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Effects of *Ceratomyxa shasta* dose on a susceptible strain of rainbow trout and comparatively resistant Chinook and coho salmon

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ABSTRACT: *Ceratomyxa shasta* infects salmon and trout, causing ceratomyxosis, a disease characterized by parasite proliferation in the intestine and death. We used laboratory challenges to investigate the infective dose for 3 fish species: a susceptible strain of rainbow trout *Oncorhynchus mykiss* and comparatively resistant Chinook *O. tshawytscha* and coho salmon *O. kisutch.* For susceptible rainbow trout, we determined the outcome of infection under conditions of varying parasite dose, fish size, and parasite concentration. A single actinospore was sufficient to cause a lethal infection in susceptible rainbow trout. The mean days to death (MDD) did not significantly decrease among doses causing 100% prevalence, indicating a minimum time required for parasites to replicate to a fatal level. When dose was constant, but delivered in a higher parasite concentration, higher infection prevalence and mortality resulted. One actinospore fish^{-1} caused 57% infection and mortality in fish challenged in 0.5 l of water, whereas 10 spores fish^{-1} resulted in an average of 49% infection and mortality in 1 l challenges. This effect is most likely due to a higher encounter rate in the smaller water volume. Neither infection prevalence nor MDD was significantly different between large trout (84.9 g) and small trout (6.3 g). Chinook salmon did not become infected even when challenged with 5000 actinospores. One fatal infection occurred in coho salmon challenged with 1000 actinospores. This study confirms that even low doses of *C. shasta* cause severe infection in highly susceptible fish, describes the dose response on MDD, and demonstrates that parasite concentration influences infection prevalence.

KEY WORDS: *Ceratomyxa shasta* · Infective dose · Rainbow trout · *Manayunkia speciosa* · Fish size · Chinook salmon · Coho salmon

INTRODUCTION

The myxozoan parasite *Ceratomyxa shasta* causes ceratomyxosis in salmonid fishes, a disease characterized by hemorrhage and necrosis of the intestine. The parasite is endemic in certain rivers of the Pacific Northwest of the United States and Canada, and infections are reported from many species of wild and hatchery-reared salmonids (Sanders et al. 1970, Margolis & Evelyn 1975, Zinn et al. 1977, Ratliff 1981, Ching & Munday 1984). The severity of infection depends on a variety of factors such as water temperature (Udey et al. 1975), the inherent resistance of the fish host (Zinn et al. 1977, Ching & Munday 1984, Hemmingsen et al. 1986, Ibarra et al. 1992b, Bartholomew 1998, Bartholomew et al. 2001), and parasite dose (Ratliff 1983). The infective dose (number of parasites required to infect the host), the lethal dose (number of parasites required to kill the host), and factors influencing susceptibility (such as fish size) are not thoroughly understood.

Until recently, infecting fish with *Ceratomyxa shasta* relied on natural challenge (exposure) to the parasite. Studies using this method demonstrated a wide range

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in *C. shasta* susceptibility between salmonid species and strains but did not allow determination of parasite dose during the exposure. Zinn et al. (1977) determined that numerous salmon and trout species are susceptible to the parasite; however, susceptibility within a species is highly variable. Strains from endemic areas, such as the Fraser, Columbia, and Klamath River Basins, were shown to have higher resistance to the parasite (reduced infection prevalence and mortality) than strains from areas free of the parasite (Zinn et al. 1977, Buchanan et al. 1983, Hendrickson et al. 1989, Bartholomew 1998). However, even strains that demonstrate resistance became infected and died when the parasite challenge was sufficiently high (Ratliff 1981, Ibarra et al. 1992b, Bartholomew et al. 2004).

Despite the limitations of using natural exposures, Ratliff (1983) estimated that one *Ceratomyxa shasta* was capable of causing fatal infection in susceptible rainbow trout *Oncorhynchus mykiss* by serially diluting infectious water until mortality no longer occurred. Similarly, Johnson (1975) and Ibarra et al. (1992a) transmitted *C. shasta* to fish through intraperitoneal injection of as few as one *C. shasta* trophozoite (developmental stage in the fish). Interpretation of these studies is limited by the inability to quantify the parasite in the former and by the artificial route of infection in the latter. However, they indicate that in susceptible strains of fish, *C. shasta* is highly pathogenic and only a small infective dose, as low as one parasite, causes fatal infection.

