Journal of Aquatic Animal Health 24:274–280, 2012

ISSN: 0899-7659 print / 1548-8667 online
DOI: 10.1080/08997659.2012.716012

ARTICLE

Predicted Redistribution of *Ceratomyxa shasta* Genotypes with Salmonid Passage in the Deschutes River, Oregon

Matthew E. T. Stinson and Jerri L. Bartholomew*
Department of Microbiology, Oregon State University, 220 Nash Hall, Corvallis, Oregon 97331, USA

Abstract

A series of dams on the Deschutes River, Oregon, act as migration barriers that segregate the river system into upper and lower basins. Proposed fish passage between basins would reunite populations of native potamodromous fish and allow anadromous fish of Deschutes River origin access to the upper basin. We assessed the potential redistribution of host-species-specific genotypes (O, I, II, III) of the myxozoan parasite *Ceratomyxa shasta* that could occur with fish passage and examined the influence of nonnative fish on genotype composition. To determine the present distribution of the parasite genotypes, we exposed eight salmonid species—three native and five stocked for sport fishing—in present and predicted anadromous salmonid habitats. We monitored fish for infection by *C. shasta* and sequenced a section of the parasite ribosomal DNA gene from fish and water samples to determine parasite genotype. Genotype O was present in both upper and lower basins and detected only in steelhead *Oncorhynchus mykiss*. Genotype I was spatially limited to the lower basin, isolated predominately from Chinook salmon *O. tshawytscha*, and lethal for this species only. Genotype II was detected in both basins and in multiple species, but only as a minor component of the infection. Genotype III was also present in both basins, had a wide host range, and caused mortality in native steelhead and multiple nonnative species. Atlantic salmon *Salmo salar* and kokanee *O. nerka* were the least susceptible to infection by any genotype of *C. shasta*. Our findings confirmed the host-specific patterns of *C. shasta* infections and indicated that passage of Chinook salmon would probably spread genotype I into the upper Deschutes River basin, but with little risk to native salmonid populations.

Noble (1950) first described *Ceratomyxa shasta* from infected hatchery trout from northern California. This myxozoan parasite, commonly found throughout the Pacific Northwest (PNW; Bartholomew 1998), causes the disease ceratomyxosis. Notable clinical signs of the disease include inflammation, necrosis, and hemorrhaging of the intestine; in severe cases the development of ascities causes a grossly distended abdomen (Wales and Wolf 1955). Fish become infected when they encounter the actinospore stage of the parasite, which is released into the water column from the invertebrate host *Manayunkia speciosa.* The myxospore stage, which develops in the salmonid fish, infects the invertebrate host (Bartholomew et al. 1997). The pathogenicity of the parasite depends upon the host species and origin, and native salmonids from endemic waters are generally less susceptible and able to survive exposure to higher parasite densities than salmonids from nonendemic waters (Bartholomew 1998). Recent studies in the Klamath River, California–Oregon, identified multiple host-specific genotypes of the parasite (Atkinson and Bartholomew 2010a, 2010b). In that river system, genotype O infects native steelhead *Oncorhynchus mykiss* (anadromous rainbow trout) and redband trout (rainbow trout subspecies), genotype I causes mortality in native Chinook salmon *O. tshawytscha*, genotype II exists as two biotypes that cause mortality in either native coho salmon *O. kisutch* or nonnative rainbow trout (Hurst and Bartholomew 2012), and genotype III was only detected at low levels, but in multiple species (Atkinson and Bartholomew 2010b). These host–parasite relationships have not been evaluated outside the Klamath River system and have implications for stocking and fish passage plans throughout the parasite's range.

Strategies for stocking salmonids in waters in which *C. shasta* is endemic have been guided by results from in-river sentinel

*Corresponding author: bartholj@science.oregonstate.edu
Received April 20, 2012; accepted July 19, 2012
Published online November 12, 2012

fish exposures. These natural challenges tested the susceptibility of particular fish strains but were often conducted in watersheds other than where stocking was proposed, with the fundamental assumption that the parasite is equivalent throughout its range (Schafer 1968; Sanders et al. 1970; Zinn et al. 1977; Ching and Parker 1989). But outcomes of stocking programs have varied, and in retrospect, the perceived resilience of some species or strains may have resulted from the absence of a pathogenic genotype (Atkinson and Bartholomew 2010a). In some instances, the stocking of nonnative salmonids has increased parasite levels with little or no consequence to native populations because the genotype that increased was specific to the introduced species. For example, stocking of susceptible nonnative rainbow trout into the upper Klamath River basin has amplified a *C. shasta* genotype that could successfully proliferate in this naïve host, yet is not infectious for native salmonids (Hurst and Bartholomew 2012). However, introducing susceptible species may also result in amplification of parasite strains that can affect native fish, as has been the case for another myxozoan *Myxobolus cerebralis* (Nehring and Walker 1996). Thus, data on the parasite genotypes present in a river system and the host specificity of these genotypes should be synthesized to develop informed stocking decisions that minimize disease risks.

