FISH DISEASE RISK ASSESSMENT: WHIRLING DISEASE AND CERATOMYXOSIS 1999 ANNUAL REPORT

PELTON ROUND BUTTE HYDROELECTRIC PROJECT
FERC No. 2030

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TABLE OF CONTENTS

LIST OF TABLES

Table | Page

INTRODUCTION

Two myxosporean parasites present a threat to the health of salmon and trout in the Deschutes River Basin. *Ceratomyxa shasta* causes ceratomyxosis, a progressive and often lethal infection of the intestinal tissues, and *Myxobolus cerebralis*, the causative agent of whirling disease, digests cartilage, especially in young fish. Infection by *M. cerebralis* often results in high mortality among young fish and causes skeletal deformities in survivors.

The purpose of this study is to assess the risk that these parasites pose for anadromous fish proposed for reintroduction above the Pelton Round Butte Project. The study also looks at possible impacts on resident fish in the Deschutes River Basin above the Project. During 1999, the study focused on the following objectives: 1) determining the presence of the alternative hosts for *M. cerebralis* and *C. shasta* above the Project; 2) determining if the strain of *Tubifex tubifex* present in the Project area can serve as an alternate host for *M. cerebralis*; 3) assessing the susceptibility of resident species to each of these parasites; and 4) examining the distribution of *C. shasta* in the area above the Project.

Current information shows that *C. shasta* is enzootic to the Deschutes River system and may significantly affect fall chinook salmon and steelhead trout survival. Studies conducted since the early 1980s have demonstrated that fish mortality increases in direct relation to the length of exposure to *C. shasta* (Ratliff 1981, 1983). During these studies, juvenile Deschutes fall chinook salmon exposed to the infectious stage of *C. shasta* for 1, 5, 10 and 25 days suffered 2, 18, 40 and 70% mortality, respectively. These data suggested that length of migration or exposure is an important variable affecting survival.

This idea — that length of time exposed to *C. shasta* affects survival — was further tested in studies conducted in the Deschutes River (Ratliff 1981), Willamette River (Oregon Department of Fish and Wildlife [ODFW], unpublished data) and Columbia River (Bartholomew 1998; Bartholomew et al. 1992). In each of these studies, juvenile salmon were captured as they migrated out of the river and were held in fresh water until the infection became patent. In the Deschutes River study, during the first study year, age-0 fall chinook collected prior to May were not seriously affected by *C. shasta*. However, mortality increased to 30% in the mid-June

collection group, and to 56% for fish collected in early July. Results were similar the second year of the study, when mortality reached 90% in the mid-July sample. These results suggest that, because peak migration occurs in June, ceratomyxosis is a significant factor affecting the survival of fall chinook (Ratliff 1981). In the Columbia River study (Bartholomew et al. 1992), mortality from *C. shasta* was lower but followed the same pattern. In this study, about 9% of the age-0 chinook salmon captured between May and September were infected with *C. shasta*; peak mortality (24%) occurred in late August. A total of 11% of the yearling chinook salmon were infected. In the Willamette River study, steelhead trout smolts were collected during outmigration in five study years. During this time, between 31% and 88% of the fish captured were infected with *C. shasta* (R.A. Holt, senior pathologist, personal communication). Given that in each of these rivers the resident salmonids are relatively resistant to *C. shasta*, the resulting mortality was higher than expected and suggests that the parasite has a substantial impact on survival.

The spread of *M. cerebralis*, the parasite that causes whirling disease, into the Deschutes River system could also threaten salmon and trout survival in the future. Although *M. cerebralis* is established in the Grande Ronde and Imnaha river of the Columbia River basin, it has only been reported in the Deschutes River in stray adult steelhead trout and adult chinook salmon. However, if spores of this parasite are distributed through the system by anadromous fish, whirling disease is likely to spread. The potential impact of this parasite, if established, could range from the devastating effects seen in the Madison River in Montana to a coexistence, which has been reported in rivers on both the east and west coasts. The outcome of introduction will depend on the interplay of variables. These include presence of the alternate host, suitability of available habitat, and susceptibility of the resident salmonids.

For ceratomyxosis and whirling disease to spread to the upper basin, alternative host organisms must present. The life cycles of *C. shasta* and *M. cerebralis* are complex and require aquatic worms as alternate hosts. For *C. shasta*, this alternate host is a freshwater polychaete worm, *Manayunkia speciosa* (Bartholomew et al. 1997). The alternate host for *M. cerebralis* is the aquatic oligochaete, *Tubifex tubifex* (Wolf and Markiw 1984). Whether these parasites become established in new areas depends not only on the introduction of spores by the fish host, but also on the presence of the alternate worm hosts. Because *C. shasta* is enzootic in the

