Running Title: Effects of water velocity on the *C. shasta* infectious cycle

The effects of water velocity on the *Ceratomyxa shasta* infectious cycle

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Abstract

Ceratomyxa shasta is a myxozoan parasite identified as a contributor to salmon mortality in the Klamath River, USA. The parasite has a complex life cycle involving a freshwater polychaete, *Manayunkia speciosa* and a salmonid. As part of ongoing research on how environmental parameters influence parasite establishment and replication, we designed a laboratory experiment to examine the effect of water flow (velocity) on completion of the *C. shasta* infectious cycle. The experiment tested the effect of two water velocities, 0.05 and 0.01 m/s, on survival and infection of *M. speciosa* as well as transmission to susceptible rainbow trout and comparatively resistant Klamath River Chinook salmon. The faster water velocity facilitated the greatest polychaete densities, but the lowest polychaete infection prevalence. Rainbow trout became infected in all treatments, but at the slower velocity had a shorter mean day to death, indicating a higher infectious dose. Infection was not detected in Chinook salmon even at a dose estimated to be as high as 80,000 actinospores per fish. The higher water velocity resulted in lower *C. shasta* infection prevalence in *M. speciosa* and decreased infection severity in fish. Another outcome of our experiment is the description of a system for maintaining and infecting *M. speciosa* in the laboratory.

Key words: *Ceratomyxa shasta*, water velocity, parasite ecology, *Manayunkia speciosa*, infection, salmonids

INTRODUCTION

The myxozoan *Ceratomyxa shasta* is enzootic among populations of salmon and trout in the larger river systems of the Pacific Northwest region of North America, including rivers in northern California (Ratliff 1983, Ching and Munday 1984, Hoffmaster, Sanders, Rohovec, Fryer & Stevens 1988, Hendrickson, Carleton & Manzer 1989). In the Klamath River, which spans the California/Oregon border, *C. shasta* infection is associated with high mortality in juvenile Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum) (Foott, Harmon & Stone 2004) and there is great interest in finding ways to mitigate the impact of this parasite on these populations.

The life cycle of *C. shasta* requires two hosts: a salmonid host and *Manayunkia speciosa*, a freshwater polychaete worm (Bartholomew, Whipple, Stevens & Fryer 1997). In the salmonid, *C. shasta* infects the intestine causing hemorrhage and necrosis. Myxospore stages are shed by the fish in the late stages of infection (usually upon death of the fish) and infect the polychaete. Completion of the cycle occurs when actinospores released from the polychaete and infect a fish.

In the fish host, *C. shasta* development is affected by temperature, the susceptibility of the fish, and the infectious dose. Infection follows a seasonal trend (Ching and Munday 1984, Hendrickson *et al.* 1989) and the rate of parasite development increases with increasing temperature (Udey, Fryer & Pilcher 1975). Fish stocks that originate or migrate through enzootic locations show the highest resistance (Bartholomew 1998), but may succumb to infection as a result of prolonged exposure and presumably high actinospore doses (Ratliff 1981). However, the threshold dose for infection in these

stocks is unknown, and other than temperature, the environmental conditions that affect the progress of disease in fish have not been investigated.

Far less is known about interactions between *M. speciosa* and *C. shasta*, yet the parasite can only become established when the polychaete is present. Although stable substrate and moderate water velocities are described as two of the defining characteristics of *M. speciosa* habitat in the Klamath River Basin (Stocking and Bartholomew 2007), little is known about factors affecting the colonization, reproduction, and lifespan of these worms (Stocking and Bartholomew 2007, Croskery 1978). The only reports of *C. shasta* infection in *M. speciosa* are from Klamath River populations where infection prevalence ranged from less than 1% to up to 8.3% (Stocking and Bartholomew 2007). Reasons for the differences in infection prevalence are difficult to determine from field studies because of the inability to separate the effects of multiple variables such as temperature, flow, proximity to a myxospore source and myxospore dose. However, the relationship between high infection prevalence in polychaetes and severe infection in fish (Stocking and Bartholomew 2007) indicates that we need to understand more about factors that facilitate parasite transmission between these hosts.

