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EVALUATION OF FISH DIPLOSTOMATOSIS IN STRAWBERRY RESERVOIR FOLLOWING ROTENONE APPLICATION: A FIVE-YEAR STUDY

Victor H. Inchausty1 and Richard A. Heckmann1,2

ABSTRACT.—Strawberry Reservoir, Wasatch County, Utah, was treated with rotenone in August 1990. For 5 yr following treatment, about 2000 fish from 5 different species were examined for eye metacercariae (Diplostomum). Incidence dropped from 88.0% before to 0.1% after treatment for cutthroat trout (Oncorhynchus clarki), from 93.0% to 0.1% for rainbow trout (O. mykiss), and from 19.0% to 0.1% for redside shiner (Richardsonius balteatus). Average numbers of metacercariae per eye also dropped from 6.8 to 0.1 for cutthroat trout, from 23.1 to 0.1 for rainbow trout, and from 18.9 to 0.1 for redside shiner. Kokanee salmon (O. nerka), introduced into the reservoir 1 yr after treatment, had a 0.9% prevalence rate and average of 0.1 metacercariae per eye. Rotenone affected almost all organisms in the system. Low incidence of diplostomatosis after treatment indicates that rotenone effectively destroyed many intermediate hosts (fish, snails), which in turn probably affected parasite burdens in definitive hosts (gulls). These changes in metacercariae per host probably occurred because of the complex life cycle of the organism, which is similar to other trematodes. Rotenone is a specific inhibitor of electron transport complex I and can be devastating to parasites with complex life cycles. Through a combination of factors, parasite numbers have decreased in Strawberry Reservoir.

Key words: rotenone treatment, eye fluke, Strawberry Reservoir, parasite life cycle, Diplostomum.

Eye fluke disease or diplostomatosis is a fish disease caused by strigeoid trematodes (Trematoda: Diplostomatidae) mainly of the genus Diplostomum. The disease’s first appearance in North America was in the New Jersey State Hatchery at Hackettstown during 1937 and 1938, where it caused considerable damage to several fish species (Palmer 1939). Diplostomatosis was first reported in Utah in Strawberry Reservoir, considered the state’s number 1 trout lake (Palmieri et al. 1976). The 1st intermediate hosts for Diplostomum spathaceum in Utah are the snails Lymnaea palustris and L. stagnalis (Palmieri et al. 1976, 1977). The 2nd intermediate host is fish, which occur within several families (Palmieri et al. 1976). The major definitive hosts in Utah are Ring-billed Gulls (Larus delawarensis) and California Gulls (L. californicus; Evans et al. 1976, Palmieri et al. 1977). Beginning early in spring and continuing through late summer, California Gulls are more prevalent. By September, Ring-billed Gulls predominate in the Strawberry Reservoir area. This causes a change in major definitive hosts from spring to autumn. More than 38 avian species in 7 families have been identified as definitive hosts of D. spathaceum throughout the world (McDonald 1969). Palmieri et al. (1976) recorded high prevalence of infection by D. spathaceum in salmonids and other fish species inhabiting Strawberry Reservoir, Utah (Table 1), and reported 6.5% and 5.7% infection prevalence in Lymnaea palustris and L. stagnalis, respectively, throughout the state.

Fisheries management in Strawberry Valley has been influenced by introductions of non-game fish for more than 30 yr. During the late 1950s, Utah chub (Gila atraria), Utah sucker (Catostomus ardens), carp (Cyprinus carpio), and yellow perch (Perca flavescens) had nearly displaced trout in Strawberry Reservoir. In October 1961 the reservoir was chemically treated with rotenone to remove undesirable fish. Reservoir volume at that time was 22,661 acre ft and surface area was 3300 acres. Treatment consisted of applying 1249.0 L of rotenone to 88.6 km of tributary streams, 13,247.5 L along the shoreline, and 27,216 kg of rotenone mixed into water-based slurry to the reservoir surface. Rotenone concentration was estimated at 1.77 parts per million (ppm). Total cost of treatment was $43,000. Utah chub reappeared in 1973 and Utah sucker had reestablished by 1978. Sources of these introductions are unknown (Wildlife Resources Division of Utah 1988).
Strawberry Reservoir was again treated with 408,240 kg rotenone to eradicate undesirable fish in August 1990. Rotenone was applied using an "aspirator" on each of the mixing barges. Large 454-kg bags of chemicals were loaded directly onto boats. The chemical was then pulled from the bags with high-pressure pumps that mixed it with lake water as the boats traversed the reservoir. Total cost was around $3.5 million (Daily Herald [Provo, Utah] 1990).

The purposes of this paper are (1) to evaluate the effect of rotenone treatment on diplostomatosis in Strawberry Reservoir and (2) to contribute to a greater understanding of host/parasite relationships in the system.