Dam removal and expanded fish passage are expected to benefit salmonid populations by increasing habitat. However, an unintended consequence may be the exposure of presently isolated fish populations to pathogens associated with introduced populations and species. In the state of Washington, increased numbers of anadromous salmonids passed above the Cowlitz Salmon Hatchery, coincided with increases in ceratomyxosis at that facility (E. Ray, Washington Department of Fish and Wildlife, personal communication). Pathogens can also exist solely above migration barriers and thus present a risk to reintroduced anadromous fishes. A survey conducted in the Elwah River, Washington, identified a myxozoan in kokanee *O. nerka* that occurred only in the upper portion of the system and could potentially affect lower-basin fish populations after removal of the migration barrier (Jones et al. 2011). To minimize risks of introducing pathogens, sentinel studies can be used to inform fisheries managers of the parasite populations present above and below migration barriers. For example, Zielinski et al. (2010) determined *M. cerebralis* is established in the lower Deschutes River basin and recommended only marked salmonids of upper Deschutes River basin origin be passed beyond barriers. Similarly, in the Klamath River, testing for *C. shasta*-associated risks has been conducted prior to anadromous fish passage. Sentinel exposures of extirpated Chinook salmon and coho salmon in the upper Klamath River basin resulted in minimal mortality because host-specific genotypes were absent (Atkinson and Bartholomew 2010a). However, the movement of fishes beyond migration barriers could expose trout and salmon to different *C. shasta* genotypes, creating new host–parasite interactions with unknown effects.

Similar to many watersheds of the PNW, the Deschutes River, Oregon, is partitioned by several dams that restrict fish migration, and recreational angling is supplemented by stocking native and nonnative salmonids. Improvements have been made to permit downstream migration, and upstream passage has recently begun (Northwest Power and Conservation Council 2004). *Ceratomyxa shasta* is endemic in the Deschutes River basin, being present in the lower basin in the river main stem and above the dams in the reservoir, several upper basin lakes, and two of the three major tributaries (Sanders et al. 1970; Ratliff 1983). To determine *C. shasta* genotype distribution and infection risks to native and nonnative salmonid populations, we collected and analyzed water samples for parasite density and genotype composition from known positive locations in the lower and upper basin. Additionally, we conducted sentinel fish exposures at these locations using salmonid species currently stocked in the upper Deschutes River and those that will gain passage beyond the barrier. We determined genotype host range by sequencing parasite DNA from infected fish and host suitability (the ability of the parasite to sporulate within a particular host) by examining for mature myxospores. Our data revealed differences in genotype composition across the basin, confirmed the host-specific patterns of genotypes observed previously in the Klamath River (Atkinson and Bartholomew 2010a, 2010b), and further defined the host range of genotype III.

METHODS

Study location.—The Deschutes River in central Oregon flows north 278 river kilometers (rkm) into the Columbia River. A series of dams, collectively referred to as the Pelton–Round Butte Hydroelectric Project (PRB), were built between 1956 and 1964, with Round Butte Dam (rkm 177) being the ultimate migration barrier that divides the Deschutes River into an upper and lower basin. Round Butte Dam impounds Lake Billy Chinook, which is fed by the Metolius, Crooked, and upper Deschutes rivers. The former two rivers were historically inhabited by anadromous fishes, but the latter has a natural migration barrier, Big Falls. *Ceratomyxa shasta* is endemic to both the upper and lower basins (Sanders et al. 1970; Ratliff 1983). Two sentinel fish exposure locations were chosen based on accessibility and were within the present and projected anadromous salmonid range: lower basin—Deschutes River at rkm 76 (45°27′30″N, 121°04′30″W), upstream of the Oak Springs State Hatchery effluent; upper basin—Crooked River at rkm 12 (44°29′45″N, 121°17′15″W) below the Opal Springs Dam (Figure 1).

Analysis of river water samples.—Triplicate 1-L river water samples were taken at the beginning and end of the exposure period (7 and 11 June 2010) from each exposure location. Water filtration, DNA extraction, and *C. shasta* quantitative PCR (qPCR) assay were done according to Hallett and Bartholomew (2006) with an additional step of using acetone to digest the nitrocellulose filter (Hallett et al. 2012). To estimate parasite density at exposure localities, we evaluated the river water quantitative

