AN ABSTRACT OF THE DISSERTATION OF

Robert Adam Ray for the degree of Doctor of Philosophy in Fisheries Science presented on September 26th, 2013.
Title: Modeling Abiotic Influences on Disease Dynamics for the Complex Life Cycle of the Myxozoan Parasite *Ceratomyxa shasta*.

Abstract approved:_____
Jerri L. Bartholomew

Most parasites and their hosts live in a balance within their environment; however a disease outbreak can occur when either the parasite, host, or environment, are perturbed. Myxozoan parasites are associated with a wide variety of cultured and wild fish populations. Most myxozoans are relatively benign to their vertebrate host; however some cause dramatic population level effects on both cultured and wild fish populations. These parasites have a complex life cycle involving a vertebrate host (fish), an invertebrate host (annelid), and two spore stages (actinospore and myxospore). Interactions between these parasites and their hosts can be strongly influenced by environmental factors, most notably by water temperature and water velocity. Given the complex life cycle of myxozoan parasites and the lack of any chemical treatments or preventatives, controlling infections and disease caused by these parasites is challenging, especially for wild populations.

The myxozoan *Ceratomyxa shasta* is endemic to many of the major rivers of the Pacific Northwest and infects all species of Pacific salmon. In the Klamath River, CA, USA, *C. shasta* infection is associated with decreased returns of adult Chinook salmon

(*Oncorhynchus tshawytscha*). The goals of this dissertation were to 1) quantify the effect that elevated water temperature has on *C. shasta*-induced disease severity and mortality rate for both Chinook and coho (*O. kitsch*) salmon, 2) identify transmission patterns and quantify transmission rates of the actinospore stage to the salmon host, 3) develop an epidemiological model of this host-parasite life cycle and assess the sensitivity of specific parameters that may act as suitable management strategies, and 4) utilize a mixture cure model, an alternative survival analysis method, to quantify the effects water temperature and discharge on the total and rate of *C. shasta*-induced mortality of both Chinook and coho salmon.

I found that, similar to disease progression naïve salmon species (i.e. from waters where *C. shasta* is absent), elevated water temperature increases the rate and overall mortality for salmon species from river systems where the parasite is endemic. Elevated water temperatures also increase the transmission rate of the actinospore stage to the salmon host. The transmission rate of the actinospore stage to the salmon host was inversely related to water velocity, and I identified a potential velocity threshold of ~0.3m/sec, above which transmission was greatly reduced. From the epidemiological model I sensitivity analyses and identified that reduction of the myxospore transmission rate from the adult salmon to the polychaete host during the winter may be the most effective management action to reduce *C. shasta*-related disease in the Klamath River. This action could potentially be achieved by increasing discharge during the winter to minimize contact between the polychaete host and myxospore stage. Lastly, I applied the mixture cure models to quantify how the daily survival rates of Chinook and coho salmon

change over time after the fish become infected with *C. shasta.* Although varied in approach, the output from both of the models presented in this dissertation can be used to guide management and conservation actions for fish populations affected by myxozoan parasites.

Modeling Abiotic Influences on Disease Dynamics for the Complex Life Cycle of the Myxozoan Parasite *Ceratomyxa shasta*.

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Robert Adam Ray, Author

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CONTRIBUTION OF AUTHORS

Dr. Jerri Bartholomew served as the major advisor and was involved in all aspects of the research presented in this dissertation. Dr. Richard A. Holt assisted with experimental design, field and laboratory data collection, and provided editorial feedback of Chapter 2. Dr. Nicholas A. Som and Dr. Russell W. Perry assisted with model development, analysis, and writing of Chapter 5.

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CHAPTER 1: INTRODUCTION

DISEASE ECOLOGY: PARASITES, HOSTS, AND ENVIRONMENT

> "Disease is any impairment that interferes with or modifies the performance of normal functions, including responses to environmental factors such as toxicants and climate, nutrition, infectious agents; inherent or congenital defects, or any combination of these factors."
> (Wobeser, 1981)

Most host-parasites live in a commensal relationship that allows for the long-term survivorship of both organisms. This relationship is generally achieved within a delicate and complex balance between the host, parasite and environment. Disease occurs when a shift occurs in the ecology of the host, the parasite, or the environment (Snieszko 1974; Hedrick 1998; Daszak, Cunningham, & Hyatt 2000; Dobson & Foufopoulos 2000; Johnson & Paull 2011; Okamura & Feist 2011). Over the last 20 years, the number and severity of disease outbreaks in wildlife populations (epizootics) has increased across a wide range of taxa (Harvell *et al.* 2002). Although not as intensely monitored or reported on as wildlife or marine environments; diseases in freshwater environments are receiving increased attention (Johnson & Paull 2011).

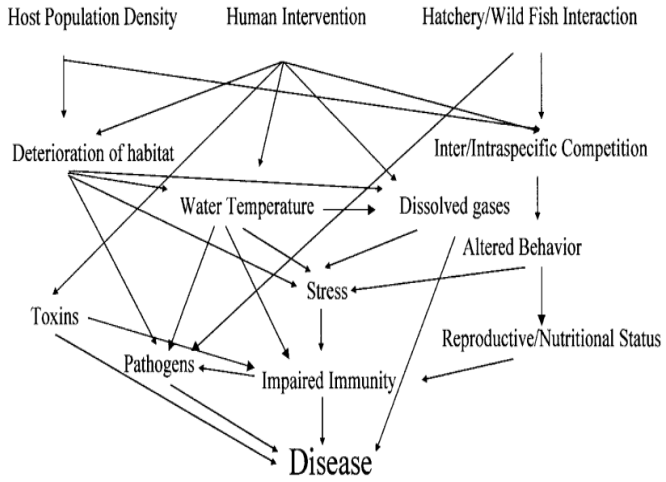

Fig. 1.1 Web of causation that identifies many of the environmental factors that when perturbed, can lead to disease in aquatic systems (Hedrick 1998).

Hedrick (1998) adapted a web of causation to highlight the complex interactions between

the host, parasite and environment that can lead to disease in the aquatic environment (Fig. 1.1). In aquatic host-parasite systems, the host is typically poikilothermic and many parasites have spore stages, each of which can be influenced by changes in the surrounding environment (Magnuson *et al.* 1997; Marcogliese 2001).

Temperature is the most influential abiotic factor and affects both the aquatic host and the parasite's spore stages (Pietrock & Marcogliese 2003). Increases in temperature are associated with increased growth, development, and maturation of poikilothermic hosts, but can also increase stress, hinder immune function, and alter behavior, thereby potentially increasing the disease risk from parasite infection (Magnuson *et al.* 1997; Richter & Kolmes 2005; Marcogliese 2008). For instance, in juvenile coho salmon (*Oncorhynchus kitsch*) a higher incidence of infection with trematodes (*Neascus sp.*) was observed as water temperatures increased (Cairns *et al.* 2005). For the parasite spore stages, elevated water temperatures may allow for earlier development and release from the invertebrate host, resulting in a longer exposure period, and in some instances year-round transmission (reviewed by Marcogliese 2001). Elevated water temperature can also promote faster replication rates and greater virulence of the parasite (Marcogliese 2001). Poulin (2006) observed for many trematode species that as water temperature increased so did the overall production of the cecariae stages. In contrast, there is an inverse relationship between water temperature and the longevity and survival of the spore stages (Pietrock & Marcogliese 2003). Although the longevity of a parasite increases with decreasing water temperature, the virulence and infectivity of the parasite are generally decreased (Pietrock & Marcogliese 2003). Water temperatures can

positively or negatively affect the infection dynamics between hosts and parasites, but regardless of the host-parasite system there is typically an optimal temperature at which the severity of infection greatly increases.

Discharge is an environmental factor comprised of two properties (water velocity and volume) that affects the transmission dynamics between aquatic hosts and parasites. Increased water velocity can result in higher exposure doses, but also decrease the probability of successful transmission. The prevalence and abundance of two different eel (*Anguilla rostrata*) parasites (*Pseudodactylogyrus anguillae* (Monogenea) and *Ergasilus celestas* (Copepoda)) were negatively correlated with stream flow (Barker & Cone 2000). In cultured channel catfish (*Ictalurus punctatus*) populations, increasing water flows proved to be a valuable management tool to lower mortality from *Ichthyophthirius multifiliis* (Bodensteiner *et al.* 2000). While no specific mechanism was identified, the authors hypothesized that the increased discharge diluted the infective stages of the parasite below a threshold where it could become established. Lowered water levels and discharge resulted in higher host and parasite densities, thus increasing the probability, intensity, and severity of infection (Izyumova 1987; Holmes 1996). The stream flow in the Platte River, NE, USA, fluctuates substantially throughout the year and even within months. Over a five year observation period in this river system, Janovy Jr & Hardin (1988) observed a negative relationship among different measures of parasite density and diversity (e.g. Shannon's H diversity index, infra assemblage diversity and species density) and decreased water levels. Habitat for invertebrates (such as snails and

annelids), many of which act as an intermediate host for aquatic parasites, increased as water levels decreased (Marcogliese 2001).

MYXOZOAN PARASITES

Myxozoans are metazoan spore-forming parasites primarily of marine and freshwater fish, but also infect amphibians, reptiles, birds, and mammals (Kent *et al.* 2001; Canning & Okamura 2003; Yokoyama, Grabner, & Shirakashi 2012). Although most myxozoans are not problematic to their vertebrate host, there are a few species that cause disease in cultured and wild fish populations. For example, *Myxobolus cerebralis* and *Tetracapsuloides bryosalmonae* have decimated trout populations around the world and *Henneguya ictaluri* is recognized as one of the most problematic pathogens in channel catfish aquaculture (Hedrick 1998; Pote, Hanson, & Shivaji 2000; Griffin *et al.* 2008; Okamura *et al.* 2010). The Myxozoa is comprised of over 2100 species; however,

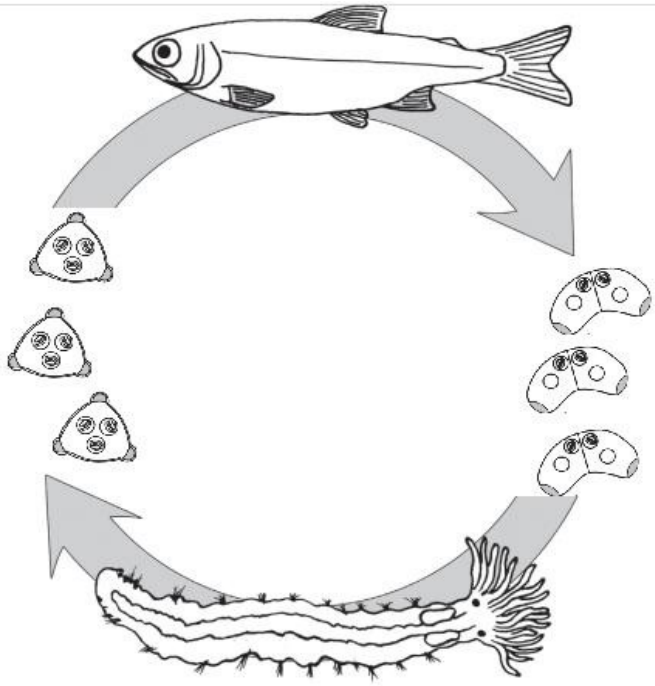

Fig. 1.2 Typical life cycle of a myxozoan parasite involving a vertebrate (fish) host, an invertebrate (annelid) host, an actinospore stage (left), and a myxospore stage (right). This is the life cycle of *Ceratomyxa shasta*, a parasite that infects several species of salmon in the Pacific Northwest of North America.

the complete life cycle is only known for ~25 species (Kent *et al.* 2001; Canning & Okamura 2003; Yokoyama *et al.* 2012).

The known life cycles demonstrate a consistent pattern of alternating between a vertebrate (fish) and invertebrate (annelid) host and two unique spore stages, an actinospore and myxospore (Fig 1.2). The myxospore stage is released from the vertebrate host and is ingested by the invertebrate host. The actinospore stage is released from the invertebrate host and infects the vertebrate host. Both spore stages have developed unique adaptations to increase the likelihood of successful transmission. Actinosporean stages typically have long processes that allow them to remain buoyant in the water column for long periods of time (Kerans & Zale 2002) to increase the probability of contacting a fish host. Myxosporean stages typically do not have elongated processes and therefore are unable to remain buoyant like the actinsporean stage; this morphological difference likely increases the probability of contacting a benthic invertebrate host.

Environmental influences on myxozoan infection

The transmission and infection dynamics between these parasites and their hosts are influenced by the environment, and it is important to understand how all three interact for effective management and control (Yokoyama *et al.* 2012). Water temperature influences the severity of infection and disease within both hosts and also the longevity and infectivity of the parasite stages. Discharge and water velocity are additional abiotic variables that can alter the transmission dynamics of the spores to their respective hosts.

Myxobolus cerebralis, described as the most notorious myxozoan species (Lom & Hoffman 1971), is also the most studied myxozoan parasite. Much of the understanding of how infection, transmission, and disease are influenced by environmental factors comes from studies conducted on *M. cerebralis*. I will focus on these studies to illustrate the environmental effects; however, when possible I will include examples from research on other myxozoans.

Water temperature affects the infection dynamics of *M. cerebralis* in both hosts, with increasing water temperature correlated with higher infection prevalence and greater disease severity in the salmon host (Halliday 1973; Baldwin *et al. 2000*). For *T. bryosalmonae* infections and disease in the salmon host were seasonally dependent and increased when water temperature was 12–18°C; however decreasing temperature was observed to suppress the effects of the disease (Clifton-Hadley, Richards, & Bucke 1986; Foott & Hedrick 1987; Hedrick, MacConnell, & De Kinkelin 1993; Okamura *et al.* 2010). Similarly, prevalence of channel catfish infected with *H. ictaluri* increased during the spring and fall when temperatures were 16–25°C (Griffin *et al.* 2008).

Water temperature influences the production and release of the actinospore stage from the invertebrate host. In the invertebrate host (*Tubifex tubifex*), *M. cerebralis* was observed from 5–15°C, but no parasites were observed in worms held at 25°C (El-Matbouli *et al.* 1999). Blazer *et al.* (2003) observed increased actinospore production from infected *T. tubifex* as water temperatures increased from 9–17°C, but again did not observe any production at 20°C. These findings suggest there was an upper thermal limit of either the parasite or the invertebrate host that prohibited completion of the life cycle.

In the bryozoan host, no upper thermal limit was observed for *T. bryosalmonae*, as the parasite increased production of overt infective stages at elevated water temperatures up to 20°C (Tops, Lockwood, & Okamura 2006; Okamura *et al.* 2010). The longevity and infectivity of the spore stages were highly variable among different species, but there was a consistent negative relationship of longevity and infectivity with increasing water temperature. In a comparison of five different species among different temperatures, actinospore viability ranged from seven days at 4°C to four days at 22°C (Ozer, Wootten, & Shinn 2002). For *M. cerebralis* the actinospore stage persisted for up to 8 days at 7°C, but viability and infectivity decreased at higher water temperatures, spores only survived 1 day at 23°C (Markiw 1992; Kallert & El-Matbouli 2008). For most myxozoan parasites the myxospore stage has hardened valves surrounding the sporoplasm making it more environmentally stable than the actinospore stage (Hoffman 1990; Hedrick *et al.* 2008), but temperature also affects this life stage. The myxospore stage of *M. cerebralis* was viable for more than 2 months at water temperatures < 10°C, but remained viable for only ~7days at 22°C (Hedrick *et al.* 2008)

Discharge and water velocity, in contrast to water temperature, influence the transmission dynamics between the free-living spore stages and their respective hosts. Compared to temperature, very little data on the effects of water velocity on transmission dynamics of myxozoan parasites are available. However, among the few relevant studies there was a consistent pattern between increased velocity and decreased transmission. Swearer & Robertson (1999) observed a lower prevalence of infection of *Kudoa ovivora* in reef fish populations in areas with high current flow. A similar trend was observed for

M. cerebralis with respect to both hosts in a controlled laboratory study. The prevalence of infection was higher in both the salmonid and the oligochaete host reared in lower flow tanks than those reared in higher flow, indicating that transmission of the spore stages increased with decreases in flow (Hallett & Bartholomew 2008).

CERATOMYXA SHASTA

Ceratomyxa shasta is a myxozoan parasite endemic to many of the major rivers throughout the Pacific Northwest and infects Pacific salmon species (Ratliff 1981; Ching & Munday 1984; Hoffmaster *et al.* 1988; Hendrickson, Carleton, & Manzer 1989; Bartholomew *et al.* 1997; Stinson 2012). The life cycle of *C. shasta* is typical for myxozoan parasites as it alternates between an invertebrate polychaete host (*Manyunkia speciosa*) and a salmon host (Fig. 1.2, Bartholomew *et al.* 1997). The actinospore stage is shed from the polychaete into the water column through mucous pores and transmitted to the salmon host. The actinospore stage fires a polar filament into the gill epithelial cells and sporoplasm migrates into the circulatory system of the salmon (Meaders & Hendrickson 2009; Bjork & Bartholomew 2010). In the salmon host, the parasite migrates through the circulatory system to the intestine where it continues to replicate and develop into the myxospore stage (Bjork & Bartholomew 2010). The myxospore stage is released from the salmon host into the water column where it is ingested by *M. speciosa* (Bartholomew *et al.* 1997). In the salmon host, severe *C. shasta* infections can lead to intestinal hemorrhaging and necrosis (ceratomyxosis or enteronecrosis) and are often lethal (Bartholomew *et al.* 1997; Hallett & Bartholomew 2011; Hallett *et al.* 2012).

The response of the salmon host to *C. shasta* infection is highly variable depending on salmon species and the "type" of the parasite. Salmon from rivers where the parasite is endemic are often less susceptible to infection, but naïve salmon hosts are highly susceptible (Conrad & Decew 1966; Sanders, Fryer, & Gould 1970; Margolis & Evelyn 1975; Zinn *et al.* 1977; Buchanan *et al.* 1983; Bartholomew 1998). Ray, Rossignol, & Bartholomew (2010) showed that *C. shasta*-induced mortality of Chinook salmon (*Oncorhynchus tshawytscha*) native to the Klamath River where the parasite is endemic, increased with exposure doses > 75,000 parasites. In contrast, naïve rainbow trout (*O. mykiss*) can succumb to infection with a single actinospore (Bjork & Bartholomew 2009a). In addition to the salmon host's varying sensitivity to *C. shasta*, the parasite also has varying specificity for the salmon host. Atkinson & Bartholomew (2010a; b) identified four unique genotypes (0, I, II, and III) of *C. shasta* that coincided with increased mortality in specific species. Most notably, genotype I is only associated with Chinook mortality; whereas genotype II is associated with coho (*O. kitsch*) and rainbow trout mortality (Hurst & Bartholomew 2012). Genotype O is commonly detected in steelhead (*O. mykiss*); however mortality is not often associated with this genotype (Stinson & Bartholomew 2012). Genotype III appears to be a generalist as it is detected in several different species, but as with genotype 0, is not associated with mortality (Stinson & Bartholomew 2012).

Environmental influence on Ceratomyxa shasta infection

As with other myxozoan life cycles, water temperature also influences all stages and interactions of the *C. shasta* life cycle. In the salmon host, disease severity and mortality rate increased with increasing water temperature (Udey, Fryer, & Pilcher 1975). The researchers also identified a lower threshold of ~6°C below which no mortality was observed; however, this study was conducted using coho and rainbow trout from areas where the parasite was not endemic and were therefore highly susceptible to infection. The spore stages demonstrate similar characteristics as other myxozoans, with the myxospore stage being more environmentally stable. The *C. shasta* actinospore stage was viable for 18 days at 4°C, but only 6 days at 20°C (Bjork 2010); however, the myxospore stage remained viable for up to 175 days at 7°C and ~50 days at 20°C (Chiaramonte 2013). Little is known about the effects of water temperature on the *C. shasta* development rate and release from the polychaete host. Currently, only qualitative data is available regarding the effects of water velocity on the transmission dynamics of spore stages to their respective hosts. Bjork & Bartholomew (2009b) observed lower prevalence of infection in both the salmon and polychaete hosts in tanks with higher velocity than those in tanks with lower velocities. As with the *M. cerebralis* study, this suggests that as water velocity increased the transmission rate of the spore stages decreased, resulting in lower levels of and less severe infections.

EPIDEMIOLOGICAL MODELS

The complex interactions between the parasite, host, and environment and the lack of chemotheraputants make controlling myxozoan infections difficult. Thus, other

approaches are required to minimize the effect of these parasites on their hosts (Yokoyama *et al.* 2012). Epidemiological (mathematical) models are valuable tools to identify and evaluate potential management actions to reduce the effects of parasites. In general, the goal of any model is to simplify complex biological process to an idealized and comprehensible series of events and relationships (Nokes & Anderson 1988). The Ross model for malaria (Ross 1911) was one of the first mathematical models developed and is the platform for all current epidemiological models (Smith *et al.* 2012). This original model, slightly altered by Macdonald (1952), consists of two differential equations that "described" the transmission of malaria from the human host to the mosquito vector. These equations are then solved for the basic reproductive number (R_0), one of the most significant concepts in disease ecology. R_0 is defined as the expected number of secondary infections caused by the introduction of an infected individual into an entirely susceptible population (Heesterbeek & Dietz 1996) and provides a threshold value for a disease: if R_0 is < 1 the

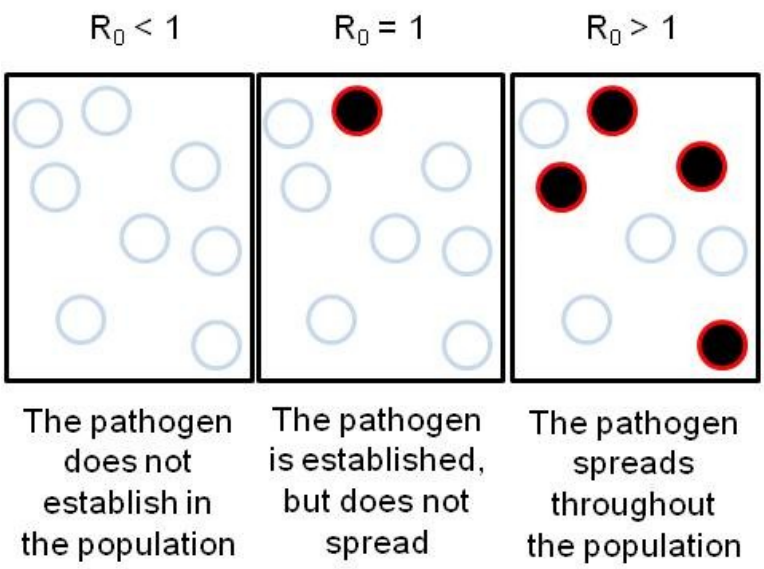

Fig. 1.3 Graphical representation of the inherent threshold value of R0 derived from epidemiological models. If R0 <1 the pathogen cannot maintain itself in the population and will eventually die out. If R0 = 1 the pathogen enters the population, but does not spread. If R0 >1 the pathogen enters and spreads throughout the population.

parasite will not become established within the population and if $R_0 > 1$ the parasite will become endemic and spread within that population (Fig. 1.3; Macdonald 1952). The significance of R_0 in terms of disease control and management is that the pathogen or the vector does not need to be entirely removed, but only reduced so that R_0 is < 1, causing the parasite to eventually die out of the population. Additionally, the equation for R_0 can be used to evaluate the risk or severity of an epidemic and also determines the appropriate control measures necessary to prevent or disrupt an epidemic (Heffernan, Smith, & Wahl 2005). For example, epidemiological models are being used to evaluate the efficacy of culling programs on reducing the spread of Tasmanian devil facial tumors and vaccine programs to reduce rabies transmission in Africa (Hampson *et al.* 2009; Beeton & McCallum 2011)

SURVIVAL ANALYSIS

Statistical modeling approaches can also be used to identify and quantify the effect of parasite infection. In many host-pathogen systems, both human and wildlife, the most common metric of interest is the time to an event (Clark *et al.* 2003). This event of interest can be applied to anything; however it typically refers to the time of infection or death. This type of data has two unique characteristics that make standard statistical methods inappropriate: 1) not all individuals experience the event and are therefore censored from the analysis, and 2) the data is typically not normally distributed (Clark *et al.* 2003; Collett 2003). These unique features require special analytical methods referred to as survival analysis.

Kaplan Meier plots, logrank test, and Cox (proportional hazard) regression are the most commonly used survival analyses (Cox 1972; Collett 2003). These standard survival analysis methods also have two important assumptions in order to be properly applied: 1) every individual is equally susceptible to the event and 2) all individuals will eventually experience the event, if the follow up period is sufficiently long (Corbière & Joly 2007). Although these analyses and assumptions are met for most survival data, data sets with long-term survivors (such as cancer research) cannot be addressed by these methods.

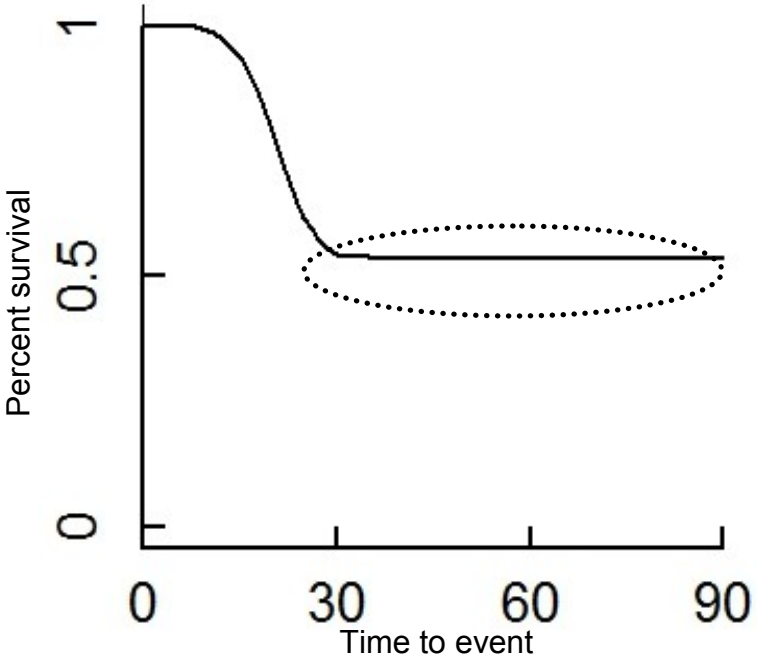

Fig. 1.4 Example of a survival curve that is not suitable for standard survival analysis. The long plateau (highlighted by the dotted circle) indicates no mortality occurred after a certain time. In this data there are both short term survivors (steep slope) and long-term survivors (plateau) which is ideally suited for analysis by cure models.

Cure models are an alternative to traditional survival analyses that are currently being applied to data on long-term human cancer survivors (Othus *et al.* 2012). In addition to cancer data, cure models provide an alternative to survival analysis of wildlife pathogens as the follow up period of wildlife studies may not be sufficiently long, and there is generally a fraction of the population that survives a disease outbreak. For

example, *Batrachochytrium dendrobatidis* can cause up to 100% mortality in some amphibian populations (Muths, Scherer, & Pilliod 2011), but in most cases there was a small proportion of the population that survived. These data tend to have long plateaus towards the end of the observation period indicating no additional mortality past a certain point in time, which violates the standard survival analysis assumptions (Fig 1.4). Cure models divide a population into 2 groups, those that experience the event (short-term) and those that are long-term survivors (Corbière & Joly 2007; Othus *et al.* 2012). One of the strengths of these models is that they allow for different parameters to be included for both short-term and long-term survivors (Corbière & Joly 2007; Othus *et al.* 2012). These models can be used to identify and quantify those factors (biotic and abiotic) that are important to either resisting or succumbing to disease and infection, and to direct management actions to reduce the overall effect of disease within a population.