Controlled challenges became possible with the establishment of the parasite's 2-host life cycle (Bartholomew et al. 1997) in the laboratory (Bjork & Bartholomew 2009). Infection of *Manayunkia speciosa,* the freshwater polychaete host of *Ceratomyxa shasta*, with myxospores from infected fish results in production of actinospores. The actinospores can be harvested from infected polychaetes for direct fish challenges.

Here we investigated the infective dose for susceptible rainbow trout, the relationship between parasite concentration and infective dose, the effects of fish size on infective dose, and the parasite dose required to cause infection in salmonids from the Klamath River, USA, where *Ceratomyxa shasta* is endemic. Klamath River redband rainbow trout (and anadromous steelhead) are highly resistant to infection (Bartholomew et al. 2001) and were not included in this study. In contrast, Klamath River Chinook salmon *Oncorhynchus tshawytscha* and coho salmon *O. kisutch* succumb to infection when parasite abundance is high (Foott et al. 1999, 2004, Stocking et al. 2006). Thus, determining the infective dose is critical to interpreting and predicting disease effects on these valued stocks.

MATERIALS AND METHODS

Polychaete infection and actinospore collection. A colony of *Manayunkia speciosa* is maintained at the John L. Fryer Salmon Disease Laboratory (SDL), Oregon State University, Corvallis, OR, USA. To establish the colony, polychaetes and associated sediment were collected with a plankton net from the Klamath River Basin in Oregon and California in April 2005, April and August 2006, and May 2007. Samples were placed in coolers supplied with oxygen using an airstone and transported at ambient river temperature. Polychaetes were maintained under static conditions with aeration for up to 1 wk in the laboratory prior to transfer to an aquarium (2005 only) or circular flow-through tanks. The aquarium was supplied with specific pathogen free (SPF) well water and oxygen. Flow-through tanks received untreated Willamette River water as a source of nutrients. Water temperature in these tanks was not controlled and changed seasonally.

To infect *Manayunkia speciosa,* rainbow trout intestinal tissues containing *Ceratomyxa shasta* myxospores were added to the tanks. To maintain a high *M. speciosa* infection prevalence, infected material was added as it became available; weekly to bi-weekly for several months. Additionally, the Willamette River supports the *C. shasta* life cycle; therefore, the untreated river water may have added myxospores to infect the colony as well. Twelve weeks after the first addition of myxospores, 10 ml sub-samples of sediment containing the polychaetes were removed and examined regularly until infected worms were detected. Dental hooks were used to separate polychaetes from the sediment and their tubes under a dissecting microscope at 60× magnification. Individual polychaetes were examined under a light microscope at 200× in a drop of water under a cover slip, and infection was confirmed by the visualization of actinospores.

Actinospore quantification. Actinospores were released from polychaete tissue by applying pressure to the worm on a slide with a cover slip. Mature, intact actinospores (characterized as having a tetrahedral shape and 3 unfired polar capsules) were enumerated by averaging the counts of three 5 µl aliquots. Actinospores were either used on the same day or stored at 4°C and used within 24 h of collection.

Fish challenges. Rainbow trout fry were obtained from Troutlodge (Winema, WA) and reared at the SDL on SPF well water. Troutlodge fish are not from an endemic river basin and are highly susceptible to ceratomyxosis. Klamath River strain Chinook and coho salmon were obtained from Iron Gate Hatchery (Hornbrook, CA), transported to the SDL, and reared on SPF well water until the time of challenge. *Ceratomyxa shasta* is endemic to the Klamath Basin, and these

stocks have evolved a degree of resistance. For all experiments, the actinospores were added to a plastic container of SPF well water at the appropriate experimental temperature: 13°C for rainbow trout and coho salmon, 13 and 18°C for Chinook salmon. Water baths were used to maintain temperatures in Chinook salmon challenges. Water in the container was mixed to distribute actinospores, and oxygen was supplied with an airstone. All challenges were for 2 h, after which fish were transferred to 25 l flow-through tanks supplied with SPF well water at the appropriate experimental temperature. A sham exposure served as a negative control for each of the challenges.

