

**ESTABLISHING BASELINE  
INFORMATION FOR ASSESSMENT OF  
FLOW MANAGEMENT ALTERNATIVES  
FOR MITIGATING EFFECTS OF  
MYXOZOAN PATHOGENS IN THE  
KLAMATH RIVER**

*Prepared For:*

**California Energy Commission**  
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*Prepared By:*

Oregon State University



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## Preface

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## Abstract

*Ceratomyxa shasta* is a myxozoan parasite identified as a significant contributor to salmon mortality in the Klamath River. The parasite has a complex life cycle with life stages developing in both a fish and a freshwater polychaete worm host, *Manayunkia speciosa*. The ecological requirements of this polychaete influence the severity of the infection in fish. An understanding of what contributes to high densities and infection of the polychaete host may provide management opportunities to reduce the parasite effects. This study investigated the effects of temperature and dewatering on the survival of the polychaete in its two primary substrates: *Cladophora* sp. (an alga) and a mixture of sand and silt. It was observed that high water temperature had an adverse effect on polychaete survival. A small percentage of polychaetes survived 24 hour dewatering in both substrates. A laboratory-based flow experiment showed that a higher water velocity (0.05 meters per second) supported greater polychaete densities. However, experimentally induced polychaete infection prevalence was greater at the slower water velocity (0.01 meters per second). 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 associated with pulsed flows from dams may decrease both *C. shasta* infection severity in the fish and infection prevalence in the polychaete host. Extrapolation of these results to explain what would occur under natural flow conditions will data require collection from field locations.

Keywords: *Ceratomyxa shasta*, *Manayunkia speciosa*, flow, temperature, Klamath River



# Executive Summary

## Introduction

The myxozoan parasite *Ceratomyxa shasta* causes severe intestinal infections in salmon and trout and has been implicated as a significant source of mortality of out-migrating juvenile Chinook salmon in the Klamath River during recent years. The parasite has a two-host life cycle requiring a salmonid host and *Manayunkia speciosa*, a freshwater polychaete worm. Another myxozoan, *Parvicapsula minibicornis*, infects the kidney of these same salmon and uses the same polychaete host to complete its life cycle. The prevalence of *P. minibicornis* infections among juvenile fall-run Chinook salmon in the Klamath River was over 90 percent in 2004. Dual infections of these myxozoans are believed to be a major factor in disease mortality. However, the shared polychaete host creates the potential for simultaneous control of both parasites by limiting populations of the polychaete or disrupting the parasite life cycles.

Proposed control measures have included manipulation of river flow to: (1) reduce habitat for the polychaete (through strategies such as scouring flows), (2) decrease numbers of polychaetes (by using low flows that would dry large areas of habitat), and (3) increase amounts of water at key times to decrease infection either in the fish or in the polychaete. Alternative control measures that are independent of flow manipulations include: (1) decreasing myxospore input back into the life cycle by removal of heavily infected fish, and (2) increasing predation on the polychaetes. However, little is known about the effect of such measures on the dynamics of the parasite life cycles.

The Klamath River and other rivers in the Pacific Northwest where this is a problem are simultaneously managed for hydropower, agriculture, and recreational use, with little understanding of how this management affects interactions between fish and pathogens. In the Klamath Basin, decisions on water allocation have been especially controversial and have been severely criticized because of the lack of scientific information to support them. Based on the author's knowledge of the effects of temperature, exposure dose, and flow on the severity of ceratomyxosis infections in salmonids, it is clear that decisions made on water use directly affect fish health. However, before the implementation of control measures, the biology of *M. speciosa* and the effects of environmental changes on this host and the *C. shasta* life cycle must be understood.

## Purpose

This study aimed to test the effects of water flows, temperature and desiccation on the survival of polychaete populations from different habitat types and to determine the factors likely to have the greatest effect on reducing alternate host densities and *C. shasta* infection levels in the Klamath River.

## **Project Objectives**

The project aims were met by a series of laboratory experiments investigating the following:

1. The effects of water temperature and dewatering (low flows that would dry polychaete habitat) on the survival of the polychaete host in two of its primary habitats.
2. The effect of water temperature on the longevity of the life stage infectious to fish.
3. The effect of two water flows (velocities) on polychaete survival and both polychaete and fish infection.

## **Project Outcomes**

Under laboratory conditions, consistent high water temperature (20°C), dewatering, and a slow flow rate of 0.01 meters per second all had an adverse effect on polychaete densities and survival. However, a higher percentage of polychaetes (14 percent) became infected in the slow (0.01 meters per second) flow experimental treatments compared with those held at a five-fold faster flow (less than 2 percent). It followed that fish infected in the slower flow challenge tanks died more quickly from *C. shasta* infections than fish infected at the fast flow, indicating they received a higher infectious dose. Interpretation of the decline in polychaete densities at the end of the flow experiment is complicated by a coincidental storm event that generated a large sediment load in the Willamette River water that was supplying the experiment. These laboratory findings thus support observations in the field, in which changes in environmental conditions affect the host-parasite balance. These data indicate that habitat disruption by way of drying may significantly reduce polychaete densities. In addition, *C. shasta* infection prevalence in the polychaete host and infection severity in the fish host was lower at the faster water velocity.

## **Conclusions**

This study's findings indicate that changing environmental parameters influence the infection prevalence and survival of both the fish and polychaete host of *C. shasta*. High water temperature has an adverse effect on polychaete survival, and habitat disturbance such as mechanical disruption or drying may have severe consequences on survival as well. Although polychaete densities increased at the faster flow, the polychaetes had lower *C. shasta* infection prevalence. In terms of mean day to death, which is a reflection of exposure dose, susceptible salmonids exposed at the fast flow (0.05 m/s) took longer to die than those exposed at the slow flow (0.01m/s). Thus, higher water velocities may decrease *C. shasta* infection prevalence in both the fish and polychaete host.

## **Recommendations**

Results of this laboratory study and research in other rivers indicate that increased flows may decrease effects of *C. shasta* by reducing infectious dose and exposure time of the fish. If the source of water that provides these increased flows are tributaries or reservoirs where the parasite is not present or is in low abundance, there would likely be additional benefit as a result of parasite dilution. Providing increased flows from cooler water sources will slow the

rate of disease in fish and may allow recovery. However, the ability to significantly reduce Klamath River temperatures in the main channel of the river above the confluence of the Trinity River is limited. Additionally, reducing water temperatures may provide simultaneous indirect benefits for the polychaete host. The effects of pulsed flows would be most beneficial for salmon that enter the main channel of the Klamath River as smolts during their peak migration in May through June. This would also benefit salmon that rear in the main channel as it spans a period of high actinospore release. Flow alterations (low or high) may also provide benefits at other times by reducing habitat for the polychaete host, although this should be tested under river conditions. Recommendations for timing and magnitude of pulsed flows that would provide optimal benefit will require long-term collection of data to identify trends or manipulation of flows to test these hypotheses.