The relationship between water flow and parasite infection dynamics have been characterized for another myxozoan, *Myxobolus cerebralis*. In a laboratory study that examined the effects of flow on the transmission and ecology of *M. cerebralis*, Hallett and Bartholomew (2008) found a significant increase in parasite numbers and infection prevalence in the invertebrate host, *Tubifex tubifex*, under the slower flow conditions. Parasite prevalence and infection severity in the fish host was also higher in the slow treatment groups. Similarly, an inverse relationship between water flow and *M. cerebralis*

infection prevalence in fish was also demonstrated in a field study where water flows were much greater (E. R. Vincent, Montana Fish, Wildlife and Parks personal communication). These studies were the first to document the effects of water flow on a myxozoan life cycle, through changes in the number of invertebrate hosts as well as the infection prevalence and severity in both hosts.

We designed a laboratory model to test the hypothesis that water velocity affects completion of the *C. shasta* life cycle as well as *M. speciosa* survival. In our model, we tested the effects of two water velocities (0.05 and 0.01 m/s) on the survival and infection prevalence in *M. speciosa*, as well as the subsequent infection of susceptible and resistant fish hosts. Understanding how changes in water velocity affect the *C. shasta* infectious cycle will provide insight on temporal changes in infection, aid in predictions about fish infection severity based on river conditions, and could lead to control measures in rivers where flow can be manipulated.

METHODS

Collection of polychaetes: Polychaetes in sediment (sand and silt) were collected from the mouth of the Williamson River, a tributary of Klamath Lake in Oregon, USA on April 28, 2006. Samples were collected using an 83 µm mesh plankton net (17 cm diameter) fitted on a telescoping handle. Material was placed in plastic bags with approximately 500 mL of river water, supplied with oxygen and transported to the John L. Fryer Salmon Disease Laboratory, Oregon State University, Corvallis, OR. Material was aerated overnight and the experiment was initiated the following day.

Experimental design: Four identical stainless steel tanks (~67 cm long) were divided into three replicate channels (each 10 cm wide) with plexiglass following the

design of Hallett and Bartholomew (2008) (Figures 1 and 2). Water from the Willamette River, Oregon, was supplied via a manifold behind the headwall to facilitate an even flow of water. The spill of water over the headwall created a plunge pool with turbulent flow in the upper one third of the channel. This hydraulic effect was decreased by the placement of a plexiglass plate fitted with twelve 0.95 cm holes drilled in a random pattern and placed 9.5 cm in front of the headwall. Polychaetes and substrate (approximately 1.7 L) were randomly distributed in each of the replicate channels to a depth of approximately 3 cm, and allowed to settle 4 h prior to the initiation of water flow. The outflow of each channel was directed into individual 19 L aquaria to accommodate fish exposures.

Use of Willamette River (WR) water insured an adequate food source for *M. speciosa*. However, because the WR supports the life cycle of *C. shasta,* we included two controls, one aquarium at each experimental velocity, to detect infection from the water source. Water was supplied at ambient river temperature for the duration of the experiment, which began April 29, 2006 and ended October 3, 2006. Temperature of the incoming WR water changed seasonally and ranged between 13.3 and 23.6 °C (USGS National Water Information System: Web Interface. http://waterdata.usgs.gov, Willamette River at Albany, OR site no. 14174000). Water temperature in each aquaria was measured during weeks 3, 9 and 14 and was within 2 °C of the mean reported temperature from the USGS monitoring station, regardless of fast or slow flow treatment.

Treatment groups: Water was supplied at 0.01 m/s to two of the tanks and one WR control creating the "slow" treatment groups. Water was supplied to the two remaining tanks and other WR control at 0.05 m/s for the "fast" treatment groups. The

experimental slow velocity was selected based on the lowest measured velocity in the Klamath River where polychaetes were documented in this substrate (Stocking and Bartholomew 2007). Although polychaetes were present in sand-silt at velocities as high as 0.15 m/s, the experimental fast flow was limited by the pump capacity (0.05 m/s). At initiation of the experiment, water velocity in the experimental channels was measured within 3 cm of the substrate level with a Marsh McBirney Flowmate 2000 (Frederick, MD) portable current velocity meter.