Deschutes system, the polychaete must also reside in the watershed; however, prior to this survey its presence had not been demonstrated. Because the distribution of *C. shasta* is limited to particular river systems in the Pacific Northwest, the polychaete likely has specific habitat requirements that have prevented the parasite from becoming widespread. The tubificid host for *M. cerebralis*, on the other hand, is a cosmopolitan worm found in many habitats where trout are present. Already, the wide distribution of *T. tubifex* has allowed the spread of whirling disease from Europe to the U.S, and between at least 22 states in the U.S. The severity of ceratomyxosis and whirling disease is also dependent on parasite dose, so that when populations of the alternate hosts are low there are likewise fewer parasites, and infections may not result in overt disease signs. As conditions become suitable for the worms, a rapid increase in parasites and clinical disease signs may occur. Consequently, it is likely that *M. cerebralis* was present in many watersheds for a long period before population declines were detected. Therefore, it is important to know the conditions that support growth and reproduction of the alternate hosts to manage the diseases.

The distribution and impact of both of these parasites is also affected by the susceptibility of the salmonid host. Resistance to *C. shasta* has been demonstrated to vary within a species, depending on historical exposure to the parasite. This resistance has been the basis for management plans prohibiting stocking of susceptible strains into enzootic waters such as the Deschutes system. However, studies of outmigrating fish, as discussed above, have demonstrated that resistance thresholds can be overwhelmed by increased exposure to the parasite. In contrast, resistance to *M. cerebralis* does not appear to be strain specific. Certain species, such as brown trout, are relatively refractory to infection, while rainbow trout are highly susceptible. To date, no resistant strains of rainbow trout have been identified. Because of the difficulties in conducting controlled laboratory challenges for *M. cerebralis*, there have been few rigorous studies evaluating disease resistance. In an unpublished study (McDowell et al. 1997 report), Deschutes rainbow trout were found susceptible to infection by a California strain of the parasite. In that same study, cutthroat and bull trout were found less susceptible to infection, but both species became infected and had lesions. This experiment needs to be repeated with the strain of parasite present in the Columbia River basin and with native salmonid strains. This portion of the fish disease risk analysis provides data necessary to assess the risk that the parasites *Myxobolus*

cerebralis and *Ceratomyxa shasta* pose for the fish species proposed for reintroduction and for the resident fish populations above the Project.

METHODS

Sampling for Oligochaete and Polychaete Hosts

In 1999, our survey focused on the presence of *T. tubifex* (host for *M. cerebralis*), to include sites where the oligochaete was previously identified, and extended the sampling to the upper watershed of the Crooked River (RM 5.5). Samples of bottom sediment and algal microhabitats were collected using a petite Ponar grab, emptied into a large pan, and examined for annelids onsite. If worm numbers were low, or a small sediment volume was obtained, subsequent samples were collected at that site. Samples were washed in a 500 m screen and were stored in doubled 1 gal recloseable plastic bags. All samples were labeled with the site number, temperature, pH, sample depth, and sediment type.

Live worms were collected in April from the Metolius arm of Lake Billy Chinook (RM 7, 10, 11 and 12) and from the Deschutes River delta (RM 119). In June, samples were collected in the lower Deschutes River at Mecca Flats campground (RM 95.4), Trout Creek campground (RM 87.2), and near Oak Springs Hatchery (RM 47.2). In July, samples were collected in the Deschutes River from the site near Oak Springs Hatchery, at the Reregulating Dam (RM 100) and at the Dry Creek campground (RM 97.3), and from the Crooked River (RM 5.5). The worms were taken to the Salmon Disease Laboratory (SDL) and placed in culture chambers in a 15 C incubator until they could be sorted for identification.

To determine if oligochaete populations collected in the Project area were capable of supporting the life cycle of *M. cerebralis*, samples collected from the following locations were sorted to enrich for tubificids by microscopic identification of worms with hair chaetae: Metolius River (RM 12) and upper Deschutes River delta (RM 119). Samples were also collected from the lower Deschutes River near Oak Springs (RM 47.2). The sorted populations were then seeded with 10^5–10^6 *M. cerebralis* spores in May (Metolius and upper Deschutes River samples) and June (Oak Springs sample). After 2 months incubation, the water from each culture was examined for triactinomyxon stages (TAMS) of *M. cerebralis*. Culture water was collected at

weekly intervals and filtered through a 20 m mesh screen (BioDesign CellMicroSieves, BioDesign Inc, New York), and the material remaining on the screens after filtration was examined for presence of TAMS by light microscopy. Monitoring will continue in this manner for 5 months or until parasite stages are identified.

Samples to be examined for presence of the alternate host for *C. shasta* were collected from six sites in the Crooked River around RM 5.5 and from the Deschutes River at RM 119. Sediment was examined under a dissecting microscope for polychaetes.