### **Benefits to California**

Salmon losses in the Klamath basin have had devastating impacts on coastal economies and tribal communities along the river. The effects of declining salmon runs throughout the region have been felt for several decades, but the reduction of the commercial catch by 90 percent in 2006 was a direct result of the weak returns of Chinook salmon to the Klamath River. Infection by myxozoan parasites has, in large part, been responsible for the declining numbers of juvenile Klamath River fall Chinook salmon and the subsequent predictions of low adult returns. Losses to coastal communities and the salmon troll industry were estimated to be 28 million dollars in 2006 alone. This highlights the need for management strategies that maximize the quantity and survival of Klamath River salmon. In the Klamath Basin, decisions on water allocation have been especially controversial and have been severely criticized because of the lack of scientific information to support them. This research provides baseline data on the effects of temperature and water flow on the *C. shasta* life cycle. Validation of the hypotheses generated in this study through field studies will be critical to making informed decisions that will allow use of these valuable resources while maintaining healthy fish populations.



# 1.0 Introduction

## 1.1. Background and Overview

*Ceratomyxa shasta*, a myxozoan parasite, has a two-host lifecycle requiring a salmonid and *Manayunkia speciosa*, a freshwater polychaete worm (Bartholomew et al. 1997). In the salmonid host, *C. shasta* infects the intestine and can cause hemorrhage, necrosis, and death. Although genetically identical, the *C. shasta* life stages in the two hosts are morphologically distinct, with myxospores developing into actinospores in polychaetes and actinospores developing into myxospores in fish exclusively. Myxospore stages of *C. shasta* are shed by the fish host, usually upon death of the fish, and they infect *M. speciosa*. *Parvicapsula minibicornis*, another myxosporean parasite, infects the kidney of salmon and uses the same polychaete host. Infection prevalence of *P. minibicornis* was over 90% in juvenile fall-run Chinook salmon in the Klamath River in 2004 (Nichols and Foott 2006). Dual infections of fish by *C. shasta* and *P. minibicornis* confound the cause of mortality; however, the shared polychaete host creates the potential for control of both parasites through the manipulation of this host. Little is known about development of either parasite into their respective actinospore stages in *M. speciosa* except that they proliferate either in the body wall (*C. shasta*) (Bartholomew et al. 1997) or the body lumen (*P. minibicornis*) (Bartholomew et al. 2006). The mode of actinospore release from the polychaete, the infectious dose, and the route of infection for both hosts remain unknown for both myxozoans. Because the effect of *C. shasta* on survival of juvenile salmon appears to be the primary mortality factor, this research focuses on this parasite. However, many of the conclusions drawn here will also apply to *P. minibicornis*, because of the similarities in their life cycles.

*Ceratomyxa shasta* is endemic to the Pacific Northwest and California where infections in wild and hatchery-reared fish have been well documented (Hendrickson et al. 1989; Ratliff 1983; Ching and Munday 1984; Hoffmaster et al. 1988). Susceptibilities of a variety of stocks have been widely reported, with those that originate or migrate through endemic locations showing the highest resistance (Bartholomew 1998). The mechanism(s) for resistance to infection and disease are not known (Ibarra et al. 1992, 1994; Bartholomew et al. 2001), although genetic loci associated with resistance have been identified (Nichols et al. 2003). Resistant stocks have, however, succumbed to infection during prolonged exposure (Ratliff 1981), suggesting that resistance may be overcome at a high actinospore dose.

In addition to the innate characteristics of the host that affect the outcome of infection, temperature is also known to influence the development of disease in the fish. Udey et al. (1975) demonstrated that the disease process was accelerated in fish held at warmer temperatures, whereas cool temperatures prolonged mean day to death or prevented development of disease. The relationship between increasing temperature and myxozoan proliferation has been demonstrated for *Myxobolus cerebralis* in *Tubifex tubifex* (Blazer et al. 2003; Kerans et al. 2005) and for *Tetracapsuloides bryosalmonae* in *Fredericella sultana* (Tops et al. 2006). However, Oezer and Wootten (2002) and El-Matbouli et al. (1999) observed a negative correlation between increasing temperature and the viability of several myxozoan actinospores. Foott et al. (2007) also found a

decrease in the infection prevalence of salmon when exposed to infectious river water at 18°C, possibly indicating the lower viability of the actinospore at this temperature. Similarly, Ratliff et al. (1983) found that the infectious stage of *C. shasta* was able to infect fish for a longer period of time after collection when held at 11°C, compared to 18°C. The effect of temperature on development and viability of the actinospore as well as the polychaete host of *C. shasta* are not well understood.

*Manayunkia speciosa* is a small freshwater tube-dwelling sabellid polychaete worm with broad distribution in the benthos of freshwater lakes, streams, and rivers across North America. Mackie and Qadri (1971) summarize the distribution of *M. speciosa* along the east coast from New Jersey down to North Carolina, across the northern Midwest in Wisconsin and Ontario, Canada, and in the west from as far north as Alaskan lakes and south to the Sacramento River, California. *Manayunkia speciosa* inhabits a versatile range of temperatures (from 4°C to > 25°C) and flow regimes (< 0.01 to 3.0 meters per second [m/s]) (Holmquist 1973; Mackie and Qadri 1971; Stocking and Bartholomew 2007). This host is generally described as colonizing soft sediments such as sand with a mix of fine benthic organic matter (FBOM) or on algae-covered rocks (Pettibone 1953; Hazel 1966; Mackie and Qadri 1971; Stocking and Bartholomew 2007). *Manayunkia speciosa* reproduces sexually or asexually, creating small broods of juveniles, but the rate of reproduction and lifespan of these worms are unknown (Croskery 1978; Stocking and Bartholomew 2007). Increased knowledge about the survival of this host and factors affecting *C. shasta* infection and actinospore development will enhance our understanding of *C. shasta* infection dynamics.

The presence and density of a *M. speciosa* population depends upon the suitability of the available habitat and food abundance. Stocking and Bartholomew (2007) report stable substrate, moderate flows, and FBOM as the defining characteristics of *M. speciosa* habitat in the Klamath River Basin. They hypothesize that the absence of *M. speciosa* populations in areas of available habitat is due to poor dispersal and insufficient food base. Seasonal changes in precipitation affect flow rate and water temperature may vary greatly between summer and winter months. Irrigation and hydroelectric demands also influence water level and flow patterns for many rivers. The effects of such changes on a *M. speciosa* population are unknown.

