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Silver Creek Whirling Disease: 1995 Survey

Final Report to:

The Nature Conservancy,
Idaho Field Office
Box 165
Sun Valley ID 83353

By:
Richard Spall, Ph.D.
Wade Foster
Jack Griffith, Ph.D.
Department of Biological Sciences
Idaho State University
Pocatello ID 83209

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The purpose of this study was to determine the presence, prevalence and intensity of infections of wild trout living within the Silver Creek Preserve by the myxozoan parasite, Myxobolus cerebralis, causative agent of whirling disease (WD). The design of the study included necropsy examinations of substantial numbers of age-0 trout, principally rainbow trout (RBT) and, to a more limited degree, other trout species [brown (BNT) and brook trout BKT)] and/or other age-classes of fish. Additionally, both wild and hatchery-reared young RBT utilized as "sentinel fish" were held in cages in the stream to monitor their survival and possible infection by WD.

Methods

Field Collection

We initially evaluated whether adult wild trout in Silver Creek might be harboring the parasite M. cerebralis and thus serve as "carriers" of WD. The heads of 50 adult trout (16 BNT and 22 RBT greater than 16 inches, and 12 RBT less than 12 inches) were collected from anglers by Idaho Department of Fish and Game personnel at Point-of-Rocks during the Memorial Day holiday weekend in 1995. They were evaluated on the bases of gross pathologic examination (necropsy), microscopic examination of tissue residue following protease digestion of the cranial skeletal elements, and/or histopathological examination of tissue samples.

Collection of juvenile trout by backpack electrofishing and seining was repeated each month during summer 1995, in conjunction with personnel from the Idaho Department of Fish and Game. In accordance with the research plan, samples containing at least 60 fish each of trout fry were collected during the summer months (June-August) and examined for the presence of M. cerebralis. Collection sites were arbitrarily designated as upstream (i.e., from tributaries and/or Silver Creek sites above the confluence of Stalker Creek and Loving Creek) or downstream (Loving Creek or Silver Creek below its confluence). A total of 387 trout were collected (Table 1). Occasionally other fish species including redside shiners, bridgelip suckers, speckled and longnose dace, and Wood River sculpin were collected for necropsy to help determine the "suitability" of Silver Creek for perpetuation of (any) myxozoan parasite life cycles and also because it has been suggested that sculpin and possibly other nonsalmonid fishes may serve as additional hosts for the specific agent of WD. Finally, multiple collections of annelid worms which serve as alternate hosts for M. cerebralis and other myxozoan parasites infecting fish were obtained from the stream sediments of Silver Creek and nearby tributaries. Hatchery fish (Kamloops strain of RBT) selected for in-cage exposure to M. cerebralis infectious agents and fish food to support the caged fish were supplied from the Hayspur Hatchery.

Standard lengths were recorded for all fish collected for necropsy. Diagnostic evaluations included gross, subgross (i.e., tissues were examined using stereoscopic microscopes) and

Table 1. Number of juvenile rainbow (RBT) and brown (BNT) collected in Silver Creek in 1995 and examined for whirling disease. In addition, 11 age-0 brook trout were examined from Grove and Loving creeks on 22 June.

		31 May		22 June		10-19 July		23 Aug	
		RBT	BNT	RBT	BNT	RBT	BNT	RBT	BNT
Site	age:	0/1+	0/1+	0/1+	0/1+	0/1+	0/1+	0/1	+0/1+
Stalker Cr.		-	-	-	-	5/0	0/0	-	-
Grove Cr.		-	-	37/4	4/0	72/0	0/0	65/0	1/0
Sullivan's slough		-	-	-	-	7/0	0/0	-	-
Loving Cr.		-	-	28/1	20/3	1/0	0/0	68/0	0/0
Purdy property		-	-	-	-	4/0	6/0	-	-
Point of Rocks		0/34	0/16	-	-	-	-	-	-
total		0/34	0/16	65/5	24/3	89/0	6/0	133/0	1/0

microscopic ("histopathologic") examinations of fish and also the protease digestion technique. Larger fish (those determined to be older than age-0 fish) were examined using both the protease digestion method and by histopathological methods. These fish were transported to the laboratory on ice and one half of their cranial tissues was resected for digestion and subsequent steps of that method. The other half of each head was fixed in an aldehyde-containing solution, subsequently decalcified and routinely prepared for histologic detection of infected tissues. Age-0 fish were preserved with aldehyde at the streamside and subsequently were prepared only for histological examination in our laboratories; they were not evaluated using the digestion method, as it is ineffective for detection of M. cerebralis infections less than three-four months in duration (i.e., before the spore-forming stage of development of the parasite). For histology, three regions of the head containing cranial skeletal tissues were embedded and sectioned for each fish.

Invertebrate samples (oligochaete worms) collected concurrently with fish were examined microscopically for the presence of actinosporean stages of myxozoan parasite life cycles. Nongame fishes collected during the course of this study were routinely examined for the presence of myxozoan "cysts" using a stereomicroscope. The cranial tissues of some nongame fish additionally were subjected to histopathologic examination, because it has been suggested that sculpin and possibly other nonsalmonid fishes may serve as hosts for WD.

