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## Dam Removal and Implications for Fish Health: *Ceratomyxa shasta* in the Williamson River, Oregon, USA

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### Abstract

The removal of dams on a river is one potential tool for the ecological restoration of native salmonid fishes. However, the removal of barriers also introduces risks, such as the introduction of fish pathogens into previously isolated populations. The proposed removal of four dams on the Klamath River, Oregon–California, provides an opportunity for examining the disease risks associated with dam removal. A salmonid pathogen endemic to the region, *Ceratomyxa shasta*, is responsible for high mortality in juvenile Chinook salmon *Oncorhynchus tshawytscha* and coho salmon *O. kisutch* below the dams. Above the dams, parasite densities are lower and not implicated in salmonid mortality, except in the Williamson River tributary, where high parasite densities raise concerns over the restoration of anadromous fish that are likely to take advantage of spawning habitat in that river. In the current study, baseline information on parasite density, distribution, and genotype composition in the Williamson River was gathered to determine how salmonid reintroduction might be affected by parasite dynamics. Assay of water samples highlighted two areas of high parasite density: between the mouth of the Williamson River and the confluence of the Sprague River tributary, and above the Spring Creek confluence. Despite these high parasite densities, mortality did not occur in sentinel coho or Chinook salmon. Genetic analyses of parasites from water samples and infected fish demonstrated that *C. shasta* genotype II was dominant and was associated with stocked nonnative rainbow trout *O. mykiss*. The absence of pathogenicity of this parasite genotype for Chinook and coho salmon suggests that reintroduction plans will not initially be adversely affected by the high parasite densities in the Williamson River. However, following dam removal, returning adult salmon will transport parasite genotypes present below the dams upstream. These genotypes are likely to become established and may reach densities that could affect juvenile Chinook and coho salmon.

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Anadromous salmonids require both freshwater and marine environments for the completion of their life history with freshwater environments providing critical spawning and early life stage rearing habitats. Over the last century, a variety of factors have led to the decline of anadromous salmonids in the Pacific Northwest, including habitat degradation brought about by agriculture, the raising of livestock, forest usage, urbanization, and dams. These effects have been further compounded by overharvest, disease, and ocean conditions (Fresh 1997; Mundy 1997; Percy 1997). One solution proposed for mitigation of these effects is dam removal, which would return the river to a more natural hydrograph and provide access to historically utilized habitats (Bednarek 2001).

Positive effects of dam removal on fish populations have been demonstrated in several rivers. On the Milwaukee River, Wisconsin, removal of the Woolen Mills Dam resulted in improved habitat and increased numbers of the native smallmouth bass while nonnative common carp *Cyprinus carpio* declined (Kanehl et al. 1997). Similarly, colonization of habitat post dam removal by anadromous salmonids occurred in the Mad River, California; Rogue River, Oregon; and Clearwater River, Idaho (Winter 1990). However, there are also risks associated with dam removal. The release of large amounts of sediment from reservoirs could negatively affect fish populations, especially if toxins have accumulated in the sediment (Stanley and Doyle 2003), and introduction or amplification of pathogens may

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occur as fish populations separated by the dams come into contact (Brenkman et al. 2008; Zielinski et al. 2010).

Few case studies have compared pathogen dynamics and associated disease risks prior to and following dam removal or fish passage. One study on the Deschutes River, Oregon, examined the risk of introducing *Myxobolus cerebralis* as a result of fish passage above the dam (Zielinski et al. 2010, 2011). Prepassage monitoring showed that *M. cerebralis* was established in at least one tributary below the dams, thus increasing the risk of the parasite being introduced above the dams. To improve the long-term success of fish passage, the authors recommended transfer of eggs or fry raised in pathogen-free water rather than passing naturally reared juveniles (Zielinski et al. 2010) and continued monitoring for *M. cerebralis* in areas where the pathogen was detected (Zielinski et al. 2011). On the Cowlitz River, Washington, passage of adult spring Chinook salmon *Oncorhynchus tshawytscha* above the Mayfield Dam began in 1996. Although no studies were conducted to assess changes in pathogen effects before and after fish passage, observational data suggests that prevalence of *Ceratomyxa shasta* has increased. Sentinel studies conducted in the late 1990s (shortly after reintroduction began) demonstrated a less than 10% prevalence of *C. shasta* in juvenile spring Chinook salmon upstream of the dam, while 70–84% infection prevalence occurred in juvenile spring Chinook collected from traps in 2010. In addition, mortality of spring Chinook salmon in Cowlitz Hatchery, located below the dam, has increased since fish passage (E. Ray, Washington Department of Fish and Wildlife, personal communication). A third study in the Elwha River, Washington, collected baseline information on multiple fish pathogens prior to dam removal and will also assess changes in pathogen dynamics post dam removal (Brenkman et al. 2008). The researchers hypothesize that the prevalence of endemic pathogens (such as *Renibacterium salmoninarum*) may increase above the dams and that nonendemic pathogens (such as infectious hematopoietic necrosis virus) could be introduced. In addition, pathogens that co-evolved with resident fish above the dams may be more virulent in naïve, reintroduced stocks (Brenkman et al. 2008). Thus, pathogens may be influenced by interactions between resident and reintroduced populations and could hinder salmonid recovery in spite of dam removal.

In the Klamath River, Oregon–California, dam removal and fish passage have been proposed for increasing salmonid habitat in the basin. However, the success of salmonid reintroduction in the Klamath River basin is contingent, in part, on their ability to cope with the endemic parasite *C. shasta*. This parasite causes ceratomyxosis, a disease of salmon and trout characterized by severe inflammation, hemorrhage, and necrosis of the intestine (Bartholomew et al. 1989). Infection often results in death, although fish native to rivers where the parasite is endemic are less likely to develop the disease (Zinn et al. 1977; Bartholomew 1998). *Ceratomyxa shasta* has a complex life cycle that requires two hosts, the salmonid host releasing the myxospore stage of the parasite and the polychaete *Manayunkia speciosa* releasing

the actinospore stage (Bartholomew et al. 1997). Parasite distribution is seasonal, with infection occurring in sentinel fish exposed between April and mid-December (Ratliff 1981; Ching and Munday 1984; Hendrickson et al. 1989), coinciding with the presence of adult spring Chinook salmon, the fish most likely to utilize upper basin habitat (Hamilton et al. 2005).