To determine the infective dose and how factors such as actinospore concentration and fish size affect infection prevalence and severity, rainbow trout were challenged in 3 infection experiments. The infective dose for Chinook and coho salmon was tested in 2 additional separate trials. The availability of actinospores was unpredictable; therefore, experiments could not be conducted simultaneously and are described below.

Expt 1: To determine the infective dose for susceptible rainbow trout, 3 replicate groups of 10 fish (mean ± SD: 0.6 ± 0.3 g) were challenged with 1, 5, 10, 20, 40, or 100 actinospores fish^{-1} in 0.5 l at 13°C.

Expt 2: The relationship between parasite concentration (equal parasite doses in different exposure volumes) and infective dose was tested by challenging 3 groups of 10 rainbow trout fry (mean 0.22 ± 0.05 g) with 1, 5, or 10 actinospores fish^{-1} in 1 l at 13°C. These doses were chosen to provide a comparison to the 0.5 l challenges in Expt 1. In a second trial, 10 fish (mean 6 ± 2.5 g) were challenged individually with 20 actinospores at 2 concentrations; 5 fish were challenged in a volume of 0.5 l (equivalent to 40 spores l^{-1}) and 5 fish in 3 l (equivalent to 6.7 spores l^{-1}).

Expt 3: To determine the effect of fish size on infective dose, 5 rainbow trout averaging 85 ± 20.5 g ('large') were individually exposed to 20 actinospores in 3 l at 13°C. This was run in parallel with the second challenge of Expt 2 ('small' fish) providing simultaneous comparison of 'large' and 'small' fish to 6.7 spores l^{-1}.

Expt 4: Chinook salmon were challenged to a range of doses and at 2 temperatures for the 2 highest doses. Groups of 5 juvenile salmon (average 1.7 ± 0.22 g) were each challenged to 10, 50, 100, or 500 spores fish^{-1} in 1 l at 13°C. Challenges were conducted in triplicate except for the 500 spore group, which was done in duplicate. To test the effect of temperature on the dose response, individual salmon (average 2.3 ± 0.25 g) were challenged to 1000 or 5000 actinospores in 1 l at 13 and 18°C. In total, 10 fish were individually challenged to each of the experimental doses at each of the experimental temperatures. Fish were then reared at the experimental temperature for the duration of the experiment.

Expt 5: Based on results of the Chinook salmon challenge, coho salmon (mean 5.6 ± 1.8 g) were challenged only at the higher parasite doses in 1 l at 13°C. Ten fish were individually challenged to 500 and 1000 actinospores, and 5 fish were challenged with 5000 actinospores. To verify the infectivity of the actinospores used in Expts 4 and 5, 5 susceptible rainbow trout were exposed to 20 actinospores fish^{-1} in 1 l in parallel to Chinook and coho challenges.

For all challenges: Fish were fed a commercial diet (Bio-Oregon) and monitored for signs of infection and mortality for 90 d after exposure for rainbow trout and 60 d for Chinook and coho salmon. Moribund fish, defined as fish showing clinical signs and unable to maintain equilibrium, were euthanized with an overdose of tricaine methanesulfonate (MS-222, Argent Laboratories) and were recorded as mortalities. A wet mount of a scraping of the intestine was examined for the presence of myxospores for 2 min (Bartholomew 2003). When myxospores were found, the fish was considered infected. If no myxospores were detected, a sample of posterior intestinal tissue was collected and frozen for assay by polymerase chain reaction (PCR; Palenzuela et al. 1999). All fish surviving the experimental period were euthanized, and intestinal tissue was collected and assayed by PCR.

Statistical analysis. S Plus version 7.0 statistical software was used (Insightful Technologies). One-way analysis of variance (ANOVA) with Bonferroni procedure (Expts 1 and 2), *t*-tests (Expt 2), and linear regression (comparison between Expts 1 and 2) were used to determine significant differences between infection prevalence and mean days to death (MDD) between the treatments.