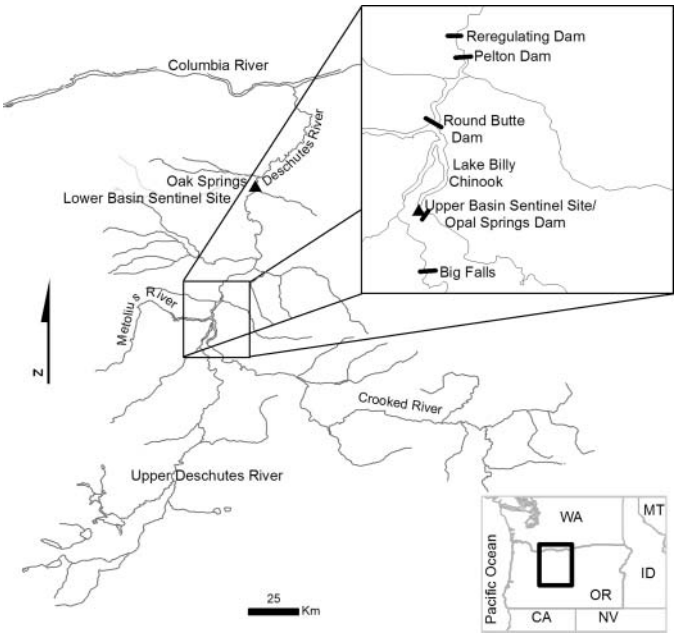

FIGURE 1. The Deschutes River basin in the Pacific Northwest showing exposure locations (triangles), migration barriers (black bars) and major tributaries.

TABLE 1. The origin (relating to the Deschutes River, Oregon) of the salmonid species used in sentinel exposures.

Species	Origin
Atlantic salmon *Salmo salar*	Nonnative
Brook trout *Salvelinus fontinalis*	Nonnative
Brown trout *Salmo trutta*	Nonnative
Cutthroat trout *Oncorhynchus clarkii*	Nonnative
Rainbow trout *O. mykiss*	Nonnative
Kokanee *O. nerka*	Native
Chinook salmon *O. tshawytscha*	Native
Steelhead (anadromous rainbow trout)	Native

cycle (Cq) results using values from previously assayed standards of 1 and 10 actinospores (Hallett and Bartholomew 2006). Parasite DNA from water samples was amplified for sequencing using *C. shasta* genotyping primers according to the protocol of Atkinson and Bartholomew (2010b). Resulting PCR products were purified using ExoSAP-IT (USB, Cleveland, Ohio) at a ratio of 5-μL PCR product to 2-μL ExoSAP-IT and sequenced at the Oregon State University Center for Genome Research and Biocomputing using an ABI Prism 3730 DNA analyzer. Sequence chromatograms were analyzed with the program 4Peaks (version 1.7.2) to determine the number of ATC repeats that define each genotype. For mixed infections, the percentage of each genotype was estimated from the average height ratios of coincident peaks at five different sequence positions (Atkinson and Bartholomew 2010a).

Fish groups and exposure.—We obtained eight salmonid species, all age 0, from Oregon Department of Fish and Wildlife (ODFW) state hatcheries (Table 1). Sizes varied between species (average weight per fish, 2–10 g) because of differences in spawning times and growth rates; however, previous studies demonstrated that salmonids of a wide range in size and age are susceptible to *C. shasta* (Zinn et al. 1977; Bjork and Bartholomew 2009). Fifty fish of each species were transported to each sentinel location in separate coolers with aeration. Species were exposed in separate cages for 5 d, 7–11 June 2010, similar to Stocking et al. (2006) but without a formalin bath treatment postexposure, then transported to the John L. Fryer Salmon Disease Laboratory (SDL), Corvallis, Oregon. A submersible temperature logger (Optic StowAway, Onset Computer Corporation, Pocasset, Massachusetts) was used at each exposure locality. Fifty control fish of each species were transferred directly from their respective hatcheries to the SDL.

Exposed and control fish were held for 60 d in separate, nonreplicated, 25-L tanks. Fish were supplied with specific-pathogen-free 18°C water and fed daily an Oregon Moist Pellet diet with 2–4% oxytetracycline (~3.5 g per 45 kg of feed; TM200, Pfizer, Atlanta, Georgia). The antibiotic reduces the chance of bacterial infection, but presumably does not affect *C. shasta* infections. We monitored the fish daily, and any moribund fish were removed and euthanized by overdose with the anesthetic tricaine methanesulfonate (MS-222; Argent Laboratories, Redmond, Washington) and counted as "mortalities." After 60 d, all remaining fish were euthanized and counted as "survivors."

Infection assessment.—For all moribund fish, clinical signs of disease were noted and fresh wet mounts from intestinal scrapes were examined microscopically at 100–250 × magnification for up to 3 min for the presence of characteristic arcuate myxospores of *C. shasta* (AFS-FHS 2010). All moribund fish were necropsied and their intestines removed and stored at −20°C. Those without myxospores were subjected to a diagnostic PCR assay. Following the methods of Stocking et al. (2006), DNA was extracted from intestines and primers specific for *C. shasta*, Cs1 and Cs3 were used to determine whether parasite DNA was present in moribund fish (Palenzuela et al. 1999). Individual fish were considered *C. shasta* positive (*Cs* +) if myxospores were identified by microscopic evaluation or if *C. shasta* DNA was amplified by diagnostic PCR; *C. shasta* negative (*Cs*−) samples were negative by both tests. Subsamples of 10 survivors per exposed and control groups were examined microscopically and molecularly similar to mortalities to determine the extent of infection. The level of susceptibility (low, moderate, or high) for each group was based on clinical signs (absent to severe) and the extent of *Cs* + mortality.

