

Klamath River Fishery Restoration Program

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**EFFECTS OF FLOW ON SEVERITY OF INFECTION BY *CERATOMYXA*
SHASTA IN KLAMATH RIVER FALL CHINOOK SALMON**

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Summary

Multiple years of data acquisition from several collaborating agencies have consistently demonstrated the parasite *Ceratomyxa shasta* as a major cause of mortality in migrating juvenile Klamath River salmonids. However, most of these studies were conducted during drought years with low water levels and high water temperatures that may have exacerbated infection severity and increased disease incidence. The studies described in this report investigate the effects of flow and parasite infectious dose on Klamath River Chinook salmon under laboratory conditions as well as in the field.

A laboratory based flow experiment showed that a higher water velocity (0.05 m/s) supported greater polychaete densities. However, experimentally induced polychaete infection prevalence was greater at the slower water velocity (0.01 m/s). Rainbow trout held in the slower flow treatments had a shorter mean day to death as a result of their infections than those held at the high flow. This difference indicates a higher infection severity, possibly a result of a higher parasite dose. Thus, at least under slow flow conditions, increased water velocities may decrease *C. shasta* infection severity in the fish and infection prevalence in the polychaete host.

Data from analysis of water samples show that, despite sharing both its vertebrate and invertebrate host with *C. shasta*, *P. minibicornis* has a different temporal and spatial distribution. *Parvicapsula minibicornis* was already present in low numbers in March 2006 and was detected at all five mainstem sites and in all four tributaries tested; *C. shasta* was not detected until early May. Both *C. shasta* and *P. minibicornis* were most abundant at the Williamson River and Beaver Creek, followed by Seiad Valley. Overall, QPCR assessment of water samples offers a faster, more cost effective means of detecting parasites, however, the assay has limitations that need to be acknowledged. The method can't distinguish between the two spore stages (actinospore and myxospore) or infective and dead organisms. Also, quantification is not absolute (due to cumulative handling effects) but rather provides a range of spore values. However, this test does provide a potential means for rapid evaluation of parasite levels. Field studies conducted in 2005-2006 provided an opportunity to compare the effects of increased flows on *C. shasta* infection severity with those recorded in previous low water years. In particular, heavy rains in May 2005 increased flows, resulting in decreased infection prevalence and mortality due to clinical ceratomyxosis in fall Chinook salmon and increased mean time to death for rainbow trout in the subsequent June 2005 exposure.

Investigation of the lethal infectious dose for Klamath River Chinook and coho salmon has provided some insight into differences in resistance between the species. The opportunity in 2005 to use ESA listed Klamath River coho salmon in one fish exposure study showed that native coho are more susceptible to infection than the fall Chinook. However, additional exposures with fall Chinook and coho salmon in May and June 2006 suggested that at reduced spore concentrations and/or lower water temperatures, coho salmon are as resistant to infection as the Chinook. This indicates that different infection tolerance thresholds exist between these two species and that water temperature and exposure dose play an important role. These observations were corroborated in laboratory challenges where Chinook and coho salmon did not become infected at doses as high as 5,000 parasites per fish at 12.8°C. The outcome of exposure at higher temperatures is currently being investigated.

INTRODUCTION

This study is part of ongoing investigations conducted by multiple agencies researching the epidemiology of salmonid diseases in the Klamath River with the goal of developing a baseline model that addresses factors regarding fish health. The need for this information was evident following the massive 2002 fish die-off of returning adult salmon as well as to explain the high yearly loss of juvenile fish during the last decade. For example, while intrinsic factors such as warm water temperatures and the proliferation of common parasites has been implicated in the 2002 fish die-off (CDFG 2004), more specialized pathogens have been implicated in the yearly losses of juveniles salmonids (Nichols and Foott 2006). The complex factors involved in the elevated incidence of salmonid diseases in the Klamath River below Iron Gate Dam are not well known, but are suspected to be associated with hydrologic changes stemming from past and present water use and water management practices. The first series of studies by Oregon State University (OSU) were conducted to document when and where fish become infected by using experimental exposure fish and identifying the sources of infection (Stocking et al. 2006). The USFWS focused on factors that influence mortality such as the immune response and water temperature and monitored the health of populations as they migrated out of the system (Foott et al 2004b).

The primary pathogen of concern in the Klamath River is the myxozoan parasite *Ceratomyxa shasta* (the causative agent of ceratomyxosis), which has been demonstrated to occur in both the upper and lower Klamath River Basin and has been implicated as a significant source of out-migrant mortality in fall Chinook salmon (*Oncorhynchus tshawytscha*) (Stocking et al. 2006; Foott et al. 2004a). Clinical signs of ceratomyxosis include abdominal distension caused by excessive ascites production, lethargy, loss of appetite, and swollen vent (Conrad & Decew 1966; Schafer 1968; Bartholomew et al. 1989). These symptoms develop as the parasite migrates to the lower intestinal tract causing inflammation and extensive tissue damage. In cases of severe infection, *C. shasta* can colonize other soft tissues including the spleen, liver and kidneys. Where the parasite is enzootic, effects on hatchery and wild populations of salmonids can range from minimal to severe depending on the susceptibility of the species (Zinn 1977; Bartholomew 1998), water temperature (Udey et al. 1975), and duration or frequency of exposure (Ratliff 1981; Ibarra et al. 1992). Another myxozoan parasite of concern, *Parvicapsula minibicornis*, co-occurs with *C. shasta* in the Klamath River (Bartholomew et al. 2006). Little

information exists regarding the impacts of *P. minibicornis* on Klamath River salmonids. However, it can cause kidney damage (glomerular nephritis) and infections with both parasites may have synergistic effects (Nichols et al. 2007). Interestingly, both parasites use the same fish hosts (salmonids) and the same invertebrate host (*Manayunkia speciosa*) in their life cycles (Bartholomew et al. 2006; Bartholomew et al. 1997).

An effective means for assessing the susceptibility of a species to infection is by sentinel fish exposures (Buchanan et al. 1983; Ratliff 1981). In these studies, species or stocks of unknown susceptibility are held at an endemic location with other stocks whose susceptibility has been demonstrated. Generally, the reference stock is highly susceptible to the parasite and will detect the presence of the infectious stage when the exposure dose is too low to infect the experimental species. Klamath River fall Chinook demonstrated a moderate to high level of resistance to *C. shasta*; however, parasite levels below Iron Gate Dam appeared to be high enough to overwhelm the resistance of this species, resulting in high infection prevalence (69%) and 49% mortality (Stocking et al. 2006). Within and downstream of this area, trap surveys conducted by the USFWS revealed that 45% of the fall Chinook population were infected with *C. shasta* and the majority of those infections were described as lethal (Foott et al. 2005). Additionally, 90% of these fish were infected with *P. minibicornis* (Foott et al. 2005). Analysis of the data suggested that the high incidence of myxozoan-related disease in out-migrants was more a function of infectious dose than water temperature (Stocking et al. 2006; Foott et al. 2004b). The hypothesis that spore densities were a primary contributor to mortality was strongly supported by study results obtained in 2004 from Hallett and Bartholomew (2006) who developed an assay for quantifying infectious spores in the water column and demonstrated high spore densities below Iron Gate Dam compared with other locations. Additionally, populations of the invertebrate host collected below Iron Gate Dam had a significant increase in infection prevalence of both parasites compared with those collected from other locations in the Klamath River (Stocking and Bartholomew 2007).

This report describes a series of laboratory experiments and field studies that examine the effect of parasite dose and flow on infection in the salmonid host. In laboratory experiments, Klamath River Chinook salmon were challenged with varying parasite doses to determine thresholds for infection. Laboratory flow studies were conducted with the aim of comparing the effects of two water flows (velocities) on polychaete survival and infection in both the fish and polychaete host. Continued field studies using sentinel rainbow trout and Klamath River

Chinook and coho salmon allowed us to examine the consequences of natural variations in flow types that occurred during May, June, and September of 2005 and April, May and June of 2006. This was a period during which a high flow event occurred (late May 2005) and forecasts for early spring of 2006 indicated that above average snow-pack and heavy rains would result in another high water year. This provided a natural experiment to assess how different water years and yearly infection levels correlate.

Note on report organization: This report is structured by objective, with results and discussion presented under each section.

Objective 1: Determine the *Ceratomyxa shasta* infectious dose for Klamath River fall Chinook salmon in laboratory studies

Methods

Challenge protocol. Challenge protocols varied for each species tested depending on size and parasite dose. To establish a lethal dose for our susceptible rainbow trout control, three groups of highly susceptible rainbow trout fry (Troutlodge strain, Winema, WA), averaging 2.7 cm and 0.3 g, were exposed to 1, 5, or 10 actinospores per fish in 1L of water. Additionally, rainbow trout with average length 3.8 cm and weight 0.49 g were challenged in groups of 5 or 10 in 500 ml to 10, 20, 30, 40 or 50 actinospores per fish. Challenge trials were repeated until a total of 2 groups (n=10) of fish were challenged at each dose.

Three groups (n=5) of Chinook salmon (Iron Gate Hatchery, CA) of approximately 5.3 cm and 1.7 g were challenged with 10, 50, or 100 *C. shasta* actinospores per fish. In a second challenge, Chinook salmon of the same size as the first exposure group were challenged to higher parasite doses (500 to 5000 actinospores per fish) and fish were exposed individually in 250 ml of water. For doses of 500 and 100 actinospores per fish, 10 fish were exposed. At the dose of 5000 actinospores/fish there were sufficient spores to expose only three fish. To assure the viability of the *C. shasta* actinospores, highly susceptible rainbow trout were exposed in parallel exposures, except that all rainbow trout were exposed in groups and challenged with 20 actinospores per fish.

Coho salmon (Cole Rivers Hatchery, OR) with average length 7.8 cm and weight 5.8 g, were challenged with 500 (n=10), 1000 (n=10), or 5000 (n=5) actinospores individually in 250 ml of

water under the same conditions as described for Chinook salmon.

Sham challenges in which no actinospores were added were performed with all fish species for each of the trials. All exposures were conducted in static 12.8 °C water supplied with an airstone for 2 h. After exposure, fish were transferred to 25 L tanks and maintained on 12.8 °C well water. Fish were fed a commercial diet and monitored for mortality daily for 90 days.

Detecting *C. shasta* infection. Moribund fish were euthanized with an overdose of MS222 (tricaine methane sulphate) and a wetmount of a scraping of the intestine was examined for the presence of myxospores for 2 minutes (AFS-FHS 2003). When myxospores were detected the fish was considered positive; if no myxospores were detected, a sample of posterior intestinal tissue was collected and frozen for assay by PCR. DNA extraction and PCR were carried out as described by Palenzuela et al (1999).

Results

One actinospore was capable of causing infection in rainbow trout and as few as 5 actinospores caused a fatal infection. There was a dose dependent response between *C. shasta* actinospore dose and infection and mean day to death in *C. shasta* infected susceptible rainbow trout (Figure 1.1). The infection prevalence of rainbow trout increased with increasing dose but as few as 20 actinospores are capable of causing 100% infection prevalence. The mean day to death decreased with increasing dose, however once prevalence reached 100% the mean day to death averaged 35 days regardless of actinospore dose.

No Chinook salmon died during the course of the experiment and none assayed positive by PCR. One coho salmon exposed to 1000 actinospores died 51 days after challenge and a wet mount of an intestinal scraping revealed the presence of myxospores. However no other coho salmon died during the experiment or assayed positive for *C. shasta* DNA by PCR. All sham challenged control fish were negative for infection.

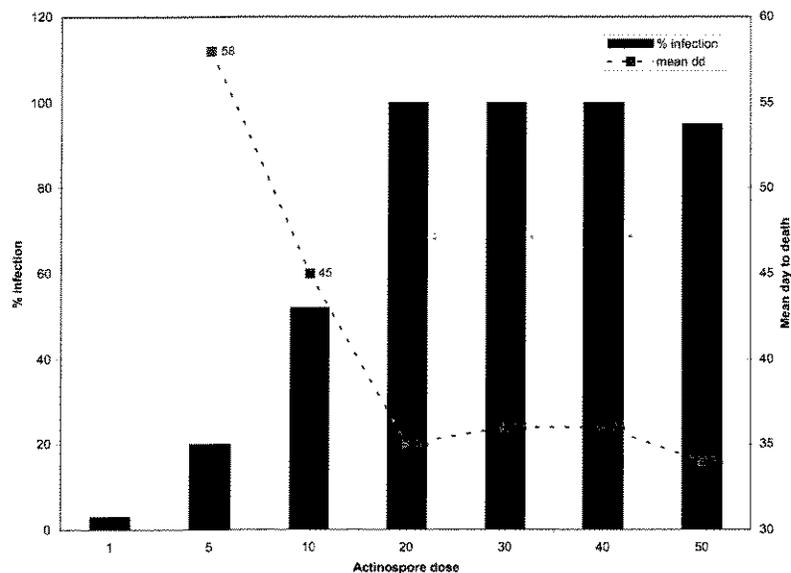


Figure 1.1. Percent infection and mean day to death of susceptible rainbow trout challenged with *Ceratomyxa shasta* actinospores.

Discussion

The exposure dose to cause infection and mortality in Klamath River Iron Gate Chinook salmon was determined to be greater than 5000 actinospores per fish when they were challenged and held at 12.8°C. In comparison, susceptible rainbow trout may be infected by as few as 1 actinospore and 5 actinospores can result in a lethal infection. A strain of coho salmon from the Rogue River system (where *C. shasta* is also endemic) appeared to have resistance similar to the Chinook salmon.

These data provide us some baseline for assessing the relationship between exposure levels measured in the field and the biological effects. However, challenges were conducted at ambient water temperatures that are lower than Klamath River temperatures during peak migration. During this time temperatures reach 18-20°C, which may have an effect on ability of the parasite to infect fish as well as on the fish to recover from the infection. Challenges are currently being repeated at these higher temperatures to determine how lethal infectious dose compares.

Objective 2. Determine the effects of flow and dilution on *Ceratomyxa shasta* infection in laboratory studies. (Note: this objective was partially funded by the California Energy Commission. Below is a synopsis of the flow data that is included in the final report for that

project; Bartholomew and Bjork 2006;

<http://animalscience.ucdavis.edu/Pulsedflow/project08.htm>)

Methods

Flow challenge protocol. Four identical stainless steel tanks (~67 cm long) were divided into 3 replicate channels (each 10 cm wide) with plexiglass following the design of Hallett and Bartholomew (submitted) (Figure 2.1). Water was supplied via a manifold behind the headwall to facilitate an even flow of water over the headwall. The spill of water over the headwall created a plunge pool with turbulent flow encompassing approximately one third of the channel. To increase the area in the channel receiving a uniform flow, this hydraulic effect was decreased by the placement of a plexiglass plate fitted with holes 9.5 cm in front of the headwall. Polychaetes and sand-silt substrate were randomly distributed to each of the replicate channels to a depth of 3 cm, and allowed to settle 4 hours prior to the initiation of water flow. Water pumped from the Willamette River supplied each tank and insured an adequate food source for *M. speciosa*. Water was supplied at ambient river temperature for the duration of the experiment, which began April 29, 2006 and was ended October 24 2006. Temperature ranged between 13.3 and 23.6 °C (USGS National Water Information System: Web Interface. <http://waterdata.usgs.gov>. Willamette River at Albany, OR site no. 14174000).

Water was supplied at 0.01 m/s to 2 of the tanks (3 replicate channels per tank) creating the “slow flow” treatment group. Water was supplied to the 2 remaining tanks (3 replicate channels per tank) at 0.05 m/s for the “fast flow” treatment group. The slow flow was selected based on the lowest measured flow in the Klamath River where polychaetes were documented in this substrate (Stocking and Bartholomew 2007). Although polychaete presence in sand-silt in the Klamath River has been documented at flows as high as 0.15 m/s, the experimental fast flow was limited by the pump volume (0.05 m/s). Field flow measurements (Stocking and Bartholomew 2007) and experimental flows were measured within 12 cm of the substrate level with a Marsh McBirney Flowmate 2000 (Frederick, MD) portable current velocity meter.

For each flow, one tank (three channels) was seeded with *C. shasta* myxospores. To create the “seeded” treatment group, a rainbow trout that had died as a result of severe ceratomyxosis was added to the head of each channel behind the eddy plate during week 3. Although the number of myxospores released from these fish may be variable, the fish were the same size, stock, and exposed under the same conditions resulting in fatal infections. Replicates were used to account

for this variability. This microcosm experiment attempts to reproduce the myxospore release rate that occurs in the natural environment. A non-infected rainbow trout was similarly added to the other tanks (three channels at each experimental flow) generating the “control” treatment group. The four treatment groups created will hereafter be identified as “slow control”, “slow seeded”, “fast control”, and “fast seeded”.

The outflow of each channel was directed to a flow-through 19 L aquarium for fish exposures. The Willamette River is known to support the life cycle of *C. shasta*, therefore an additional aquarium supplied directly with Willamette River water (Willamette River control) was added to distinguish fish infections caused by infectious particles in the Willamette River supply water from those induced by exposure to the treatment groups. Ten *C. shasta*-susceptible rainbow trout (Troutlodge strain, Troutlodge, Sumner, WA; average length 7.5 cm, weight 6.1 g) were held in each aquarium, including the Willamette River control, from experiment initiation to wk 6. At weeks 6 and 11 the fish in each aquarium were replaced with 10 susceptible rainbow trout (Shasta strain, Sinnhuber Aquatic Research Laboratory, Oregon State University, Corvallis, OR; average length 8.2 cm, weight 8.1 g and 7.7 cm and 5.7 g respectively).

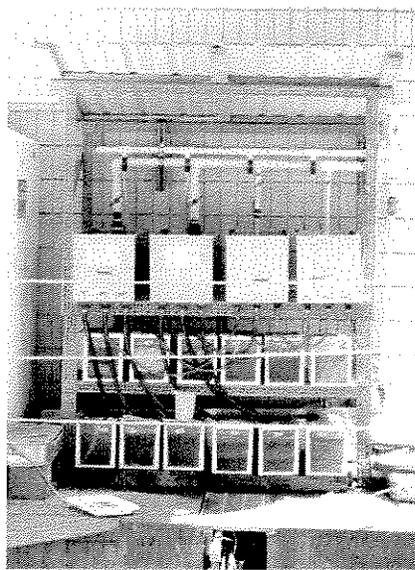
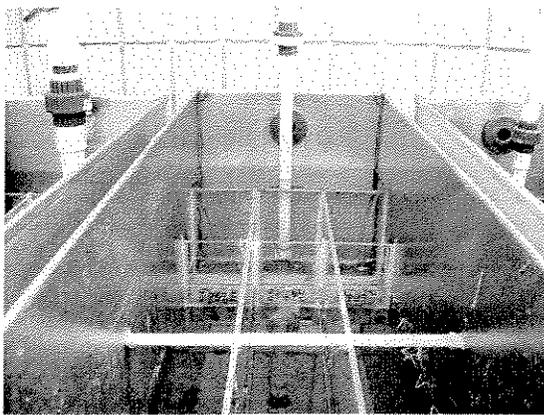
To test the effect of the treatments on infection in a *C. shasta*-resistant fish strain, Klamath Chinook salmon (Irongate strain, Irongate Hatchery, CA; average size 5.3 cm 1.7 g) were added to the aquaria in place of rainbow trout at week 16. These fish were exposed for one week, but because of loss resulting from bacterial infections, the remaining fish were transferred to SPF water and replaced with a second group from the same cohort. This group received a daily prophylactic treatment of TM-100 (4% oxytetracycline) medicated feed (Bio-Oregon, Longview, WA) and was exposed for 4 weeks. After removal from the aquaria at each time point, all surviving fish were held for 90 days in 12.8 °C SPF water. Moribund fish were euthanized with Tricaine methanesulfonate (MS222). Aquaria were disinfected between exposure groups. Dead and moribund fish were examined for *C. shasta* myxospores in a wet mount of an intestinal scraping at 200x for 3 minutes (AFS-FHS 2003). Intestinal tissue was collected from visually negative fish for assay by a *C. shasta*-specific polymerase chain reaction (PCR) (Bartholomew et al. 2004, Palenzuela et al. 1999). DNA extraction and PCR were performed as described by Palenzuela et al. (1999). Fish surviving 90 d were euthanized with MS222 and intestinal tissue samples removed and frozen until processed for analysis of *C. shasta* DNA by PCR. Fish infection prevalence was compared amongst the treatment groups and with the Willamette River control group.