RESULTS

Infectious dose for susceptible rainbow trout

The mean infection prevalence (±SD) in groups challenged in 0.5 l with 1 spore was 57 ± 5.8%; at all other doses, 100 ± 0% of the fish were infected (Fig. 1). In the sham exposure, all fish survived to the end of the experiment and all fish were negative by PCR. One fish from one of the replicates of the 10-spore dose died within the first week after challenge and was not included in the analysis.

The MDD of rainbow trout challenged in 0.5 l decreased as the dose increased (Fig. 1). There was a significant difference between the MDD of fish challenged with 1 spore and all other doses (1-way ANOVA with Bonferroni procedure on all Expt 1 chal-

lenges, $p < 0.01$). There was no significant difference in MDD between the 5- and 10-spore doses, although the MDD of fish challenged with 5 spores was significantly different from fish challenged with the other doses. Significant differences also existed between MDD of fish challenged with 10 and 100 spores. Fish that survived to the end of the 90 d experiment (all from the 1-spore dose) were all negative by PCR.

Fish challenged in 1 l with 1, 5, or 10 actinospores $fish^{-1}$ also showed increasing infection prevalence as the actinospore dose increased (Fig. 2). Fatal infections occurred in all treatment groups, except for fish challenged with 1 actinospore, where a single fish was infected (positive by PCR) but survived to the end of the experiment. Although the mean prevalence of infection was higher as dose increased, the difference was not significant among fish challenged in 1 l (1-way ANOVA, $p = 0.06$). There was no significant difference between the MDD of fish challenged in 1 l (*t*-test of 5- and 10-spore doses, $p = 0.22$).

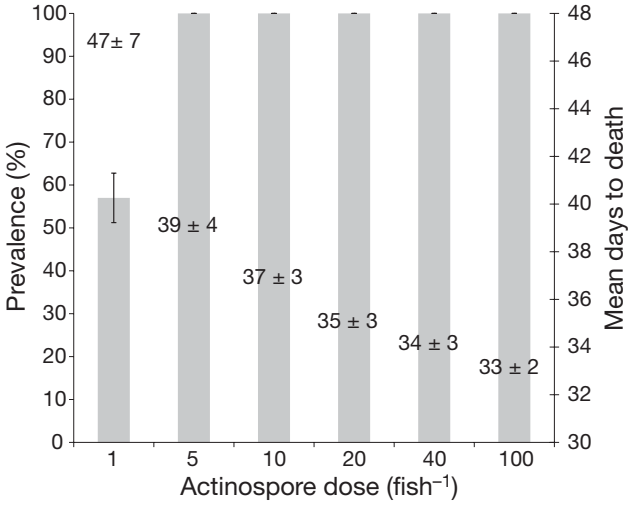

Fig. 1. *Ceratomyxa shasta* infecting *Oncorhynchus mykiss.* Infection prevalence (mean ± SD of 3 replicates), mortality, and mean days to death of susceptible rainbow trout after challenge to a range of *C. shasta* actinospore doses (1 to 100) in 0.5 l

Relationship between parasite concentration and dose

The mean infection prevalence among fish challenged in 0.5 l was significantly higher than the 1 l challenge at equivalent doses (linear regression with interaction between volume and dose, $p < 0.01$). When fish were challenged in 0.5 l, the mean infection prevalence in groups challenged with 1, 5, and 10 spores $fish^{-1}$ was 57 ± 5.8, 100 ± 0, and 100 ± 0 %, respectively. When the same parasite dose was administered in a challenge volume of 1 l, infection prevalence was 3 ± 5.8, 20 ± 17.3, and 49 ± 24.4 %, respectively. At each of the doses, the fish challenged in 0.5 l had a lower MDD than fish challenged in 1 l at the same dose (Fig. 2).