To determine if the infecting *C. shasta* genotypes were able to persist and cause mortality, we randomly sampled five fish from each group at 7 and 21 d postexposure (dpe) and froze portions of their intestines. Chinook salmon exposed in the lower basin were sampled only at 7 dpe as insufficient fish remained at 21 dpe

(26 fish escaped from their tank early in the monitoring period). Ten visually *Cs*+ fish mortalities per group were selected for genotyping; if groups had fewer than 10 *Cs*+ fish mortalities, 7 and 21 dpe samples were genotyped. As described above DNA was extracted for fish intestines and samples were genotyped similar to water samples.

Percent total mortality and *C. shasta* cumulative mortality (visual and PCR *Cs*+) were calculated as the number of mortalities divided by the starting count (minus fish sampled at 7 and 21 dpe) and multiplied by 100. To determine the mean days to death (MDD) we averaged the number of days postexposure for *Cs*+ cumulative mortalities for each group. Kaplan–Meier survivorship curves show the probability of survival at a given time and were drawn using R (version 2.9.2 for Macintosh), censoring visually and PCR *Cs*− mortalities and survivors. Survival of a species between basins was not compared because of differences in dose and genotype, or between species because of the lack of replication.

RESULTS

Water Samples

Water was ~13°C throughout the exposure at both exposure localities. At the beginning of the exposure, parasite density was higher in water samples from the lower basin (1–10 spores/L) than the upper basin (<1 spore/L). When fish were collected 5 d later, parasite densities at both exposure localities were ~1 spore/L. Genotypes O (33%) and III (67%) were identified from three upper basin water samples at the end of the exposure period. The quantity of parasite DNA in earlier samples from the upper basin was insufficient for performing the genotyping assay. Five out of six lower basin water samples were genotyped with an average composition of 89% genotype I and 11% genotype III.

Sentinel Fish: Upper Basin

Genotype III was isolated from all eight fish species and was the dominant genotype detected in all mortalities (Table 2). Genotype II was detected as a minor component (<29%) in multiple species, and genotype 0 was detected only from steelhead sampled at 21 dpe. Survivorship curves reflected the susceptibility of each species to genotypes O and III and identified categories with varying degrees of susceptibility (Figure 2).

High susceptibility.—Nonnative rainbow trout and cutthroat trout had 100% *Cs*+ cumulative mortality. Clinical signs were severe and often systemic with lesions and myxospores observed in the liver, kidney, spleen, and epidermis, and abdominal swelling was common. Brook trout also had 100% mortality, but 15 mortalities occurred prior to the onset of ceratomyxosis and were presumably caused by *Flavobacterium columnare* (based on 18°C water temperatures and clinical signs). When these fish were censored from the analysis, *Cs*+ cumulative mortality was 58%. Positive brook trout mortalities had typical clinical signs of ceratomyxosis (myxospores, swollen intestines).

TABLE 2. Species exposed in the lower and upper Deschutes River basin: total mortality (number and % morts), *Ceratomyxa shasta* positive cumulative mortality (number and % *Cs*+), mean days to death (MDD), and genotype (O, I, II, III) results from mortalities and 7 and 21 d samples. Genotypes that comprise less than 29% of the infection are underlined and in bold.

				Genotype (morts)				Genotype (7 and 21 d)			
Species	Morts (%)	*Cs*+ (%)	MDD	I	II	III	O	I	II	III	O
Upper basin											
Rainbow trout	41 (100)	41 (100)	25		**1**	9					
Cutthroat trout	38 (100)	38 (100)	24		**1**	10					
Brook trout	39 (100)	14 (58)[a]	25			12				10	
Brown trout	21 (57)	18 (49)	41		**2**	12					
Steelhead	7 (18)	4 (11)	33			2				1	3
Kokanee	3 (8)	2 (6)	33			1			**1**	4	
Atlantic salmon	7 (18)	1 (3)	36						**1**	10	
Chinook salmon	1 (3)	0 (0)							**2**	10	
Lower basin											
Rainbow trout	31 (100)	31 (100)	26		**2**	10					
Cutthroat trout	38 (100)	35 (92)	24			12					
Brook trout	39 (100)	22 (92)[a]	24	4	**1**	11		**10**		10	
Brown trout	12 (29)	6 (15)	41			4				8	
Steelhead	0 (0)	0 (0)								1	6
Kokanee	4 (9)	1 (2)	48			1			**2**	4	
Atlantic salmon	8 (21)	0 (0)							**1**	10	
Chinook salmon	19 (100)	15 (79)	19	10	**1**			4		**4**	

[a]Data were censored to exclude mortalities that occurred prior to the detection of *C. shasta* by PCR.

