Compilation of Information Relating to Myxozoan Disease Effects to Inform the Klamath Basin Restoration Agreement

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1. INTRODUCTION

This technical report describes how myxozoan disease effects on juvenile Chinook and coho salmon are predicted to differ between the scenarios of current conditions and removal of the four Klamath project dams. We begin by summarizing what we know about the effects of myxozoan pathogens on Chinook salmon, the parasite life cycles, their distribution in the Klamath River and characteristics of the polychaete host that may be affected by changes in river management. We then look at how the two proposed scenarios will affect each of these parameters, supporting our predictions with available data. It must be taken into account that parasite infectious dose is the primary metric that will influence disease severity.

This assessment is structured to prioritize the factors we believe are most important for predicting where disease effects will occur under current conditions and where future areas of high infections might occur. In order for an area to develop as an infectious zone, several factors need to coincide, including:

- polychaete habitat (physical habitat: pools, eddies, periphyton, sediment)
- microhabitats with low velocity, stable flows
- close proximity to spawning areas (myxospore input)
- temperatures above 15˚C (rate of disease development in fish)

Because of the lack of data on how some of these parameters might change under the two scenarios and the lack of information on certain aspects of host:pathogen dynamics, we have had to make certain assumptions. These will be clearly stated under each assessment and are listed below so that if future research provides additional data our predictions can be reassessed.

Assumptions and definitions

- Disease mortality = overt death due to physiological imbalance associated with ceratomyxosis and kidney pathology (*Parvicapsula minibicornis*) or predation associated with disease morbidity.
- Reach definitions used in this assessment:
    - R1: Estuary to Indian Creek
    - R2: Indian Creek to Iron Gate Dam (IGD) – includes area of current high parasite abundance
    - R3: IGD to Keno – project area
    - R4: Above Klamath Lake
- Sediment transport and deposition – Assumptions on sediment release effects are based on modeling results from the Stillwater Sciences Technical Report (2009). With dam removal, deposition of sediment will increase in the short-term if flushing flows are provided to reduce macrophyte establishment; if no high flow events occur sediment levels will increase. In the long term, a more variable flow regime

would reduce stability of sediments in the system with the potential outcome of destabilizing polychaete habitat. To describe predicted disturbance thresholds we have used data from the report by Ayres Associates (1999) and a technical memorandum by Varyu and Greimann (DRAFT Sediment Mobilization Analysis at Little Bogus Creek and Beaver Creek for Klamath Dam Removal Studies, May 2010). Although the hydrograph will not change significantly with dam removal, the reduction of median substrate size means that lower flows are required for bed mobilization. Refer to **Sections 3 and 4**.

- Hydrograph – Flow assumptions following removal of PacifiCorp's Klamath River dams are based on the USFWS Arcata Settlement Technical Report (Hetrick et al. 2009) using the WRIMS Run-32 Refuge model. Stream gradients within the current project area are relatively steep compared with the river below IGD and thus would provide higher velocity flows. Refer to **Section 5**.

- Temperature - Temperature predictions following removal of the dams are based on the USFWS Arcata Settlement Technical Report (Hetrick et al. 2009), using supplemental projections for dam removal scenarios based on the 2000 water year provided by PacifiCorp/CH2M Hill. Refer to **Section 6**.

- Myxospore input – We assume primary myxospore contribution is from adult salmon. Juvenile fish are likely to die prior to spore formation or be removed by predation. Mortality in juvenile Chinook salmon occurs as a result of infection by a specific parasite genotype that is perpetuated only by this species. Under current conditions there would be no significant change in the numbers of adult Chinook salmon spawning in the river reach below IGD. With dam removal, the percent of adult Fall-run Chinook salmon likely to migrate to and spawn in reaches in the current project area will increase, as will spawning in the Williamson and Sprague Rivers. Refer to **Section 7**.
- Nutrient levels – Assumptions of current nutrient (nitrogen and phosphorus) levels are informed by the North Coast RWQCB (Asarian et al. 2009). However, currently, there is little information that would provide a direct measure of polychaete diet availability (e.g. chlorophyll a) and how that would change. Refer to **Section 8**.
- Life stages affected by *C. shasta.*
    - egg-fry - assume little impact due to seasonality of infection, no vertical transmission
    - fry-smolt –population most at risk.
    - returning adults – assume these fish become reinfected when entering the KR, potential for impacts on pre-spawn mortality
- Smolt life histories
    - R1 and 2: Fall Chinook salmon type I life history, with slow migration arriving at the estuary by June-July; Spring Chinook salmon from Salmon

River exhibit type II life history, arriving at the estuary in fall (this population in low numbers).

  - R3: Fall Chinook salmon type I predominates, some tributary spawners may shift to type II but this is expected to be a low proportion of the population
  - R4: Potential for types I and III
  - Diversity within and between life history types is expected to increase over time in response to reintroduction of anadromy into the upper basin.

- Hatchery production at Iron Gate dam is uncertain with dam removal. Alternatives include full mitigation for 6 years (6 million fish annually), expansion of the Fall Creek program, and construction of a conservation hatchery. For this assessment we will:
  - Assume 2-3 years of hatchery returns – short term
  - After 3 yrs natural production only

2. BACKGROUND

2.1 MYXOZOANS IN THE KLAMATH RIVER AND FACTORS THAT INFLUENCE DISEASE

Two **myxozoan parasites,** *Ceratomyxa shasta* and *Parvicapsula minibicornis,* have been associated with disease and mortality in Klamath River salmon. Infection by *P. minibicornis* may occur at a prevalence of greater than 90% in Chinook salmon and over 50% of coho salmon emigrants. These infections are often associated with clinical pathology but it is unknown if they cause direct mortality. There is some experimental evidence that these fish may recover; however, the anemia associated with the infection my weaken these fish and make them more susceptible to other infections and stressors. Infection by *C. shasta* occurs at a lower prevalence; however, the parasite has a more direct effect on mortality of Klamath River salmon. Because *P. minibicornis* and *C. shasta* share the same invertebrate host, environmental variables (temperature, flow, nutrients etc.) are expected to affect their abundance similarly. For these reasons, we have chosen to use *C. shasta* as an indicator of mortality as a result of myxozoan infection.

2.2 MYXOZOAN LIFE CYCLES

Myxozoan parasites have complex life cycles, with an annelid typically serving as the definitive host. For both *P. minibicornis* and *C. shasta*, this host is the freshwater polychaete worm *Manayunkia speciosa* (Figure 2.1)(Bartholomew et al. 1997, 2006). Thus, unlike bacterial pathogens and external parasites, transmission of myxozoan parasites is limited to areas where the invertebrate host is present.

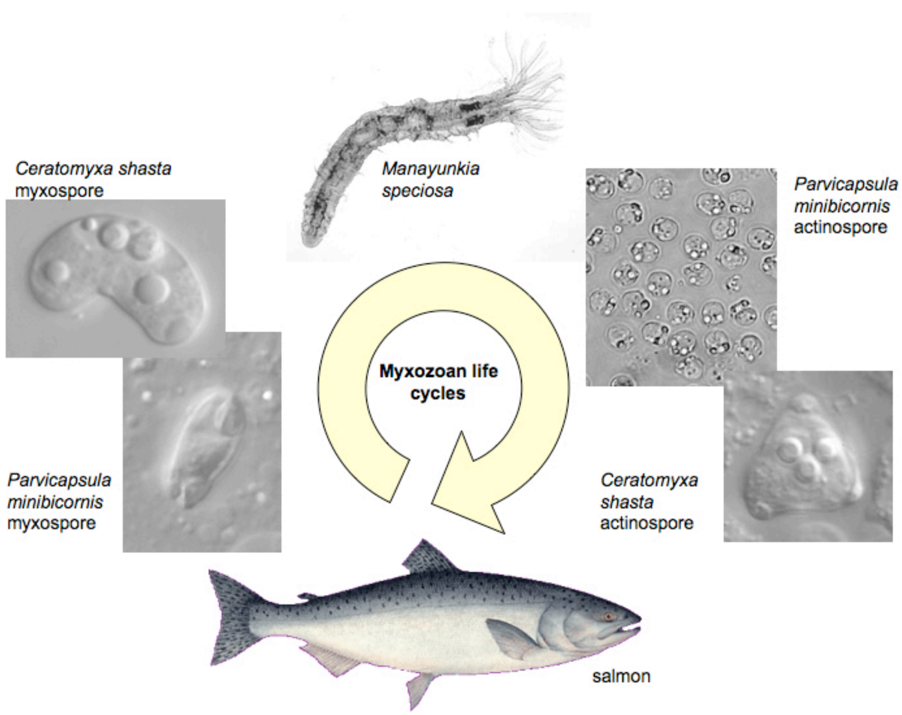


Figure 2.1. Life cycles of *Ceratomyxa shasta* and *Parvicapsula minibicornis*

Ceratomyxa shasta actinospore stages are released from infected polychaetes into the water column as temperatures rise above 10˚C in late March/early April. These stages are neutrally buoyant (Foott et al. 2007; Bartholomew, unpublished results), relatively short-lived (days to several weeks; Bjork 2010) and die unless encountering a susceptible fish host. Fish become infected when the parasite invades the gills (Bjork and Bartholomew, 2010), traveling through the bloodstream to reach the intestine. Here, the parasite continues to replicate, causing tissue damage and eventually maturing to the myxospore stage. This stage is assumed to be released upon death of the host; however, shedding of myxospores has also been observed from infected juvenile fish (Holt and Bartholomew, unpublished data). Myxospore stages have a higher density than actinospores, allowing them to sink and be more readily ingested by polychaetes. The polychaete is presumably the definitive host, and here the parasite develops in the body wall of the worm (Meaders and Hendrickson 2009) and is released in a non-lethal fashion through pores in the body wall (Bjork 2010).

2.3 MYXOZOAN PREVALENCE AND DISTRIBUTION WITHIN THE MAINSTEM KLAMATH RIVER

The geographic distribution of *C. shasta* is limited to the Pacific Northwest of the U.S. and Canada, where it is present in many of the larger river systems. In the Klamath River, a study by Hendrickson et al. (1989) demonstrated that the parasite was present in the mainstem Klamath River, but that fish did not become infected in the tributaries below Klamath Lake. Hemmingson (Oregon Department of Fish and Wildlife, personal communication) reported *C. shasta* distribution in the Williamson River More recently, results of a study that measured *C. shasta*-related mortality, infection prevalence and mean day to death in susceptible rainbow trout held for 3 days at locations in the Klamath mainstem and tributaries demonstrated that *C. shasta* prevalence is increased in the Klamath River downriver of Iron Gate Dam compared with the mainstem river above the dam (Stocking et al. 2006), with the exception of the lower Williamson River. Data from sentinel studies exposing Klamath Chinook and coho salmon simultaneously with the susceptible rainbow trout (Bartholomew and Holt, unpublished data) show a finer resolution, with high mortality occurring in fish held above Beaver Creek and at Seiad Valley and low mortality in fish held at upriver and downriver sites. An example of these trends is depicted in Figure 2.2.

Water monitoring data also provides a picture of spatial distribution of the parasite at any particular time. Although this method does not differentiate between the actinospore and myxospore stages, we assume that during the period of sentinel fish exposure the primary life stage detected is the actinospore. Mortality in sentinel fish is generally supportive of this assumption. Water samples collected from four index sites below Iron Gate Dam since 2005 and assayed for parasite density (Hallett and Bartholomew 2006) allow comparison of parasite levels between sites (Figures 2.3, 2.4). This study, and subsequent water monitoring, demonstrates that parasite abundance (i.e. actinospore stage) is low at the outflow of Iron Gate Reservoir but increases in the main stem Klamath between the Interstate 5 bridge (I-5) and the confluence of the Scott River. This general pattern has remained stable, but the size of the infectious zone and the magnitude of parasite densities change seasonally and annually. For example, examination of water samples from three sites in the infectious zone in 2009 showed that parasite densities were higher and peaked earlier in 2009 (Figure 2.5). Parasite densities in water samples can be used to help

interpret sentinel fish data. However, the susceptibility of the fish species affects the threshold level for mortality. For example, for susceptible rainbow trout the mortality threshold is < 1 parasite/liter compared with > 10 parasites/liter for Klamath River Chinook salmon. Additionally, water sample data, as currently determined, does not differentiate between parasite genotypes (see Section 2.7). Thus, as sites such as Orleans, which have moderate parasite densities, infection in Chinook salmon might be lower than predicted as a result of a shift in parasite genotype.

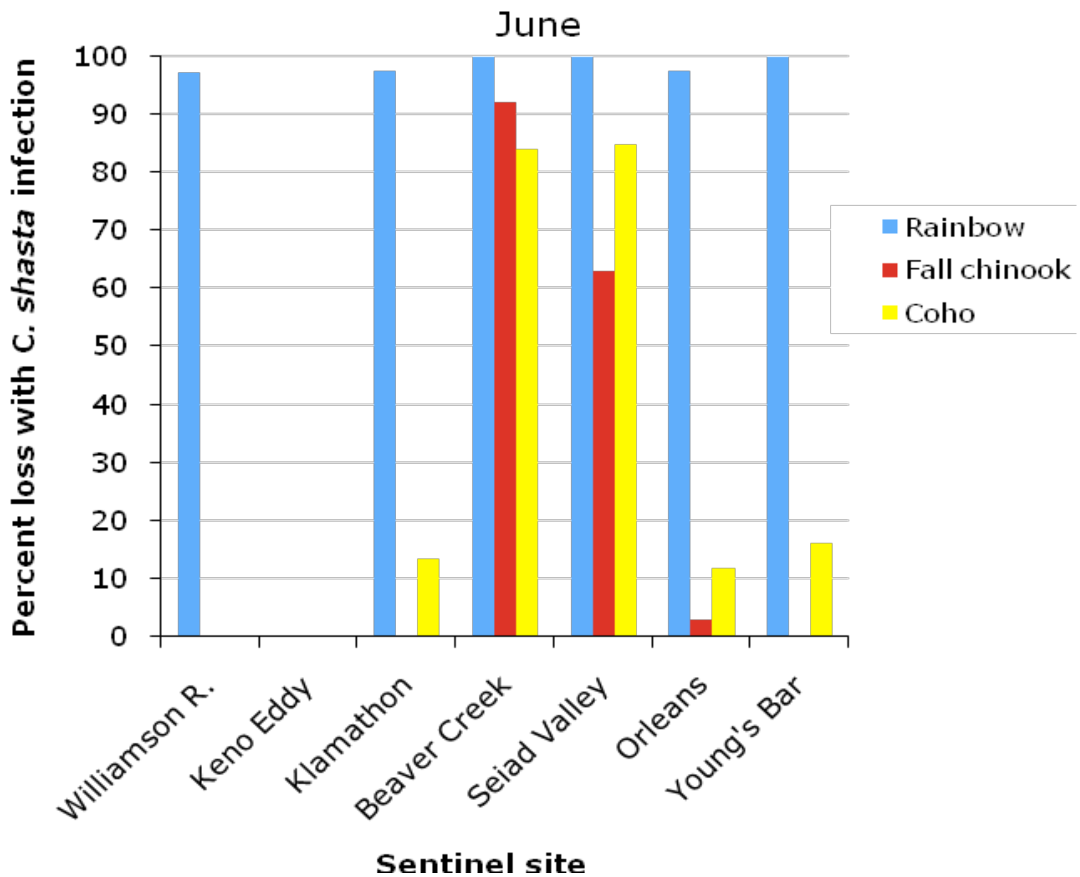


FIGURE 2.2. Percent *Ceratomyxa shasta* mortality in susceptible rainbow trout and Iron Gate Hatchery Chinook and coho salmon held at exposures sites in the Klamath River in June 2008. Coho salmon were not held at the Williamson River and Keno sites in the upper basin. (Bartholomew, unpublished data).

Release of actinospores, and thus infection of fish, is seasonal, with spring release being the highest (Ratliff 1983). This is reflected in both sentinel exposures and water sampling data. In the Klamath River, release begins as water temperatures increase in late April and generally peaks in late June. During late July through early September parasite densities decrease (Figure 2.6). Whether this is a result of decreased longevity of the parasite or the polychaete host at increased water temperatures or an adaptive strategy on the part of the parasite to target fish migration timing is unknown. We make an assumption that parasites detected by QPCR in late spring through late summer are predominantly actinospores, which is supported by fish infection studies.

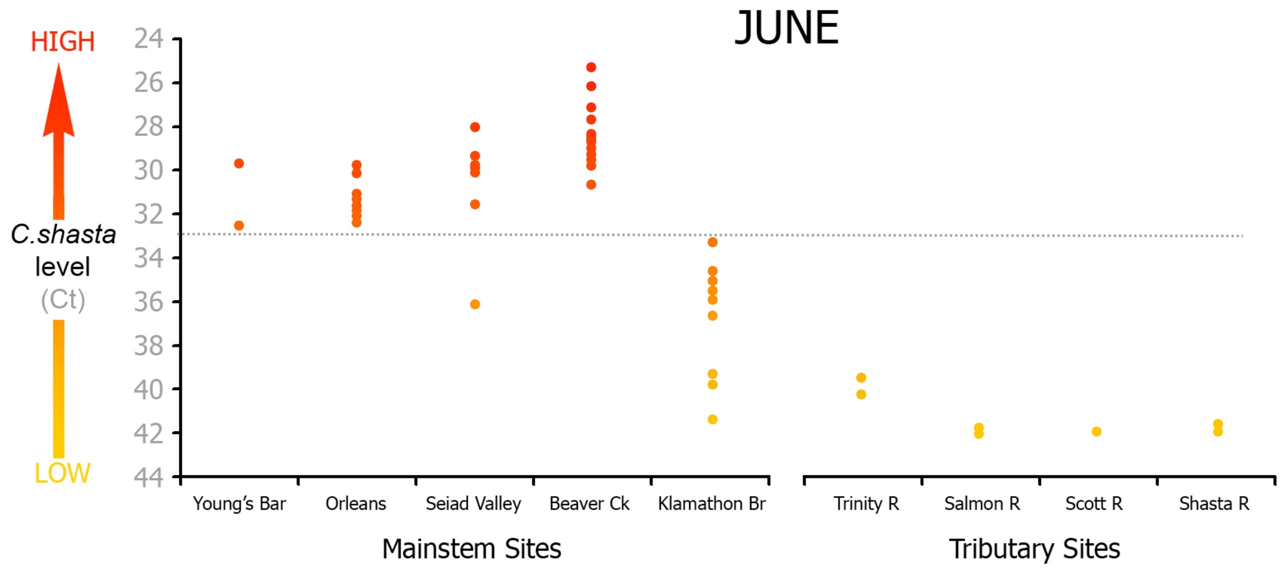


Figure 2.3. Comparison of parasite levels between sites. Abundance of *Ceratomyxa shasta* actinospores in water samples in June (month with highest abundance) 2008 at various mainstem and tributary sites. Sites are displayed from west to east. Each data point on the graph represents the average of the 3 water samples collected at that time point. Most sites were sampled multiple times during the month. The dotted line represents approximately one spore per liter. (Hallett and Bartholomew, unpublished data).