To examine the effect of flow on polychaete survival, three 30 ml samples of sediment were randomly collected at the initiation of the experiment prior to the distribution of sediment to the tanks. Three 30 ml sub-samples were randomly collected weeks 6, 11, 15, and 22 from below the inflow, the middle and above the outflow of each channel of each tank. These samples were fixed in 95% ethanol for polychaete density determination and the mean polychaete density of each replicate channel was estimated.

To determine whether flow had an effect on the infection prevalence in polychaete populations exposed to *C. shasta* myxospores during the experiment, the polychaetes collected for density determination in each treatment were assayed by PCR in a pooled prevalence assay (Palenzuela et al. 1999; Stocking and Bartholomew 2007). The Ausvet pooled prevalence calculator was used to calculate infection prevalence (Sergeant 2004). Ausvet uses various sample sizes (pools) to estimate the prevalence for each replicate in the treatment groups. Pool size refers to the number of polychaetes that were pooled and assayed by PCR for presence of infection. The number of pools tested is the total number of pools for that treatment group used to determine the estimate. Infection prevalence was compared between treatment groups and over time.

Polychaete density determination. Polychaetes were quantified at 65x magnification using a dissecting microscope. The entire 30 or 50 ml samples collected from the temperature, dewatering, and flow experiments were examined. The preserved substrate was emptied into a Petri dish in 10 ml increments and modified dental tools were used to separate polychaetes from the substrate. Higher magnification was used as necessary to confirm polychaete identification. Data based on polychaete age and size was not collected.

Statistical Analysis. All statistical analysis was performed using SPlus version 7.0 (Insightful Corporation, Seattle, WA). Square root and arcsine transformations were used to provide equal variance and meet the assumptions of the statistical tests. ANOVA (with Bonferroni procedure when appropriate) tests were used to compare survival in the temperature, dewatering, and experiments and to compare percent fish mean day to death of exposed fish in the flow. Linear regression analysis was used to analyze infection prevalence trends over time.



polychaete
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polychaete

Figure 2.1. Stainless steel tanks used to test fast and slow flow conditions. 1a) The common inflow water is split and flows into a header section of each tank, then spills equally over a plexiglass divider into the three replicate channels. The outflow passes over a dam wall and down an outflow pipe. 1b) The outflow of each of the replicate channels flows into a 19 L aquarium where fish were held during the exposure period.

RESULTS

Polychaete densities fluctuated throughout the course of the experiment (Figure 2.2). At week 0, there was a mean of 49 polychaetes per 30 ml sample (SE = 5.7). The average polychaete density declined to 24 or fewer worms per sub-sample in all treatment groups at wk 6. Although these densities were not statistically different from the initiation of the experiment, mean polychaete densities were lower at the faster flows in comparison with slow flows at wk 6. This change may represent an acclimation shock prior to the initiation of the water flow. Loss of worms that disassociated from the substrate and washed out of the tanks could not be estimated due to the presence of fish held in the outflow of these tanks. By week 10, densities at the faster flows exceeded those at the slower flows, with the fast control group having the highest mean density of 60 polychaetes/sample \pm 27 (SD) followed by fast seeded with mean density 46 polychaetes/sample \pm 26 (SD), slow seeded with mean density 35 polychaetes/sample \pm 10 (SD) and slow control with mean density 28 polychaetes/sample \pm 17 (SD). The densities of all groups, except for fast control, peaked at week 10 then declined. Densities in the fast control treatment group continued to increase to a mean 103 polychaetes/sample \pm 53 (SD) at wk 15 then declined. At week 22, the mean density was less than 16 polychaetes for all of the treatment groups. Only the trends in fast control and slow control are statistically significant from each other, with the largest difference occurring at week 15 ($p = 0.017$ linear regression). Although there were no differences in the water temperature of the tanks at fast and slow flow, there was a seasonal warming of river temperature that peaked between weeks 15 and 22. The decline in polychaetes seen in week 22 may have been associated with this high temperature.

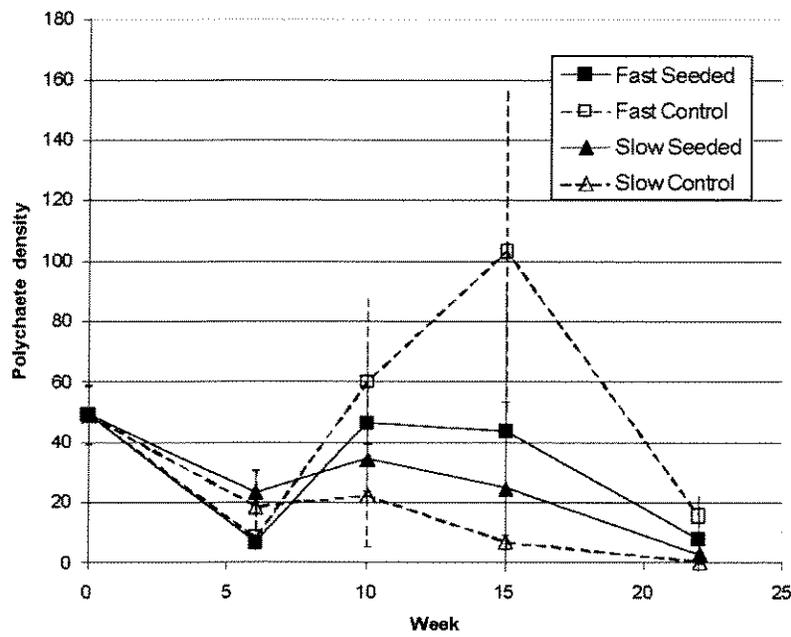


Figure 2.2. *Manayunkia speciosa* densities at slow (0.01m/sec) and fast (0.05 m/sec) water velocities, with and without addition of *Ceratomyxa shasta* myxospores. Error bars indicate standard deviation.

Table 2.1 illustrates the pool size, pool number, and number of infected polychaetes. The infection prevalence among polychaetes at week 0 was 0.7%. At wk 6, the infection prevalence for the fast control group averaged $1.3\% \pm 2.2$ (SD), $1.5\% \pm 1.3$ (SD) for slow seeded, and $2.7\% \pm 0.54$ (SD) for the slow control group (Figure 2.3, Table 2.1). No infected polychaetes were detected in the sub-samples collected from the fast seeded group at wk 6. As myxospores were added only to the seeded groups, the infection prevalence of the control groups can be considered to represent the natural range of infection in these populations. Infection prevalence in both fast and slow control groups declined to $0.4\% \pm 0.61$ (SD) and $0\% \pm 0$ (SD), respectively, at week 10. Infection in the fast seeded group peaked at $2.8\% \pm 2.6$ (SD) at this time point, and the slow seeded group increased to $2.7\% \pm 2.4$ (SD), both within the range of natural infection in these populations. Mean infection prevalence in the slow seeded group reached $14\% \pm 8.4$ (SD) at week 15, whereas mean prevalence in all other treatment groups was below 2%. This peak in the slow seeded group is the only significantly different observation amongst all of the sample periods ($p = 0.009$ ANOVA after arcsine transformation). None of the polychaetes assayed at wk 22 were positive for *C. shasta* infection.

Table 2.1. Prevalence of polychaetes infected with *C. shasta* from the Ausvet pooled prevalence calculator.

Treatment	Week	Pool size and number of pools tested (n)	Pools positive	Total polychaete	% Prevalence
None	0	1(10), 5(10), 8(1), 10(8)	0,0,0,1	161	0.7
Fast Seeded	6				
1		1(10), 4(1)	0,0	14	0
2		1(10), 4(1), 5(1)	0,0,0	19	0
3		1(10), 4(1), 5(3)	0,0,0	29	0
Fast Control	6				
1		5(3), 6(1)	0,0	21	0
2		1(2), 5(6)	0,0	31	0
3		3(1), 5(5)	0,1	28	3.86
Slow Seeded	6				
1		1(10), 5(11), 10(2)	0,1,0	75	1.21
2		1(10), 4(1), 5(6)	0,0,1	44	2.38
3		1(10), 5(10), 10(2)	0,0,0	80	0
Slow Control	6				
1		5(8)	1	40	2.64
2		2(1), 5(11), 10(4)	0,2,0	97	2.15
3		3(1), 5(6)	0,1	33	3.23
Fast Seeded	10				
1		1(10), 4(1), 5(7)	0,0,1	49	2.13
2		1(10), 3(1), 5(5(10), 10(10), 20(1)	0,1,0,0,0	193	0.55
3		1(10), 5(11), 10(11)	0,2,6	175	5.7
Fast Control	10				
1		5(10), 10(11), 20(2)	0,2,0	180	1.05
2		3(1), 5(10), 10(1)	0,0,0	63	0
3		5(10), 8(1), 10(9), 20(5)	0,0,0,0	248	0
Slow Seeded	10				
1		1(10), 3(1), 5(10), 10(5)	0,0,4,0	113	3.82
2		1(10), 5(10), 9(1), 10(7)	0,3,0,2	139	4.39
3		1(10), 5(11)	0,0	65	0
Slow Control	10				
1		1(1), 5(2)	0,0	11	0
2		2(1), 5(10), 10(3)	0,0,0	82	0
3		5(10), 10(6)	0,0	110	0

Table 2.1. Continued

Treatment	Week	Pool size and number of pools tested (n)	Pools positive	Total polychaete	% Prevalence
Fast Seeded	15	1(10), 4(1), 5(11),	0,0,0	69	0
		1(10), 5(3),	0,0	25	0
		1(10), 5(20),10(5)	0,2,0	160	3.42
Fast Control	15	5(11), 10(10), 20(5)	0,1,0	255	0.4
		3(1), 5(10), 10(9)	0,1,0	143	0.7
		5(10), 10(10), 20(5)	0,1,1	250	0.9
Slow Seeded	15	1(10), 3(1), 5(22), 10(5)	0,1,5,2	173	5.28
		1(20), 3(1), 5(1)	2,1,1	28	16.3
		1(21), 2(1)	5,0	23	21.74
Slow Control	15	4(1), 5(5)	0,0	29	0
		5(4)	0	20	0
		5(3)	0	15	0
Fast seeded	22	1(1)	0	1	0
		1(10), 3(1), 5(9), 10(1)	0,0,0,0	68	0
		1(2)	0	2	0
Fast Control	22	2(1),10(3)	0	32	0
		10(7)	0	70	0
		10(4)	0	40	0
Slow Seeded	22	1(4)	0	4	0
		1(8)	0	8	0
		1(10)	0	10	0
Slow Control	22	2(1)	0	2	0
		4(1)	0	4	0
		0	0	0	0

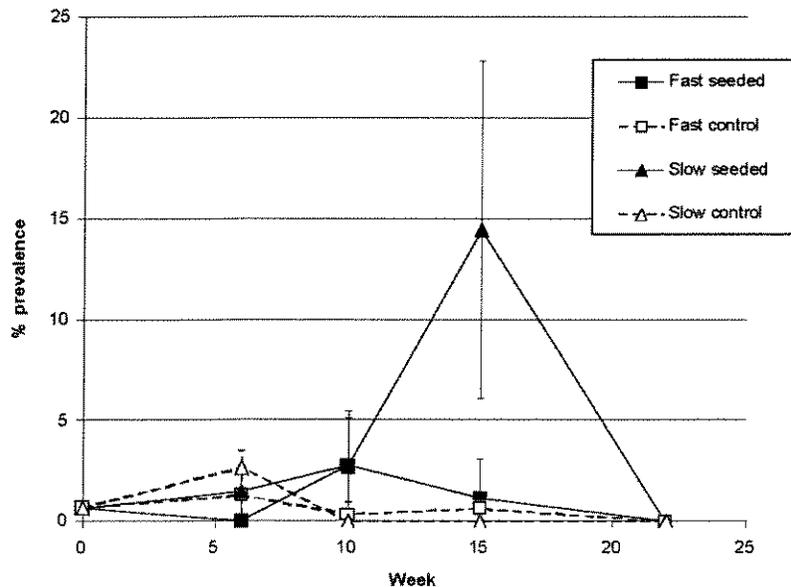


Figure 2.3. *Manayunkia speciosa* infection prevalence at slow (0.01 m/sec) and fast (0.05 m/sec) water velocities, with and without the addition of myxospores at three weeks. Error bars indicate standard deviation.

Susceptible rainbow trout became infected during all exposures and mortality was high in all exposure groups and exposure periods (Table 2.2). None of the Chinook salmon died from

ceratomyxosis and none assayed positive for *C. shasta* by PCR. Due to fish loss through escape (through the standpipe or tank lid), the number of fish assayed by PCR did not equal ten for all groups. To ensure that exposure time was equal for the groups compared; only groups that had at least 5 surviving fish at the time of the first *C. shasta* mortality were included in the analysis. Only 1 fish held in the Willamette River control tank during weeks 1-6 was positive by PCR. The infection prevalence in this group (10%) was significantly lower than the treatment groups (100%) for the first exposure period ($p < 0.001$ one way ANOVA with Bonferroni procedure on arcsine transformed data) and no Willamette River exposed fish became infected after the first 6 wk exposure.

All of the susceptible rainbow trout exposed during the first 6 weeks were *C. shasta* positive. In weeks 6-10, the infection prevalence of rainbow trout exposed in the fast seeded treatment (82%) was significantly less than fish exposed in the other treatment groups during the same time (99.6 to 100%) in the other exposure groups ($p = 0.014$ from ANOVA after arcsine transformation). Mortality between groups exposed during weeks 11 to 15 was not significantly different ($p = 0.09$) although the slow flow groups experienced 19% higher mortality on average than the fast flow groups.

The mean day to death of susceptible fish exposed to both slow flow treatments was significantly lower than those exposed to the fast flow during all exposure periods ($p < 0.001$) (Table 2.2). In the first exposure period, fish exposed in the slow flow treatment groups died an

Table 2.2. Percent *Ceratomyxa shasta* infection \pm SD and mean day to death \pm SD of susceptible fish during the first three exposure periods. Data represent average for triplicate group

	wk 1-6		wk 6-10		wk 11-15	
	% Infected	Mean Day to Death	% Infected	Mean Day to Death	% Infected	Mean Day to Death
Fast seeded	100 \pm 0	51.5 \pm 0.7	81.7 \pm 10.1	26 \pm 0	74.2 \pm 13.8	21.3 \pm 1.5
Fast control	100 \pm 0	47 \pm 1.4	96.7 \pm 5.8	27.7 \pm 0.6	66.7 \pm 57.7	24 \pm 1.4
Slow seeded	100 \pm 0	38.6 \pm 1.5	100 \pm 0	23.7 \pm 1.2	96.7 \pm 5.8	17.7 \pm 2.1
Slow control	100 \pm 0	38.6 \pm 2.1	100 \pm 0	23.7 \pm 2.1	88.7 \pm 9.8	17.7 \pm 0.6
Willamette Slow	10	*	0	*	0	*
Willamette Fast	0	*	0	*	0	*

* terminated at 90 days

average of 11 days earlier than the fast flow exposure groups. For the second and third exposure periods this difference decreased to 3 and 4 days, respectively. Differences between the seeded and control treatments at either flow were not significantly different.

No Chinook salmon succumbed to *C. shasta* infection during either a 1 week or 4 week exposure. No Chinook assayed were positive by PCR.

DISCUSSION

In the experimental channels, flow had a significant effect on polychaete survival with higher mean polychaete densities occurring in the fast treatment groups at 0.05 m/s than at the slow rate of 0.01 m/s. This finding is consistent with the peak densities found in sand-fine benthic (FBOM) organic matter at 0.05m/s in the Klamath and Ottawa rivers (Stocking and Bartholomew 2007, Mackie and Qadri 1971). The 0.05 m/s flow is a modest flow when considering the range at which the *M. speciosa* has been reported (0.01 to 0.15 m/s in FBOM and 0.01 to >0.3 m/s in *Cladophora* sp.) (Stocking and Bartholomew 2007). This flow is apparently high enough to transport nutrients and carry away wastes without disturbing the habitat substrate. Although *M. speciosa* did survive in the low flow treatment groups, peak densities were not as high as in the fast flow groups. Polychaete density at the slow flow was greater than the fast flow groups only at wk 6 when all of the populations appear to have been experiencing a decline, perhaps as a result of acclimation to experimental conditions. Thereafter, the fast flow treatment groups had higher mean polychaete densities at all time points, indicating that reproduction was occurring in these groups.

Polychaete infection prevalence was affected by flow, with prevalence significantly higher (average prevalence 14.4%) in the slow seeded flow treatment 12 weeks after the addition of spores. Infection prevalence in all other treatments ranged from 0-3.86%, and there was no significant difference between the controls and seeded group at the fast flow. Variation within replicates of the slow seeded treatment at 15 weeks was high, but this may be a reflection of the natural variation in the rate of myxospore dispersal from a dead fish. Although this relationship could be clarified by seeding with an exact number of myxospores (to deliver an equal dose at the same time), a whole infected fish was chosen for this experiment to deliver a steady dose of myxospores, as a more accurate representation of the natural release that would occur in the river.

Infection in susceptible fish exposed over the course of the study was high when exposed at either flow and at all levels of polychaete infection prevalence. Because different species, strains, and sizes of fish were exposed at each time period, fish infection cannot be compared across time. However, mortality as a result of *C. shasta* infection was high among all groups of susceptible rainbow trout, and Irongate Chinook salmon were resistant to infection regardless of exposure time. The Willamette River was not a significant contributor of actinospores or myxospores and therefore *C. shasta* infections in both *M. speciosa* and exposed fish can be attributed to the conditions of the experiment.

The high mortality that occurred in all rainbow trout exposure groups is a result of the natural background infection prevalence of *M. speciosa* (0.7 %) and the high susceptibilities of the fish. Mortal infections in susceptible rainbow trout occur at a dose as low as 5 actinospores (Obj 1). Thus, it is not surprising that rainbow trout became infected in the non-seeded groups due to this background infection prevalence.

Chinook salmon were held in treatment outflows in effort to see a dose response effect.

However, no Chinook salmon succumbed to *C. shasta* infection during either a 1 week or 4 week exposure. Infection prevalence was measured at 90 days after exposure, possibly enough time for the fish to have cleared the infection. Based on the polychaete density and infection prevalence, it is estimated that an average of 207 infected polychaetes were present in the slow seeded treatment group at week 15. Although infection severity and actinospore maturity in the polychaetes may vary, a single heavily infected polychaete may produce more than 200 actinospores (personal observation). When this estimate is applied to the average number of infected polychaetes, it indicates that Chinook salmon may be resistant to as high as 8,200 actinospores per fish.

Although there was no difference in the number of rainbow trout infected between treatments, the mean day to death for fish exposed in the slow flow treatment was lower than for fish exposed in the fast flow. This suggests these fish received a higher infectious dose or experienced greater stress reducing their ability to resist infection. An increased intensity of parasite infection at slow flows has been demonstrated in field (Vincent 2002) and laboratory (Hallett and Bartholomew, submitted) studies in both fish and annelid hosts of *Myxobolus cerebralis*. Vincent (2002, 2003) attributed the inverse relationship between flow and intensity of infection to a dilution effect of the infectious agent in large volumes of water as would occur at

high flow rates.