When considering the epidemiology of *C. shasta*, the infection prevalence in the polychaete population is as important as population density. The number of infected polychaetes in a population determines the number of actinospores that may be released and thus, the infectious dose for fish. The only reports of *C. shasta* infection prevalence in *M. speciosa* are from Klamath River populations (Stocking and Bartholomew 2007). Infection prevalence in the 13 populations tested was generally below 1%, with the exception of two locations where prevalence was 4.9% and 8.3%. No correlation was demonstrated between flow and natural *C. shasta* infection prevalence in that study. The effect of flow alone on parasite abundance is difficult to determine from field studies because of the inability to separate the effects of flow from other variables. However, in a laboratory study that examined the effects of flow alone on the ecology of another myxozoan, *Myxobolus cerebralis*, Hallett and Bartholomew (in preparation) found a significant increase in parasite numbers and infection prevalence in the invertebrate host *Tubifex tubifex* under low flow conditions.

Although the spatial overlap of infected polychaete and fish hosts may increase infection prevalence, it is not required for infection to occur. High *C. shasta* associated mortality has been reported from sites at which no polychaetes were found and no parasite DNA was detected in water samples collected from the exposure site (Stocking et al. 2006; Hallett and Bartholomew 2006). These studies attribute upstream populations of polychaetes as the sources of infection. When comparing fish infection records with polychaete population presence and infection prevalence, Stocking and Bartholomew (2007) estimated that actinospores may travel as far as 327 river kilometers (Rkm) over a period of five days. Thus, although water flow may transport the actinospore downstream, the viability of the actinospore determines if infection occurs. Currently, few studies have investigated the relationship between time and *C. shasta* actinospore viability. Ratliff (1983) demonstrated that the infectious stage is viable for up to one week at ambient summer river temperature. However, because temperature was not constant during any of these observations, the viability of actinospores at any given temperature is not known. During a spring 2005 survey of fall Chinook infection prevalence, Foott et al. (2007) observed a decline in *C. shasta* infection prevalence when fish were exposed to infectious water that was 18°C. They hypothesize that this warmer temperature may have decreased the viability of the actinospore.

## **1.2. Project Objectives**

The study presented here investigates the relationship between changing environmental parameters and survival of *M. speciosa*, as well as the effect of these changes on the *C. shasta* infectious process. Specifically, laboratory models were developed to investigate the following:

1. Determine the survival of the polychaete host, *M. speciosa*, and the actinospore stage of the parasite at three different temperatures that are expected to represent the extremes that would be experienced in the Klamath River.
2. Measure the effects of a simulated low flow event, or draw-down on polychaete survival in the two habitats in which it is found.
3. Determine how water flow affects polychaete population density and the prevalence of infection in both the polychaete and fish host.

## **1.3. Report Organization**

The methods and results will be presented in the subsequent sections, providing details of experimental design, data, and data analysis. Tables and figures pertinent to each section are provided at the end of that section. Both sections will be organized under the objectives stated above. Conclusions and discussion of the results will follow in Section 4, with general overall conclusions presented, then discussion of the specific objectives addressed, and finally other outcomes of the study.



## 2.0 Methods

### 2.1. Determination of the Survival of the Polychaete Host, *M. Speciosa*, and the Actinospore Stage of the Parasite, at Three Different Temperatures

#### 2.1.1. Collection and Maintenance of Polychaetes

Polychaetes were collected from the Klamath River system from two substrates in which they are commonly found. The epiphytic algae *Cladophora* was collected at river kilometer (RKm) 292 (a site near Interstate 5). The sand-silt substrates were collected from the mouth of the Williamson River (RKm 0), a tributary of Klamath Lake. These collection sites are geographically distant, and due to this separation, these populations may be genetically distinct. However, environmental variables such as temperature and precipitation in each of the locations are similar; therefore it is expected that the physiological responses of these populations would be comparable. To date, there are no reports of differences in the tolerance of *M. speciosa* to changing environmental parameters from different locations. Samples were collected using an 83 micrometer ( $\mu\text{m}$ ) mesh plankton net (17 centimeter [cm] diameter) fitted on a telescoping handle. A 2 cm metal flange was attached to the rim of the net to facilitate scraping of hard substrates. A plastic kitchen spatula was also used to scrape *Cladophora* off of rocks. Material was placed in plastic bags with approximately 500 milliliter (mL) of river water, supplied with oxygen via an airstone, and transported back to the John L. Fryer Salmon Disease Laboratory (SDL) at Oregon State University in Corvallis, Oregon. All experiments were conducted at the SDL using specific pathogen free (SPF) well water unless indicated otherwise. The temperature and dewatering experiments were run in tandem.

#### 2.1.2. Temperature Effects on *Manayunkia Speciosa*

Individual bags of each substrate (*Cladophora* sp. and sand-silt) were pooled according to substrate type and mixed for randomization. Polychaete density within *Cladophora* is highly variable, so this substrate was homogenized by breaking the algae into 2 cm squares and mixing before distribution into 500 mL containers. Equal amounts of each substrate type and associated biota (30 mL aliquots of *Cladophora* containing an average of 147 (with a standard deviation [SD] of 18) polychaetes per container and 50 mL of sand-silt with an average of 197 (SD 52) polychaetes per container) were distributed in shallow plastic containers in triplicate. Two additional replicates for each temperature were created for determination of polychaete density at four and eight weeks (only one replicate for each of these time points). Each container was fitted with a hole to facilitate water flow and a low flow (approximately 100 mL/min) of water at 5°C, 12.8°C, or 20°C was supplied to each of the containers for the duration of the three month experiment or until termination. The equivalent of one replicate was fixed in ethanol at weeks four and eight for polychaete density determination. At termination, all material was fixed in ethanol for polychaete density determination. Due to the possibility of polychaete replication and inability to monitor the survival of individual polychaetes, the relative change in polychaete population density (RPD) was used to measure changes in population densities throughout the experiment. The RPD was calculated for each sample by comparing the density

of polychaetes in each treatment group and time point with the mean density of the initial sample.

### **2.1.3. Temperature Effects on Actinospore Longevity**

Polychaetes in sand-silt substrate were transported to the laboratory as described and maintained in 19L aquaria with no flowing water, but oxygen was supplied via an airstone. Intestinal tissue and serous fluid in the abdominal cavity from *C. shasta*-infected rainbow trout were added to the aquaria as a source of myxospores. After three to four weeks, polychaetes were examined by light microscopy for infection. Actinospores from infected polychaetes were released by applying pressure to the polychaete on a slide with a cover slip. Actinospores were pooled and mixed in nanopure water, aliquoted to microcentrifuge tubes and held at 5°C, 12°C, or 20°C. Only one replicate of actinospores was held at each temperature due limited availability. On days 0, 3, 6, 10, and 12, the contents of each tube were mixed and 10 µl was removed, put onto a slide with a coverslip and examined for the presence of actinospores using a light microscope. Actinospore morphology was the only criterion measured and although it is not a measure of actual viability (the ability to infect fish was not measured) it does provide some initial data on the longevity of this life stage.