RESEARCH OBJECTIVES

The objective of this dissertation is to assess the effect of environmental factors on *C. shasta* infection and increased disease severity in the salmonid host. I achieve this objective through a series of field and laboratory experiments and the development of both mathematical and statistical models. The following chapters are manuscripts that have been published or submitted and that address the objective of this dissertation.

- **Chapter 2 e**xamines the effect of elevated water temperature on the disease progression of resistant Klamath River Chinook and coho salmon and compares disease severity across years.

R. Adam Ray, Richard A. Holt, and Jerri L. Bartholomew (2012) Relationship between temperature and *Ceratomyxa shasta*–induced mortality in Klamath River salmonids. Journal of Parasitology: June 2012, Vol. 98, No. 3, pp. 520-526. **doi:** http://dx.doi.org/10.1645/JP-GE-2737.1

- **Chapter 3** presents two field and two laboratory experiments that investigate and quantify the influence of exposure duration, water velocity and temperature on the transmission dynamics of the actinospore stage to the salmonid host.

  Ray, R. A., & Bartholomew, J. L. (2013). Estimation of transmission dynamics of the *Ceratomyxa shasta* actinospore to the salmonid host. Parasitology 140:907-916.

- **Chapter 4** Develops and conducts a sensitivity analysis of an epidemiological model designed to represent the complete life cycle of the *C. shasta* parasite and its hosts. This model can be used to identify and assess potential management actions to reduce the disease effects on the population. As myxozoan parasites have a similar lifecycle, involving vertebrate and invertebrate hosts, this model can be applied to other systems that are affected by these parasites to evaluate potential control strategies.

  R. Adam Ray and Jerri L. Bartholomew. Epidemiological model and sensitivity analysis of a myxozoan parasite, *Ceratomyxa shasta*. Formatted for Ecological Modeling

- **Chapter 5** Presents the novel use of a cure model for survival analysis. This model estimates the daily mortality rate and the total parasite-induced mortality for Chinook and coho salmon. These estimates can then be incorporated into salmon production models to improve escapement goals and harvest quotas.

R. Adam Ray, Russell W. Perry, Nicholas A. Som, and Jerri L. Bartholomew. Using cure models for analyzing the influence of pathogens on salmon survival. Transactions of American Fisheries Society (Accepted)

- **Chapter 6** Concludes with an overall summary and discusses the findings of this research with respect to disease management in the Klamath River and in wildlife populations in general.

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CHAPTER 2: RELATIONSHIP BETWEEN TEMPERATURE AND *CERATOMYXA SHASTA*–INDUCED MORTALITY IN KLAMATH RIVER SALMONIDS

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ABSTRACT

Water temperature influences almost every biological and physiological process of salmon, including disease resistance. In the Klamath River (California), current thermal conditions are considered sub-optimal for juvenile salmon. In addition to borderline temperatures, these fish must contend with the myxozoan parasite *Ceratomyxa shasta*, a significant cause of juvenile salmonid mortality in this system. This paper presents 2 studies, conducted from 2007-2010, that examine thermal effects on *C. shasta*-induced mortality in native Klamath River Chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon. In each study, fish were exposed to *C. shasta* in the Klamath River for 72 hr then reared in the laboratory under temperature-controlled conditions. The first study analyzed data collected from a multi-year monitoring project to assess the influence of elevated temperatures on parasite-induced mortality during the spring/summer migration period. The second study compared disease progression in both species at 4 temperatures (13, 15, 18, and 21 C) representative of spring/summer migration conditions. Both studies demonstrated that elevated water temperatures consistently resulted in higher mortality and a faster mean day to death. However, analysis of data from the multi-year monitoring showed that the magnitude of this effect varied among years and was more closely associated with parasite density than with temperature. Also, there was a difference in the timing of peak mortality between species; Chinook incurred high mortalities in 2008 and 2009 whereas coho was greatest in 2007 and 2008. As neither temperature nor parasite density can be easily manipulated, management strategies should focus on disrupting the overlap of this parasite and its

obligate hosts to improve emigration success and survival of juvenile salmon in the Klamath River.

INTRODUCTION

Environmental temperature is a critical factor that affects the function and efficiency of biological and physiological processes of poikilothermic animals. Prolonged exposure to either cold or warm temperature extremes can result in the cessation of these processes and eventually lead to death. The thermal tolerances of salmonids vary, depending on life stage and biological process, e.g. incubation, development, smoltification, spawning, etc. Optimal temperatures for rearing and growth of juvenile Chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon are similar (12.2 – 20.0 C and 11.8 – 17.0 C, respectively), with Chinook capable of handling slightly higher temperatures (see review by Richter and Kolmes 2005). The upper incipient lethal temperature (UILT) for both species is approximately 25 C (Brett, 1952). Although the UILT provides an accurate assessment of temperature-induced mortality, it fails to capture the compounding effects of other stressors, and thus chronic and acute temperature thresholds have been identified for juvenile salmon. Temperatures at the chronic (16 C) threshold can result in negative effects on salmon behavior and physiology (Sullivan, 2000; Campbell et al., 2001); at the acute (22 C) threshold, negative effects on the salmon are intensified and mortality can occur from temperature alone (Campbell et al., 2001). In the Klamath River below Iron Gate dam (IGD), Chinook and coho salmon encounter water temperatures ranging from 3 – 6 C in January to 20 – 22.5 C in July and August (Bartholow 2005), thus exceeding both the chronic and acute thresholds.

In addition to direct effects on fish physiology, increased temperatures also decrease the ability of fish to cope with pathogens and other stressors (Fryer and Pilcher, 1974; Wedemeyer et al., 1980; Wedemeyer and McLeay, 1981). Indeed, as temperatures exceed 15 C, mortality from many salmonid pathogens increases, as does the rate at which fish succumb to these pathogens (Richter and Kolmes, 2005). In the Klamath River there are several enzootic salmonid pathogens, including the myxozoan parasites *Parvicapsula minibicornis* and *Ceratomyxa shasta* (Nichols and Foott, 2006). Of these, *C. shasta* is considered a significant cause of juvenile salmonid mortality (Foott and Stone, 2004; Fujiwara et al., 2011). A relationship between increasing water temperature and elevated and accelerated mortality from *C. shasta* was first demonstrated by Udey et al. (1975). That study used strains of rainbow trout (*O. mykiss*) and coho salmon from river systems where *C. shasta* is not established and therefore are considered highly susceptible to *C. shasta*, with a lethal infectious dose as low as a single parasite (Bjork and Bartholomew, 2009). In contrast, as a result of evolving with the parasite, Klamath River salmonids have developed a degree of genetic resistance and therefore are considered less susceptible, with a lethal threshold dose approaching 75,000 actinospores for Chinook salmon (Ray et al., 2010). To examine the effect of water temperature on disease progression in these more resistant salmon strains, Foott and Stone (2004) exposed native Klamath River Chinook salmon to the parasite and then reared the fish at 16 C and 20 C. Despite the increased resistance of this strain, mortality in that study was unexpectedly high at both temperatures. Thus, while the findings of Udey et al. (1975) demonstrate a strong positive relationship between temperature and the rate of disease,

the results of the second study suggest some other factor (i.e. parasite density), once exceeded, may overwhelm any temperature related effects.

Temperature also affects the developmental rate and timing of release of the actinospore stage of *C. shasta* from its obligate invertebrate polychaete host, *Manayunkia speciosa* (Bartholomew et al., 1997). In the Klamath River, *C. shasta* infections were observed in fish held in the river beginning in April and increased through July. Although infections declined in late summer and fall, fish became infected as late as December, at water temperatures between 7 and 22 C (Hendrickson et al., 1989; Stocking et al., 2006), indicating the presence of the actinospore stage in the water column. Direct measurement of *C. shasta* in water using a quantitative molecular assay (Hallett and Bartholomew, 2006) showed a similar trend, with actinospore stages in the upper Klamath Basin detected between 10 and 22 C, and peak production occurring at approximately 17 C (Hurst et al., 2011). These studies illustrate a strong seasonal relationship between parasite density and salmonid infection.

This study explores the relationship between temperature, parasite density and *C. shasta*–induced mortality in Klamath River salmonids. First, we analyzed data from sentinel fish exposures conducted from 2007 to 2010, to determine the relative influence of temperature on parasite related mortality between years. Second, we conducted a temperature experiment to determine the relationship between temperature and *C. shasta*-induced mortality for native Klamath River Chinook and coho. The findings of this study will facilitate better predictions of disease related mortality and provide directions for

research and management efforts to reduce the effects of this parasite on out-migrating juvenile salmonids in the Klamath River system.

MATERIALS AND METHODS

Fish exposures and handling

Juvenile (0+ age class) Chinook and coho salmon were obtained from California Department of Fish and Game, Iron Gate Hatchery. Both species varied in size between study mo and years, but coho were consistently smaller than Chinook. Chinook averaged approximately 2.0 g in May and 4.5 g in June and the coho averaged 1.0 g in May and 3.5 g in June. Exposures were conducted in May and June 2007-2010, in the main stem Klamath River in California approximately 1 river km (Rkm) up-river from the confluence with Beaver Creek (259.1 Rkm, from the Pacific Ocean). Fish were transported to the study site in aerated coolers containing approximately 38- L of specific pathogen free (SPF) well water. Holding cages were anchored to the river's edge using cables, then submerged approximately 1 m from the bank in about 1 m deep water. Infection by *C. shasta* was accomplished by holding fish in the river for 72 hr, after which the exposure groups were transferred to separate aerated coolers and transported to the Oregon State University-John L. Fryer Salmon Disease Laboratory (SDL), Corvallis, Oregon. At the SDL, exposure groups were divided approximately in half and placed in 25-L aquaria with SPF water at appropriate experimental temperatures (see subsequent sections). Preventative treatments for bacterial infections and external parasites were administered (Stocking et al., 2006). Fish were fed and observed twice daily. Moribund fish were removed and killed with an overdose (20 ml/L) of tricane methansulfonate

(MS-222) and either immediately examined for infection or frozen for future examination. Surviving fish were killed 90 days post-exposure (DPE) with an overdose of MS-222 and 10 fish from each tank were immediately examined for infection. All moribund and a subset of up to 25 surviving fish were microscopically examined for the myxospore stage of *C. shasta* by collecting material from the posterior intestine with a sterilized inoculating loop and smearing the material in a drop of water on a microscope slide. The material was examined at 200x magnification for 3 min or until the myxospore stage was identified (Bartholomew, 2002). For each of the experiments described below, 20 control fish of each species were not exposed, but were otherwise handled the same as the experimental fish.

Multi-year monitoring

Chinook and coho salmon were exposed to *C. shasta* in the Klamath River in May and June from 2007 to 2010, during the period when hatchery fish are released and migrating. After exposure fish were reared at two temperatures to assess the effects of temperature at different parasite densities. Due to differences in fish size between May and June, different sized cages were used (May: 67.3 cm by 17.8 cm covered with 0.3 cm mesh; June: 61.0 cm by 38.1 cm covered in 0.6 cm mesh). In May 2007 to 2009, June 2007 and June 2009, 80 each of Chinook and coho salmon were exposed as described above. In June 2008, data from the duplicate groups of fish held at 13 and 18 C in the temperature study were combined for this data point. In May and June 2010, 80 Chinook were used, but due to poor adult returns the previous year only 60 coho were available. Following exposure, all fish were transported to the SDL and equally partitioned into 25

L aquaria at either ambient (A) laboratory conditions (13 C) or elevated (E) temperature, which varied between 16 and 20 C to best represent river conditions during the exposures. In May 2010, in-river conditions were 13 C; however, to continue with the thermal comparison a group was reared at 16 C.

Relationship between temperature and C. shasta-induced mortality

In June 2008**,** Chinook and coho salmon were exposed to *C. shasta* and then reared at a range of temperatures to test the relationship between temperature and ceratomyxosis-induced mortality in resistant fish. For each species, 320 fish were placed in large holding cage (123.2 cm by 40.6 cm with 0.3 mesh). After exposure, fish were transported to the SDL and duplicate groups of 40 fish were randomly distributed into 25-L aquaria with water at 13 C. After acclimation, duplicate tanks were supplied with water at 13, 15, 18 or 21 C.

Parasite density measurement

Three 1L samples of water were collected when exposures were initiated and concluded to approximate the density of *C. shasta* during sentinel fish exposure. In 2007, these samples were collected manually at the start and end of the exposure. In 2008 – 2010, an automated ISCO water sampler (Teledyne Isco, Inc. Lincoln, NE) was used to collect 1L of water every 2hr into a 15L composite chamber. From that composite sample, 3 1L subsamples were manually collected to estimate the average parasite density over the first and last 24 hrs of exposure. Samples were processed and analyzed by qPCR using methods described by Hallett and Bartholomew (2006) with modifications described in Hurst et al. (2011). The Cq values reported from this assay

are inversely proportional to the amount of parasite DNA detected in the sample. Standard samples were used to determine the Cq value of 1 and 10 actinospores/L.

Water temperature measurement

From 2008-2010, water temperature was recorded at the exposure site from April through August (153 days). Temperature was recorded every 15 min by a submerged HOBO temperature recorder (Onset computer corporation, Pocasset, Massachusetts). For each mo, daily temperature averages were compared to chronic and acute temperature threshold values to determine the number of days above each threshold. Monthly temperature averages were compared between years to determine inter-annual differences.

Data analysis

Days post exposure (DPE) to death (morbidity/moribund) were recorded for all fish that were visually *C. shasta* positive and was used as a metric of the virulence and intensity of infection. The geometric mean of DPE was used to determine the mean day to death (morbidity/mortality, MDD) for groups when more than 1 fish succumbed to infection. Cumulative mortality was measured as the total number of fish that were visually positive for *C. shasta*, divided by the total number of fish in the aquaria. Overtly morbid and moribund fish were used as a proxy for each of these metrics. Mortality attributed to factors other than *C. shasta* was minimal and was not included in our analysis.

Statistical analyses were conducted using TIBCO Spotfire S+ (TIBCO Software Inc., Palo Alto, California). For the observational study, a three-way ANOVA was used

to analyze overall differences in parasite induced mortality and the rate of mortality between mo, year, and temperature. For the parasite induce mortality analysis the reported *P*-values are based on the arcsine square root transformed data. At each individual site a Chi Square analysis was used to determine differences in mortality between temperatures within a mo and between the same rearing groups within a year. For the temperature experiment, non-parametric Kaplan Meier survival curves and Cox-proportional hazard tests were used to determine differences in cumulative and rate of mortality within and between temperature groups. We report the resulting Score [logrank] test *P*-values from this analysis. A Wilcoxon rank-sum test was conducted to determine differences in parasite density between years and mo within a year from 2007 to 2010.

RESULTS

Multi-year monitoring

Ceratomyxa shasta induced mortality differed between year for both Chinook and coho salmon, but within a year mortality generally increased from May to June (Fig. 2.1). Chinook salmon mortality was driven by inter-annual differences (ANOVA $P = 0.002$), but within a year there was no difference in mortality for similar rearing groups (ANOVA $P = 0.995$). Differences in mortality between ambient (A) and elevated (E) treatments were significant when A mortality was low, as in May ($X^2 = 5.118$, d. f. = 1, $P = 0.024$) and June ($X^2 = 15.428$, d. f. = 1, $P = 0.0001$) 2007 and May ($X^2 = 4.878$, d. f. = 1, $P = 0.027$,) and June ($X^2 = 6.806$, d. f. = 1, $P = 0.009$) 2010. Unlike the overall observed cumulative mortality trends, the rate at which Chinook succumbed to infection did not

statistically differ between years (ANOVA $P = 0.114$), but there were differences between rearing temperature (ANOVA $P < 0.0001$) and exposure mo (ANOVA $P < 0.0001$, Fig. 2.2). The MDD was faster in the E groups and also in June than in May for each year of observations. At either rearing temperature, Chinook mortality was highest in 2008 and 2009 and lowest in 2010.

Like Chinook, there was a significant inter-annual difference in mortality of coho (ANOVA $P = 0.014$, Fig. 1). Coho mortality did not differ between mo within a year for similar rearing groups, with the exception of 2009 E groups ($X^2 = 7.168$, d. f. = 1, $P = 0.007$). Differences in mortality between temperatures were observed in May 2007 ($X^2 = 46.484$, d. f. = 1, $P < 0.0001$), June 2007 ($X^2 = 48.580$, d. f. = 1, $P < 0.001$) and June 2009 ($X^2 = 26.062$, d. f. = 1, $P < 0.001$). The rate at which coho died from *C. shasta* infection was statistically different between years, mo, and temperature (ANOVA $P < 0.001$, all). Faster mortality rates were observed for all the E groups compared to A and June exposures resulted in shorter time to death in 2008 and 2009 (Fig. 2.2). In 2007 and 2010 the May E groups had a slightly shorter time to death than the June E groups, despite a 2 C increase in temperature. For coho, parasite induced mortality differed greatly between years with the highest mortality occurring in 2007 and 2008.

Thus, *C. shasta* related mortality for both species differed between years; however the timing and severity of infection differed between species. No *C. shasta*-related mortalities were observed in the unexposed control fish for either species in any mo or year.

Relationship between temperature and C. shasta-induced mortality

Increases in rearing water temperature led to elevated and accelerated mortalities from *C. shasta* in both Chinook and coho salmon, with Chinook being more affected than coho. Cumulative mortality from *C. shasta* in Chinook increased with temperature from 68.8% at 13 C to 97.7% at 21 C (Fig. 2.3). The MDD was inversely correlated to temperature and decreased by almost 50% between 13 C and 21 C groups (30.5 and 15.9 days, respectively). There were no differences in cumulative mortality between replicates at either 13 or 21 C (Score [logrank] test $P = 0.529$ and 0.698, respectively). However, differences were detected between the duplicates of both 15 C and 18 C treatments (Score [log rank] test $P = 0.03$ and 0.01, respectively). The mortality curve of only one 15 C duplicate was statistically different from the 13 C treatment (Score [logrank] test $P = .005$). Each 18 C duplicate was significantly different from both the 15 C (Score [logrank] test $P < 0.0001$, for both) and 21 C groups (Score [logrank] test $P < 0.0001$, for both). Therefore, each 15 C duplicate and the combined duplicates of 18 C were graphically represented. Overall, the cumulative and rate of mortality were significantly different between all temperature groups (Score [logrank] test $P < 0.0001$). The risk of succumbing to infection increased disproportionately between adjacent temperature groups, i. e. 2.2-fold between 13 and 15 C (averaged for each 15C group), 4.7-fold between 15 and 18 C, and 6.9-fold between 18 and 21 C.

Similar trends were observed for coho salmon, although cumulative mortality was slightly lower at most temperatures (Fig. 2.4). As observed in Chinook, cumulative mortality increased with increased rearing temperature from 66.7% at 13 C to 87.8% at 21 C and the MDD for coho decreased by almost 50% between 13 and 21 C (35.0 and

17.6 days, respectively). There were no significant differences in cumulative and rates of mortality between replicates at each temperature group (Score [logrank] test $P = 0.116$, 0.375, 0.609, and 0.176, 13, 15, 18, and 21 C respectively). Between adjacent temperature groups, i.e. 13 and 15 C, 15 and 18 C, 18 and 21 C, there were significant differences (Score [log rank] test $P = 0.02$, < 0.01, and < 0.01, respectively). As observed in Chinook, the risk of morality increased unequally between adjacent rearing groups, i. e. 2.4-fold between 13 and 15C, 3.5-fold between 15 and 18 C, and 5.5-fold between 18 and 21 C.

Chinook cumulative mortality was higher for each temperature group, except 15 C, and was more variable, especially at the lower temperatures, than observed in coho. While there was no difference in mortality and MDD between the 2 species at either 13 or 15 C, Chinook incurred higher mortality and faster MDD than coho at 18 and 21 C (Score [log rank] test $P < 0.0001$ for both groups). The overall and rate of mortality for both Chinook and coho increased with increasing water temperatures. No *C. shasta*-related mortalities were observed in the unexposed control fish of either species.

Parasite density measurement

In 2008 and 2009 parasite densities were greatest and consistently at or above 10 parasites/L. Individual water sample collection, which occurred in 2007, resulted in greater variability between samples and larger standard deviations (Fig. 2.5). Due to this within sample variation, 2007 was not significantly different from any of the other years. However, when similar sampling techniques (ISCO) were compared there were differences between 2008 and 2009, 2008 and 2010, and 2009 and 2010 ($P = 0.039$, $<$

0.001, and < 0.001, respectively). Densities were generally higher in June than May, but were only significantly different in 2008 ($P = 0.005$).

Water temperature

Temperature measurements were compared to the chronic (16 C) and acute (22 C) thresholds to distinguish differences between mo and years (Fig. 2.6). The chronic threshold was not exceeded in April for any year; the highest average temperature during that mo was in 2009 (11.4 C). The average daily temperature in May 2008 and 2009 was 15.3 and 16.2 C, respectively, and the chronic threshold was exceeded 12 days in 2008 and 16 days in 2009. In May 2010 the average daily temperature was 13.7 C and the chronic threshold was not exceeded during this month. The chronic threshold was exceeded 100% of the days in June, July, and August for all 3 years. Daily temperatures for July and August averaged at or above the acute thermal threshold. Of the 153 daily averages recorded for each year from 2008-2010, the chronic threshold was exceeded a total of 104, 108, and 88 days (respectively) and the acute was surpassed 44, 36, and 47 days (respectively). Although the temperature patterns were consistent between most of the mo for all 3 year observed, May 2010 was approximately 1.5-2 C cooler than previous years.

DISCUSSION

Temperature influences almost every aspect of the *C. shasta* life cycle, from effects on overall salmon physiology, including stress and disease resistance, to developmental rate and longevity of the parasite in the aquatic environment (Richter and Kolmes, 2005; Foott et al., 2007; Bjork, 2010). Both of our studies demonstrated a

relationship between increasing water temperature and *C. shasta*-related mortality in native Klamath River Chinook and coho salmon. Although elevated temperatures consistently resulted in higher mortality and quicker mean day to death, the magnitude of this relationship was not consistent between mos within a year or between years, and also differed between species. The findings of these studies using Klamath River salmonids were consistent with the trends described by Udey et al. (1975) for the effects of temperature on *C. shasta* related mortality of more susceptible salmonids strains. They also provided further support for the threshold of high infectivity and mortality from *C. shasta* in Klamath River salmonids when densities exceed ~10 spores/L (Hallett and Bartholomew, 2006; Ray et al., 2010). These findings also suggest a hierarchy of density, then temperature, with respect to the relative importance of factors that affect ceratomyxosis.

The complex relationship between temperature and environmental conditions and the *C. shasta* life cycle were best exemplified by the multi-year observations. The water years 2007 to 2010 were very similar in the Klamath River Basin, with no major flooding events (USGS water data, gauging station 11516530). In May 2008-2009, water temperatures exceeded 16 C and intermittently surpassed 18 C, coinciding with the highest observed mortality for both species. In contrast, in 2010 water temperatures did not exceed 16 C until 1 June, 2 to 3 wk later than previous years. Mortalities of both species were low compared with previous years and remained low even as June temperatures increased. A strong relationship between thermal time (degree days) and growth and development has been shown for many ectothermic organisms, including

invertebrates (Mullens et al., 1995; Honek et al., 1996; Trudgill et al., 2005) and also for the production of the actinospore stage of *C. shasta* (Hurst et. al., 2011). Therefore, it is possible that the delay of warmer water temperatures in 2010 hindered the development of the polychaete host and/or the development of the actinospore stage of *C. shasta* within this host, postponing release of the parasites. These cooler temperatures in conjunction with the lower parasite density may have decreased the infection prevalence and allowed the salmon adequate time to recover from infection. As there were no discernable differences in water years over the course of this study, the delayed warming in 2010 provides at least circumstantial evidence for the importance of the interaction between water temperature and the ceratomyxosis disease cycle.

Although juvenile salmonids may experience a wide range of temperatures, our experimental study focused on the effects of temperature during the peak period of salmon migration. Study temperatures overlapped the coolest temperature during this period (13 C), the chronic threshold (16 C), and approached the acute threshold (22 C) established by Campbell et al. (2001). The risk of mortality was greatest as temperatures increased from 18 to 21 C (6.9 – Chinook and 5.5 – coho), which neared the acute thermal threshold, and lowest as they increased from 13 to 15 C (2.2 – Chinook and 2.4 – coho), which does not exceed any thermal limits. This disproportionate increase in risk at temperatures near, or above, the different thermal thresholds, emphasizes the compounding influences of increased thermal stress and decreased ability to cope with the infection for both species.

Since Udey et al.'s (1975) original experiments describing the relationship between water temperature and mortality from ceratomyxosis, there have been advances in parasite detection and our understanding of the biology of *C. shasta*. Two of the most significant were the elucidation of the parasite life cycle (Bartholomew et al. 1997) and the development of molecular assays that could detect *C. shasta* DNA in fish (Palenzuela et al., 1999) and quantify parasite DNA in water (Hallett and Bartholomew, 2006). The latter assay allows for the estimation of parasite density in a given amount of water, which can be extrapolated to estimate the exposure dose. Variation in density between years provides an explanation for the differences in mortality observed, at similar temperatures, during the temporal study, as well as between this and previous studies. In 2007 and 2010, parasite density measured less than the 10 spores/L threshold. In these years mortality was lowest in groups held at 13 C and mortality in groups held at elevated temperatures was significantly higher, except for coho in 2010, where low fish numbers may have limited the statistical sensitivity. In 2008 and 2009 parasite density exceeded the 10 spores/L threshold and high mortality was observed regardless of rearing temperature, except for 2009 coho, which will be further discussed below. Thus, temperature effects were most significant below 10 spores/L, supporting the lethal threshold density identified by Hallett and Bartholomew (2006).

Another recent development in our understanding of this parasite was the identification of *C. shasta* genotypes associated with different salmonid hosts. Atkinson and Bartholomew (2010a, b) identified 4 unique genetic types (genotypes 0, I, II, III) of *C. shasta* in the Klamath Basin. It was observed that mortality in Chinook was

consistently associated with genotype I and in coho with genotype II, even though most genotypes were simultaneously detected in the river during exposure. These specific host associations may explain some of the disparities we observed in the temporal monitoring and temperature experiment. For example, between 2007 and 2009 there was a switch in cumulative mortality between the species, with higher mortality in coho in 2007 and higher mortality in Chinook in 2009. Parasite density was similar between these years, thus differing proportion of host-specific genotype may provide an explanation for the differential mortality.

Coho are generally considered more sensitive to elevated temperatures and this is supported in the June 2007 exposure. During this exposure, Atkinson and Bartholomew (2010a) estimated an approximate 1:1 ratio of *C. shasta* genotype I (Chinook) to genotype II (coho) in water samples. Mortality in exposure groups held at 13 C was equally low for both species, but coho held at the higher temperature died at a rate double that of Chinook. This suggests that as temperature increases, coho became less capable of preventing the onset of ceratomyxosis. However this trend is not repeated in the temperature experiment. During this exposure parasite densities were higher than in 2007 and mortality was significantly lower in coho at both 18 and 21 C. This suggests that during this exposure a greater proportion of the total dose was genotype I, associated with Chinook. These examples emphasize the importance of understanding how all 3 factors, e.g. temperature, density, and genotype, in combination, can affect the survival of juvenile salmonids as they migrate towards the ocean.

The magnitude of the relationship between temperature and mortality was affected by parasite density during exposure: when densities were high thermal influences were dampened. In the Klamath River, current environmental conditions are marginal for out-migrating and over-summering juvenile salmonids based solely on temperature , which is predicted to increase 0.3 – 0.6 C per decade (Bartholow, 2005). The compounding stress of *C. shasta* infections likely further impedes restoration of these commercially and culturally important species. Use of cool water refugia provided by tributaries and groundwater-influenced hyporheic zones by salmonids may mitigate some of the adverse effects of increasing temperature (May and Lee, 2004; Hatch et al., 2006; Sutton et al., 2007). However, these areas may provide only minimal relief for salmonids if the density of *C. shasta* remains at or near the densities observed over the course of our study. As this is a hatchery driven system, a potential management action would be to release salmon earlier in the spring or later in the fall to avoid both peak parasite production and higher temperatures. To restore and stabilize the salmonid population in the Klamath River, river-wide modifications, such as dam removal and habitat restoration, have been proposed as part of the Klamath Basin Restoration Agreement (Klamathrestoration.org, accessed 10-28-2011). Even if these projects do not alter in-river temperatures, they could disrupt the spatial or temporal overlap of hosts and parasite currently observed in the Klamath. Temperature and parasite density are difficult parameters to control; however our findings provide avenues for research and management actions to potentially circumvent or lessen the adverse affects of *C. shasta* on the juvenile salmon population.