In the upper Klamath basin, high densities of both *C. shasta* and the polychaete host occur in the Williamson River, an area of critical spawning habitat. From the mouth of the Williamson River to the Sprague River confluence (river kilometer [rkm] 19), 90% parasite-induced mortality has been observed in nonnative (naïve) rainbow trout *O. mykiss* (RBT; freshwater form) exposed in cages in the river (Hemmingsen et al. 1988; Buchanan et al. 1989; Hallett and Bartholomew 2006; Stocking et al. 2006). Assay of water samples collected during sentinel studies in 2004 demonstrated that parasite densities in the Williamson River were similar to those in the lower Klamath River where severe disease occurs (Hallett and Bartholomew 2006). In addition, a survey for the polychaete host identified a large population at the Williamson River confluence with Klamath Lake (Stocking and Bartholomew 2007). These studies demonstrated that appropriate conditions exist for *C. shasta* to persist in the Williamson River.

Complicating predictions of how *C. shasta* will affect reintroduction plans is our changing understanding of the parasite. Four distinct genotypes of *C. shasta* (0, I, II, and III) occur in sympatry with their salmonid hosts (Atkinson and Bartholomew 2010a, 2010b) and are host-specific (Hurst 2010). Thus, the ability of the parasite to establish and reproduce is influenced by the species and strain of salmonid hosts available. In the Williamson River, anadromous salmon have been extirpated since 1917 (Hamilton et al. 2005), leaving redband trout *O. mykiss newberrii* as the only native host for *C. shasta*. However, since 1925, nonnative RBT have been stocked into the Spring Creek tributary of the Williamson River to supplement recreational fishing. Fish stocking occurs during the peak months of parasite production—from May through August—and because of their high susceptibility to *C. shasta*, these fish are assumed to die if not caught by anglers (W. Tinniswood, Oregon Department of Fish and Wildlife [ODFW], personal communication). This strategy ensures that these fish will succumb to ceratomyxosis before interbreeding with native redband trout but may also amplify certain parasite genotypes in the Williamson River.

Understanding how biotic factors such as fish host species and polychaete host densities, and abiotic factors such as temperature and management practices influence parasite dynamics is important for predicting the success of reintroduced anadromous salmonids in the upper Klamath basin. The first objective of this study evaluates the risk of disease resulting from infection with *C. shasta* in the Williamson River. The approach was to determine distribution, density, and genotype composition of *C. shasta* in fish and polychaete hosts and water samples collected throughout the Williamson River. The second objective examines how stocking practices may alter parasite dynamics. Here

the approach was to compare parasite genotype composition, parasite density, and mortality of native and nonnative sentinel fish in the Williamson River and at a site downstream in the main-stem Klamath River where stocking does not occur.

## METHODS

### Density, Distribution, and Genotype Composition of *Ceratomyxa shasta* in the Williamson River

**Study location.**—The Klamath River is 480 rkm and extends from the Williamson River in Oregon to the Pacific Ocean in California. The upper and lower portions of the river are divided by a series of five dams. Copco I was completed in 1917, blocking fish passage above rkm 314. With installation of Iron Gate dam in 1962, upstream fish passage was further restricted to the lower 307 rkm (Hamilton et al. 2005). The Klamath Basin Restoration Agreement (Klamath Restoration 2010a) and Klamath Hydrologic Settlement Agreement (Klamath Restoration 2010b) propose increasing fish habitat in the upper basin by the removal of the four lower dams (Iron Gate, Copco I and II, and J. C. Boyle) and providing fish passage facilities at the fifth dam (Keno) beginning in 2020. Historically, the ranges of spring Chinook salmon and coho salmon *O. kisutch* and steelhead (anadromous rainbow trout) extended into the upper basin (Hamilton et al. 2005), and after conditions in the above agree-

ments are met these fish are expected to repopulate their historic ranges, the Williamson River providing critical habitat.

The Williamson River is a headwater of the Klamath River basin and flows into upper Klamath Lake. This river is approximately 120 rkm and is predominately a spring-fed system with inputs from two major tributaries, Sprague River and Spring Creek, with confluences at rkm 19 and 27.5, respectively. Water sources for the Sprague River include a combination of snowmelt and cool springs, while Spring Creek is largely influenced by cool springs (Gannett et al. 2007). For this study, the main-stem Williamson River was divided into three reaches based on geomorphology. Reach 1 comprised the area from the mouth of the Williamson River (rkm 0) to the Sprague River confluence (rkm 19), reach 2 included the area between the Sprague River and Spring Creek (rkm 27.5), and reach 3 consisted of the area above Spring Creek to rkm 42. Twenty sites were selected within the three reaches: six sites in reach 1, three sites in reach 2, and seven sites in reach 3. In addition, two sites were sampled in each tributary. Sites were selected based on accessibility and spatial representation of the reaches.

**Water sampling.**—Four 1-L samples of water were collected from each site (Figure 1) in September 2008, June 2009, and May and June 2010. These months were selected because parasite density is highest from late spring to late summer (Hendrickson et al. 1989). Thermometers were placed about 6 in below the surface, and Global Positioning System latitudes

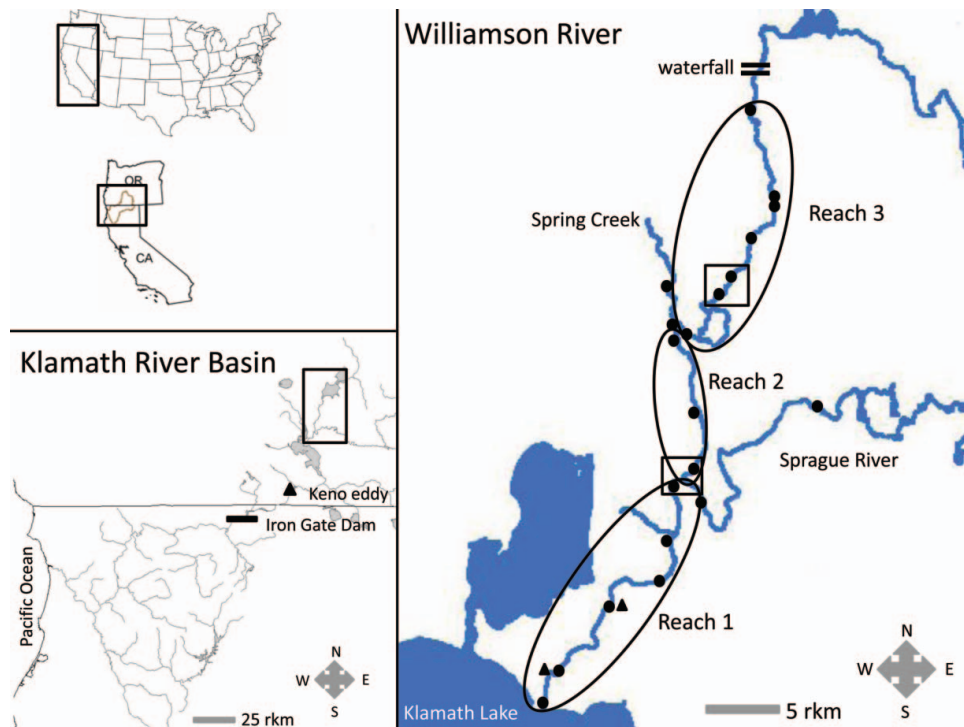


FIGURE 1. Klamath basin in relation to the United States on the left; Williamson River and tributaries on the right, where oval boundaries indicate river reaches, filled circles depict water sample sites, triangles represent sentinel fish exposure sites, and hollow boxes indicate collection sites for polychaete (*Manayunkia speciosa*). [Figure available in color online.]

and longitudes were recorded for each site. Sites were the same at all time points with the exception of rkm 36 in June 2009 to replace sites at rkm 36.3 and 36.6 as high water levels prevented access. All samples were kept on ice during transport to the John L. Fryer Salmon Disease Laboratory, Oregon State University, Corvallis, Oregon (SDL).