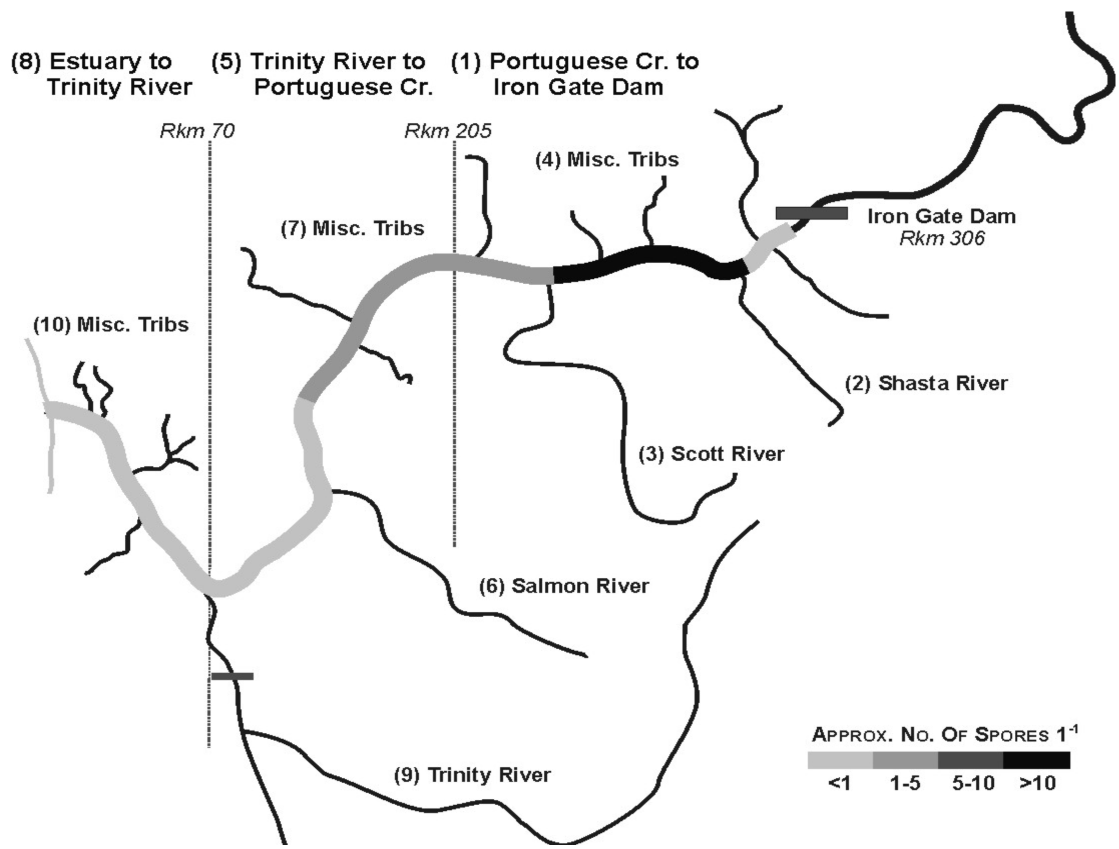


Figure 2.4. Map of Klamath River below Iron Gate dam showing approximate numbers of *Ceratomyxa shasta* detected per liter of water collected during May-June, 2005. Parasite stages assumed to be actinospores released from polychaete worms (interpreted using data from Hallett and Bartholomew 2006).

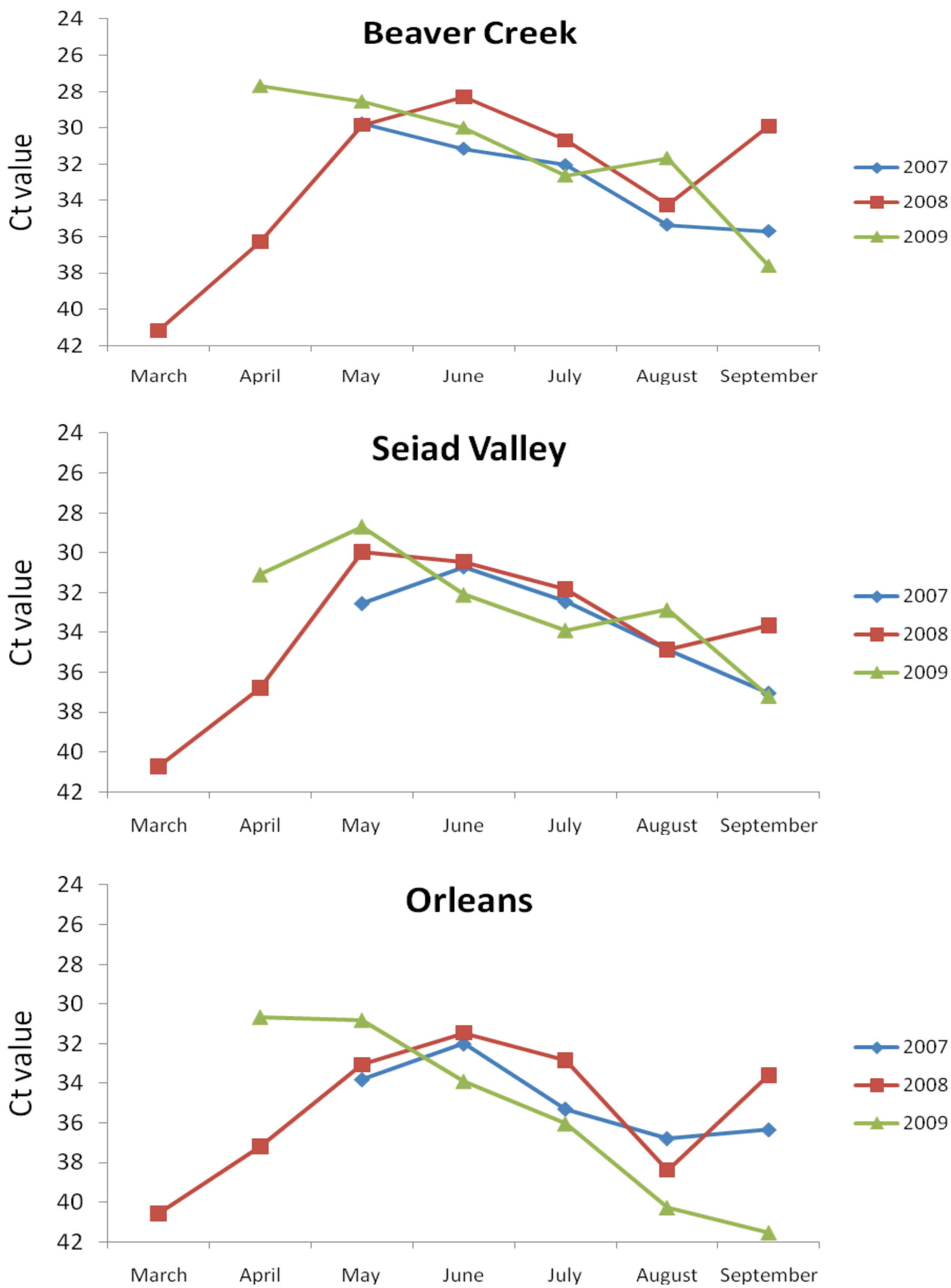


Figure 2.5. Abundance of *Ceratomyxa shasta* in water samples from three sites within the infectious zone in the main stem Klamath River. Data points are averages of multiple samples taken each month. Values for 2007 and 2009 were adjusted to be comparable with 2008 Ct values; 1 spore/L =~ Ct 34, a ten-fold increase in abundance is approximately equal to a decrease of 3 Ct values. (Hallett and Bartholomew, unpublished data).

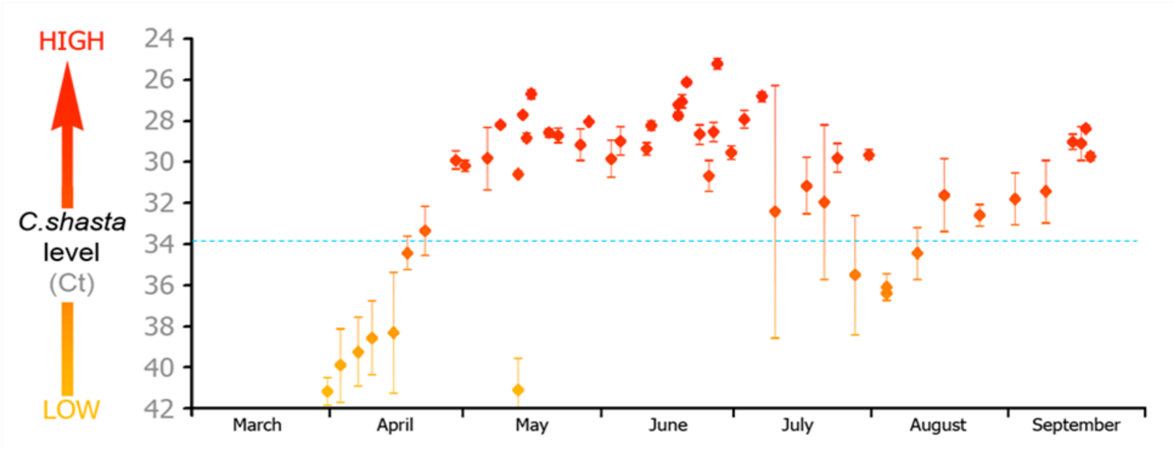


Figure 2.6. Seasonal abundance of *Ceratomyxa shasta* in water samples from the Klamath River near Beaver Creek collected March through September 2008. Each data point on the graph represents the average of the 3 water samples at that time point; error bars display the standard deviation. The dotted line represents approximately one spore per liter. (Hallett and Bartholomew, unpublished data).

Prevalence of parasite infection in polychaete host populations in this section of the river is also high compared with samples from other sites (Figure 2.7; Stocking and Bartholomew 2007). Although data is limited, it is notable that infection prevalence in polychaete host populations was an order of magnitude greater at river kilometer (Rkm) 290 (near I-5) and 278 (Tree of Heaven) than at any other site throughout the river.

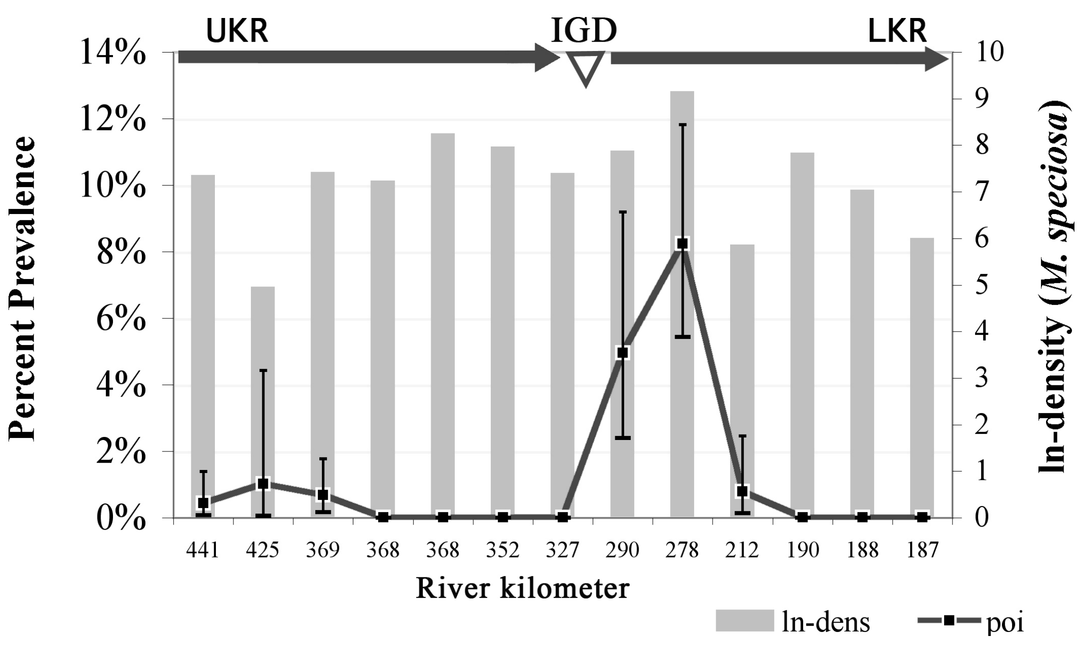


Figure 2.7. Estimates of *Ceratomyxa shasta* infection prevalence (poi) and associated 95% confidence intervals within selected populations of *Manayunkia speciosa* collected from the Klamath River. Sites sorted on the x-axis from Upper Klamath Lake (Rkm 441) going downriver towards the mouth. Abbreviations UKR = Upper Klamath River, IGD = Iron Gate Dam, and LKR = Lower Klamath River (from Stocking and Bartholomew 2007).

2.4 INVERTEBRATE HOST DISTRIBUTION AND ABUNDANCE IN THE KLAMATH RIVER

A qualitative survey for *M. speciosa* demonstrated the polychaete is present throughout the main stem Klamath River from Klamath Lake to the estuary (Stocking and Bartholomew 2007). The polychaete was present in a variety of macrohabitats such as runs, pools, riffle edge-water, and reservoir inflow zones. The polychaete was also found within a variety of microhabitats such as sand, gravel, boulder and bedrock, freshwater sponge, aquatic vegetation, and frequently with a non-vascular periphyton identified as a species of *Cladophora*. Slow flowing habitats such as runs and eddy-pools had the highest relative densities and frequency of occurrence of *M. speciosa*. Within runs, *M. speciosa* tended to occur within a protective microhabitat, such as *Cladophora* sp. when the algae was infused with fine organic matter. Pools/eddies often contained both sand-silt and *Cladophora* sp., the 2 microhabitats identified with the highest densities of *M. speciosa*, explaining why the polychaete was detected most frequently in this habitat. The authors speculate that the capacity for a habitat to buffer against disturbances will be a critical factor determining the distribution and abundance of *M. speciosa* in the riverine environment.

In reservoirs and Klamath Lake, *M. speciosa* was consistently present at the riverine inflow areas (lotic-lentic interface) at moderate (804/m^2) to high (16,054/m^2) densities. Thus, the creation of reservoirs has likely altered the distribution of *M. speciosa* in the Klamath River by creating conditions that support unusually large populations at the inflow areas. This interface is the most stable macrohabitat where *M. speciosa* was documented. Here, polychaete populations receive a constant supply of flowing water with extreme flow events buffered by the reservoir. Refer to sections 3 and 4 for additional data on polychaete habitat.

The unique feeding abilities of *M. speciosa* may be a key factor to its persistence in the river environments. In the study by Stocking and Bartholomew (2007), tubes containing *M. speciosa* were observed on vertical rock walls, projecting out into the water column. At the mouth of the Williamson River, populations of *M. speciosa* were found freely roaming the sediment. These traits are characteristic of this polychaete family, which are both suspension feeders and facultative surface deposit feeders, utilizing a diet of fine organic detritus and micro-algae (Rouse and Pleijel, 2001; Fauchald and Jumars, 1979) and switching feeding modes during fluxes of food quantity and quality (Taghon and Green, 1992). Refer to sections 3 and 4 for additional data on polychaete habitat and feeding characteristics.

2.5 SOURCES OF MYXOSPORE INPUT FOR THE LIFE CYCLE

The spatial overlap of both hosts is a key factor in predicting where parasite abundance will be increased, and the formation of an infection nidus between the Shasta River and Indian Creek could be explained by a high concentration of spawning adult salmon in the reach below the dam, which provides myxospores to infect the dense polychaete populations in the reach below. A parasite strategy using the adult salmon as the primary source of myxospore production would have minimal effect on the salmon host (as it will die in

either case) and takes advantage of a means of upstream dispersal. Current data on myxospore sources are presented in Section 7.

2.6 DISEASE RESISTANCE OF KLAMATH RIVER CHINOOK AND COHO SALMON

Differences in susceptibility between salmonid strains are well documented, with strains of salmon native to waters endemic for the parasite generally developing a high degree of resistance to *C. shasta* (reviewed in Bartholomew 1998). Klamath River strains of salmon and steelhead do have a high degree of resistance to the parasite in comparison with the non-native trout used in sentinel studies to detect parasite presence. For this rainbow trout strain, a lethal challenge dose is less than 5 actinospores (Bjork and Bartholomew 2009). That study unsuccessfully attempted to determine the infectious dose for Chinook and coho salmon using controlled challenge doses of 5 x 10^3 parasites/fish.

A more recent attempt to quantify the infectious dose for Chinook salmon was done using natural challenges in the Klamath River. In this study fish were held for 16 – 72 hr in the infectious zone and the exposure dose was calculated using measurements of flow rate and parasite density during exposure. Results demonstrated a non-linear mortality threshold for Iron Gate Hatchery Chinook salmon that ranged from 5.6 - 9.9 x 10^4 total parasites. Below this threshold no mortality occurs, yet above it mortality dramatically increases (Ray and Bartholomew, accepted)(Figure 2.8).