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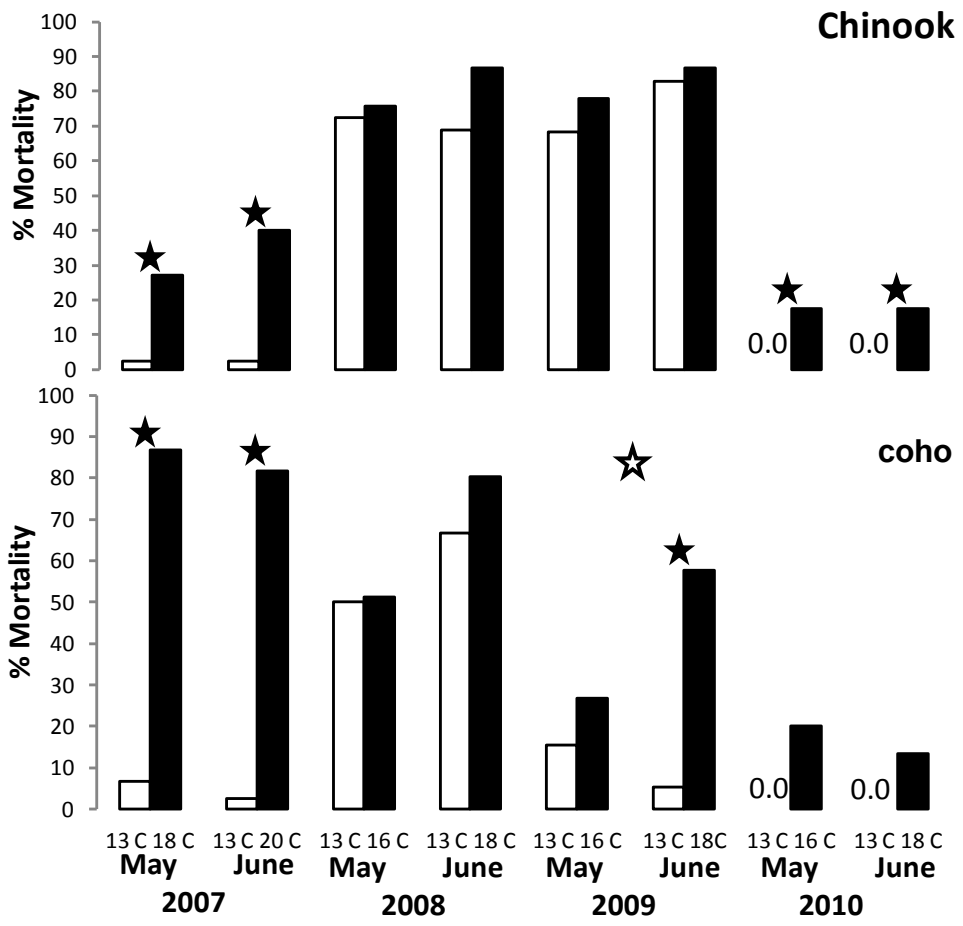

FIGURE 2.1. *Ceratomyxa shasta* related mortality in Chinook (*Oncorhynchus tshawytscha)* and coho (*O. kisutch*) exposed at KBC from 2007 to 2010. Fish were reared at either ambient (13 C) or elevated temperatures (16, 18, or 20 C) in the laboratory. Statistically significant differences between ambient and elevated temperatures, within a mo are indicated by ★ and between elevated temperatures within a year are indicated by☆.

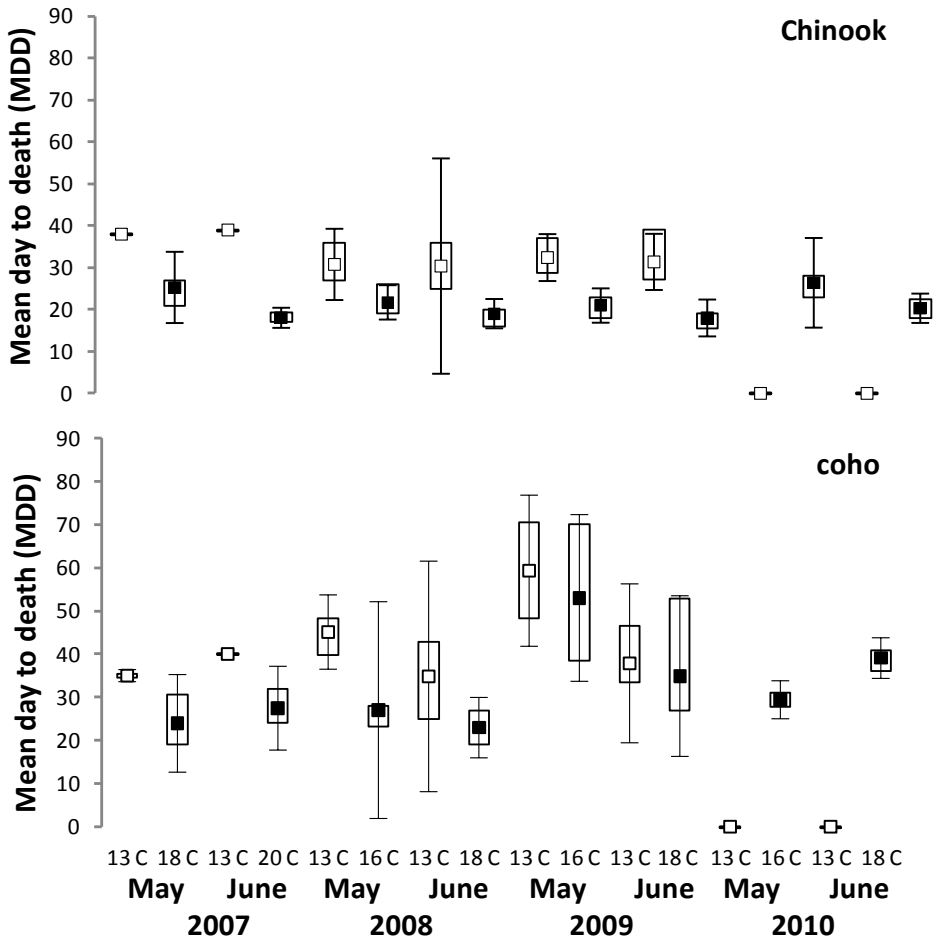

Figure 2.2 *Ceratomyxa shasta* related mean day to death (MDD) observed for Chinook (*Oncorhynchus tshawytscha)* and coho (*O. kisutch*) exposed at KBC from 2007 to 2010. Fish were reared at either ambient (13 C) or elevated temperatures (16, 18, or 20 C) in the laboratory.

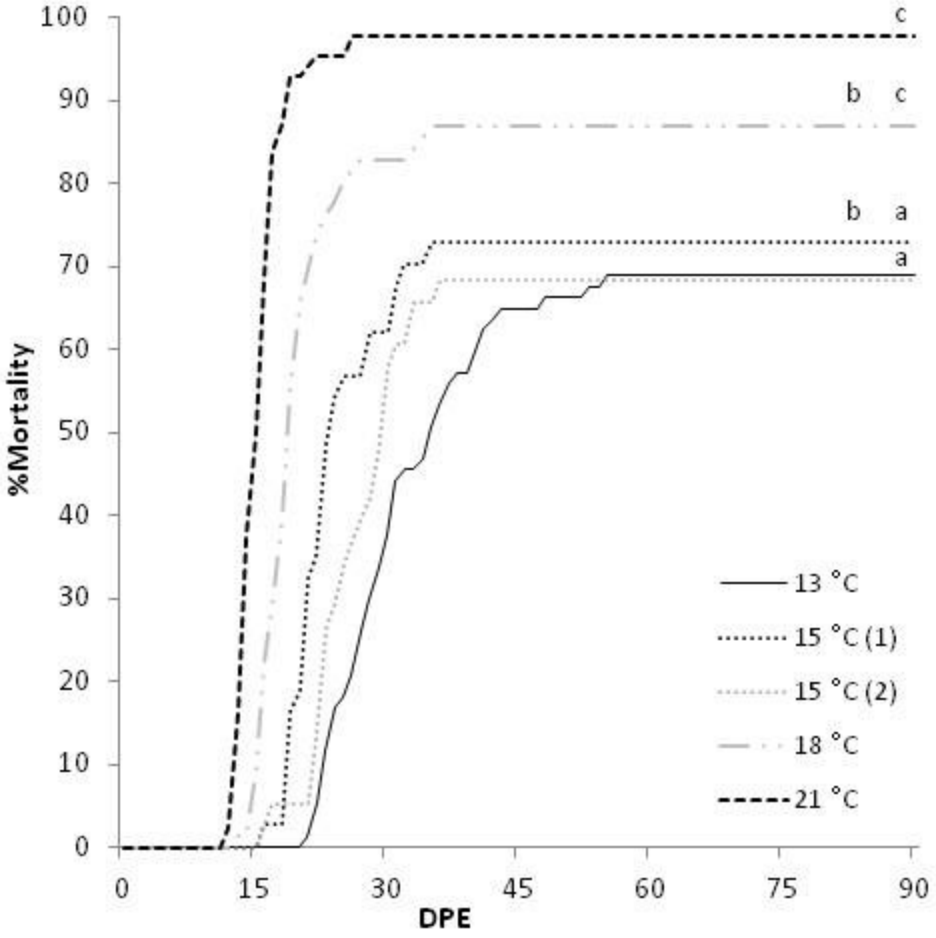

FIGURE 2.3. *Ceratomyxa shasta* related mortality curves for Chinook (*Oncorhynchus tshawytscha)* held at 13, 15, 18 and 21 C. The 15 C groups were statistically different from each other and only one 15 C differed from 13 C. Both 15 C replicates were different from 18 C, which, in turn, was statistically different from 21 C; a, b, and c represent statistically different relationships. (DPE = Days post exposure)

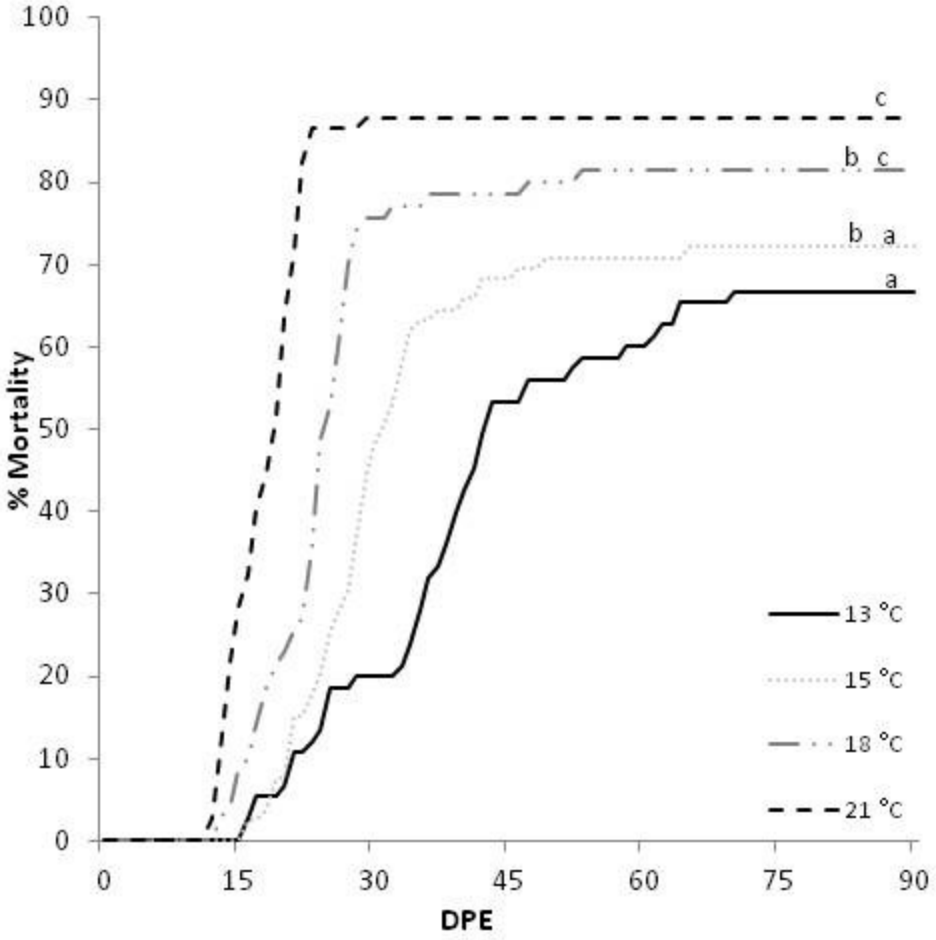

FIGURE 2.4. *Ceratomyxa shasta* related mortality curves for coho (*Oncorhynchus kisutch*) held at 13, 15, 18, and 21 C. Mortality curves for each adjacent temperature groups (13-15 C, 15-18 C and 18-21 C) were statistically different; a, b, and c represent statistically different relationships. (DPE = Days post exposure)

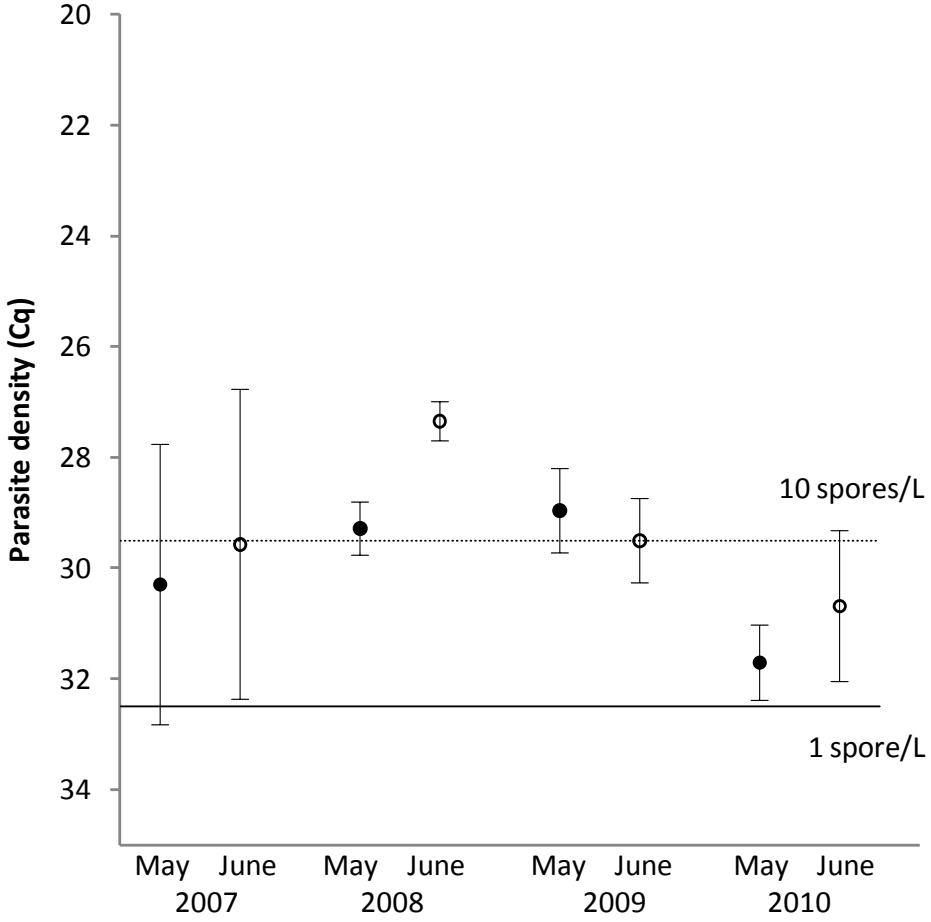

FIGURE 2.5. Average parasite density measurements from water samples collected at beginning and ending of fish exposures. Cq values are inversely related to density. Solid and dashed lines represent standard values of 1 and 10 spores/L, respectively.

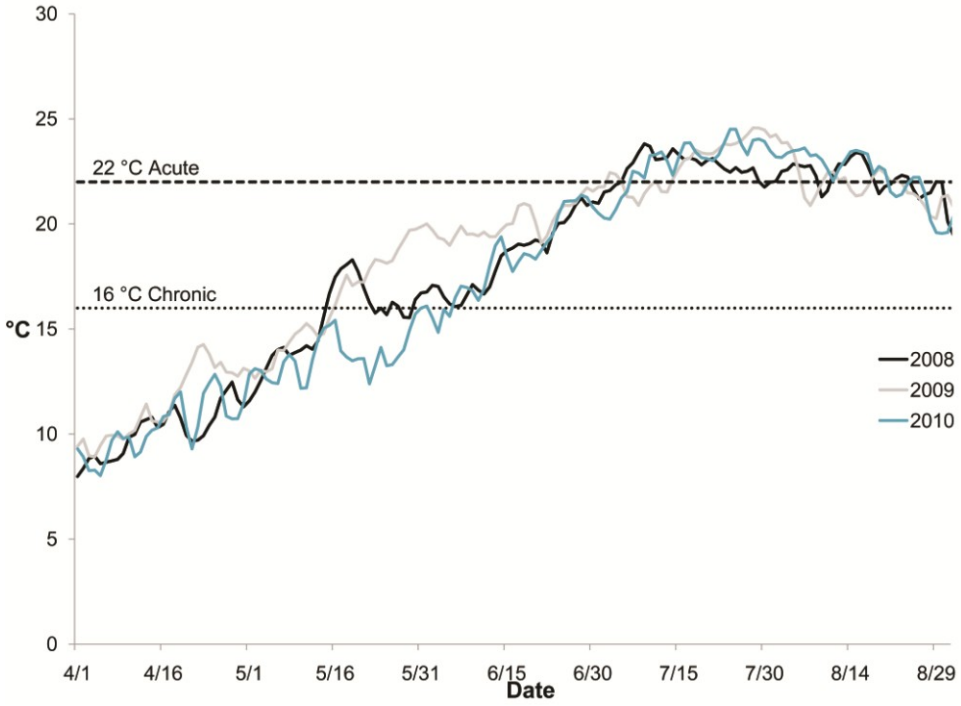

FIGURE 2.6. Klamath River temperature trends monitored at KBC sentinel site from March through August 2008-2010. Dotted and dashed lines represent chronic and acute thresholds for thermal tolerance of Chinook (*Oncorhynchus tshawytscha)* and coho (*O. kisutch)* salmon.

CHAPTER 3: ESTIMATION OF TRANSMISSION DYNAMICS OF THE *CERATOMYXA SHASTA* ACTINOSPORE STAGE TO THE SALMONID HOST

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SUMMARY

Transmission dynamics of the actinospore stage of *Ceratomyxa shasta* to the salmonid host were investigated under field and laboratory conditions. The number of parasites transmitted and the transmission rate were compared between two different exposure durations and also among different water velocities, by means of field exposures. Under laboratory conditions, the number of parasites transmitted and the transmission rates were compared across a broader range of water velocities and also at different water temperatures. Transmission rate was not constant over time as the number of parasites transmitted increased non-linearly between the two exposure durations. Transmission was also inversely related to water velocity and there was a threshold to transmission between 0.2- 0.3 m/sec. Lastly, transmission rate increased with water temperature up to 18 °C then decreased at 23 °C. These experiments provide a range of values of transmission that will be incorporated into an epidemiological model to simulate effectiveness of different management strategies. Additionally, these experiments provided novel information on the effects of environmental conditions (i.e. water velocity and water temperature) on the transmission dynamics between the salmonid host and the actinospore stage.

Key words: myxozoan, *Ceratomyxa shasta,* transmission dynamics

INTRODUCTION

Transmission is the driving force in host-parasite interactions for most epidemiological models; however it is generally the most difficult to directly quantify (McCallum *et al.* 2001). Transmission rates vary depending on the pathogen type, host(s) involved, and mode of infection (direct, indirect, or vector). This rate has been directly quantified for highly infectious pathogens of humans (i.e. malaria, measles, and several sexually transmitted diseases) (Earn *et al.* 2000; Lloyd-Smith *et al.* 2004; Smith *et al.* 2007). However, there are few examples of directly quantified transmission rates in wildlife populations. Instead, transmission rates are indirectly quantified by means of statistical or mathematical models (Anderson *et al.*1981; Begon *et al.*1999). Some scientists have used small-scale laboratory challenges to estimate transmission rates in wildlife populations (for example Dwyer 1991; Bouma *et al.* 1995; Karvonen *et al.* 2003). Many of these small-scale experiments examined the effects of altering densities (host, pathogen, or both) on transmission; yet there are factors in addition to density that can have significant effects on host − pathogen transmission dynamics.

Transmission of parasites with free-living stages in the aquatic environment is influenced by a plethora of abiotic factors, including water temperature and velocity (Marcogliese 2001; Pietrock and Marcogliese 2003; Thieltges *et al.* 2008). In many aquatic host-pathogen relationships there is evidence of an optimal temperature range where transmission is maximized (El-Matbouli *et al*. 1999; Pietrock and Marcogliese 2003). In contrast, water velocity appears to have a more consistent inverse relationship with transmission of non-motile spore stages, although for some parasites there is evidence of

a velocity threshold above which transmission is greatly reduced or inhibited (Radke *et al.* 1961; Barker and Cone 2000; Hallett and Bartholomew 2008).

Ceratomyxa shasta, a myxozoan parasite of salmon and trout, has a complex lifecycle requiring salmonid and polychaete (*Manayunkia speciosa*) hosts and 2 non-motile waterborne stages, an actinospore and a myxospore (Bartholomew *et al.* 1997). Transmission to the fish host occurs when the actinospore penetrates the gill epithelial cells (Bjork and Bartholomew 2010). This parasite is endemic in the Pacific Northwest of the United States and Canada (Bartholomew 1998) and has recently been associated with high mortality in out-migrating juvenile salmonids and lower abundance of returning adults in the Klamath River (Fujiwara *et al.* 2011; Hallett *et al.* 2012). The goal of this study is to quantify the transmission of the waterborne actinospore to the salmonid host with respect to different water velocities and temperatures.

To estimate transmission rates of actinospore stages to the salmonid host we conducted a series of 4 experiments (2 field and 2 laboratory). Field experiments tested the hypotheses that transmission rate is 1) constant with respect to exposure duration and 2) inversely related to water velocity. Laboratory experiments tested the hypotheses that 3) transmission is reduced above a threshold water velocity and 4) that higher temperatures result in higher transmission rates. Based on the empirical results, we defined a functional relationship between parasite transmission and environmental factors (i.e. parasite density, water velocity, and water temperature). We also attempted to identify whether actinospore transmission was frequency or density dependent and if the trends

were consistent between field and laboratory experiments. The estimated range of values and predictive model defined from these experiments can be incorporated into the epidemiological model developed by Ray *et al.* (2010) to improve the estimates of the basic reproductive number (R_0) and better understand host – parasite dynamics.

MATERIALS AND METHODS

FIELD EXPOSURES

Field exposures

For both field exposures, juvenile (0+ age class, ~5- 8.0 g) Chinook salmon (*Oncorhynchus tshawytscha*) were obtained from the California Department of Fish and Game, Iron Gate Hatchery. Each exposure was conducted in June (2009 and 2010) when parasite densities are generally highest (Hallett *et al.* 2012; Ray *et al.* 2012). Fish were transported from the hatchery to the exposure site in aerated coolers and exposed to *C. shasta* in the main stem Klamath River, California, USA, ~1 river kilometer upstream from the confluence of the Beaver Creek tributary (41° 52.1'N, 122° 48.6'W) . Fish were held in cylindrical PVC live cages, 0.28 x 1 m with 0.64 cm mesh screening on each end and placed on the river bottom parallel to flow. Each cage contained 25 fish. During the exposure, water velocities were measured every 30 min with a Marsh-McBirney FLO-MATE (Marsh-McBirney Inc. Frederick, MD, USA). Three 1 L samples of water were collected upstream of the cages twice per h and combined into a large collection bucket. From this collection, a subset of 3 – 1 L samples was collected and assayed by quantitative PCR (qPCR) as described by Hallett *et al.* (2012) to estimate the density of

parasite/L.

Exposure duration experiment

This first study compared the effect of exposure duration on the number of parasites attached to the gill and the resulting transmission rate. In June 2009, 8 cages were placed side by side in 4 pairs; 2 m from the bank in water 0.5 m deep with 1 m between each pair of cages. The cages were then randomly assigned exposure durations of either 3 h (cages 1, 2, 5, and 6) or 6 h (cages 3, 4, 7, and 8).

Velocity experiment

In June 2010, 3 replicate cages were exposed at 1 of 3 different water velocities (0.03, 0.10, and 0.19 m/sec) for 3 h to determine the relationship between velocity and actinospore transmission rates. Replicate cages were placed in a line with the flow and parallel to the bank, with 1 m between each cage. Cages were sited at different distances from the bank and depths to achieve the different velocities. The cages with the slowest velocity were 3 m from the bank in 0.5 m deep water, the medium velocity cages were 4 m from the bank and 1.5 m deep, and the highest velocity cages were 9m out and 2 m deep. For each velocity, water samples were collected from in front of the first cage of each set.

Post field exposure sample collection and fish care

Following river exposure, a subset of 10 and 5 fish from each cage (duration and velocity experiment, respectively), were euthanized with 400 mg/L of tricane methanesulfonate

(MS-222, Argent Laboratories, Redmond, WA). The entire gill from the left side of the fish was removed and stored in 95% ethanol (EtOH) to be assayed by qPCR. The remaining fish were transported to the Oregon State University - John L. Fryer Salmon Disease Laboratory (SDL) Corvallis, Oregon in aerated coolers and observed for signs of morbidity associated with parasite infection. At the SDL, fish were placed into 25 L aquaria, fed daily (Bio-Oregon, Longview, WA) and preventative treatments for bacterial infections and external parasites were administered as described by Stocking *et al.* (2006). After 60 days, fish were euthanized using MS-222 and examined for *C. shasta* myxospores as described by Ray *et al.* (2012).

LABORATORY EXPOSURES

Velocity experiment

Iron Gate Hatchery juvenile (0+ age class, ~10-18.0 g) Chinook salmon were exposed to the actinospore stage of *C. shasta* at 4 velocities (0.05, 0.18, 0.32, and 0.43 m/sec) to determine the effect of velocity on the transmission rate of the actinospore stage. For each of the 4 velocities, 10 trials of 2 fish each were conducted. Effluent from laboratory colonies of infected polychaete hosts (*M. speciosa*) provided a source of actinospore stages for these challenges. For each trial, 38 L of this effluent was added to 310 L of specific-pathogen free well water in a 350 L fiberglass tank. From this reserve, 3 – 1 L samples were collected for qPCR assay to estimate parasite density. For each challenge, two fish were held in a swim tube (28 x 5.1 cm). For the two slowest velocities, water was pumped from the large aquarium through a series of 1.9 cm PVC piping connected to

an Odyssea WP 500 water pump (Odyssea Aquarium Co., China), for the two highest velocities, a Little Giant Water Wizard 5-MSP (Franklin Electric Co., Bluffton, IN) was used. Each end of the swim tube was funneled from 5.1 cm to a 1.9 cm opening and attached to the PVC. Mesh screen (0.64 cm) was glued to the inner rim to aide in diffusing the water and preventing the fish from leaving the tube. Velocities were controlled using a valve on the PVC pipe and measured in the center of the swim tube before each trial with a Marsh McBriney Flo-Mate. After each trial, fish were immediately euthanized with MS-222 and half of the entire gill was removed and frozen in a 2 mL centrifuge tube for assay by qPCR.