Water was filtered 24–48 h after collection, and the filter and its retentate were frozen at  $-20^{\circ}\text{C}$  until processed (Hallett and Bartholomew 2006). The processing procedure was modified in 2010 to use acetone to dissolve the filter membrane instead of cutting the filters, leading to an increase in the sensitivity of the molecular assay described below. We extracted DNA from three of the four replicate samples from each site during each collection time. Each of the three samples was assayed in duplicate by quantitative polymerase chain reaction (qPCR; Hallett and Bartholomew 2006), along with negative (molecular grade water) and positive controls (*C. shasta*-infected tissue), using an ABI7300 qPCR sequence detection system. Interassay variability was assessed from the standard deviations of the positive control samples. Quantitative cycle threshold (Cq) values (a measurement of parasite DNA) for the three site replicates were averaged, and tests for inhibition were conducted on one sample from each site during each collection time (Hallett and Bartholomew 2009). Samples were considered inhibited if the sample fluoresced later than the negative control in the presence of a known amount of synthetic DNA. Inhibited samples were reanalyzed with a 1:10 dilution of parasite DNA to molecular grade water if a difference of greater than one cycle occurred between the sample and the negative control. We determined parasites per liter based on a one-spore standard reference sample with a Cq value of 32.5. Sites with less than 1 parasite/L were considered negative. Parasite density threshold values of 10 parasites/L and 100 parasites/L were extrapolated from a one-spore standard and on previous correlations (Hallett and Bartholomew 2006).

*Spore stage identification.*—To determine the parasite stage (myxospore or actinospore) at locations where water samples tested positive (the qPCR assay does not differentiate between spore stages), 5 L of water were collected at rkm 18.2 and 33 in June 2009, in conjunction with the water sampling. Sites were selected based on results from water samples collected in September 2008. Samples were transported on ice to the SDL, and water was poured into separate 25-L fiberglass tanks with air stones. Ten *C. shasta*-susceptible rainbow trout (RBT; Roaring River Hatchery, Scio, Oregon) were placed in each tank. After 72 h of exposure, flow into each tank was resumed with pathogen-free water and fish were monitored daily for signs of disease. Moribund fish were euthanized using tricaine methanesulfonate (MS-222; Argent Laboratories, Redmond, Washington), and a swab of the intestinal tissue was examined under a compound microscope at  $200\times$  for the presence of myxospores. Intestinal tissues were collected for sequencing of the parasite DNA (see below). Surviving fish at 60 d postexposure were euthanized and processed as above.

*Polychaete density and infection prevalence.*—Areas surveyed for polychaetes were selected based on water sample data (parasite densities of  $> 1$  parasite/L within the area and no parasite detected above the area indicate infected host populations) and suitable polychaete habitat (fine benthic organic matter, boulders, or *Cladophora* spp.; Stocking and Bartholomew 2007). Two areas fit these criteria: rkm 18.2–19.2, which straddles the Sprague confluence (reaches 1 and 2), and rkm 33–34, above Spring Creek (reach 3). To focus our search efforts within each area, two 1-L water samples were collected  $\sim$ every 0.1 rkm and processed using qPCR as described above. Water samples from one 0.1-rkm segment within rkm 33–34 and two segments within rkm 18.2–19.2 contained parasite densities greater than 1 parasite/L. Ten substrate samples were collected from the segment within rkm 33–34 and seven samples from the two segments within rkm 18.2–19.2 on July 20–21, 2010. Within rkm 18.2–19.2, where water was too deep to wade, two divers with scuba collected benthic invertebrates. For sample collection, a scrub pad was used to dislodge benthic invertebrates and associated substrate contained within a Hess Sampler (256 cm<sup>2</sup>). Material was then collected in the sampler's 80- $\mu\text{m}$  aquatic mesh. Samples were transferred to Whirl-Pak plastic bags, immediately preserved with a 95% solution of ethanol, and placed on ice for transport to the SDL.

Preserved samples were emptied into a  $30 \times 20\text{-cm}^2$  180-D10 tray (Wildco, Yulee, Florida) subdivided into 15 sections. Three 5-cm<sup>2</sup> subsamples were randomly selected, and material within each section was collected using a disposable pipette and placed in 5-mL glass vials with a 1:3 ratio of Rose Bengal dye (20 mg/L 95% solution of ethanol) to a 95% solution of ethanol. After 24 h, the sample was filtered through an 80- $\mu\text{m}$  sieve to remove dye and then stored in a 95% solution of ethanol. Polychaetes were sorted using a dissecting microscope at  $100\times$  magnification. This procedure was repeated for all three subsamples. Individual polychaetes were placed into 1.5-mL microcentrifuge tubes with  $\sim 100\ \mu\text{L}$  of a 95% solution of ethanol and refrigerated until processing. Polychaete densities were calculated by averaging the three subsamples and extrapolating to polychaetes per square meter.

We extracted DNA from individual polychaetes according to Stocking and Bartholomew (2007) with the following modifications: DNA extraction buffer was reduced to 100  $\mu\text{L}$ , proteinase K was reduced to 5  $\mu\text{L}$ , and following the addition of RNase, sample incubation at  $37^{\circ}\text{C}$  was extended to 1 h. After boiling, pools of five polychaetes were created by adding 2  $\mu\text{L}$  of each sample into a tube (10  $\mu\text{L}$  total), then adding 2  $\mu\text{L}$  of the pooled DNA into 198  $\mu\text{L}$  of distilled water. Pooled DNA was assayed for *C. shasta* by qPCR (Hallett and Bartholomew 2006). Polychaetes from positive pools were then assayed individually by qPCR using a 1:100 dilution of the original sample to determine infection prevalence.