To test the effects of water velocity on *M. speciosa* infection, one tank of triplicate channels at each of the experimental velocities was seeded with *C. shasta* myxospores. To simulate natural myxospore introduction, a rainbow trout (average wt. 8.0 g) that had died as a result of severe ceratomyxosis was added to the head of each channel behind the eddy plate during week 3 as a source of myxospores, creating a "seeded" treatment. A non-infected rainbow trout was similarly added to the head of each of the channels of the other tanks at each velocity generating the "control" treatment. The four treatment groups will hereafter be identified as "slow control", "slow seeded", "fast control", and "fast seeded". WR controls will be identified as "WR fast" and "WR slow".

Myxospore estimation: To estimate the number of myxospores added to the system, the viscera and gills of similarly infected rainbow trout were removed from the fish. A modified centrifugation technique based on the procedure described by Bartholomew, Rohovec & Fryer (1989) was used to purify and quantify myxospores from each fish. Briefly, the tissues were mixed with 100 mL of well water and pulverized in a stomacher until they reached a slurry (about 5 minutes) to release myxospores. The

solution was filtered through 0.83 cm mesh screen to remove larger pieces of fish tissue, and rinsed with 125 mL water. This step was repeated using 0.088 cm mesh screen. The total 350 mL spore solution was centrifuged at 1500 g, 4°C for 20 minutes. Supernatant was removed and the pellet suspended in 200 mL water. This step was repeated and the final pellet suspended in 5 -10 mL of water. Myxospore concentration was estimated by four separate counts on a hemacytometer, then multiplied by the volume to obtain the total myxospores from one fish. The estimates from four individual fish were averaged to provide the myxospore contribution from one fish in the seeded tanks.

Polychaete survival: Initial polychaete densities were determined from three 30 mL samples of sediment, randomly collected prior to the distribution of sediment to the tanks. To estimate the mean polychaete density of a channel, 30 mL sub-samples were randomly taken from three locations in each channel (below the inflow, in the middle and above the outflow) at weeks 6, 11, 15, and 22, and were fixed in 95% ethanol for polychaete density determination. The preserved substrate was emptied into a Petri dish in three 10 mL increments and under a dissecting microscope; polychaetes were separated from the substrate using modified dental tools. Polychaetes were counted at 65x magnification using a dissecting microscope. Higher magnification was used as necessary to confirm polychaete identification. Each channel was considered a replicate of the treatment group and densities from the three channels were averaged to estimate the polychaete density of that treatment group.

Polychaete infection: To determine whether water velocity 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 (Stocking and Bartholomew 2007). In tthat study, the infection prevalence of *C. shasta* in *M. speciosa* from the Williamson River was estimated to be 0.45% (Stocking and Bartholomew 2007). This estimate, and the estimated prevalence (0.7%) in *M. speciosa* at the time of collection for this experiment (week 0), was used to develop the pooling scheme for the non-seeded groups. Due to the addition of myxospores, the prevalence was expected to be higher in the seeded groups and the pooling scheme was adjusted to assay more individual worms (Williams and Moffitt 2001). Table 1 illustrates the pool size, and number of pools used for comparison of infection prevalence between treatments and over time. The AusVet pooled prevalence calculator (Sergeant 2004), which uses various pool sizes (number of *M. speciosa* in a pool) and pool numbers (pools), was used to calculate the infection prevalence in *M. speciosa*.