Temperature experiment

Juvenile rainbow trout (Troutlodge, Sumner, WA, USA) were exposed to the actinospore stage of *C. shasta* at 4 temperatures (11, 14, 18, and 22 °C) to determine if there was a relationship between transmission rate and water temperature. Although this was a different species than the previous experiments, we assumed the transmission dynamics should not be different between the parasite and salmonid hosts. Effluent from infected colonies of the polychaete host was continuously collected into 189 L aquarium. From this reservoir, 3 – 1 L samples were collected every hour for assay by qPCR to estimate parasite density. Water was pumped from the reservoir aquarium into 3 – 25 L fiberglass tanks using an Odyssea WP 500 water pump, at ~ 0.5 L/min. Twenty-four hours before each exposure, 30 rainbow trout were acclimated to the desired temperature. Ten fish were randomly placed into each aquarium and exposed for 3 h. Elevated water

temperatures were maintained using two submersible aquarium heaters in each replicate tank. Cooler water temperatures were maintained by placing 1 L and 2 L Nalgene® bottles of ice in both the replicate tanks and the reservoir aquarium. Temperatures were recorded every 30 min and control devices were adjusted to maintain a constant a temperature. After each temperature trial, fish were immediately euthanized with MS-222. The left gill was removed and frozen in a 2 mL microcentrifuge tube for assay by qPCR.

Gill qPCR assay

Gills preserved in EtOH were air-dried overnight to allow the EtOH to evaporate before processing. The gill tissue was digested using 495 µL ATL and 5 µL Proteinase K in a shaker overnight at 37 °C. After digestion, all gills samples were processed for qPCR assay using the Qiagen Qiaquick PCR Purification kit and protocol (Valencia, CA, USA): 60 µL of genomic DNA was eluted. Samples were then assayed in duplicate using the qPCR protocol established by Hallett and Bartholomew (2006). If the difference between the duplicate samples was greater than 1 quantification cycle (Cq), the samples were re-assayed. In addition to a non-template control of molecular grade water, two different positive controls were included on each plate; these also informed inter-plate variation.

To relate the qPCR output to actual number of actinospores detected on the gills, biologically relevant calibration standards were established by spiking unexposed gills with 1 and 8 actinospores. Actinospores were obtained by squashing an infected polychaete on a microscope slide and diluting the material with PBS. Then, under a

microscope slide, spores were individually collected using a modified glass pipette (thin drawn) and capillary action and extruded into a microcentrifuge tube prior to extraction (Hallett and Bartholomew 2006). These calibration standards were analyzed by qPCR, as described above, in triplicate and fit with a logarithmic regression. Once *C. shasta* attaches to the gill, a binucleated sporoplasm penetrates into the epithelial cells (Bjork and Bartholomew 2010). The binulceate sporoplasm constitutes ¼ of the DNA of the eight nuclei actinospore (Hallett and Bartholomew 2006). From this assumption we multiplied the estimated number of actinospores, based on the calibration standards, by 4 to approximate the number of sporoplasms transmitted; which will be referred to throughout as parasites transmitted.

Predictive model

We constructed a statistical model to define a functional relationship between transmission rate, water velocity, water temperature, and parasite density using data from all 3h field exposures. The full model consisted of three main effects (average velocity, average parasite density, and average exposure temperature) and pair-wise interactions between each term. A generalized linear model (GLM) was developed to identify which of the environmental parameters (velocity, parasite density, or water temperature) and their interactions were most important with respect to the transmission rate. To achieve a more normalized distribution of values, we negative log transformed the transmission rates. This model was developed using a Gamma distribution because our variances

increased with increasing mean values. The best model was selected comparing Akaike Information Criterion (AIC) scores (Akaike 1973; Burnham and Anderson 2002).

Data analyses

Statistical analyses were conducted using the R software package (2.14, R development core team 2011). The exposure dose for each trial was estimated from the product of the water velocity, the volume of water, and density of parasite in the water as determined by qPCR assay. In each experiment we quantified 2 metrics: total parasites transmitted - the number of sporoplasms in the gill based on estimates from the reference samples and transmission rate - estimated for each fish from the number of parasites transmitted to the gill divided by the total actinospore dose. The prevalence of infection (POI), based on parasite detection on the gills, was also calculated for each experiment. All estimates of parasite transmitted and transmission rates were normalized by a log transformation prior to statistical analyses and back transformed for display in tables and figures. One-way ANOVAs were used to determine differences in exposure dose, parasites transmitted, and transmission rates among treatments for each experiment.

RESULTS

Calibration standards

Three sets of *C. shasta*-negative gills were spiked with 1 or 8 actinospores and analyzed by qPCR to translate the sample values to actual parasite numbers. There was minimal variation between the replicate samples; the average Cq value of 1actinospore per gill

was 35.2 ± 0.5 and 8 actinospores per gill was 33.0 ± 0.2. The data were best represented as a logarithmic relationship (Eq. 1) between the Cq and the number of parasites spiked onto the gill standards, with an adjusted R^2 = 92.8%. As we assume that a sporoplasm has the same DNA as a ¼ of an actinospore, we back calculated using Eq. 1 to estimate the Cq value of a single sporoplasm (36.6). We rounded this up to 37 then added an extra cycle to establish our positive sample threshold: a sample with a Cq value greater than 38 was considered negative for parasite DNA (uninfected). The positive controls indicated minimal variation; average Cq = 34.9 ± 1.2 SD and the coefficient of variation among plates was 3.5%.

$$\text{Actinospores} = 10^{(Cq - 35.16) / -2.4102} \qquad \text{Eq. 1}$$

Effects of exposure duration

The average hourly parasite density during the 3h exposure, as estimated from qPCR assay of water collected during exposure, was 13.2 parasites/L. For the remaining 3h of the 6h exposure, the average density nearly tripled to 37.6 parasites/L for an hourly average of 25.4 parasites/L for the 6h exposure. Velocities in the cages ranged from 0.05 – 0.07 m/sec. Given the longer exposure and higher parasite density, there was almost a 2 order of magnitude difference in the average number of parasites transmitted to the gills of the fish exposed for 6 h (1.2 X 10^4 parasite) compared with fish exposed for 3 h (1.0 X 10^2) (F_1 = 281.5, $P < 0.0001$) (Table 3.1, Fig. 3.1). There was no difference in the number of parasites transmitted among the 6 h cages (F_3 = 0.866, P = 0.4680). There was

a difference in parasites transmitted among the 3 h cages ($F_3 = 4.884$, $P = 0.006$) due to differences observed between cage 2 and cages 5 and 6 (Fig. 3.1a).

There was also a difference in transmission rate among cages exposed for 3 h ($F_3 = 4.083$, $P = 0.0136$), as transmission estimated in cage 2 was again lower than the other cages (Table 3.1). There was no difference in the transmission rates among the 6 h cages ($F_3 = 1.008$, $P = 0.4$). The estimated average transmission rate was almost 20-fold higher for the 6h than the 3h exposure ($F_1 = 111$, $P < 0.0001$) (Fig. 3.1b). A total of 5 fish from all cages died from *C. shasta* infections, 2 from the 3 h exposure and 3 from the 6 h. Although there was a doubling of parasite density over the last 3 h of exposure, the number transmitted and transmission rates estimated for the 6 h cages was more than double those of the 3 h cages, suggesting that transmission was not constant over time.

Effects of water velocity

In the field study, average parasite density increased as velocity increased, but we did not detect differences among treatments ($F_2 = 0.928$, $P = 0.446$) (Table 1, Fig. 3.2). Among the 3 cages at each velocity, there was no difference either in the number of parasites transmitted ($Fs_2 = 0.118$, $P = 0.89$, $Fm_2 = 0.512$, $P = 0.612$, $Ff_2 = 1.024$, $P = 0.389$) or in the transmission rate ($Fs_2 = 0.224$, $P = 0.803$, $Fm_2 = 0.652$, $P = 0.539$, $Ff_2 = 1.01$, $P = 0.393$). More parasites were transmitted at the faster velocity compared to the slow and medium velocities ($F_2 = 4.229$, $P = 0.0212$) (Fig. 3.2a). Although the average number of parasites transmitted differed by 3-fold between the slow and fast velocity trials, the transmission rate was highest at the slow velocity ($F_2 = 6.404$, $P = 0.0038$) (Fig. 3.2b).

These results suggest an inverse relationship between velocity and transmission rate of *C. shasta*, yet they might be confounded by the large differences in exposure dose among the different velocities.

Even under laboratory conditions, holding dose constant among the different velocities proved difficult and parasite densities of the 0.05, 0.18, and 0.32 m/sec were nearly double that of the 0.43 m/sec trial ($F_3 = 25.18$, $P < 0.0001$). However, this difference ($3.90 – 6.90 \times 10^3$) was much smaller than what was observed in the field experiment ($0.12 – 1.86 \times 10^6$) (Table 1, Fig 3.3). The total number of parasites transmitted differed between velocities ($F_3 = 10.55$, $P = <0.0001$) and was higher at the slower velocities compared to the faster velocities (Fig. 3.3a). Not only were fewer parasites transmitted at higher velocities, but the prevalence of infection was almost 3-fold lower at the higher velocities (27 and 20%) than at the slower velocities (75% at both lower velocities). The average transmission rates were greatest and approximately equal at 0.05 and 0.18 m/sec and decreased at the 2 higher velocities ($F_3 = 9.226$, $P < 0.0001$) (Fig. 3.3b). As the doses were more consistent in the laboratory trials, we were able to directly observe an inverse relationship between velocity and transmission. We also identified a threshold between 0.18 and 0.32 m/sec where transmission efficiency was greatly reduced.

Effects of water temperature

Densities ranged from 170 to 241 parasites/L and we did not detect differences in parasite density among temperature treatments ($F_3 = 2.846$, $P = 0.105$) (Table 1, Fig. 3.4). Total number of parasites transmitted was low in the 11 °C, intermediate in the 14 and 22 °C

and high in the 18 °C treatments ($F_3 = 10.78$, $P < 0.0001$) (Fig. 3.4a). As with total parasites transmitted, there was a difference in transmission rates among the temperature groups ($F_3 = 11.03$, $P < 0.0001$) (Fig. 3.4b). The highest transmission rate occurred in the 18 °C group and, interestingly, the 14 and 22 °C groups had very similar mean transmission rates. Both parasites transmitted and transmission rate appear to be maximized around 18 °C.

Predictive model

The full model had 7 parameters, including the intercept (Table 3.2) and an AIC score of 130.74. The final model had 4 parameters with a similar AIC value to the full model (127.47). This final model consists of the main effect terms parasite density ($P < 0.0001$) and exposure temperature ($P < 0.0001$), and the interaction between parasite density and exposure temperature ($P < 0.0001$). Although water velocity was not a significant parameter ($P = 0.8887$), it was left in the model as it was a parameter of interest and removing this parameter did not significantly improve the AIC score (125.5). This model explains 71.9% of the variation observed, suggesting there are other factors that influence the transmission rate that are not addressed in this model.

Transmission determination

We attempted to define the transmission process for the actinospore stage of *C. shasta* by comparing the number of parasites transmitted and transmission rates against density and exposure dose (Fig. 3.5). As transmission was does not appear to be constant over time, observations were standardized by using only experimental results from 3 h exposures.

The number of parasites attached increased with increasing density in both field and laboratory experiments.

DISCUSSION

Transmission is one of the key parameters of the basic reproductive number (R_0), yet there are few pathogens of wildlife populations for which it has been directly measured. We quantified a range of transmission rates of the actinospore stage of the myxozoan parasite *Ceratomyxa shasta* to the salmonid host and defined a functional relationship between transmission and biotic (parasite density) and abiotic factors (water velocity and temperature). Through a series of field and laboratory experiments we determined that water velocity was inversely related to transmission and that there was an optimal temperature range for transmission, around 18 °C.

Our prediction of an inverse relationship between water velocity and transmission rate was supported in both the field and laboratory experiments. The true transmission rates in the field study were obscured because exposure dose increased with velocity. There was almost a 7-fold increase in the average dose between the slow and medium velocities and over a 15-fold increase between the slow and fast velocities. In the laboratory experiment we were able to control the exposure dose and better observe the inverse relationship between velocity and transmission. This inverse relationship has been observed for other host-pathogen systems (Barker and Cone 2000; Bodensteiner et al. 2000). For another myxozoan parasite, *Myxobolus cerebralis*, Hallett and Bartholomew (2008) observed decreased disease severity, a proxy for transmission, in rainbow trout

(*Oncorhynchus mykiss*) in experimental channels with higher velocity (0.02 m/sec) than in channels with slower velocity (0.002m/sec). Rowan and Gram (1959) found higher transmission rates of *S. mansoni* at slow velocities; however as velocities increased above 1.30 m/sec transmission rates declined (Radke *et al.* 1961). We also observed a velocity threshold as both the transmission rate and prevalence of infection dramatically decreased between 0.18 and 0.32 m/sec. This suggests that fish (i.e. larger or older) that spend more time in the thalweg of the channel, where flows are > 0.3 m/sec, may become less heavily infected than those that inhabit areas of slower velocities (e.g. proximal to banks, slow flowing pools, etc.).

We hypothesized that increasing water temperature would cause an increase in transmission as a function of increased ventilation of the fish at higher temperatures. Increased ventilation would cause more water to move over the gills, increasing the number of actinospores potentially contacting the gill surface. Although this hypothesis was not fully supported, we did define an optimal temperature for transmission (around 18 °C). The presence of an optimal thermal range for *C. shasta* transmission is consistent with many other parasites with free-living stages (Anderson et al. 1982; Evans 1985; Pietrock and Marcogliese 2003). There is also evidence of this thermal optimum for the parasite within the fish host; Ray et al. (2012) observed consistently higher *C. shasta*-induced mortality in Chinook that were reared at 18 °C than 13 °C. In the present study, when fish were held at 18 °C, nearly 4-fold more parasites were detected than in those fish held at 14 or 22 °C, suggesting some additional indirect effects of temperature on the host-parasite dynamics. Increased temperatures have been shown to reduce the salmonid

host immune response (Richter and Kolmes 2005) and the viability of the actinospore (Markiw 1992; El-Matbouli *et al.* 1999), but it can also potentially increase the replication rate of pathogen within a host, all indirectly affecting the transmission rate.

There was no support for our hypothesis that attachment rate would be constant with respect to exposure duration. Rather than a linear relationship between the 3 h and 6 h exposures, the number of parasites transmitted was 3 to 9 times greater than predicted, even when accounting for the 3-fold increase in parasite density during the last 3 h of exposure. If transmission was constant, independent of density, then a 2-fold increase in the number of parasites transmitted would be expected. If we assume some relationship with density, then we would expect to see 6-fold higher parasites attached (2x for the duration and 3x for the increased density). Thus, either transmission was not constant or some other factor (e.g. underestimation of exposure dose, in-host replication) affected these values.

As a result of the challenges experienced conducting the field experiments (i.e. inconsistent parasite densities, large differences in exposure doses), we attempted to minimize confounding factors in the laboratory studies. However, one of the factors we were unable to account for was replication of the parasite in the gill tissue. The effects of this replication may contribute to variation in transmission rates observed in the duration and temperature experiments. For a similar myxozoan parasite, *M. cerebralis*, replication within the salmonid host occurred within 10 min of penetration, and peak replication occurred between 2-4 h post-exposure (Markiw 1989; El–Matbouli *et al.* 1995). If *C.*

shasta has similar timing of replication then this could explain the 2 orders of magnitude difference between the 3 h and 6 h exposed fish. It has also been suggested that higher temperatures also affect parasite replication within the salmonid host (Bartholomew 1998). In the temperature experiment, the number of parasites transmitted in the 18 °C group was almost 4-fold higher than the adjacent temperature groups, providing some evidence that 18 °C may be a thermally optimal temperature for both replication and transmission in rainbow trout. Additionally, the only difference between the 3h duration experiment and the 2 slower velocities from the field exposures was that the water temperature during the duration experiment was ~2 °C warmer (18 °C); yet the estimates of parasites transmitted were an order of magnitude higher.

Because of the uncertainty about the effects of in-host replication, the predictive model was developed only using data from the 3h field exposures of Chinook salmon (duration and velocity experiments). As expected, water temperature and parasite density were both significant parameters for transmission; however water velocity was not. The lack of significance of water velocity was unexpected and we feel this is most likely due to the very limited range of velocities used (0.03 – 0.19 m/sec), which do not exceed the potential threshold observed in the laboratory challenge.

Identifying the transmission process (frequency or density dependent) is vital to understanding host-pathogen dynamics, not only for improving model accuracy, but also for applying the model to other systems (Thrall *et al.* 1995; McCallum *et al.* 2001). We observed patterns indicative of both types of transmission processes, dependent on the

scale of observation. In the laboratory experiment, transmission rate was positively correlated with both parasite density and exposure dose, which suggests density dependent transmission. However, this pattern may not be truly indicative of natural transmission, as the densities in the laboratory experiment exceeded all field measurements (Hallett *et al.* 2012; Ray *et al.* 2012). McCallum *et al.* (2001) discuss the difficulties in applying transmission rates or patterns identified from small-scale homogeneous experiments to large-scale heterogeneous landscapes. Our controlled laboratory studies identified important relationships between transmission, water velocity, and water temperature, but direct extrapolation of these rates to a natural river would be unrealistic because of the high parasite densities achieved in the laboratory. The field experiments were more representative of the natural conditions encountered by juvenile salmonids, and the patterns observed in these studies suggest that the in-river transmission of the actinospore to the salmonid host is frequency-dependent. Although tentative, this is an important observation as it has been shown that frequency-dependent transmission nullifies the importance of the host density threshold for a pathogen to persist within a population (Getz and Pickering 1983).

In the Klamath River, CA, *C. shasta* infections have been linked to high mortality rates in emigrating juvenile salmon and reduced abundance of returning adults (Fujiwara *et al.* 2011). Although both water velocity and temperature affect the transmission dynamics of *C. shasta*, velocity is the more crucial environmental factor and also potentially easier to manipulate in this system. Thus, fish that can tolerate higher velocities (> 0.3 m/sec) could experience lower transmission of the actinospore stage. The range of estimated

transmission values and the predictive model based on velocity, density, and temperature will be incorporated into the epidemiological model to provide improved simulations of disease dynamics under different environmental conditions (Ray *et al.* 2010).

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Table 3.1. Average values and standard deviations of all biotic and abiotic factors measured in all four experiments.

Experiment	Duration (h)	Parasite Density (/L)	Velocity (m/sec)	Exposure Temperature (°C)	Parasite Transmitted	Total dose ($X10^4$)	Transmission Rate ($X10^{-4}$)	Prevalence of infection
Field Duration	3	13.2 ± 10.0	0.057 ± 0.012	18.4 ± 0.24	98.9 ± 69.2	51.0 ± 7.7	1.9 ± 1.3	100.0%
	6	25.4 ± 16.2	0.064 ± 0.013	18.9 ± 0.64	1856.6 ± 1108.6	169.6 ± 19.5	11.2 ± 7.3	100.0%
Field Velocity	3	8.8 ± 9.2	0.03 ± 0.0	15.8 ± 0.12	10.5 ± 10.7	12.4 ± 1.1	0.9 ± 0.9	92.3%
		13.2 ± 3.5	0.10 ± 0.01	16.5 ± 0.30	9.0 ± 8.9	85.7 ± 6.0	0.1 ± 0.1	86.7%
		15.0 ± 1.3	0.19 ± 0.01	15.9 ± 0.17	48.1 ± 72.0	186.4 ± 13.3	0.3 ± 0.4	100.0%
Laboratory Velocity	1.1	19.7 ± 5.0	0.05	13.0 ± 0.2	2.0 ± 3.3	0.7 ± 0.2	3.4 ± 6.3	75.0%
	0.25	18.9 ± 2.2	0.18		2.6 ± 3.1	0.7 ± 0.1	3.9 ± 4.4	75.0%
	0.17	18.2 ± 2.6	0.32		0.4 ± 0.7	0.6 ± 0.1	0.6 ± 1.1	27.3%
	.07	11.1 ± 4.2	0.43		0.2 ± 0.6	0.4 ± 0.1	0.8 ± 1.7	20.0%
Laboratory Temperature	3	172.7 ± 13.0	< 0.01	10.5 ± 0.5	37.9 ± 41.9	1.9	20.3 ± 22.4	96.7%
		170.3 ± 61.9		13.5 ± 0.5	94.8 ± 92.7	1.8	51.5 ± 50.4	100%
		241.2 ± 31.8		17.5 ± 0.5	412.5 ± 405.0	2.6	158.4 ± 155.5	97.4%
		218.6 ± 12.1		21.6 ± 1.5	97.5 ± 98.7	2.4	41.3 ± 41.8	100%

Table 3.2. Summary of parameters dropped from global model and coefficients of significant parameters for final model predicting log (transmission).

Parameters	Δ AIC	Coefficient	S. E.	*P*-value
Intercept	130.74	16.94	4.73	0.0006
Velocity * Temperature	-1.44	–	–	–
Velocity * Density	-2.43	–	–	–
Average Velocity	0.00	-0.01	0.08	0.8887
Parasite Density	0.00	-1.29	0.36	0.0006
Water Temperature	0.00	-1.05	0.30	0.0007
Density * Temperature	0.00	0.08	0.02	0.0006

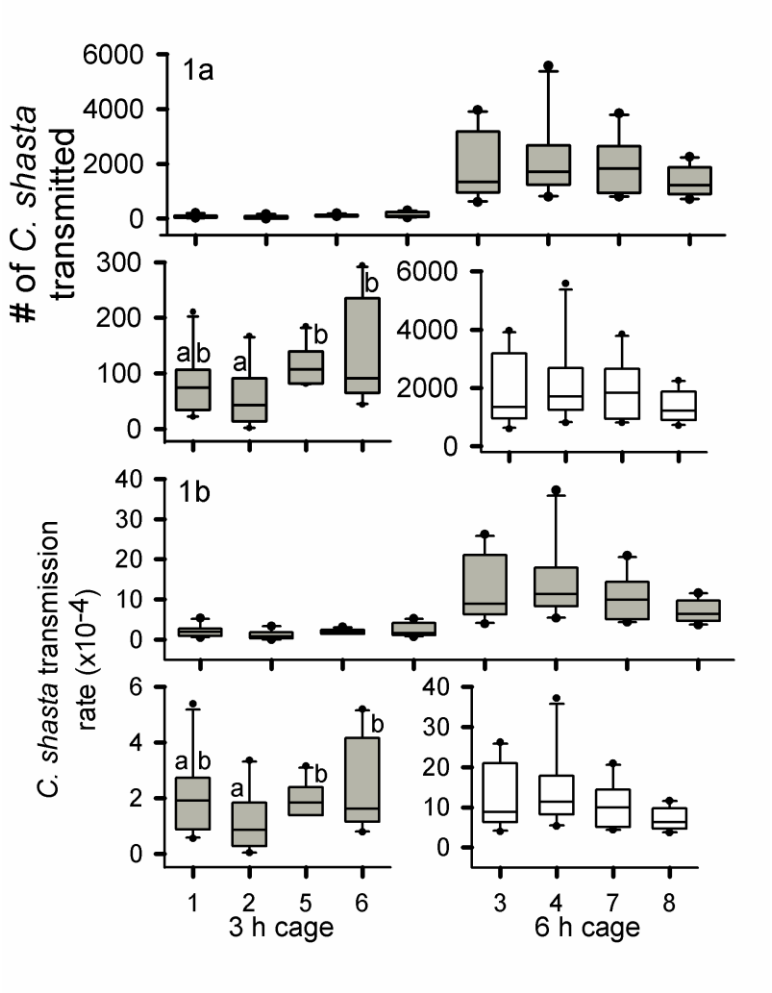

Fig. 3.1. The number of *Ceratomyxa shasta* transmitted (a) and the transmission rates (b) for Chinook salmon (*Oncorhynchus tshawytscha*) exposed for 3 and 6h in the Klamath River. For each, the top graph shows overall comparison between exposure durations and the bottom graphs are scaled to show differences among cages for each duration. The solid lines are the medians, the boxes cover the inter-quartile range, the bars the 5% to 95 % ranges, and the dots are outside two standard errors. Letters represent statistically significant detected differences by Tukey comparison of means ($\alpha = 0.05$).

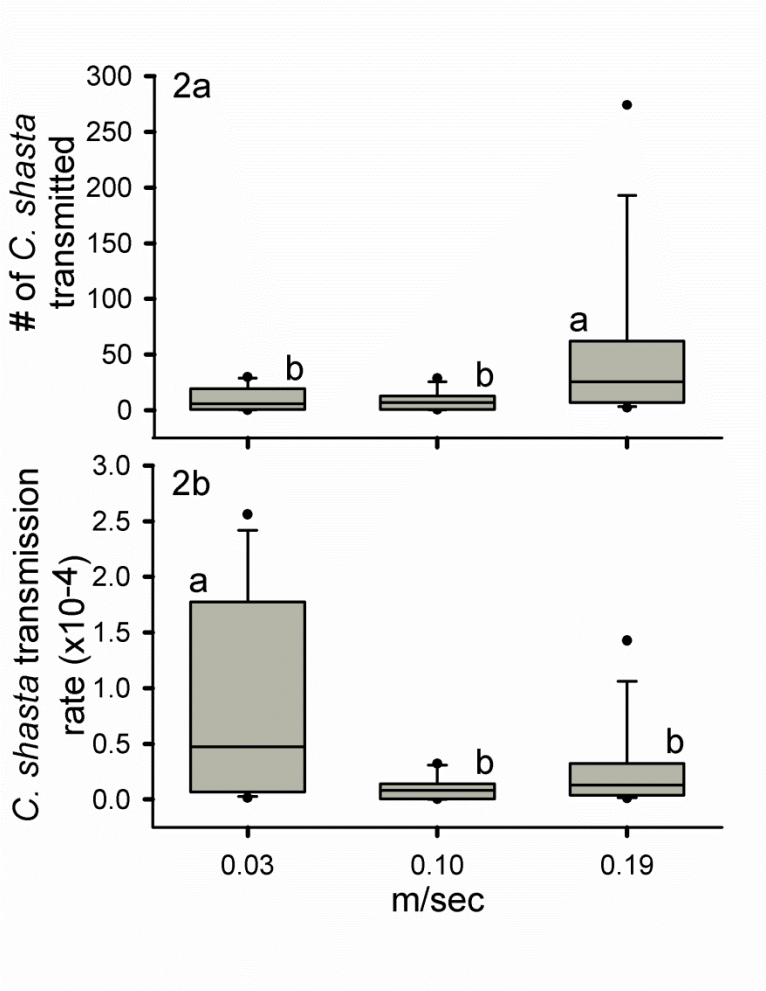

Fig. 3.2. The number of *Ceratomyxa shasta* transmitted (a) and transmission rates (b) for Chinook salmon (*Oncorhynchus tshawytscha*) exposed to the parasite at three different velocities (0.03, 0.10, and 0.19 $m \cdot sec^{-1}$) in the Klamath River. The solid lines are the medians, the boxes cover the inter-quartile range, the bars the 5% to 95 % ranges, and the dots are outside two standard errors. Letters represent statistically significant differences detected by Tukey comparison of means ($\alpha = 0.05$).