*Sequencing.*—We extracted *C. shasta* DNA from water samples, polychaetes, and fish intestinal tissues as above. Aliquots of 1  $\mu\text{L}$  of the final eluate for water samples, 1  $\mu\text{L}$  of a 1:100

dilution for fish samples, and 2  $\mu\text{L}$  of a 1:100 dilution for polychaete samples was used in the genotyping polymerase chain reaction (PCR) assay (Atkinson and Bartholomew 2010a). Samples were purified using ExoSAP-IT (USB, Cleveland, Ohio) and submitted to the Center for Genomics and Bioinformatics at Oregon State University for sequencing using an ABI Prism 3100 genetic analyzer. Parasite genotype was determined according to the number of trinucleotide repeats in the internal transcribed spacer region I, and genotype proportions in mixed samples were identified by comparing chromatogram peak heights (Atkinson and Bartholomew 2010a).

**Statistical analyses.**—Statistical significance was determined with an  $\alpha = 0.05$  for all tests. Temperature differences among river reaches for June 2009 and 2010 were assessed using a one-way analysis of variance (ANOVA). Significant effects were examined using Tukey's tests. The relationship between temperature and mean parasite density was examined using a polynomial regression. Both the one-way ANOVA and polynomial regression were performed using S-plus version 8.1 statistical software (Tibco, Palo Alto, California). Differences in parasite density (Cq values) among river reaches (1, 2, and 3) and sampling times (month-year combination) were examined using a nested ANOVA (PROC GLM, SAS version 9.2, Cary, North Carolina), where site was nested within reach, and month and year were tested as one variable because we were not able to sample all months over all years. Significant results were followed by Tukey's tests.

### Sentinel Fish Studies in the Upper Basin

**Fish exposures.**—Sentinel fish exposed as part of a comprehensive parasite monitoring study from 2006 to 2010 were used to assess differences in RBT mortality at rkm 7.6 in the Williamson River ( $42^{\circ}30.820'N$ ,  $121^{\circ}55.021'W$ ), a system in which stocking of RBT occurs and at Keno eddy ( $42^{\circ}8.9835'N$ ,  $122^{\circ}0.9332'W$ ), a site 47 rkm downstream of the Williamson River where stocking does not occur (Figure 1). In 2009, the Williamson River site was moved 6 rkm downstream for accessibility reasons ( $42^{\circ}29.661'N$ ,  $121^{\circ}56.095'W$ ). Nonnative RBT (Roaring River Hatchery) were held at both sites in May and June 2006–2010. Chinook and coho salmon (Iron Gate Hatchery strains, Hornbrook, California) were exposed to determine the effects of upper basin parasite dynamics on native anadromous fish strains. Fall Chinook salmon were exposed at both sites in May and June 2006–2010. Coho salmon exposures were only conducted in the Williamson River and took place in May and June 2009. Fish exposures and subsequent monitoring followed methods in Stocking et al. (2006).

**Water sampling.**—To determine parasite density during sentinel fish exposures, three 1-L samples of water were taken at the beginning and end of fish exposure at each site, except in June 2008 when water samples were only collected at the beginning of the exposures. Water samples were processed and assayed using qPCR, as above.

**Sequencing.**—To determine if parasite genotype differed between the two upper basin sites, parasite DNA was sequenced from 10 *C. shasta*-positive RBT exposed at each location in June 2006–2010. When possible, fish were selected from both the mortality and survivor groups for parasite genotype comparison. Sequencing was performed as detailed above.

**Statistical analyses.**—Differences in Cq values between sentinel sites (Williamson River and Keno eddy), months, and years were analyzed using a one-way ANOVA with statistical significance determined with an  $\alpha = 0.05$ . The data met the assumption of normality, and significant effects were tested using a Tukey's honestly significant difference (HSD) test. Sentinel fish exposures were one component of a comprehensive monitoring study, and as a result we did not have an appropriate experimental design for statistical analysis of fish mortality between sentinel sites. We used S-plus version 8.1 (Tibco) to perform statistical analyses.

## RESULTS

### Density, Distribution, and Genotype Composition of *Ceratomyxa shasta* in the Williamson River

Parasite density differed between reaches and sites, and also by month-year combination (Table 1). Parasite density was highest in reach 1, there being greater than 10 parasites/L at all sites. Densities in reach 2 were less than 1 parasite/L, except at rkm 19.2 in June 2009 where parasite densities exceeded 1 parasite/L. In reach 3, parasite densities at two sites were 1–10 parasites/L: just above Spring Creek at rkm 28 and 33 (Tukey's HSD:  $P < 0.05$ ). At the remaining five sites, parasite densities were less than 1 parasite/L (Figure 2). The month-year variable also affected parasite density, densities in May 2010 being lower than in September 2008, June 2009, and 2010 (Tukey's HSD:  $P < 0.05$ ). In both the Spring Creek and Sprague River tributaries, parasite densities were less than 1 parasite/L during all sampling times. Variability between qPCR assays was low, as standard deviations for both reference samples were 1.1 and 0.9 cycles.

Water temperatures were significantly different (Figure 2) between river reaches but did not differ between June 2009 and June 2010 (one-way ANOVA:  $F_{2,24} = 21.0$ ,  $P < 0.001$ ;  $F_{1,24} = 2.8$ ,  $P = 0.11$ ). June temperatures in reach 2 ranged from  $5^{\circ}\text{C}$

TABLE 1. Results of ANOVA for the effects of site nested within reach, reach, and month-year combination on parasite density in the Williamson River.

Source	Type III sum of squares	df	F	P-value
Site (reach)	716.68	13	8.99	<0.0001
Month-year	65.57	3	3.56	0.015
Error (subplot)	1,024.49	167		
Reach	2,216.83	2	20.16	0.0001
Error (whole plot)	715.32	13		