Fish infection: To test the effect of water velocity on infection in susceptible fish, groups of either Troutlodge (Troutlodge, Sumner, WA) or Shasta strain (Sinnhuber Aquatic Research Laboratory, Oregon State University, Corvallis, OR) rainbow trout *Oncorhynchus mykiss* (Walbaum) were exposed in aquaria under the outflow of each channel. Rainbow trout averaged 7.6 cm (± 0.4) and 5.9 g (± 1.3) at exposure. Groups of 10 fish were sequentially held in the aquaria for three separate exposure periods: weeks 1-6, weeks 6-10 and weeks 11-15. After exposure, surviving fish were held for 90 days in 12.8 ºC specific pathogen free (SPF) water. Aquaria were disinfected with Ido-Sept II (Mt. Hood Chemical, Portland, OR) between exposure groups.

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, CA; average size 5.3 cm

± 0.4, 1.7 g ± 0.2) were added to the aquaria in place of rainbow trout at week 16. These fish were exposed only for one week due to high mortality caused by bacterial infections. The fish that survived this one week exposure were transferred to SPF water and replaced with a second group from the same cohort. This second group received a daily prophylactic treatment of TM-100 4% oxytetracycline medicated feed (Bio-Oregon, Longview, WA) and was exposed for 4 weeks. Moribund fish were euthanized with tricaine methanesulfonate (MS222) (Argent Laboratories, Redmound, WA).

Dead and moribund fish were examined for *C. shasta* myxospores in a wet mount of an intestinal scraping at 200x for 3 minutes (Bartholomew 2002). Intestinal tissue was collected from visually negative fish for assay by a *C. shasta*-specific polymerase chain reaction (PCR) as described by Palenzuela, Trobridge & Bartholomew (1999). Fish surviving 90 days were euthanized with MS222 and intestinal tissue samples removed and frozen until processed for PCR analysis. Fish infection prevalence was compared between treatment groups.

Statistical Analysis: All statistical analyses were performed using S Plus version 7.0 (Insightful Technologies). Square root transformations were used to provide equal variance and meet the assumptions of the statistical tests. ANOVA (with Bonferroni procedure when appropriate) tests were used to compare polychaete survival. ANOVA tests were also used to compare percent fish infection and mean day to death. Linear regression analysis was used to analyze polychaete infection prevalence trends over time.

RESULTS

Polychaete survival (Figure 3): Polychaete densities fluctuated throughout the course of the experiment. At week 0, there was a mean of 2837 *M. speciosa* per channel

(SD = 567). The only significant difference between polychaete densities at any sampling time was between the fast and slow control groups at week 15. The average polychaete density declined to less than 1500 polychaetes per channel in all treatments at week 6. Although there was a trend towards lower polychaete densities in the fast groups at this time, they were not significantly different from the slow groups. By week 10, densities in all treatments had increased, but densities in the fast channels exceeded those of the slower channels, with the fast control treatment having the highest mean density of 3465 (SD = 1574) polychaetes per channel, followed by the fast seeded treatment with a mean density of 2664 (SD = 1503). The mean density of the slow seeded group was 1987 (SD = 596) per channel at week 10, and the slow control channels had a mean density of 1297 (SD = 978). The densities of all groups, except for fast control, peaked at week 10 then declined. Densities in the fast control treatment group continued to increase to a mean 5948 (SD = 3052) polychaetes per channel at week 15 then declined. At week 22, the mean density was less than 1000 polychaetes per channel for all of the treatment groups. Due to high variability between sub-samples and channels, only the trends in fast control and slow control are significantly different from each other, with the largest difference occurring at week 15 ($P = 0.017$ linear regression). As the experiment progressed, there was a seasonal warming of river temperature that peaked (23 °C) between weeks 8 and 22 (Figures 3 and 4). However, there were no observed differences in water temperature of the tanks at fast and slow flow.

Myxospore estimate: The average myxospore estimate was 4.9×10^7 myxospores per fish (range 5.2×10^5 - 1.1×10^8).