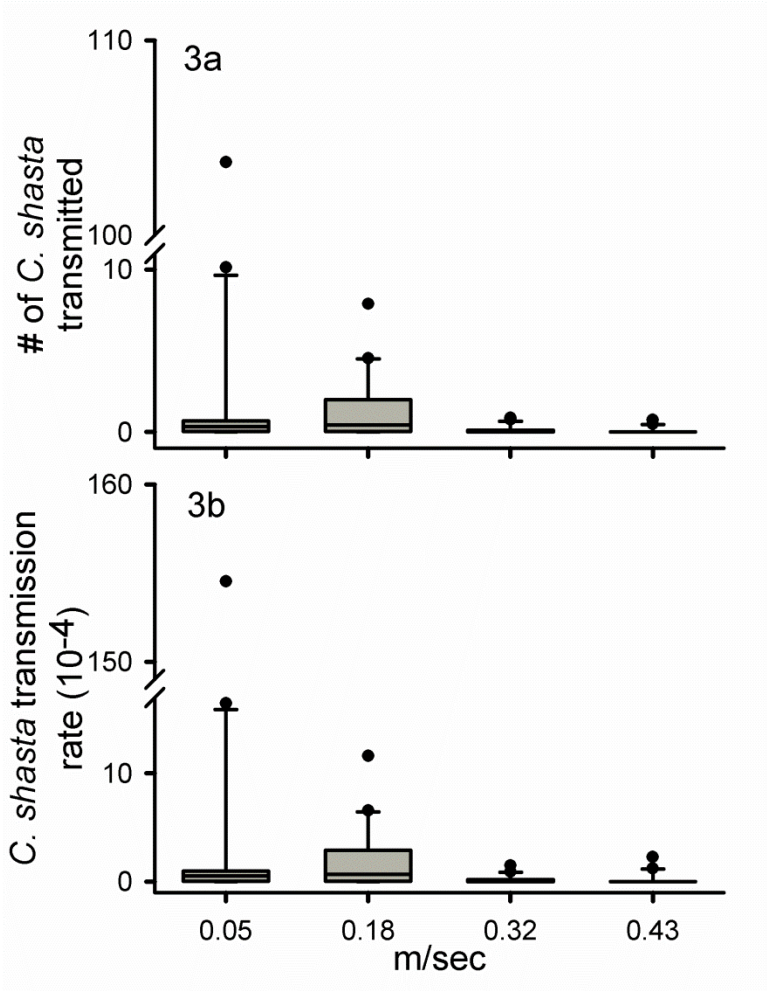

Fig. 3.3. The number of *Ceratomyxa shasta* transmitted (a) and transmission rates (b) for Chinook salmon (*Oncorhynchus tshawytscha*) at four different velocities (0.05, 0.18, 0.32, and 0.43 $m \cdot sec^{-1}$) in a laboratory challenge. The solid lines are the medians, the boxes cover the inter-quartile range, the bars the 5% to 95 % ranges, and the dots are outside two standard errors.

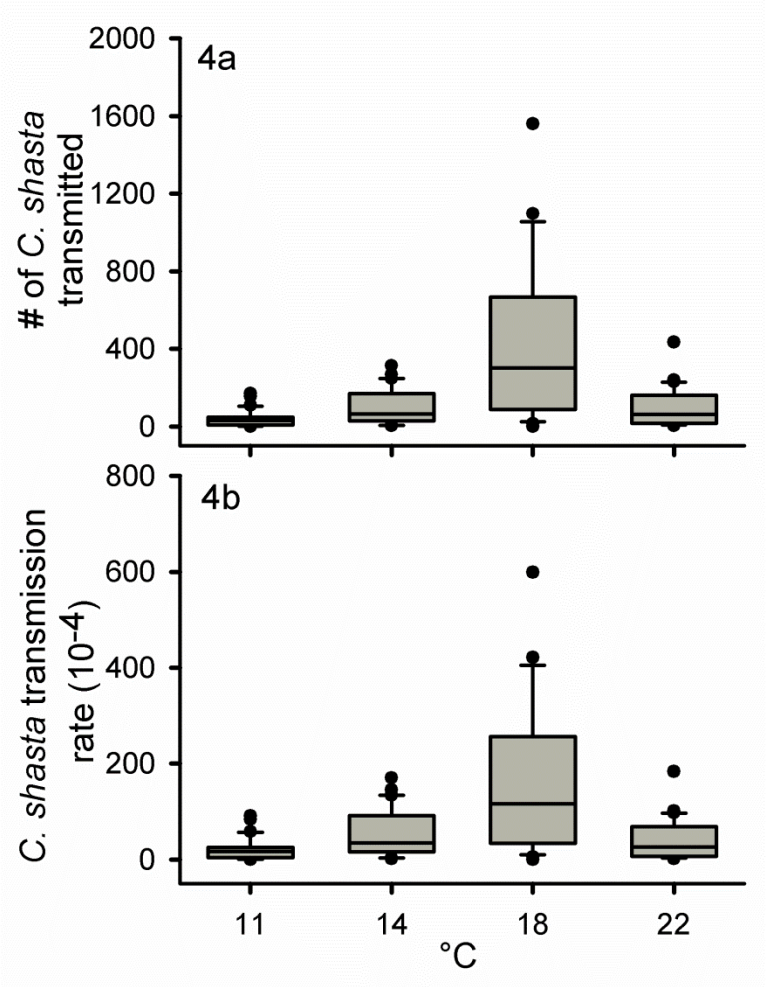

Fig. 3.4. The number of *Ceratomyxa shasta* transmitted (a) and transmission rates (b) for rainbow trout (*Oncorhynchus mykiss*) at four different temperatures (11, 14, 18, and 22 °C) in a laboratory challenge. The solid lines are the medians, the boxes cover the inter-quartile range, the bars the 5% to 95 % ranges, and the dots are outside two standard errors.

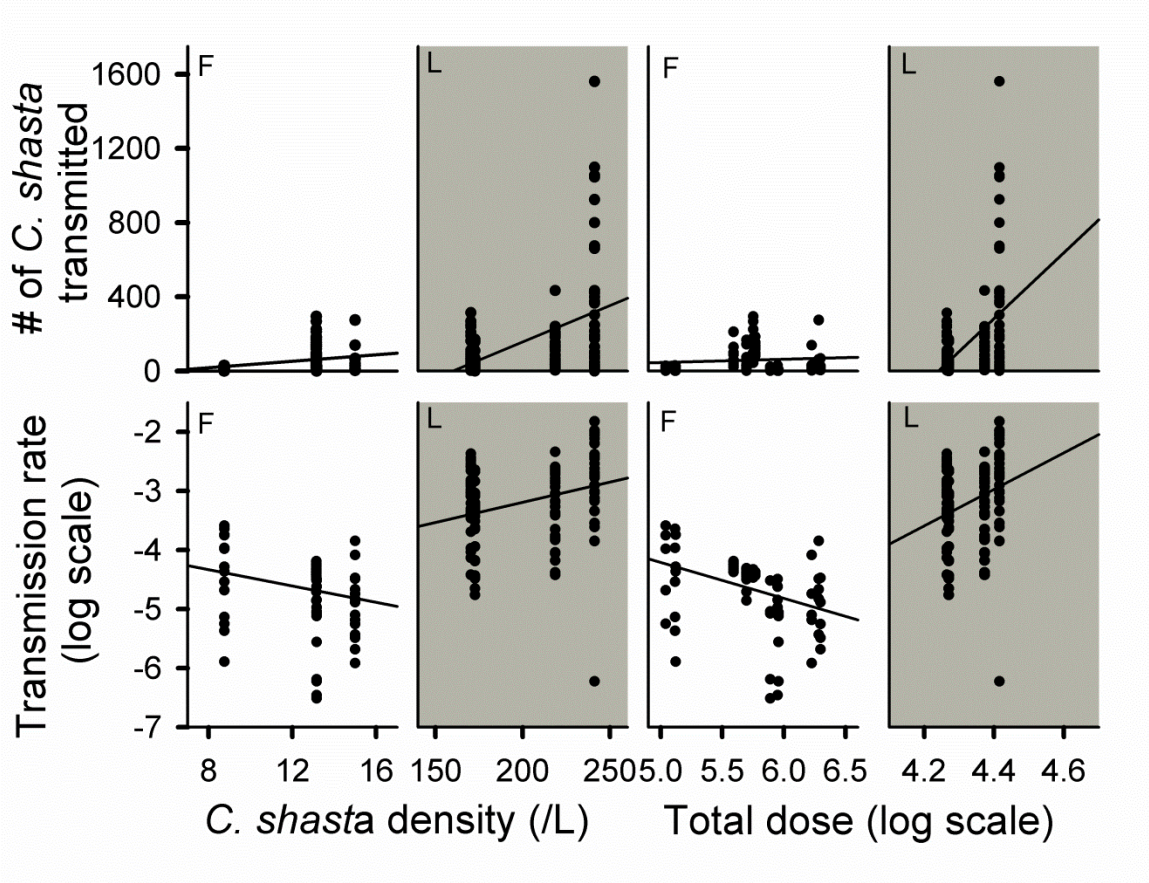

Fig. 3.5. Comparison of parasites transmitted (top row) and transmission rate (bottom row) with parasite density (left) and log total dose (right) for all 3h field (F) and laboratory (L) exposures.

CHAPTER 4: USING AN EPIDEMIOLOGICAL MODEL TO QUANTIFY SENSITIVITY OF MYXOZOAN DISEASE DYNAMICS TO DEMOGRAPHIC PARAMETERS: APPLICATION TO *CERATOMYXA SHASTA* IN THE KLAMATH RIVER.

R. Adam Ray and Jerri L. Bartholomew

Formatted for Ecological Modeling

ABSTRACT

Myxozoan parasites cause disease in a wide variety of cultured and wild fish populations. These parasites have a complex life cycle involving a vertebrate host (fish), an invertebrate host (annelid), and two spore stages (actinospore and myxospore). As no vaccines or chemotherapeutants exist for these parasites, epidemiological models can provide critical insights about management actions capable of disrupting disease dynamics. By deriving an analytical expression for the basic reproductive number (R_0), sensitivity analyses can be used to evaluate management actions on the epidemiological parameters that reduce R_0. In the Klamath River, CA, USA the myxozoan parasite *Ceratomyxa shasta* is linked to decreased returns of adult Chinook salmon. This host-parasite relationship is unique due to the anadromous and semelparous life history traits of the salmon host and the ability of the parasite to infect spatially and temporally distinct life stages of that host. We develop an epidemiological model of the *C. shasta* life cycle and conduct a sensitivity analysis to identify parameters that may be sensitive to potential management actions. We demonstrate that reducing the transmission rate of the myxospore (β_M) from the adult salmon to the invertebrate host population during the winter is one of the most influential parameters on R_0. In contrast, reducing the densities of either the polychaete or adult salmon host alone had the smallest effect on R_0. This model can be applied to other systems affected by myxozoan parasites and possibly for other pathogens involving complex life cycles in aquatic systems.

KEYWORDS

Epidemiological model, Sensitivity analysis, Parasites (Myxozoan), Chinook salmon

INTRODUCTION

Containment, control, and elimination of wildlife diseases are central goals for many wildlife managers (Heisey *et al.*, 2006). One of the challenges is identifying the most effective and efficient approach, especially when considering that control measures are often controversial (e. g. culling, sterilization) or provide mixed results (Lloyd-Smith *et al.* 2005). Vaccines are effective against certain wildlife pathogens (e.g. rabies, Buddle *et al.*, 2011; *Mycobacterium bovis*, Cleaveland *et al.*, 2003); however programs to apply these treatments are often cost prohibitive. Controlling diseases of aquatic wildlife poses additional challenges, as the underlying cause of an outbreak is often difficult to identify (Subasinghe, 2005). While vaccines and chemotherapeutants are available to control some pathogens of cultured aquatic animals (www.fws.gov/fisheries/aadap/home.htm, accessed 07/01/2013), these options are often infeasible in wild fish populations and therefore alternative control measures must be identified.

Epidemiological models are a commonly used tool to investigate complex interactions between a host and pathogen and can be used to evaluate the efficacy of different control measures (May and Anderson, 1991). One of the most important concepts in disease ecology derived from these models is the basic reproductive number (R_0), defined as the expected number of secondary infections caused by the introduction of an infected individual into an entirely susceptible population (Heesterbeek and Dietz, 1996). R_0 has an inherent threshold value of one, below which the pathogen is unable to persist within a host population (Dietz, 1993). Given an analytical expression for R_0 derived from an epidemiological model, control measures that drive R_0 below one can be identified, leading to insights about actions that may prevent or disrupt an epidemic (Diekmann *et al.*, 2010; Heffernan *et al.*, 2005). Originally

applied to human pathogens, these models are now being applied to pathogens of fish and wildlife populations (Dobson and Foufopoulos, 2000; Murray, 2009; Reno, 1998).

Myxozoan parasites infect a wide range of marine and freshwater fishes (Kent *et al.*, 2001; Lom and Dyková, 2006; Yokoyama *et al.*, 2012). Although most are not problematic for their fish host, infections by some species cause severe disease. In cultured populations of channel catfish (*Ictalurus punctatus*), *Henneguya ictaluri,* the cause of proliferative gill disease, causes high mortality and economic loss (Beecham *et al.*, 2010; Pote *et al.*, 2000; Pote *et al.*, 2011). In the marine environment, *Enteromyxum leei* and *Kudoa thyrsites* adversely affect a wide range of cultured and wild caught species (Diamant, 1997; Moran *et al.*, 1999; Yasuda *et al.*, 2002). Globally, *Myxobolus cerebralis* and *Tetracapsuloides bryosalmonae* affect cultured and wild populations of rainbow trout (*Oncorhynchus mykiss*) and other salmonid species (Hedrick, 1998; Canning *et al.*, 1999; Okamura *et al.*, 2010). In the Pacific Northwest of North America, *Ceratomyxa shasta* and *Parvicapsula minibicornis* infect several species of cultured and wild salmon (*Oncorhynchus spp*.; Bartholomew, 1998; Buchanan *et al.*, 1983; Jones *et al.*, 2003; Zinn *et al.*, 1977). High mortality in returning adult sockeye salmon (*O. nerka*) in the Frasier River, B.C. Canada (Jones *et al.*, 2003) has been directly attributed to infection by *P. minibicornis,* while decreased adult Chinook (*O. tshawytscha*) salmon returns in the Klamath River, USA have been linked to *C. shasta*-induced mortality in emigrating juveniles (Fujiwara *et al.*, 2011).

Although < 2% (~25/2100) of myxozoan life cycles have been described, there is a consistent pattern involving a vertebrate (fish) host, an invertebrate (annelid) host, and two spore forming stages (actinospore and myxospore; Kent *et al.*, 2001). The inability to grow these parasites in culture has impeded development of control measures such as vaccines or other

chemotheraputants. Therefore, to minimize the population level effects of these parasites management actions should focus on disrupting their complex life cycle (Yokoyama *et al.* 2012). Epidemiological models provide an approach for identifying and evaluating potential control measures by depicting how changing specific factors can influence the infection dynamics between the parasite and its host.

In this paper, we present a biological and mathematical description of the *C. shasta* life cycle, define equations for R_0, and evaluate the sensitivity of R_0 to changes in certain parameters. Altering parameter values is a proxy used to represent potential management actions aimed at reducing the effect of this parasite on Klamath River Chinook salmon populations. This model may also be applied to other myxozoan life cycles to evaluate management actions to reduce the population level effects of these parasites.

Life cycle

The *C. shasta* life cycle involves a salmonid host, a polychaete (*Manayunkia speciosa* Leidy) invertebrate host, and two spore stages (Fig. 4.1; Bartholomew *et al.*, 1997). Infected polychaetes release the actinospore stage through mucus pores into the water column (Bartholomew *et al.*, 1997; Meaders, 2008). The actinospore stage is transmitted to the salmonid host when it penetrates the gill epithelium (Bjork and Bartholomew, 2010). The parasite proliferates as it migrates through the circulatory system to the intestinal tissue, where it continues to multiply and mature into the myxospore stage (Bjork and Bartholomew, 2010). Severe infections result in enteronecrosis and mortality of both juvenile and adult salmon (Hallett *et al.*, 2012; Hendrickson *et al.*, 1989) and the myxospore stage is shed into the water column (upon the death of the salmon host), where it is ingested by the polychaete. The

dynamics of this complex host–pathogen cycle are further complicated by the anadromous life cycle of the salmon host.

In the Klamath River, CA, juvenile Chinook salmon emigrate over 300 km from Iron Gate Dam (a barrier to salmon migration) to the Pacific Ocean. During their spring migration, these fish pass through a river reach where high densities of parasites are consistently observed (Hallett *et al.*, 2012). Juvenile Chinook appear tolerant of this parasite, as mortality does not become significant (> 40%) until densities exceed 10 parasites/L (Hallett *et al.*, 2012). Infection prevalence in the out-migrant salmon population varies annually, but is typically less than 40% (Bolick *et al.*, 2012).

After rearing in the ocean for ~2–5 years, adult salmon return uninfected and encounter the parasite during their upstream migration (Slezak, 2009). Although parasite densities in the water are decreased in the fall, infection prevalence in adult salmon is > 80%, presumably owing to their weakened immune systems (Foott *et al.*, 2009). Despite this high infection prevalence, mature myxospore stages are observed only in naturally spawned carcasses (Slezak, 2009) and there is no evidence of parasite-induced pre-spawn mortality (Fujiwara *et al.*, 2011). Consequently, infected adult carcasses are the most likely source of myxospores in the system as they serve as a vehicle to return the parasite to the "top" of the system, Iron Gate Dam.

There is limited demographic information for the polychaete and even less information on the infection dynamics between the parasite and the invertebrate host (Meaders and Hendrickson, 2009; Stocking and Bartholomew, 2007). The polychaete host population structure and infection prevalence fluctuate seasonally (Jordan, 2012). In the summer and early fall, polychaete populations are comprised primarily of juvenile and immature stages and occur at

higher densities than in the winter. In the winter and early spring, adult stages (>3mm) are dominant, but overall densities are low. Prevalence of infection in the polychaete host is generally < 2% (Bartholomew, unpublished data), but infection prevalence >8% has been observed in at least one population (Stocking and Bartholomew, 2007).

MODEL DEVELPOMENT

Mathematical model description

To address the spatially and temporally distinct interactions between *C. shasta* and the juvenile and adult salmonid hosts we expand the model introduced by Ray *et al.* (2010) to include a total of eight differential equations. We let $A_{sp}(t)$ denote the actinospore stage in the water column during the spring, $A_c(t)$ represent the actinospore stage in the juvenile salmonid host, $M_{su}(t)$ denote the myxospore stage in the water column during the summer, $M_{Ps}(t)$ represent the myxospore stage in the polychaete host during the summer, $A_f(t)$ denote the actinospore stage in the water column during the fall, $A_C(t)$ represent the actinospore stage in the adult salmonid host, $M_w(t)$ denote the myxospore stage in the water column during the winter, and $M_{Pw}(t)$ represent the myxospore stage in the polychaete host during the winter (equations 1–8). The parameters and values used in these equations are described in section 2.1–2.4 and also defined in Table 4.1.

$$\frac{dA_{sp}}{dt} = \theta_{Asp}P_w - \gamma_{sp}A_{sp} - \beta_{cA}cA_{sp} \quad (1)$$

$$\frac{dA_c}{dt} = \beta_{cA}cA_{sp} - (\pi_c + \delta_c + \eta_c)c \quad (2)$$

$$\frac{dM_{su}}{dt} = \lambda_{cM}c - \gamma_{Ms}M_{su} - \beta_{cM}P_S M_{su} \quad (3)$$

$$\frac{dM_{Ps}}{dt} = \beta_{cM}P_S M_s - \left(\mu_{\mathrm{Ps}} + \varepsilon_{\mathrm{Ps}} + \nu_{Ps}\right)P_s \quad (4)$$

$$\frac{dA_f}{dt} = \theta_{Af}P_s - \gamma_f A_f - \beta_{CA}CA_f \quad (5)$$

$$\frac{dA_C}{dt} = \beta_{CA}\mathrm{C}A_f - (\pi_C + \delta_C + \eta_{\mathrm{C}})C \quad (6)$$

$$\frac{dM_{\mathrm{w}}}{dt} = \lambda_{CM}C - \gamma_{Mw}M_w - \beta_{CM}P_w M_w \quad (7)$$

$$\frac{dM_{Pw}}{dt} = \beta_{CM}P_w M_w - \left(\mu_{\mathrm{Pw}} + \varepsilon_{\mathrm{Pw}} + \nu_{\mathrm{Pw}}\right)P_w \quad (8)$$

These differential equations represent the transmission (β) rates between parasite (A and M) and hosts (c, C, P_s, and P_w), production rates of the free-living spore stages (θ and λ), and instantaneous mortality rates of the hosts (π, δ, η, ν, μ, and ε) and spore stages (γ_A and γ_M) (Dobson 1988). For example, equation 1 describes the rate of production of actinospores in the spring from polychaetes infected in the winter ($\theta_{Asp}P_w$), less those actinospores that die before encountering and transmitting to a salmon host ($\gamma_{sp}A_{sp}$) and those that are successfully transmitted ($\beta_{cA}cA_{sp}$).

To derive expressions for R_0, we first divide the system of equations into two biologically unique host-pathogen interactions: 1) *C. shasta* interacting with the juvenile salmonid host (eqs 1, 2, 7, and 8), and 2) *C. shasta* interacting with the returning adult host (eqs 3–6). We then mathematically evaluate each group of equations using vector notation as described by Van den

Driessche and Watmough (2002). Vector f represents the production of infective stages (actinospore or myxospore) and infected hosts and v is the rate of transfer of infective stages (actinospore or myxospore) or hosts from one step of the life cycle to the next. The juvenile salmon–parasite (9) and adult salmon–parasite (10) interactions are rewritten as:

$$\frac{d}{dt}\begin{bmatrix} A_{sp} \\ A_c \\ M_w \\ M_{Pw} \end{bmatrix} = f - v = \begin{bmatrix} \theta_{Asp} P_w \\ \beta_{cA} c A_{sp} \\ \lambda_{CM} C \\ \beta_{CM} P_w M_w \end{bmatrix} - \begin{bmatrix} \gamma_{sp} A_{sp} + \beta_{cA} c A_{sp} \\ (\pi_c + \delta_c + \eta_c) c \\ \gamma_{Mw} M_w + \beta_{CM} P_w M_w \\ (\mu_{Pw} + \varepsilon_{Pw} + \nu_{Pw}) P_w \end{bmatrix} \tag{9}$$

$$\frac{d}{dt}\begin{bmatrix} M_{su} \\ M_{Ps} \\ A_f \\ A_C \end{bmatrix} = f - v = \begin{bmatrix} \lambda_{cM} c \\ \beta_{cM} P_S M_s \\ \theta_{Af} P_s \\ \beta_{CA} C A_f \end{bmatrix} - \begin{bmatrix} \gamma_{Ms} M_{su} + \beta_{cM} P_S M_{su} \\ (\mu_{Ps} + \varepsilon_{Ps} + \nu_{Ps}) P_s \\ \gamma_{Af} A_f - \beta_{CA} C A_f \\ (\pi_C + \delta_C + \eta_C) C \end{bmatrix} \tag{10}$$

We next derive the corresponding Jacobian matrices, **F** (11 and 13) and **V** (12 and 14), which describe the linearization of these systems about a disease-free equilibrium where the population persists in the absence of disease (Van den Driessche and Watmough, 2002).

$$\boldsymbol{F_j} = \begin{bmatrix} 0 & 0 & 0 & \theta_{Asp} \\ \beta_{cA} c & 0 & 0 & 0 \\ 0 & \lambda_{CM} C & 0 & 0 \\ 0 & 0 & \beta_{CM} P_w & 0 \end{bmatrix} \tag{11}$$

$$\boldsymbol{V_j} = \begin{bmatrix} \gamma_{sp} + \beta_{cA} c & 0 & 0 & 0 \\ 0 & \pi_c + \delta_c + \eta_c & 0 & 0 \\ 0 & 0 & \gamma_{Mw} + \beta_{CM} P_w & 0 \\ 0 & 0 & 0 & \mu_{Pw} + \varepsilon_{Pw} + \nu_{Pw} \end{bmatrix} \tag{12}$$

$$\boldsymbol{F_a} = \begin{bmatrix} 0 & 0 & 0 & \lambda_{cM} c \\ \beta_{cM} P_S & 0 & 0 & 0 \\ 0 & \theta_{Af} & 0 & 0 \\ 0 & 0 & \beta_{CA} C & 0 \end{bmatrix} \tag{13}$$

$$\boldsymbol{V_a} = \begin{bmatrix} \gamma_{Ms} + \beta_{cM}P_S & 0 & 0 & 0 \\ 0 & \mu_{Ps} + \varepsilon_{Ps} + \nu_{Ps} & 0 & 0 \\ 0 & 0 & \gamma_{Af} + \beta_{CA}C & 0 \\ 0 & 0 & 0 & \pi_C + \delta_C + \eta_C \end{bmatrix} \quad (14)$$

The dominant eigenvalue of the next generation matrix for these systems ($\mathbf{FV}^{-1}$) is used to define R_0 (15 and 16; Van den Driessche and Watmough, 2002).

$$R_0^{juvenile} = \sqrt[4]{\frac{\theta_{Asp} * \beta_{cA}c * \lambda_{CM}C * \beta_{CM}P_w}{(\gamma_{sp} + \beta_{cA}c) * (\pi_c + \delta_c + \eta_c) * (\gamma_{Mw} + \beta_{CM}P_w) * (\mu_{\mathrm{Pw}} + \varepsilon_{\mathrm{Pw}} + \nu_{\mathrm{Pw}})}} \quad (15)$$

$$R_0^{adult} = \sqrt[4]{\frac{\lambda_{cM}c * \beta_{cM}P_S * \theta_{Af} * \beta_{CA}\mathrm{C}}{(\gamma_{Ms} + \beta_{cM}P_S) * \left(\mu_{\mathrm{Ps}} + \varepsilon_{\mathrm{Ps}} + \nu_{Ps}\right) * \left(\gamma_{Af} + \beta_{CA}C\right) * (\pi_C + \delta_C + \eta_{\mathrm{C}})}} \quad (16)$$

In general, R_0 equations produced by next generation models are presented as a square root function because of the two generations required for an infected host or infected vector to complete the life cycle (Van den Driessche and Watmough, 2002). However, in our system both R_0 equations appear as a fourth root function to account for the two hosts and the two spore stages required for completion of each portion of the life cycle.

Parameter values and sensitivity analysis

Host density parameters

We assume that host densities remain relatively constant among years, but that the densities of hosts in relation to each other vary by orders of magnitude. Iron Gate Hatchery produces and releases ≈5 x 10^6 juvenile Chinook salmon (c) in May/June. From the confluence with the Shasta River to the confluence with the Scott River (~24km), we assume a density of 1 x 10^6 fish /24km during this time period. For our analysis we set juvenile Chinook salmon

densities at 1 and scale the other host densities relative to this value. Estimates for numbers of adult salmon (C) spawning in the main stem Klamath River below Iron Gate Dam to the Shasta River from 2002-2010 averaged ≈1 x 10^3 (Klamath Basin Megatable, https://nrm.dfg.ca.gov/documents/ContextDocs.aspx?cat=KlamathTrinity accessed 07/20/2013). Estimates for polychaete densities were based the range of densities observed at sites located on the Klamath River mainstem between the confluence with the Shasta and Scott rivers by Stocking and Bartholomew (2007) and Jordan (2012). For our estimates the winter and summer polychaete populations we multiply the area of our reach of interest (≈24km long and ≈50m wide =1.2 x 10^6 m^2) by the densities during the respective seasons,10–100/m^2 (winter) and 1000–10000/m^2 (summer; Jordan, 2012). We approximate the winter polychaete (P_w) population in this reach at 10^7 or 10-fold the juvenile salmon population and the summer polychaete (P_s) population at 10^9 or 1000-fold the juvenile salmon population.

Transmission parameter values

The transmission rate of the actinospore to juvenile Chinook salmon (β_c) was estimated from a 3-hr exposure in the Klamath River (Ray and Bartholomew, 2013). Although the authors observed a non-linear transmission rate during a 6-hr exposure duration, interpretation of these results were confounded by fluctuating parasite densities during the exposure period and by in-host parasite replication. Transmission is most likely constant due to the passive interactions between the actinospore and salmon host, but is influenced by varying in-river parasite densities. Therefore, we assumed this rate was constant and converted it to a daily transmission rate. Due to a lack of data and the expectation of a similar passive transmission process to the adult salmon host; we assume the same value for transmission to adult salmon (β_C).