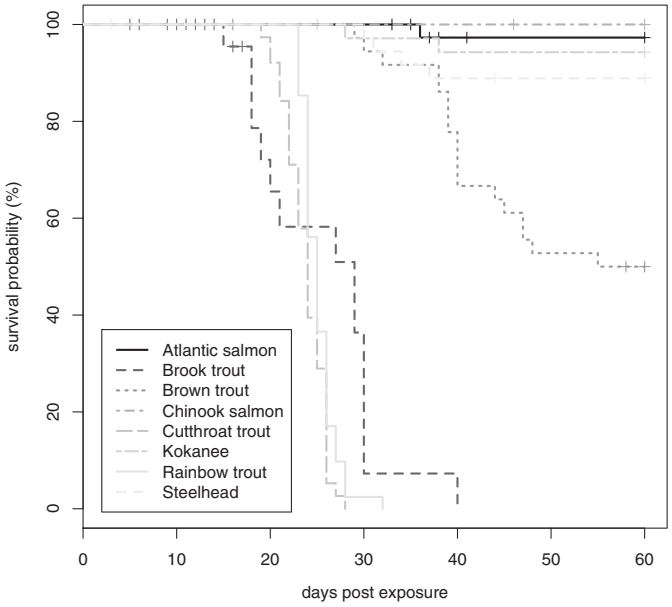

FIGURE 2. Kaplan–Meier survivorship curves of salmonids exposed to Deschutes River water at the upper basin location. Visually and PCR-assayed negative mortalities were censored and are denoted with vertical tick-marks.

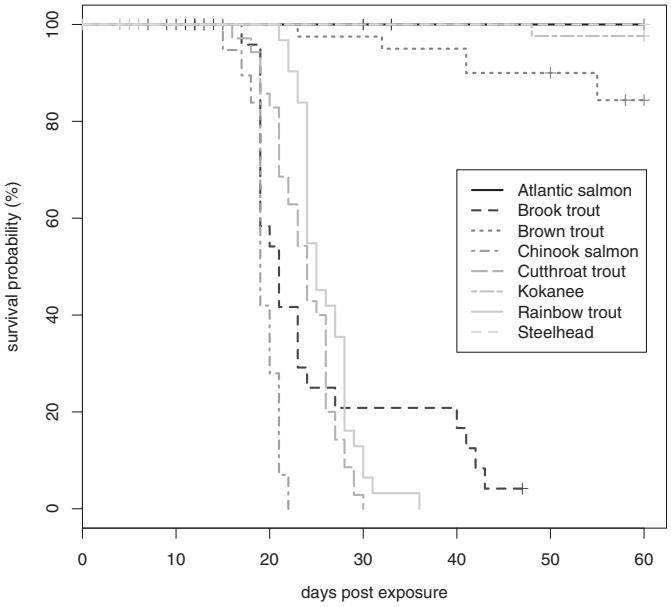

FIGURE 3. Kaplan–Meier survivorship curves of salmonids exposed to Deschutes River water at the lower basin location. Visually and PCR-assayed negative mortalities were censored and are denoted with vertical tick-marks.

Moderate susceptibility.—Brown trout had 49% *Cs*+ cumulative mortality. Mortalities had slightly swollen intestines with mostly underdeveloped or deformed myxospores visible in intestinal scrapes. Of the upper-basin survivors, only brown trout tested positive for *C. shasta* by PCR assay (20%), but myxospores were not observed.

Low susceptibility.—Steelhead had 11% *Cs*+ cumulative mortality and clinical signs were less apparent than in other species, but included myxospores and slightly swollen intestines. None of the exposed kokanee, Atlantic salmon, or Chinook salmon developed clinical signs of disease and *Cs*+ cumulative mortality was 6%, 3% and, 0%, respectively. The two visually *Cs*+ kokanee also had a heavy infection with another myxozoan in the intestines and kidneys that resembled *Myxidium minteri*. Kokanee were the only species that tested negative after 21 dpe.

Sentinel Fish: Lower Basin

Similar to the upper basin, the dominant genotype isolated from all mortalities was genotype III, except for Chinook salmon. Genotype I was predominant in Chinook salmon mortalities and was also present as a minor component in brook trout mortalities and in 7- and 21-dpe samples. Genotype O was detected only in steelhead at 7 and 21 dpe. As in the upper basin, mixed infections with <22% genotype II were detected in several species. Survivorship curves reflected the susceptibility of each species to genotypes I and III (Figure 3).