Sample Sorting and Identification of Aquatic Annelids

Oligochaetes sorted from sediment samples were preserved in 10% buffered formalin. For identification, worms were removed from the formalin and placed in 70% ETOH. They were then mounted on a microscope slide using the mounting and clearing medium CMCP 10 (Polysciences Inc.). If fewer than 30 worms were sorted from a sample, they were all mounted; if greater than 30, a subsample was mounted. Worms were identified to the lowest taxa possible, using a key for aquatic annelids (Brinkhurst 1986).

Collection of Myxozoan Spores

M. cerebralis spores used to infect the populations of *T. tubifex* were obtained from fish collected at enzootic sites in Oregon and from fish previously infected in experimental challenges. To harvest the spores from infected fish, entire fish heads, or cores from large fish, were ground in a blender and screened through gauze under a vacuum. To concentrate the spores, the filtrate was processed by the plankton centrifuge method as described in the AFS Fish Health Blue Book (Thoesen 1994). Spores were counted under a compound microscope using a hemocytometer, percent viability was determined by methylene blue vital staining (0.25% stock solution; 1:2 working dilution), and the spores were stored fresh at 4°C.

Spores of *C. shasta* for testing the efficacy of UV treatment were harvested from the intestines of rainbow trout infected by exposure in the Willamette River. Infected intestines were removed upon death of the fish, the tissue processed in a blender and the spores partially purified

by centrifugation. Spores were counted using a hemocytometer; viability was determined, and they were stored fresh at 4°C.

Maintenance of the *M. cerebralis* Life Cycle in the Laboratory

Conducting virulence challenges for whirling disease necessitates that the life cycle of the parasite be established in the laboratory. Populations of *T. tubifex* from the following sites have been maintained to support the parasite life cycle: Spring Creek, Oregon (tributary of the Wallowa River); Wallowa Hatchery, Oregon; a private trout farm on the Lostine River, Oregon; and Whitney Hatchery, California. *T. tubifex* collected in 1997 from a drainage ditch in the Willamette Valley are being used as a negative control population. Methods for maintenance of the worms, infection with *M. cerebralis* spores, and collection of TAM stages were described previously (Bartholomew 1998b, 1999).

Efficacy of UV Irradiation for Killing Myxozoan Spores

To insure that our work with *M. cerebralis* does not contribute to the dissemination of whirling disease, we have taken every precaution to insure that live spores do not leave our research facility. In addition to assessing spore killing by vital stain as reported previously (Bartholomew 1999), *M. cerebralis* spores treated at the two UV doses and untreated control spores were seeded on populations of uninfected *T. tubifex*. These populations are monitored for release of TAM stages to determine if the life cycle was completed.

This year we continued these investigations by examining the efficacy of UV irradiation for killing spores of *C. shasta*. Protocols for testing the effects of UV irradiation on spore viability were described previously (Bartholomew 1999). Briefly, tests were conducted using a field model liquid UV sterilizer (Ideal Horizons Model IH-4L). Killing was tested at flow rates of 12, 18, and 24 l/min at wavelengths of 185 and 254 nm. All treatments were tested in replicate. Negative control samples were not treated. A volume of 54 liters (2 replacement volumes) of effluent from the unit was collected and the spores concentrated by continuous flow centrifugation (8,000 rpm). Spores were stained with methylene blue vital stain. Percent viability

was determined by microscopic examination and scoring 100 spores from each treatment, with a minimum of six replicate counts. One hundred untreated spores were counted as a control. In vivo testing of spore viability was not done for *C. shasta* because we are unable to maintain populations of the polychaete host in the laboratory.

Detection of *M. cerebralis* by PCR

As described previously (Bartholomew 1999), the polymerase chain reaction (PCR) assay developed by Andree et al. (1997) at U.C. Davis was adapted for use on adult tissues by modifications in sample preparation and processing procedures to ensure detection of the parasite in core samples from adult heads. This modified assay has been used to confirm identification of spores from samples provided through ODFW's monitoring program. In 1999, we began preliminary tests to determine if the PCR could be adapted for non-lethal sampling applications. Fish infected with *M. cerebralis* in the laboratory were used for this test. Fish received either a dose of 200 or 2000 parasites, and the infection was allowed to progress for 5 months. At this point, the following samples were collected: opercular, caudal fin, and gill filaments. Tissue samples were collected using a hole punch or scissors. These instruments were sterilized in 20% bleach for 2 minutes between each fish to eliminate cross-contamination. After the samples were collected, the fish were killed and the head divided for PCR, histological analysis, and digestion for spore counts. Tissue samples were assayed by PCR and these results compared with infection data obtained using visual diagnostic methods (histology and spore counts).

M. cerebralis Sentinel Studies

To determine if *M. cerebralis* has become established in the lower Deschutes River, sentinel rainbow trout were exposed at a site just above Oak Springs Hatchery (RM 47.2). Stock 66 rainbow trout and summer steelhead reared at Oak Springs Hatchery were used because of their resistance to *C. shasta*, which is enzootic at this site. One hundred fish of each strain (average weight 1g) were exposed for a period of 2 weeks; rainbow trout were exposed from June 3 to June 17; and steelhead trout were exposed from June 17 to July 1. After exposure, all fish were transported to the SDL to be held for 5 months and observed for clinical disease signs.