Transmission rates (β_M) of the myxospore to the polychaete have not been directly quantified, and we therefore assume this transmission rate to be equal to the actinospore transmission rate. This assumption is based on the hypothesis that the ratio of myxospore production from the salmon host (high) to the polychaete densities (high) is similar to that of the actinospore production from the polychaete host (low) and the densities of the salmon host (low).

Mortality parameter values

Daily mortality rates are calculated from the inverse of the life expectancy at each stage. In the Klamath River, the juvenile daily instantaneous mortality rate (δ_c) was assumed to be 0.0136 (Bradford, 1995; Hendrix *et al.*, 2011), but can range between $0.3 – 2.3 \text{ X } 10^{-2}$ (Connor *et al.*, 2003; Quinn, 2011). Daily parasite induced mortality rates (π_c) for juveniles were based on sentinel surveys conducted in the Klamath River upstream from the confluence with Beaver Creek (Hallett *et al.*, 2012). Although the overall mortality varies among years, Ray *et al.* (2012) observed that the mortality rate only varied among temperatures. For this analysis we use the mortality rate observed at 18°C, which is representative of the Klamath River during emigration of hatchery juveniles (Hallett *et al.*, 2012; Ray *et al.*, 2012). We assume that both natural (δ_C) and parasite-induced (π_C) mortality of adult salmon have minimal effect on the production and release of *C. shasta,* as all adult salmon die after spawning and death is required for release of the myxospore stage (Fujiwara *et al.*, 2011).

We define the parameter parasite mortality in the salmon host (η_c and η_C) as the ability of the salmon host to stave off the initial infection. For juvenile salmon, there is a consistent pattern of increased mortality as parasite densities exceed 10 parasite/L (Hallett and Bartholomew, 2006; Hallett *et al.*, 2012). Therefore we assume that parasite mortality rate in the

juvenile salmon host (η_c) is 0.1. We assume no parasite mortality in the adult salmon host (η_C) as infection prevalence is generally >80%. This high infection prevalence suggests that the adult salmon are unable to cope with the initial infection, possibly due to their weakened immune system.

Willson *et al.* (2010) provided evidence that *M. speciosa* has an annual life cycle. Adult polychaetes dominate the population during the winter and early spring and produce offspring in the late spring/early summer. We therefore have two values for the natural mortality rate for polychaete hosts that are defined by the season and overlap with each salmon host. In winter we assume a polychaete has a life expectancy of ≈90 days, which correlates to a daily mortality rate (ε_w) of 1/90 days. This is based on the winter population being mostly adults that reproduce and die in the spring. In summer we assume the polychaete has a life expectancy of ≈300 days and therefore a daily mortality rate (ε_s) of 1/300 days. This population is the offspring of the previous winter adult population and will mature throughout the summer. We assume no parasite-induced mortality in the polychaete host (μ_w and μ_s) as the parasite is discharged through mucus pores in the body wall resulting in minimal damage to this host (Bartholomew *et al.*, 1997; Meaders, 2008).

We define parasite mortality within the polychaete host (ν_w and ν_s) the same as in the salmon host: the ability to stave off initial infection. Based on evidence of a sigmoid relationship for *M. cerebralis* described by Elwell *et al.* (2009), who demonstrated 100% prevalence of infection in the invertebrate host when exposed to >10 myxospores, we assume a similar relationship between *C. shasta* and the polychaete host.

The longevity of both spore stages is highly correlated to temperature (Bjork, 2010; Chiaramonte, 2013; Foott *et al.*, 2007). Bjork (2010) observed that actinospores remain viable for ~ 15 days at 12°C. We use this estimate for both the spring (γ_a) and fall (γ_A) actinospore mortality rates. Chiaramonte (2013) observed that myxospores remain viable for ~ 30 days at ~20°C and >150 days at ~4°C. We use these estimates for myxospore mortality rates in the summer (γ_m) and winter (γ_M), respectively.

Production parameter values

The estimate of total myxospore production from juvenile salmon was $\approx 5 \times 10^5$ and ranged from $<1 \times 10^3$–>1×10^6 (Ray, 2009). Estimates of myxospores in adult salmon ranged from 0 to 10^7; we therefore set production to $\approx 3.5 \times 10^5$ (Fogerty *et al.* 2012; Foott *et al.* 2009, 2010, 2013). Myxospore release from juvenile salmon carcasses occurred over 20 days (Hendrickson and Benson, 2011). We set the daily myxospore production rate for both juvenile (λ_c) and adult (λ_C) salmon by dividing the production estimates by the observed 20 day release period and assume release is equal over this time. Meaders and Hendrickson (2009) estimated actinospore production (θ) in the polychaete host to be $>1.3 \times 10^3$ over a 14 day period, which we assume for both winter/spring and summer/fall populations.

Developmental delay parameter

Developmental delay (ψ) of *C. shasta* occurs after the host has become infected, but before the development of the next stage. For example, a delay occurs between the time a polychaete ingests a myxospore and the time when actinospores are released. During this delay period, parasites are subject to both natural and parasite-induced host mortalities (Dobson and Hudson, 1992). Therefore prolonging development time can result in higher parasite mortality,

potentially decreasing the likelihood that the parasite becomes established in a host population. For each R_0 equation there are two points in the life cycle where hypobiosis can occur: actinospores in the polychaete host (ψ_A, 17) and myxospores in the salmon host (ψ_M, 18). Each parameter is incorporated into the numerator for each R_0 equation, but we separately analyze the sensitivity of R_0 to these parameters. Developmental delay is implemented as follows:

$$\psi_A = \exp [- (\mu + \varepsilon + \nu) * \text{days}] \quad (17)$$

$$\psi_M = \exp [- (\pi + \delta + \eta) * \text{days}] \quad (18)$$

Sensitivity analysis

Due to the complexity of this life cycle and existing data gaps, we conduct a sensitivity analysis of R_0 with respect to changes in different model parameters values (Table 1) that could arise as a result of potential management actions. Sensitivity analyses quantify changes in R_0 in response to changes in the input parameter values (Iman and Helton, 1988). Most parameter values in Table 1 are obtained from studies conducted in the reach of the Klamath River between the confluences of Shasta River and Beaver Creek (≈24 Rkm), where high parasite densities, polychaete infection prevalence, and parasite induced mortality were observed (Hallett and Bartholomew, 2006; Hallett *et al.*, 2012; Stocking and Bartholomew, 2007).

We evaluate the sensitivity of R_0 to changes in the values of six different parameters: 1) reducing polychaete populations (P), 2) reducing adult salmon carcass density (C), 3) decreasing actinospore transmission rates to the salmon host (β_A), 4) decreasing myxospore transmission rates to the polychaete host (β_M), 5) the effect of developmental delay for both the spore stages (ψ_A and ψ_M), and 6) decreasing both winter polychaete densities (P_w) and myxospore

transmission (β_M). These parameters were selected based on their potential for be altered by proposed management actions.

RESULTS

In both equations the R_0 values are insensitive to reductions in polychaete density (Fig. 4.2). To achieve $R_0 < 1$ for both winter and summer populations of the invertebrate host would need to be reduced 100-fold (Figs. 4.2a and 4.2b). In addition there is a relatively gentle slope in the response until the densities are ≈10% of the original population. This finding indicates that reducing polychaete populations is not an ideal parameter for reducing disease in either equation as it requires almost complete removal of the invertebrate host. Reducing adult salmon densities had a similar effect on R_0 as did reducing polychaete host density (Fig. 4.3). R_0 does decrease with decreasing adult salmon densities; however, almost complete removal of all carcasses is required in order to drive R_0 below the threshold value of 1.

The responses of R_0 in both equations reacted similarly to decreases in actinospore transmission (β_A; Fig. 4.4), but there were differences between the juvenile and adult responses to decreases in myxospore transmission (β_M; Fig. 4.5). Both the juvenile and adult R_0 values responded similarly to decreasing actinospore transmission rate. To achieve an equivalent decrease for the juvenile R_0 value required a greater reduction in transmission rate than the adult R_0. The juvenile R_0 value had a steeper response to decreasing myxospore transmission rate than the adult R_0, which had an almost linear response (Fig. 4.5). As the juvenile R_0 is based on transmission of myxospores from adults to the less dense winter polychaete population, decreasing the transmission rate over the winter has a greater effect on reducing disease than in the summer even though polychaete densities are greater. To achieve $R_0 < 1$ requires at least two

orders of magnitude decrease in either transmission rate for both the juvenile and adult equations.

The developmental delay parameter (ψ) produced the steepest response on the R_0 values (Fig. 4.6). The lone exception is that the delay of the myxospores from the adult had no effect on the juvenile R_0 equation as adult mortality has little effect on the *C. shasta* life cycle. We therefore assess the delay effect on actinospore development at current spring temperatures (14°C) and elevated spring temperatures (21°C, Fig. 4.6a). At current temperatures, a delay of ≈60 days in actinospore development and transmission resulted in $R_0 < 1$, and at elevated temperatures the delay effect was reduced by one third (≈45 days) for $R_0 < 1$. R_0 values for the adult equation responded almost identically for delays of either actinospore or myxospore development and transmission; however a shorter delay of myxospore production (≈25 days) than actinospore production (≈40 days) was required for $R_0 < 1$ (Fig. 4.6b).

Altering the value of two parameters simultaneously, winter polychaete densities (P_w) and myxospore transmission rate (β_M) did not have any multiplicative or synergistic effect on the juvenile R_0 values (Fig. 4.7). To achieve $R_0 = 1$ requires almost 50% reduction in both the polychaete densities and transmission rates. As with the previous polychaete examples, almost complete removal is required to have an effect on R_0, unless the transmission rate is essentially zero.

DISCUSSION

We present an epidemiological model for *C. shasta* and demonstrate the relative sensitivity of R_0 to different parameters that could be affected by potential management actions. Although epidemiological models exist for other aquatic pathogens, these models have been

developed only for directly transmitted pathogens in cultured settings or under laboratory experimental conditions (Georgiadis *et al.*, 2001; Ögüt, 2003). In contrast, we develop and evaluate an epidemiological model for the complex life cycle of a myxozoan parasite in a natural system and provide a framework within which to evaluate disease dynamics in other myxozoan parasite systems. We then examine the effect of decreasing values of parameters that could be influenced by management actions. In our model, developmental delay was the most influential parameter on R_0; however, it is also the most challenging to modify as it would require a large and prolonged decrease in temperature. Of the parameters we analyzed, the next most sensitive was the transmission rate of the myxospore from the adult salmon to the winter polychaete population. In a managed system, such as the Klamath River, this parameter could be affected by intentional releases of water after peak spawning has occurred.

Because the complex life cycle of *C. shasta* involves essentially three hosts (invertebrate, adult salmon, and juvenile salmon) over a long temporal scale, our model is a simplification of the actual dynamics. One simplifying assumption is that adult salmon myxospore production is constant among individuals, whereas estimates of myxospore production obtained from carcass surveys indicate that a small proportion of the adults produce a majority of the myxospores (Foott *et al.* 2013). This pattern is often observed in macroparasite infections, leading Woolhouse *et al.* (1997) to propose the "20/80" rule, where 20% of the hosts are responsible for 80% of transmissions. Data supports this in the Klamath River, as 3–9% of adult carcasses each contribute > 5×10^6 myxospores into the system (Foott *et al.* 2013). Unfortunately, methods for identifying these "high contributor" carcasses are not available for use in the field. To significantly reduce the input of myxospores and consequently R_0, a successful management action would require removing almost all the carcasses from the river. A similar conclusion was

reached from a multi-year study where adult carcasses were removed from one section of Bogus Creek (a Tributary to the Klamath River) in an attempt to detect differences in myxospore production; however the scientists were unable to detect any such difference (Fogerty *et al.* 2012; Foott *et al.* 2009, 2010, 2013).

For most parasites, parasite-induced host mortality may be undesirable as the premature death of a host theoretically removes parasites from the system. As salmon have a semelparous life history and the adults will die regardless of infection status, mortality of the vertebrate host may actually be favored for *C. shasta* spore production and dispersal. In the Klamath system, there is no evidence of *C. shasta*-induced mortality of returning adults (Fujiwara *et al.*, 2011); however in other systems this parasite has been linked to pre-spawning mortality (Ching and Munday, 1984; Ratliff, 1981; Zinn *et al.*, 1977). Understanding the factors that determine myxospore development and release from the adult carcass may help to focus management actions on areas where high concentrations of myxospores and dense populations of polychaete are likely to overlap.

Parasite transmission rates are one of the more difficult parameters to quantify and incorporate into a model. For our model we had to make two assumptions about the transmission dynamics. First, we assumed that the rate of myxospore transmission (fish to polychaete) was equal to the rate of actinospore transmission (polychaete to fish). The spatial overlap between adult carcasses and polychaete populations' is likely the determining factor of a myxospore successfully infecting a polychaete after release from the adult salmon. We hypothesize that successful transmission of the myxospore stage may be a function of a carcass settling near a polychaete population and directly depositing spores on or near that population. Strobel *et al.* (2009) observed only minor (≈1km) movement of salmon carcasses in Clear Fork

Sandy River and Clear Creek, OR, but the discharge of these streams was ≈10-fold less than the minimum flow in the Klamath River. Thus, to test our hypothesis we suggest tagging and monitoring the movement of carcasses in the main stem Klamath River and comparing the infection prevalence between polychaete populations where carcasses settled and populations with no carcasses. Second, we assumed a linear function for the transmission parameters. A sigmoidal response was observed between myxospore dose and prevalence of infection in *Tubifex tubifex* with *M. cerebralis* (Elwell *et al.*, 2009). Logan *et al.* (2012) also utilized a sigmoid function to estimate the transmission rate of gregarine parasites to the invertebrate host. As *C. shas*ta has similar transmission dynamics to *M. cerebralis* and gregarine parasites, incorporating a sigmoid function into the transmission parameters may improve the model, but additional data is needed to identify the parasite saturation densities for both host species. As we better understand the complexities of these host-parasite interactions, they can be incorporated to improve model predictions and increase the efficacy of management actions.

The Klamath River provides a unique opportunity for experimental manipulations aimed at reducing disease in Chinook salmon. Implementation of experimental management actions is facilitated by two characteristics of this system: 1) it is highly regulated through a series of hydroelectric and irrigation control dams and 2) the Chinook salmon population is supplemented by hatchery production. For example, the transmission dynamics of either spore stage to their respective host may be influenced by different (elevated, reduced, or pulsed) flow regimes (Bjork and Bartholomew, 2009), which can be achieved by releasing or withholding water from Iron Gate Dam (Bunn and Arthington, 2002). Results from our model demonstrate that reducing the transmission of myxospore from the adult salmon to the winter polychaete populations produced the second steepest decrease in R_0. One way to accomplish this would be by

increasing the variability and magnitude of winter discharge in the Klamath River. Another example may be to essentially remove the juvenile salmon host from the system during peak actinospore production, or at least alter the timing of their presence in the river, by releasing the hatchery produced fish earlier in the spring or later in the summer to disrupt the spatial and temporal overlap with the actinospore stage. This strategy was initially suggested by Hendrickson *et al.* (1989) and again by Hallett *et al.* (2012). Although this strategy directly benefits the hatchery produced fish, it may also indirectly benefit the naturally produced salmon by lowering parasite densities later in the year, resulting in lower infection severity and myxospore production from returning adult salmon. Another strategy for disrupting the disease dynamics is currently being pursued as part of the Klamath Basin Restoration Agreement (Klamathrestoration.gov, accessed 09/12/ 2013): the removal of four of the lower dams, including Iron Gate Dam. Not only will this provide access to ≈100 km of spawning habitat, but it could disrupt the spatial-temporal overlap by diffusing the concentration of myxospore densities directly below Iron Gate Dam or reducing the likelihood of an infected carcass settling near large polychaete populations, thereby decreasing the myxospore transmission rate.

Dam removal would not only disrupt the spatial overlap between the parasites and hosts, but is expected to change the thermal profile of the river (Perry *et al.*, 2011). The Klamath basin is currently near the upper thermal limit for salmon and water temperatures are expected to increase 0.5°C per decade (Bartholow, 2005), potentially increasing the effect of *C. shasta* on the emigrating juvenile salmon population. Water temperature plays an important role in the disease dynamics as well as the life histories (e.g. growth, development, stress, disease resistance) of all the organisms involved in this life cycle. Increasing water temperature can increase the rate and total *C. shasta*-induced mortality of the salmon host (Udey *et al.*, 1975; Ray *et al.*, 2012) and can

decrease the survival rate of both the actinospore and myxospore stages (Bjork, 2010; Chiaramonte, 2013). There is also evidence that temperature can affect both the actinospore transmission rate to, and replication rate in, the salmon host (Ray and Bartholomew, 2013). In Fig. 4.6a, we compare the effect of increased actinospore mortality between normal spring temperature (15 days at 14°C) and elevated temperature (3 days at 21°C) with respect to developmental delay parameter (ψ). In this example, elevated water temperatures and increased actinospore mortality result in a shorter developmental delay period to achieve $R_0 = 1$ than at normal temperatures. This analysis does not account for all the possible effects of elevated water temperature, such as increased overall salmon mortality, earlier actinospore production, increased transmission, or a shorter or shifted migration period that will also indirectly reduce parasite exposure. In the Klamath River it may be challenging to delay the development of the parasite, but it may be possible to accelerate actinospore development in both winter and summer polychaete populations by releasing warmer water from Iron Gate Dam. Increasing the water temperatures could increase the actinospore mortality rate, thereby reducing the disease severity on the salmon hosts. Conversely, increased water temperatures could also exacerbate disease by allowing for earlier release and development of the actinospore stage and potentially producing multiple generations within season and/or year-round transmission (Chiaramonte, 2013; Marcogliese, 2008). Increasing water temperatures can either amplify or diminish the infection intensity and disease severity of *C. shasta* on its salmon host; however, identifying how the different parameters respond requires more empirical research.

Although we assess several potential management actions with this epidemiological model, there are a couple alternative actions that we did not examine: 1) restoring the Chinook salmon to its historically dominant spring-run population and 2) allowing lower summer flows.

Prior to dam construction in the Klamath River, the majority of adult Chinook salmon returned to the river in the spring; currently the predominant run has a fall return timing (Hamilton *et al.*, 2005). Reintroducing a spring-run Chinook population may simplify our model as both salmon hosts would overlap spatially and temporally and the adults could act as an actinospore filter, reducing the effect of *C. shasta* on the out-migrating juveniles. Although this may be theoretically possible, current thermal conditions in the Lower Klamath River may be near the upper thermal limit for a spring-run population that generally over-summers in freshwater. The second alternative action not assessed is allowing for decreased summer discharge. Current reservoir operations in the Klamath allow for a minimum flow of 25.5 m^3/sec in July and August (NOAA, 2013); however, if a lower discharge were allowed it could affect multiple parts of the life cycle. Decreasing the discharge may reduce summer polychaete populations due to desiccation, increase actinospore mortality due to increased water temperature and reduce myxospore transmission (from juvenile salmon) due to slower water movement. However this action may have detrimental effects not assessed in the model, such as decreasing salmon summer habitat and increasing water temperatures that could increase disease severity in the fish host. Additionally this action may increase the density of polychaetes in certain areas (e.g. pools) allowing for more successful myxospore transmission and potentially causing an increase in disease severity. Before assessing either of these alternative management actions in our model we need to ascertain if a Chinook spring-run population can survive in the current conditions of the Klamath River, how these fish respond to *C. shasta* infections, how the polychaete population reacts to desiccation and what areas would be most affected by decreased summer discharge.

Epidemiological models are useful for highlighting where and to what extent control efforts may be best used to mitigate effects of disease on fish populations. In our model, we demonstrated that developmental delay was the most influential parameter on R_0. Although influential, this parameter is difficult to alter in natural systems as is requires prolonged durations of cooler water temperatures. Two of the most commonly identified control strategies, reducing polychaete or adult salmon densities, would require almost 100% removal of either to result in $R_0 < 1$. Consequently, these are not ideal management actions when used alone, but may prove effective if additional parameters are affected. The example shown in Fig. 4.7 reinforces earlier concepts that transmission is one of the most important parameters, and attempting to reduce parasite numbers or host densities alone does not greatly affect R_0 values. To improve the chances of alleviating disease in the Klamath River, managers and biologist should focus on control strategies that affect multiple parameters, such as altered flow regimes, which could alter polychaete densities and influence spore transmission rates. Our epidemiological model also provides the general framework to highlight effective control strategies for other myxozoan systems, especially for those that affect cultured fish populations (e.g. *H. ictaluri*, Griffin *et al.*, 2008; *T. bryosalmonae*, Okamura *et al.*, 2010). As these populations are generally confined to ponds or raceways, managers may have more control over many of the parameters (e.g., water temperature, flow, invertebrate host populations) directly influencing parasite success than in natural river systems.

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Table 4.1. Parameter descriptions and daily values for the *Ceratomyxa shasta* – Chinook salmon epidemiological model.

Parameter	Definition	Host	Season	Value
C	Juvenile salmon density		Early spring – summer	1
C	Adult salmon density		Summer / Fall	.01
P_s	Polychaete density		Summer / Fall	1000
P_w	Polychaete density		Winter / Spring	10
β_{ca}	Actinospore transmission rate	Juvenile salmon	Spring	2.0×10^{-5}
π_c^1	Parasite induced mortality (13 °C)	Juvenile salmon	Spring	2.9×10^{-2}
π_c^1	Parasite induced mortality (18 °C)	Juvenile salmon	Spring	4.3×10^{-2}
δ_c	Natural mortality rate	Juvenile salmon	Spring	1.39×10^{-2}
η_c	In-host parasite mortality	Juvenile salmon	Spring	1.0×10^{-1}
λ_{cm}	Myxospore production	Juvenile salmon	Summer	2.5×10^{4}
γ_m	Myxospore mortality rate	Myxospore	Summer	3.3×10^{-2}
β_{CM}	Myxospore transmission rate	Polychaete	Summer	2.0×10^{-5}
μ_p	Parasite induced mortality	Polychaete	Summer	0
ε_p	Natural morality rate	Polychaete	Summer	3.3×10^{-3}
ν_s	In-host parasite mortality	Polychaete	Summer	1.0×10^{-1}
θ_{CA}	Actinospore production	Polychaete	Summer / Fall	9.3×10^{3}
γ_A	Actinospore mortality rate	Actinospore	Fall	6.7×10^{-2}
β_{CA}	Actinospore transmission rate	Adult salmon	Fall	2.0×10^{-5}
π_C	Parasite induced mortality	Adult salmon	Fall	0
δ_C	Natural mortality rate	Adult salmon	Fall	0
η_C	In-host parasite mortality	Adult salmon	Fall	0
λ_{CM}	Myxospore production	Adult Salmon	Fall / Winter	1.8×10^{4}

γ_M	Myxospore mortality rate	Myxospore	Winter	6.0×10^{-3}
β_{CM}	Myxospores transmission rate	Polychaete	Winter	2.0×10^{-5}
μ_P	Parasite induced mortality	Polychaete	Winter	0
ε_P	Natural morality rate	Polychaete	Winter	1.0×10^{-2}
ν_P	In-host parasite mortality	Polychaete	Winter	1.0×10^{-1}
θ_{ca}	Actinospore production	Polychaete	Winter	9.3×10^{3}
γ_a	Actinospore mortality rate	Actinospore	Spring	6.7×10^{-2}
ψ_a	Developmental delay	Actinospore	Winter/Spring	$Exp^{(-(\varepsilon P+\mu P+\nu P) \times days)}$
ψ_m	Developmental delay	Myxospore	Summer	$Exp^{(-(\pi c+\delta c+\eta c) \times days)}$
ψ_A	Developmental delay	Actinospore	Summer/Fall	$Exp^{(-(\varepsilon p+\mu p+\nu p) \times days)}$
ψ_M	Developmental delay	Myxospore	Winter	$Exp^{(-(\pi C+\delta C+\eta C) \times days)}$

[1]– parasite induced mortality in juvenile Chinook salmon ranges from $1.2 – 4.3 \times 10^{-2}$ at 13°C and from $2.0 – 6.3 \times 10^{-2}$ at 18°C (Hallett *et al.*, 2012; Ray *et al.*, 2012)

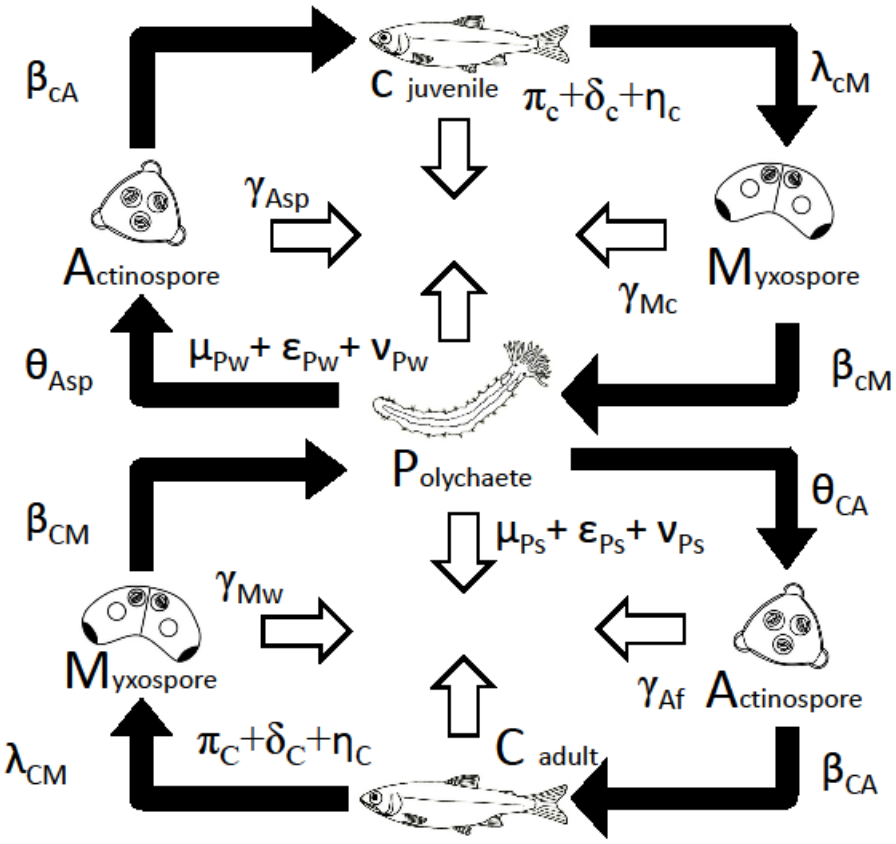

Fig. 4.1 Life cycle of the myxozoan parasite *Ceratomyxa shasta* showing involvement of juvenile (c) and adult (C) Chinook salmon and the polychaete (P) hosts.