**MATERIALS AND METHODS**

Fish were collected from Strawberry Reservoir 3 times a year by the Central Division of Utah Wildlife Resources to assess the state of the fisheries. Eyes of fish in these collections were checked for parasite infection following standard biological evaluation by state fisheries biologists. Approximately 5–10% were examined on site using dissecting microscopes; the remainder were fixed in 70% ethyl alcohol and examined in the laboratory, where each eye was dissected, the lens placed in 1 container, and the vitreous humor and retinal tissue in another container. We recorded which eye(s) were infected and the number and location of parasites. Metacercariae were isolated by location, stained with Semichon's carmine, destained with acid-alcohol, mounted with Permount (Lillie 1991), and identified following the procedure and keys of Shigin (1986).

Eyes from infected fish were processed by standard histological techniques (Sheehan and Hrapchak 1973, Lillie 1991), 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 evaluate the host/parasite interaction.

**RESULTS**

During the 5-yr period (1989 and 1992–1995), 3496 eyes from 5 fish species were examined for metacercariae. These included rainbow trout (Oncorhynchus mykiss), cutthroat trout (O. clarki), kokanee salmon (O. nerka), redside shiner (Richardsonius balteatus), and Utah chub (Gila atraria; Table 2). Metacercariae were found infecting the lens, vitreous humor, or both (mixed infections). In eyes examined in situ, metacercariae were actively motile when extracted. Metacercariae in the lens were identified as *D. spathaceum* (Fig. 1); those floating free in the vitreous humor were identified as *D. baeri* (Fig. 2).

Histological preparations did not show alterations in the lens capsule. Metacercariae of *D. spathaceum* were situated near the periphery of the lens and invaded the liquified portion representing that year's growth of tissue (Fig. 3). The number of metacercariae in the lens was low (1–20) for 1992–1995, and the overall infection rate for the 5 fish species was low (0.96%) for 1993–1995. No inflammatory response or retinal detachment was observed associated with *D. baeri* infections (Fig. 4b).
Table 2. Data for fish examined from Strawberry Reservoir, Utah, during 1989 and 1992–1995. Compare with Table 1 (pretreatment levels). Note: 1989 is a pretreatment sample.

<table>
<thead>
<tr>
<th>Host</th>
<th>Year</th>
<th>Eyes examined</th>
<th># of eyes infected</th>
<th>% infected</th>
<th>Mean/meta/eye</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fixed</td>
<td>Fresh</td>
<td>Fixed</td>
<td>Fresh</td>
</tr>
<tr>
<td>Cutthroat trout</td>
<td>1989</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><em>Oncorhynchus clarki</em></td>
<td>1992</td>
<td>0</td>
<td>86</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>1993</td>
<td>0</td>
<td>82</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1994</td>
<td>282</td>
<td>34</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>1995</td>
<td>106</td>
<td>340</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>1989</td>
<td>0</td>
<td>112</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td><em>Oncorhynchus mykiss</em></td>
<td>1992</td>
<td>0</td>
<td>94</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>1993</td>
<td>0</td>
<td>62</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>1994</td>
<td>608</td>
<td>52</td>
<td>0</td>
<td>4</td>
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<tr>
<td></td>
<td>1995</td>
<td>170</td>
<td>154</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Kokanee salmon</td>
<td>1992</td>
<td>0</td>
<td>118</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td><em>Oncorhynchus nerka</em></td>
<td>1993</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1994</td>
<td>415</td>
<td>22</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>1995</td>
<td>115</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Mix salmonids*</td>
<td>1994</td>
<td>505</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Utah chub</td>
<td>1994</td>
<td>107</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Gila atraria</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redside shiner</td>
<td>1994</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>Richardsonius balteatus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>2318</td>
<td>1178</td>
<td>9</td>
<td>93</td>
</tr>
</tbody>
</table>

*Not able to identify species from head samples; no labels in sample bags.

**Discussion**

Assuming that most fish were eradicated, diplomatosis has reentered Strawberry Reservoir following rotenone treatment as exemplified by infected fish. However, post-treatment rates are much lower than pretreatment levels (Tables 1, 2). We do not observe a definite pattern in parasite dynamics. This is the first report of *D. baeri* from Strawberry Reservoir.

Rotenone is a well-known pesticide used widely in home gardens. Its use as fish control as part of water-body management is restricted by the Environmental Protection Agency (Ray 1991, Walter and Keith 1992). Rotenone readily breaks down when exposed to sunlight with most of the toxicity being lost in 5–6 d of spring sunlight or 2–3 d of summer sunlight (Walter and Keith 1992). The compound can be neutralized before entering other bodies of water. Rotenone metabolizes rapidly in soil and in water. The half-life of rotenone in both environments is 1–3 d, and the compound does not readily leach from soil; thus it is not expected to be a groundwater pollutant (Walter and Keith 1992). Rotenone is slightly toxic to wildfowl and highly toxic to fish and aquatic invertebrates that have a wide range of sensitivity.