At termination, all fish will be examined by PCR, and a digest will be performed to examine for spores.

M. cerebralis Susceptibility Studies

The *M. cerebralis* virulence challenge of Crooked River redband rainbow trout, initiated in October 1998, was terminated in March 1999. At termination, clinical signs of disease were recorded. All surviving fish were killed with MS-222, and the heads were severed from the body and then halved. One half was fixed for histological examination and the other half processed for determination of numbers of spore in the cartilage.

In March, Metolius River kokanee salmon were tested for relative susceptibility to whirling disease in comparison to susceptible Mt. Lassen rainbow trout fry. At time of challenge, the kokanee fry weighed 1.5 g, and the rainbow trout controls weighed 1.8 g. Replicate groups of 25 fish of each species were exposed to a low dose of 200 TAMS/fish and a high dose of 2,000 TAMS/fish. Two additional replicate groups were unexposed as negative controls. The fish were placed in 500 ml containers with aeration, then an appropriate number of parasites were added to the container; and the fish were exposed for 2 hours. After exposure, the fish were transferred to 17 l tanks in the isolation facility. Fish were fed and water was changed daily. Behavioral or clinical signs of whirling disease were recorded, and the challenge was terminated at 5 months. All surviving fish were killed with MS-222, and the heads were severed from the body. One half of each head was fixed for histological examination and the other half processed for determination of numbers of spore in the cartilage.

On April 11, replicate groups of 25 Rapid River chinook salmon (average wt.0.6 g; obtained from Rapid River Hatchery), and control Mt. Lassen rainbow trout (average wt. 4.1 g), were exposed to a high and low dose of TAMS and maintained as described. These fish will be monitored for clinical signs for 5 months and examined as described. On April 21, replicate groups of 25 wild steelhead trout (spawned at Round Butte Hatchery), and control Mt. Lassen rainbow trout, were exposed to a high and low dose of TAMS and maintained as described. Both strains were at an average weight of 1g at the start of the challenge. These fish will be monitored for clinical signs for 5 months and examined as described.

Ceratomyxa shasta Sentinel Studies

The distribution of *C. shasta* was investigated in the Metolius, Deschutes, and Crooked Rivers by exposure of susceptible rainbow trout (stock 72; Roaring River stock) obtained from Wizard Falls Hatchery. Fish were exposed in each of the Crooked and Deschutes rivers at the inflow to LBC, at a depth of 2 m (Crooked River -RM 5.5; Deschutes River – RM 120), and at a location approximately 1 mi. up each arm, at a depth of 33 m (Crooked River -RM 0.8; Deschutes River – RM 116). In the Metolius River, fish were exposed at RM 4, at a depth of 33 m, and at the inflow to LBC (RM 12), at a depth of 2 m. Two additional exposure locations were in the Metolius River above and below the confluence of Lake Creek (RM 40.5 and RM 39, respectively). One hundred fish were exposed at each site for 1 week between June 7–14. After exposure, all fish were returned to the SDL and observed for development of clinical signs of ceratomyxosis. At termination, all fish are examined for spores.

C. shasta Susceptibility Studies

Relative susceptibility to *C. shasta* was determined for kokanee salmon, Crooked and Metolius River redband rainbow trout, and bull trout by natural exposure to the parasite in the Willamette River. The histories of each group exposed are as follows: kokanee salmon were from adults spawned in the Metolius River in September 1998; Crooked River redband rainbow trout were from adults captured at Bowman Dam in June 1997; Metolius River redband rainbow trout were captured as fry above Camp Sherman in May 1998; and bull trout were from adult fish spawned in Canyon and Jack Creeks in September 1997. The number of fish exposed and the average weight at exposure for each group was: kokanee salmon — 30 fish exposed, wt 19 g; Metolius River redband rainbow — 24 fish exposed, wt 119 g; Crooked River redband rainbow trout, 16 fish exposed, wt 289 g; bull trout — 20 fish exposed, wt 275 g. Each group was exposed for 4 to 7 days along with a susceptible rainbow trout strain as a control. After exposure, all groups were transferred to holding tanks at the SDL, where they were maintained on 12°C fish pathogen-free water. Mortalities were recorded, and all fish were examined for

presence of *C. shasta* in wet mounts of intestinal tract scrapings. After the fish were held for 90 days, the experiment was terminated.