Objective 3. Determine how parasite numbers detected in the Klamath River between Iron Gate dam and Orleans relate to the lethal infectious dose.

Methods

Collection of field water samples. Three groups of water samples were collected. The first ('sentinel samples') were obtained in coordination with the 2006 fish sentinel exposures in April, May and June. The second group ('ISCO samples') was obtained at set intervals focusing on one site, Beaver Creek (in the Klamath River above the Beaver Creek confluence, Rkm 259.1). For this collection, the USGS provided two ISCO 3700 portable samplers that collected two 500 ml samples with a single purge cycle every 2.0 hrs over a 24 hr period during the May and June 2006 exposures. The third set ('tribal samples') were collected bi-weekly from March through August by the Karuk and Yurok tribes at five mainstem sites below Iron Gate Dam: R-Ranch (Rkm 300.2), Beaver Creek (Rkm 259.1), Seiad Valley Gage (Rkm 207), and Tully Creek (Rkm 61.9). Samples were also collected at monthly intervals at the mouths of four tributaries: Shasta River, Scott River, Salmon River, Trinity River.

Sentinel and tribal samples were collected in triplicate at each site. The water samples were filtered and assayed by quantitative PCR (QPCR) using methods described by Hallett and Bartholomew (2006). These were modified to deal with increased levels of inhibition present in 2006 water samples and to detect both myxozoan parasites, *C. shasta* and *Parvicapsula minibicornis*, simultaneously (see methods below). The amount of target DNA (i.e. 18S rDNA gene) present in a sample is visualized as a Ct-value, which can translated to the number of parasite spores present using a standard curve. The QPCR machine had been upgraded since determination of the published 2004 standard regression formula, thus this formula was re-calculated for 2006 samples from a combination of the original and new reference standards.

Note that the three duplicate samples for each sentinel site in May 2006 were inadvertently combined and thus there is only a single value (not a mean) without a standard deviation. A Ct value of 42 was designated when there was no detection of parasite.

Quantification of *C. shasta* and *P. minibicornis* spores in water samples. Environmental water sample collection, filtration and analysis protocols were developed in 2004 to detect and quantify *C. shasta* in the Klamath River. Samples were collected along the length of the river from the Williamson to the mouth of the Trinity in conjunction with placements of sentinel cages to investigate spatial distribution of the parasite and temporal density patterns. The methods and our findings were published in *Diseases of Aquatic Organisms* in 2006. Briefly, the procedure used is as follows.

Water samples were filtered through a 5µm cellulose membrane filter (Millipore) within 24h of collection. The filter disc was then folded to fit into a 2.0mL microfuge tube and frozen. Prior to extraction using the Qiagen DNeasy protocol for animal tissues, the filter paper was cut into strips and lysed with 360µL ATL buffer with final elution in 120µL buffer AE.

In the QPCR assay, the 20µL reaction volume comprised 800µM of each primer (Applied Biosystems, CA), 200µM of the probe (Applied Biosystems), 10µL TaqMan Universal PCR Master Mix (Applied Biosystems), 0.5µL stock BSA (10mg/mL; final concentration of 250ng/µL) and 1µL DNA template. Reactions were performed in an ABI PRISM 7300 or 7500 Sequence Detection System located in the Center for Gene Research and Biotechnology (OSU) in ABI MicroAmp AE optical 96-well reaction plates, with 50 cycles of standard thermal cycling conditions. A positive control (parasite DNA) and a negative control (non-template = MG water) were included in each reaction run. Results of the QPCR were interpreted against a standard curve developed using known parasite numbers.

In 2005, the CA/NV Fish Health Center developed an assay for the detection of *P. minibicornis* in tissue samples which we trialed with the water samples. However, the discovery of different genetic strains of *P. minibicornis* (S. Atkinson, Oregon State University, unpublished data) and problems with specificity and sensitivity of the assay when used with water samples required redesign of the primers and probes and reassessment of this new combination for use with water samples. The MGB FAM labelled probe is: 5' TGTCCACCTAGTAAGGC; the forward primer: 5' AATAGTTGTTTGTCGTGCACTCTGT; and the reverse primer: 5' CCGATAGGCTATCCAGTACCTAGTAAG which produce an amplicon of 72 bases. We prepared reference standards of single actinospores, freshly obtained from infected polychaetes, which were added directly to sample tubes and also onto filter paper. These references were processed as per the water samples for direct comparison.

The original water extraction protocol and *C. shasta* QPCR assay required modification in 2006 to deal with the higher levels of inhibitors in samples which impeded the DNA analyses and thus detection and quantification of the parasites (Figure 3.1). Inhibitors (impurities such as humic acid and tannins) can partially to totally interfere with the QPCR assay such that final parasite values are reduced or even absent. River conditions vary considerably from site to site and thus each site must be individually assessed for the presence of inhibitors and the final parasite values adjusted accordingly. In 2004, inhibitors were present in relatively few samples and these could be repurified using a kit (Qiagen) and then rerun. In later years, however, most samples showed some degree of inhibition and the repurification method proved too labor intensive and costly. Thus we investigated alternative procedures to minimize inhibition.

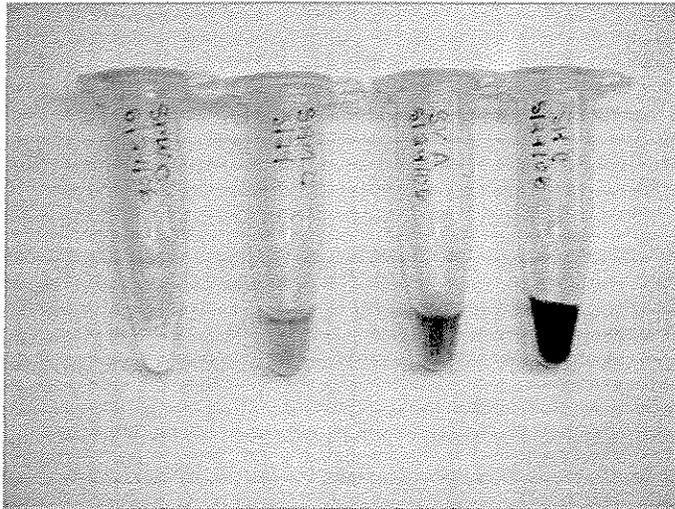


Figure 3.1. Four water samples collected in 2006 from the Klamath River showing a range in inhibition levels which are proportional to the level of discoloration of extracted, purified DNA (sample processed with QIAGEN DNeasy tissue kit). The sample on the left is uninhibited whereas the far right sample is totally inhibited in a QPCR and must be processed further.

The first method we tested for reducing the effects of inhibition was addition of the chemical GeneReleaser (BioVentures Inc.). We assessed its effectiveness at a range of concentrations and included water as a control. The product was mixed with extracted DNA and the sample incubated, centrifuged, and the ‘cleaned’ supernatant used in subsequent QPCRs. A

ratio of sample to GeneReleaser of 1:6 was most effective at reducing inhibition, however this concentration affected the Internal Positive Control (IPC; see below). Surprisingly, dilution with water proved most effective. We therefore further assessed dilution of samples to find the balance between sufficient reduction of inhibition and sufficient presence of parasite DNA to enable detection. Using 4 μ L of a 1:40 dilution of the stock DNA eluate (120 μ L) proved successful for *C. shasta*. However, this approach was unsuitable for *P. minibicornis* because each spore possesses fewer copies of the target 18S rDNA gene. We are therefore pursuing alternative methods (new chemicals and extraction kits) suitable for both myxozoan parasites.

Until recently, we ran three separate QPCR assays: one assay to detect each parasite and a third assay to assess inhibition. Reducing the number of assays would reduce time to obtain data, cost and labour. Therefore, another avenue we pursued in 2006 was the development of a multiplex assay that would enable simultaneous testing for both parasites and inhibition. Unfortunately, use of a triplex QPCR assay would jeopardise our quantification capability (consultant recommendation, Applied Biosystems), especially at the lower concentrations relevant to levels seen in water samples, and we were directed instead to focus on a duplex assay: either Cs / Pm, Cs / inhibition, or Pm / inhibition. We assessed all three combinations, adjusting primer and DNA concentrations.

To assess inhibition, we used a commercially available primer and probe combination (Internal Positive Control, Applied Biosystems). This acted as both an assay positive control and inhibition indicator. Previously, if a sample gave no reading, it could be either because no parasite was present or the reaction was inhibited. We experimented with different combinations and concentrations of the three primer and probe sets and different concentrations of the target DNA. We found that *C. shasta* was incompatible with either *P. minibicornis* or the IPC. When used in combination with one of these other sets, *C. shasta* Ct values differed to those obtained when *C. shasta* was used alone and there was no consistent relationship that would allow us to use a correction factor. *Parvicapsula minibicornis*, on the other hand, performed equally as well in a duplex assay with the IPC (at a reduced concentration of 0.5 strength) as it did in its singleplex assay. Thus, we used the Pm and IPC assay on a subset of samples (sample B from each site) to determine inhibition, followed by dilution of the samples if necessary prior to *C. shasta* assessment. The final Ct values were then adjusted for both dilution and inhibition. To adjust for inhibition, the difference between the IPC Ct values of the pure water controls and

water samples is subtracted from the water sample parasite Ct values; i.e. if the difference is 1.5 cycles and the parasite Ct value is 35, then the final value adjusted for inhibition is 33.5.

The reduced concentration of the IPC renders it more susceptible to inhibition but also to increased concentrations of other organisms, including our target parasites. We determined the threshold *P. minibicornis* concentration for the IPC was exceeded by samples fluorescing before 26 cycles and which therefore contained more than 1000 actinospores (extrapolation of 1 spore reference). This effect is outside the usual detection levels of the parasite which have never been less than Ct 28. Samples presenting low Ct values and abnormal IPC values are diluted 1:100 and rerun.

Results and Discussion

Methodology. The new *P. minibicornis* assay was sensitive enough to detect 1 actinospore: 1 μ L of extracted DNA (120 μ L eluate) of the filter paper samples fluoresced at 37/38 cycles (Figure 3.2) whereas 1 μ L of extracted DNA (120 μ L eluate) from spores placed directly into the sample tube fluoresced at 32/34, indicating a 10-fold loss in DNA caused by the necessary filtration method for environmental samples. A dilution series showed that the detection limit of the assay is about 40 cycles.

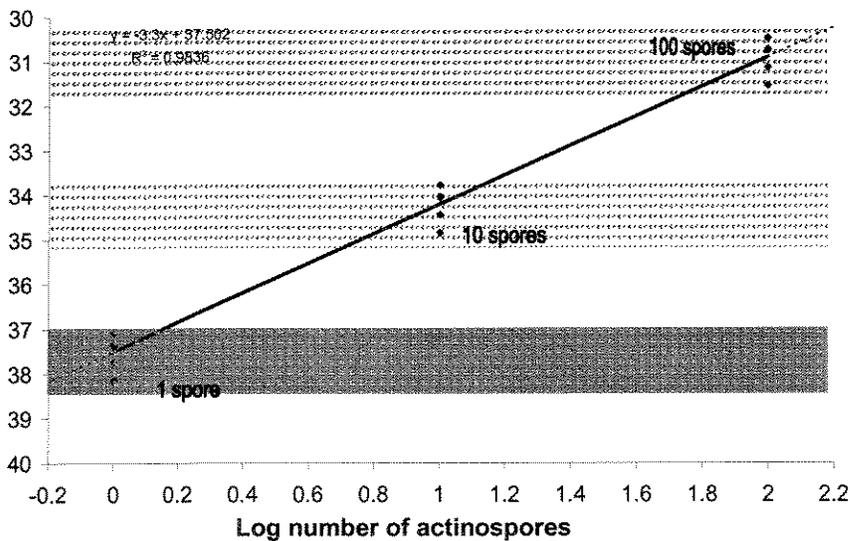


Figure 3.2. QPCR standard curve for *Parvicapsula minibicornis* in water samples. The

experimental values for one spore/liter are extrapolated by the theoretical cycle threshold difference of 3.3 for a 10-fold difference in DNA content to give the projected 10 and 100 spore values.

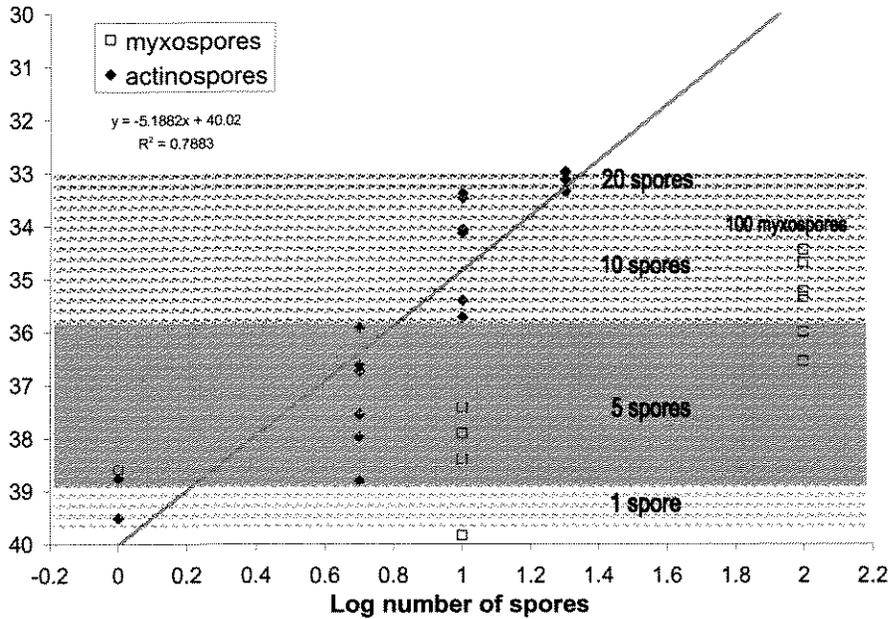
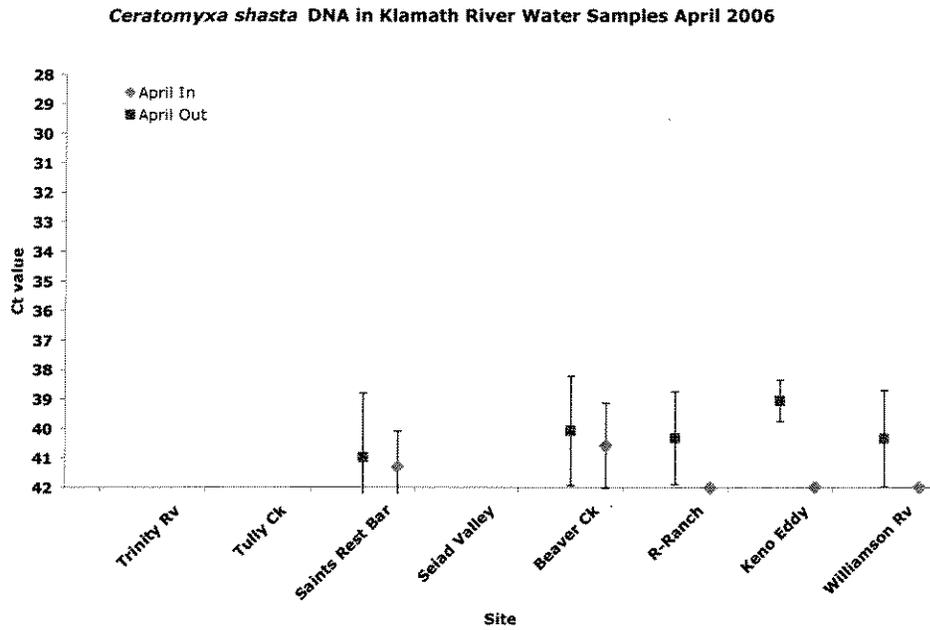


Figure 3.3. QPCR standard curve for water samples containing known numbers of *Ceratomyxa shasta* spores.

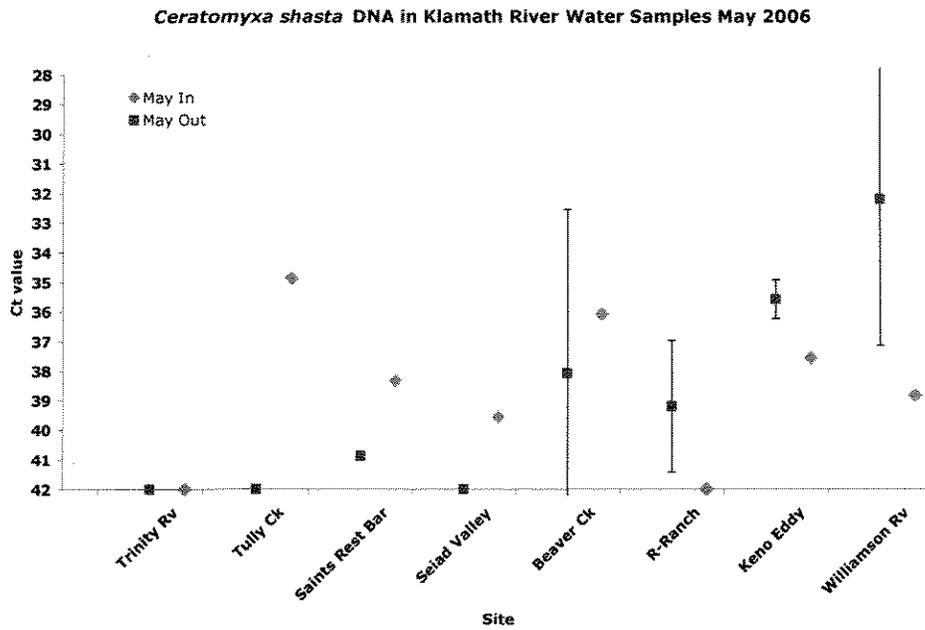
To assign spore numbers to Ct values generated by the QPCR we need reference samples which have been processed identically to the river samples but to which known numbers of parasites have been added. A comprehensive reference set for *C. shasta* was prepared in 2004, however when these original samples and freshly prepared 100 myxospore and 1 actinospore samples were assayed in 2006 we found all presented values about 10 fold higher. Since both the original and fresh samples were affected, the calibration shift is not caused by degradation of DNA but may be caused by the different QPCR machine used in 2006. This observation has highlighted the need for reference samples to be included with each sample group and for fresh reference samples to be processed alongside water samples whenever possible to provide both extraction and QPCR standards.

Ceratomyxa shasta - Sentinel field samples. In April, very low levels of *C. shasta* were present in the river (one spore or less) (Figure 3.4). This increases over the following two months to around 100 spores per liter in the Williamson River and Beaver Creek and decreases downstream from both these sites.