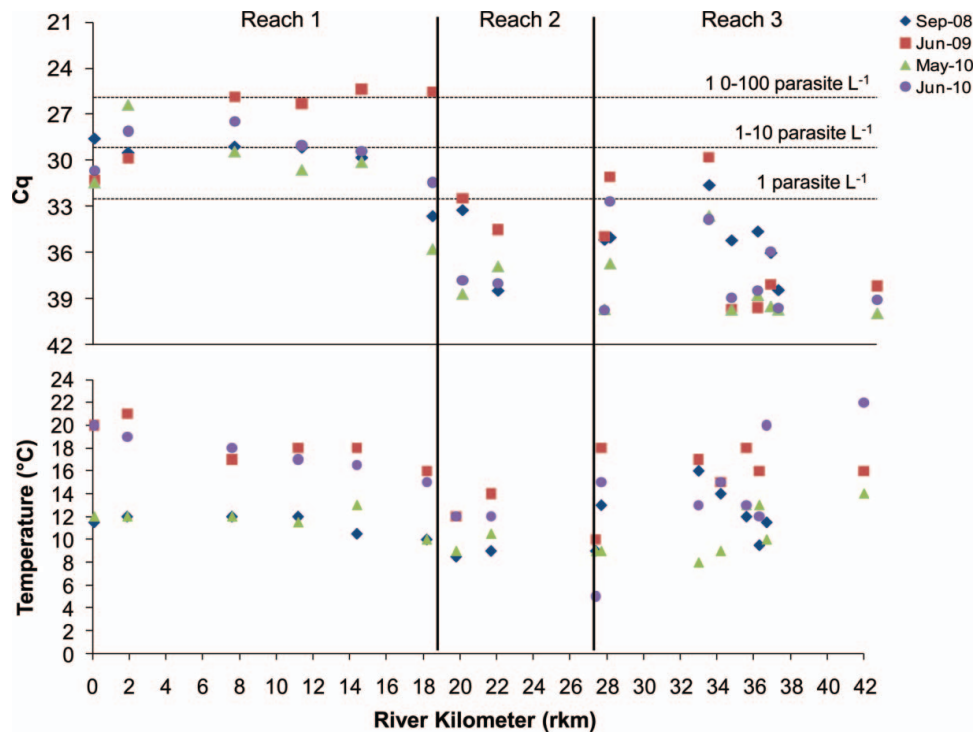


FIGURE 2. Cycle threshold (Cq) values and temperatures of *C. shasta* for Williamson River water samples collected in 2008, 2009, and 2010 by reach. Spring Creek and Sprague River confluences of the Williamson River occur at rkm 27.5 and 19, respectively, as indicated by the vertical lines. Note: two sites occur between rkm 26 and 28, the lower Cq and temperature values correlating with the site in reach 2. [Figure available in color online.]

to 14°C and were lower than temperatures in reaches 1 and 3, which ranged from 15–21°C and 12–22°C, respectively. A wider temperature range was observed across the three reaches in June (5–22°C) than in September (8.5–16°C) and May (8–14°C). Temperatures in the Sprague River were warm: 23°C in June and 16°C in May and September. Spring Creek maintained a relatively constant cool temperature of 5–8°C regardless of month. In the Williamson River main stem, parasite DNA was detected between 10°C and 21°C. A polynomial regression ( $y = 0.0641x^2 - 2.1108x + 50.144$ ,  $R^2 = 0.1976$ ,  $P < 0.001$ ) showed that approximately 20% of the variability in parasite density was explained by temperature (Figure 3).

Two of the four known genotypes of *C. shasta* were detected in the Williamson River. The proportions of parasite genotypes II (pathogenic for nonnative RBT) and 0 (associated with native redband trout) in water samples changed spatially and temporally, larger proportions of genotype 0 being detected in reach 1 than in reach 3. Genotype 0 generally accounted for 10–30% of the parasites present in water samples from all sampling times in reach 1, although in May 2010 water collected from rkm 18.2 contained 82% genotype 0. This was the only time when the proportion of genotype 0 was greater than genotype II. Genotype 0 was not detected at any sites in reach 3, except in June 2009 when it comprised approximately 10%. Parasite densities in reach 2 were below the sequencing threshold, except in June 2009 when genotype II accounted for 100% of the parasites detected.

Infection occurred in RBT held in water collected from either reach 1 (100%) or reach 3 (20%), demonstrating presence of the actinospore stage of the parasite. All fish were infected with genotype II; genotype 0 was not detected.

One polychaete assemblage was identified at rkm 18.3 in reach 1 and one assemblage at rkm 33.2 in reach 3. Polychaete density at the site below the Sprague River was calculated as  $4.08 \times 10^3$  polychaetes/m<sup>2</sup>, and infection prevalence was 1.1% ( $n = 185$ ). Only genotype II was detected in the polychaetes. In the site within reach 3, polychaete density was estimated at 78 polychaetes/m<sup>2</sup> and infection prevalence could not be determined.

### Sentinel Fish Studies in the Upper Basin

Mortality of sentinel nonnative RBT differed between the Williamson River and Keno eddy, while Chinook salmon mortality was consistently below 5% at both sites. No mortality occurred in coho salmon exposed in the Williamson River. From 2006 to 2010, mortality in RBT held at the Williamson River sentinel site during May and June ranged from 97% to 100%, and infection was from genotype II. Mortality in RBT exposed at Keno eddy ranged from 0% to 75% (Figure 4) and fish were infected with either genotype 0 (50%) or genotype II (50%), while exposure survivors were infected with genotype 0. Chinook salmon were infected with genotypes II and III (Atkinson and Bartholomew 2010a).

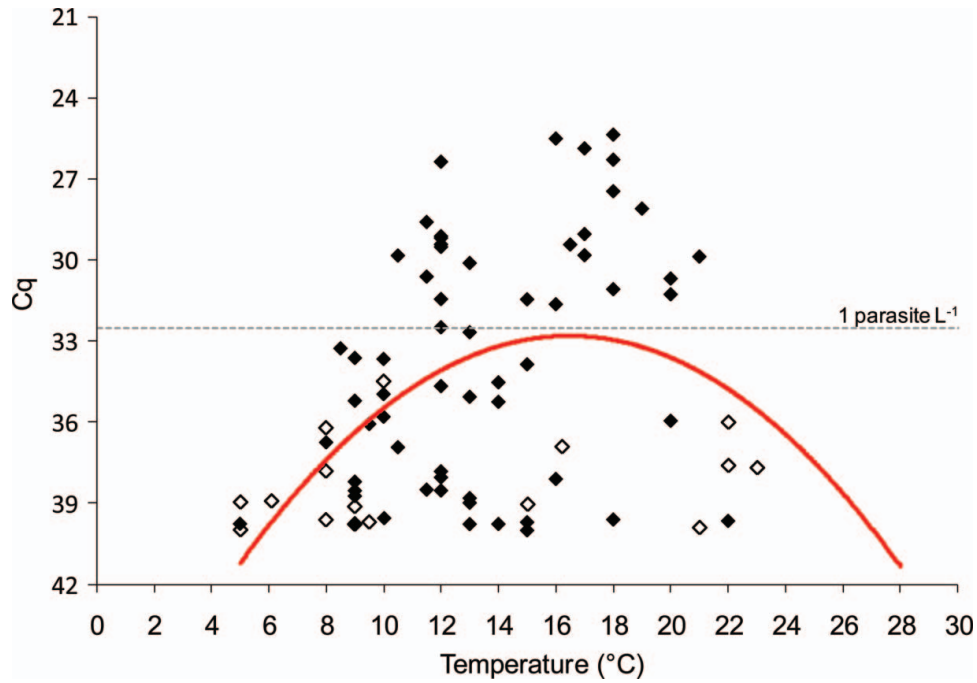


FIGURE 3. Polynomial regression plot of temperature versus *C. shasta* cycle threshold (Cq) values for all months and reaches. Main-stem Williamson River samples are indicated using filled squares, and tributary data are depicted with hollow squares. The dashed horizontal line indicates parasite production (1 parasite/L). [Figure available in color online.]