Polychaete infection (Figure 5): The estimated infection prevalence among polychaetes at week 0 was 0.7%. Myxospores were added only to the seeded treatments, therefore the infection prevalence in the control channels throughout the experiment, and at week 0 for all treatment groups is considered to be the background infection prevalence and was estimated to be as high as 3.86%. At week 6, there was not a significant difference in infection between any treatments, although the slow seeded group had a higher prevalence than the fast seeded treatment (Figure 5, Table 1). Infection in the fast and slow seeded treatments at week 10 was 2.8% ± 2.6 (SD) and 2.7% ± 2.4 (SD), respectively, with no significant difference between either group or the controls. At week 15 mean infection prevalence in the slow seeded group reached 14% ± 8.4 (SD), 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.

Fish infection (Figure 6): Susceptible rainbow trout became infected during all exposures and mortality was high in all exposure groups and exposure periods (Figure 6). 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 for that exposure period were included in the analysis; one replicate of the fast seeded treatment was excluded during the first and third exposure periods.

All susceptible rainbow trout exposed during the first 6 weeks had fatal *C. shasta* infections, except for those in the WR groups. Only one fish held in the WR control slow tank during this period was positive by PCR but the infection was not fatal. The infection prevalence in this group (10%) was significantly lower than all of the treatment groups ($p<0.001$ one way ANOVA with Bonferroni procedure on arcsine transformed data).

In weeks 6-10, *C. shasta* associated mortality in rainbow trout exposed in the fast seeded treatment (mean 82%) was significantly less than in fish exposed in the other treatment groups during the same time (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 ($p= 0.09$). No WR exposed fish became infected after the first 6 week exposure. Only fish from the same exposure period were compared due to the variation in temperature between exposure groups.

The mean day to death of susceptible fish exposed in the slow treatments was significantly lower than those exposed to the fast velocity during all exposure periods ($p<0.001$) (Figure 6). In the first exposure period, fish exposed in the slow treatment groups died an average of 11 days earlier than the fast exposure groups. For the second and third exposure periods this difference decreased to 3 and 4 days, respectively. Differences between mean day to death of the seeded and control treatments at either velocity were not significantly different.

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

DISCUSSION

Environmental conditions are inextricably linked to a multitude of processes vital to the *C. shasta* host parasite relationship. Water velocity can affect water temperature, which affects the rate of myxospore development in the fish host (Udey *et al.* 1975). Thus, it has the potential to affect parasite concentration, extent of distribution, and residence time of the parasite in an area. Although the relationship between water velocity and salmon migration rate is complex, multiple studies report an association between decreased water velocity or water flow and increased migration times (Smith, Muir, Williams & Skalski 2002, Plumb, Perry, Adams & Rondorf 2006). Longer migration times likely increase the exposure time of fish to the parasite. In this laboratory study, we investigated the effects of water velocity on the invertebrate host population density and *C. shasta* infection prevalence in the invertebrate and fish hosts.

In the experimental channels, flow had a significant effect on polychaete density, 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. Although *M. speciosa* did survive at the slow flow, peak densities were not as high except at week six. At this time, all of the populations appear to have been experiencing a decline, perhaps as a result of acclimation to experimental conditions or handling stress after collection from the river. Thereafter, the fast flow treatment groups had higher mean polychaete densities at all time points, indicating that reproduction was occurring in these groups.

Higher *M. speciosa* densities at the faster flow is consistent with the peak densities found in sand-fine benthic organic matter at 0.05m/s in the Klamath (Stocking and Bartholomew 2007) and Ottawa rivers (Mackie and Qadri 1971). The fast flow (0.05 m/s) represents a modest flow when considering the range at which *M. speciosa* is

reported in this substrate (0.01 to 0.15 m/s). The ability of *M. speciosa* to colonize other habitats (i.e.*Cladophora* sp.) appears to facilitate their ability to survive higher velocities and to persist after high flow events (Stocking and Bartholomew 2007). The higher population density of *M. speciosa* at the fast velocity in this experiment suggests that polychaetes require sufficient flow to transport nutrients and carry away wastes without disturbing the microhabitat.