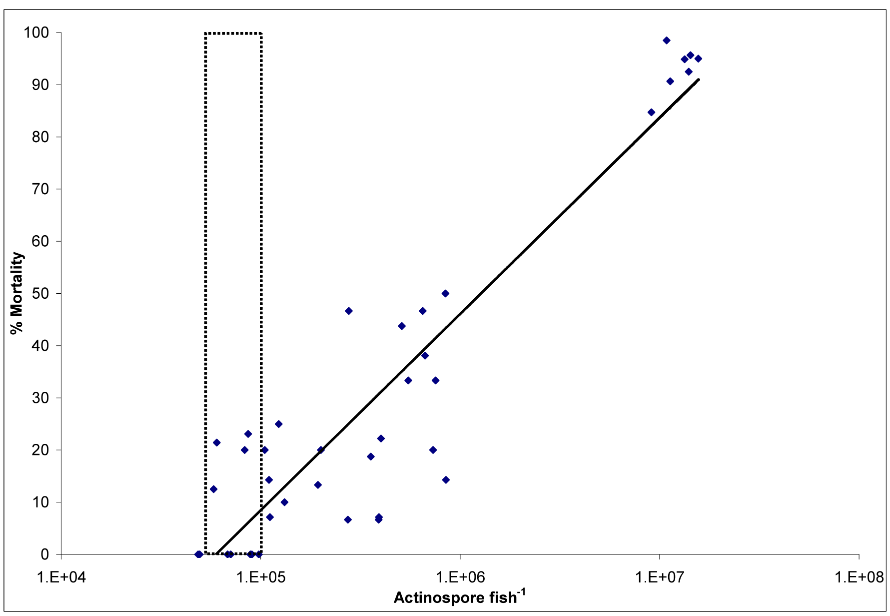


Figure 2.8. Relationship between *Ceratomyxa shasta* induced mortality in Iron Gate Hatchery Chinook salmon and estimated actinospore dose fish^{-1}. The bars represent the standard deviations on the percent mortality and actinospore dose. The x-axis is log transformed to better represent the wide range of actinospore doses. The R^2 value is 0.969 and the regression equation is y = 17.883 * Ln(x) - 199.08. The dotted box represents the range of values quantified for the mortality threshold.

Challenges to determine the relative susceptibility of Klamath River Chinook and coho salmon have shown equivocal results, and we now believe this is a result of differences in abundance of host-specific parasites strains (Section 2.7). Mortality in both species varies between years as a result of differences in temperature and infectious dose, with 3 day exposures resulting in mortality in both species as high as 98% (Figure 2.2).

Differences in susceptibilities of Klamath River stocks of both species have also been examined, and although data is not comprehensive (Figures 2.9 and 2.10), Chinook and coho salmon originating from areas above or within the infectious zone appear to be more resistant than Trinity River Hatchery stocks. This suggests that fish migrating longer distances in the mainstem river are subject to greater selection pressure by the parasite.

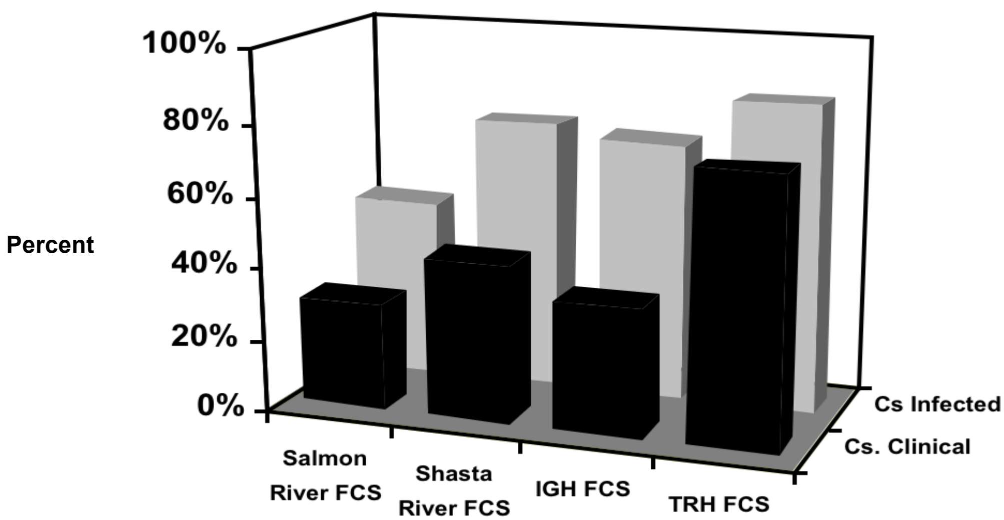


Figure 2.9. Percent *Ceratomyxa shasta* infection and clinical disease in four stocks of fall Chinook salmon [FCH: Salmon River, Shasta River, Iron Gate Hatchery (IGH) and Trinity River Hatchery (TRH)] exposed for 3 d at Beaver Creek (Rkm 262) in June, 2006 (R. Stone and J. Scott Foott, unpublished data). All groups were exposed in replicate.

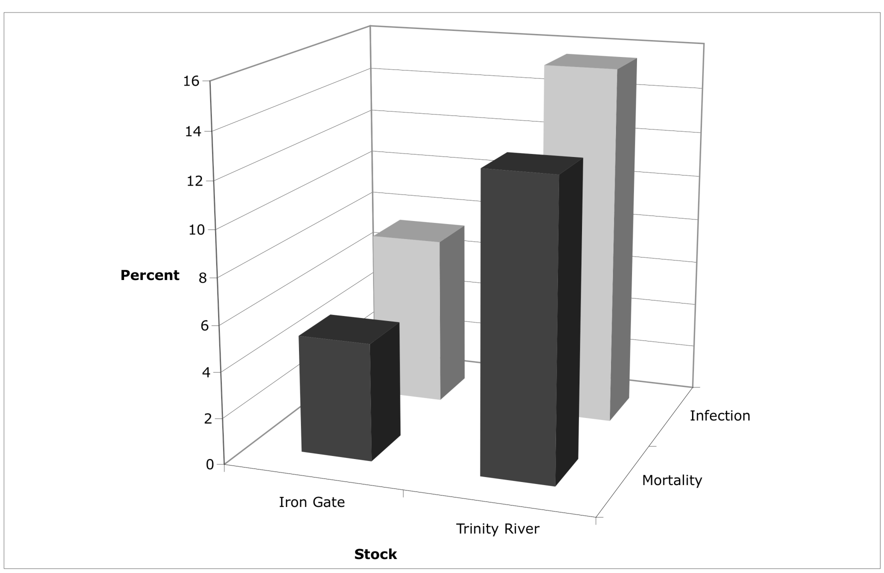


Figure 2.10. Comparison of infection prevalence and mortality between Trinity River and Iron Gate stocks of coho salmon following a 3 d exposure to *Ceratomyxa shasta* near Beaver Creek (Rkm 259) in June 2006 (Holt and Bartholomew, unpublished data).

Analysis of data from sentinel studies and water sampling permits establishing relationships between parasite density and mortality. In general there is evidence for mortality in Chinook and coho salmon when parasite abundance >10 actinospores/l during May and June. In September, presence of myxospores likely contributes to the overall parasite density but does not result in infection in fish (Figure 2.11)

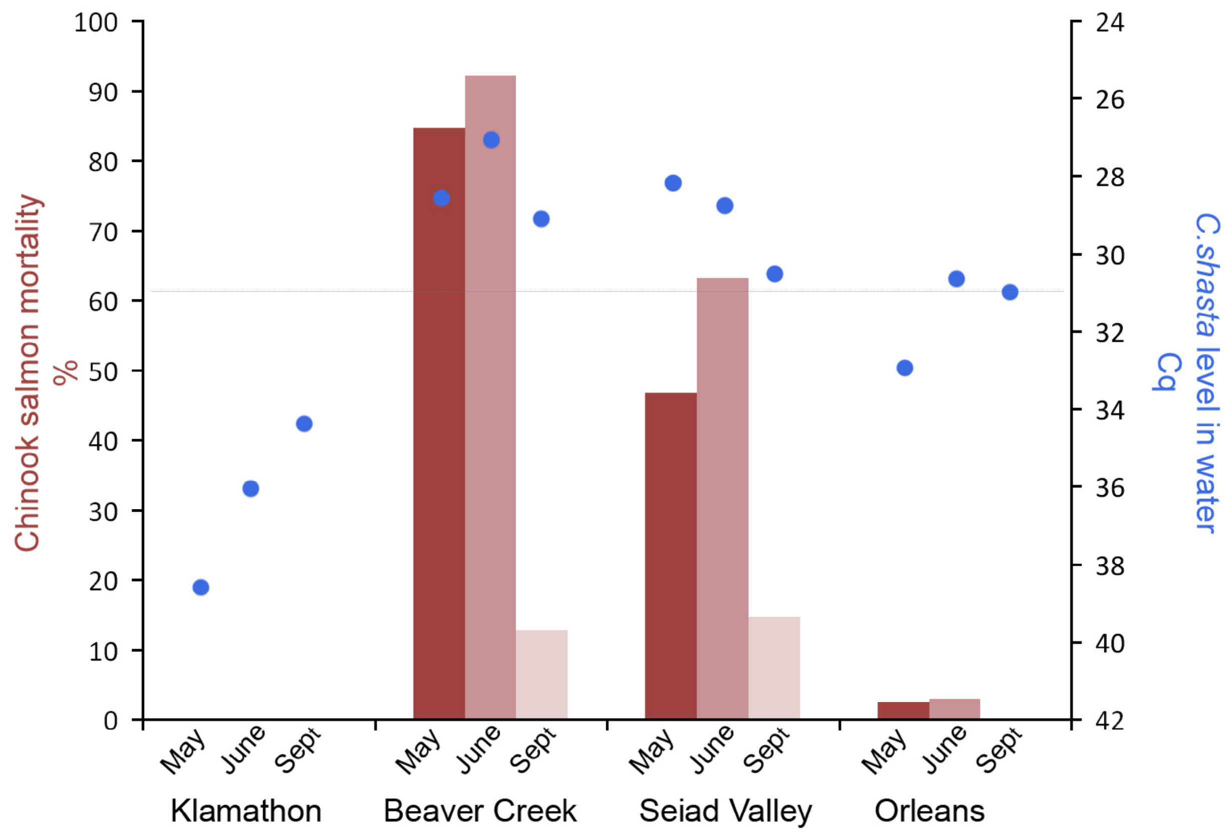


Figure 2.11. Sentinel mortality from *Ceratomyxa shasta* infection in Chinook versus parasite abundance in water at four lower Klamath River index sites,in 2008. Values were adjusted relative to 2008; 1 spore/L =~ Cq (Ct) 34, a ten-fold increase in abundance is approximately equal to a decrease of 3 Cq values. The line is approximately 10 spores/L

2.7 GENETICS OF CERATOMYXA SHASTA

The inability to conclusively demonstrate differences in susceptibility between the species and the inability to infect Chinook salmon in the Williamson River, where parasite densities are high, caused a closer examination of the parasite for an explanation. Atkinson and Bartholomew (2010) hypothesized that these disparate infection patterns correlated with genetic variation in the parasite. To test this, they designed specific primers for the small subunit ribosomal RNA gene (SSU) and the neighboring, more variable Internal Transcribed Spacer region 1 (ITS-1). The SSU sequences of 50 isolates, which encompassed both locations and species, were identical. However, the ITS-1 had a trinucleotide repeat that allowed discrimination between four principal genotypes: 0, I, II

and III. Non-native rainbow trout, regardless of location, were infected with genotype II and a low level of III. The few Chinook salmon infected in the upper basin had types II and III, but in contrast, the lower basin fish were infected with type I. Analysis of several hundred Chinook and coho salmon, steelhead and rainbow trout further emphasized the profound genetic structure of the *C. shasta* population in the Klamath river basin. Coho salmon became infected with type II and native rainbow trout and steelhead were exclusively infected with type 0. In water samples from the Williamson River, type II predominates, with type 0 at a lower level. Between the dams, type 0 predominates, likely reflecting that redband trout is the host species present. The genotype specific for Chinook salmon, type I, is detected only below the dams; the genotype specific for coho salmon appears to be present in both the Williamson (possibly maintained by the introduced rainbow trout) and in the lower river, but has not been detected between the dams. Genotype III is present at low levels and thus is only detected from fish, which are a more sensitive detector than water samples. This resolution of *C. shasta* into host-specific genotypes (Atkinson and Bartholomew 2010b; Table 2.1) provides some explanation for the perceived differences in susceptibility.

Table 2.1. Distribution of *Ceratomyxa shasta* ITS1 genotypes in the different fish species and water in the upper and lower Klamath River basin. n/a = data not available. Predonimant genotype in bold.

	Water	Non-native Rainbow	Redband/Steelhead	Chinook salmon	Coho salmon
Upper (Williamson)	**II,** 0	**II**	**0**	II, III	n/a
Lower	**I, II**	**II**, III	**0**	**I**, II, III	**II**

3. SEDIMENT AND DAM REMOVAL

3.1. SUMMARY OF ASSUMPTIONS ON SEDIMENT

3.1.2 SUSPENDED SEDIMENT (SHORT-TERM INFLUENCE)

Assumptions on total suspended sediment (TSS) effects are based on modeling results from the Stillwater Sciences Technical Report (2009). In this assessment, the primary impact from dam removal is predicted to result from release of fine sediment during the four to eight months following reservoir drawdown, with little sediment release after drawdown is complete. The composition of sediment behind the dams is approximately 79% clay or silt, 18% sand and the remaining 3% gravel or larger material. Upper reservoirs are characterized by higher proportions of gravel, sand and silt, whereas the lower impoundments have higher proportions of silt and clay (PacifiCorp 2004).

Eroding sediments would dramatically increase suspended sediment concentrations immediately downstream of IGD for the period of time required to draw down the reservoirs. The transport rate of suspended sediment during the drawdown period is anticipated to stabilize shortly after draw down is complete, with significantly smaller pulses of sediment waves accompanying storms during the bank stabilization and re-vegetation period (Stillwater Sciences 2008)

3.1.2. SEDIMENT TRANSPORT

There is expected to be minimal sediment deposition downstream of Iron Gate following dam removal, with most of the dam-derived sediment transported to the ocean. There will be areas of deposition of silt and clay along the channel margins and in vegetated areas. These deposits, depending on elevation and succeeding flows, may persist, especially in densely vegetated areas (Stillwater Sciences Technical Report 2009). Following removal of the dams, coarse sediment (gravel or larger) stored behind the dams would be mobilized and transported downstream of IGD. Along with fines liberated by dam removal, coarse sediments may have short-term impacts such as filling of pools, covering of roughness elements, fining of the bed, and large-scale morphological adjustments such as braiding or changes in the river planform.

Recovery from potential fine sediment deposition and pool filling will be rapid, with chronic effects not anticipated beyond 1 year. The higher flows modeled in WRIMS R-32 Refuge during the late winter and spring months, when combined with tributary accretions below Keno that are currently being regulated, will increase the frequency of flows that mobilize sediment. Morphological effects will be driven by the supply of sediment and the frequency and magnitude of flows to transport that sediment.

Under current conditions, analyses of flows required to flush sand sediments from pool habitats at six sites below Iron Gate Dam suggest that this occurs under normal conditions except at the two uppermost sites (Beaver Creek and Little Bogus Creek) (Ayres 1999). In addition, most of the gravel-cobble-boulder bars downstream of Weitchpec undergo frequent movement (Ayres 1999). Comparison of estimated sediment grain sizes under both scenarios predicts that the bed will be coarser under the dams in alternative, requiring higher mobilization flows; with dam removal, bed material will be finer and thus

mobilization will occur more frequently. These effects will be most significant near Iron Gate Dam but will decrease significantly by Beaver Creek (David Varyu and Brian Greimann, DRAFT Sediment Mobilization Analysis at Little Bogus Creek and Beaver Creek for Klamath Dam Removal Studies, May 2010).

3.2. EFFECTS OF SUSPENDED AND DEPOSITIONAL SEDIMENT ON DISEASE

3.2.1 POLYCHAETES

One component of polychaete microhabitat is sediment, and this is considered separately from other habitat features because of the direct effect that dam removal will have on the amount and distribution of sediment within and below project waters. Polychaetes are associated with areas of sand-silt embedded with fine benthic organic matter (FBOM), which may occur at low velocities in habitats such as pools and eddies, and at higher velocities when associated with the algae *Cladophora* (Section 5, Figure 5.1; Stocking and Bartholomew 2007). FBOM is an important nutritional source for both microorganisms and invertebrates (Cummins et al. 1989).

Between Iron Gate Dam and Cottonwood Creek the bedload is sediment starved; from Cottonwood Creek downstream, tributary input results in flashy run-off and high sediment load (PacifiCorp 2004). *Cladophora* is present below the Trinity, but it contains less FBOM and polychaete populations are patchy and densities are generally low (500-250,000/m^2). Benthic sand and *Cladophora* in the river below Cottonwood Creek are enriched with FBOM and polychaete population densities ranged from 250,000-2,500,000/m^2 (Figure 3.1) (R. Stocking, ODFW, personal communication).

Areas of abundant fine sediment also occur behind the dams (GEC 2006); however, much of this sediment is too anoxic (dissolved oxygen concentrations < 3 ppm), or flows are too low, to serve as suitable polychaete habitat (Stocking and Bartholomew 2007). The inflow areas to eutrophic lakes and reservoirs (lotic-lentic interface) provide optimal habitat characteristics in terms of food availability, flows and dissolved oxygen and are among the most stable macrohabitat types where the polychaete has been documented (Figure 3.2) (Stocking and Bartholomew 2007; Hiltunen 1965). On a smaller scale, this habitat is replicated in pools and eddies throughout the river. Removal of the dams would eliminate these reservoir inflow zones and either eliminate or redistribute the large polychaete populations that occur there to suitable small-scale habitats downstream. However, sand-silt habitats at these smaller scales are more susceptible to disturbance and thus less stable.