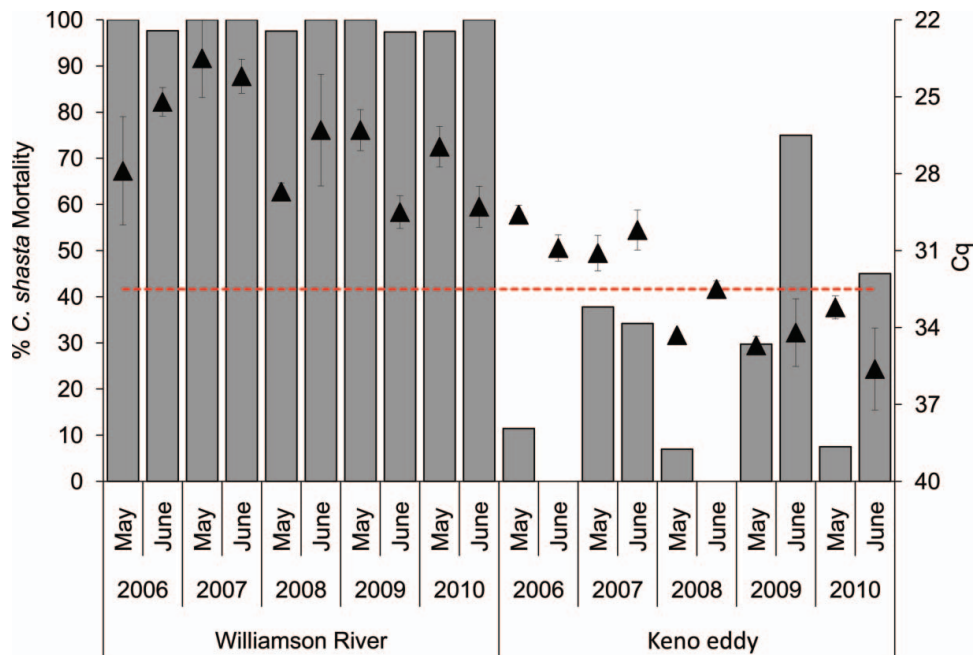


FIGURE 4. Mortality of nonnative RBT (bars) and average *C. shasta* cycle threshold (Cq) values (triangles) for the Williamson River and Keno eddy sentinel sites for years 2006–2010. The dashed line designates the Cq value that corresponds with 1 parasite/L. [Figure available in color online.]

Parasite density was affected by year and site, but we did not detect effects of month. Densities in 2006 and 2007 were higher than those in 2008–2010 (one-way ANOVA:  $F_{4, 99} = 20.2$ ,  $P < 0.0001$ ). Variation among reference samples on assay plates was up to three cycles or a 10-fold difference in parasite density and likely affected yearly comparisons in 2006–2007, whereas variation in 2008–2010 was generally less than 1 cycle and did not equate to a ten-fold change in density. Parasite density at Keno eddy was typically less than 1 parasite/L, which is 10–100-fold less than in the Williamson River (where density was usually  $> 10$  parasites/L; one-way ANOVA:  $F_{1, 99} = 232.3$ ,  $P < 0.0001$ ).

## DISCUSSION

The success of salmon restoration in the Klamath River is dependent on the availability of spawning and rearing habitat in the upper basin and on the level of exposure to pathogens that cause high mortality. In the Williamson River high densities of the myxozoan *C. shasta* reflect the influence of water temperature variability, location of polychaete populations, and movement of the fish hosts on parasite dynamics. Only two of the three parasite genotypes present in the upper basin were detected: genotype II (associated with nonnative RBT) was dominant in the Williamson River, and at Keno eddy (located 47 rkm downstream in the main-stem Klamath River) genotype 0, associated with native redband trout, was dominant. This genotype distribution likely reflects the presence of stocked nonnative RBT in the system, which has provided a new host for the parasite.

With dam removal, modification, or both, the migration of anadromous salmonids into the upper Klamath basin is likely to alter parasite dynamics by providing new hosts for existing parasite genotypes and introducing new genotypes. Chinook salmon that historically inhabited the Williamson and Sprague rivers (Hamilton et al. 2005) are the focus of reintroduction efforts (Hooten and Smith 2008). The short-term effects of the high parasite densities in the Williamson River are likely to be negligible for juvenile Chinook salmon as disease is not associated with exposure to the genotypes present (0 and II; Atkinson and Bartholomew 2010a; Hurst 2010). However, adult Chinook salmon will introduce genotype I as they migrate into new habitats (Atkinson and Bartholomew 2010a). As conditions in the Williamson River below the Sprague River confluence are already conducive to parasite propagation, this introduced parasite genotype is likely to become established. Thus in the long-term, we predict juvenile Chinook salmon that migrate through this area of the river after late May could encounter increased disease risk from *C. shasta*. Introduction of adult salmon infected with genotype I does not present a risk for the native redband trout as this species does not become diseased after exposure to this parasite genotype (Atkinson and Bartholomew 2010b).

In addition to risks from establishment of new parasite genotypes, reintroduction of anadromous fish into the upper Klamath

basin potentially allows for an expansion of the distribution of *C. shasta*. Chinook salmon historically utilized spawning habitats in both the Williamson and Sprague rivers (Hamilton et al. 2005), and the recent removal of the Chiloquin Dam on the Sprague River in 2008 is expected to increase salmonid habitat. Although *C. shasta* was not detected in the Sprague River (this study), this may have been a result of warm temperatures that may limit both host utilization of the habitat (Cherry et al. 1977) and the parasite's reproduction. However, Chinook salmon returning in the spring are likely to utilize the Sprague River when water temperatures are lower and are more favorable for both the parasite and the host.

The short-term effects of *C. shasta* on coho salmon reintroduction success are likely to be minimal. The historical distribution of coho salmon likely extended to Spencer Creek, located less than 1 rkm upstream of Keno eddy (Hamilton et al. 2005), suggesting it is unlikely these fish will migrate into the Williamson River. Even if migration into the Williamson River were to occur, the survival of coho salmon after exposure to high doses of genotype II in this study suggests that this genotype may be adapted to different host species in the upper and lower Klamath River basin. This differential pathogenicity has also been supported in laboratory challenges (Hurst 2010). The risk of disease from *C. shasta* for coho salmon migrating into the vicinity of Keno eddy is currently low based on low parasite densities and predominance of genotype 0 (Atkinson and Bartholomew 2010b). However, long-term effects of coho reintroduction would likely include the introduction and establishment of a lower-basin genotype II specific for coho salmon, potentially increasing disease risk over time.

