...
infected by *D. spathaceum* (Fig. 1) in the lens only. Three percent of Yellowstone cutthroat trout had mixed infections, 5% were infected in the lens by *D. spathaceum*, and 92% were infected by *D. baeri* (Fig. 2) in the vitreous humor. Eight percent of lake trout had mixed infections, and 92% were infected by *Diplostomum* spp. (Fig. 3) in the vitreous humor (Table 1).

Discriminant analysis of significant morphological measurements correctly classified 83% of the metacercariae according to occurrence in lens or vitreous humor. The same statistical analysis tabulated 93% accuracy in distinguishing among metacercariae infecting lens in longnose sucker and Yellowstone cutthroat trout. Discriminant analysis had 79% accuracy in distinguishing among metacercariae infecting vitreous humor in Yellowstone cutthroat trout and lake trout.

**DISCUSSION**

Besides upsetting an established ecological balance for the biota in Yellowstone Lake, Yellowstone National Park, the introduced lake trout may also introduce new parasites into the aquatic system. The change in parasitofauna may impact native fish in the lake. There is an established life cycle for diplostomiasis in the lake with fish containing metacercariae, a larval stage in the cycle.

Discriminant analysis using significant morphological measurements was 83.3% accurate in distinguishing between metacercariae infecting the 3 fish species localized in the lens or vitreous humor. Metacercariae from the different host species thus appear somewhat related. The same statistical analysis was 93% accurate in distinguishing metacercariae infecting the lens in longnose sucker and Yellowstone cutthroat
<table>
<thead>
<tr>
<th>Host</th>
<th>Number (Infected)</th>
<th>Prevalence (%)</th>
<th>Metacercariae (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mixed A</td>
</tr>
<tr>
<td>Longnose sucker</td>
<td>40 (31)</td>
<td>78</td>
<td>7</td>
</tr>
<tr>
<td>Catosomus catosomus</td>
<td></td>
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</tr>
<tr>
<td>Cutthroat trout</td>
<td>101 (95)</td>
<td>94</td>
<td>3</td>
</tr>
<tr>
<td>Oncorhyncus clarki</td>
<td></td>
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<tr>
<td>Lake trout Salvelinus namaycush</td>
<td>27 (25)</td>
<td>93</td>
<td>8</td>
</tr>
</tbody>
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*Includes Diplostomum host, both lens and vitreous humor infected.

trout, emphasizing the close taxonomic relationship these metacercariae have and the long-time coexistence of the 2 hosts in the lake. Thus, morphological characters differentiate the 2 different groups. For Yellowstone cutthroat trout and lake trout, discriminant analysis was 79.4% accurate in distinguishing metacercariae infecting the vitreous humor. These data are marginal for a close taxonomic relationship.

Statistical analysis using morphological characteristics of metacercariae substantiated similarity of those infecting lens of longnose sucker and Yellowstone cutthroat trout; thus they can be considered a single species (*D. spathaceum*). Morphological data also substantiated that metacercariae infecting Yellowstone cutthroat trout and lake trout can be considered different species. Preliminary DNA studies using standard techniques (Shiozawa et al. 1992) and arbitrary primers substantiated the morphological conclusions; additional DNA research is needed to strengthen this conclusion. Yellowstone cutthroat trout and longnose sucker are long-time residents in Yellowstone Lake; therefore, a nonspecific parasite such as *D. spathaceum* can infect both species. Lake trout, which has its own species of *Diplostomum* and other parasites, was caught and identified for the first time in Yellowstone Lake during 1993 and 1994. Impact of this newly introduced parasite is unknown and, depending on the definitive host (bird) and 1st intermediate host (mollusk), may impact native fish populations such as the Yellowstone cutthroat trout. Additional samples during future years will add important information to this assumption. The same possible impact may occur for the introduced species if the native *Diplostomum* and other parasites found in established Yellowstone Lake fishes become parasites of the lake trout population. Yearly surveillance of fish and parasites in Yellowstone Lake should be considered for proper ichthyofauna management. This will substantiate or refute damage of new hosts and parasites.

ACKNOWLEDGMENTS

Thanks are extended to the Ezra Taft Benson Agriculture and Food Institute, Brigham Young University, for funding provided for the research. Fisheries biologists at Yellowstone National Park provided specimens for study, and we thank them for their help and hospitality.

LITERATURE CITED


Received 29 April 1996
Accepted 17 March 1997