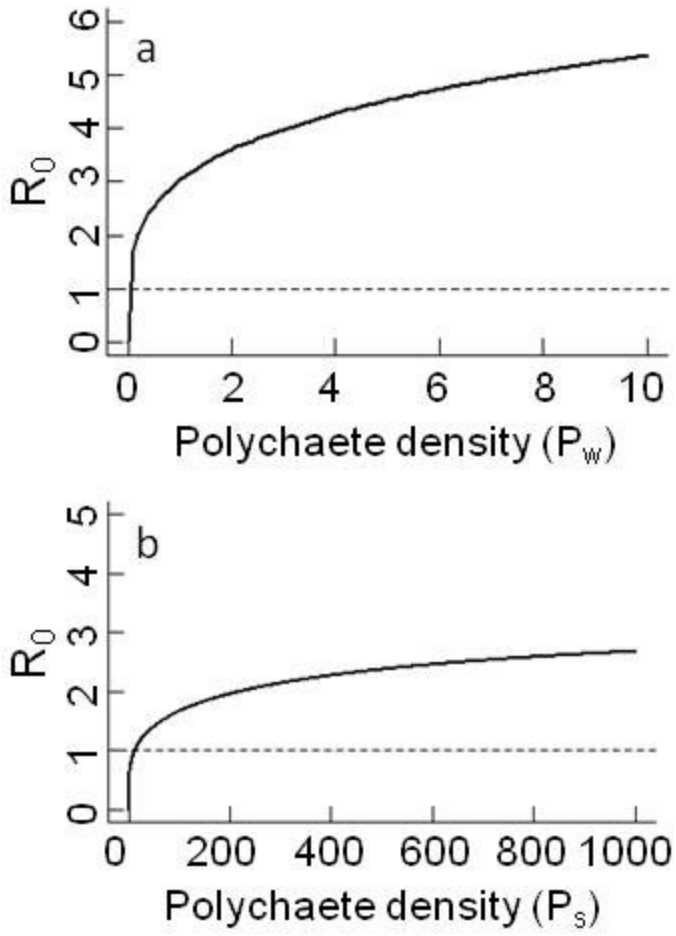

Fig. 4.2 Response of juvenile (a) and adult (b) Chinook salmon R_0 values to changes in polychaete density. The R_0 threshold value of 1, below which the parasite cannot persist in the populations is represented by the dashed line.

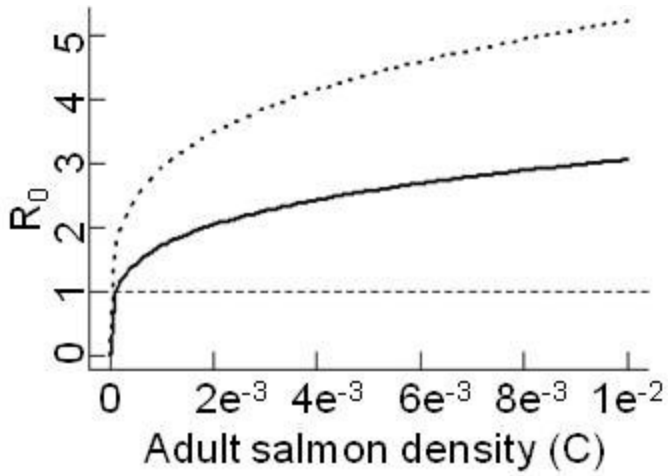

Fig. 4.3 Response of juvenile (solid) and adult (dotted) Chinook salmon R_0 values to changes in adult salmon density (C). The R_0 threshold value of 1, below which the parasite cannot persist in the populations is represented by the dashed line.

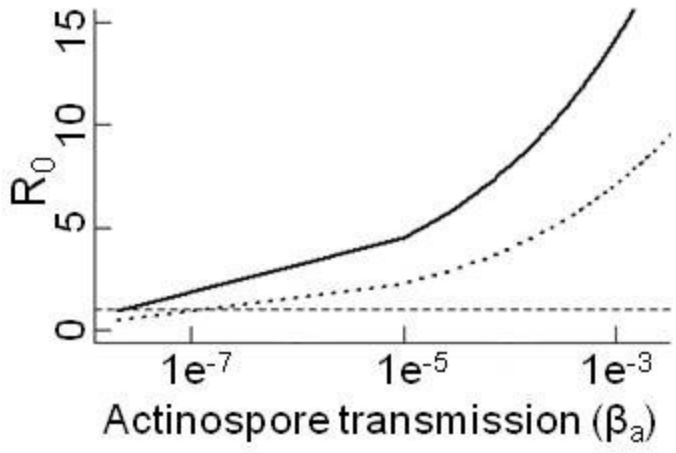

Fig. 4.4 Response of juvenile (solid) and adult (dotted) Chinook salmon R_0 values to changes in actinospore transmission rates (β_A). The R_0 threshold value of 1, below which the parasite cannot persist in the populations is represented by the dashed line.

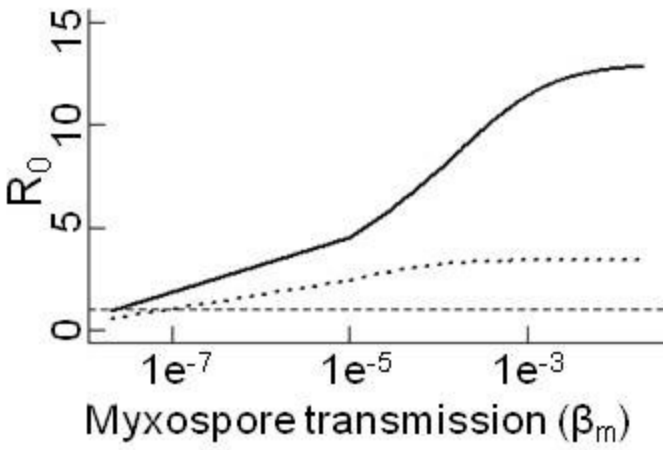

Fig. 4.5 Response of juvenile (solid) and adult (dotted) Chinook salmon R_0 values to changes in myxospore transmission rates (β_M). The R_0 threshold value of 1, below which the parasite cannot persist in the populations is represented by the dashed line.

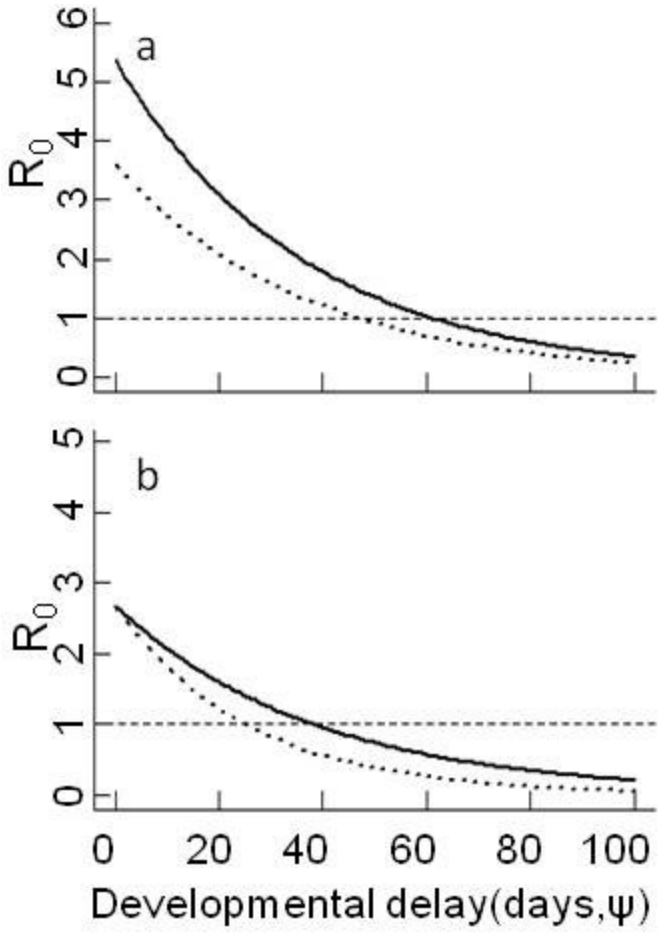

Fig. 4.6 Response of juvenile (a) and adult (b) Chinook salmon R_0 values to effects of developmental delay (ψ). Juvenile R_0 values (a) compare actinospore mortality at normal spring (14°C, solid) and elevated (21°C, dotted) water temperatures. Adult R_0 values (b) are compared between developmental delays of actinospores (solid) and myxospores (dashed). The R_0 threshold value of 1, below which the parasite cannot persist in the populations is represented by the dashed line.

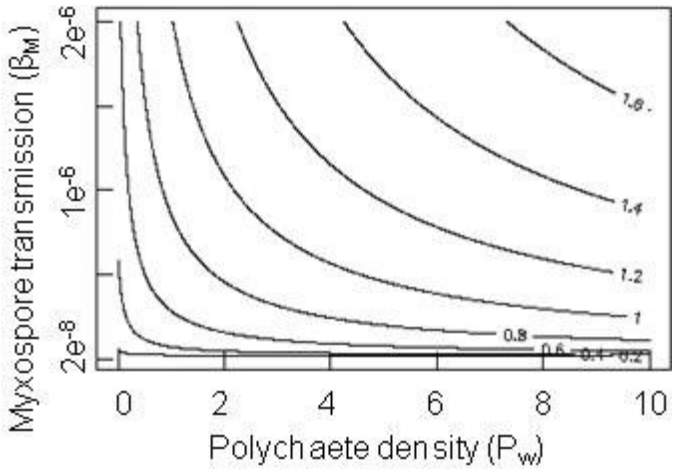

Fig. 4.7 Contour plot comparing changes in both winter polychaete density (P_w) and myxospore transmission (β_M) on juvenile Chinook salmon R_0 values.

CHAPTER 5: USING CURE MODELS FOR ANALYZING THE INFLUENCE OF PATHOGENS ON SALMON SURVIVAL

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ABSTRACT

Parasites and pathogens influence the size and stability of wildlife populations, yet many population models ignore the population-level effects of pathogens. Standard survival analysis methods (e.g. accelerated failure time models) can be used to assess how survival rates are influenced by disease. However, an assumption of these models is that each individual is equally susceptible and will eventually experience the event of interest and this is not typically satisfied with regard to pathogens of wildlife populations. Mixture cure models provide an alternative to standard survival analysis that may be more appropriate for wildlife populations when a fraction of the population survives a disease outbreak. These models are comprised of a logistic regression and survival analysis component, and allow for different covariates to be entered into each part of the model. We fit mixture cure models to the host-pathogen dynamics of Chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon and the myxozoan parasite *Ceratomyxa shasta*. In our models, we used total parasite concentration, water temperature and discharge as covariates to predict the observed parasite-induced mortality in juvenile salmonids collected as part of a long-term monitoring program in the Klamath River, CA, USA. The mixture cure models predicted the observed total mortality well, but there was more unexplained variability in the observed mortality rates that the models were not able to capture. Parasite concentration and water temperature were positively associated with both the total mortality and rate of mortality for both Chinook and coho salmon. Discharge was positively associated with total mortality for both species, but only affected the mortality rate for coho. The mixture cure models provide insights into how daily survival rates of Chinook and coho salmon change over time after becoming infected with *C. shasta.*

INTRODUCTION

Parasites and pathogens influence the size and structure of host populations (Anderson and May 1978; May and Anderson 1978; Dobson 1988; Hudson et al. 1998), and wildlife populations reduced to low levels may be particularly susceptible to effects of disease. Dramatic population declines have occurred across a range of species (e.g. corals, amphibians, birds, and mammals) as a result of more frequent and severe disease outbreaks (Harvell et al. 2002). Scientists use population models (e.g. population viability analysis) to synthesize biological and environmental data to predict the long term stability of a population (Boyce 1992). However, many of these models ignore population-level effects of pathogens and parasites (Haydon et al. 2002). Incorporating host-pathogen dynamics into these models can provide critical insights into a population's viability, particularly for species having high cultural, economic, and conservation value (e.g. salmonids).

Predicting a population's long-term viability requires accurate estimates of survival (Gilroy et al. 2012). Standard survival analysis methods (e.g. Cox proportional hazard and accelerated failure time models) assume that each individual in the population will eventually experience the event of interest (Corbière and Joly 2007). Although infections by some wildlife pathogens result in 100% mortality, in most disease outbreaks some fraction of the population survives. For example, *Flavobacterium columnare* outbreaks can result in up to 100% mortality in salmonid and catfish culture and other pathogens (e.g. viral hemorrhagic septicemia virus (VHSv) and M*yxobolus cerebralis*) have similar wide ranging affects on both cultured and natural populations (Holt *et al.* 1975; Thompson *et al.* 1999; Thomas-Jinu & Goodwin 2004; Kim & Faisal 2010). A different analytical approach is required to quantify factors that may affect the surviving fraction of a population. Cure models are an extension of survival analysis

methods that can be used to investigate heterogeneity between individuals by analyzing the population as two distinct groups: those that succumb to disease and those who are long-term survivors (Othus et al. 2012).

An example of a heterogeneous population response to a pathogen occurs in populations of juvenile salmon infected by the myxozoan parasite *Ceratomyxa shasta*. This parasite, although enzootic in major river systems throughout the Pacific Northwest (Margolis and Evelyn 1975; Ratliff 1981; Ching and Munday 1984; Bartholomew 1998), is well studied in the Klamath River, CA, USA. Data analyzed from a long-term pathogen monitoring study identified a link between *C. shasta* infection and lower abundance of returning adult salmon (Fujiwara et al. 2011). This monitoring program also provided evidence of individual heterogeneity in response to parasite-induced mortality as this ranged in juvenile Chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon from 0–98% (Hallett et al. 2012; Ray et al. 2012). *Ceratomyxa shasta*-induced mortality in juvenile salmon has three characteristic traits that are difficult to capture using traditional survival analysis methods: 1) a delayed onset of mortality after exposure to *C. shasta*, 2) a period of high mortality rate during which most susceptible fish die, and 3) a plateau in the survival curve where no additional mortality occurs (Fig. 5.1).

The goal of this paper are two-fold (1) introduce a novel application of a cure model to pathogens in wildlife populations, and (2) develop a mixture cure model to predict *C. shasta*-induced mortality for Chinook and coho salmon in the Klamath River. Our application of the mixture cure model will help us understand the complex interactions between host, parasite, and the environment. Ultimately, these insights will help to guide management and conservation actions for these salmon populations.

METHODS

Sentinel trials

The data analyzed in this paper were collected during a long-term project to monitor the spatial and temporal distribution of *C. shasta* and associated parasite-induced mortality in the Klamath River basin. Sentinel trials were conducted by holding juvenile Chinook and coho salmon (0+ age class obtained from Iron Gate Hatchery Hornbrook, CA, USA) in cages in the Klamath River for 3 days where they were exposed *in situ* to varying parasite densities, as described by Ray et al. (2012). Although sentinel trials were conducted at multiple locations, this analysis focuses on data from one site above the confluence with Beaver Creek (see Hallett et al. 2012 for map) where high parasite densities and fish mortalities were consistently observed (Hallett and Bartholomew 2006; Fujiwara et al. 2011; Hallett et al. 2012). Data were analyzed from Chinook (n = 33 trials; 1463 fish exposed) and coho salmon sentinel trials (n = 30 trials; 1238 fish exposed) conducted during the summers of 2006–2010 (Table 5.1).

During the exposure, river temperatures were recorded every 15 min with a HOBO temperature logger (Onset Computer Corporation, Pocasset, Massachusetts, USA) and then averaged over the 3-day exposure period. To estimate total parasite concentration during exposure, we collected three 1-L samples of river water at the start and end of each sentinel trial, which were subsequently filtered and assayed using a *C. shasta*-specific qPCR as described by Hallett et al. (2012). The proportion of parasite genotypes specific to Chinook (type I) and coho (type II) were also quantified from these samples as they can influence the severity of infection (Atkinson and Bartholomew 2010*a*; *b*; Hallett et al. 2012). We estimated discharge (m^3/s) during the sentinel trials by subtracting tributary discharge (Scott River USGS gauging station 11519500) from the main stem Klamath River (USGS gauging station 11520500).

Following exposure, we held fish at two temperatures: 13°C (the ambient lab water temperature) and an elevated temperature that best represented the in-river conditions during the sentinel trial (15–21°C), except for trials conducted in 2006 when only the ambient temperature was available. Fish were observed for signs of *C. shasta*-induced mortality up to 90 days, and time to mortality (days) was recorded for each fish. We visually examined each fish for the myxospore stage of *C. shasta* following the methods described in American Fisheries Society, Fish Health Section Blue Book (2012). When myxospores were not visually identified, a section of intestine was assayed using a *C. shasta*-specific PCR to determine whether fish were infected (Palenzuela and Bartholomew 2002). Fish that survived to the end of the observation period or died from causes other than *C. shasta* were right-censored in the analysis. For our statistical analysis, fish that succumbed to infection were assigned a value of 1; whereas the right-censored fish were assigned a value of 0.

Cure model

For this analysis we selected a mixture cure model as it directly models the survival of two distinct groups: those that experience the event of interest (susceptible individuals) and those that will never experience the event (non-susceptible or cured individuals; Othus et al. 2012). In our application, the event of interest is death caused by *C. shasta.* Susceptible individuals are those that die due to *C. shasta*, and cured individuals are those that survive the 90-day holding period. We assumed salmon could be "cured" by either failing to be infected during the exposure period or by recovering from infection.

A mixture cure model is a survival distribution function that combines a logistic model for the probability of death with a standard survival model for the time to death of uncured individuals (Othus et al. 2012):

$$S(t \mid \boldsymbol{x}, \boldsymbol{z}) = [1 - \pi(\boldsymbol{z})] + \pi(\boldsymbol{z})\, S(t \mid U = 1, \boldsymbol{x}) \qquad \text{(Eq. 1)}$$

where $S(t \mid \boldsymbol{x}, \boldsymbol{z})$ is the survival distribution function for time t given covariate vectors $\boldsymbol{x}$ and $\boldsymbol{z}$; S $(t \mid U = 1, \boldsymbol{x})$ is the survival distribution function for time t conditional on individuals that died due to *C. shasta*, U is an indicator function of *C. shasta*-induced mortality (1 for those that died due to *C. shasta* and 0 otherwise); and $\pi(\boldsymbol{z})$ is the probability of death due to *C. shasta* (Corbière and Joly 2007). We used the Weibull distribution for $S(t \mid U = 1, \boldsymbol{x})$ because it is flexible and contains a number of other distributions as special cases (e.g. the exponential distribution).

Covariates can be included in the mixture cure model, where $\boldsymbol{z}$ is the covariate vector for the proportion of individuals that die and $\boldsymbol{x}$ is the covariate vector for the timing and rate of mortality among susceptible individuals. The same covariates can be included in both $\boldsymbol{x}$ and $\boldsymbol{z}$, but this is not required. For the logistic part of the model, the logit of $\pi(\boldsymbol{z})$ is expressed as a linear function of the covariates. For the survival distribution part of the model, $S(t \mid U = 1, \boldsymbol{x})$ is implemented as an accelerated failure time model where log (t) is modeled as a linear function of the covariates (Peng et al. 1998).

The covariates of interest for our models are: total amount of host-specific parasite/L (genotype I for Chinook (TI) or II for coho (TII)), holding temperature at the laboratory (HT), water temperature during the 3-day exposure period (ET), the average discharge during the 3-day sentinel trial (Q), and the interactions TI x HT and TI x Q for Chinook salmon, and TII x HT and TII x Q for coho salmon. We developed separate global models for Chinook and coho salmon that included all the covariates and interaction terms in both components of the model (Table 2). To improve model convergence, we centered the covariates by subtracting the mean from each observation.

Covariates and interaction terms were selected based on their hypothesized effect on *C. shasta*-induced mortality. The concentration of species-specific parasite (TI for Chinook and TII for coho) was calculated by multiplying the total concentration of parasite DNA by the proportion of each genotype present in the water (Hallett et al. 2012). Water temperature is associated with higher total mortality and faster mortality rates in both Chinook and coho salmon, and so we included two covariates for water temperature: 1) ET during the exposure period and 2) HT during the holding period (Udey et al. 1975; Hallett et al. 2012; Ray et al. 2012). We included Q as a proxy for velocity because Ray et al. (2013) identified an inverse relationship between water velocity and parasite attachment to the gills. The interactions TI x HT (for Chinook) and TII x HT (for coho) account for the known compounding effects of parasite concentration and water temperature on the mortality rate and total mortality (Ray et al. 2012), while TI x Q (for Chinook) and TII x Q (for coho) act as a proxy for the total exposure dose (Hallett et al. 2012).

Parameters of candidate models were estimated via maximum likelihood with the gfcure package in R (Zhang and Peng 2007; R developmental core team 2011). The weight of evidence for each model was then assessed using Akaike information criterion (AIC) to identify the most parsimonious model (Akaike 1973; Burnham and Anderson 2002). Candidate models were constructed by first separately removing the ET covariate from the logistic component and then from the survival component, as the exposure period comprised of only a small fraction of the entire monitoring period. Selecting the model with the lowest AIC value, we then individually removed each interaction term. After removing a covariate we would select the model with the lowest AIC value and repeat this process until the model with the lowest AIC score was selected as the final model.

We present the results of the final models in two ways. First, we plot the estimated Kaplan-Meier (KM) survivor function for each sentinel trial (Collett 2003) and compare the predicted curves from the final models. To assess the accuracy of the logistic regression component we calculated a Brier score that ranges from 0 (perfect fit) to 0.25 (poor fit) and assumed a score < 0.125 to be a good fit (Steyerberg et al. 2001). We assess the fit of the survival model by plotting 95% confidence intervals of the predicted cure model curves. Second, we evaluate the relative influence of the HT and Q covariates, and their interactions, with TI (for Chinook) and TII (for coho) from the cure model. We accomplish by plotting the minimum, mean, and maximum observed values of each environmental covariate (HT and Q) against the minimum, mean, and maximum observed values of parasite concentrations.

RESULTS

Chinook salmon

We found the best fitting model, based on AIC, excluded ET in the logistic component and TI x HT in the survival component (AIC = 1068.34, Table 5.2). Removal of the ET covariate from the logistic component (model 2) resulted in a lower AIC value than removal from the survival component (model 1) or the global model (AIC = 1069.87). Removing the TI x HT term in the survival component (final model) produced the lowest AIC value. Individually removing the remaining interaction terms (model 6–8) resulted in higher AIC scores than the final model.

In general, the Chinook salmon mixture cure model was able to capture all three of the mortality characteristics (delayed mortality onset, a period of high mortality, and a plateau in which no further mortality occurs) for a majority of the sentinel trials (Fig. 5.2). The model correctly predicted the mortality onset for almost all sentinel trials in which mortality was

observed. The logistic component of the model accurately (Brier score < 0.125) reproduced 84.8% (28/33) of the estimated total mortalities. Three trials (i.e. 11, 20, and 23) had Brier scores between 0.125 and 0.25, suggesting an adequate fit between predicted and observed data, but the remaining two trials (i.e. 3 and 22) had a Brier score > 0.25, indicating a poor model fit. The survival component replicated 69.7% (23/33) of the observed mortality rates (slopes). In general, the mixture cure model best reproduced the slopes of the KM curves when mortality was > 50% (e .g. trials 12-16). The model always predicted mortality, even when none was observed (e.g. trials 1, 2, and 30). Our model was able to reproduce at least one of the three mortality characteristics for all the sentinel trials, except for trial 3 where none of the observed patterns were captured.

In the final model, all the logistic regression covariates were positively associated with probability of mortality due to *C. shasta* and all the survival analysis covariates were negatively associated with the survival rate (Table 5.3). Overall, both the total and rate of mortality increased as TI increased (from left to right, Fig. 5.3); however this response differed with the interacting covariate, either HT (Fig. 3 a, b, and c) or Q (Fig. 3 d, e, and f). Although the total mortalities were similar between HT and Q for each of the different values of T, increasing values of Q resulted in higher total mortality than HT. The mortality rate was more influenced by increasing values of HT than Q.

Coho salmon

We found the best fitting coho model, based on AIC, excluded ET and TII x HT in the logistic component and TII x Q in the survival component (AIC = 1351.75; Table 5.2). Removal of the ET covariate in the logistic regression component (model 2) resulted in a lower AIC score than its removal from the survival component (model 1; AIC = 1357.591) or the global model

(AIC = 1355.02). Dropping the TI x HT interaction term in the logistic component and Dropping the TI x Q term from the survival component resulted in the lowest AIC (final model). Removal of the two remaining interaction terms increased the AIC score (models 9 and 10).

Although coho salmon respond differently than Chinook salmon to *C. shasta* infection, the coho mixture cure model was able to capture all three mortality characteristics for a majority of these sentinel trials (Fig. 5.4). The model predicted the mortality onset for a majority of sentinel trials; except for when no mortality was observed (e.g. trial 28) or when mortality onset occurred much later than average (e.g. trial 25). The logistic component of the model accurately (Brier score < 0.125) reproduced observed total mortality for 93.3% (28/30) of the sentinel trials. Unlike the Chinook model, all Brier scores were < 0.25, but there were two trials (i.e. 11 and 17) that exceeded our threshold score of 0.125. The survival component was able to replicate 56.7% (17/30) of the estimated KM curves, especially when total mortality was > 50% (e.g. trials 12-16). The model was able to predict zero and low mortality in some cases (e.g. trials 5, 19, and 32), but was inconsistent (predicting either faster or slower) for other trials. The coho mixture cure model was able to predict the overall mortality for almost all the trials (93.3%); however it did not perform as well as the Chinook model at replicating the mortality rate of the observed trials. However, the coho model was able to reproduce at least one mortality characteristics for all observed sentinel trials.

As with the Chinook model, HT, TII, and Q in the logistic component of the model were positively associated with probability of total *C. shasta*-induced mortality; however the interaction term TI x Q was associated with a decreased probability of total mortality (Table 5.3). In the survival analysis component, all the covariates, except ET and TI x HT, were negatively associated with survival rate. Overall, increasing values of TII resulted in increases in both the

total and rate of mortality and as with Chinook salmon the pattern of this response also differed between HT (Fig. 5.5 a, b, c) and Q (Fig. 5.5 d, e, f) covariates. The total and rate of mortality were greater at minimum values of Q than HT across all values of TII. However, increasing values of HT resulted in greater differences in both total and rate of mortality than increasing values of Q.

DISCUSSION

Cure models provide an analytical tool to model and predict survival rates in wildlife populations, especially in populations whose abundance is constrained by pathogens. Cure models can be analyzed by parametric or semi-parametric methods allowing for use in a wide array of host–pathogen systems with varying amounts of empirical data. In this paper we present a parametric version of the model, as it allows for more precise estimates of the covariates of interest (Collett 2003). Another advantage of using cure models over other survival analysis methods is the ability to incorporate different covariates in the survival and logistic components, further expanding the applications to different disease systems. We used a mixture cure model to analyze the effects of the parasite, *C. shasta*, on the survival rates of Chinook and coho salmon, as it allows us to divide our populations into two distinct groups, those that succumb to infection and those that survive infection ("cured").

In this host-pathogen system, the "cured" proportion of the salmon host can arise in two different ways. First, not all individuals may become infected with *C. shasta*. When parasite densities are relatively low (< 10 parasite/L), parasite-induced mortality is often lower and highly variable (Hallett et al. 2012). Ray and Bartholomew (2013) observed density independent transmission dynamics between *C. shasta* and salmon host when parasite densities were low (< 10 parasite/L) suggesting that random chance encounters led to infection. However as parasite

densities increased, density-dependent transmission dynamics were observed, suggesting that at some parasite concentration threshold, almost all salmon become infected. Second, salmon may be able to recover from the infection. Evidence for the ability to recover from infection was reported in Ray et al. (2010), who conducted caged exposures in the Klamath River when total parasite densities exceeded 100 parasites/L. Over 90% mortality was observed yet no evidence of infection, by either visual inspection or PCR, was detected among the survivors.

In our study, the mixture cure models had slightly different structures in terms of the covariates retained, indicating biologically different responses to infection between Chinook and coho salmon. Although there are a couple comparable models (within 2 ΔAIC) for both Chinook and coho, they share an overall similar structure to the final model selected. The logistic component of the models differed in that the TI x HT interaction was retained in the Chinook model, but dropped in the coho model. This finding suggests that *C. shasta* type I (Chinook) could be more virulent than type II (coho) and that this virulence is compounded by increased water temperatures, as hypothesized by Hallett et al. (2012). The survival component of the model was similarly structured for the two species, except that TI x Q was retained in the Chinook model whereas TII x HT was retained in the coho model. As type I (Chinook) is the dominant genotype in this system, the TI x Q term provides an exposure dose estimate for Chinook salmon. The TII x HT interaction for coho salmon indicates that rising water temperature increases the severity and rate of *C. shasta*-induced mortality (Udey et al. 1975; Ray et al. 2012), and inclusion of this term in the coho model suggests that they appear to be more sensitive to warmer temperatures than Chinook salmon in the Klamath River (Richter and Kolmes 2005; Hallett et al. 2012).