Because of the specificity of *C. shasta* genotypes for their fish host species, the fish host(s) responsible for parasite amplification can be inferred from the genotype ratio. Native redband trout become infected with *C. shasta* genotype 0 but do not often develop ceratomyxosis (Buchanan et al. 1989; Atkinson and Bartholomew 2010b). In contrast, nonnative RBT are highly susceptible to infection and disease with genotype II (Atkinson and Bartholomew 2010a, 2010b). The low proportion of genotype 0 in the Williamson River suggests that native redband trout are not responsible for the high parasite densities. Thus, the stocking of nonnative RBT to supplement recreational fishing is the most likely explanation for the amplification of parasites in the Williamson River. This explanation is supported by higher mortality of RBT and higher density of genotype II in the Williamson River as compared with Keno eddy, where stocking does not occur. This result has prompted the ODFW to reevaluate the RBT stocking plan (Tinniswood, personal communication).

Patterns of fish movement can also be inferred from the genotype ratio. The presence of *C. shasta* genotypes 0 and II throughout the main-stem Williamson River suggests that both nonnative RBT and native redband trout are found throughout the main-stem river. Conversely, the inability to detect genotype II from water samples at Keno eddy indicates that the nonnative RBT are unlikely to migrate this distance downstream.



Following dam removal, lower basin genotypes I and II (associated with coho salmon) will likely be detected in the Williamson River and at Keno eddy as both Chinook and coho salmon migrate into this area.

Trends in parasite density and distribution are affected by temperature throughout the main-stem Williamson River and its tributaries. Water temperatures in the lower Williamson River (reach 1) are influenced by input from the Sprague River, where land use practices have decreased natural riparian shading, resulting in warmer temperatures (Boyd et al. 2002). Thus, in the Williamson River below the Sprague River confluence, temperatures rise above 10°C in April, peak in July, then continue to decline, falling below 10°C in October (USGS; <http://waterdata.usgs.gov/or/nwis/rt>; this study). Consequently, parasite densities below the Sprague River confluence were high during all sample times. Water temperatures above the Sprague River confluence were largely influenced by Spring Creek, a constant source of cold water (5–10°C) even during the summer months. Although both hosts may be present, temperatures are below the threshold of both actinospore production in the polychaete host (Bjork 2010) and parasite proliferation in the fish host (Schafer 1968; Johnson 1975; Udey et al. 1975; Ratliff 1981; Hendrickson et al. 1989). Therefore, the cool-water input from Spring Creek likely limits parasite production in reach 2.

Parasite density above Spring Creek (reach 3) may be limited by water flow, as temperatures were similar to those recorded below the Sprague River confluence. The upper portion of reach 3, at rkm 42, is ephemeral, with no flow from late summer through winter (USGS; <http://waterdata.usgs.gov/or/nwis/rt>) and reduced flows occur further downstream where polychaetes were identified. This flow variability may naturally constrain polychaete populations, which require a certain amount of flow for filter feeding and to prevent desiccation (Stocking and Bartholomew 2007; Bjork and Bartholomew 2009). Thus, water flow may restrict the range of the parasite in the upper reaches of the Williamson River.

With dam removal proposed as a tool in many restoration efforts, it is important to consider how pathogen dynamics in a system may change once dams are removed (Brenkman et al. 2008). In cases where fish passage above the dams has never occurred, resident fish have been isolated from pathogens present below the projects and may have decreased resistance. Conversely, isolated resident fish could transmit novel pathogens to reintroduced fish stocks. Thus, when considering the disease risk from an endemic parasite such as *C. shasta*, some level of parasite-induced effects should be factored into reintroduction efforts. Dam removal may be expected to reduce some pathogen risks below dams as a result of improved flows and temperature as well as increased movement of fish. However, in areas upriver of the effects of dam removal, environmental conditions may not change significantly and reintroduction may have unintended consequences. Thus, disease risks should be evaluated by establishing baseline information on pathogens above and below the current barriers and by monitoring high-risk loca-

tions, such as the Williamson River, following reintroduction. Results of this study also suggest that current management practices, such as stocking nonnative fish, should be reevaluated as restoration plans progress.

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## REFERENCES

- 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 ITS-1 sequence variation in the parasite. *International Journal of 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., J. S. Rohovec, and J. L. Fryer. 1989. *Ceratomyxa shasta*, a myxosporean parasite of salmonids. U.S. Fish and Wildlife Service Fish Disease Leaflet 80.
- Bartholomew, J. L., M. J. Whipple, D. G. Stephens, 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.
- Bednarek, A. T. 2001. Undamming rivers: a review of the ecological impacts of dam removal. *Environmental Management* 27:803–814.
- Bjork, S. J. 2010. Factors affecting the *Ceratomyxa shasta* infectious cycle and transmission between polychaete and salmonid hosts. Doctoral dissertation. Oregon State University, Corvallis. Available: [ir.library.oregonstate.edu/xmlui/handle/1957/15435](http://ir.library.oregonstate.edu/xmlui/handle/1957/15435). (December 2010).
- 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.
- Boyd, M., S. Kirk, M. Wiltsey, and B. Kasper. 2002. Upper Klamath Lake drainage total maximum daily load (TMDL) and water quality management plan (WQMP). Oregon Department of Environmental Quality, Portland.
- Brenkman, S. J., S. L. Mumford, M. House, C. Patterson. 2008. Establishing baseline information on the geographic distribution of fish pathogens endemic in Pacific salmonids prior to dam removal and subsequent recolonization by anadromous fish in the Elwha River, Washington. *Northwest Science* 82(Special Issue):42–52.