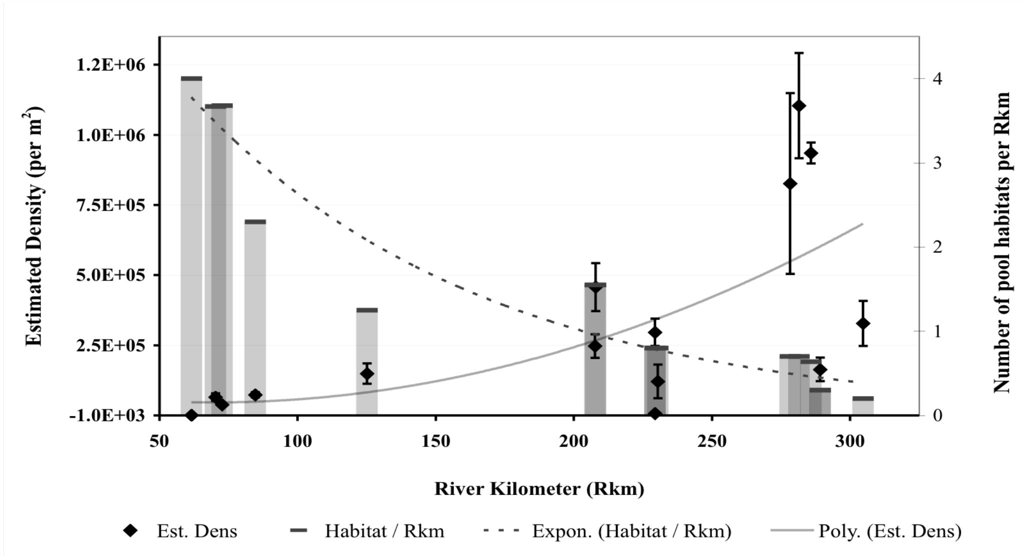


Figure 3.1. Preliminary results of 2006 sampling to document the pool-habitat distribution as well as the abundance of *Manayunkia speciosa* in the lower Klamath River from Iron Gate Hatchery to Tully Creek. Density estimates given in standard scientific notation (1.2E+06=1,2000,000 polychaetes per m^2) with standard deviation. (Stocking and Bartholomew, unpublished data)

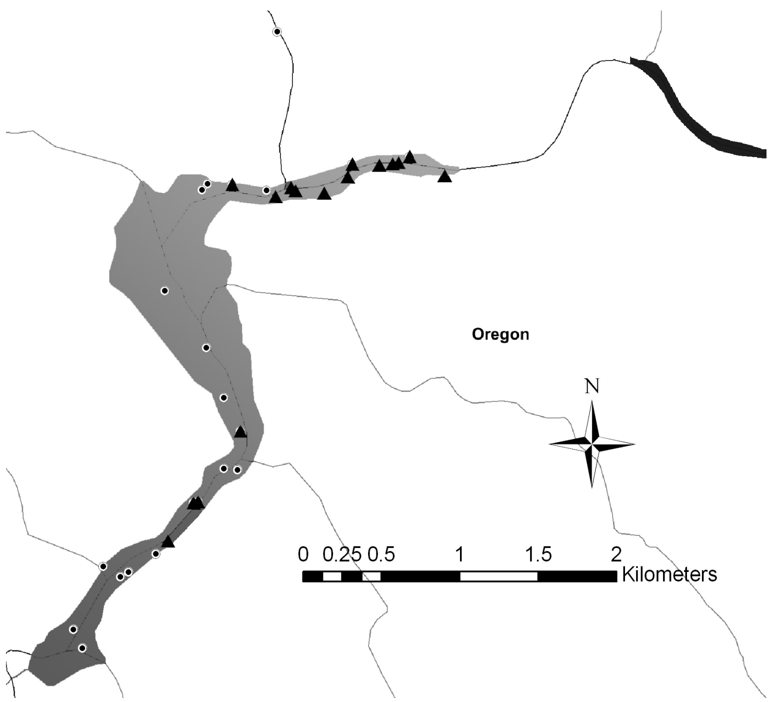


Figure 3.2. Samples collected June 2003 from the J.C. Boyle reservoir. Circles are sample sites where *Manayunkia speciosa* was not identified and triangles are sites where *M. speciosa* was identified. The inflow to the reservoir is located in the northeast corner and flows to the southwest. (From Stocking and Bartholomew 2007)

Similar to predictions of 100% mortality of salmon eggs and amphibian eggs in the mainstem Klamath River immediately following dam removal (Stillwater Science Report 2009), it is likely that sediment deposition will have the initial effect of smothering some polychaete populations below Iron Gate. Polychaete populations in sand-silt habitats will

be most vulnerable, while those in *Cladophora* or on vertical surfaces (bedrock) may receive some protection from these effects. Observational data from a laboratory study indicated that a sedimentation event in late fall that covered a population with several centimeters of silt decreased polychaete numbers dramatically, but that worms survived in areas where scour occurred (S. Bjork, personal communication). This suggests that sedimentation following dam removal will result in population declines, but that habitat complexity (logs, cobble, boulders etc) will create scour zones where sediment effects would be decreased. If peak appearance of juvenile polychaetes occurs during late spring and early summer as shown in a study by Willson et al. (2010), population effects as a result of a drawdown in November may not have a great effect. Recolonization is likely to occur rapidly as not all populations will be affected and there are established populations in the upper river that are likely to seed areas of suitable habitat below, as well as individuals that survived in these scour zones. However, success of recolonization will be dependent on the stability of the microhabitat and we anticipate colonization will be negatively influenced by the implementation of a variable flow regime that increases the frequency of fine sediment mobilization.

It is not known how increased TSS will affect food availability and polychaete feeding efficiency. However, as *M. speciosa* forages on deposited and suspended sediment (Stocking and Bartholomew 2007) there is likely to be a direct effect. A laboratory study on marine spionid polychaetes showed that sediment resuspension as a result of increased flows greatly increases relative growth rates (Hentschel 2004). They concluded that the combination of enhanced deposition and the transport of sediment in bedload or suspension could enhance the growth of these interface-feeding worms. In a study conducted with *M. speciosa*, polychaete populations maintained at a flow rate of 0.05 m/sec reached higher densities than populations at a lower flow rate of 0.01 m/sec (Bjork and Bartholomew 2009), although subsequent siltation resulted in a decrease in densities. These studies provide some explanation as to why polychaetes are not present at the very low flows that occur in reservoirs (0.02 m/sec).

With dam removal, additional habitat will become available for polychaetes in areas where reservoirs existed. Currently, within reservoirs polychaetes are restricted to reservoir inflow and areas where the channel becomes more constricted (Figure 3.2). Although polychaete populations here are large, they do not contribute to disease in the river below IGD, presumably because of the long residence time of water in the reservoir and the absence of myxospore contribution from adult salmon. Densities of populations in the current project waters after dam removal will be variable and dependent on nutrient levels as well as flow and degree of sand-silt or *Cladophora* and other aquatic macrophyte habitats.

3.2.2 PARASITE TRANSMISSION

Direct effects of TSS on actinospore and myxospore stages are unknown, but it could be expected that transmission rate to fish and polychaete hosts may be negatively affected if dam removal occurs during periods of transmission to either host. Actinospores, which are more fragile, may be more directly affected than myxospores. Indirectly, actinospore transmission would be decreased if significant reduction of polychaetes occurred as a result of sediment deposition.

3.2.3 DISEASE PROCESSES IN FISH

Sediment effects on the disease process itself will be indirect, acting as a stressor on the fish. There are no data for effects of suspended sediment on fish immune function and the only related study on a myxozoan disease evaluated the effects of multiple stressors (gas supersaturation, elevated water temperature and a bacterial pathogen) on fish infected with *Myxobolus cerebralis* (Schisler et al. 2000). That study reported that significant disease effects depended on the stressor (i.e., temperature had a direct effect while gas supersaturation had little effect), but that the occurrence of multiple stressors increased morbidity. However, the extent to which high TSS levels may influence disease is uncertain.

3.3. ASSESSMENT EFFECTS OF DAM REMOVAL ON SEDIMENT AND DISEASE

The short-term increase in sediment will have the greatest effect on the polychaete host itself, causing mortality in the high-density populations below IGD during the drawdown event. This decrease is expected to be limited as not all populations may be affected and recolonization will likely be rapid. However, recolonization may not occur to the same extent as under conditions prior to dam removal and implementation of the KRBA as a result of the shift to variable flow management and restoration of a more natural sediment regime. In areas of the river where there is increased mobilization of FBOM we expect that polychaete populations will be destabilized.

Under current conditions, the level of mobilization of fine and coarse sediment suggest that under normal conditions (non-drought years) FBOM would be disturbed from all locations except those above Beaver Creek, and cladophora would be disturbed downstream of Weitchpec. With removal of the dams there will be additional disturbance of FBOM below IGD, but this effect will diminish by Beaver Creek. This may result in further limiting the infectious zone.

Overall, physical polychaete habitat in the project area will increase with the return of reservoirs to a riverine environment (Section 4). The degree of colonization of these habitats is uncertain and will depend on available sediment, nutrients and flow variability. There is a need for additional data on how these parameters will interact following dam removal before predictions can be made for locations and abundance of high-density polychaete populations in the current project area. The most likely area for colonization will be in the reach below Copco dam; however, the extent of suitable habitat is not likely to be as extensive in the current infectious zone because of the steeper gradient in the area bounded by the projects. In addition, because the KRBA provides flexibility to manage flows to respond to real-time climatic and biological conditions we expect that this will create variability in flows and resulting habitat conditions and reestablish natural instability and disturbance of microhabitats preferred by polychaetes.

4. PHYSICAL HABITAT AND DAM REMOVAL

4.1. SUMMARY OF INFORMATION AND ASSUMPTIONS ON POLYCHAETE HABITAT AND IMPLICATIONS OF DAM REMOVAL

Dams have altered the geomorphology of the river, with the most prominent effects in the area bounded by the projects. This part of the river (R3) lies within volcanic terrain, in a region with less rainfall, lower sediment and more bedrock-controlled channel than the river below the projects (NRC 2008; PacifiCorp 2004). Reservoirs provide poor habitat for polychaetes for a number of reasons, including low dissolved oxygen levels and flows too low to facilitate feeding. Thus removal of the dams and the subsequent return of much of this section to riverine habitats could potentially increase polychaete habitat in the current project area. Habitat in the Williamson River (R4) would not change significantly with dam removal.

Stream gradients are steeper between IGD and Keno Dam than in R1 or R2, however runoff is groundwater derived and thus is relatively steady compared with the flashy runoff of lower basin tributaries. This reduced flood runoff could result in less active bed scour, erosion, deposition and channel migration, thus reducing fresh sediment surfaces. Alternately, there would be less frequent scouring of established sediment (PacifiCorp 2004). Within the project area there is a higher proportion of riffle habitats in the Copco bypass reach, whereas the Boyle Bypass reach is characterized by long pools and runs alternating with riffles (PacifiCorp 2005). Pool habitats are abundant in the project area, particularly in the Boyle bypass reach. It might be expected that with dam removal the proportions of these habitats would remain similar but the amount would increase.

4.2. EFFECTS OF ALTERERED PHYSICAL HABITAT ON DISEASE

4.2.1. POLYCHAETES

In a study by Stocking and Bartholomew (2006), slow flowing lotic habitats such as runs and eddy-pools had the highest relative densities and frequency of occurrence of *M. speciosa*. The polychaete was identified in 52% of pools, 47% of eddies, and 40% of runs sampled (Figure 4.1); however, eddies were not as common a habitat feature as pools and runs. The polychaete was only present in 20% of the riffles sampled and here only a few polychaetes were collected along the edge-water. Polychaete populations were always located outside of the main flow along the margins of the riverbanks and most often within protective structures such as a bedrock ledge or boulder out-crops. Within runs, *M. speciosa* tended to occur within a protective microhabitat, such as *Cladophora* sp. Eddy-pools often contained both sand-silt and *Cladophora* sp., the 2 microhabitats identified with the highest densities of *M. speciosa*, explaining why the polychaete was detected most frequently in this habitat. The geomorphic reach that encompasses the infectious zone below IGD (R2) is a low gradient reach characterized by a confined bedrock channel with cobble-gravel bed and well-developed pool-riffle morphology (PacifiCorp 2004), with pools representing approximately 42% of the habitat (PacifiCorp 2005).

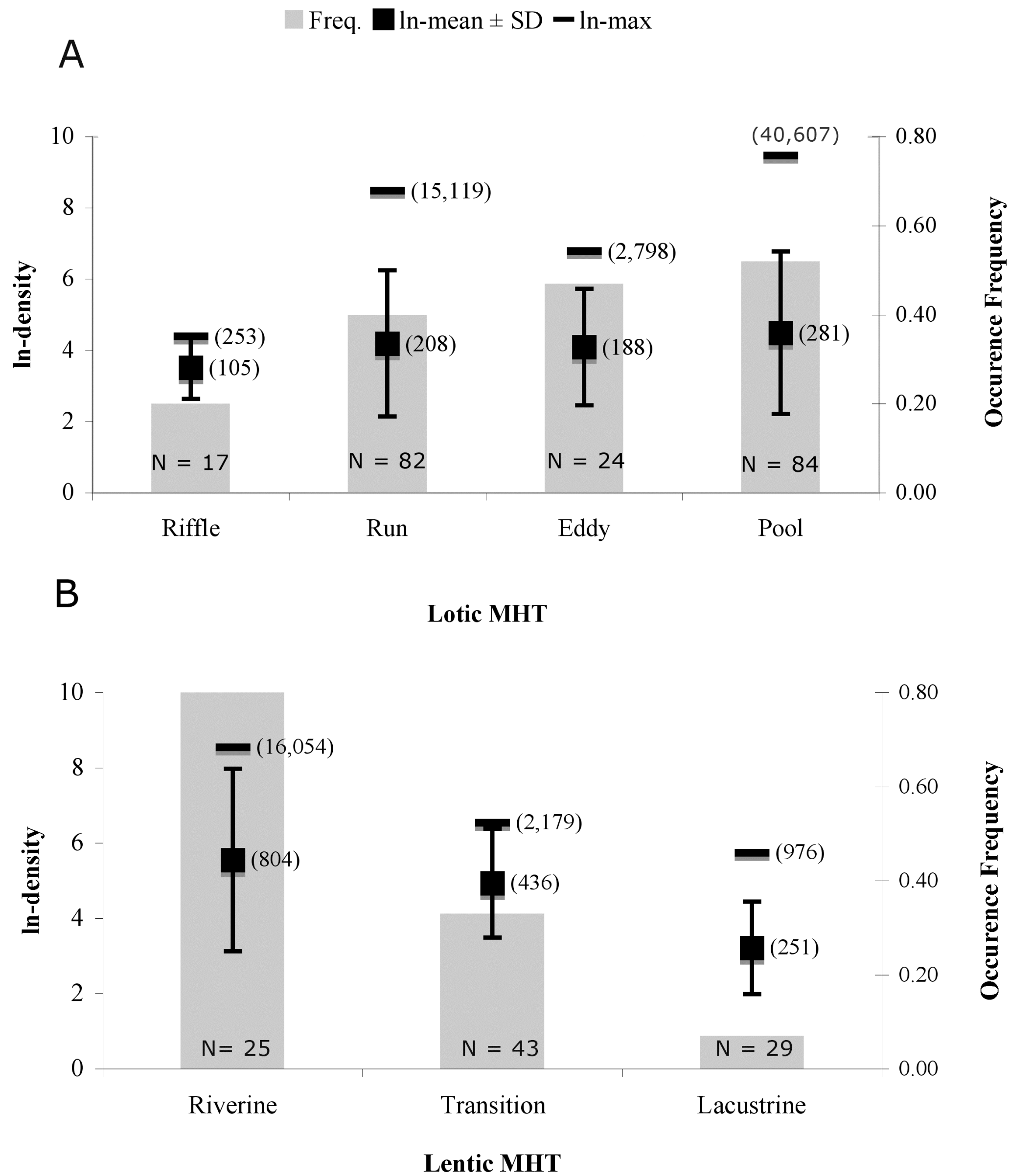


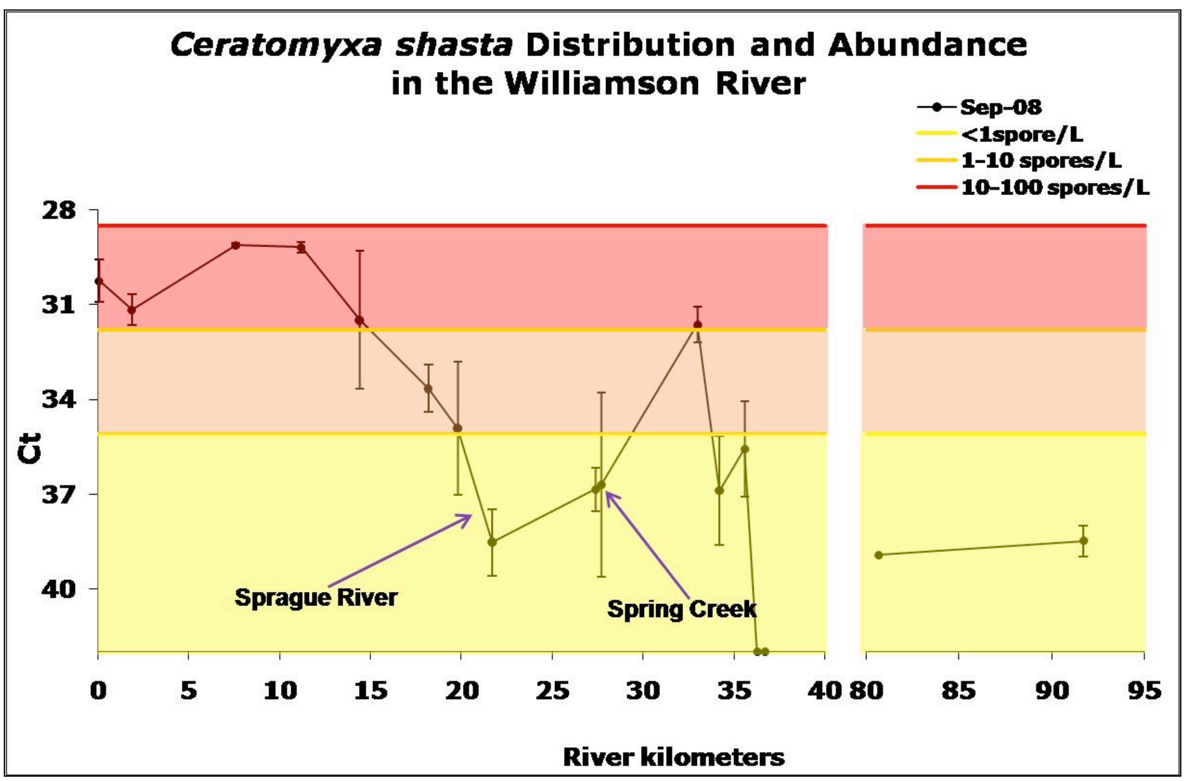
Figure 4.1. Mean density (natural log-transformed), max density, and frequency of occurrence of *Manayunkia speciosa* by (A) lotic macrohabitat (MHT) and by (B) lentic MHT. Values in parenthesis are estimated mean and maximum field densities m^{-2}. Minimum values are not shown since all MHTs had at least one minimum value of zero (From Stocking and Bartholomew 2007).

In the project area (R3), polychaetes have been documented from the slow-flowing reservoir inflow zones, as well is in the intervening river reaches (Stocking and Bartholomew 2007). Moderate parasite densities were detected in in the JC Boyle bypass reach by both sentinel fish exposures (Rkm 354) (Stocking et al. 2006) and water sample analysis (Hallett and Bartholomew 2006). This reach is generally moderate gradient and encompasses channel bed dominated by boulders and cobbles; however, it also

encompasses areas of long pools (PacifiCorp 2004) that may provide suitable polychaete habitat. It is expected that the intermittent thermal refugia created in this reach as a result of coldwater tributaries will benefit a variety of aquatic biota (Hetrick et al. 2009). If this results in increased diversity of organisms that provide competition for habitat or increase predation, it could reduce polychaete populations; alternatively, it could also provide benefit to already existing polychaete populations.