Polychaete infection prevalence was affected by water velocity, with prevalence significantly higher (average prevalence 14.4%) in the slow seeded treatment group, 12 weeks after the addition of myxospores. This finding parallels that of Hallett and Bartholomew (2008), who found a higher infection prevalence of *M. cerebralis* in *T. tubifex* when exposed to an infected fish in a slow flow compared to a ten-fold faster flow. In our study, variation in infection prevalence within treatment replicates was high, but this may be a reflection of the natural variation in the rate of myxospore dispersal from a dead fish. Although the infectious dose for a polychaete is unknown, at least 180 myxospores were available per polychaete and this resulted in a significant increase in infection prevalence under slow flow conditions.

Polychaete densities were not significantly different between the seeded and control groups of the same water velocities, indicating that infection had little effect on survival. However, the inability to monitor individual polychaetes during the course of an infection makes it difficult to interpret the effect of *C. shasta* infection on polychaete survival. Studies utilizing higher polychaete infection rates and more frequent monitoring may yield more conclusive results.

Infection in susceptible fish exposed over the course of the study was high at either velocity and at all levels of polychaete infection prevalence. The natural background infection prevalence in the *M. speciosa* collected from the Williamson River (3.86 % or lower) was sufficient to cause mortal infections in susceptible rainbow trout. At week 0, this equates to as many as 110 infected polychaetes in each channel. One infected polychaete has the potential to produce several thousand actinospores (author's personal observation), therefore even the low number of infected polychaetes in the control (non-seeded) treatments was capable of producing sufficient actinospores to overcome the low resistance of the rainbow trout. The overall high mortality (except in the controls) and low variability within treatment groups made it unlikely that exclusion of one replicate (based on low fish numbers) in the first and third exposure periods biased the analysis.

No Chinook salmon became infected by *C. shasta* during either a 1 week or 4 week exposure. Based on the polychaete density and infection prevalence at the time of exposure, it is estimated that an average of 207 infected polychaetes per channel were present in the slow seeded treatment group. The number of a actinospores released from an infected polychaete will vary based on the degree of infection, however, we have observed over 2,000 mature actinospores in a single heavily infected polychaete (author's unpublished data). When this estimate is applied to the average number of infected polychaetes, the group of Chinook salmon had a cumulative exposure of greater than 410,000 actinospores. Although this is a gross estimate, it indicates that this stock may be resistant to as high as 82,000 actinospores per fish. This level of resistance is supported by a field study in which 37% of Trinity River Chinook salmon did become

infected when exposed to at least 300,000 actinospores (J. S. Foott, personal communication). Controlled studies utilizing higher actinospore concentrations are needed to determine the infectious dose for Chinook salmon and characterize the conditions (such as water velocity and temperature) that facilitate infection.

Although there was no difference in the number of fish infected, the mean day to death was lower for rainbow trout exposed in the slow velocity treatments, indicating these fish received a higher infectious dose. In a study conducted in a natural system, E. R. Vincent, Montana Fish, Wildlife and Parks (personal communication) demonstrated an increase in intensity of parasite infection in fish at slow flows. In that study, the inverse relationship between flow and intensity of infection was attributed to a dilution effect of the infectious agent in large volumes of water, as would occur at high flow rates. Contrary to this conclusion, another *C. shasta* study reported that a three fold increase in water flow did not coincide with either a reduction in parasite DNA detection in water samples or fish infection prevalence (Foott, Stone, Wiseman, True & Nichols 2007). Hallett and Bartholomew (2008) also found evidence of increased *M. cerebralis* actinospore and fish interactions at a slower water flow. These data indicate that factors other than dilution, such as the ability of the parasite to find and interact with the fish host, may be affected by water velocity.

One additional outcome of our research was the establishment of a laboratory culture system for *M. speciosa.* Research on *M. cerebralis* progressed rapidly once populations of the alternate hosts could be sustained and the life cycle completed in the laboratory (Wolf and Markiw 1983). This was accomplished with some degree of success in this study. The difficulties in maintaining *M. speciosa* led to pilot tests of different

food and water sources, with stable populations established in sand-silt through the use of Willamette River water as a source of nutrients. Under these conditions, polychaete populations were maintained in the laboratory for up to six months and in the fast control treatment, population densities increased significantly.