A



B



C

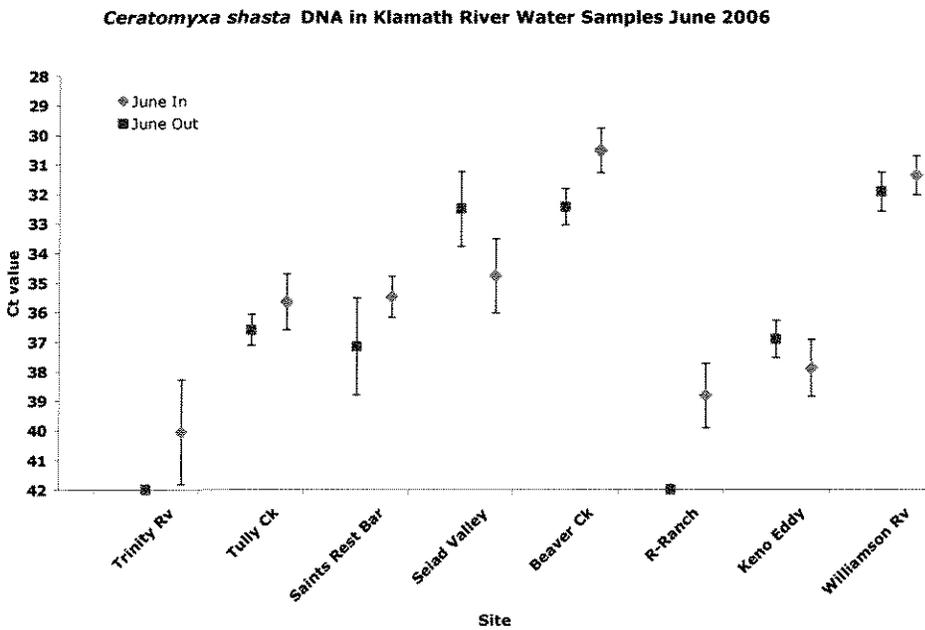


Figure 3.4. *Ceratomyxa shasta* DNA in Klamath River water samples collected with sentinel fish exposures in April (A), May (B) and June (C) 2006. The lower the Ct value, the more parasite DNA is present.

Comparison of *C. shasta* infections in fish and water sample data in 2006. In April, mortality was the highest when the parasite was detected in both the water samples taken at the beginning and end of the exposure period (i.e. Beaver Creek and Saints Rest Bar). In May there is an increase both in spore loads and in fish infections but the site pattern differs between the two detection methods. Two of three highest infection (mortality and prevalence) sites (Beaver Creek and Williamson River), also had the highest spore counts; however, low spore counts at Seiad Valley did not predict the high infection at this site. Trinity River was negative by both methods. In June, the lowest mortality (none to some) occurred at R-Ranch, Keno Eddy and Trinity River and these were the three sites with the lowest spore loads.

The June data indicates that rainbow trout have a low threshold to infection by *C. shasta*: Spore levels at R-Ranch and Keno Eddy are insufficient to cause high mortality (<50%) but once levels cross a threshold (around 5 spores per liter) as seen at Tully Creek and Saints Rest Bar, mortality in the susceptible trout is significant (>50%). There was a clear correlation of *C. shasta* infection levels in Chinook salmon (exposed at Beaver Creek) and parasite levels in water samples. In May, when spore numbers were around 10 per liter, we recorded 0% mortality and 15% prevalence of infection, whereas in June, when there were approximately ten times more parasites in the water, there was 8% mortality and 82% prevalence of infection (fish held at 13°C after exposure).

ISCO samples. The portable, automated ISCO 3700 proved a viable method for collection of water samples. The average spore load in May was 39.2 Ct (values ranged from 34-42 Ct, standard deviation 2.65) and in June averaged 33.4 Ct (range 32-37 Ct, standard deviation 1.72) (Figure 3.5). This equates approximately to 0 - 10 spores/liter in May and 5- 35 spores/liter in June. Although spore levels vary over a 24 hour period, there is no consistent pattern; the consecutive May samples differ as do the May and June samples. The May sentinel water samples (36-38 Ct) had higher spore levels than the ISCO average but fell within the ISCO ranges. However, in June, the sentinel samples (30-32 Ct) had parasite levels above the ISCO samples (there is no identical time point to compare).

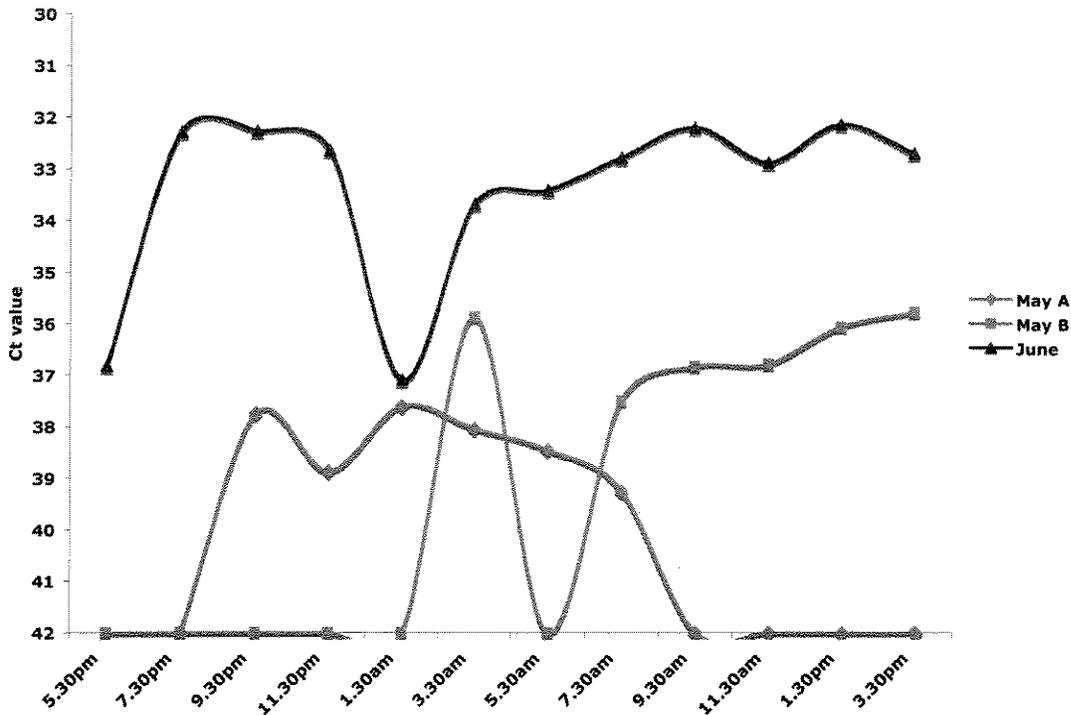
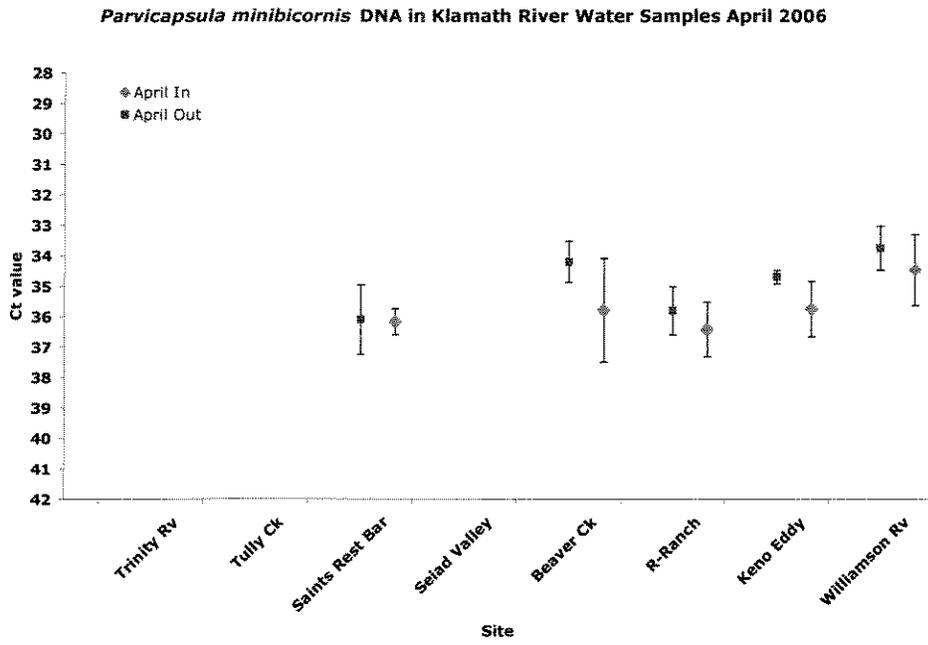


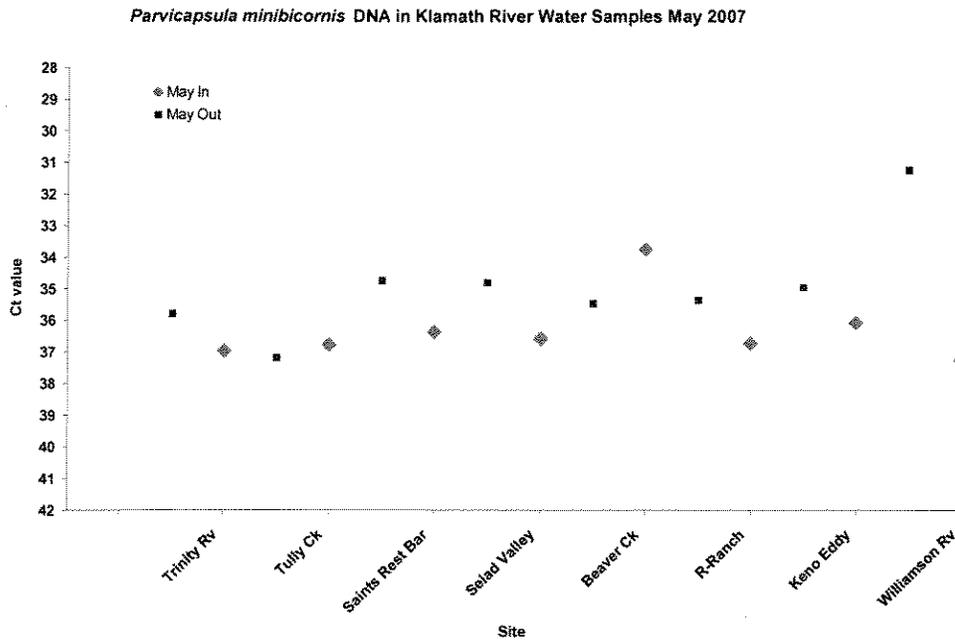
Figure 3.5. Results of automated 24-hr water samples collected by an ISCO 3700 to quantify *Ceratomyxa shasta* spores in the Klamath River above Beaver Creek during the May and June 2006 fish exposures. May was sampled for 48 hours (May A represents the first 24 hours of sampling and May B the second 24 hours) whereas June was sampled for 24 hours.

Parvicapsula minibicornis – Field water samples. In contrast to *C. shasta*, all sites tested for *P. minibicornis* were positive (Figure 3.6). As for *C. shasta*, numbers increased from April to June: levels ranged from 1 to 10 spores in April and May to 100 spores per liter in June. The most pronounced site variation was in June with lowest numbers at Trinity River (1 spore or less) and the highest at Beaver Creek (100 spores). In each month, the Beaver Creek and Williamson River samples contained the most parasites. In June, there is a similar spatial pattern for both myxozoans with the exception of R-Ranch: levels remain very low at this site for *C. shasta* whereas there were 10 times more *P. minibicornis* parasites.

A



B



C

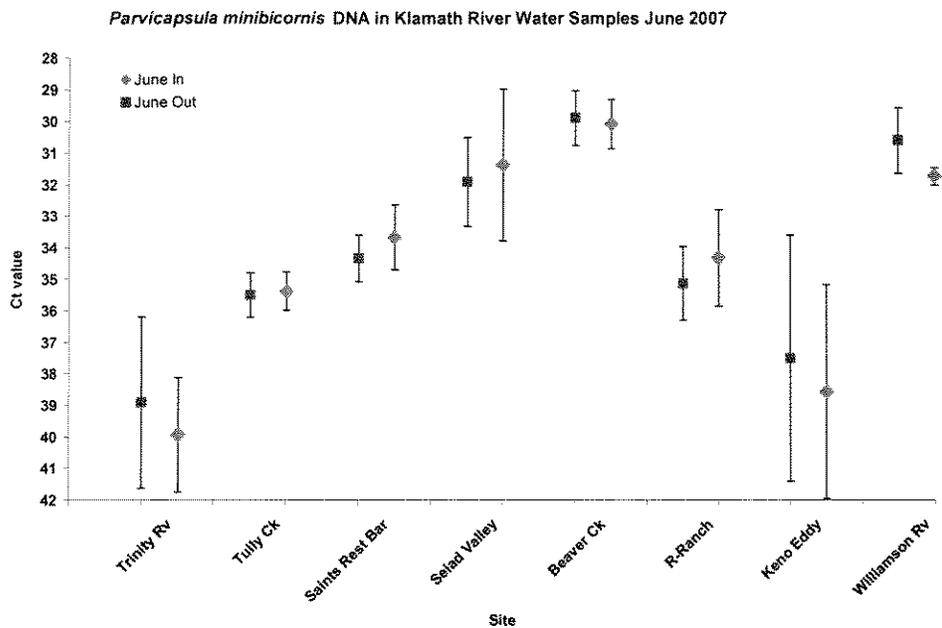


Figure 3.6. *Parvicapsula minibicornis* DNA in Klamath River water samples collected with sentinel fish exposures in April (A), May (B) and June (C) 2006. The lower the Ct value, the more parasite DNA is present.

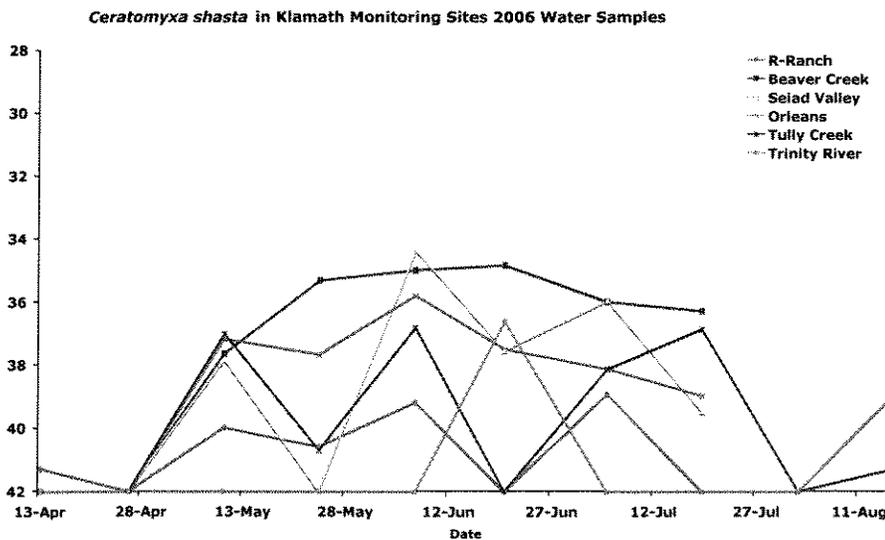
Tribal Field Water Samples. *C. shasta* was detected in all five mainstem sites and one (Trinity River) of the four tributaries tested (Figure 3.8A). All mainstem sites were positive by mid-May. All sites peaked in parasite numbers in June, with the Beaver Creek and Seiad Valley samples containing some 10 spores/liter. This value is 10 times less than that measured for Beaver Creek in the sentinel field samples taken that month but the ISCO sample data (Figure 3.7) show that both value sets are within the daily range recorded for this site.

Comparison of water sample results from sentinel and tribal collections for other sites shows a fairly high consistency with the following exceptions: in April no parasites were detected in the tribal samples whereas very low levels (one spore/liter or less) were detected in the sentinel samples; in May, the only outlier was Beaver Creek, which presented lower levels in the tribal samples; in June, Tully Creek was negative for the tribal samples but positive for the sentinel samples and similarly, Seiad Valley was lower in the tribal samples. At both these sites,

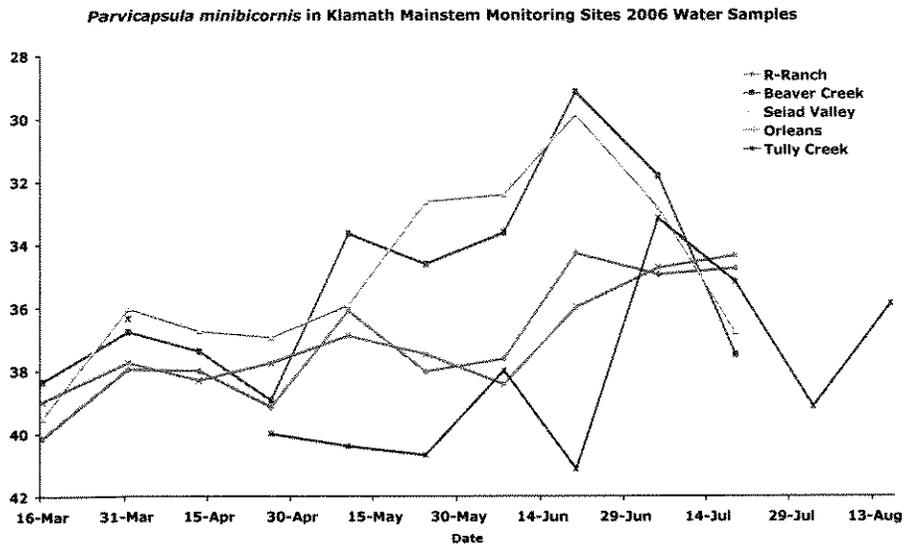
the parasite was present in higher numbers in the tribal samples taken on either side of the sentinel dates which highlights the short term variability seen in spore abundance.

These data show that, despite sharing both its vertebrate and invertebrate host with *C. shasta*, *P. minibicornis* has a different temporal and spatial distribution. It was already present in low numbers in March and was detected at all five mainstem sites and in all four tributaries tested. As for *C. shasta*, *P. minibicornis* was most abundant at Beaver Creek (100 spores/liter detected), followed by Seiad Valley. It was only present in low numbers (< 10 spores/liter) in the tributary samples. *Parvicapsula minibicornis* peaked at three mainstem sites in June (Beaver Creek, Seiad Valley and R-Ranch) and in July at the remaining two sites (Tully Creek and Orleans). Comparison of sentinel and tribal water samples shows a fairly high consistency: in April there were lower numbers in the tribal samples than in the sentinel samples (but the tribal samples taken on either side of the sentinel samples presented higher values, similar to those of the sentinel samples, as for Tully Creek, below); in May and June the different sites were comparable with the exception of Tully Creek which presented a lower level in the tribal samples but again.

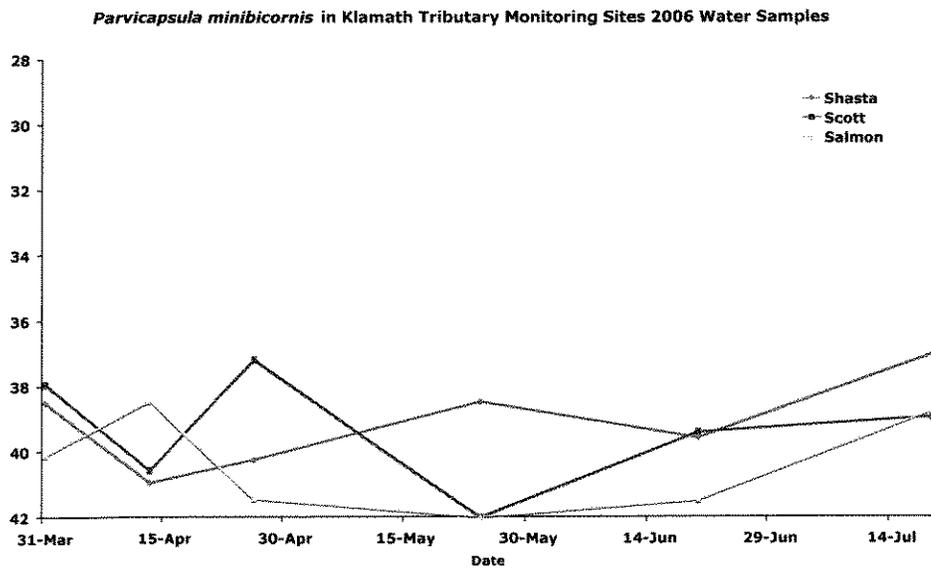
A.



B.



C.



D.