Above the project area (R4), polychaetes have been documented in high densities from the inflow zone of the Williamson River into upper Klamath Lake. Additionally, the high prevalence of infection and severe mortality in sentinel rainbow trout exposed in the lower Williamson River indicate that the life cycle is well established within the lower 18 Rkm. The lower Williamson River is a low gradient system dominated by pools. Recent analysis of water samples collected from the Williamson and Sprague Rivers demonstrate high parasite densities between the mouth of the Williamson River and Rkm 11.4, and above the confluence of Spring Creek at Rkm 33. The parasite was not detected from either the Sprague River or Spring Creek (Figure 4.2 and 4.3) (Hurst and Bartholomew, unpublished data)

Figure 4.2. Cycle threshold (Ct) for qPCR analysis of water samples collected from the Williamson River. The lower Ct values indicate higher concentrations of parasite DNA (Charlene Hurst, unpublished data).



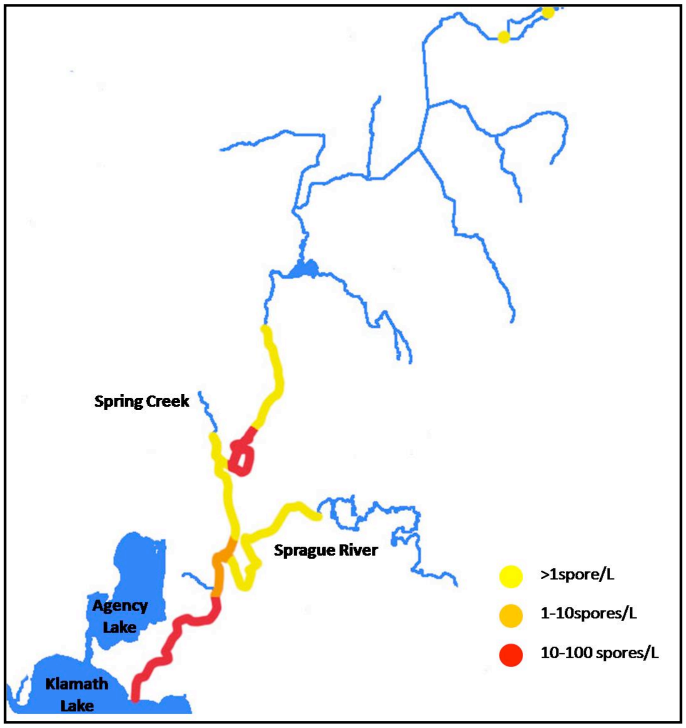


Figure 4.3 Summary figure of the distribution and abundance of *Ceratomyxa shasta* along the Williamson River, September 2008

4.2.2. PARASITE TRANSMISSION

Removal of the projects will result in a free-flowing system with no barriers to parasite transmission.

4.2.3. DISEASE PROCESSES IN FISH

Changes in habitat features would not be expected to directly alter disease progression in fish. Thermal refugia created at the inflow of coldwater tributaries in the current project area (Hetrick et al. 2009) may benefit juvenile salmon, as parasite densities are often reduced or undetectable in coldwater tributaries (e.g. Spring Creek, Williamson River tributary; Hurst and Bartholomew unpublished data). However, tributary inflows that result in a decrease of only a few degrees below high ambient mainstem temperatures (e.g. 18˚C versus 23˚C) may not alleviate *C. shasta* infection and can also serve to congregate fish (potentially resulting in increased columnaris transmission).

Similarly, benefits may accrue for adult salmon by decreasing the rate of parasite replication in fish infected while migrating through the lower river (R2), although this would depend on the amount of temperature reduction.

4.3. ASSESSMENT EFFECTS OF DAM REMOVAL ON PHYSICAL HABITAT AND DISEASE

Habitats that support polychaetes currently exist within the project area and removal of the dams will likely increase the amount of physical habitat available for colonization. There will be little direct alteration of habitats above the project area, where the parasite is also established. Additional coldwater refugia may become available within the project area, potentially providing relief to migrating juvenile and adult salmon and increasing the diversity of aquatic biota.

5. HYDROLOGY AND DAM REMOVAL

5.1. SUMMARY OF FLOW ASSUMPTIONS

Flows in the area that encompass the infectious zone are most directly influenced by IGD and the Shasta River. It is recognized that low flows over sustained drought periods increase deposition of fine sediments, leading to increased siltation of pools and riffles and establishment of rooted macrophytes that in turn trap sediment (KRBFTF 1991).

Flow assumptions following removal of PacifiCorp's Klamath River dams are based on the USFWS Arcata Settlement Technical Report (Hetrick et al. 2009) using the WRIMS Run-32 Refuge model. Under this model, output flows would exceed historical IGD flows during March – June and are similar to the Hardy Phase II recommendations for the 30% and greater exceedences during critical periods for Chinook salmon fry and juvenile rearing. At 10% exceedence, the model flow outputs are similar to historical IGD flows and higher than the Hardy Phase II baseflow recommendations. For dry years the model flow outputs are lower than Hardy Phase II recommendations for the fall and winter (Hardy et al. 2006). Following removal of PacifiCorp's Klamath River dams, hydraulic residence time through reaches occupied by the PacifiCorp dam complex would decrease from several weeks to less than a day. Removal of the dams is expected to result in a flow pattern more characteristic of pre-dam conditions, with greater intra and inter-annual variability. Stream gradients within the current project area are relatively steep compared with the river below IGD and thus would provide higher velocity flows.

5.2. EFFECTS OF FLOW AND VELOCITY ON DISEASE

5.2.1. POLYCHAETES

The feeding strategy of the polychaete, suspension feeding using delicate feeding appendages, restricts its colonization to slower flowing habitats in areas with moderate to high sediment loads. In the Klamath River *M. speciosa* is found across a narrow range of velocities in two primary habitat types: sand-silt with FBOM and *Cladophora*. Populations found in FBOM occur at low velocities (0.02-0.05 m/sec) in habitats such as pools and eddies, and at higher velocities when associated with the algae *Cladophora* (Figure 5.1; Stocking and Bartholomew 2007).

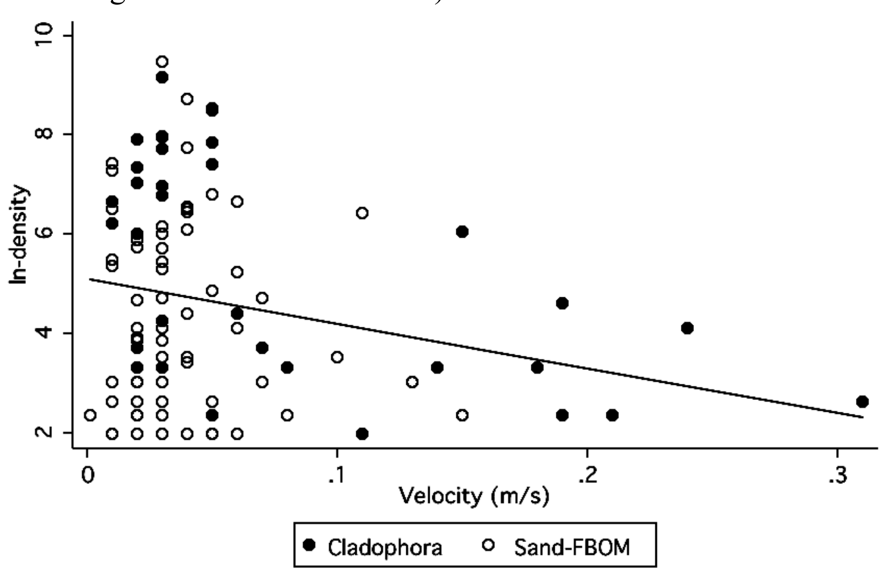


Figure 5.1. Relationship between *Manayunkia speciosa* density (natural log-transformed) and flow velocity within two microhabitats: *Cladophora* sp. (a mat-forming, epilithic algae) and sand with fine benthic organic matter (FBOM) collected from the Klamath River. Simple linear regression: within cladophora (r^2 = 0.30, P <0.01, n = 32) and sand (r^2 = .02, P = 0.26, n = 69). (From Stocking and Bartholomew 2007)

Because these two habitat types differ in their capacity to provide a buffer from increased flows, some populations of polychaetes may be more susceptible to disturbances such as high flow events. One example of this occurred in a population in sand-silt at the Tree of Heaven: a survey of this site in March 2005 recorded a polychaete density of 40,607/m^2; when sampled in July following a high flow event (> 5000 cfs) in May-June 2005, this population was absent. A reference polychaete population in *Cladophora* sp., upstream of the Tree of Heaven site was not impacted by the high flow event (Stocking and Bartholomew 2007).

Cladophora, and other macrophytes, provide a buffer that allows polychaetes to persist at higher flows. Polychaetes likely persist within a narrow range of water velocities, the high end of which can be observed within mats of *Cladophera*. Optimal growth of *Cladophora* has been reported between 0.5 and 0.8 m/sec (Schönborn 1996). Additionally, the dense filaments of this algae are colonized by epiphytic algae and diatoms (Higgins et al. 2008) and trap sediment, providing food and a source of tube-building material. Thus, the ability of *M. speciosa* to persist is influenced by microhabitat complexity and stability.

5.2.2 PARASITE TRANSMISSION

The effect of flow on transmission of myxozoan parasites between hosts has been demonstrated for *Myxobolus cerebralis*, a myxozoan that infects *Tubifex tubifex* and trout (Hallett and Bartholomew 2008). A similar laboratory study design was used to examine the effect of flow on *C. shasta*:host interactions (Bjork and Bartholomew 2008). When the parasite life cycle was established in sand-silt at two velocities (0.05 m/s and 0.01 m/s), a higher proportion of the polychaete population became infected at the slower velocity. Fish that were held in the outflow of the slower velocity treatments consequently had a shorter mean day to death than those held in the faster velocities, indicating an increased exposure dose. This suggests a relationship between flow and polychaete infection, resulting in a higher infectious dose for fish.

The effect of parasite dilution on transmission to fish was examined in another laboratory study (Bjork and Bartholomew 2009). Water volume during challenge (parasite concentration) significantly affected infection prevalence. When water volume was 0.5L, fatal infections occurred in 60% of fish challenged with 1 actinospore and 100% of the fish challenged with 5 actinospores. However, when fish were exposed to 1 and 5 actinospores in a 1L volume, mortality was 3.3% and 20%, respectively (Figure 5.2). One explanation for this difference is that in larger water volumes fish have fewer encounters with the parasite than in smaller volumes. This provides some explanation for the decreased infection that occurs with input of the major tributaries. However, the contribution of dilution to the patterns of infection and actinospore abundance in the river below Seiad Valley is difficult to assess because of the addition of new parasites by infected polychaetes and the loss of parasites by natural mortality.

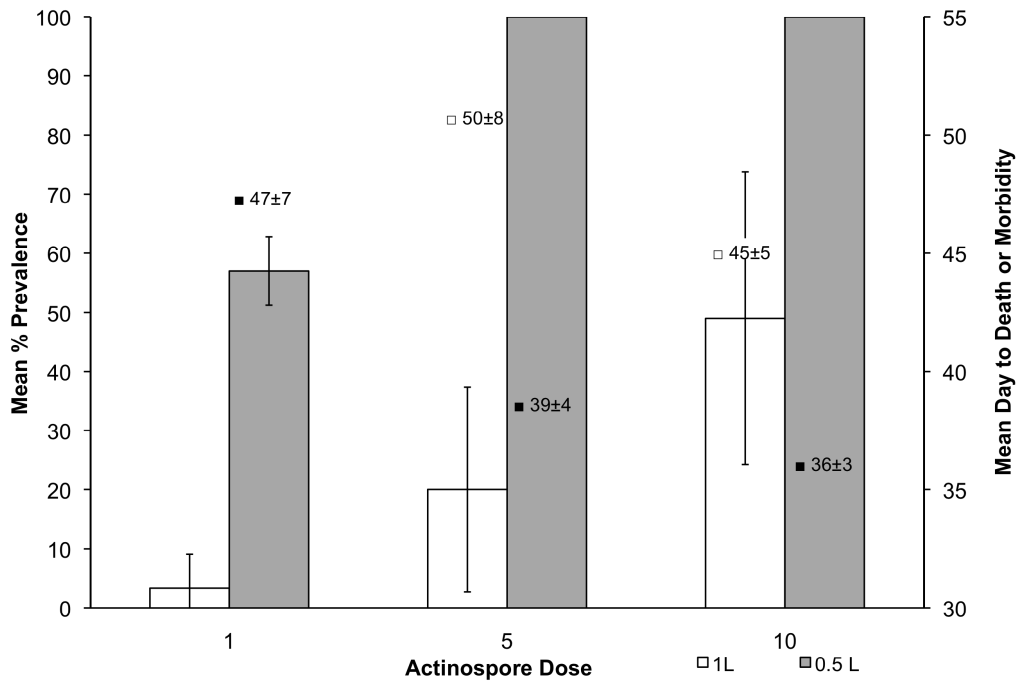


Figure 5.2. Infection prevalence and mean day to death of fish challenged to 1, 5, or 10 actinospores in 0.5 or 1L water.

In field studies, the effects of flow are difficult to separate from those of infectious dose, temperature and exposure time of migrating smolts. However, a study in the Willamette River, OR did provide some insight into the cumulative effect of these variables. Outmigrant steelhead smolts (hatchery and wild) were collected near the mouth of the Willamette River during four years when flows varied. During low flow years, mortality from *C. shasta* was increased compared with high flow years (Figure 5.3 shows two representative flow years). The low flows were associated with more consistently high temperatures and an increased length of time migrating through the section of the river harboring the parasite (Mamoyac et al. 2000). Under these conditions the natural resistance of the resident steelhead was overwhelmed. It is important to note that flow and temperature were highly correlated in this study (i.e. temperatures were cooler when flows were higher), so the cause of different mortality levels cannot be assigned directly to one or the other factor. Given the demonstrated effect of temperature on *C. shasta* virulence in controlled environments, it might be assumed that temperature rather than flow was the direct influencing factor over infection rates and that effects of flow in this study are indirect through their influence on temperature, exposure time of migrating smolts and the potential effects of parasite concentration. However, in contrast to the Willamette River, temperatures in the Klamath River may not be expected to have such a direct relation to flow events.

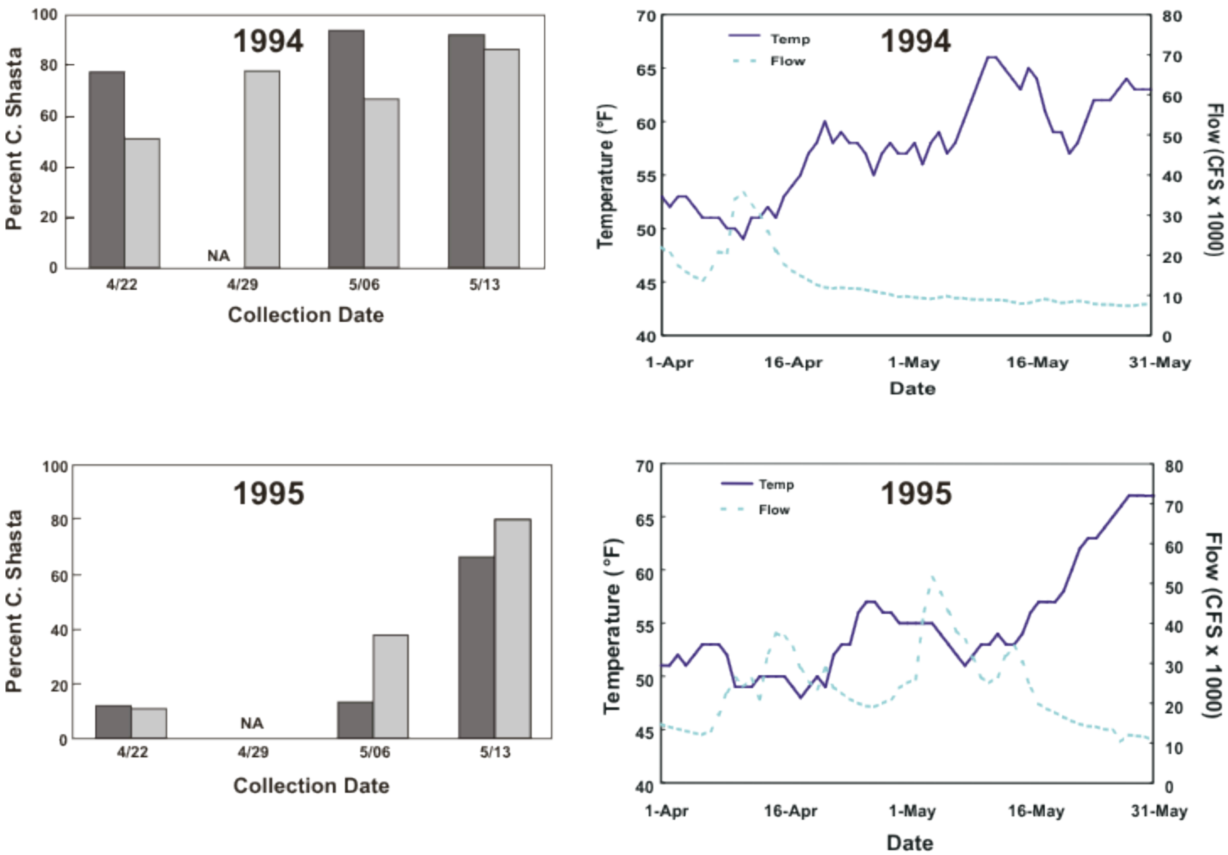


Figure 5.3. Data on *Ceratomyxa shasta* infection prevalence in wild (dark gray) and hatchery (light gray) outmigrant smolts collected at Willamette Falls during 1994 (left column) and 1995 (right column). Corresponding data on flow (light blue) and temperature (dark blue) are shown for each year. From Mamoyac et al. 2000.