C. shasta Screening in Captured Fish

In April, ODFW personnel collected 60 mountain whitefish and 60 redband rainbow trout from the lower Deschutes River at Maupin (RM 52). All fish were age 1–2. Samples of the posterior intestine from these fish were collected and assayed for presence of *C. shasta* by PCR using methods described by Palenzuela et al. (1999). The Redband Trout Life History group provided intestinal samples of fish collected in their study for detection of *C. shasta* using the PCR. The fish were collected beginning in mid-May by either Merwin trap or angling. Samples from 85 fish were assayed by PCR. The collecting group supplied pertinent data and identified each fish as a resident, hatchery, or steelhead form.

RESULTS

Identification of Alternate Hosts

Oligochaetes have been sorted from the samples collected between March and July. All, or a portion of, the worms have been mounted, and identification is ongoing. This year, further identification was made only on worms with the characteristic hair chaetae of the tubificidae. *T. tubifex* was tentatively identified from the following sites: Metolius RM 12; the lower Deschutes at RM 47.2 and 97; and the upper Deschutes at RM 119. Identification of oligochaetes collected from other sites is ongoing. Live worm populations collected in 1998 continued to be monitored for production of triactinomyxons, and the only actinosporean identified was confirmed not to be *M. cerebralis* by PCR analysis and morphological characteristics. Populations of worms collected in March, 1998, from the Metolius River (RM 11, Box Canyon) and seeded with *M. cerebralis* spores in June, 1998 and April, 1999 began releasing triactinomyxon stages of *M. cerebralis* in August, 1999. Identity of the parasite was determined visually and confirmed by PCR assay. Exposures using these triactinomyxons to infect rainbow trout have been initiated to complete the

parasite life cycle. Tubificids collected in May, 1999 (Deschutes delta, RM 119; Metolius River,RM11) and June, 1999 (Oak Springs Hatchery, RM 47.2) and seeded with *M. cerebralis* spores are being monitored but have not yet produced triactinomyxons.

Samples for identification of polychaetes collected from sites in the lower Crooked River and the Deschutes River delta have been sorted. Only one polychaete worm was identified, in a sample collected from the Deschutes River.

Efficacy of UV Irradiation for Killing Myxozoan Spores.

In vivo experiments to determine viability of *M. cerebralis* after UV irradiation by the ability of the spores to complete their life cycle in *T. tubifex* are in progress.

The minimum UV dose necessary for killing 100% of *C. shasta* spores, as assessed by vital staining, was 185nm at a flow rate of 12 l/min (Table 1). However, the data for spore killing was inconsistent between treatment trials. Possible explanations for this may include differences in the viability of the spore preparations used or inaccuracy of assessing viability using vital stains.

Table 1. Viability of *Ceratomyxa shasta* spores after irradiation at different wavelenths and flow rates, as assessed by methylene blue staining.

Wavelength (nm)	Flow (liters/min)	Percent of Spores Viable after Treatment
	Pre-UV — Controls	70%
185	24	63%
		62%
	18	52%
		64%
	12	47%
		53%
	Pre-UV — Controls	76%
245	24	73%
		63%
	18	75%
		62%
	12	22%
		40%

Detection of *M. cerebralis* by PCR

A portion of the fish collected by ODFW have been processed and assayed for the presence of *M. cerebralis* by PCR. In 1998–1999, samples from 60 fish presumptively positive for *M. cerebralis* were provided by ODFW for PCR analysis. Of these, 23 were confirmed positive for the infection by PCR, the DNA from 3 fish was not recovered, and 19 fish have not yet been assayed. Fifteen fish presumptively positive in the visual examine were not confirmed positive by the PCR. The inability to detect the parasite in these fish may indicate either that the level of infection was low and spores were not well distributed in the sample or that the spores were of another myxobolid species. Modifications have been made by ODFW personnel in the procedure to insure that spores are distributed in the sample provided for PCR analysis.

Analysis of the results of the non-lethal PCR assay for detection of *M. cerebralis* suggest that gill filaments may be suitable for non-lethal detection of the parasite. In a study using rainbow trout infected in the laboratory, parasite DNA was detected from 32% of gill filaments from fish exposed to a low parasite dose (200 TAMS/fish) and from 68% of gills from fish exposed to a high parasite dose (2,000 TAMS/fish). Parasite DNA was detected in only 5% of samples of operculum and caudal fin using the PCR (Table 2).

Table 2. Summary of PCR-based non-lethal sampling results of different samples from fish infected at either a low dose or high dose of *Myxobolus cerebralis*.

Sample Type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Caudal fin — low dose			+																
Gill Filament — low dose					+							+	+	+	+	+		+	+
Opercular — low dose		+																	
Head digest — low dose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Caudal fin — high dose										+	+								
Gill Filament — high dose			+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+
Opercular — high dose		+																+	+
Head digest — high dose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

M. cerebralis Sentinel Studies

Fish held as sentinels for detection of *M. cerebralis* in the lower Deschutes River are being monitored for development of infection.