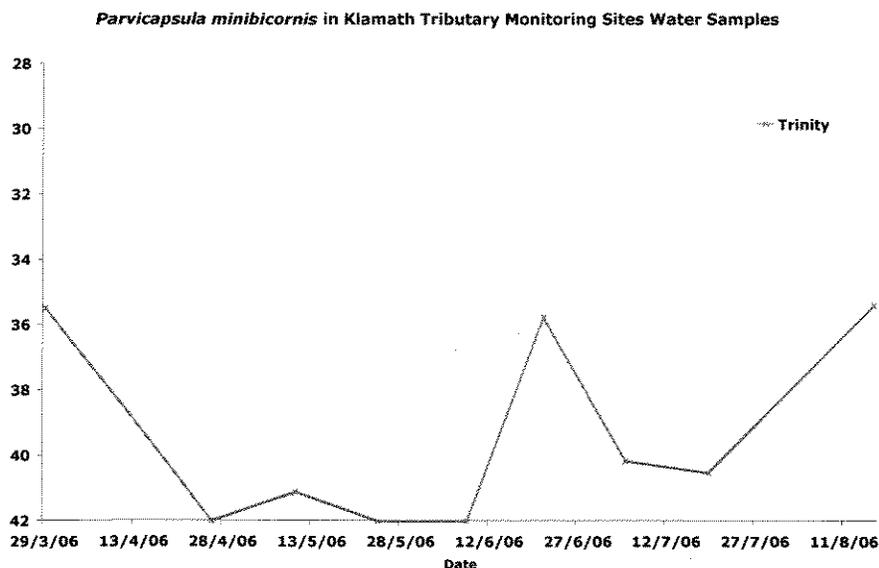


Figure 3.7. Distribution and abundance of *Ceratomyxa shasta* (A) and *Parvicapsula minibicornis* (B-D) in the Klamath River mainstem and tributaries as determined from water samples collected from March through August 2006. The lower the Ct value, the more parasite DNA is present.

Overall, QPCR assessment of water samples offers a faster, more cost effective means of detecting parasites, however, the assay has limitations that need to be acknowledged. The method can't distinguish between the two spore stages (actinospore and myxospore) or infective and dead organisms. Also, quantification is not absolute (due to cumulative handling effects) but rather provides a range of spore values. Furthermore, as highlighted above, the assay is subject to inhibition. This last consideration can be addressed and is the focus of current tests. However, it does provide a potential means for rapid evaluation of parasite levels. Further research linking these levels to biological effects on Chinook and coho salmon should allow estimates of mortality and the opportunity for rapid management responses.

Objective 4. Examine the effects of flow by exposing fish during periods of natural flow variation in the Klamath River. (*Note: this objective includes data from 2005 exposures that was analyzed as part of this study. The initial study was also extended in scope with support from the Hoopa Valley and Karuk Tribal Fisheries programs. In order to present a comprehensive discussion of this material we have included these data below. However, additional discussion appears in reports submitted to those programs*)

Methods

Fish handling. Susceptible rainbow trout (*O. mykiss*; stock 72.04 and 72.05) were obtained from Roaring River Hatchery, Oregon; fall Chinook and coho salmon were obtained from Iron Gate and Trinity River hatcheries. Fish weighed between 2.5 and 26.0 grams. Fish transport permits were filed and issued by the Oregon Department of Fish and Wildlife (ODFW) and California Department of Fish and Game (CDFG). Rainbow trout were held at the OSU–Smith Farm facility until transport to the exposure sites whereas the Chinook and coho salmon were obtained from hatcheries on the day of exposure. As a prophylactic treatment against bacterial infections, rainbow trout were administered a diet containing 2–4% oxytetracycline (approximately 3.5 g oxytetracycline/45 kg fish/day) in the form of TM100 (Pfizer, Atlanta, GA) for 10 d prior to exposure.

All fish were transported to the exposure sites (Figure 4.1) and placed in 0.3 x 1.0 m cylindrical holding cages and held for 3 d (72 hrs) in the river without feeding. Although exposure sites differed seasonally and annually depending on collaborator interests, logistics and accessibility, exposures were consistently conducted above Beaver Creek, in the Keno Eddy and in the Williamson River for comparison.

In 2005, exposures in May (23-26), June (20-23) and September (19-22) were conducted using groups of 70 fish per cage. Locations in May and June were Williamson River, Keno Eddy, Boyle bypass Reach and Klamath River above Beaver Creek (Figure 4.1; Table 4.1). In May, both rainbow trout and Iron Gate Hatchery (IGH) fall Chinook were exposed at all locations with additional exposure of IGH coho and redband rainbow trout obtained from Spencer Creek, occurring at Beaver Creek. In June, rainbow trout and IGH fall Chinook were held at each location (Table 4.2). Exposure locations for September were Williamson River, Keno Eddy, R-Ranch, Beaver Creek and Seiad Valley and both Chinook and rainbow trout were

placed at all sites (Table 4.3).

In 2006, exposures in April (24 – 27) were conducted at five locations: Williamson River, Keno Eddy, R-Ranch, above Beaver Creek, and Saints Rest Bar above the Trinity River confluence (Table 4.4). Rainbow trout were exposed at all locations and fall Chinook salmon were exposed at only Beaver Creek. In addition to the exposure sites used in April, in May (15-18) and June (20-24) rainbow trout were also held at Seiad Valley and near Tully Creek in the Klamath River, and in the Trinity River at Hoopa (Tables 4.5 and 4.6; Figure 4.1). In May and June, Iron Gate Hatchery (IGH) and Trinity River Hatchery (TRH) stocks of fall Chinook and coho salmon were exposed at selected sites to determine the effects of exposure on these stocks and compare their susceptibilities (Tables 4.5 and 4.6).

After exposure, fish and cages were retrieved from the river, and the exposure groups placed in individual coolers supplemented with bubbled oxygen. The fish were then transported to the OSU–Salmon Disease Laboratory (OSU-SDL) where each exposure group was held in a 100-L tank receiving 13 °C specific pathogen free (SPF) well-water until termination at approximately 90 days post exposure (dpe).

Treatment. Preventative treatments for bacterial infections were administered within 1dpe and included a two-week diet consisting of TM100 (Bio-Oregon, Warrenton, OR) medicated feed and 1.0 mg l⁻¹ Furanase (Aquarium Products, Glenburnie, MD) bath treatment 1 hr daily for 3 d. After two weeks, fish received a 1 hr formalin bath at 125 – 170 mg l⁻¹ for three consecutive days to remove external parasites. Dead or moribund fish were collected daily and examined for signs of infection.

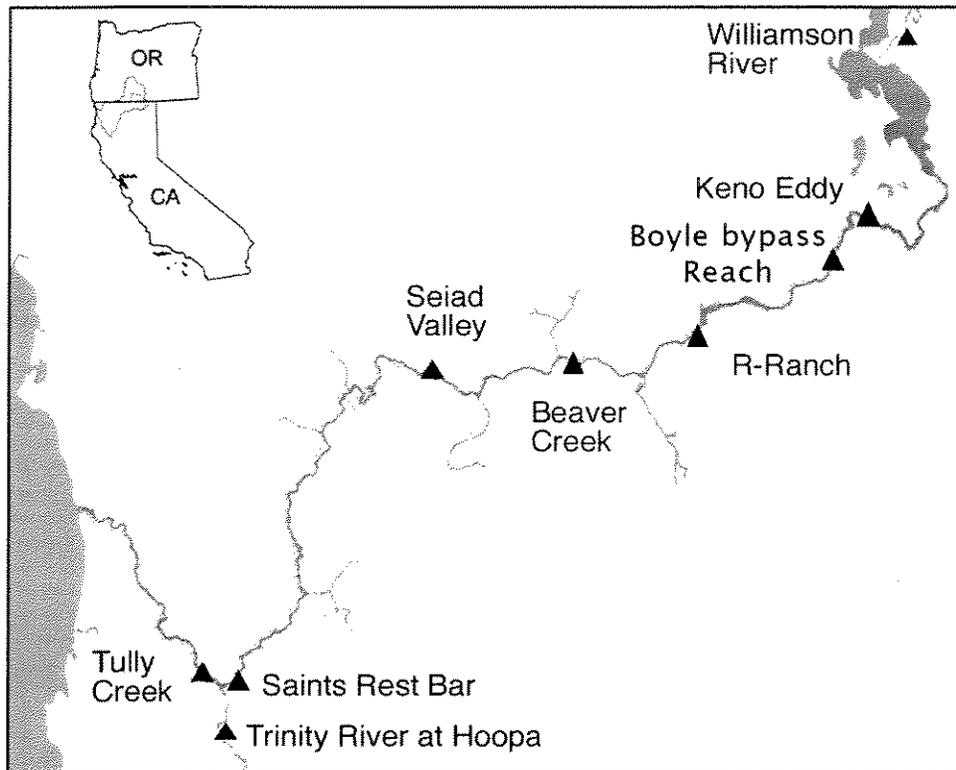


Figure 4.1. Sites used in the 2005 and 2006 fish exposure studies to detect the salmonid parasite *Ceratomyxa shasta* in the Klamath River Basin.

Diagnosics. All dead or moribund fish were visually examined for *C. shasta* by microscopy. At termination, all fish, including unexposed controls, were killed with a lethal dose of MS222 (tricaine methanesulfonate) and 10 fish per exposure group were visually examined for spores. To detect spores, a wet-mount was prepared by inserting a sterilized inoculating loop of the appropriate diameter into the anogenital pore to a depth of approximately 1.0 -1.5 cm. The sample collected was smeared onto a glass microscope slide and observed at 100 X or 250 X magnifications for 3 min. Fish were considered positive if the characteristic kidney bean-shaped myxospore was observed. If any fish from a termination group was positive, an additional 15 fish were examined by microscopy. If spores were not observed, intestinal tissue was excised, digested, the DNA extracted, and assayed by a single round polymerase chain reaction (PCR) using methods described by Palenzuela and Bartholomew (2002). The following modifications were made to the protocol: an additional 2 – 3 mm segment of the alimentary canal, just posterior to the pyloric caeca attachment, was excised and included with the 5.0 mm segment of the posterior intestine.

Percent prevalence of infection was calculated as the number of exposed fish that tested

positive for infection (by microscopy and/or PCR analysis), including euthanized fish and mortalities, divided by the total number of fish examined for infection (X 100). Percent mortality was calculated as the number of fish that died during the 90 d holding period that were visually positive for *C. shasta* by microscopy, divided by the total number of fish that had survived the treatment period (X 100). The mean day to death for each exposure group was calculated as the geometric mean of all days with *C. shasta* positive mortalities within the 90 d holding period. To test for differences in death rates between 2005 and 2006, the rainbow trout exposures at Beaver Creek were used as the standard. The cumulative mortalities for May and June of 2005 were averaged and compared against the averages for May and June of 2006 using a paired t-test and graphed.

RESULTS

Water flow conditions below Iron Gate in early 2005 suggested that the low flow conditions of 2004 would continue into 2005. However, a sharp increase in water levels occurred due to heavy rains, lasting approximately two weeks (Figure 4.2). Fish were exposed at four locations in May 2005 during this high flow condition and were retrieved without complications. Water temperature during exposure was approximately 16°C at all sites, except at the Boyle bypass Reach, which was cooler (13°C). Infection prevalence in rainbow trout groups was high (92 – 100%) at all locations, but significant mortality (100%) occurred only in the Williamson River and Beaver Creek groups (Table 4.1; Figure 4.3). The mean time to death for the rainbow trout was similar for these two groups at 40.1 ± 9.0 dpe (Figure 4.4). Fall Chinook became infected with *C. shasta* only at the Boyle bypass Reach (12% infection prevalence) and Beaver Creek (11.5% infection prevalence); there was no mortality in the group held in the Boyle bypass reach and 1.6% mortality in the Beaver Creek exposure group (Figure 4.3). Coho salmon were exposed only at the Beaver Creek location; infection prevalence was 39.4% and 26.2% died with *C. shasta* infections (Figure 4.3). Redband rainbow trout, being wild and unaccustomed to artificial feeds, were more difficult to hold after the exposure. All of these fish survived the exposure period with only one fish succumbing to *C. shasta* infection. However, infection prevalence in this group was 31.7% and mature myxospores were observed in these fish (Figure 4.3). In June of 2005, exposure cages at the Williamson River were tampered with and no fish were recovered (Table 2). All other groups were retrieved without complications. Redband rainbow

trout and coho salmon were not exposed in June 2005. Water temperatures ranged from 17.0 – 18.1°C at Keno Eddy and Beaver Creek with the lowest water temperatures occurring at the Boyle bypass Reach (13.4°C). Infection prevalence in rainbow trout was high for all groups (100%) with mortality occurring at Boyle bypass Reach (7.9%) and Beaver Creek (98.6%). A low number of fall Chinook salmon became infected (4.8%) at Beaver Creek without resulting mortality (Figure 4.5). The mean time to death for the Beaver Creek rainbow trout was slightly greater in June than in May (43.7 ± 14.4 vs. 40.1 ± 9.5) (Figure 4.6; Table 4.2).

Fall Chinook salmon and rainbow trout were exposed at five locations in September 2005 and all fish were retrieved without complications except for fall Chinook salmon exposed at Seiad Valley, which were lost during transport (Table 4.3). *Ceratomyxa shasta* was not detected in any of the remaining fall Chinook exposure groups (Figure 4.7). Infection prevalence for rainbow trout groups ranged from 61.1 – 100% with 88.1% mortality occurring in the R-Ranch group and >90.0% occurring in groups exposed at all other sites except for Keno Eddy, where no mortality occurred (Table 4.3; Figure 4.7). The cumulative mortality curve for rainbow trout (Figure 4.8) demonstrates that, where mortality occurred, the rate of death was lowest in the R-Ranch exposure group. However, it should be noted that significant mortality has not been demonstrated at this site in previous exposures (Tables 4.1 and 4.2). For purposes of comparison, the mortality curves of rainbow trout exposed at Beaver Creek for all three months were examined (Figure 4.9) demonstrating that the rate of death decreased from May to June and then increased slightly in September.

Another high flow event occurred in spring of 2006, however, this time the event lasted for several months (Figure 4.10). We report here the results of the April, May and June exposures of 2006, which were also summarized in a report to the Hoopa Valley Tribal Fisheries (results from the July and September exposures will be summarized in a report to the Karuk Tribal Fisheries Program). Fish exposed in April 2006 (Table 4.4, Figure 4.11) were retrieved successfully. Water temperatures at all sites during the exposure period were fairly uniform with an average temperature of 11.9°C (Table 4.4). Prevalence of infection was highest in the Williamson River rainbow trout exposure group (96.3%), followed by the Keno Eddy group (96.2%) and least (22.5%) in the Saints Rest Bar group. Mortality was less than 10% in all exposure groups, with the highest mortality (9.8%) occurring at Saints Rest Bar (Figure 4.11). Infection was not detected in the fall Chinook group exposed at the Beaver Creek location (Table

4.4).

Fish exposed in May 2006 (Table 4.5) were successfully exposed and retrieved. The average water temperatures during exposure decreased from the Williamson River (19.3°C) to Tully Creek (13.8°C). Compared to April exposure results, infection in the rainbow trout groups increased markedly, with high infection prevalence occurring in all rainbow trout groups (except the Trinity River group) and high mortality occurring in all but the Keno Eddy and R-Ranch groups (Figure 4.12). The highest mortality occurred in the Williamson River (97.5%), Beaver Creek (92.3%) and Seiad Valley (91.7%) exposure groups. The prevalence of infection for rainbow trout held at the Keno Eddy (50.0%) was less than the April infection prevalence (96.0%) at the same site, yet mortality was greater in May (7.1%) compared with April (0%). The mean day to death for the Williamson River rainbow trout was less than 32 days, yet it was greater than 50 days for all other sites (Table 4.5). A pattern of decreasing infection prevalence and mortality was observed from Beaver Creek and Seiad Valley to Tully Creek (Figure 4.12). Infection was not detected in the Trinity River exposure group (Table 4.5). The highest rate of death occurred in the Williamson River rainbow trout exposure group followed by Beaver Creek, with the lowest rate of death occurring in the R-Ranch group (Figure 4.13). Fall Chinook became infected at a low prevalence at Beaver Creek (TRH = 15.4%; IGH = 7.4%) and Tully Creek (TRH = 20.0%) with no resulting mortality (Figure 4.14). Coho salmon were exposed only at Beaver Creek (IGH) and Tully Creek (TRH) with *C. shasta* infection (7.4%) and mortality (5.1%) occurring only at the Beaver Creek site (Figure 4.15).

In June 2006 the average water temperature during exposure in the Williamson River was almost 2°C cooler than during the May exposures (Table 4.6; Table 4.5). The highest water temperatures (20.0°C) were recorded at the Beaver Creek site (Table 4.6) and again there was a trend of decreasing temperature with distance downriver; however, the lowest recorded mainstem temperature was 18.5°C. Water temperature in the Trinity River during exposure averaged 15.4°C. *Ceratomyxa shasta* infection prevalence was high (>82.0%) for all rainbow trout exposure groups (Figure 4.16) except the Trinity River group where infection was not detected (Table 4.6). Percent mortality for rainbow trout was also high (>95.0%) except for groups exposed at R-Ranch (21.2%) and the Keno Eddy (0.0 %) (Figure 4.16). The mean day to death for rainbow trout was the least at the Williamson River (32.1±4.5 dpe) with 100% mortality and Seiad Valley (35.7±3.8 dpe) with 97.6% mortality, followed by the Beaver Creek

site (37.9 ± 3.2 dpe) with 96.2% mortality. The mean day to death for all other rainbow trout groups, except Trinity River and Keno Eddy, was >50 dpe (Figure 4.17; Table 4.6). Prevalence of infection in the fall Chinook groups was lower than that of the rainbow trout groups. Fall Chinook salmon exposed at Williamson River, Trinity River, R-Ranch and Saints Rest Bar had no detectable infection with *C. shasta* (Table 4.6). The highest prevalence of infection for both stocks of Chinook salmon occurred at Beaver Creek: TRH = 82.8% and IGH = 37.5% (Figure 4.18). The percent mortality and mean day to death for these two stocks at Beaver Creek were: TRH = 8.1% (50.3 ± 12.2 dpe) and IGH = 16.7% (46.1 ± 15.1 dpe). Low infection prevalence ($< 7.5\%$) and mortality ($< 3.5\%$) was recorded for the Seiad Valley (IGH stock) and Tully Creek (TRH stock) Chinook salmon exposure groups. The two hatchery stocks of coho salmon were exposed at the Beaver Creek site and TRH stocks of coho were also exposed at Saint Rest Bar and Tully Creek (Table 4.6). Infection prevalence for coho salmon was highest in the TRH stocks at Beaver Creek (15.6%) and Saints Rest Bar (13.8%) and low ($\leq 7.4\%$) for the other groups (Figure 4.19). Percent mortality was close to percent prevalence in all coho salmon exposure groups. In general, infection prevalence and mortality in the coho salmon was less than that of the fall Chinook salmon. Negative controls (unexposed fish) all tested negative for infection in 2005 and 2006 except for one IGH coho, which tested positive for infection in June 2006.

Comparison of 2005 and 2006 rainbow trout exposures at Beaver Creek indicate that cumulative mortality had significantly decreased in 2006 ($p < 0.5$) (Figure 4.20).