## **2.2. Measurement of the Effects of a Simulated Low Flow Event, or Draw-down, on Polychaete Survival in the Two Habitats**

### **2.2.1. Effects of Dewatering on *Manayunkia Speciosa***

Polychaetes in sand-silt and *Cladophora* substrates were collected, transported, and divided into five replicates in the same volumes as in the temperature experiment. Water was poured off of each substrate, and they were allowed to dry under a grow light (Sylvania Gro-Lux Standard 1.5 inch diameter, 40 watts) for 24 hours, however, the residual moisture content was not determined. During this time the temperature of the sediment reached 25°C. After 24 hours, 12.8°C water was added at the same low flow as the temperature experiment. Initial polychaete densities were the same as in the temperature experiment described in Section 2.1.2. Due to the limited availability of substrate, only one replicate was fixed in ethanol at weeks four and eight; at 12 weeks the triplicate groups were also preserved for density determination.

## **2.3. Determination of How Water Flow Affects Polychaete Population Density and the Prevalence of Infection in the Polychaete and Fish Host**

### **2.3.1. Effects of Flow**

Four identical stainless steel tanks (~67 cm long) were divided into three replicate channels (each 10 cm wide) with Plexiglas following the design of Hallett and Bartholomew (in preparation) (Figure 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 Plexiglas 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 four 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°C and 23.6°C (USGS National Water Information System: Web Interface. <http://waterdata.usgs.gov>. Willamette River at Albany, Oregon, site no. 14174000).



1 a



1 b

**Figure 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 Plexiglas divider into the three replicate channels. The outflow passes over a dam wall and down an outflow pipe. (1b) The out flow of each of the replicate channels flows into a 19 L aquarium where fish were held during the exposure period.**

Water was supplied at 0.01 m/s to two of the tanks (with three replicate channels per tank) creating the “slow flow” treatment group. Water was supplied to the two remaining tanks (with three 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, Maryland) 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 three. Although the number of myxospores released from these fish may be variable, the fish were the same size and 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 liter (L) aquarium for fish exposures. The Willamette River is known to support the life cycle of *C. shasta*, therefore two additional aquaria supplied directly with Willamette River water at the two experimental flows created the “Willamette Fast” and “Willamette Slow” control treatment groups. These control groups were 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, Washington; average length 7.5 cm, weight 6.1 grams [g]) were held in each aquarium, including the Willamette River control, from experiment initiation to week six. 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, Oregon; average length 8.2 cm, weight 8.1 g [week 6] and 7.7 cm and 5.7 g [week 11]).

To test the effect of the treatments on infection in a *C. shasta*-resistant fish strain, Klamath Chinook salmon (Iron Gate strain, Iron Gate Hatchery, California; 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, Washington) and was exposed for four 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 with iodophor between exposure groups.

Dead and moribund fish were examined for *C. shasta* myxospores in a wet mount of an intestinal scraping at 200x for three 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 polymerase chain reaction assay were performed as described by Palenzuela et al. (1999). Fish surviving 90 days were euthanized with MS222 and intestinal tissue samples were removed and frozen until they could be processed for analysis of *C. shasta* DNA by polymerase chain reaction. Tissue from the posterior intestine was also collected from the Chinook salmon and preserved in 10% neutral buffered formalin for histology. 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. At weeks 6, 11, 15, and 22, three approximately 30 mL sub-samples were randomly taken 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. Table 1 illustrates the pool size, pool number, and number of infected pools that were put into the Ausvet pooled prevalence calculator.

### **2.3.2. 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.

**Table 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(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 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

## 2.4. Statistical Analysis

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



### 3.0 Project Results

#### 3.1. Determination of the Survival of the Polychaete Host, *M. Speciosa*, and the Actinospore Stage of the Parasite at Three Different Temperatures

##### 3.1.1. Temperature Effects on *Manayunkia Speciosa*

Polychaete densities in the sand-silt substrate at 5°C and 12°C remained relatively stable during the first four weeks of the experiment, with a mean relative change in polychaete density (RPD) of 66% and 93%, respectively (Figure 2). Densities of polychaetes held at 5°C remained stable over the experimental period compared to the 12°C and 20°C treatment groups. The mean RPD at week 12 for each of the temperature treatments in sand silt were significantly different ( $p = 0.011$  from ANOVA on arcsine transformed data,  $p < 0.05$  for individual t-tests). The RPD of the three replicate groups at 5°C at 12 weeks was 39%, 62%, and 69% with a mean of 57% (SD 16). Survival at 12°C decreased more markedly, with RPD of 14%, 23%, and 29% and a mean of 22% (SD 8) at 12 weeks. Polychaetes held at 20°C had low survival during the initial four weeks (27% RPD), and at 12 weeks the mean survival was 1.5% (SD 0.9) for the three replicates.