M. cerebralis Susceptibility Studies

Data from challenge of the Crooked River redband rainbow trout has been analyzed and the infection levels compared with that of the control Mt. Lassen strain rainbow trout (Table 3). Challenge results indicate that redband rainbow trout are susceptible to infection by *M. cerebralis* and will develop clinical signs of disease. All redband rainbow trout became infected with *M. cerebralis*; however, they did not develop disease signs to the same degree as the susceptible control trout, and the mean spore count was lower than in the susceptible strain at the high exposure dose.

Challenges of Metolius River kokanee demonstrated these fish are more resistant to infection than the rainbow trout (Table 4). In the low dose exposure group of rainbow trout, spores were detected from 75% of the fish, while at that same exposure level only 19% of the kokanee had detectable spores, and the number per fish was lower. At the high exposure dose, 98% of the rainbow trout and 23% of the kokanee had detectable spores. Clinical signs did develop in the kokanee as well as the rainbow trout at the high exposure dose. However, mean spore loads in the rainbow trout were lower in this challenge than in the previous experiment.

Challenges with Deschutes River steelhead trout and Rapid River chinook salmon were successfully completed and tissues are being processed to determine mean number of spores and severity of microscopic lesions.

Table 3. Numbers of infected fish, presence of clinical signs of whirling disease, and concentration of *Myxobolus cerebralis* spores in Crooked River redband rainbow trout (Redband) and Mt. Lassen rainbow trout 4-1/2 months after exposure to the infectious stage of *M. Cerebralis.*

Species	Exposure Dose (TAMS/fish)	# Survivors (25 exposed)	Clinical Signs	Mean Spore Count (total spores/half head)
Mt. Lassen	controls	17	none	--
Redband	controls	16	none	--
Mt. Lassen	200	10	none	1.2×10^5
	200	15	none	1.3×10^5
Redband	200	16	none	1.8×10^5
	200	12	2 fish	9×10^4
Mt. Lassen	2000	17	15 fish	5.8×10^5
	2000	19	18 fish	8.2×10^5
Redband	2000	14	3 fish	4.1×10^5
	2000	17	2 fish	4.6×10^5

Table 4. Numbers of infected fish, presence of clinical signs of whirling disease, and concentration of Myxobolus cerebralis spores in Metolius River kokanee salmon and Mt. Lassen rainbow trout 4-1/2 months after exposure to the infectious stage of *M. Cerebralis.*

Species	Exposure Dose (TAMS/fish)	# Survivors (25 exposed)	Clinical Signs	Mean Spore Count (total spores/half head)
Mt. Lassen	controls	17	none	--
Kokanee	controls	23	none	--
Mt. Lassen	200	20	none	3 x 104
	200	24	none	2.2 x 104
Kokanee	200	21	none	4.3 x 103
	200	22	none	1.4 x 103
Mt. Lassen	2000	25	none	1.1 x 105
	2000	25	7 fish	5.7 x 104
Kokanee	2000	23	none	2.6 x 103
	2000	24	5 fish	6.6 x 103

C. shasta Sentinel Studies

Results of exposures in the Crooked River demonstrate dilution of *C. shasta* infectious stages from the upriver site to the confluence with the Deschutes River, while in the Deschutes River, mortality in fish held at a depth of 30 m near its confluence with the Crooked River was nearly as high (81%) as at the upriver site (100%). Exposures in the Metolius River resulted in a low prevalence of infection among fish exposed at both at the inflow into LBC (2 fish) and downriver near the forebay (1 fish), with a higher prevalence among fish held below the confluence with Lake Creek (19 fish). No fish were infected in the groups held above the confluence with Lake Creek (Table 5).

Table 5. Prevalence of Ceratomyxa shasta infection among sentinel fish held in locations in the Crooked, Deschutes, and Metolius Rivers.

Location	Prevalence of *C. shasta* Infection
Crooked River	
RM 0.8	0%
RM 5.5	100%
Deschutes River	
RM 116	81%
RM 120	100%
Metolius River	
RM 4	2%
RM 12	1%
Above Lake Creek	0%
Below Lake Creek	23%

* Fish surviving 90 d not yet examined

C. shasta Susceptibility Studies

Experiments to determine the relative susceptibility of Metolius River kokanee, bull trout and redband rainbow trout from the Crooked and Metolius Rivers are in progress. To date, no *C. shasta*-related mortality has occurred in bull trout (age-2) or kokanee salmon (age-1) exposed in the Willamette River. Redband rainbow trout from the Crooked River (age-2) have not had any *C. shasta*-related mortality while 30 % of those from the Metolius River (age-1) have died with clinical signs of infection.

C. shasta Screening of Captured Fish

Mountain whitefish collected in the lower Deschutes River at Maupin in April were not infected with *C. shasta*, as determined by PCR analysis. The prevalence of infection among the redband rainbow trout collected at same site was 25% (15 of 60 fish).