High susceptibility.—Rainbow trout and cutthroat trout were highly susceptible, with clinical disease similar to their cohorts exposed in the upper basin. Brook trout had 100% mortality, with 92% of those *Cs*+; again, there was a complicating infection with *Flavobacterium columnare* (15 mortalities prior to the first *Cs*+ detection). The *C. shasta*-infected brook trout had clinical signs of ceratomyxosis and visible myxospores. In contrast to their low susceptibility in the upper basin, Chinook salmon suffered high *Cs*+ cumulative mortality (79%) and had myxospores and swollen intestines.

Low susceptibility.—Brown trout mortality was 15% with mild clinical signs, and again, the majority of myxospores were underdeveloped or deformed. No mortality occurred in steelhead and no *Cs*+ mortality was recorded for Atlantic salmon. One kokanee mortality tested PCR positive. Again, kokanee were the only species that tested negative at 21 dpe, and brown trout were the only surviving species in the lower basin that were *Cs*+ by PCR assay (20%), with myxospores observed in 10% of fish.

Mean Days to Death

Chinook salmon exposed in the lower basin had the most rapid *Cs*+ mortality rate (19 MDD) among all groups. Brown trout from both exposure locations had the slowest rate of death (41 MDD). Only a single kokanee exposed in the lower basin died at 48 dpe. For all other species, the MDD was 24–33 (Table 2).

Controls

All control mortalities (13% of Atlantic salmon, 28.6% of brown trout, 32.4% of cutthroat trout, and 2.4% of steelhead) and the 10-fish subsets of each species were visually and PCR *Cs*−.

DISCUSSION

This Deschutes River study confirmed the host-specificity patterns of *C. shasta* genotypes observed in the Klamath River (Atkinson and Bartholomew 2010b). In both rivers, genotype I had a narrow host range, predominately infecting Chinook salmon. During this short study period, genotype I was not detected from the upper basin of either system, presumably as a result of the extirpation of Chinook salmon after construction of the dams. Similar to the Klamath River, genotypes O, II, and III were able to persist above and below migration barriers in current populations of suitable hosts. We examined the host range of genotype III, and while this genotype infected all eight salmonid species, steelhead were the only native species to develop clinical signs of disease. These results suggest native salmonids in the upper basin, such as kokanee and redband trout, would probably have low susceptibility to genotype I if the parasite was reintroduced by Chinook salmon.

Genotype I was the only parasite strain with a restricted distribution. Historically, spring Chinook salmon migrated into the Metolius and Crooked rivers of the upper basin and would have carried genotype I to their spawning grounds. Our inability to detect genotype I in the Crooked River suggests that without a suitable host, this genotype was unable to persist after fish migration was blocked by construction of the PRB and the failure of fish passage. We predict that passing spring Chinook salmon over the dam into the upper basin will eventually reestablish genotype I in the Crooked River, but this genotype presents a low disease risk for native upper-basin salmonids due to its strict host specificity. In this study, the only detection of genotype I in a species other than Chinook salmon was as a mixed infection in brook trout (a nonnative species) exposed in the lower basin. The inability to detect genotype I in fish or water from the Crooked River, despite brook trout being present in this river since their introduction in 1918, suggests this species is not a suitable host for genotype I. Previous studies presented conflicting results on brook trout susceptibility (Schafer 1968; Zinn et al. 1977), and this may reflect that this species can become infected by multiple *C. shasta* genotypes.

The presence of genotype O in the Crooked River indicated that a host other than steelhead is proliferating this genotype. Atkinson and Bartholomew (2010b) documented native Great Basin redband trout *O. mykiss newberrii* infected with genotype O in the Klamath River basin; hence, we presume that in the upper Deschutes River basin, ubiquitous, native Columbia River basin redband trout *O. mykiss gairdneri* perpetuate genotype O. In this study we exposed two *O. mykiss* forms (native steelhead and nonnative rainbow trout) and only steelhead became infected with genotype O; we did not test native redband trout. We did not observe pathological changes associated with genotype O in native *O. mykiss*, which was consistent with other studies (Atkinson and Bartholomew 2010b; Stinson 2012), although water samples had only low amounts of genotype O and this low exposure dose would be expected to have reduced pathogenic effects.

The only parasite genotype not detected in water samples was genotype II. However, low levels of genotype II were detected in many salmonid species and in both basins. No mortality was associated with this genotype despite using the same nonnative rainbow trout stock used in the Klamath River exposures where high mortalities resulted from genotype II infection (Atkinson and Bartholomew 2010a). This difference in genotype composition between the Deschutes and Klamath rivers may reflect differences in the salmonid species native to each basin. Coho salmon, the presumed natural host for genotype II (Atkinson and Bartholomew 2010b), are native and extant in the Klamath River but are extinct in the Deschutes River (Williams et al. 1991). Genotype II was documented in a wide range of hosts surveyed throughout the PNW (Stinson 2012) and could be proliferating at low levels in other salmonids residing in the Deschutes River.