Although this study was successful in completing the *C. shasta* life cycle, there were limitations in the experimental design. First, the relationships determined in this study are limited to *M. speciosa* populations in sand-silt and at relatively slow velocities when compared with the range occurring in real river systems. We limited our studies to this habitat because of the poor survival of *M. speciosa* in *Cladophora* sp. in pilot studies, likely as a result of damage to the worm when this material is scraped from rocks and boulders. Given the occurrence of *M. speciosa* populations in *Cladophora* sp. at velocities greater than 0.2 m/s (Stocking and Bartholomew 2007), the trend toward higher population densities at higher velocities as demonstrated in this experiment may be even more distinct in this substrate. Second, the experimental velocities were limited by the pump capacity, and both are relatively slow compared to velocities in which *M. speciosa* populations are reported. Third, the culture system relies on polychaete collection from wild populations, which often have a background level of *C. shasta* infection. This background level was responsible for causing lethal *C. shasta* infections in susceptible fish even when the polychaete populations were not seeded with myxospores. This natural infection prevalence prevented us from distinguishing the effect of the two velocities on fish infection prevalence.

The open design of the system was also vulnerable to changes in the Willamette River. It is unclear if factors such as temperature and sediment load influenced the

survival of *M. speciosa* or the rate of actinospore production. Although the use of Willamette River water introduced the risk of adding myxospores and actinospores to the system, there was no significant increase in *M. speciosa* infection prevalence or fish infection prevalence in any of the controls indicating that this risk was not realized. In spite of these limitations, this system maintained polychaetes at relatively high infection levels and offers many opportunities to answer questions about the *C. shasta* life cycle. For example, the peak in polychaete infection prevalence 12 weeks after the addition of an infected fish 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.

The goal of this study was to determine the effect of water velocity on *M. speciosa* populations and the *C. shasta* infectious cycle. The faster water velocity was associated with decreased *M. speciosa* infection prevalence and longer survival of infected susceptible fish. At the slower velocity, *M. speciosa* had higher infection prevalence and poorer survival. As a means to control *C. shasta*, water velocity manipulation has the potential to alter *M. speciosa* population densities, *M. speciosa* infection prevalence and fish infection severity. Water flow manipulations may influence fish residence and migration times thus further affecting the likelihood of infection. The velocities tested in this experiment were limited by the capacity of the system, allowing a comparison of only a five-fold difference in velocity. Therefore, the differences in infection and survival demonstrated in this experiment may be even more distinct at higher water velocities in the wild.

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Figures

Figure 1. Stainless steel tanks used to test fast and slow flow conditions. A) Experimental flow tanks divided into replicate channels of the treatment groups (fast seeded [FS], fast control [FC], slow seeded [SS] and slow control [SC]) containing sediment and polychaetes. B) Aquaria in the outflow of the polychaete tanks for fish exposures.

Figure 2. A treatment tank with replicate channels. The common inflow water is split and flows into a header section of each tank, then spills equally over a plexiglass divider (A) into the three replicate channels (B).

Figure 3. *Manayunkia speciosa* densities at slow (0.01 m/s) and fast (0.05 m/s) water velocities, with and without addition of *Ceratomyxa shasta* myxospores. Error bars indicate standard deviation. Asterisks indicate observations that are significantly different.

Figure 4. Willamette River temperature during the experiment obtained from USGS Daily Surface Water at Albany, Oregon (14174000).

Figure 5. *Manayunkia speciosa* infection prevalence at slow (0.01 m/s) and fast (0.05 m/s) water velocities, with and without the addition of myxospores at three weeks. Error bars indicate standard deviation. Asterisks indicate observations that are significantly different.

Figure 6. Survival curves for susceptible rainbow trout from the three exposures groups. No *Ceratomyxa shasta* associated mortality occurred in the Willamette River groups in any exposure period.

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