Examination of data from fish collected by the Redband Life History study (Table 6) shows that in 1999, the prevalence of *C. shasta* infection was higher in fish collected in the Deschutes River above the Project, (70%) than among fish collected from the Crooked River (30%) and the Metolius River (20%). A high prevalence of infection also occurred in fish captured in the forebay (75%); however, this data was based on only four fish. Although infection prevalence was consistently higher among the hatchery/steelhead forms compared with resident forms for each river, there were few of these fish collected in the traps, and the significance of this observation has not been determined. Comparison of this year's data with that from fish collected in 1998 (Table 6) shows an increased prevalence of *C. shasta* among resident fish from the Deschutes River.

Table 6. Distribution of fish collected from the Rainbow Trout Life History study positive for *Ceratomyxa shasta* (determined by PCR) for 1998 and 1999.

1998

Trap Location	Total # Collected	Percent Positive	Percent Positive for *Ceratomyxa shasta* (total # fish assayed)		
			Hatchery	Resident	Steelhead-like
Crooked River	36	39%	100% (2)	36 % (31)	33% (3)
Deschutes River	28	36%	43% (7)	37% (19)	0% (2)
Metolius River	48	33%	57% (14)	16% (25)	22% (9)
Forebay	24	54%	75% (4)	62% (13)	29% (7)

1999

Trap Location	Total # Collected	Percent Positive	Percent Positive for *Ceratomyxa shasta* (total # fish assayed)		
			Hatchery	Resident	Steelhead-like
Crooked River	36	30	55% (9)	22% (27)	--
Deschutes River	30	70	100% (2)	66% (27)	100% (1)
Metolius River	15	20	100% (1)	8% (13)	100% (1)
Forebay	4	75	--	75% (4)	--

DISCUSSION

During the third year of this study, the focus has been establishing if the resident strains of *T. tubifex* can serve as alternate hosts for *M. cerebralis* and examining the impact of both parasites on resident salmonids. Survey data from the first two study years demonstrated the presence of *T. tubifex* from sites in the Deschutes, Crooked, and Metolius rivers. However, because recent studies on the susceptibility of *T. tubifex* to infection by *M. cerebralis* have indicated that all populations of the worm are not equally susceptible (Ron Hedrick, U.C. Davis, personal communication), we have gone beyond the morphological characterization of the worms to determine if they are capable of serving as alternate hosts. In laboratory experiments conducted this year, tubificids collected from the Metolius River and exposed to *M. cerebralis* in

the laboratory were found capable of acting as an alternate host for the parasite. A number of these worms have been provided to researchers at the National Biological Sciences Center, Seattle, who are developing molecular markers to identify those populations of *T. tubifex* that can serve as alternate hosts. These markers will make it possible to compare *T. tubifex* collected in the Metolius River with worms from other regions. Establishing if resident forms of *T. tubifex* are susceptible to infection was critical to the risk analysis because introduction of *M. cerebralis* has occurred periodically with infected adult salmon and steelhead trout straying from watersheds in the upper Columbia River basin (Engelking 1998, 1999a 1999b). To determine if this introduction may have already resulted in establishment of the parasite in the lower river, susceptible sentinel fish were exposed in the lower Deschutes River. These fish are being monitored for development of clinical signs and will be assayed by PCR to determine if infection has occurred.

Development of the PCR assay as a method for confirming the presence of *M. cerebralis* was a high priority because of the increased sensitivity this techniques affords and the necessity to confirm results of other, less sensitive methodologies. Although it is the current diagnostic standard, the pepsin-trypsin digest method has the disadvantage of relying on morphological characteristics to separate *M. cerebralis* spores from those of other similarly shaped myxosporeans. The PCR assay is both sensitive and specific, and we have used it to confirm suspect infections. However, there have been a number of fish presumptively positive for *M. cerebralis* in which the infection could not be confirmed by PCR. This is likely a result of low numbers of spores that are unevenly distributed in the sample material, and steps have been taken to insure the samples provided for PCR analysis are representative of the sample examined.

A priority for further development of diagnostic methods is development of non-lethal sampling techniques. In the previous study year (Bartholomew 1999), we reported development of a non-lethal PCR assay for detection of *C. shasta*. This year, we initiated trials to test the feasibility of a non-lethal assay for detection of *M. cerebralis*. Because the parasite infects cartilage tissue, this method will necessarily be more invasive than the assay developed for *C. shasta*. However, preliminary results demonstrate that *M. cerebralis* DNA can be detected in gill filaments from infected fish, although detection frequency is lower than in lethally collected

cartilage tissues. This work needs to be continued using tissue from adult fish to determine if this method will be sufficiently sensitive for screening.