Genotype III had a wide host range, infecting all native and nonnative salmonids tested, resulting in varying levels of disease. All *Cs+* mortalities, except Chinook salmon, were predominately infected with genotype III. Many of these were nonnative salmonids, representing new hosts for *C. shasta*, thus highlighting the nonspecific nature of genotype III. The only native host that died from an infection by genotype III was steelhead. The only species in which genotype III myxospores did not develop were Atlantic salmon and Chinook salmon, but even these species were positive for genotype III at 7 and 21 dpe.

Although we could not statistically compare the susceptibilities between species because of the lack of replication for the both exposure in the river and subsequent holding at the SDL, these experimental design constraints probably had minimal influence on study conclusions. Closely positioned sentinel cages have been demonstrated to receive a similar exposure dose (Ray et al. 2010) and postexposure holding conditions in this study were identical for all species. Additionally, intertank variation in disease severity should not have been a factor with *C. shasta*, which has a two-host life cycle and no fish-to-fish transmission, in contrast to directly transmissible agents that spread rapidly within aquaria, such as bacteria and viruses (Bricknell 1995).

The ability of some myxozoans to infect a wide range of hosts was recognized by Shul'man (1966), who hypothesized that the level of development within the host is a measure of the compatibility of the host–parasite relationship. Considering the development of myxospores as evidence that the parasite can complete its fish host phase, we can make some observations on the suitability of each species as hosts for *C. shasta* genotype III. Kokanee, Chinook salmon, and Atlantic salmon were unsuitable hosts as they were either able to clear the infection or no myxospores developed. Under the current study conditions, the high MDD and incomplete development of myxospores in brown trout suggest this species is also not a suitable host for genotype III. Nonnative brook, rainbow trout, and cutthroat trout were highly susceptible and suitable hosts, but they probably represent incidental hosts for this genotype. The only native species in which genotype III matured was steelhead. Thus, although there

is only evidence for one native host, genotype III has adapted to a suite of hosts.

In addition to supporting the host–parasite relationships first observed in the Klamath River, results of this study can inform stocking and fish passage plans in the Deschutes River and suggest strategies for similar river systems. The likely reintroduction of genotype I into the upper basin would present a low risk to native salmonids due to its high host specificity; a similar conclusion was made by Hurst and Bartholomew (2012) in the Klamath River. In contrast to the Klamath River, genotype III is widely distributed in the Deschutes River and infects a wide range of salmonids; thus, it could be amplified through stocking of susceptible species. We predict that passage of steelhead would not introduce novel genotypes, as genotypes O and III are already established in the Crooked River and are potentially present in other endemic areas of the upper Deschutes River basin. Ultimately, reintroduced anadromous salmonids will overlap with resident salmonids (mountain whitefish *Prosopium williamsoni*, bull trout *Salvelinus confluentus*, and redband trout) in major tributaries of the Deschutes River, but for *C. shasta*, the disease risk associated with introduction will be limited by the host specificity of each genotype.

ACKNOWLEDGMENTS

This work was funded by the Oregon Department of Fish and Wildlife (ODFW), who also provided juvenile sentinel fish. We thank the Lyle Curtis (ODWF) and Gary Lytle (Deschutes Valley Water Department) for allowing access to sentinel locations. Adam Ray, Luciano Chiaramonte, Annie Horton, Richard Holt, and Edson Adriano assisted with sentinel exposures. Stephen Atkinson and Charlene Hurst provided training in genotype assessment and valuable contributions to this manuscript.

REFERENCES

AFS-FHS (American Fisheries Society-Fish Health Section). 2010. FHS blue book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2010 edition. AFS-FHS, Bethesda, Maryland.

Atkinson, S. D., and J. L. Bartholomew. 2010a. Disparate infection patterns of *Ceratomyxa shasta* (Myxozoa) in rainbow trout *Oncorhynchus mykiss* and Chinook salmon *Oncorhynchus tshawytscha* correlate with internal transcribed spacer-1 sequence variation in the parasite. International Journal for Parasitology 40:599–604.

Atkinson, S. D., and J. L. Bartholomew. 2010b. Spatial, temporal and host factors structure the *Ceratomyxa shasta* (Myxozoa) population in the Klamath River basin. Infection Genetics and Evolution 10:1019–1026.

Bartholomew, J. L. 1998. Host resistance to infection by the myxosporean parasite *Ceratomyxa shasta*: a review. Journal of Aquatic Animal Health 10:112–120.

Bartholomew, J. L., M. J. Whipple, D. G. Stevens, and J. L. Fryer. 1997. The life cycle of *Ceratomyxa shasta*, a myxosporean parasite of salmonids, requires a freshwater polychaete as an alternate host. Journal of Parasitology 83:859–868.

Bjork, S. J., and J. L. Bartholomew. 2009. Effects of *Ceratomyxa shasta* dose on a susceptible strain of rainbow trout and comparatively resistant Chinook and coho salmon. Diseases of Aquatic Organisms 86:29–37.