Sentinel Fish

Construction of ca. 4 cubic yard cages for sentinel fish studies was completed in early July 1995 and they were placed in Silver Creek on 8 July. Two cages were placed in the water beneath the bridge at Stalker Creek (the "upper site") and two others were put in Silver Creek at a site on private property ca. two miles east of Picabo (Swanson (?) Ranch, the "lower site"). On 10 July, wild age-0 fish were captured from Stalker Creek and from the outlet of Sullivan's slough using electrofishing or a seine. Seventy-two RBT and 5 BNT were placed in one cage at the Stalker creek site. An additional 11 fish collected from these sites were sacrificed at that time for parasite examinations; these fish are included in the July sample indicated in Table 1. Attempts were made to obtain a similar number of age-0 trout from four sites in Silver Creek downstream from the Loving Creek confluence, but no juvenile fish were captured from those areas. Idaho Department of Fish and Game personnel returned on 11 July and obtained only four trout (lower Loving Creek) suitable for placement in cages at the lower cage site.

On 19 July, additional samples of fish were captured from a portion of Silver Creek on the Purdy ranch and placed in the cage at the lower site. They included 75 age-0 RBT and 4 age-0 BNT. An additional 6 BNT and 4 RBT collected at the Purdy site were sacrificed for histological examination for WD. Also on 19 July, approximately 230 WD-free hatchery-reared RBT fingerlings were obtained from Hayspur Hatchery and were roughly split into cages at the two sites.

Cages were maintained (scrubbed) and the fish were fed pelleted food daily by The Nature Conservancy personnel for the three months they remained in the water. Cages were removed on 20 October 1995 and the surviving fish were prepared for gross and microscopic evaluations for the presence of M. cerebralis and WD. Caged fish that died during the experiment were collected and held for analysis where possible, but their remains proved too decomposed to be usable.

Results and Discussion

Field Collection

The adult trout collected during the Memorial Day weekend were all determined to be free of the myxozoan parasite that causes WD. It is noteworthy that a single brown trout specimen from this collection was found to be infected with the nonpathogenic myxozoan parasite Henneguya zschokkei, indicating that conditions in Silver Creek are at least satisfactory for continuation of that myxozoan life cycle (and that our methods for detection of myxozoan infections in large trout were appropriate.)

Four of the juvenile trout collected in June from Grove and Loving creeks outwardly displayed skeletal deformities consistent with a diagnosis of WD. However, no evidence of infection could be detected upon microscopic examination of their cranial skeletal tissues, even after repeated sectioning of these suspicious samples. Using the same approaches, juvenile trout collected in

July and August showed no evidence of infection of their cranial (or vertebral) skeletal tissues by M. cerebralis, even in those three trout that showed gross skeletal deformities. While both methods of detection for M. cerebralis infections might produce "false negative" results, more than 1,000 histologic sections from the 387 fish processed for histology were evaluated, leading us to conclude that if M. cerebralis is established in trout populations in Silver Creek, it occurs only rarely in the past year's class of fish (i.e., either incidence or intensity, or both, of infection among young trout is low).

Necropsy of nongame fish showed the following: speckled dace were heavily infected with a previosly undescribed species of Myxobolus. This parasite principally was found encysted around the heart in the pericardial and epicardial tissues; it did not appear to contribute to significant morbidity. None of the other fish species, including trout, harbored this myxozoan. The bridgelip suckers were heavily infected with another Myxobolus species, apparently also undescribed, which encysted in their gill tissues. Sculpin were infected with a species of Myxidium in their gallbladders. Also, one additional species of undescribed myxozoan parasite (Myxobolus) was found encysting in the skin of the speckled dace but, like the others we found in Silver Creek fish, had no resemblance to the organism associated with WD in trout. It is noteworthy that the "heart parasite" so common in speckled dace was not found infecting longnose dace. Additionally, none of these non-trout fish species provided evidence of having been infected by the agent of WD.

Sentinel Fish

For sentinel fishes, the presence of M. cerebralis infection was not detected in wild fish that survived the summer and fall. Survival of the wild trout placed into cages was low as they apparently did not adapt well to the pelleted diet, and natural food was evidently mostly excluded from entering the cages due to small mesh size of the cage walls. Only 11 from a stocking number of 78 trout wild survived in the upstream cage and 28 of 75 wild fish survived in the downstream cage. However, any conclusions from this result must be guarded, as caged fish were not placed into the creek ("exposed") until mid July and thus may have avoided exposure to actinosporean stage spores. It presently is not known when the maximum shedding of actinosporean spores occurs in natural habitats such as the Silver Creek system, Also, it is generally thought that older trout fry (two or more months past the swim-up stage) are less vulnerable to infection by these infectious stages of M. cerebralis regardless of their abundance.

For hatchery-reared sentinel fishes, the presence of M. cerebralis infection also was not detected in fish that survived. While survival of "control" hatchery fish placed in cages moored at both sites was better (88 and 123 fish, respectively, were recovered) circumstances dictated that the only hatchery-reared fish available to us for placement in cages were of advanced age and size (ca. 6-month-old fingerlings derived from a fall-spawning Kamloops strain of RBT), again providing inconclusive evidence regarding the risks for contracting WD that wild trout fry face in Silver Creek.

Conclusions

The results of this study do not necessarily lead to the conclusion that Silver Creek is free of the agent that causes WD in trout, but they do lend support for a conclusion that WD presently is not a threat to the trout resources in the Preserve. Sampling of trout populations by Idaho Department of Fish and Game personnel during the summer and fall 1995 indicated substantial numbers of juvenile rainbow trout present at some sites on the Preserve (S. Elle, personal communication) and lend additional support to that conclusion. Also, during Idaho State University electrofishing surveys and snorkeling observations in Silver Creek in 1992-1994, there were no obvious signs of infected fish of any size or species (R. Wilkison, personal communication). Numbers of juvenile rainbow trout in 1992-1994 (in those sites where brown trout had not dramatically increased in abundance) were similar to their abundance in 1986-1987.

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