Difficulties in establishing the whirling disease life cycle in the laboratory and in constructing an isolation facility for conducting whirling disease challenges were overcome this year, and the first virulence challenges have been completed. Redband rainbow trout from the Crooked River (near Opal Springs) were tested for their susceptibility to infection by *M. cerebralis*. Results of that challenge demonstrated that this strain is susceptible to infection. Spores were isolated from all fish exposed to a low dose of 200 TAMS/fish and to a high dose of 2,000 TAMS/fish. However, fewer of the redband rainbow trout developed clinical signs of whirling disease compared with the susceptible Mt. Lassen rainbow trout controls (at the high dose, 5 redband rainbow displayed whirling behavior compared with 33 of the Mt. Lassen strain). Total spore counts in the redband rainbow trout exposed to a high parasite dose were also lower (4.4 x 105) than in Mt. Lassen rainbow trout (7 x 105).

Results of virulence challenges using Metolius River kokanee salmon demonstrate that these fish are less susceptible to infection than the rainbow trout controls. Fewer kokanee had detectable spores at either the low (19%) or the high (23%) parasite dose compared with the rainbow trout at the low (75%) and high dose (98%) exposures. However, clinical signs were evident in kokanee at the high exposure dose. This suggests that establishment of *M. cerebralis* would have a lesser effect on kokanee salmon than on the redband rainbow trout, however, if conditions occurred where the parasite proliferated to high levels this species would suffer some effects.

Sampling for the alternate host for *C. shasta* was more limited in scope this year, and the polychaete was identified from only one sample. Because of the high incidence of ceratomyxosis in the Project area, it was expected the alternate host would be more prevalent. However, the polychaete may occupy different habitats than encountered in other rivers, and it may have a patchy distribution. This is supported by exposure studies, which indicate parasite distribution is scattered.

The impact of *C. shasta* on resident populations was examined by assessing the prevalence of infection in populations captured in the Project area, by exposure of susceptible sentinel fish,

and by virulence challenge with the parasite. Samples of intestinal tissues of redband rainbow trout captured in the Project area were provided in 1998 and 1999 by the Rainbow Life History study as part of the study's survey for parasites. These tissues were assayed using the *C. shasta*-specific PCR to determine the prevalence of infection in these fish. Results from 1998 showed a high prevalence of infection in fish collected in each of the tributaries (39% in the Crooked River, 36% in the Deschutes River, and 33% in the Metolius River), with a slightly higher prevalence in fish collected in the forebay (54%). This year, the prevalence of infection was higher in fish captured in the Deschutes River (70%) compared with the Crooked (30%) and Metolius (20%) River. It is difficult to extrapolate severity of disease from presence of infection, but clinical signs of ceratomyxosis and *C. shasta* spores were seen in these fish (Kristy Groves, Rainbow Life History study, personal communication). Although data from the previous year suggests that the prevalence of infection is higher among hatchery fish than resident fish (Bartholomew 1999), there were too few hatchery fish collected in 1999 to support this conclusion.

Data from exposures of sentinel fish in the Metolius, Crooked and Deschutes Rivers demonstrate high numbers of infectious stages of *C. shasta* in both the Crooked and Deschutes rivers at their inflow into LBC. In the Deschutes River, infections also occurred in the sentinel fish held near the confluence with the Crooked River at a depth of 30 m. However, in the Crooked River, fish exposed near the confluence with the Deschutes River at that depth did not become infected. This may indicate either that initial parasite numbers are higher in the Deschutes River, masking the effects of dilution, or, that temperature stratification occurs in the Crooked River, affecting vertical distribution of the parasite. In the Metolius, prevalence of infection was low both at the inflow and near the forebay. Exposure of fish in the Metolius River above and below the confluence of Lake Creek demonstrated that Lake Creek is a source of infection in the upper Metolius, but that fish were not exposed upstream of its confluence.

In collaboration with ODFW, intestinal samples from mountain whitefish and rainbow trout were collected from fish trapped in the lower Deschutes River. *C. shasta* was not detected in the 60 whitefish assayed; however, 25% of the rainbow trout were infected. There is no published data on the susceptibility of whitefish to infection by *C. shasta*, and this may indicate that they are resistant to infection or that they reside in areas where the parasite is not established. *C. shasta* virulence challenges of kokanee salmon and bull trout show these fish are resistant to the parasite.

Challenges comparing the susceptibility of Crooked River and Metolius River redband rainbow trout are still underway, but data indicates the Metolius River strain is more susceptible to infection than the Crooked River strain. If true, this suggests that resistance to *C. shasta* is not as important a selection factor in the Metolius River and that these fish would be more severely affected if parasite levels increased. However, this experiment was conducted using only a limited number of fish from different year classes and needs to be repeated. Data from captured fish in this study demonstrates that infection occurs at a relatively high frequency, and records of clinical signs of infection provided by the Rainbow Life History study indicate that there is some impact on fish health. A question that remains to be answered is if infection in resident rainbow trout in the Metolius River (33% in 1998, 20% in 1999) occurs as a result of exposure in the Metolius River or because these fish stray into the Deschutes and Crooked Rivers, where the parasite life cycle is established.

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