In the second challenge of Expt 2, the infection prevalence of fish challenged with 20 actinospores in 0.5 l (concentration: 40 spores l^{-1}) was 100 % with 80 % mortality, whereas the infection prevalence of fish challenged at the same dose in 3 l (concentration: 6.7 spores l^{-1}) was 60 % with 40 % mortality (Fig. 3). In 0.5 l, 4 of the 5 fish died, 1 infected fish survived, and

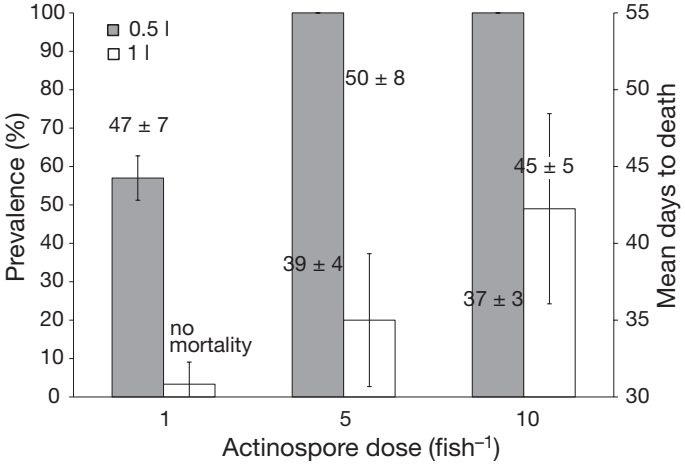

Fig. 2. *Ceratomyxa shasta* infecting *Oncorhynchus mykiss.* Infection prevalence (mean ± SD of 3 replicates) and mean days to death of rainbow trout challenged in 1, 5, or 10 *C. shasta* actinospores in 0.5 or 1 l of water

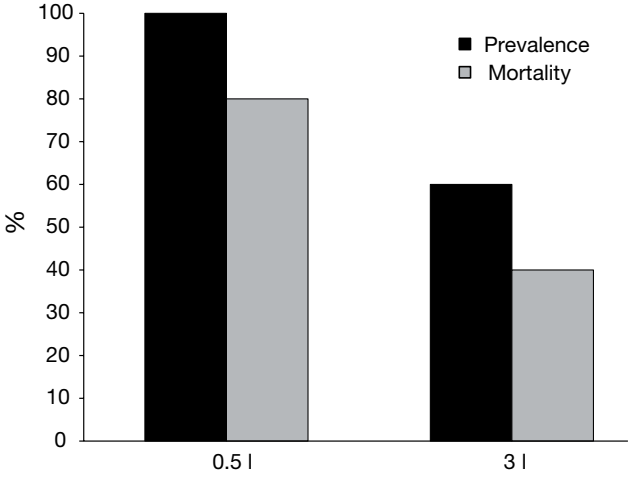

Fig. 3. *Ceratomyxa shasta* infecting *Oncorhynchus mykiss.* Infection prevalence and mortality of susceptible rainbow trout challenged with 20 *C. shasta* actinospores in either 0.5 (40 actinospores l^{-1}) or 3 l (6.7 actinospores l^{-1}) for 2 h

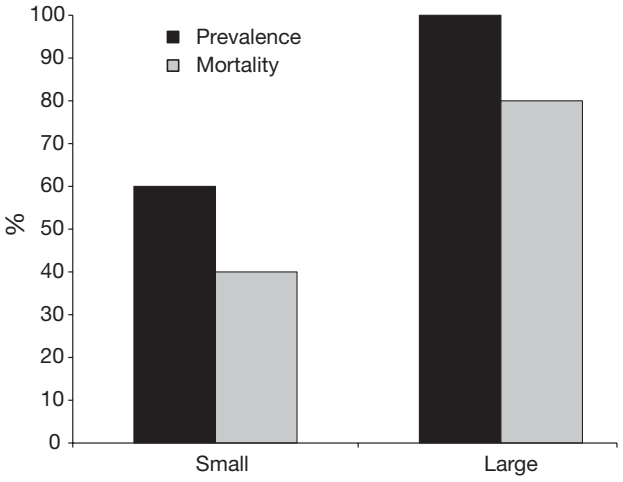

Fig. 4. *Ceratomyxa shasta* infecting *Oncorhynchus mykiss.* Infection prevalence and mortality of susceptible rainbow trout challenged at 2 sizes, small (6 ± 2.5 g) and large (85 ± 20.5 g), with 20 *C. shasta* actinospores in 3 l (equivalent to 6.7 actinospores l^{-1})

the MDD was 63 ± 13. Only 2 fish had fatal infections in the 3 l challenge, 1 infected fish survived, and the MDD was 59 ± 6. As there is only one data set for each of the 20 actinospore challenge groups from Expt 2, no statistical analysis was done.