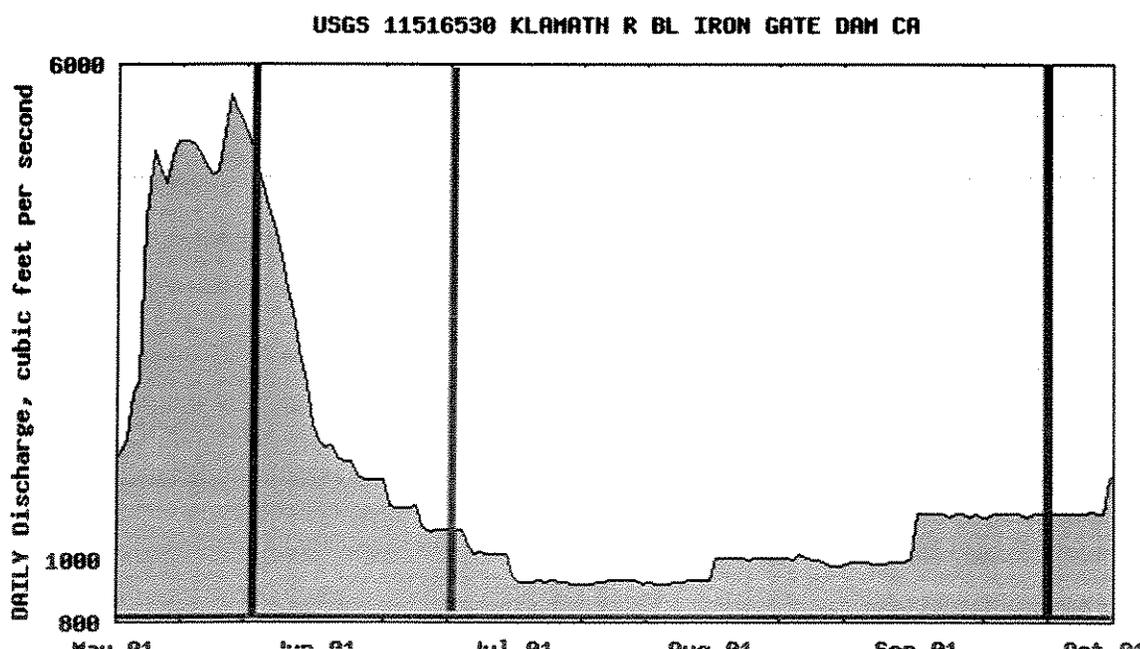


Table 4.1 *Ceratomyxa shasta* infection prevalence, percent mortality and mean day to death for fish exposed for 3-days (72 hr) in May 2005 at eight locations in the Klamath River.

Site	Species	Rkm	Avg Temp \bar{u}C (SD)		% Prev	% Mort	Avg day to death (SD)	
Williamson River	Trout	7.7	16.6	0.89	100	100	40.4	9.2
Williamson River	Chinook IGH	7.7	16.6	0.89	0.0	0.0	0.0	0.0
Keno Eddy	Trout	368.5	16.5	1.01	100	1.8	83.0	0.0
Keno Eddy	Chinook IGH	368.5	16.5	1.01	0.0	0.0	0.0	0.0
Boyle bypass Reach	Trout	354.8	13.3	1.09	92.0	0.0	0.0	0.0
Boyle bypass Reach	Chinook IGH	354.8	13.3	1.09	12.0	0.0	0.0	0.0
Beaver Creek	Trout	259.1	16.0	1.14	100	100	40.1	9.48
Beaver Creek	Chinook IGH	259.1	16.0	1.14	11.5	1.59	38.0	0.0
Beaver Creek	coho IGH	259.1	16.0	1.14	39.4	26.2	50.6	12.2
Beaver Creek	redband trout	259.1	16.0	1.14	31.7	1.6	93.1	0.0

Note: A total of 70 fish per exposure group.

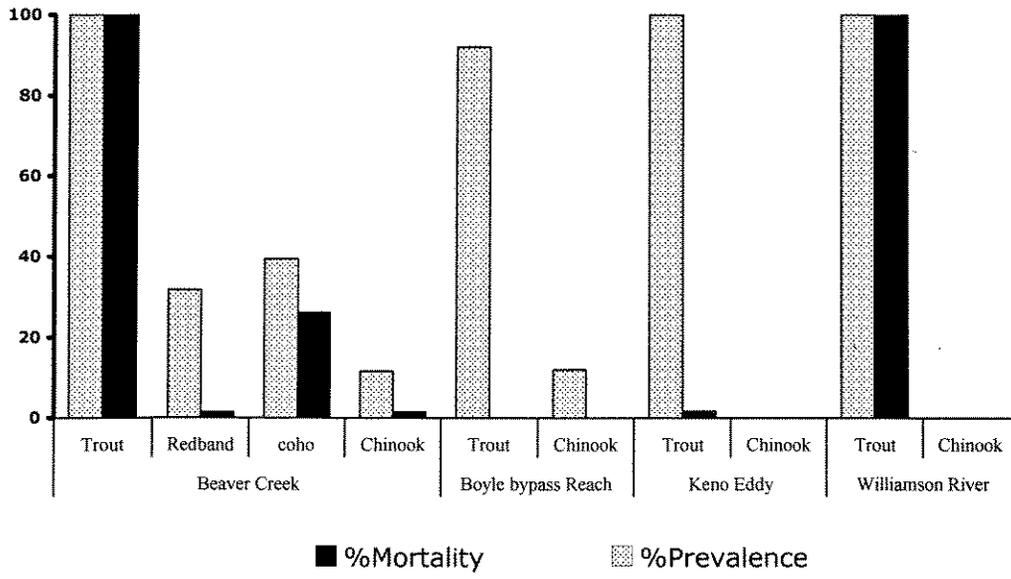


Figure 4.3. Results from the May 2005 fish exposures to *Ceratomyxa shasta* in the Klamath River basin. Redband trout are a native Klamath River strain of rainbow trout. Fall Chinook and coho salmon were obtained from Iron Gate Hatchery.

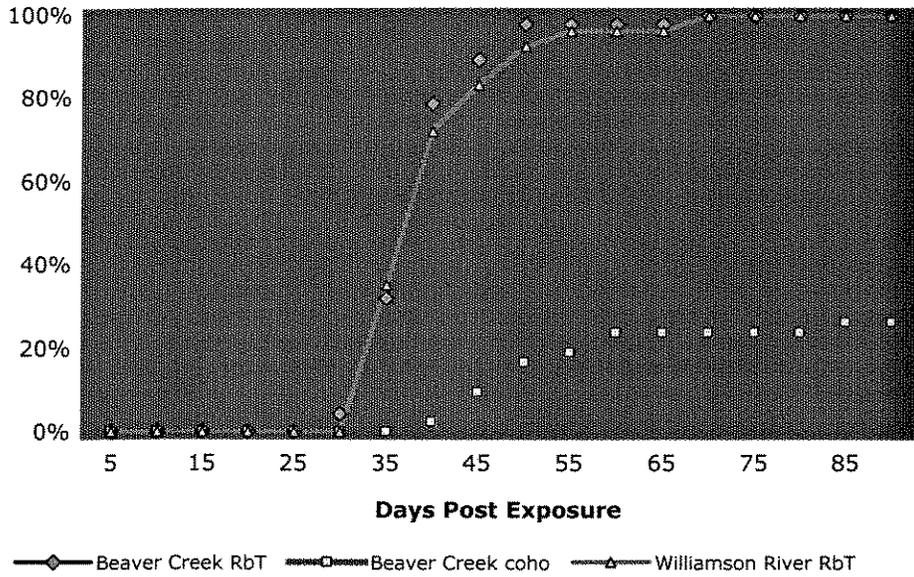


Figure 4.4. Cumulative mortality curve for fish following a 3-day exposure to *Ceratomyxa shasta* in the Klamath River in May 2005 (See also Table 4.1 and Figure 4.3). Rainbow trout labeled as RbT.

Table 4.2. *Ceratomyxa shasta* infection prevalence, percent mortality and mean day to death for fish exposed for 3-days (72 hr) in June 2005 at four locations in the Klamath River.

Site	Species	Rkm	Avg		% Prev	% Mort	Avg day	
			Temp	ūC (SD)			to death (SD)	
Williamson River	Trout	7.69
Williamson River	Chinook IGH	7.69
Keno Eddy	Trout	368.50	17.0	1.20	100	1.4	85.0	0.0
Keno Eddy	Chinook IGH	368.50	17.0	1.20	0.0	0.0	0.0	0.0
Boyle bypass Reach	Trout	354.8	13.4	0.91	100	7.9	72.1	9.8
Boyle bypass Reach	Chinook IGH	354.8	13.4	0.91	0.0	0.0	0.0	0.0
Beaver Creek	Trout	259.10	18.1	0.69	100	98.6	43.7	14.4
Beaver Creek	Chinook IGH	259.10	18.1	0.69	4.8	0.0	0.0	0.0

Note: A total of 70 fish per exposure group. Data for Williamson River was lost due to tampering.

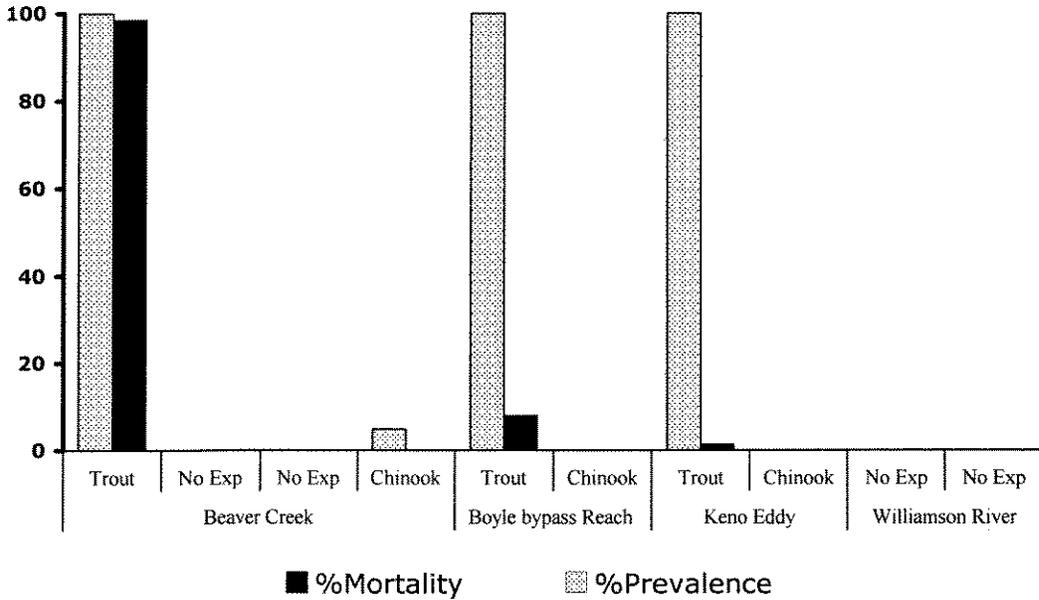


Figure 4.5. Results from the June 2005 fish exposures to *Ceratomyxa shasta* in the Klamath River basin. *No Exp* means that no exposure occurred (Beaver Creek) or that the cages were tampered with (Williamson River). See also Table 4.2.

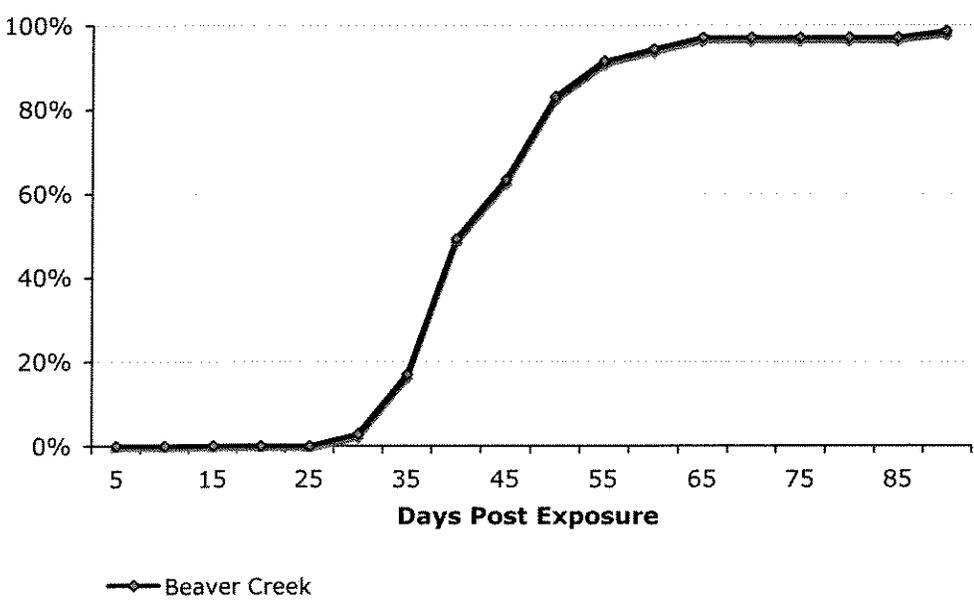


Figure 4.6. Cumulative mortality curve for fish following a 3-day exposure to *Ceratomyxa shasta* in the Klamath River at Beaver Creek in June 2005. Only rainbow trout shown (see also Table 4. 2).

Table 4.3. *Ceratomyxa shasta* infection prevalence, percent mortality and mean day to death for fish exposed for 3-days (72 hr) in September 2005 at five locations in the Klamath River.

Site	Species	Rkm	Avg Temp °C (SD)		% Prev	% Mort	Avg day to death (SD)	
Williamson River	Trout	7.69	11.7	0.85	100	90.3	39.7	17.5
Williamson River	Chinook IGH	7.69	11.7	0.85	0.0	0.0	0.0	0.0
Keno Eddy	Trout	368.50	16.0	1.10	61.5	0.0	0.0	0.0
Keno Eddy	Chinook IGH	368.50	16.0	1.10	0.0	0.0	0.0	0.0
R-Ranch	Trout	300.19	12.7	0.73	88.1	100	55.5	13.1
R-Ranch	Chinook IGH	300.19	12.7	0.73	0.0	0.0	0.0	0.0
Beaver Creek	Trout	259.10	18.5	0.40	100	98.6	40.7	6.5
Beaver Creek	Chinook IGH	259.10	18.5	0.40	0.0	0.0	0.0	0.0
Seiad Valley	Trout	206.96	18.3	0.61	100	98.4	42.0	6.4
Seiad Valley	Chinook IGH	206.96	18.3	0.61

Note: A dot means that data is not available.

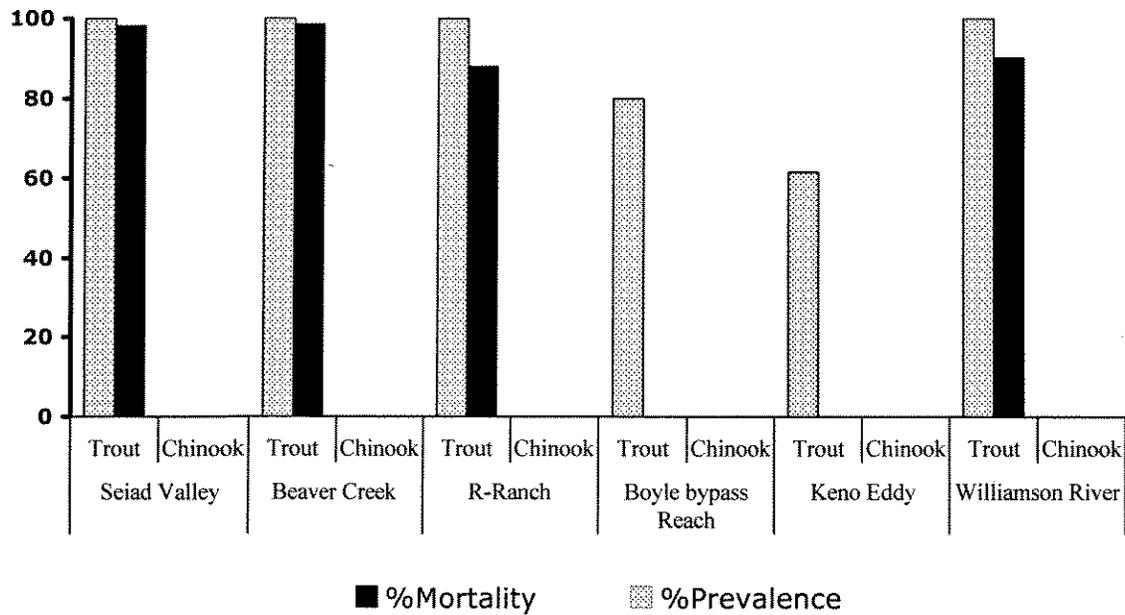


Figure 4.7. Results from the September 2005, 3-day fish exposures to *Ceratomyxa shasta* in the Klamath River basin. See also Table 4.3.

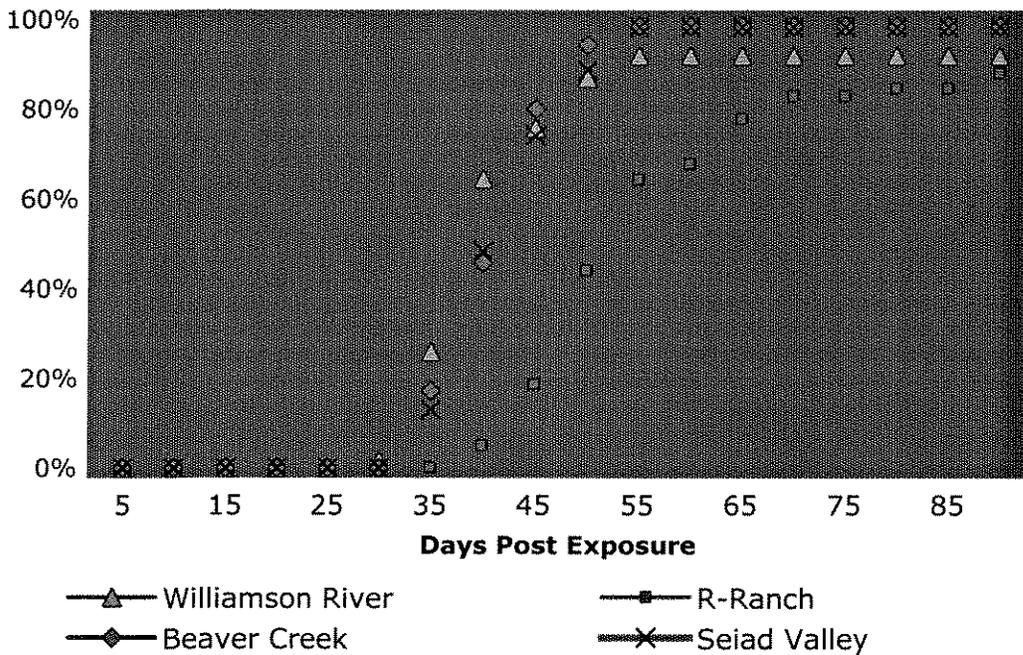


Figure 4.8. Cumulative mortality curve for fish following a 3-day exposure to *Ceratomyxa shasta* in the Klamath River in June 2005. Only rainbow trout shown (see also Table 4.3).