During the treatment of the reservoir the minimum concentration of rotenone obtained was 0.022 ppm (Wildlife Resources Division of Utah 1988). At this concentration most organisms living in or closely related to the reservoir were affected. Fish eradication was considered successful, and detoxification of the reservoir occurred in the estimated time. During the rotenone treatment period, birds consumed dead fish. No documentation is available about the effect of rotenone on birds, mollusks, and other invertebrates during the treatment period at Strawberry Reservoir. Nishiuchi and Yoshida (1972) found that rotenone caused contraction in snails, and almost all died after 3–4 d in test water. Because the minimum concentration of rotenone was above lethal levels for almost all aquatic organisms (Anonymous 1991), we assume most intermediate hosts for *Diplostomum* spp. were eliminated.

Pretreatment infection rates for salmonids ranged from 80% to 100% (Evans et al. 1976, Palmeri et al. 1976). Following treatment, salmonids were restocked in the reservoir. All fish used for stocking came from Utah state fish hatcheries, most of which are diplomatosis-
Fig. 1. Line drawing and SEM micrograph of metacercariae of Diplostomum spathaceum in the lens of fish from Strawberry Reservoir, Utah: h = holdfast, n = blind intestine, o = oral sucker; p = pseudo sucker, pr = primordium, v = ventral sucker. Bar = 100 μm.

Fig. 2. Line drawing and SEM micrograph of metacercariae of D. baeri in the vitreous humor of fish from Strawberry Reservoir, Utah: h = holdfast organ, o = oral sucker, p = pseudo sucker; v = ventral sucker. Bar = 100 μm.
Fig. 3. Metacercariae (D. spathaceum) infecting the lens of fish: L = lens, m = metacercariae. Note the separation of the retinal layer from the choroid layer. Mag. 400X. Bar = 100 μm.

Fig. 4. Metacercariae (D. baeri) floating free in the vitreous humor of the infected fish (4a) and next to the retina (4b): m = metacercariae, R = retina, V = vitreous humor. Mag. 400X. Bar = 100 μm.

From 1990 to 1994, 15,234,362 cutthroat trout, rainbow trout, and kokanee salmon were reintroduced into Strawberry Reservoir (Central Division, Utah Division of Wildlife Resources, personal communication). Stocking was to reestablish a quality sport fishery. Four years after the treatment, nongame fish such as Utah chub and redside shiner were captured in the reservoir. Incidence of diplostomatosis is still low compared to pretreatment levels (Table 2); however, due to reservoir size and the abundance of (intermediate and definitive) hosts, diplostomatosis may again reach a high prevalence.

Palmieri et al. (1976) and Evans et al. (1976) noted that D. spathaceum had only 2 molluscan intermediate hosts and 2 definitive avian hosts in Utah. Because no further data are available for this area, we do not know all the dynamics of the parasite/host relationship prior to treatment in 1990. A possible source of reestablishing the Diplostomum life cycle in Strawberry Reservoir is nearby streams and lakes. There also is a chance that reintroduced fish were carriers of the parasite.

Major definitive hosts in Utah are Ring-billed Gulls and California Gulls. Beginning early in the spring and continuing through late summer, the California Gull is most prevalent. From September the Ring-billed Gull predominates until the reservoir ices. This accounts for a constant supply of Diplostomum eggs from spring to fall each year. Because of the
abundance of dead fish during the treatment period. Seagulls, mainly California Gulls, ate an unusually large amount of fish and may also have suffered toxic effects of the rotenone (Feldman and Kruckenberg 1975). Even though no data are available, the possibility exists that rotenone reached the birds' intestines and affected the parasites there. Because the treatment was done during late August, the Ring-billed Gull was probably the most affected. We assume that all stages in the parasite life cycle were somewhat altered.

Inasmuch as most fish reintroduced into the reservoir were from parasite-free hatcheries, the parasite will require some time to become established in these fish. The sample of cutthroat trout for 1989 was only 2 fish, both of which were infected. Rainbow trout for that year had an infection rate of 22%, which dropped to 0% during subsequent years. An explanation for these data may be that fish planted in the reservoir following treatment came from hatcheries, such as the Midway, Utah, fish hatchery, which has earth-lined raceways. Earth-lined raceways are conducive to establishing the life cycle of Diplostomum. The fish may have been infected with Diplostomum metacercariae previous to planting in the reservoir.

Diplostomum spathaceum metacercariae were in the liquified portion of the lens, and the pathology correlates with reactions described by Shariff et al. (1980). Infections in the lens of the fish are visible for 1 yr, after which the infected lens tissue changes color and absorbs the fluke (Palmieri et al. 1977). Histological studies showed no alterations caused by D. baeri found in the vitreous humor or adjoining epithelial tissue and retina for this study. After 1990 there were very few infected fish in the reservoir, indicating a lack of re-establishment of the cycle. It would be advantageous to start long-term studies on the parasitic disease for fish in Strawberry Reservoir to evaluate host/parasite dynamics and to record future changes in the infection.

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