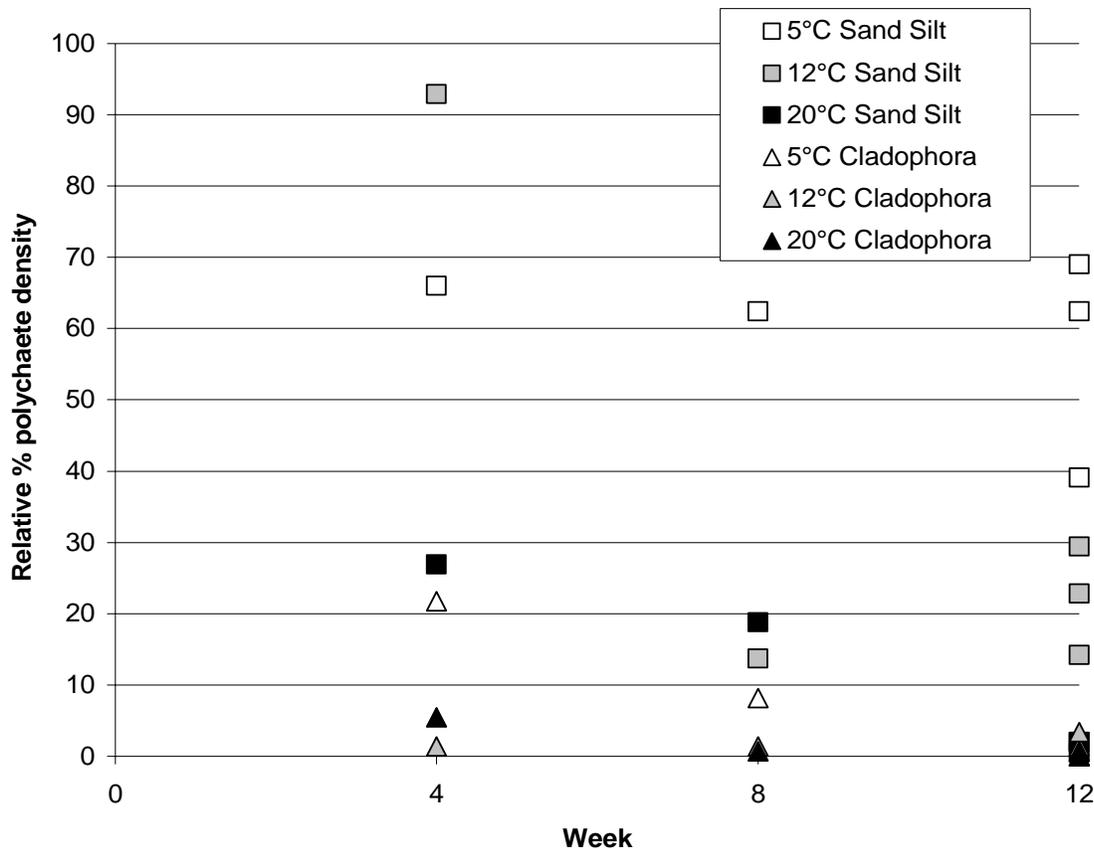


Figure 2. Relative change in population density of *Manayunkia speciosa* in *Cladophora* and sand-silt substrates at 5°C, 12°C, and 20°C

There was a large decline in polychaetes in *Cladophora* between the initiation of the experiment and the first sampling at week four at all three temperatures (Figure 2). At this time, RPD was 22% for those held at 5°C while RPD at 12°C and 20°C was less than 5%. By week 12 no significant recovery was observed at any temperature in this substrate with mean RPD less than 2% for all temperatures ( $p = 0.492$  from ANOVA on arcsine transformed data).

### 3.1.2. Temperature Effects on Actinospore Longevity

*Ceratomyxa shasta* actinospores were seen on day 0 and day 3 at all of the experimental temperatures (Table 2). No actinospores were seen in the sample held at 20°C on day 6, whereas actinospores could still be seen in the 4°C and 12°C treatments on this day. Thereafter, actinospores could only be seen in the 4°C treatment on days 10 and 12.

**Table 2. The presence of intact *Ceratomyxa shasta* actinospores at 4°C, 12°C, and 20°C over time**

Treatment	Days				
	0	3	6	10	12
4°C	+	+	+	+	+
12°C	+	+	+	-	-
20°C	+	+	-	-	-

+ indicates presence, - indicates absence

## 3.2. Measurement of the Effects of a Simulated Low Flow Event, or Draw-down on Polychaete Survival in the Two Habitats in Which It Is Found

### 3.2.1. Effects of Dewatering on *Manayunkia Speciosa*

Following a 24 hour period of dewatering and high temperature, there was a significant decline in the number of surviving polychaetes in both substrates (Figure 3). At four weeks, the RPD of *M. speciosa* in *Cladophora* was only 3%, compared with 42% for the sand-silt treatment. The RPD was less than 2% from subsequent samples of the *Cladophora*. Although densities of polychaetes in sand-silt continued to decline, 25% remained at eight weeks and RPD at 12 weeks was 3%, 13%, and 19% with a mean of 11% (SD 8). The RPD at week 12 is not statistically different between the substrates ( $p = 0.075$  from a t-test on arcsine transformed data). Additionally, the decline in polychaete survival may also be an effect of the 12°C water temperature that was used after the 24 hour challenge. From the previously described temperature experiment, the mean survival in water at 12°C was 92% at four weeks in sand-silt and less than 5% in *Cladophora*. At the same water temperature, the 24 hour dewatering challenge resulted in 50% less population density in sand-silt. Polychaete densities in *Cladophora* were low overall.