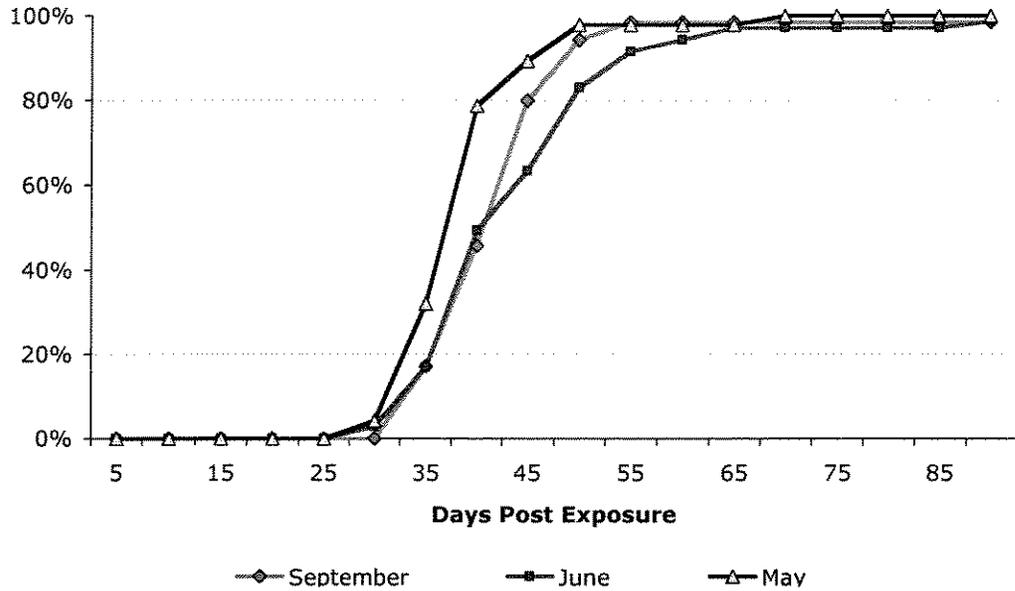


Figure 4.9. Comparison of the cumulative mortality curves for rainbow trout following a 3-day exposure to *Ceratomyxa shasta* in the Klamath River at Beaver Creek in May, June and September 2005 (See also Tables 4.1, 4.2 and 4.3).

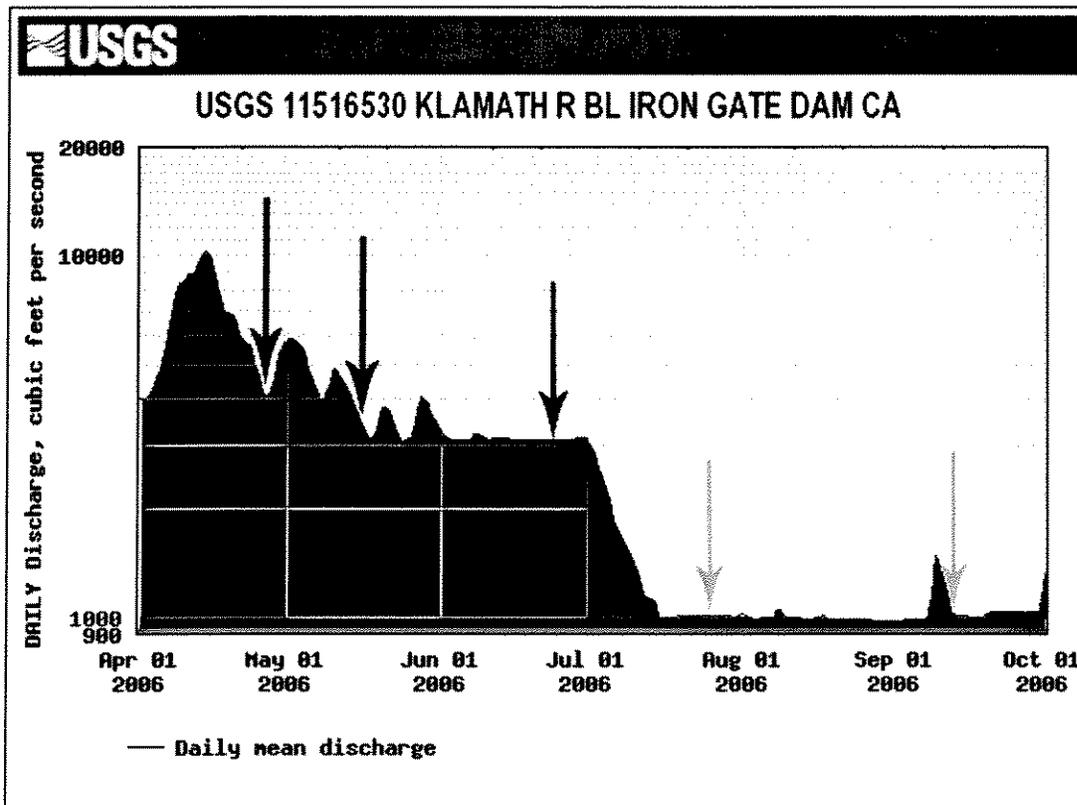


Figure 4.10. Hydrograph of the Klamath River below Iron Gate Dam. Time period ranges from early April 2006 to early October with fish exposure periods represented as arrows. Fish exposures represented as dark arrows are reported here and the fish exposures represented as grey arrows will appear in a report to the Karuk Tribe who funded that portion of the project.

Table 4.4. *Ceratomyxa shasta* infection prevalence, percent mortality and mean day to death for fish exposed for 3-days (72 hr) in April 2006 at five locations in the Klamath River.

Site	Species	River Kilometer	Avg Temp °C (SD)		% Prev	% Mort	Avg day to death (SD)	
Williamson River	Trout	7.69	12.2	1.09	96.3	2.5	49.0	0.0
Keno Eddy	Trout	367.29	12.3	0.96	96.0	0.0	0.0	0.0
R-Ranch	Trout	300.19	11.8	0.71	52.0	0.0	0.0	0.0
Beaver Creek	Chinook IGH	259.10	12.2	0.99	0.0	0.0	0.0	0.0
Beaver Creek	Trout	259.10	12.2	0.99	53.3	4.7	46.5	0.7
Saints Rest Bar	Trout	72.58	11.4	0.30	28.1	9.8	54.3	8.2

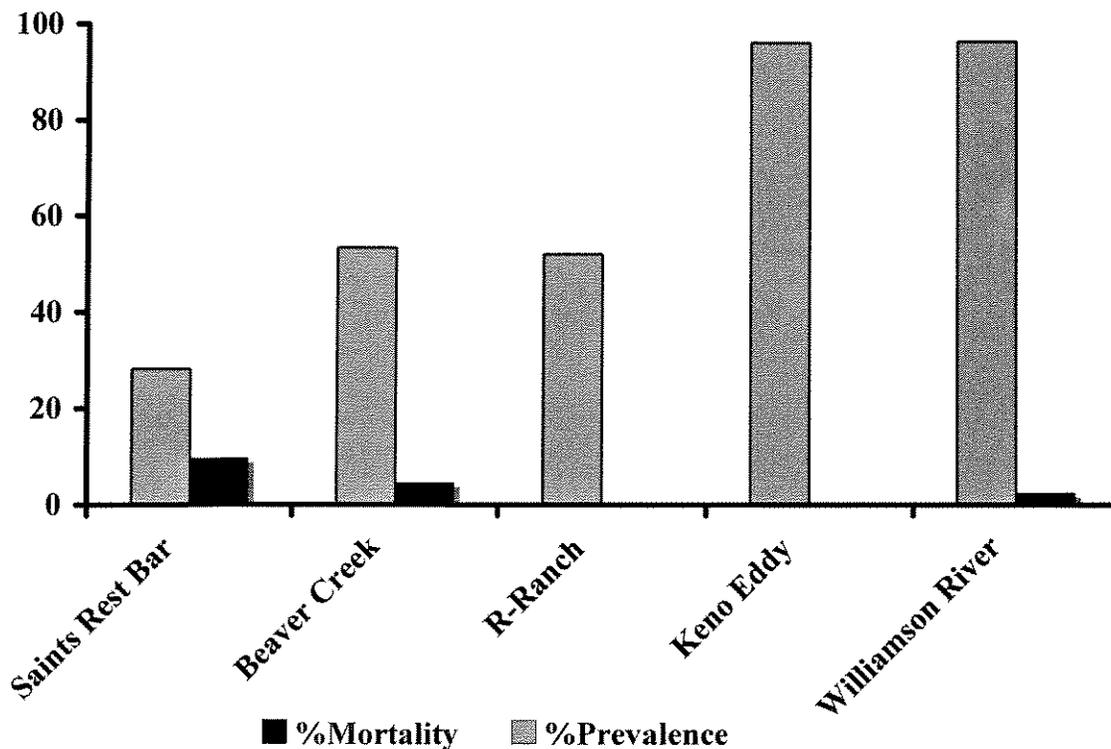


Figure 4.11. Percent mortality and prevalence of infection in rainbow trout as a result of *Ceratomyxa shasta* following a 72-hr exposure in the Klamath River in April 2006. The x-axis is arranged from uppermost exposure site (Williamson River) to the lowermost (Saints Rest Bar).

Table 4.5. *Ceratomyxa shasta* infection prevalence, percent mortality and mean day to death for fish exposed for 3-days (72 hr) in May 2006 at eight locations in the Klamath River.

Site	Species	River Kilometer	Avg Temp °C (SD)		% Prev	% Mort	Avg day to death (SD)	
Williamson River	Trout	7.69	19.3	0.96	100	97.5	31.8	5.4
Keno Eddy	Trout	368.50	19.5	0.77	50.0	7.1	80.4	7.6
R-Ranch	Trout	300.19	17.3	0.65	74.3	15.4	72.4	10.9
R-Ranch	Chinook IGH	300.19	17.3	0.65	0.0	0.0	0.0	0.0
Beaver Creek	Chinook IGH	259.10	18.2	0.61	7.4	0.0	0.0	0.0
Beaver Creek	Chinook TRH	259.10	18.2	0.61	15.4	0.0	0.0	0.0
Beaver Creek	Coho IGH	259.10	18.2	0.61	7.4	5.1	54.4	3.5
Beaver Creek	Trout	259.10	18.2	0.61	100	92.3	52.0	14.6
Seiad Valley	Trout	206.96	14.9	0.47	100	91.7	56.9	14.4
Saints Rest Bar	Chinook TRH	72.58	14.1	0.33	0.0	0.0	0.0	0.0
Saints Rest Bar	Trout	72.58	14.1	0.33	78.9	64.1	61.9	13.3
Trinity at Hoopa Valley	Trout	21.16	13.5	0.81	0.0	0.0	0.0	0.0
Tully Creek	Chinook TRH	61.88	13.8	0.30	20.0	0.0	0.0	0.0
Tully Creek	Coho TRH	61.88	13.8	0.30	0.0	0.0	0.0	0.0
Tully Creek	Trout	61.88	13.8	0.30	63.6	56.8	58.9	13.9

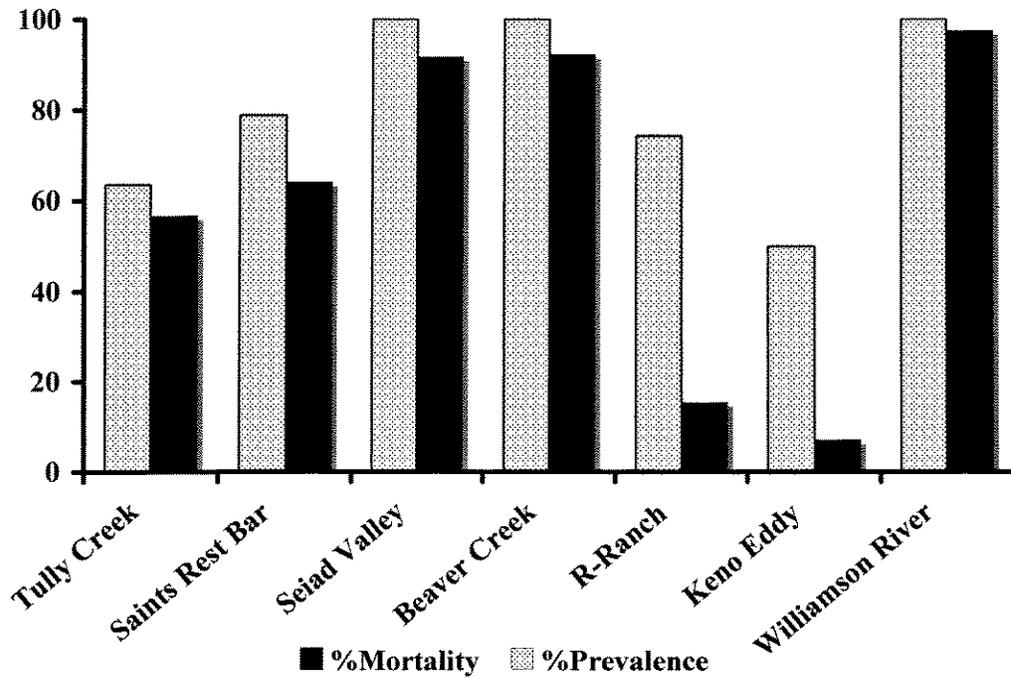


Figure 4.12. Percent mortality and prevalence of infection in rainbow trout following a 72-hr exposure to *Ceratomyxa shasta* in the Klamath River Basin in May 2006. The x-axis is arranged from uppermost exposure site (Williamson River) to the lowermost (Klamath River at Tully Creek).

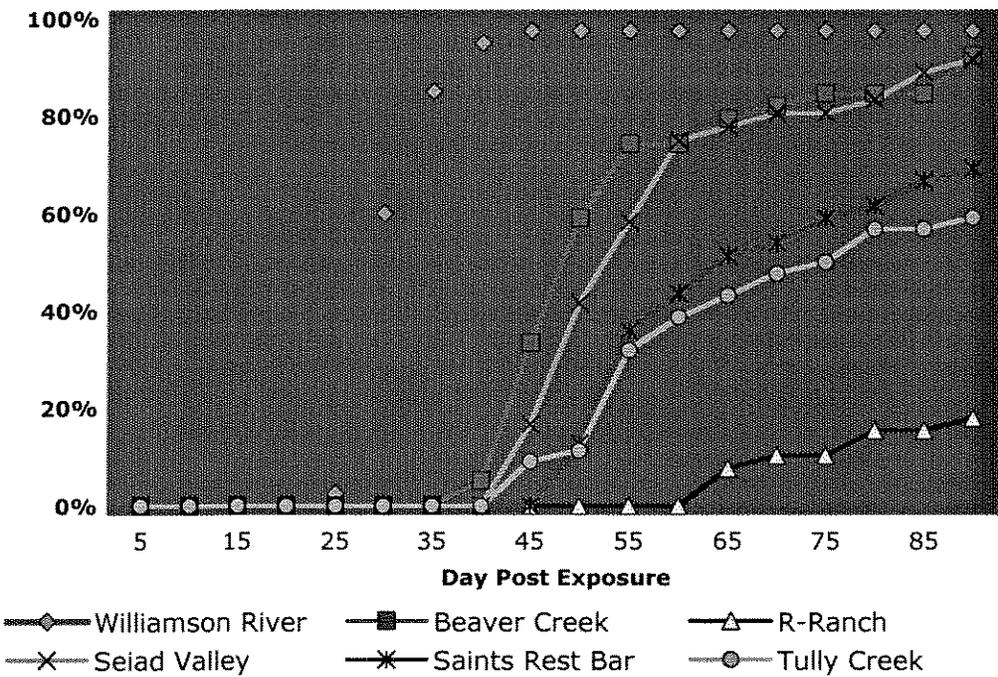


Figure 4.13. Cumulative mortality curve for rainbow trout exposed to *Ceratomyxa shasta* in the Klamath River for 3-days in May 2006.

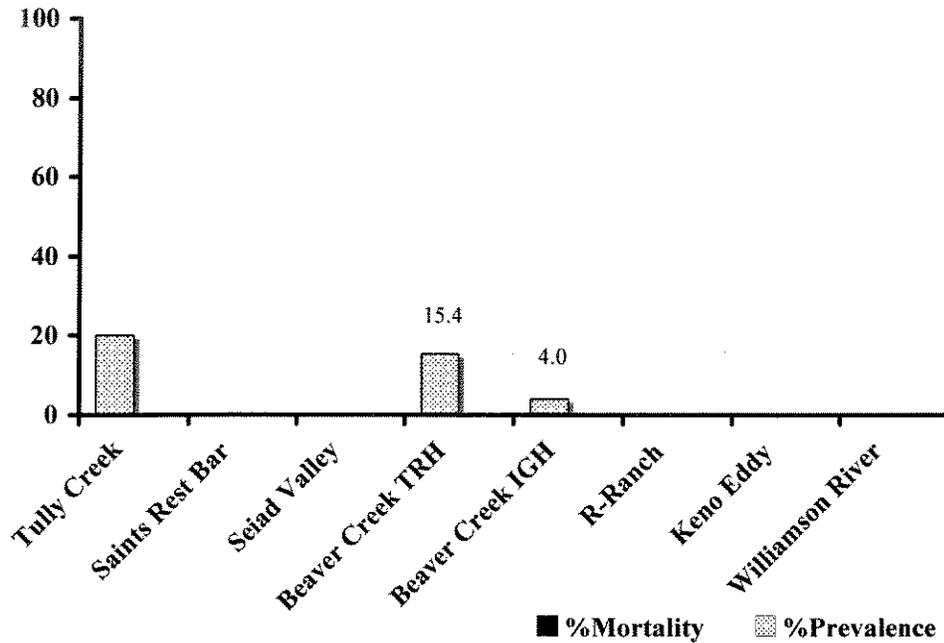


Figure 4.14. Percent mortality and prevalence of infection in fall Chinook salmon as a result of *Ceratomyxa shasta* following a 72-hr exposure in the Klamath River in May 2006. Note in Table 4.2 that May fall Chinook exposures only occurred at R-Ranch, Beaver Creek with Trinity River Hatchery (TRH) and Iron Gate Hatchery (IGH) stocks, Saints Rest Bar (TRH) and Tully Creek (TRH).

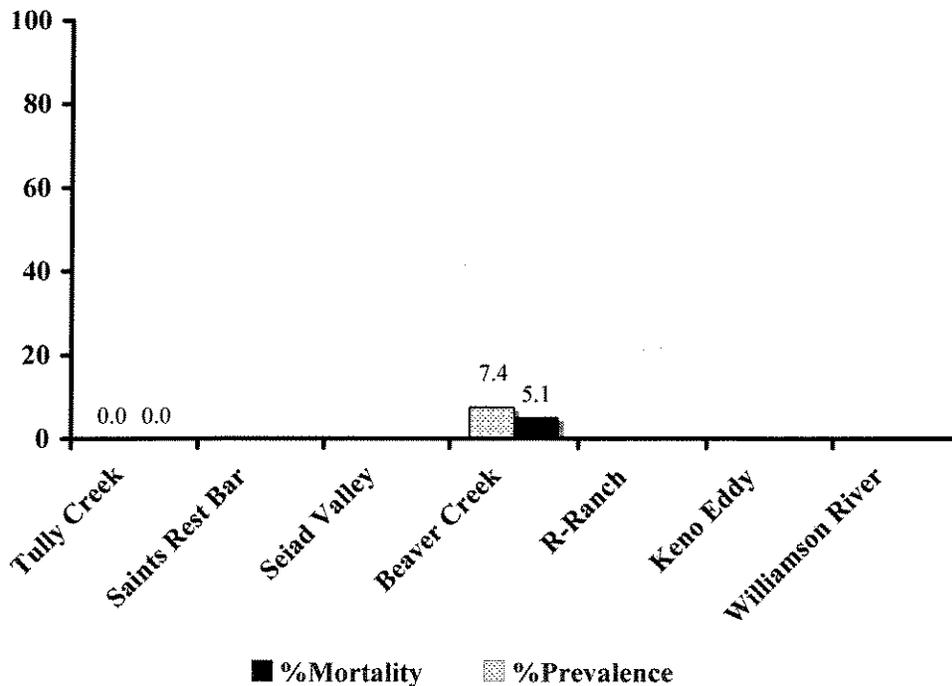


Figure 4.15. Percent mortality and prevalence of infection in coho salmon as a result of *Ceratomyxa shasta* following a 72-hr exposure in the Klamath River in May 2006. Note in Table 4.2 that May coho exposures only occurred at Beaver Creek with Iron Gate Hatchery (IGH) stock and Tully Creek with Trinity River Hatchery (TRH) stock.