For the Klamath River, when flow and temperature during exposure are compared with mortality of sentinel Chinook salmon, no clear pattern emerges (Table 5.1). Temperatures during all exposures were greater than 19˚C and did not vary by more than 2˚C between years. Flows during exposure did vary, but there was no correlation with mortality. This illustrates the complicated interaction between flow and disease, and suggests that flows at other times of the year may be important because of their effects on polychaete density or parasite transmission. For example, high winter flows may influence polychaete densities and infection prevalence (myxospore transmission efficiency) while high spring flows may influence fish migration rates, actinospore concentration and attachment efficiency.

Table 5.1. Data for average temperature and flow during exposure of Chinook salmon above Beaver Creek during June 2006-09. Fish were exposed for 3 d then held at approximately ambient river temperature following exposure.

Exposure Year	Temperature	Flow (cfs)	Percent mortality
2004	20.6	805	48.6
2005	18.1	1120	0
2006	19.9	3050	16.7
2007	20.8	1540	2.4
2008	19.1	1960	68.4
2009	20.9	1530	82.9

When the sentinel fish data are compared with the long-term flow events over the past six years some patterns begin to emerge. Figures 5.4, 5.6 and 5.7 show Chinook salmon mortality from sentinel exposures overlaid on the annual hydrograph. Because the longest data set was for fish held at 13˚C following exposure, mortality is lower than would have occurred at actual river temperatures; however, this reduced mortality makes the differences between years more apparent. The high flows of 2005-06 correlate with reduced infection in sentinel fish and low infection levels continued through 2007, possibly as a result of impacts on polychaete populations that took time to recover. However, flows during 2008-09 were reduced, resulting in higher disease effects than in any previous years. A similar trend of reduced infection during 2006-07, increasing in 2008-09 can be seen in the histology data obtained from juvenile Chinook salmon migrating in the river (Figure 5.5). Although the origin of these fish and the length of time spent in the infectious zone are not known, these two data sets show similar results. One difference is in 2005, where reduced infection in sentinel fish would have predicted reduced infection in migrating fish. However, the sentinel data may reflect the immediate effects of the high flow event that overlapped the exposure periods, while captured fish are more representative of the average exposure.

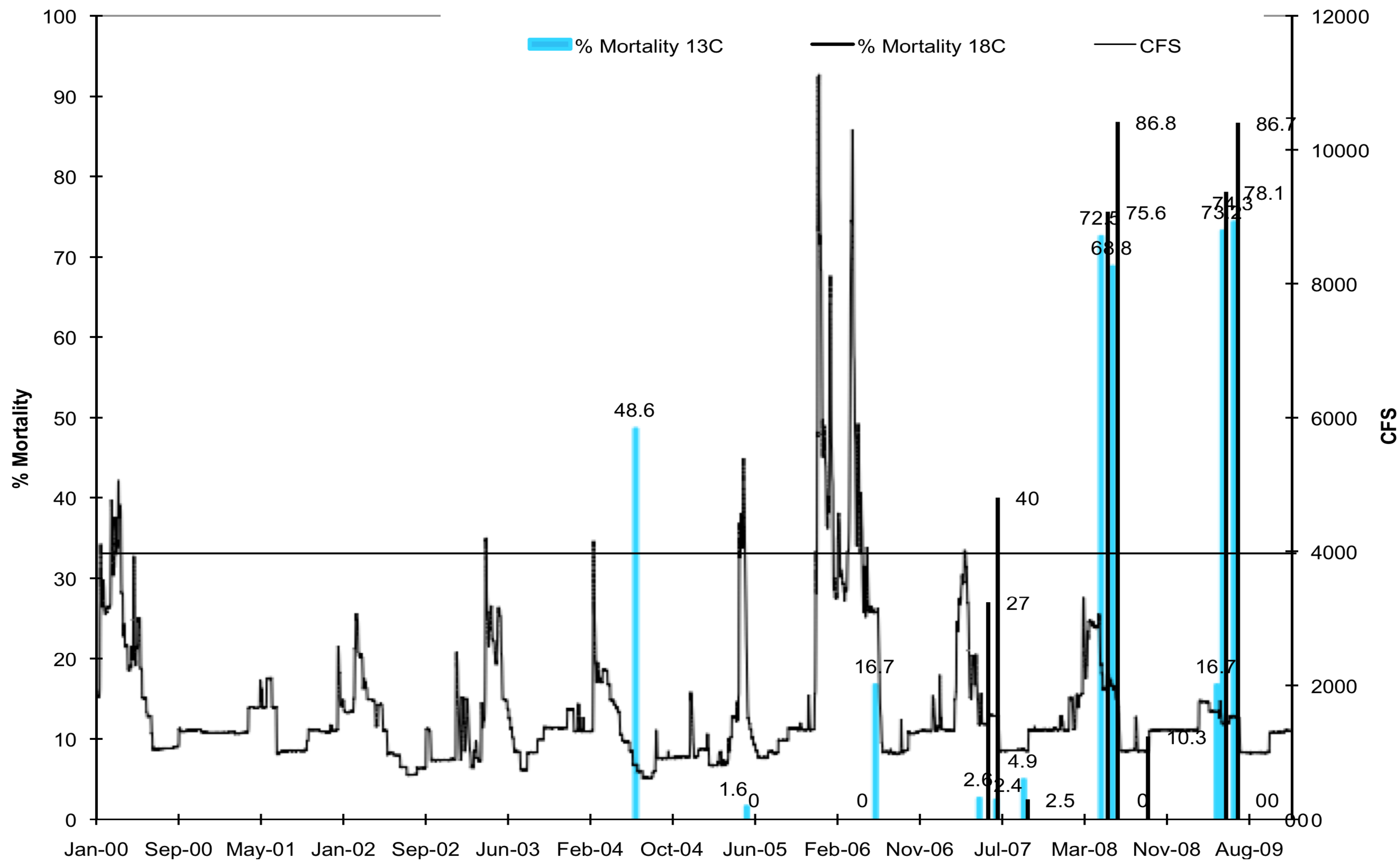


Figure 5.4. Daily flows (cfs) below Iron Gate Dam from June 2000 – August 2009 (http://waterdata.usgs.gov/nwis/rt). Mortality data for Chinook salmon exposed above Beaver Creek is overlaid for the periods of exposure in 2004-2009. All fish were exposed for 3 days and held at 13 or 18˚C.

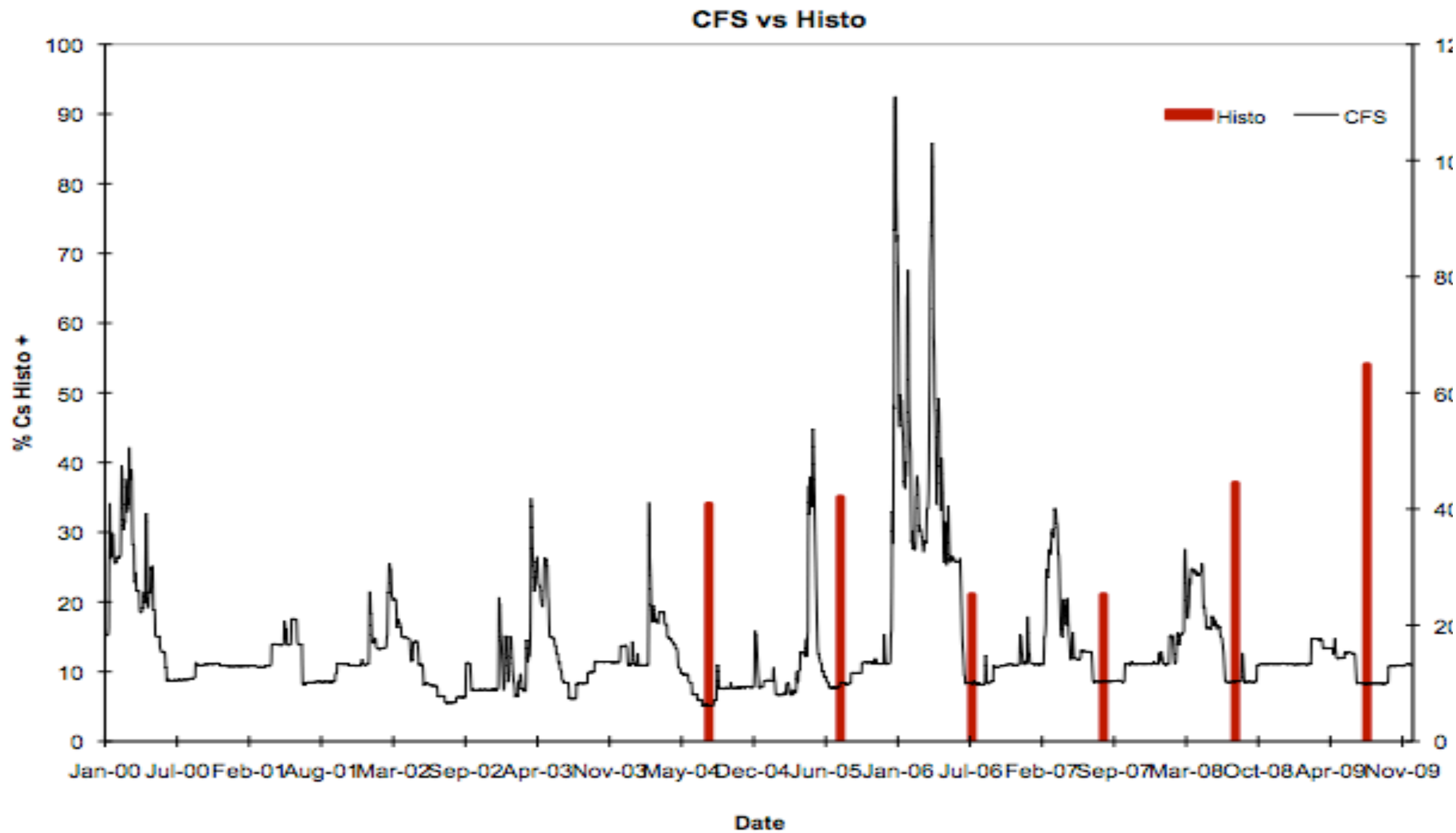
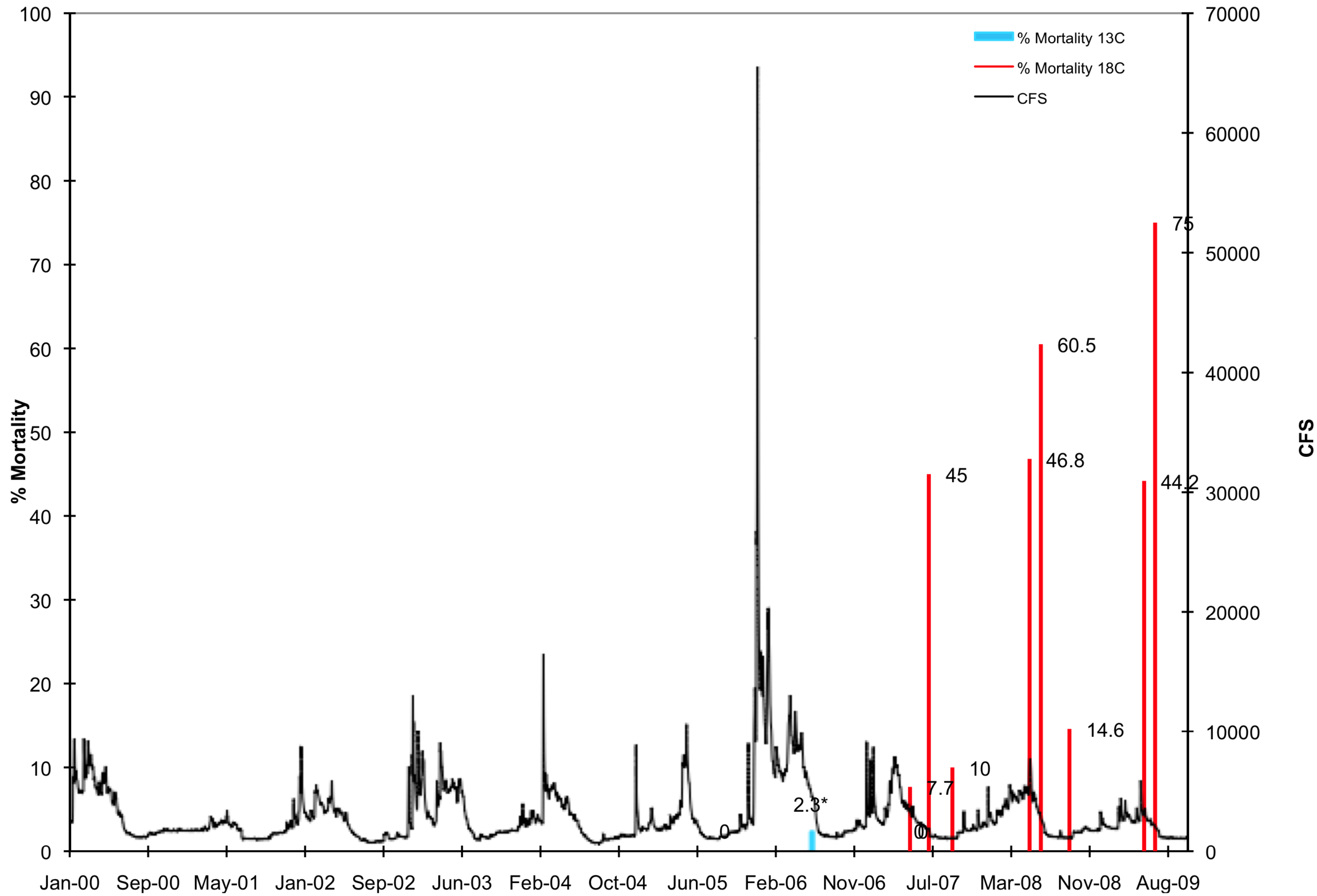


Figure 5.5. Daily flows (cfs) below Iron Gate Dam from June 2000 - August 2009 (http://waterdata.usgs.gov/nwis/rt). Histological detection of the parasite in juvenile Chinook salmon trapped during migration is plotted for each 2004-2009.



% Mortality 13C
% Mortality 18C
CFS
% Mortality
CFS
45
60.5
46.8
75
44.2
14.6
10
7.7
2.3*
0
0
Jan-00
Sep-00
May-01
Jan-02
Sep-02
Jun-03
Feb-04
Oct-04
Jun-05
Feb-06
Nov-06
Jul-07
Mar-08
Nov-08
Aug-09

Figure 5.6. Daily flows (cfs) at Seiad Valley from June 2000 – August 2009 (http://waterdata.usgs.gov/nwis/rt). Mortality data for Chinook salmon exposed at Seiad Valley is overlaid for the periods of exposure in 2005-2009. All fish were exposed for 3 days and held at either 13 or 18°C.

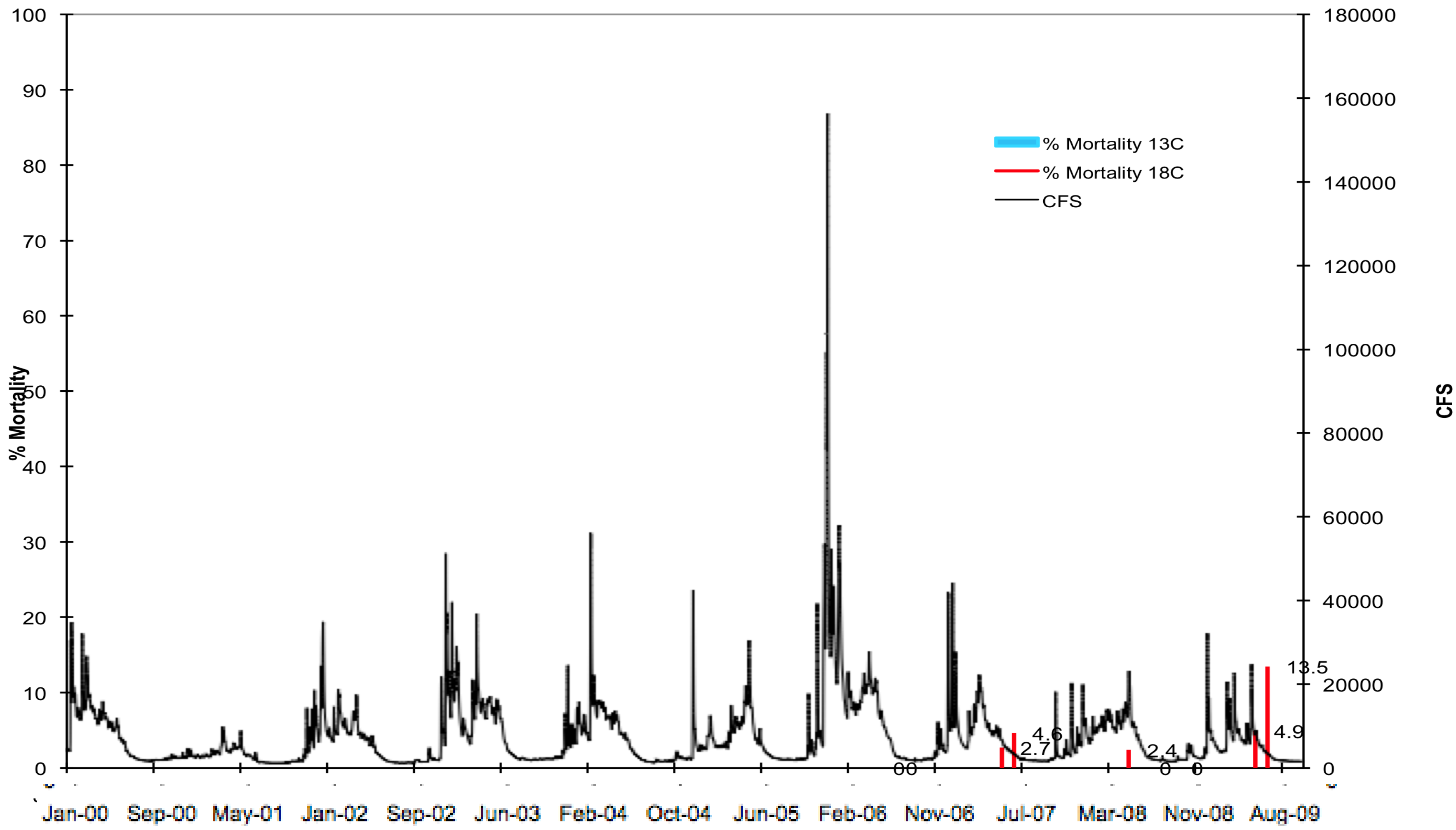


Figure 5.7. Daily flows (cfs) at Orleans from June 2000 – August 2009 (http://waterdata.usgs.gov/nwis/rt). Mortality data for Chinook salmon exposed at Orleans is overlaid for the periods of exposure in 2005-2009. All fish were exposed for 3 days and held at 13 or 18°C.