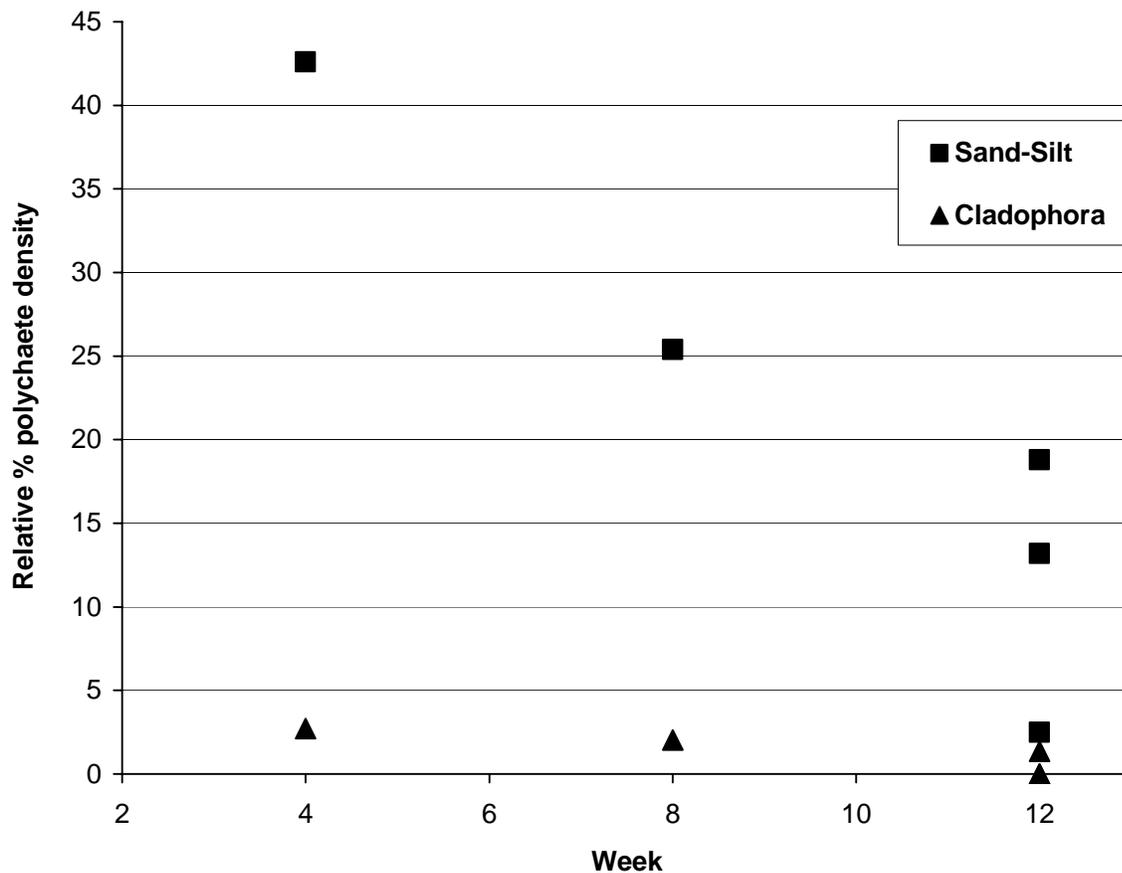


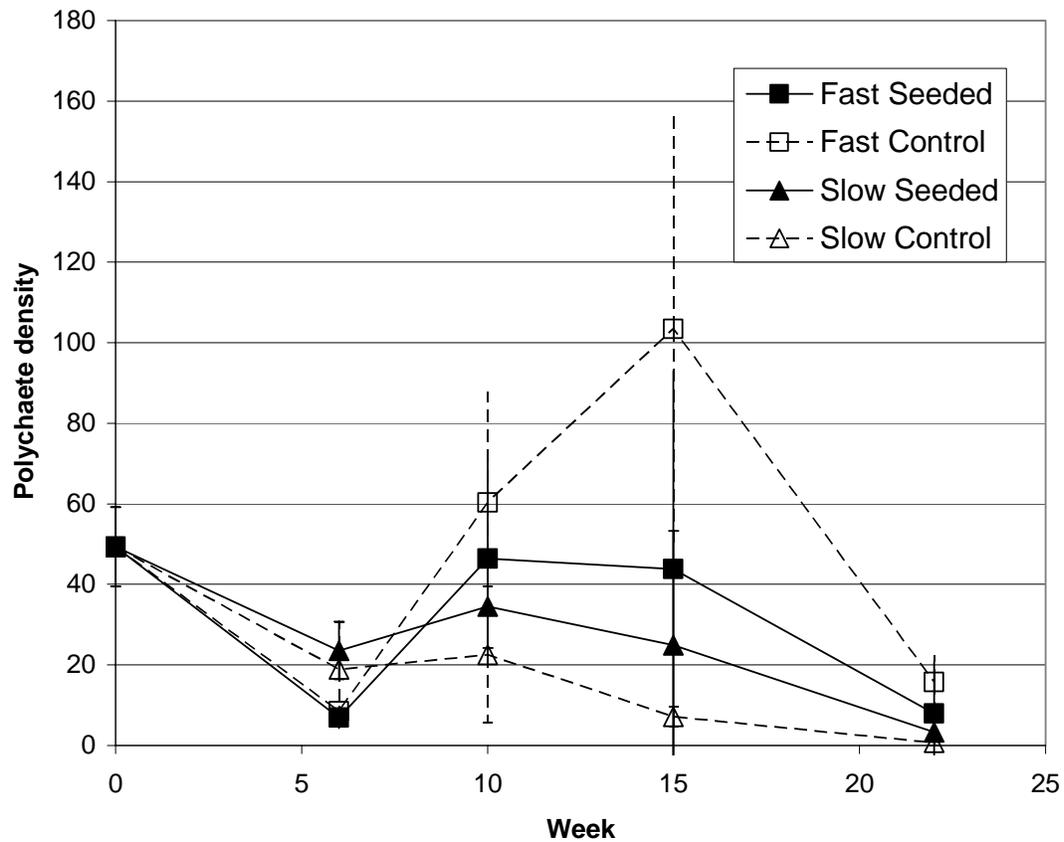
Figure 3. Relative change in the density of *Manayunkia speciosa* after dewatering for 24 hours

### 3.3. Determination of How Water Flow Affects Polychaete Population Density and the Prevalence of Infection in Both the Polychaete and Fish Host

#### 3.3.1. Effects of Flow

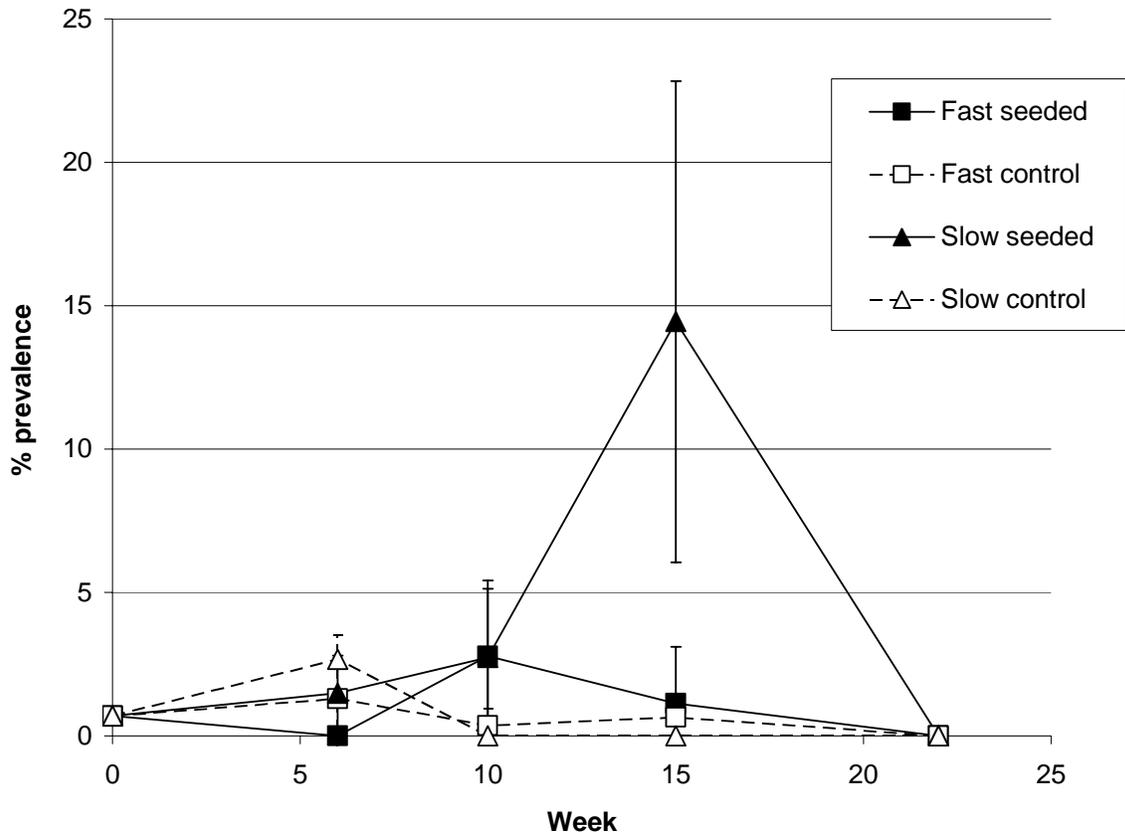
Polychaete densities fluctuated throughout the course of the experiment (Figure 4). At week 0, there was a mean of 49 polychaetes per 30 mL sample (Standard Error = 5.7). The average polychaete density declined to 24 or fewer worms per sub-sample in all treatment groups at week six. Although these densities 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 week six. 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 (SD 27) followed by the fast seeded group with a mean density of 46 polychaetes/sample (SD 26), the slow seeded group with a mean density of 35 polychaetes /sample (SD 10), and the slow control group with a mean density of 28 polychaetes/sample (SD 17). The densities of all groups, except for the fast control group, peaked at week 10, then declined. Densities in the fast control treatment group continued to increase to a mean 103 polychaete/sample (SD 53) at week 15, then declined. At week 22, the mean density was less than 16 polychaetes for all of the treatment groups. Only the trends in the fast control and slow control groups 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 also been associated with this high temperature.