Relation between fish size and parasite dose

The prevalence of *Ceratomyxa shasta* in 'large' fish challenged with 20 actinospores in 3 l (Expt 3) was 100% with 80% mortality, and the MDD was 63. The prevalence of *C. shasta* in 'small' fish challenged with 20 actinospores in 3 l (Expt 2) was 60% with 40% mortality, and the MDD was 59 (Fig. 4). One additional fish from the survivors of each challenge was positive for *C. shasta* DNA by PCR. No statistical analysis was done on this data set because fish were challenged individually.

Infectious dose for Chinook and coho salmon

No Chinook salmon became infected at any of the doses or temperatures tested. There were no fatalities, none showed signs of clinical infection, and none assayed positive by PCR. Rainbow trout that were exposed in parallel became infected in each of the exposure trials.

A fatal infection occurred in 1 coho salmon challenged with 1000 actinospores $fish^{-1}$. This fish produced myxospores that were visualized in a wet mount. However, no coho salmon had fatal infections at the higher 5000 actinospore dose, and none assayed positive by PCR at this dose.

DISCUSSION

Control over *Ceratomyxa shasta* actinospore dose, concentration, and fish size in the laboratory facilitated the determination of the infective dose for susceptible rainbow trout. These fish developed fatal infections when challenged with as few as 1 *C. shasta* actinospore $fish^{-1}$. Thus, 1 actinospore is both an infective and lethal dose for this rainbow trout strain. This study confirms the findings of Ratliff (1983), who was unable to quantify actinospores in natural challenges, but nonetheless estimated that 1 *C. shasta* was capable of causing fatal infection in a susceptible strain of rainbow trout.

However, susceptibility to infection varies within a species, and rainbow trout from endemic rivers such as the Pit (CA), Skamania (OR), Deschutes (OR), and Klamath River (OR-CA), are more resistant (Buchanan et al. 1983, Ibarra et al. 1991, 1992b, 1994, Bartholomew et al. 2001) and are likely to require a high infective dose. Further evidence for resistance of Klamath River rainbow trout is from 3 d exposures in the Klamath River where native Chinook (Hallett & Bartholomew 2006, Stocking et al. 2006) and coho salmon suffered mortality, yet simultaneously exposed native redband trout and steelhead remained uninfected (authors' unpubl. data).

Due to the low infective and lethal dose for susceptible rainbow trout, the effects of increasing dose could only be observed at very low doses. Infection prevalence was approximately 50% when exposed to 1 spore and increased to 100% when challenged with ≥5 actinospores (in 0.5 l challenge). The maximum infection prevalence and mortality were achieved at the second to lowest dose (5 spores), which prevents these parameters from accurately describing a dose response. Therefore, the MDD is used as a measure of infection severity, based on the assumption that fish with more severe infections die earlier. In this study, the MDD decreased when dose was increased from 1 to 10 to 100 actinospores. However, there were no significant differences between the MDD at doses greater than 20 actinospores. Therefore, once a fatal dose is acquired, additional parasites do not increase infection severity in terms of MDD. In this study, parasite severity is described only by MDD; histopathology (Bartholomew et al. 2004) and estimating myxospore abundance in affected fish could further clarify the effect of dose on the progress of infection.