5.2.3. FISH

One aspect of transmission that has not been examined is the efficiency of parasite attachment to the host at different velocities, although it might be expected that efficiency would decrease at high velocities.

5.3. ASSESSMENT OF EFFECTS OF DAM REMOVAL ON HYDROLOGY AND DISEASE

The restoration of variable flow conditions as a result of dam removal is expected to result in a dynamic flow regime that will decrease the stability of the microhabitats that support polychaete populations and thus decrease polychaete abundance in the infectious zone (Stocking and Bartholomew 2007). This may result from direct flushing of sediment habitats as well as scouring with gravel/sand that occurs with flood flows. Higher stream flows during winter and spring could flush myxospores from the mainstem river channel. These spores, being negatively buoyant, accumulate in areas of slow flow and would be susceptible to mobilization and transport during high flow events. Although elimination of reservoirs will expose new habitats in the project area, the amount of new habitat and its characteristics will be dependent both on the bathymetric characteristics of the reservoirs and on flows. Variable flows resembling natural flow regimes could prevent establishment of high-density polychate populations. Threshold flows critical for reducing habitat are those that would 1) result in transport of FBOM and 2) disturb attached periphyton and embedded macrophytes. We expect that the frequency of these flows as well as the magnitude of flow during the previous year would have an effect on polychaete populations (See Section 3).

The predicted increased flows during March – June in the WRIMS Run-32 Refuge model may decrease juvenile migration time and dilute parasites, potentially resulting in decreased exposure, especially for fish originating from lower Klamath River tributaries. However, these effects would be interactive with temperature effects (Section 6) and with smolt behavior.

Similar to effects on fish, potential effects of low flows include concentrating the parasite, thus enhancing contact potential between myxospores and the polychaete. The outcome of both is an increased infectious dose. Thus provisions for flows during drought years will be important. Extreme low flows likely occurred historically and, like high flows, would have a controlling effect on polychaete populations by drying habitat on vertical surfaces and stagnating pools and marginal habitats.

6. TEMPERATURE AND DAM REMOVAL

6.1. SUMMARY OF ASSUMPTIONS ON PREDICTED TEMPERATURE REGIMES

Temperature predictions following removal of the dams are based on the USFWS Arcata Settlement Technical Report (Hetrick et al. 2009), using supplemental projections for dam removal scenarios based on the 2000 water year provided by PacifiCorp/CH2M Hill. Removal of the dams will result in a thermal regime that exhibits natural diurnal and seasonal fluctuations rather than the phase shift in thermal regimes that exists today with the project reservoirs in place. Temperature reductions ranging between 2 and 10° C would occur from mid- to late August through mid-November. Generally, temperatures would reach 12˚C earlier in the spring and a small increase is expected in summer temperatures (18˚C versus 17˚C).

At increasing distance downstream from Iron Gate Dam, the effect of dam removal becomes less pronounced and both maximum and minimum daily temperatures increase to the point where during mid-June to Aug temperatures are consistently above 20˚C under each scenario assessed. In the current project area, with dam removal temperatures will fluctuate more than under current conditions, with maximum daily temperatures increased and minimum temperatures decreased, resulting in decreased mean daily temperatures. However, throughout this reach, with the exception of the bypass reach above the JC Boyle Powerhouse (Rm 221.4; 220.4) maximum daily temperatures are projected above 15˚C during May-Oct under either current conditions or dam removal.

6.2. EFFECTS OF TEMPERATURE ON DISEASE

6.2.1. POLYCHAETES

Little is known about the effects of temperature on polychaetes. In one laboratory study where polychaetes were held in at 4, 10 or 20˚C, survival after 30 days was greater than 60% at all temperatures and there was no significant difference between survival at any temperature (Bjork 2010). It is clear that polychaetes thrive across a broad temperature range in the Klamath River and thus the slight increase in summer temperature is not expected to have an effect, particularly if there are diurnal fluctuations that provide some buffer. Earlier warming will likely result in earlier reproductive activity of the polychaete and more rapid colonization, potentially increasing and prolonging the period of actinospore release in spring.

The effect of cooler temperatures in the early fall is unknown. Reduction in temperature could have the result of reducing reproductive rates. Peak polychaete reproduction has been reported to occur in late spring-early summer (Willson et al. 2010), although observations by Stocking (2006) suggest that reproduction may be less synchronous. These differences may reflect the importance of other variables in influencing polychaete reproductive timing, such as food availability.

6.2.2. PARASITE TRANSMISSION

Actinospore release from the polychaete host occurs in spring as temperatures rise. In the Klamath River, *C. shasta* can be detected from water samples beginning in March, with *P. minibicornis* detected somewhat earlier (Hallett and Bartholomew 2009). Infection in sentinel fish has been detected in late March/early April and the river remains infectious for fish through the late fall until temperatures decline below approximately 7˚C (Bartholomew, unpublished data; Hendrickson et al. 1989).

Temperature is known to affect parasite development in the annelid hosts of other myxozoans. For example, *Myxobolus cerebralis* triactinomyxons develop in *Tubifex tubifex* at a greater rate in warmer temperatures (El-Matbouli et al. 1999). In a laboratory study that investigated the rate of actinospore development in infected polychaetes acclimated to different temperatures, Bjork (2010) demonstrated that polychaetes held at 20˚C released actinospores earlier and in greater numbers than polychaetes held at 4 or 10˚C. This suggests that actinospore release will likely occur earlier in the spring, resulting in a prolonged release period.

The effects of reduced temperatures in the fall on myxospore production in the fish are unknown and arguments could be made to support either increased or decreased numbers of myxospore released from the fish. Myxospore development in juvenile fish in concurrent with mortality and is inversely related to temperature (see Section 6.2.3.). Thus, lower temperature would likely result in decreased parasite replication rates and decreased myxospore numbers in the adult fish; however, this may be offset by the increased migration distances in the mainstem river. Because myxospore production varies widely between individual adult fish (Foott et al. 2009) it is unlikely that we will be able to predict the effect of temperature on transmission from the fish host.

Free-living stages of *C. shasta* are also vulnerable to temperature, and increases in summer temperatures may result in a decrease in their period of infectivity. In a study that examined the effects of temperature on the actinospore, spores survived approximately 15 d at 12˚C; this decreased to 9 d at 20˚C (Bjork 2010; Foott et al. 2006). Effects of temperature on myxospore stages are likely to be more subtle, especially as myxospore release from adult salmon occurs during periods of colder water temperatures.

6.2.3. DISEASE PROCESSES IN FISH

Water temperature plays a role in the outcome of exposure to *C. shasta,* and the relationship between temperature and progress of ceratomyxosis is well described. Fish may become infected with *C. shasta* at water temperatures as low as 4°C (Ratliff 1983; Ching and Munday 1984) but progress of the disease is temperature dependent. Udey et al (1975) reported that susceptible rainbow trout held at water temperatures from 7 to 23°C had limited ability to resist infection. Mean time to death was inversely correlated to temperature, increasing from 14 d at 23°C to 155 d at 7°C. The decreased time to death with increased temperature most likely results from increased replication of the parasite at these higher temperatures. A susceptible strain of coho salmon appeared to have a greater capacity to resist infection at the lower temperature, but mean time to

death remained temperature dependent (Figure 6.1; Udey et al 1975). That study did not examine the disease dynamics in resistant strains of fish under varying temperatures.

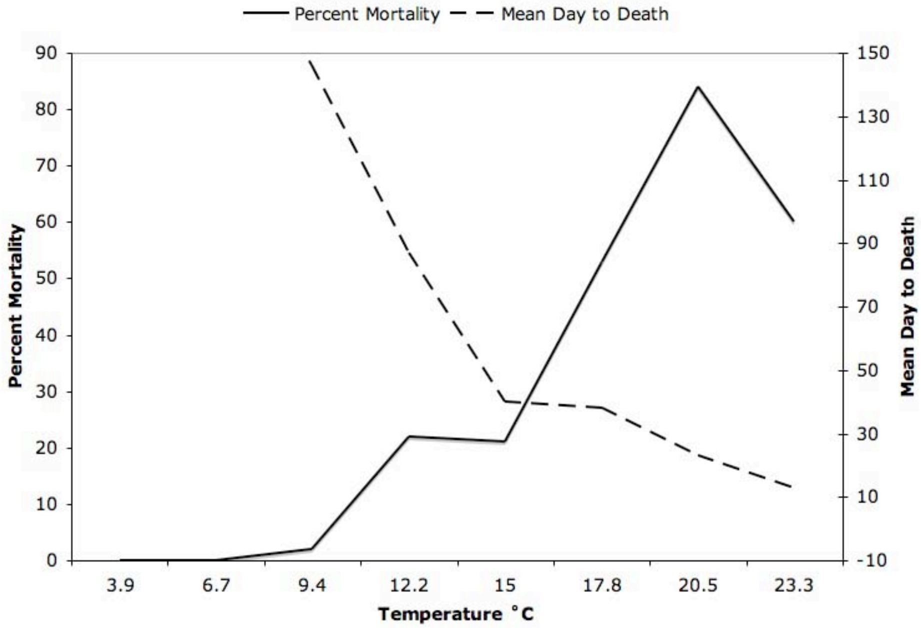


Figure 6.1. Relationship between water temperature, *Ceratomyxa shasta*-related mortality and mean day to death in a susceptible strain of coho salmon (from Udey et al. 1975).

When infectious dose is high, temperature is not a strong predictor of survival. A study by Foott et al. (2004) examined the effects of water temperature on resistance to *C. shasta* in juvenile Klamath River fall Chinook salmon and steelhead following a 3 d exposure near Beaver Creek. In that study, Chinook salmon experienced clinical disease resulting in 83% mortality of fish held at 16˚C and 90% of those held at 20˚C following exposure. This was supported by a more recent study, in which mortality in both Chinook and coho salmon increased from approximately 70% to 98% with a rise in temperature from 13 – 21˚C (Figure 6.2). Although mortality was high at all temperatures, more dramatic differences occurred in mean day to death (MDD), with the MDD for both species held at 13˚C >30 d, and < 18 d at 21˚C (Table 6.1).

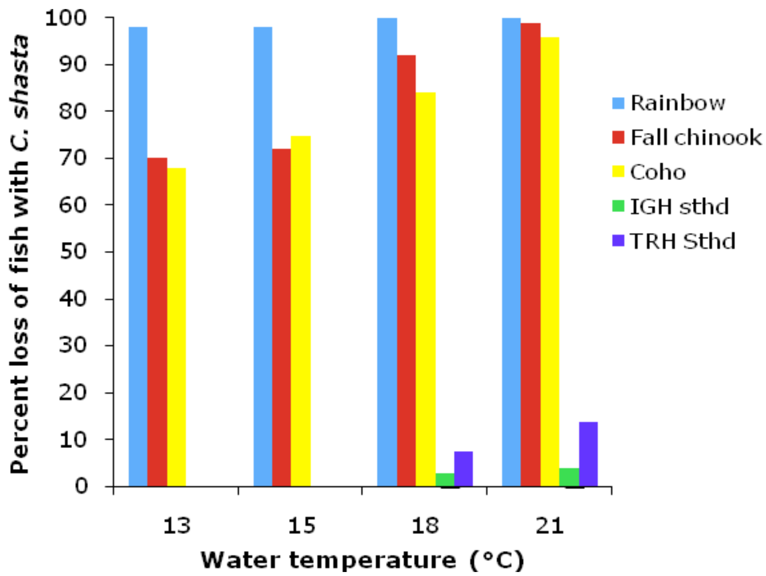


Figure 6.2. Effect of post-exposure water temperature rearing on *Ceratomyxa shasta* infections in stocks of fish exposed near Beaver Creek on June 17-20, 2008.

Table 6.1. Effect of water temperature on the mean day to death during post-exposure holding at the Salmon Disease Laboratory for stocks of fish held for 72 hr in the Klamath River near Beaver Creek during June 17-20, 2008. IGH = Iron Gate Hatchery, TRH = Trinity River Hatchery.

Water Temp. (°C)	Rainbow Trout	IGH fall chinook salmon	IGH coho salmon	IGH steelhead	TRH steelhead
13	32.7	30.6	34.9	ND	ND
15	30.5	24.1	26.7	ND	ND
18	24.5	19.3	25.2	40.0	35.0
21	18.2	16.4	17.5	35.0	34.2

At locations or times when infectious dose is low temperature plays a greater role in survival. Exposure levels at Beaver Creek in 2007 were approximately 10-fold lower than in 2008. Mortality among sentinel Chinook salmon held at 13˚C following exposure was less than 5%, whereas when they were held at ambient river temperatures (20˚C), mortality was 40%. The difference was even more pronounced for coho salmon, with mortality increasing from less than 5% to 80% at the higher temperature (Figure 6.3). Results of the temperature studies in 2007-08 suggest a temperature threshold for mortality at lower infectious doses lies at approximately 15˚C. Thus during periods when temperatures remain below this threshold, additional mortality as a result of disease is likely to remain low. When temperatures surpass 15˚C, disease-related mortality increases.

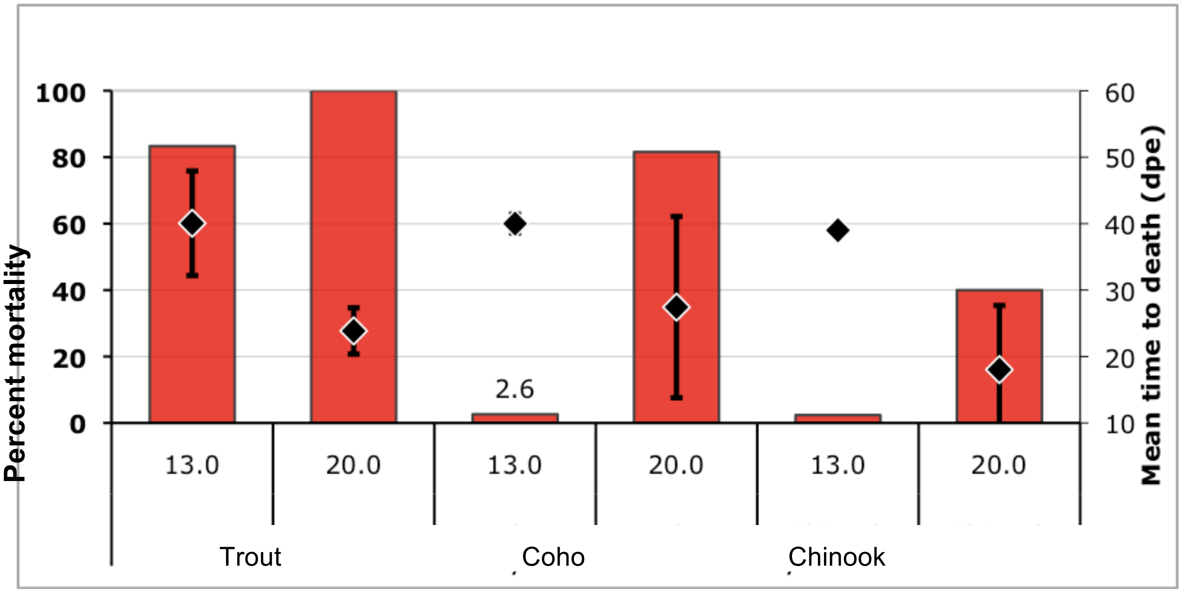


Figure 6.3. Percent mortality and mean day to death for non-native rainbow trout and Klamath River coho and Chinook salmon exposed at Beaver Creek for 3 days in June 2007 and held at 13 and 18˚C.

In addition to influencing disease severity directly, temperature changes may result in alterations in fish behavior that could indirectly affect disease dynamics. Earlier increase in spring temperatures will result in earlier parasite release. It is predicted that these changes would affect migration timing, with fish migrating out earlier than under current conditions (Hetrick et al. 2009). Thus an earlier increase in water temperature may not result in increased exposure.

Earlier cooling in the fall will likely benefit returning adult fish. These fish become infected with the parasites during their return migration. Generally, parasite levels are lower during September than in May and June, and they decrease further as fall progresses. A lower infectious dose may be especially critical for those fish migrating further distances upriver, and which will be more vulnerable to the effects of disease.

6.3. ASSESSMENT OF EFFECTS OF DAM REMOVAL ON TEMPERATURE AND DISEASE

It would be expected that disease in juveniles migrating through the current infectious zone (R2) may escalate earlier in the spring when temperatures reach a threshold of approximately 15˚C. It has been suggested that the effects of temperature increase may be offset by earlier migration with dam removal; however, water sampling data from 2009 (Figure 2.5) demonstrates that parasite levels may increase above 10 spores/l as early as April. It is likely that a greater diversity of salmon life histories will evolve, with some of those types more likely to avoid parasite exposure by migrating earlier or overwintering in tributaries and migrating in the fall.

One of the changes in temperature with dam removal will be increased daily temperature ranges (increased variability). Although the effects of fluctuating temperatures on the disease process are not known, it might be expected that fish would avoid migrating

during periods when temperatures are high and would utilize tributaries and the large springs as temperature refuges. In the area of the current project (R3) temperatures are permissive for disease effects with the exception of the cooler waters of the bypass reach above the JC Boyle Powerhouse. However if the current infectious zone is not altered following dam removal, it is likely that with longer migration distances from these upriver rearing areas, fish will be migrating through this zone throughout the period when temperatures are high.

The earlier decrease in temperature during fall will have a positive effect on adults that have increased migration distances upriver, thus reducing potential for prespawn mortality as a result of parasite infection.

7. ADULT SPAWNING DISTRIBUTION AND DAM REMOVAL

7.1. SUMMARY OF INFORMATION AND ASSUMPTIONS ON ADULT SPAWNING DISTRIBUTION FOLLOWING DAM REMOVAL

Removal of IGD will remove the barrier to migration for anadromous salmonids. It is expected that these fish will migrate into the upper basin and spawn in the KR mainstem and tributaries encompassed by the current project area, and in the Williamson River system. Numbers of adults spawning in the KR mainstem between the Shasta River and Bogus Creek might be expected to decrease with changes in hatchery operation, but this reach will continue to provide spawning habitat**.**

7.2. EFFECTS OF ALTERERED SPAWINING DISTRIBUTION ON DISEASE

7.2.1. POLYCHAETES

Effects of altered adult salmon distribution on infection in polychaetes are covered under the discussion of infection prevalence in that population under parasite transmission; it is not expected that altered salmon populations would directly affect polychaete densities.