**Figure 4. *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.**

The infection prevalence among polychaetes at week 0 was 0.7%. At week six, the infection prevalence for the fast control group averaged 1.3% (SD 2.2) and was 1.5% (SD 1.3) for the slow seeded group and 2.7% (SD 0.54) for the slow control group (Figure 5 and Table 1). No infected polychaetes were detected in the sub-samples collected from the fast seeded group at week six.



**Figure 5. *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.**

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% (SD 0.61) and 0% (SD 0), respectively, at week 10. Infection in the fast seeded group peaked at 2.8% (SD 2.6) at this time point, and the slow seeded group increased to 2.7% (SD 2.4), both within the range of natural infection in these populations. Mean infection prevalence in the slow seeded group reached 14% (SD 8.4) 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 among all of the sample periods ( $p = 0.009$  ANOVA after arcsine transformation). None of the polychaetes assayed at week 22 were positive for *C. shasta* infection.

Susceptible rainbow trout became infected during all exposures and mortality was high in all exposure groups and exposure periods (Table 3). None of the Chinook salmon died from ceratomyxosis and none assayed positive for *C. shasta* by PCR, therefore tissue sections were not analyzed histologically. 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 five living fish at the time of the first *C. shasta* mortality were included in the analysis. Only one fish held in the Willamette River control tank during weeks one through six was identified as 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 six week exposure.

**Table 3. The percent *Ceratomyxa shasta* infection and mean day to death of susceptible fish during the first three exposure periods. Standard deviations are provided in parentheses. Data represent average for triplicate groups.**

	Week 1–6		Week 6–10		Week 11–15	
	% Infected	Mean Day to Death	% Infected	Mean Day to Death	% Infected	Mean Day to Death
<b>Fast seeded</b>	100 (0)	51.5 (0.7)	81.7 (10.1)	26 (0)	74.2 (13.8)	21.3 (1.5)
<b>Fast control</b>	100 (0)	47 (1.4)	96.7 (5.8)	27.7 (0.6)	66.7 (57.7)	24 (1.4)
<b>Slow seeded</b>	100 (0)	38.6 (1.5)	100 (0)	23.7 (1.2)	96.7 (5.8)	17.7 (2.1)
<b>Slow control</b>	100 (0)	38.6 (2.1)	100 (0)	23.7 (2.1)	88.7 (9.8)	17.7 (0.6)
<b>Willamette Slow</b>	10	*	0	*	0	*
<b>Willamette Fast</b>	0	*	0	*	0	*

\* terminated at 90 days

All of the susceptible rainbow trout exposed during the first six weeks were *C. shasta* positive. In weeks 6 through 10, the infection prevalence in rainbow trout exposed in the fast seeded treatment group (82%) was significantly less than fish exposed in the other treatment groups during the exposure period (99.6% to 100%) ( $p = 0.014$  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 3). In the first exposure period, fish exposed in the slow flow treatment groups died an average of 11 days earlier than the fast flow exposure groups. For the second and third exposure periods, this difference decreased to three and four 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 one week or four week exposure. No Chinook assayed were positive by PCR.

## 4.0 Conclusions and Recommendations

### 4.1. Conclusions and Results Discussion

#### 4.1.1. Summary

This study's findings indicate that the changing parameters of the environment influence the infection prevalence and survival of both the fish and polychaete host of *C. shasta*. Temperature has an inverse relationship with polychaete survival, and habitat disturbance such as mechanical disruption or drying may have severe consequences on survival as well. Although mean polychaete densities increased at the faster flow, the polychaetes at this flow had a lower *C. shasta* infection prevalence. In terms of mean day to death, which is a reflection of exposure dose, susceptible salmonids exposed at the fast flow had longer mean day to death than those exposed in the slow flow. Thus, high water velocities may decrease *C. shasta* infection prevalence in both the fish and polychaete host.

#### 4.1.2. Specific Conclusions Related to Project Objectives

##### ***Determination of the Survival of the Polychaete Host, M. speciosa, and the Actinospore Stage of the Parasite at Three Different Temperatures that are Expected to Represent the Extremes that Would Be Experienced in the Klamath River***

In this study, the effect of temperature on polychaete survival was evident in populations collected from sand-silt substrate, where there was an inverse relationship between temperature and relative percent survival of the polychaetes. Populations held at 5°C remained stable over the 12 week experimental period compared with almost no survival at 20°C at 12 weeks. In its natural habitats, *M. speciosa* occurs over the range of all of the temperatures tested (Holmquist 1973; Mackie and Qadri 1971; Stocking and Bartholomew 2007). In the river, water temperatures are not likely to be consistently high, as in our laboratory challenge, which may explain their survival during the summer months. Additionally, assessment of polychaete survival in the laboratory was affected by lack of food available through the experiment.

Temperature and actinospore longevity had an inverse relationship. Actinospores remained intact the longest at 4°C, but were short-lived at 20°C. These findings are consistent with Oezer and Wooten (2002) and El-Matbouli et al. (1999) who found an inverse relationship between temperature and the longevity of other myxozoan actinospores. Although, this study only looked at the presence or absence of the actinospore, temperatures at or above 12°C appear to limit the presence of the actinospore to less than six days. The actinospores remained intact for at least three days at all of the temperatures tested. When this data is applied to Stocking and Bartholomew's (2007) estimates for rate and distance traveled by *C. shasta* actinospores in the Klamath River (327 Rkm over five days), actinospores at 4°C, 12°C, or 20°C are viable long enough to travel from Iron Gate dam (the barrier to fish passage) to the estuary. As actinospore viability increases, so may actinospore distribution, increasing the infectious dose for fish over a larger area of the river. Actinospores may survive longer than 12 days at 4°C, but accurate quantitative monitoring and viability studies involving fish infection are needed to fully understand the relationship between temperature and actinospore viability.

### **Measurement of the Effects of a Simulated Low Flow Event, or Draw-down on Polychaete Survival in the Two Habitats in Which It Is Found**

It has been noted that polychaetes do not inhabit edge habitats where water levels fluctuate (Stocking and Bartholomew 2007), and it could be expected that an organism with the apparent fragility of the polychaete would be unable to survive even a brief period of drying. Although dewatering for 24 hours significantly reduced the number of polychaetes that survived in the sand-silt habitat, nearly 43% survived the event. Because 12°C water was supplied at low flow for the remainder of the experiment, changes in survival after the initial dewatering challenge may have been influenced by water temperature as well. From the temperature experiment, polychaetes in sand-silt held at 12°C experienced a 10% decline in density in the first four weeks. If temperature caused the same effect in the dewatering experiment, only 50% of the total loss in sand-silt can be attributed to dewatering, and the other 10% may be a temperature effect. Previous observations indicate that there are no resting stages of *M. speciosa* (Holmquist 1973), so survival under these drying conditions is remarkable. Although the substrate in this experiment was not desiccated and some moisture remained, the polychaetes did survive static conditions at temperatures as high as 25°C. The polychaete's tube may offer some protection or buffering from adverse conditions in its environment, such as maintaining moisture within the tube. Because the rate of maturity and lifespan of *M. speciosa* is not known, it is unclear whether one life stage is more vulnerable to these conditions than another.