Table 4.6. *Ceratomyxa shasta* infection prevalence, percent mortality and mean day to death for fish exposed for 3-days (72 hr) in June 2006 at eight locations in the Klamath River.

Site	Species	River Kilometer	Avg Temp °C (SD)		% Prev	% Mort	Avg day to death (SD)	
Williamson River	Trout	7.69	17.4	0.33	100	97.6	32.1	4.5
Williamson River	Chinook IGH	7.69	17.4	0.33	0.0	0.0	0.0	0.0
Keno Eddy	Trout	368.50	19.8	0.69	92.0	0.0	0.0	0.0
R-Ranch	Trout	300.19	19.7	0.44	87.2	21.2	60.4	15.9
R-Ranch	Chinook IGH	300.19	19.7	0.44	0.0	0.0	0.0	0.0
Beaver Creek	Trout	259.10	20.0	1.08	98.1	96.2	37.9	3.2
Beaver Creek	Coho TRH	259.10	20.0	1.08	15.6	12.8	47.1	6.8
Beaver Creek	Coho IGH	259.10	20.0	1.08	7.1	2.6	49.0	0.0
Beaver Creek	Chinook IGH	259.10	20.0	1.08	37.5	16.7	46.1	15.1
Beaver Creek	Chinook TRH	259.10	20.0	1.08	82.8	8.1	50.3	12.2
Seiad Valley	Trout	206.96	19.1	0.55	100	97.6	35.7	3.8
Seiad Valley	Chinook IGH	206.96	19.1	0.55	7.1	2.3	73.0	0.0
Saints Rest Bar	Trout	72.58	19.1	0.56	100	95.0	50.3	11.5
Saints Rest Bar	Coho TRH	72.58	19.1	0.56	13.8	7.9	56.2	11.5
Saints Rest Bar	Chinook TRH	72.58	19.1	0.56	0.0	0.0	0.0	0.0
Trinity at Hoopa Valley	Trout	21.16	15.3	1.02	0.0	0.0	0.0	0.0
Trinity at Hoopa Valley	Chinook TRH	21.16	15.3	1.02	0.0	0.0	0.0	0.0
Tully Creek	Trout	61.88	18.5	0.52	97.8	95.6	50.3	6.5
Tully Creek	Coho TRH	61.88	18.5	0.52	7.4	4.7	49.8	5.7
Tully Creek	Chinook TRH	61.88	18.5	0.52	7.4	3.4	42.0	0.0

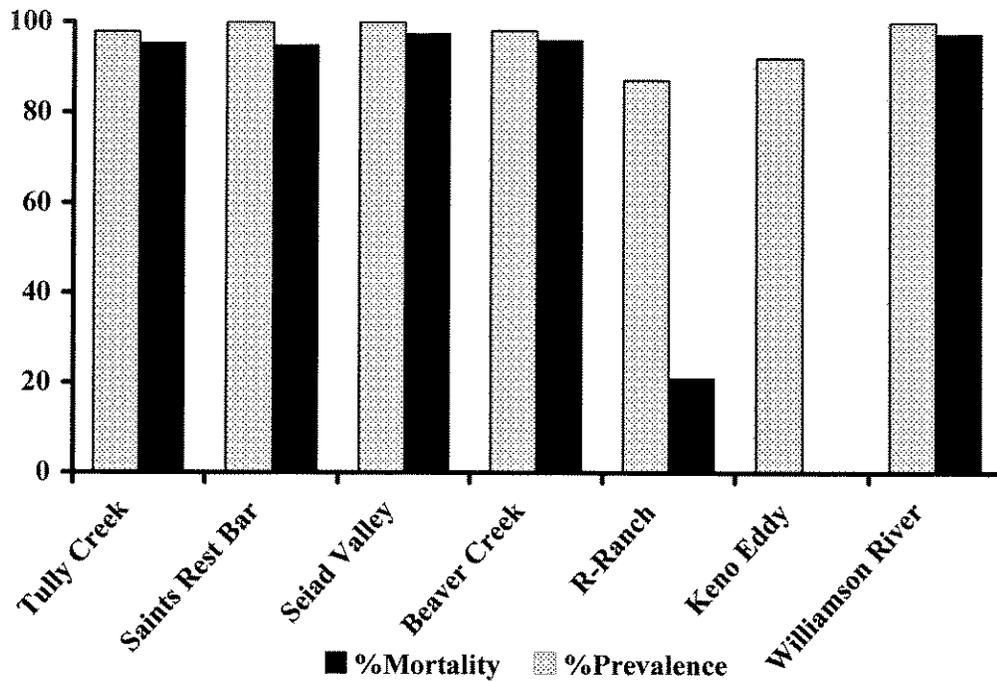


Figure 4.16. Percent mortality and prevalence of infection in rainbow trout as a result of *Ceratomyxa shasta* following a 72-hr exposure in the Klamath River in June 2006.

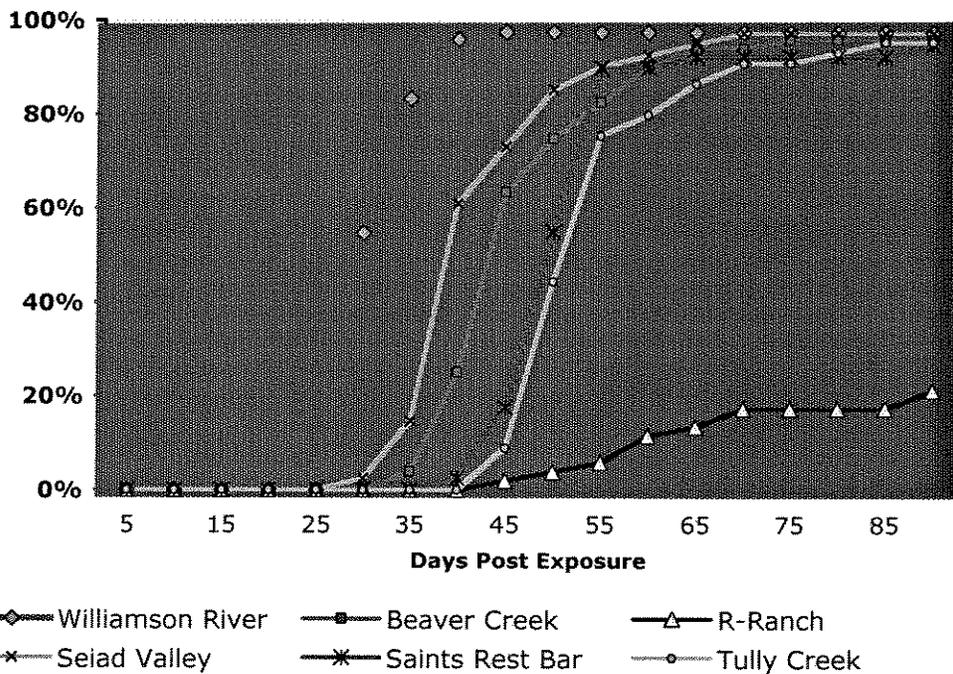


Figure 4.17. Cumulative mortality curve for rainbow trout exposed to *Ceratomyxa shasta* in the Klamath River for 3-days in June 2006.

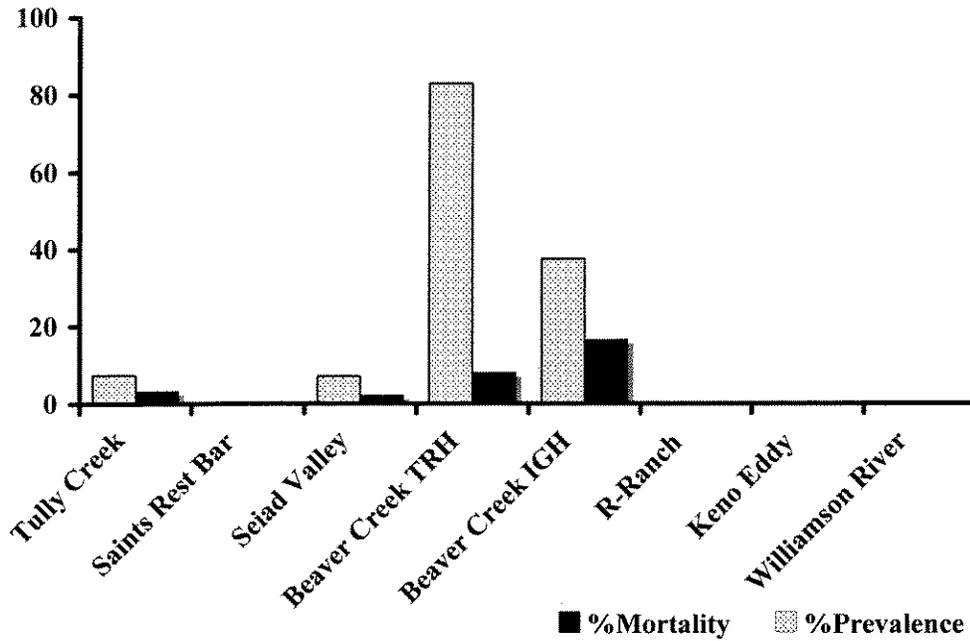


Figure 4.18. Percent mortality and prevalence of infection in fall Chinook salmon as a result of *Ceratomyxa shasta* following a 72-hr exposure in the Klamath River in June 2006. Note that fall Chinook exposures did not occur at Keno Eddy (Table 4.6). Iron Gate (IGH) and Trinity River hatchery (TRH) stocks used.

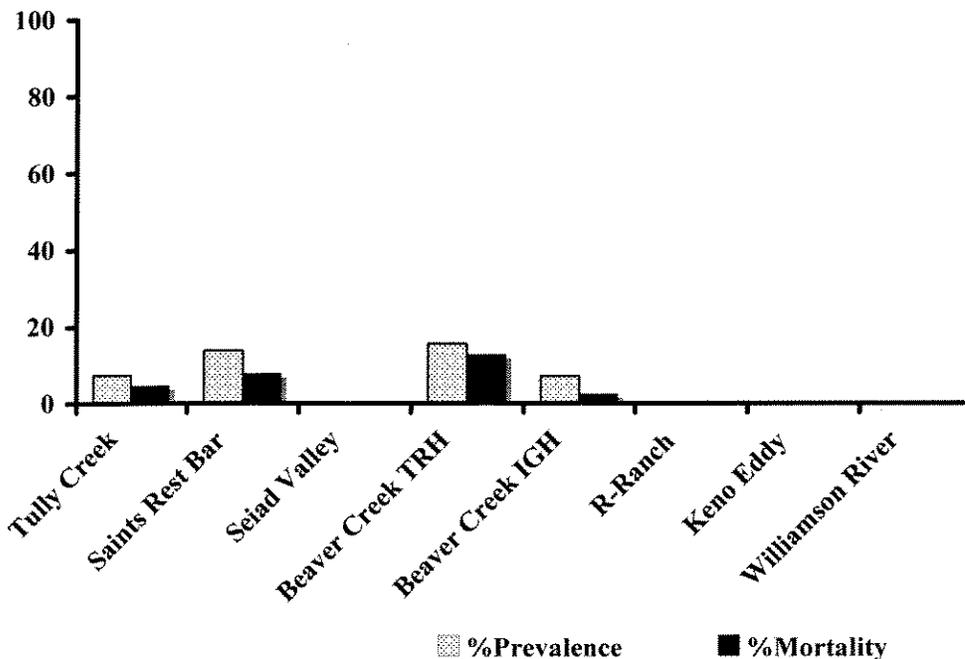


Figure 4.19. Percent mortality and prevalence of infection in coho salmon as a result of *Ceratomyxa shasta* following a 72-hr exposure in the Klamath River in June 2006. Note that coho salmon exposures occurred only at Beaver Creek with both Iron Gate (IGH) and Trinity River hatchery (TRH) stocks used, Saints Rest Bar (TRH) and Tully Creek (TRH) (see also Table 4.6).

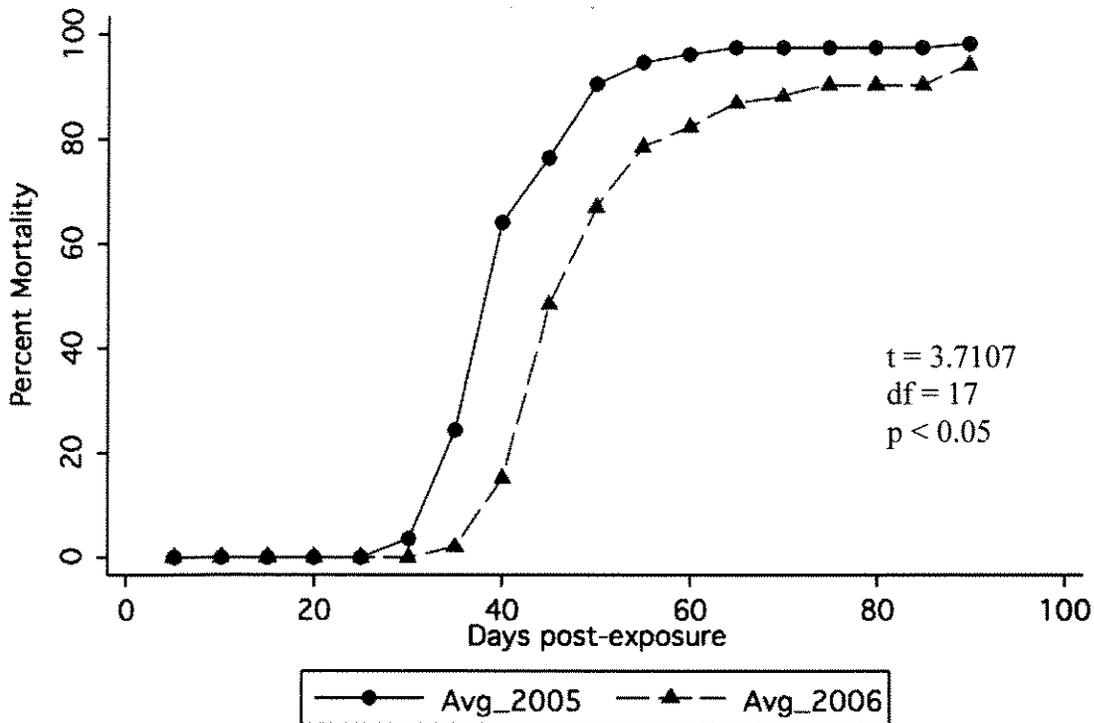


Figure 4.20. Comparison of 2005-2006 cumulative mortality for rainbow trout exposures to *Ceratomyxa shasta* in the Klamath River near Beaver Creek. May and June mortality was averaged for 2005 and for 2006 and then compared using a paired t-test.

DISCUSSION

Results of May 2005 exposures suggested that the incidence and severity of *C. shasta* in fall Chinook salmon would increase and peak in early to mid-June as observed in previous years. However, the high flow event of May 2005 appears to be responsible for a decline in *C. shasta*-related mortality in Chinook salmon, and for an increase in the mean day to death for susceptible rainbow trout exposed in June 2005. Out-migrant data obtained by the USFWS also describes a pattern of decreasing incidence of infection between May and June, 2005, during the period of increased flows and decreased water temperature, although overall infection prevalence was similar to previous years (Nichols et al. 2007).

Sentinel exposures have been useful for determining locations of high infectivity and in comparing parasite exposure between years, seasons and sites. However, these exposures are only for 3 d; thus, we cannot directly extrapolate these results to reflect outmigrant survival. Additionally, post-exposure holding temperatures for exposures in 2005-06 were 12.8°C, well

below ambient Klamath River water temperatures during May and June. In exposures conducted by the USFWS at Beaver Creek with post-exposure holding temperatures of 18 – 20°C, mortality in fall Chinook salmon was higher than was observed in our fall Chinook exposures (J. S. Foott, Personal Communication). At the much colder holding temperatures (12.8°C) used by the OSU lab, it is likely that the fall Chinook and coho salmon can resist progression of disease to a clinical state and may be able to overcome infection altogether. Indeed, preliminary data from recent (May 2007) experiments suggests that holding exposed fall Chinook and coho salmon at ambient (12.8°C) and 18°C temperatures alters not only the death rate, but overall mortality. Udey et al. (1975) were the first to notice that Fall Creek Hatchery coho salmon exposed to *C. shasta* in the Willamette River were able to successfully combat infection when held at colder water temperatures. As temperatures increased, susceptibility of the coho salmon also increased and they speculated this may be a result of the defense mechanisms of the fish rather than of increased pathogenicity of the parasite.

An exception to this is the Williamson River exposure location above the Upper Klamath Lake. Results from this location are somewhat perplexing given that rainbow trout exposed at this site in 2006 demonstrated the highest mortality in the least amount of time and yet we were not able to infect a single fall Chinook at that location. In fact, the Williamson River has always been one of the most infectious locations for *C. shasta* and experimental fall Chinook have never developed infection at that site. At present, the only explanation for this is the possibility of a strain difference.

The consistency of OSU exposure-holding protocols does permit analysis of details between the fall Chinook and coho salmon in 2005 and 2006. The data suggests that the coho salmon, at a low infectious dose, are as resistant to infection as the fall Chinook. However, the threshold at which the coho resist infection seems fairly narrow compared to the fall Chinook. Note that in the 2006 coho exposures, infection prevalence and mortality were always similar whereas the fall Chinook appear to be a little more tolerant of infection. It is suggested here that at a higher infectious dose and higher holding temperatures, the death rate and overall mortality of the Klamath River coho salmon would exceed that of the Fall Chinook salmon. This tolerance threshold is likely a function of the exposure dose, water temperature and the resistance mechanisms employed by each species and each stock. For example, the two stocks of fall Chinook used in 2006 consistently exhibit different susceptibilities to infection with the Trinity

River stock slightly more susceptible but also more tolerant to infection.

Although the 2005 and 2006 high flow events mark the transition from the high infection and mortality of 2002 to 2004, the actual mechanisms responsible for that decrease are not clearly understood and could include a dilution of spore quantities due to increased water volume, faster flows inhibiting attachment of the spores to the fish, strong flows assisting juvenile fish out of the highly infective areas and/or removal of heavily infected polychaete populations. An example of this latter event is one population at the Tree of Heaven that was removed during the May–June 2005 high flow event (Stocking and Bartholomew 2007). However, the removal of one polychaete population is probably of limited value since numerous polychaete populations of significant size were still identified within the “hot-zone” during the 2006 surveys for *M. speciosa* and infection prevalence within these populations was also significantly decreased from 2004 estimates (Stocking and Bartholomew 2007; Authors unpublished data supported by Hoopa Tribal Fisheries). The low levels of *C. shasta* infection prevalence within invertebrate host populations tested during June, July and August 2006 suggest yet other mechanisms: a reduction of spore input from spawning adult salmon and/or the removal of spore-laden sediments that the invertebrate host is known to feed on. To better understand the mechanisms responsible for yearly infection levels, it may be necessary to quantify the parasite loads carried by returning adult salmon and determine where those parasites over winter.

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PROJECT BUDGET

a. Salaries (including benefits)	\$27,099.00
b. Operating Expenses	\$31,401.00
c. Administrative Overhead @ 15%	<u>\$8,775.00</u>
TOTAL PROJECT COST TO USFWS	\$67,275.00
TOTAL COST SHARE:	\$25,218.00
TOTAL PROJECT COST:	\$92,493.00

COST SHARE INFORMATION:

Oregon State University: Cost share provided by OSU will consist of the difference between the overhead rate of 41.5% and the allowed rate of 15%. Total cost share: \$14,257.00.

Karuk Tribe: The Karuk Tribe contributed cost share in the form of personnel (\$4,673 wages and fringe), sampling equipment (\$1,500) and travel (\$4,788). Total cost share: \$10,961.00.