7.2.2. PARASITE TRANSMISSION

Samples from adult salmon spawning at Iron Gate and Trinity River Hatcheries collected by CDFG personnel have been examined for myxozoan infection by PCR and histology. Table 7.1 presents data for these fish showing percent infection for *P. minibicornis* and *C. shasta* for two spawning years, as determined using specific molecular assays for each parasite. In general, infection prevalence was high for both parasites with the exception of low *C. shasta* infection in Iron Gate coho salmon and Trinity River Fall Chinook salmon sampled in 2005-06. However, subsequent sampling the following year detected high infection prevalence in these stocks. These discrepancies may be a result of the limited numbers of fish sampled (3 coho; 19 Chinook salmon), or a reflection of differences in infection during the run. We have no data on how long these fish were in the river and exposed to these parasites. These data do not reflect severity or stage of infection; however, the histological assessments conducted by the USFWS demonstrated severe myxozoan infections in greater than 50% of the fish.

Data from adult carcass removal conducted on Bogus Creek in 2008 and 2009 found that 30 and 60% of adults had mature *C. shasta* myxospores, respectively, with an average of 1.28×10^6 per fish (Bartholomew et al. 2009; Foott et al. 2009). Taking into account the number of adult salmon spawning in the mainstem and tributaries, even at the lower % prevalence this extrapolates to an approximate release of 4.6×10^9 myxospores between IGD and the Scott River confluence (~ 75 Rkm) (Foott et al. 2009). Water samples were also assayed to obtain a rough estimate of actual parasite numbers coming from Bogus Creek. Using the average of 1 myxospore/liter and flow rate of 660 liters/sec, this extrapolates to 5.7×10^7 myxospores/day.

Table 7.1. Prevalence of *Parvicapsula minibicornis* and *Ceratomyxsa shasta* in adult salmonids returning to Iron Gate and Trinity River Hatcheries determined by PCR assay (% PCR +). .- = assay not done.

Year	Hatchery	Species	No. Sampled	% Positive for *P. minibicornis*	% Positive for *C. shasta*
2005-06	Iron Gate	Fall Chinook	20	100	70
		Coho salmon	3	100	33
		Steelhead	10	100	50
	Trinity	Fall Chinook	19	100	0
		Coho salmon	19	47	100
		Steelhead	20	95	85
2006-07	Iron Gate	Fall Chinook	20	100	85
		Coho salmon	20	55	95
	Trinity	Fall Chinook	20	100	90
		Spring Chinook	19	100	75
		Coho salmon	20	80	90
		Steelhead	20	90	-

Data from a similarly conducted study of infected juvenile Chinook salmon demonstrated that these fish also contribute myxospores (Ray, 2009). In that study, the estimated contribution from juvenile fish (using IGH production numbers) ranges from 1.8×10^9 (at 30% prevalence of infection (POI) & minimum myxospore production) to 1.6×10^{13} myxospores (at 85% POI & maximum myxospore production). The low-end calculation is about half of the value estimated for adult salmon (Foott et al 2009); whereas the high end is approximately 3000 fold greater. This indicates that juvenile salmon could be an important source of myxospores. However, it is our opinion, that infected juvenile Chinook salmon do not contribute significant numbers of *C. shasta* myxospores to infect polychaetes within the infectious zone. We base this hypothesis on the 4 lines of evidence: 1) rare detection of myxospores in histological samples of salmon collected within the reach (biased to active fish), 2) laboratory data indicating that vast majority of infected fish die prior to 18 – 25d post-infection time required for myxospore production at ≥ 18˚C, 3) the assumption that moribund salmon would be consumed by predator prior to myxospore production and 4) the assumption that infected fish will continue to migrate and thus be downriver of the infectious zone before clinical disease develops.

Myxospores released by tributary populations of spawning adult fish likely have a lower impact on infection prevalence in mainstem populations of polychaetes. It is more likely that mainstem spawning adult salmon play a larger role, as adult carcasses tend to deposit in pools and eddies where polychaete populations are prevalent, thus facilitating transmission of the parasite to its next host. Data from USFWS surveys illustrate that the majority of mainstem spawning occurs in the reach just upstream of the infectious zone (Figure 7.1). However, there does not appear to be a direct relationship between numbers of adults returning and disease severity in juvenile fish in the infectious zone during the

following spring (Figure 7.2; USFWS). This suggests that a low number of infected adult salmon (>3000) are sufficient to maintain high infection prevalence in polychaetes.

Movement of adult salmon into the upper basin will result in introduction of parasite genotypes that have been restricted to the lower river (Section 2.8). While the effects of these introductions is uncertain, there appears to be at least some degree of host-specificity that should limit impacts on native redband populations.

7.2.3. DISEASE PROCESSES IN FISH

Returning adult salmon become infected as they enter the Klamath River in the fall and disease likely progresses as a function of temperature and infectious dose. Returning adult salmon have a low infection threshold as demonstrated by the high infection prevalence among Trinity River salmon. Thus high infection prevalence in adult fish will likely be maintained even if *C. shasta* levels are reduced in the infectious zone. It could be expected that prespawn mortality as a result of myxozoan infection will be increased for fish migrating further in the mainstem KR. Prespawn mortality as a result of *C. shasta* infections have been documented in summer Chinook salmon migrating to upper reaches of the Columbia River basin (Chapman 1986) and *P. minibicornis* has been documented as a cause of prespawn mortality in upper Fraser River stocks of sockeye salmon (Jones et al. 2003).

The potential effect of adult redistribution on disease in juvenile salmon is unclear, however, it will result in longer migration periods. Establishment of infectious zones in the project area would increase exposure, and if the infectious dose is sufficient to overwhelm the juvenile salmon, this could result in further dispersal of parasites. Under current conditions, any myxospore release from juvenile fish is assumed to occur below areas of high polychaete densities. Juvenile fish infected in upper reaches could release myxospores during their migration if they survive predation. However, observations of clinically infected juvenile fish suggest that these fish die prior to myxospore formation and thus are not a major contributor of myxospores.

7.3. ASSESSMENT OF EFFECTS OF DAM REMOVAL ON ALTERED ADULT DISTRIBUTION AND DISEASE

The opening of habitat above the projects will mean greater dispersal potential for myxospores as adult salmon migrate into new habitats. There is a great deal of uncertainty with these predictions. This would potentially have the immediate effect of decreasing myxospore input in the area immediately below IGD, which is currently hypothesized to be the primary source of infection for down river polychaete populations. If these fish migrate into upriver tributaries to spawn there may be a reduction in myxospore availability in this reach, although it is uncertain what threshold is required to maintain infection in polychaetes.

Adult salmon are expected to disperse into R3 and 4, which will provide spawning habit both in the main stem and tributaries such as Spencer and Jenny Creek in R3, and the Williamson and Sprague Rivers in R4. Where these fish spawn will likely influence the occurrence of additional infectious zones, as polychaete populations are already well

established in these reaches. The creation of an infectious zone in the Williamson River as a result of stocking susceptible hatchery fish might provide some insight into what might be expected to occur in that system.

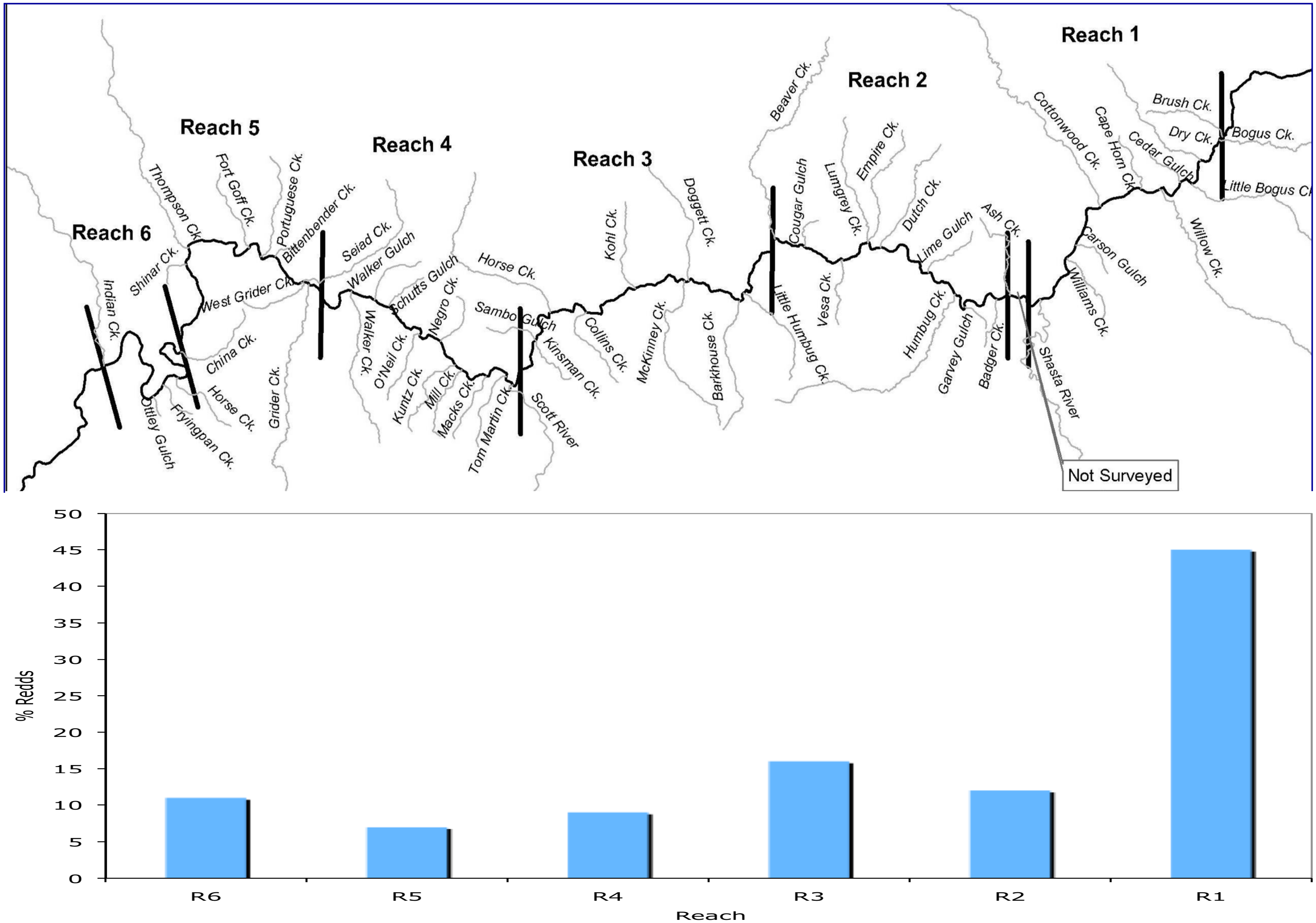


Figure 7.1. Location of adult spawning redds by river reach. Data and map from USFWS report http://www.fws.gov/arcata/fisheries/projectUpdates/KRSpawningSurvey/KlamathSpawnSummary2009.pdf.

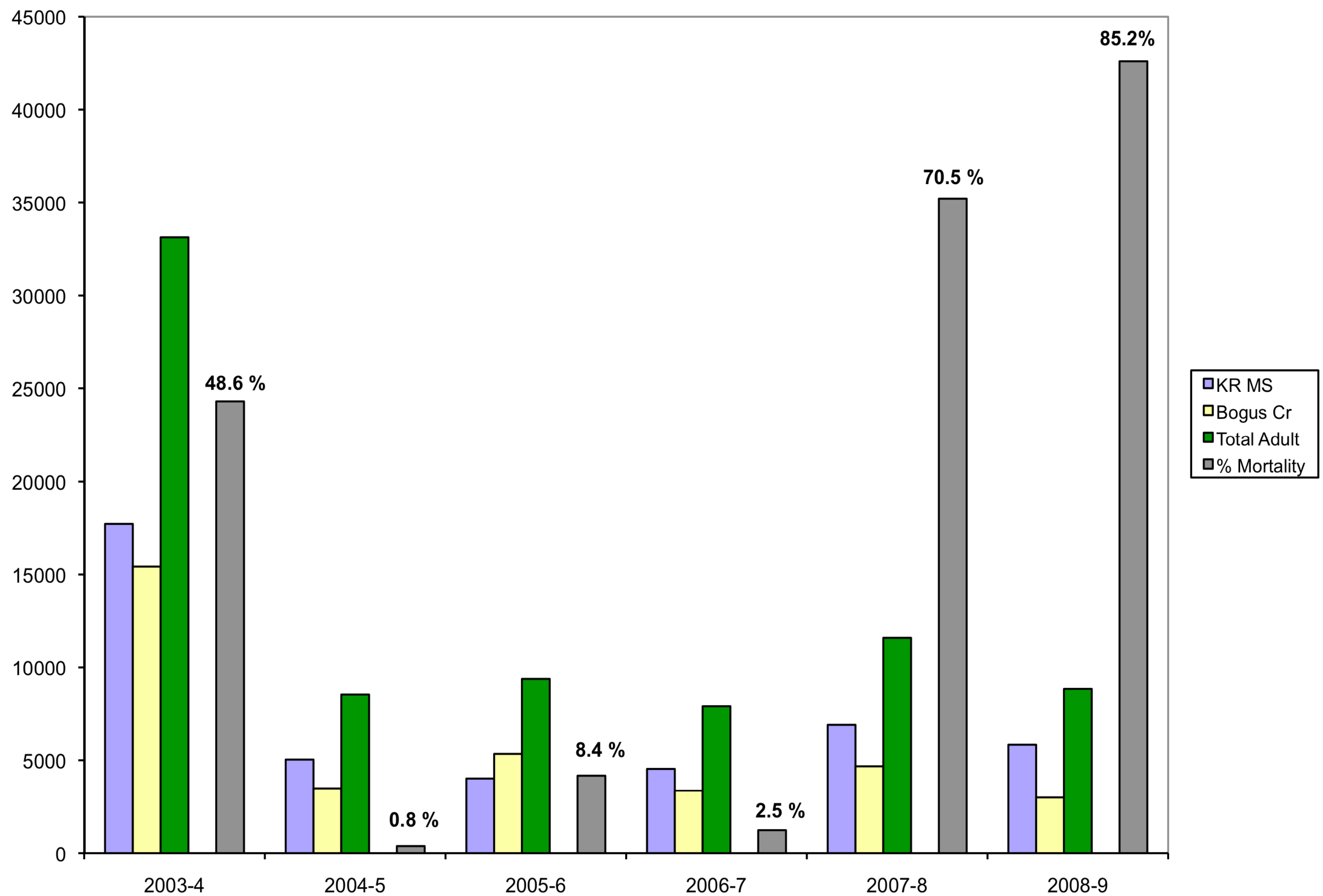


Figure 7.2. Adult spawning data for 2003-04 through 2008-09, separated into numbers of fish spawning in Bogus Creek, Klamath River main stem (KR MS). The % mortality is that of sentinel juvenile Chinook salmon held in the infectious zone the following spring.

8. WATER QUALITY AND DAM REMOVAL

8.1. SUMMARY OF INFORMATION AND ASSUMPTIONS ON WATER QUALITY FOLLOWING DAM REMOVAL

Assumptions of current nutrient (nitrogen and phosphorus) levels are informed by the North Coast RWQCB (Asarian et al. 2009). The Klamath River was likely a highly production ecosystem prior to the current anthropogenic impacts. However, degraded habitat, increased sediment load, altered flow conditions and impoundments, have resulted in increased levels of nutrient and organic loading. Elevated nutrient levels stimulate the growth of periphyton (benthic algae), which serves as habitat for polychaetes. Similarly, levels of suspended algae and diatoms, which provide a food source for the polychate host, are elevated as a result of reservoirs. We assume that dam removal would reduce nutrient levels by restoring the river to something closer to pre-project conditions, with sediment passing through the system during the winter and early spring and providing a more constant nutrient load during the peak growing season.

Because of the unknowns associated with changes in nutrient levels and our incomplete knowledge of the variety of food sources utilized by polychaetes we assume that polychaete densities will be directly correlated with presence of fine benthic organic matter, which provides both habitat and a food source (see Section 3).

8.2. EFFECTS OF WATER QUALITY ON DISEASE

8.2.1. POLYCHAETES

Polychaetes are both suspension feeders and facultative surface deposit feeders, utilizing a diet of fine organic detritus and micro-algae (Rouse and Pleijel 2001; Fauchald and Jumars 1979). This strategy is referred to as "interface" feeders, as the worms switch feeding modes during flux of seston quantity and quality (Taghon and Green 1992). Because they require large quantities of organic matter, interface feeders are considered food limited (Lopez and Levinton 1987). To optimize food intake, these polychaetes are able to select particulates of the appropriate size for ingestion while larger sizes are either rejected or used as material for tube construction (Lewis, 1968b). Elevated nutrient concentrations resulting in increased periphyton and suspended algae thus would simultaneously result in increased habitat and food availability. The distribution of polychaetes described in Section 3 (Figure 3.1) is likely a function of food availability and habitat, with decreased food availability below the Trinity River resulting in low population densities.

8.2.2. PARASITE TRANSMISSION

The effects of nutrients on parasite transmission are likely indirect, as these waterborne stages are spores and not actively feeding until they encounter a host. Low dissolved oxygen and pH may have an effect on spore longevity, but this has not been demonstrated.

8.2.3. DISEASE PROCESSES IN FISH

Improvements in water quality would reduce a primary stressor on fish and this will likely result in improved fish health. Whether this would in turn result in increased resistance to disease is unknown.

8.3. ASSESSMENT OF EFFECTS OF DAM REMOVAL ON WATER QUALITY AND DISEASE

There is a great deal of uncertainty regarding the effects of nutrient levels post dam removal on polychaete densities. Elimination of the reservoirs would decrease the levels of suspended algae, thus reducing a food source for polychaetes in the river reaches below the dam. In addition, it would reduce abundance of the fine benthic organic matter that serves as polychaete habitat and as a foraging substrate. Thus it is likely that dam removal, by reducing nutrients, will result in some reduction in polychaete densities. However, as the system is inherently nutrient rich it is uncertain whether polychaete population decreases, at least in the short term, would be significant enough to reduce disease. Another limitation on predicting water quality effects on polychaete populations is the lack of correlative data that would provide a useful measure of their diet.

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