Parasite concentration significantly affected infection prevalence. Fatal infections occurred in 100% of

the fish challenged with 5 actinospores in 0.5 l water, but in only 20% of the fish challenged in twice this volume. This effect was also evident at a higher infectious dose, although the differences were not as marked. One explanation for this difference is that fish have fewer encounters with the parasite in larger water volumes than in smaller volumes. Fish were exposed to the parasite for only 2 h in this study, and an increase in exposure time may also have resulted in an increase in parasite encounter and subsequently higher infection prevalence. However, a longer exposure would be more indicative of a static system than a flowing river. Interestingly, when larger fish were exposed to 20 actinospores in 3 l, infection prevalence and mortality mirrored that of smaller fish in the 0.5 l challenge. In this case, the larger fish occupied a higher proportion of the water and were therefore more likely to encounter the parasites.

In natural systems, predicting the effects of dilution on parasite prevalence is much more complex. Factors such as continuous release of actinospores from polychaetes in the system, fluctuating water flow rates, and the ability of the parasite to attach to the host under those conditions must be considered. Several studies have noted a decrease in myxozoan parasite infection prevalence or severity in faster water flows (Vincent 2002, Hallett & Bartholomew 2008, Bjork & Bartholomew 2009). Conversely, Foott et al. (2007a) reported no decline in fish infection prevalence or parasite concentration in water samples after a 3-fold increase in river flow. In the river, increased flow may stimulate actinospores to be released from their polychaete host or increase the rate by which actinospores are transported from an infectious area upstream. Both of these effects may add more actinospores to the river per unit time. In this study, we demonstrated that dilution of the parasite results in decreased infection prevalence; however, parasite dilution in a natural setting is highly dependent on the presence and proximity of an infected polychaete population.

For susceptible rainbow trout, fish size did not affect infective dose. When fish were challenged in a volume proportional to their size (small fish in 0.5 l and large fish in 3 l), infection prevalence and mortality were equal. However, when large and small fish were challenged at the same spore dose in the same volume, the effect of parasite concentration was evident. This age-independent mortality contrasts with the relationship between age and infective dose for another myxozoan parasite, *Myxobolus cerebralis*, in which younger and hence smaller fish are more susceptible to disease (Markiw 1991, Sollid et al. 2003, Ryce et al. 2004, 2005). *M. cerebralis* proliferates in the cartilage of its fish host, and as fry mature and cartilage ossifies into bone, the amount of target tissue decreases. The intestine is the target tissue of *Ceratomyxa shasta*; therefore, it is not surprising that fish size did not affect infection prevalence.

It is interesting that there were no obvious effects of fish size on MDD. Large fish have more intestinal tissue than small fish, which could influence infection severity and the rate of disease. The infection dynamics of *Ceratomyxa shasta*, specifically the location and reproductive rate, would determine whether a greater amount of target tissue would affect infection severity. If parasite proliferation is finite, for example 1 actinospore produces 1000 myxospores, a larger intestine would result in a smaller percentage of the tissue infected compared to smaller fish. If parasite proliferation is unlimited, a larger intestine would provide more host tissue to infect, and parasite proliferation in a larger intestine could exceed that of a smaller one. The latter explanation appears to fit the data from this study. It is also possible that the high susceptibility of the rainbow trout strain used in this study may have masked the relationship between size and infection severity that may be more evident in a less susceptible fish strain.

Studies on the relationship between fish size and susceptibility to infection by other myxozoan parasites are more difficult to interpret because they were not conducted under controlled conditions. An increase in infection prevalence and parasite abundance with increasing fish size was reported for *Enteromyxum scophthalmi* (Quiroga et al. 2006) but not *Kudoa* spp. (Shaw et al. 1997, Wang et al. 2005). Fish in those studies were collected from natural populations or from aquaculture centers, where older, larger fish are likely to have had longer exposure to the parasite in comparison to younger, smaller fish. This increased exposure time presents a size-independent explanation for differences in prevalence and infection intensity. Additionally, these studies are confounded by the exclusion of smaller fish that were infected and died prior to data collection. In our study, there were no differences in infection prevalence between large and small fish; therefore, in susceptible rainbow trout at least, these factors do not appear to play a significant role.