Bricknell, I. R. 1995. A reliable method for the induction of experimental furunculosis. Journal of Fish Diseases 18:127–133.

Ching, H. L., and L. Parker. 1989. Experimental exposure of trout and salmon from 12 British Columbian stocks to the Myxozoan parasite *Ceratomyxa shasta*. Journal of Aquatic Animal Health 1:205–208.

Hallett, S. L., and J. L. Bartholomew. 2006. Application of a real-time PCR assay to detect and quantify the Myxozoan parasite *Ceratomyxa shasta* in river water samples. Diseases of Aquatic Organisms 71:109–118.

Hallett, S. L., R. A. Ray, C. N. Hurst, R. A. Holt, G. R. Buckles, S. D. Atkinson, and J. L. Bartholomew. 2012. The density of the waterborne parasite, *Ceratomyxa shasta*, and biological effects on salmon. Applied Environmental Microbiology 78:3724–3731.

Hurst, C. N., and J. L. Bartholomew. 2012. *Ceratomyxa shasta* genotypes cause differential mortality in their salmonid hosts. Journal of Fish Diseases 35:725–732.

Jones, S., I. Fiala, G. Prosperi-Porta, M. House, and S. Mumford. 2011. *Sphaerospora elwhaiensis* sp. n. (Myxosporea: Sphaeroporidea) from landlocked sockeye salmon *Oncorhynchus nerka* (Salmoniformes: Salmonidae) in Washington State, USA. Folia Parasitologica 58:87–97.

Nehring, R. B., and P. G. Walker. 1996. Whirling disease in the wild: the new reality in the Intermountain West. Fisheries 21(6):28–30.

Noble, E. R. 1950. On a myxosporidian (protozoan) parasite of California trout. Journal of Parasitology 36:457–460.

Northwest Power and Conservation Council. 2004. Deschutes subbasin plan. Northwest Power and Conservation Council, Portland, Oregon. Available: http://www.nwcouncil.org/fw/subbasinplanning/deschutes/plan/EntirePlan.pdf. (April 2012).

Palenzuela, O., G. Trobridge, and J. L. Bartholomew. 1999. Development of a polymerase chain reaction diagnostic assay for *Ceratomyxa shasta*, a myxosporean parasite of salmonid fish. Diseases of Aquatic Organisms 36:45–51.

Ratliff, D. E. 1983. *Ceratomyxa shasta*: longevity, distribution, timing, and abundance of the infective stage in central Oregon. Canadian Journal of Fisheries and Aquatic Sciences 40:1622–1632.

Ray, R. A., P. A. Rossignol, and J. L. Bartholomew. 2010. Mortality threshold for juvenile Chinook salmon *Oncorhynchus tshawytscha* in an epidemiological model of *Ceratomyxa shasta*. Diseases of Aquatic Organisms 93:63–70.

Sanders, J. E., J. L. Fryer, and R. W. Gould. 1970. Occurrence of the myxosporidan parasite *Ceratomyxa shasta*, in salmonid fish from the Columbia River basin and Oregon coastal streams. Pages 133–144 *in* S. F. Snieszko, editor. A symposium on diseases of fishes and shellfishes. American Fisheries Society, Special Publication 5, Bethesda, Maryland.

Schafer, W. E. 1968. Studies on the epizootiology of the myxosporidan *Ceratomyxa shasta* Noble. California Fish and Game 54:90–99.

Shul'man, S. S. 1966. *Myxosporidia* of the USSR. Nauka, Moscow-Leningrad. Translated from Russian by Amerind Publishing Company. Amerind Publishing Company, New Delhi, India.

Stocking, R. W., R. A. Holt, J. S. Foott, and J. L. Bartholomew. 2006. Spatial and temporal occurrence of the salmonid parasite *Ceratomyxa shasta* in the Oregon–California Klamath River basin. Journal of Aquatic Animal Health 18:194–202.

Stinson, M. E. T. 2012. Re-examining *Ceratomyxa shasta* in the Pacific Northwest. Master's thesis. Oregon State University, Corvallis.

Wales, J. H., and H. Wolf. 1955. Three protozoan diseases of trout in California. California Fish and Game 41:183–187.

Williams, J. E., W. Nehlsen, and J. A. Lichatowich. 1991. Pacific salmon at the crossroads: stocks at risk from California, Oregon, Idaho, and Washington. Fisheries 16(2):4–21.

Zielinski, C. M., H. V. Lorz, and J. L. Bartholomew. 2010. Detection of *Myxobolus cerebralis* in the lower Deschutes River basin, Oregon. North American Journal of Fisheries Management 30:1032–1040.

Zinn, J. L., K. A. Johnson, J. E. Sanders, and J. L. Fryer. 1977. Susceptibility of salmonid species and hatchery strains of Chinook salmon *Oncorhynchus tshawytscha* to infections by *Ceratomyxa shasta*. Journal of the Fisheries Board of Canada 34:933–936.