- Buchanan, D. V., A. R. Hemmingsen, D. L. Bottom, R. A. French, K. P. Currens, and F. C. Shrier. 1989. Native trout project. Oregon Department of Fish and Wildlife, Fish Research Project, Annual Progress Report F-136-R, Portland.
- Cherry, D. S., K. L. Dickson, J. Cairns Jr., and J. R. Stauffer. 1977. Preferred, avoided, and lethal temperatures of fish during rising temperature conditions. *Journal of the Fisheries Research Board of Canada* 34:239–246.
- Ching, H. L., and D. R. Munday. 1984. Geographic and seasonal distribution of the infectious stage of *Ceratomyxa shasta* Noble, 1950, a myxozoan salmonid pathogen in the Fraser River system. *Canadian Journal of Zoology* 62: 1075–1080.
- Fresh, K. L. 1997. The role of competition and predation in the decline of Pacific salmon and steelhead. Pages 245–276 in D. J. Stouder, P. A. Bisson, and R. J. Naiman, editors. *Pacific salmon and their ecosystems: status and future options*. Chapman and Hall, New York.
- Gannett, M. W., K. E. Lite, Jr., J. L. LaMarche, B. J. Fisher, and D. J. Polette. 2007. Ground-water hydrology of the upper Klamath basin, Oregon and California. U.S. Geological Survey, Scientific Investigations Report 2007-5050, version 1.1, Washington, D.C. Available: [pubs.usgs.gov/sir/2007/5050/](http://pubs.usgs.gov/sir/2007/5050/). (October 2010).
- 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 water samples. *Diseases of Aquatic Organisms* 71:109–118.
- Hallett, S. L., and J. L. Bartholomew. 2009. Development and application of a duplex QPCR for river water samples to monitor the myxozoan parasite *Parvicapsula minibicorni*. *Diseases of Aquatic Organisms* 86:39–50.
- Hamilton, J. B., G. L. Curtis, S. M. Snedaker, and D. K. White. 2005. Distribution of anadromous fishes in the upper Klamath River watershed prior to hydropower dams: a synthesis of the historical evidence. *Fisheries* 30(4): 10–20.
- Hemmingsen, A. R., D. V. Buchanan, D. L. Bottom, R. A. French, K. P. Currens, and F. C. Shrier. 1988. Native trout project. Oregon Department of Fish and Wildlife, Fish Research Project Annual Progress Report F-136-R, Portland.
- Hendrickson, G. L., A. Carleton, and D. Manzer. 1989. Geographic and seasonal distribution of the infective stage of *Ceratomyxa shasta* (Myxozoa) in Northern California. *Diseases of Aquatic Organisms* 7:165–169.
- Hooten, B., and R. Smith. 2008. A plan for the reintroduction of anadromous fish in the upper Klamath basin. Oregon Department of Fish and Wildlife, draft report, Klamath Falls. Available: [www.dfw.state.or.us/agency/commission/minutes/08/05\\_may/C\\_2\\_Draft%20Plan%20for%20the%20Reintroduction%20of%20Anadromous%20Fish%20in%20the%20Upper%20Klamath%20Basin.pdf](http://www.dfw.state.or.us/agency/commission/minutes/08/05_may/C_2_Draft%20Plan%20for%20the%20Reintroduction%20of%20Anadromous%20Fish%20in%20the%20Upper%20Klamath%20Basin.pdf). (September 2010).
- Hurst, C. N. 2010. *Ceratomyxa shasta*-related concerns for reintroduced anadromous salmonids into the upper Klamath basin California/Oregon, USA. Master's thesis. Oregon State University, Corvallis. Available: [ir.library.oregonstate.edu/xmlui/handle/1957/19652](http://ir.library.oregonstate.edu/xmlui/handle/1957/19652). (December 2010).
- Johnson, K. A. 1975. Host susceptibility, histopathologic and transmission studies on *Ceratomyxa shasta*, a myxosporidian parasite of salmonid fish. Doctoral dissertation. Oregon State University, Corvallis.
- Kanehl, P. D., J. Lyons, and J. E. Nelson. 1997. Changes in the habitat and fish community of the Milwaukee River, Wisconsin, following removal of the Woolen Mills Dam. *North American Journal of Fisheries Management* 17:387–400.
- Klamath Restoration. 2010a. Klamath Basin restoration agreement for the sustainability of public and trust resources and affected communities. Klamath Restoration, Yreka, California. Available: [klamathrestoration.gov/sites/klamathrestoration.gov/files/Klamath-Agreements/Klamath-Basin-Restoration-Agreement-2-18-10signed.pdf](http://klamathrestoration.gov/sites/klamathrestoration.gov/files/Klamath-Agreements/Klamath-Basin-Restoration-Agreement-2-18-10signed.pdf). (November 2010).
- Klamath Restoration. 2010b. Klamath hydrologic settlement agreement. Klamath Restoration, Yreka, California. Available: [http://www.kbrt.org/files/Klamath\\_Hydroelectric\\_Settlement\\_Agreement.pdf](http://www.kbrt.org/files/Klamath_Hydroelectric_Settlement_Agreement.pdf). (January 2012).
- Mundy, P. R. 1997. The role of harvest management in the future of Pacific salmon populations: shaping human behavior to enable the persistence of salmon. Pages 315–330 in D. J. Stouder, P. A. Bisson, and R. J. Naiman, editors. *Pacific salmon and their ecosystems: status and future options*. Chapman and Hall, New York.
- Pearcy, W. G. 1997. Salmon production in changing ocean domains. Pages 331–351 in D. J. Stouder, P. A. Bisson, and R. J. Naiman, editors. *Pacific salmon and their ecosystems: status and future options*. Chapman and Hall, New York.
- Ratliff, D. E. 1981. *Ceratomyxa shasta*: epizootiology in Chinook salmon of central Oregon. *Transactions of the American Fisheries Society* 110:507–513.
- Schafer, W. E. 1968. Studies on the epizootiology of the myxosporidian *Ceratomyxa shasta* (Noble). *California Fish and Game* 54:90–99.
- Stanley, E. H., and M. W. Doyle. 2003. Trading off: the ecological effects of dam removal. *Frontiers in Ecology and the Environment* 1:15–22.
- Stocking, R. W., and J. L. Bartholomew. 2007. Distribution and habitat characteristics of *Manayunkia speciosa* and infection prevalence with the parasite, *Ceratomyxa shasta*, in the Klamath River, Oregon/California, USA. *Journal of Parasitology* 93:78–88.
- 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.
- Udey, L. R., J. L. Fryer, and S. K. Pilcher. 1975. Relation of water temperature to ceratomyxosis in rainbow trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). *Journal of the Fisheries Research Board of Canada* 32:1545–1551.
- USGS (United States Geological Survey). 2010. USGS real-time water data for Oregon. USGS Water Resources, Washington, D.C. Available: [waterdata.usgs.gov/or/nwis/rt](http://waterdata.usgs.gov/or/nwis/rt). (December 2010).
- Winter, B. D. 1990. A brief overview of dam removal effects in Washington, Oregon, Idaho, and California. NOAA Technical Memorandum NMFS F/NWR-28.
- 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.
- Zielinski, C. M., H. V. Lorz, S. L. Hallett, L. Xue, and J. L. Bartholomew. 2011. Comparative susceptibility of Deschutes River, Oregon, *Tubifex tubifex* populations to *Myxobolus cerebralis*. *Journal of Aquatic Animal Health* 23: 1–8.
- 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 Research Board of Canada* 34:933–936.