We were unable to determine the infective dose of *Ceratomyxa shasta* for Klamath River Chinook and coho salmon. In contrast to a lethal dose of a single parasite for susceptible rainbow trout, the inability to infect Klamath River salmon with 5000 parasites highlights the selective pressure this parasite has played in rivers where it is endemic. The infection of a single coho salmon at a dose of 1000 actinospores suggests that this fish may have been compromised or represented individual variation in parasite resistance.

Although we were unable to surpass the threshold for infection of Klamath River salmon in the laboratory,

our challenges do corroborate estimates of parasite abundance in the Klamath River. Using molecular methods to measure parasite abundance, Hallett & Bartholomew (2006) detected >20 spores l^{-1} of water at some sites. A concurrent 3 d exposure of Klamath Chinook salmon resulted in 68.6% infection prevalence and 48.6% mortality (Stocking et al. 2006). This equates to fish being exposed to >1 million spores in a day when water flow (USGS National Water Information System: Web Interface. http://waterdata.usgs.gov; Klamath River, Orleans site no. 11523000) is used to extrapolate the dose. Foott et al. (2007b), measured water flow and parasite concentrations, and estimated that in 6 h, nearly 360 000 spores infected 46% of the Klamath Chinook salmon challenged.

The ability of a single parasite to cause mortality in a susceptible rainbow trout suggests that *Ceratomyxa shasta* is a highly efficient pathogen. Bartholomew et al. (1997) described the actinospore as having 1 sporoplasm, indicating the presence of 1 infective germ cell. In contrast, *Myxobolus cerebralis* has an infective dose (resulting in clinical signs) of 10 actinospores and a lethal dose of 100 parasites in susceptible rainbow trout (Ryce et al. 2004). *M. cerebralis* has 64 germ cells in each triactinomyxon (El-Matbouli & Hoffmann 1998), thus delivering 64 times the infectious material of a *C. shasta* actinospore. *Tetracapsuloides bryosalmonae*, like *C. shasta*, has a small number of infectious sporoplasms, and just 1 parasite can cause fatal infections in non-native rainbow trout (McGurk et al. 2006). Thus, the high pathogenicity of *T. bryosalmonae* and *C. shasta* may compensate for the low number of infective germ cells. This high pathogenicity explains the role of *C. shasta* as a selection factor, driving the evolution of resistance in native stocks.

Although this study is the first to challenge fish to a range of *Ceratomyxa shasta* actinospore doses, there were some limitations. Due to the asynchronous development of actinospores in *Manayunkia speciosa*, the number of mature actinospores available to perform infection experiments could not be determined until worms were sorted and observed. Actinospore collection methods could also be improved, as mechanical collection of actinospores from infected hosts is both time consuming and labor intensive. However, the small size of the *C. shasta* actinospore and the inability to distinguish it from other particles complicates actinospore collection by the filtration methods that have been used successfully for *Myxobolus cerebralis.* Both of these factors prevented the simultaneous challenge of all fish at all doses and is the reason for the staggered experiments. Another limitation was the inability to obtain sufficient actinospores to infect Chinook and coho salmon. The number of actinospores collected in this study is small in comparison to the thousands (4.6×10^4) of *M. cerebralis* actinospores collected from a single *Tubifex tubifex* (Gilbert & Granath 2001). Because of the small size of *M. speciosa* (approximately $^1/_{10}$ that of *T. tubifex*) and the location of parasite development in the dermis of the polychaete (Bartholomew et al. 1997), there is inherently less area for actinospores to develop and thus fewer spores are produced per worm.

Despite these limitations, we developed a *Ceratomyxa shasta* infection model for fish in the laboratory that will be critical for examining the confounding factors that exist during natural exposures. For *Myxobolus cerebralis,* one of the most studied myxozoans, the laboratory infection model facilitated investigation of a multitude of host parasite interactions including the route of infection (El-Matbouli et al. 1995), fish response to various doses (Markiw 1992, Ryce et al. 2004), and host specific responses to the parasite (Baerwald et al. 2008). In the *C. shasta* host–parasite relationship, these factors have not been thoroughly investigated, but with a laboratory infection model in place (at least for rainbow trout) they may be addressed in the future.

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