While the effects of temperature and dewatering on polychaetes in the sand-silt habitat were clear, this study's researchers were unable to draw conclusions on how these parameters might affect populations in *Cladophora* (algae), another primary habitat. The immediate decline in polychaete densities in the *Cladophora* habitat in both experiments is likely to have been affected by other factors. Substrate disturbance is one possible explanation for the large difference in polychaete survival observed between the substrates. The sand-silt mixture settles more quickly than *Cladophora*, possibly generating a more stable environment. The removal of *Cladophora* from rocks and boulders in the river, followed by the division of this mat-like algae to reduce variations in polychaete density, likely damaged polychaete tubes and marginalized its habitat. Because few polychaetes survived under any conditions in *Cladophora*, confounding variables such as these may have overshadowed the effects of temperature and dewatering in this study.

In both the temperature and dewatering experiments, the absence of an adequate food source may have also contributed to the decline of populations in both substrates. Algae and bacteria that grew in the sample container were the only food sources available to the polychaetes in this experiment. If food was indeed the factor limiting survival, the increased survival at 5°C could be due to a reduction in metabolic rate at this temperature. Considering the survival of *M. speciosa* in shallow Alaskan lakes where water reaches or remains just above freezing in the winter (Holmquist 1973), such a strategy seems plausible for over-wintering.

### **Determination of How Water Flow Affects Polychaete Population Density and the Prevalence of Infection in Both the Polychaete and Fish Host**

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

organic matter (FBOM) 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 sand 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 only greater than the fast flow groups at week six 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 Iron Gate 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 five actinospores (authors' personal observation). Thus, it is not surprising that rainbow trout became infected in the non-seeded groups due to this background infection prevalence. For this reason, Chinook salmon were incorporated into the experiment in effort to see a dose-response effect.

No Chinook salmon succumbed to *C. shasta* infection during either a one week or four week exposure. Infection prevalence was measured in the Chinook at 90 days after exposure, giving enough time for the fish to have possibly 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, the Chinook salmon may have been exposed to more than 41,000 actinospores, yet none became infected. This data indicates that this stock may be resistant to as high as 8200 actinospores per fish.

Although there were not detectable differences in the number of fish infected, the mean day to death for fish exposed in the slow flow treatments was lower than for fish exposed in the fast flow treatments, indicating 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 both field (Vincent (2002) and laboratory (Hallett and Bartholomew, in preparation) studies in both fish and annelid hosts of *M. cerebralis*. Vincent (2002 and 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. In a study in which fish were held in a volume of Klamath River water Foott et al. (2007 in press) collected during April, May, and June 2005, infection prevalence was higher after a natural high flow event that occurred between the April and May collection. However, water flows continued to increase through the end of May, and June infection prevalence was decreased. Actinospore density was also measured from these samples and tended to increase from April to June, regardless of variations in river flow. Sentinel exposures conducted by the Oregon State University laboratory before and after the high flow event between May and June 2005 did result in decreased mortality of Chinook salmon and a higher mean day to death of susceptible rainbow trout, suggesting a decreased infectious dose (unpublished data). Comparison of these studies is difficult because they were designed to address different questions, but they do illustrate the complex relationships between temperature and flow.

#### **4.1.3. Additional Study Findings**

One of the unstated objectives of this research was to establish a culture system for maintaining *M. speciosa* under laboratory conditions. This was accomplished with some degree of success in this study. The difficulties in maintaining populations in the temperature study led to pilot tests of different food and water sources, with stable populations established through the use of Willamette River water as a source of nutrients. *Manayunkia speciosa* densities in the flow experiment were much higher (greater than 100% of the starting density) than the temperature experiment, likely a result of the constant inflow of Willamette River water. However, this model system was also susceptible to the adverse effects of an open system, with the eventual decline in polychaete numbers in the flow experiment after 20 weeks, likely a result of storms that occurred in the latter weeks of the experiment that increased sediment load in the tanks. Alternatively, the decline may also be a natural seasonal trend associated with the transition from summer to autumn accompanied by decreased daylight and water temperatures. Regardless of the reason for the decreased survival at the end of the experiment, this system maintained polychaetes at relatively high infection levels and offers many opportunities to study *C. shasta* infection in *M. speciosa* as well as the actinosporean life stage. The peak in polychaete infection prevalence at week 15 (12 weeks after the addition of an infected fish) is the first report of a laboratory infection and provides some indication of the amount of time it takes for myxospores to be released from a dead fish and infection in *M. speciosa* to develop.

## **4.2. Recommendations**

Results of this laboratory study, and research in other rivers (Ratliff 1981; Mamoyac et al. 2000), indicate that increased flows may decrease effects of *C. shasta* by reducing infectious dose and exposure time of the fish. If the source of water that provides these increased flows are tributaries or reservoirs where the parasite is not present or is in low abundance, there would likely be additional benefit as a result of parasite dilution. Providing increased flows from cooler water sources will slow the rate of disease in fish and may allow recovery, although the ability to significantly reduce mainstem Klamath River temperatures in the river above the confluence of the Trinity River is limited. Additionally, reducing water temperatures may provide simultaneous indirect benefits for the polychaete host. The effects of pulsed flows would be most beneficial for salmon that enter the mainstem Klamath River as smolts during their peak migration in May through June. This would also benefit salmon that rear in the mainstem as it spans a period of high actinospore release. Flow alterations (low or high) may also provide benefits at other times by reducing habitat for the polychaete host, although this should be tested under river conditions.

This pilot study examined the effects of flow using a laboratory model. Studies on the effects of flow on polychaete populations should be continued using field sites established in the Klamath River, where populations of this host can be monitored over three to five years during periods of varying flow regimes. This strategy would provide data on how populations fluctuate in response to natural variations in flows, which is essential for developing models to predict when timed pulsed flows would provide the maximum benefit.

## **4.3. Benefits to California**

The Klamath River and other major rivers in California and the Pacific Northwest are simultaneously managed for hydropower, agriculture, and recreational use, with little understanding of how this management affects interactions between fish and their pathogens. In the Klamath Basin, decisions on water allocation have been especially controversial and have been severely criticized because of the lack of scientific information to support them. This research, in combination with related field research, provides baseline data critical to making informed decisions that will allow use of these valuable resources while maintaining healthy fish populations.



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