Overall, mixture cure models captured the observed mortality characteristics for both Chinook and coho salmon; however the lack of fit among certain trials could be improved by further refining our measurements of environmental variables and improving our understanding of infection dynamics in the salmon host. The estimates for total parasite (TI for Chinook and TII for coho) were based on three 1–L samples collected at that start and end of the exposure period. Continuously collecting water samples during the 3-day exposure period may help improve the estimates of this important covariate. Although Q (discharge) was a significant covariate in both models, it is a relatively coarse proxy for water velocity and does not capture variation in velocity among other locations in the river (e.g. eddies, pools, riffles). Ray and Bartholomew (2013) observed an inverse relationship between velocity and actinospore transmission with a threshold at ~0.3m/s, above which transmission was greatly reduced. Therefore, a more fine-scale measurement such as near-cage velocity may improve the fit of these models to the observed data. In addition to more fine scale measurements of total parasite and velocity, the use of replicate cages would aide in capturing variation in the mortality response, especially in years of low to moderate (< 50%) mortality. Atkinson and Bartholomew (2010*a*) demonstrated a link between the relative proportion of species-specific genotype and mortality. However, it is not known how co-infection with both genotypes influences the severity of disease. When type I (Chinook) was the dominant genotype in the water column, Chinook salmon mortality was generally high (e.g. trials 22 and 23). Yet, when there was a similar proportion of each genotype (e.g. trials 5 and 7), Chinook salmon mortality was lower, suggesting that higher proportions of type II (coho) may lessen the lethal effects of type I on Chinook salmon. In addition to multiple genotypes of *C. shasta* these salmon are also exposed to other pathogens (e.g. *Flavobacterium columnare, Aeromonas salmonicida)* and parasites (e.g.

Nanophyetus salmincola) that could affect the overall and rate of mortality. Refining our measurement techniques of environmental factors and including interactions between *C. shasta* genotypes, other pathogens, and the salmon host may improve the predictive capabilities of these mixture cure models.

As the incidence of epizootics and diseases affecting wildlife populations are increasing across all taxonomic classes (Harvell et al. 2002), disease ecologists employ a variety of statistical methods and models to understand and quantify host-pathogen dynamics. However, standard survival analysis methods largely ignore the importance of heterogeneity in a population's response to infection. The heterogeneity displayed in wildlife populations makes cure models an attractive alternative to traditional survival methods. The mixture cure models developed in this paper provide daily survival rates and estimates of population level parasite-induced mortality that can be incorporated into salmon population and production models. These models allow for detailed understanding of juvenile salmon survival in the Klamath River; which in turn will allow managers to better account for the effects of disease dynamics on this stock. Although these mixture cure models were for an aquatic pathogen they could also be applied to other host-pathogen systems, such as sylvatic plague (*Yersinia pestis*) in prairie dog colonies **(***Cynomys sp)***,** *white-nose syndrome (G. destructans) in bats, or chytridiomycosis (B. dendrobatidis) in amphibians* (Cully Jr. and Williams 2001; Frick et al. 2010; Muths et al. 2011). Cure models provide a flexible yet powerful analytical tool that can be applied to a wide range of host-pathogen systems to identify and quantify significant biotic and abiotic factors to both the affected and surviving fractions of the population.

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Table 5.1. Summary of covariate values and observed *Ceratomyxa shasta*-induced mortality for each Chinook and coho salmon sentinel trial used in the mixture cure models. HT = Holding water temperature, ET = Water temperature during sentinel trial, TI = Total Chinook specific parasite/L, TII = Total coho specific parasite/L, and Q = Discharge during sentinel trial (m^3/s). Blank cells indicate no sentinel trial was conducted

Trial #	Year	Month	HT (°C)	ET (°C)	TI	TII	Q (m^3s^{-1})	Number of Chinook Exposed	% Observed Mortality (Chinook)	Number of coho Exposed	% Observed Mortality (coho)
1	2006	April	13	12.2	0.4	0.1	194.9	37	0.0		
2	2006	May	13	18.2	0.0	10.7	168.1	39	0.0	39	5.1
3	2006	June	13	20.0	63.2	16.9	118.7	36	19.4	38	2.6
4	2006	September	13	20.0	0.2	0.0	34.2	39	0.0		
5	2007	May	13	17.6	4.8	4.8	65.1	39	2.6	30	6.7
6	2007	May	18	17.6	4.8	4.8	65.1	37	27.0	45	86.7
7	2007	June	13	20.8	9.7	9.3	53.1	42	2.4	38	2.6
8	2007	June	20	20.8	9.7	9.3	53.1	40	40.0	38	81.6
9	2007	September	13	20.8	3.2	2.6	31.3	41	7.3	40	2.5
10	2007	September	18	20.8	3.2	2.6	31.3	40	2.5	34	35.3
11	2008	May	13	16.2	16.2	6.3	100.2	40	75.0	48	52.1
12	2008	May	16	16.2	16.2	6.3	100.2	41	85.4	41	65.9
13	2008	June	13	19.0	42.2	24.8	81.4	77	72.7	75	68.0
14	2008	June	15	19.0	42.2	24.8	81.4	75	72.0	79	84.3
15	2008	June	18	19.0	42.2	24.8	81.4	76	92.1	70	73.4
16	2008	June	21	19.0	42.2	24.8	81.4	86	98.8	74	95.5
17	2008	September	13	19.1	7.3	24.5	31.8	41	2.4	35	8.6
18	2008	September	18	19.1	7.3	24.5	31.8	39	12.8	19	79.0
19	2009	April	13	12.1	37.8	0.0	58.2	36	16.7	42	0.0
20	2009	May	13	14.9	22.0	5.1	62.8	41	73.2	39	12.8
21	2009	May	16	14.9	22.0	5.1	62.8	41	78.1	41	24.4
22	2009	June	13	20.8	13.5	3.4	53.8	35	74.3	38	5.3

23	2009	June	18	20.8	13.5	3.4	53.8	45	86.7	45	57.8
24	2009	September	13	19.9	0.0	0.0	31.1	39	0.0	25	0.0
25	2009	September	18	19.9	0.0	0.0	31.1	40	0.0	30	3.3
26	2010	April	13	12.6	0.1	0.0	63.8	42	0.0	29	0.0
27	2010	April	18	12.6	0.1	0.0	63.8	41	17.1		
28	2010	May	13	13.6	4.0	1.7	70.5	35	0.0	48	0.0
29	2010	May	16	13.6	4.0	1.7	70.5	45	15.6	39	15.4
30	2010	June	13	18.2	9.0	1.1	60.4	39	0.0	27	0.0
31	2010	June	18	18.2	9.0	1.0	60.4	40	20.0	39	10.3
32	2010	September	13	17.0	0.1	0.0	36.0	39	0.0	30	0.0
33	2010	September	18	17.0	0.1	0.0	36.0	40	0.0	23	0.0

Table 5.2. Model selection results for Chinook and coho mixture cure models based on Weibull distribution. All covariates are shown for the global model, with other models showing terms removed from the global model. Blank cells indicate that no covariates were removed. π (z) = logistic model, S (t) = survival model, k = number of estimated parameters, MLL = maximized log-likelihood, AIC = Akaike's Information Criterion

Number	Component	Model	k	MLL	AIC	ΔAIC
		Chinook salmon				
Global	π (z)	HT+ET+TI+Q+TI*HT+TI*Q	15	-519.93	1069.87	1.53
	S (t)	HT+ET+TI+Q+TI*HT+TI*Q				
1	π (z)		14	-561.66	1151.32	82.98
	S (t)	- ET				
2	π (z)	- ET	14	-520.54	1069.08	0.74
	S (t)					
3	π (z)	- (ET + HT * TI)	13	-533.40	1092.81	24.47
	S (t)					
4	π (z)	- (ET + TI * Q)	13	-525.68	1077.36	9.02
	S (t)					
5	π (z)	- ET	13	-616.85	1259.70	191.36
	S (t)	- TI * Q				
Final	**π (z)**	**- ET**	**13**	**-521.17**	**1068.34**	**0.00**
	S (t)	**- TI * HT**				
6	π (z)	- (ET + HT * TI)	12	-533.96	1091.92	23.58
	S (t)	- TI * HT				
7	π (z)	- (ET + TI * Q)	12	-526.30	1076.60	8.26
	S (t)	- TI * HT				
8	π (z)	- ET	12	-619.32	1262.65	194.31
	S (t)	- (TI * HT + TI * Q)				
		Coho salmon				
global	π (z)	HT+ET+TII+Q+TII*HT+TII*Q	15	-662.51	1355.02	3.27
	S (t)	HT+ET+TII+Q+TII*HT+TII*Q				
1	π (z)		14	-664.79	1357.59	6.36
	S (t)	- ET				
2	π (z)	- ET	14	-663.48	1354.96	3.73
	S (t)					
3	π (z)	- (ET + TII * Q)	13	-667.01	1360.02	8.79
	S (t)					
4	π (z)	- (ET + TII * HT)	13	-665.51	1353.01	1.78

	S (t)					
5	π (z)	- ET	13	-674.55	1357.10	5.87
	S (t)	- TII * HT				
6	π (z)	- ET	13	-663.61	1353.21	1.98
	S (t)	- TII * Q				
7	π (z)	- (ET + TII * HT + TII * Q)	12	-667.06	1358.12	6.89
	S (t)					
8	π (z)	- (ET + TII * HT)	12	-665.80	1355.60	4.37
	S (t)	- TII * HT				
final	**π (z)**	**- (ET + TII * HT)**	**12**	**-663.62**	**1351.23**	**0.00**
	S (t)	**- TII * Q**				
9	π (z)	- (ET + TII * HT + TII *Q)	11	-667.06	1356.13	4.90
	S (t)	- TII * Q				
10	π (z)	- (ET + TII * HT)	11	-666.60	1355.19	3.96
	S (t)	- (TII * Q + TII * HT)				

Table 5.3. Parameter coefficients for final mixture cure models for both Chinook and coho salmon

Component	Covariate	Coefficient	SE
	Chinook salmon		
Logistic			
	Intercept	-0.280	0.101
	HT	0.389	0.045
	TI	0.123	0.010
	Q	0.022	0.005
	TI x HT	0.019	0.004
	TI x Q	0.001	0.0003
Survival			
	Intercept	3.424	0.013
	HT	-0.084	0.003
	ET	-0.037	0.005
	TI	-0.007	0.001
	Q	-0.003	0.001
	TI x Q	-0.001	0.0001
	Log(scale)	-1.561	0.030
	Coho salmon		
Logistic			
	Intercept	-0.555	0.091
	HT	0.524	0.045
	TII	0.115	0.009
	Q	0.012	0.003
	TII x Q	-0.001	0.0004
Survival			
	Intercept	3.907	0.034
	HT	-0.134	0.018
	ET	0.038	0.153
	TII	-0.020	0.003
	Q	-0.006	0.001
	TII x HT	0.003	0.001
	Log(scale)	-1.136	0.034

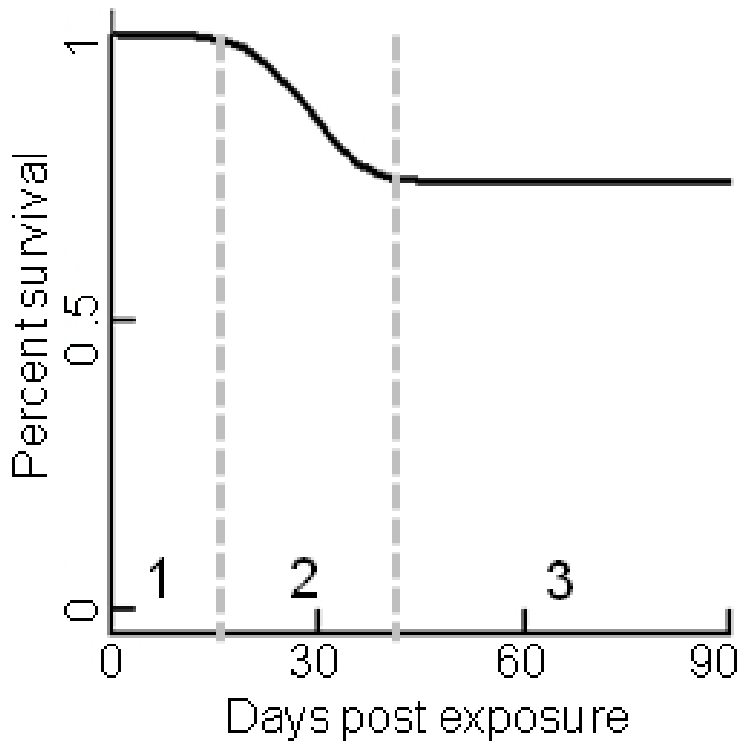

Fig. 5.1. Conceptual model depicting the three characteristic traits of *Ceratomyxa shasta*-induced mortality in juvenile salmon: 1) Delayed onset of mortality after exposure to *C. shasta*, 2) period of high mortality rate, and 3) plateau in the survival curve where no additional mortality occurs.

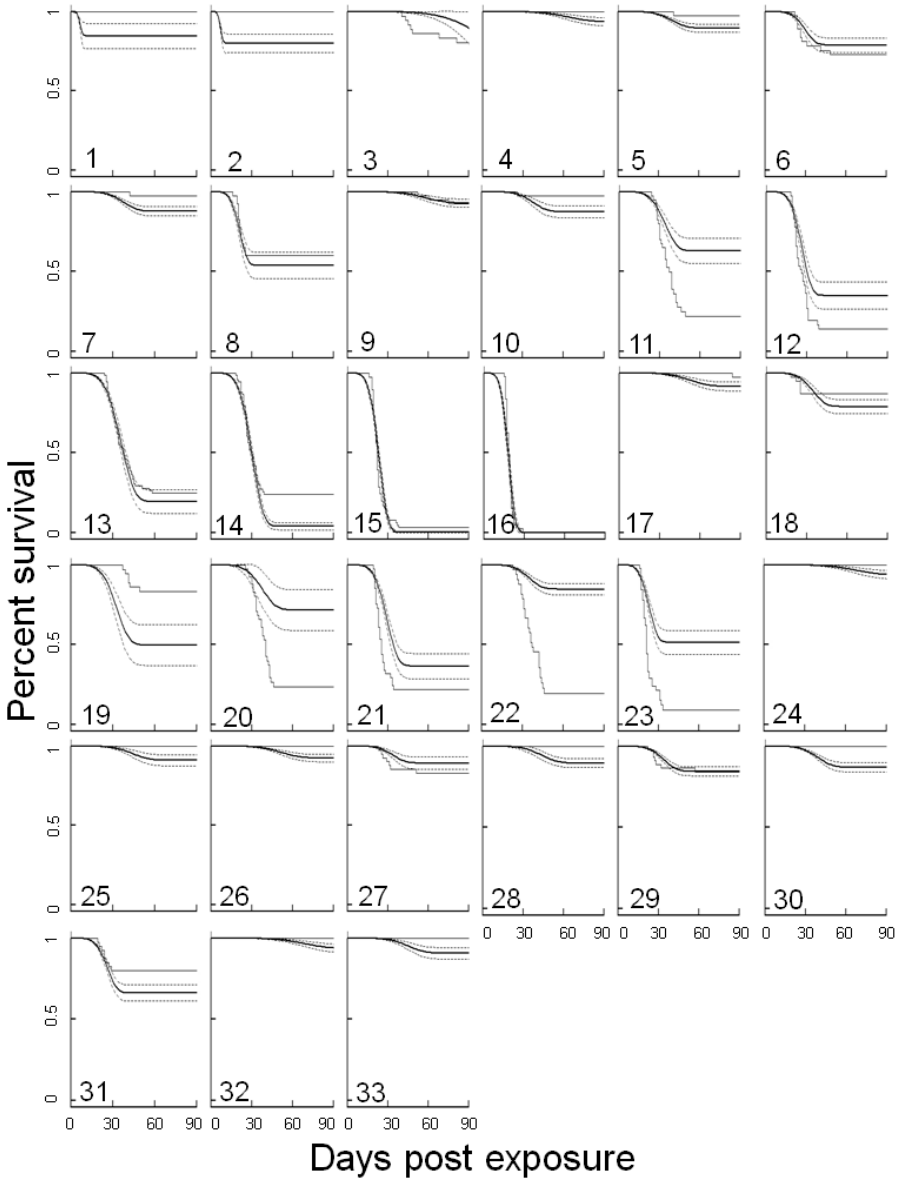

Fig. 5.2. Estimated Kaplan-Meier (thin) and mixture cure model (thick) survival curves for Chinook sentinel trials conducted in the Klamath River, with 95% confidence intervals (dashed). Numbers in the lower left corner correspond with trial numbers in Table 5.1.

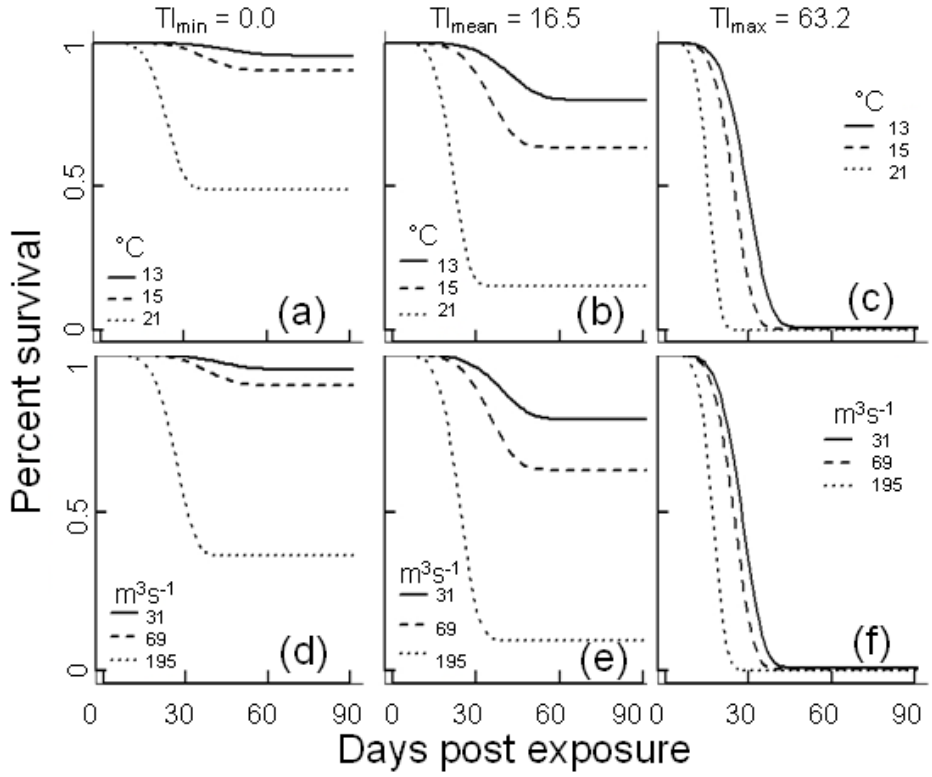

Fig. 5.3. Response of predicted survival probability from the Chinook mixture cure model at minimum (a and d), mean (b and e), and maximum (c and f) values of Chinook-specific *Ceratomyxa shasta* L^{-1} (TI). The top row (a, b, and c) represents the interacting effect of holding temperature (HT) and TI on the predicted survival probability. The bottom row (d, e, and f) represents the interacting effect of discharge (Q) and TI on the predicted survival. The lines within each panel correspond with minimum (solid), mean (dashed), and maximum (dotted) observed values of HT and Q.

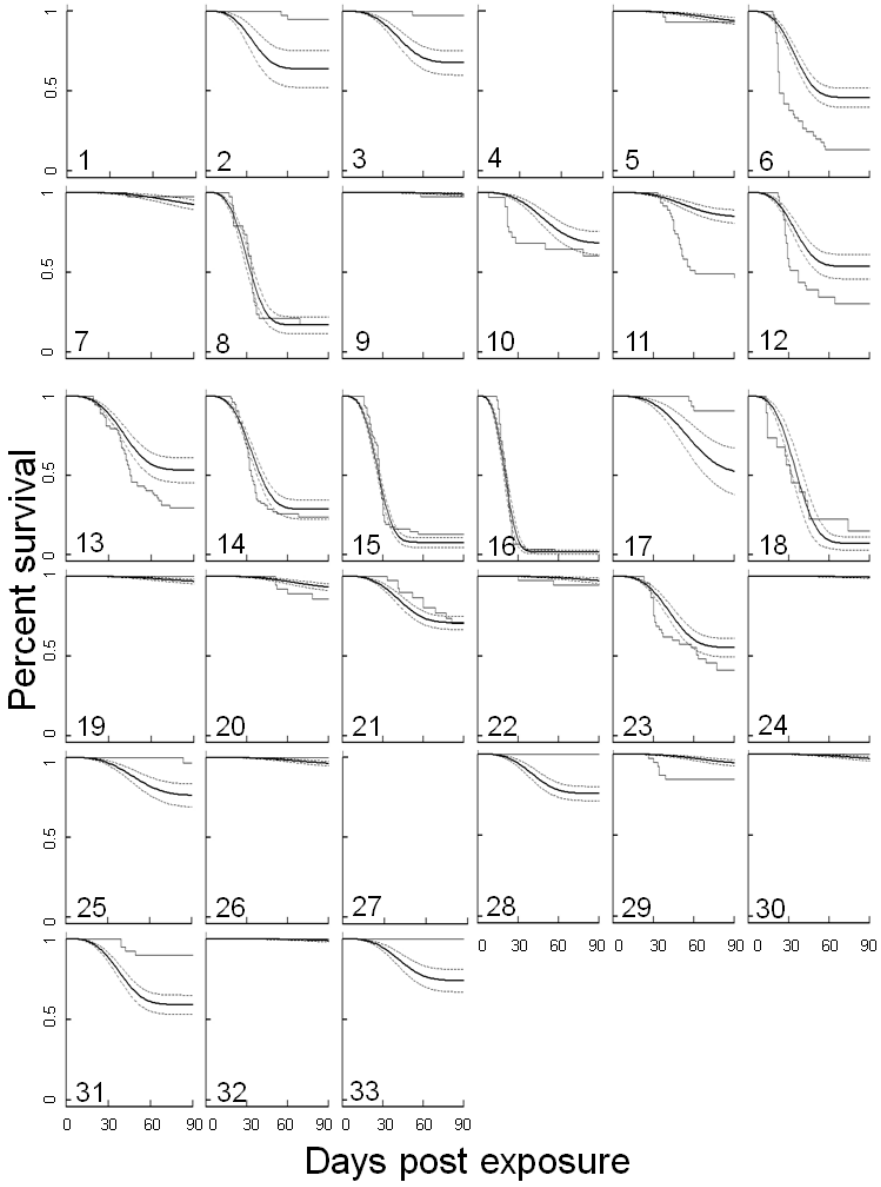

Fig. 5.4. Estimated Kaplan-Meier (thin) and mixture cure model (thick) for coho sentinel trials conducted in the Klamath River, with 95% confidence intervals (dashed). Numbers in the lower left corner correspond with trial numbers in Table 5.1. No coho trials were conducted at 1, 4, and 27.

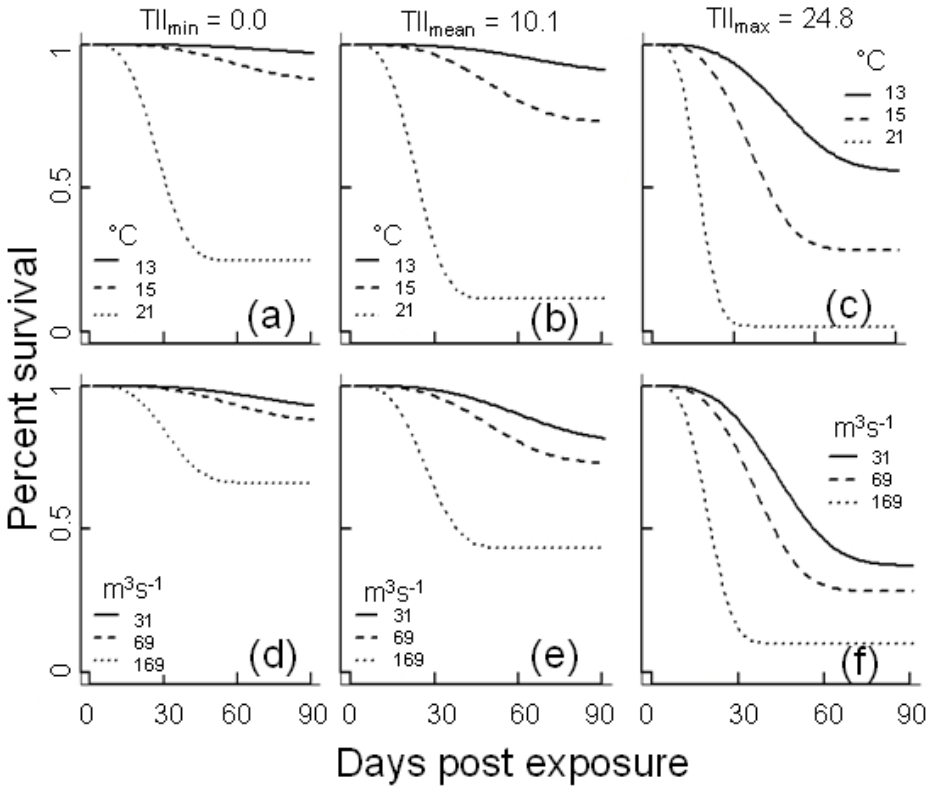

Fig. 5.5. Response of predicted survival probability from the coho mixture cure model at minimum (a and d), mean (b and e), and maximum (c and f) values of coho-specific *Ceratomyxa shasta* L^{-1} (TII). The top row (a, b, and c) represents the interacting effect of holding temperature (HT) and TII on the predicted survival probability. The bottom row (d, e, and f) represents the interacting effect of discharge (Q) and TII on the predicted survival. The lines within each panel correspond with minimum (solid), mean (dashed), and maximum (dotted) observed values of HT and Q.

CHAPTER 6: SUMMARY

Chapter 2: Relationship between temperature and *Ceratomyxa shasta*–induced mortality in Klamath River salmonids.

- Elevated water temperature resulted in increases of both the rate and severity of ceratomyxsis for both Klamath River Chinook and coho salmon.
- The effect of increased water temperature on disease severity was more significant when total density was <10 parasite/L. At higher densities (>10 parasite/L) the salmon host incurs high (>50%) mortality regardless of temperature.
- The risk of mortality approximately doubled for both species as water temperature increased from 13°C to 15°C. However, the greatest increase in the risk of mortality was observed between 18°C and 21°C, which is near the acute thermal threshold (22°C) for salmon.
- *Ceratomyxa shasta*-induced mortality varied among years between Chinook and coho. In 2007, coho incurred the highest mortality; whereas in 2009 the highest mortality was observed in Chinook. In 2008, parasite induced mortality was higher (> 60%) for both species. This shift in mortality is mostly attributed to shifts in the proportion of species-specific genotypes.

Chapter 3: Estimation of transmission dynamics of the *Ceratomyxa shasta* actinospore to the salmonid host.

- Actinospore transmission increased non-linearly with exposure duration and varying exposure dose, although in-host replication of the parasite may have confounded this response.
- Transmission rate of the actinospore stage was inversely related to water velocity and there appeared to be a velocity threshold (~ 0.3 ms^{-1}) above which transmission was reduced.
- Water temperature may have influenced the transmission rate as the transmission rate increased from 13–18°C but then decreased at 23°C. The increase in transmission could be confounded by increased in-host replication rate of *C. shasta*.
- We observed transmission patterns that exemplified both density-dependent and frequency-dependent transmission. Under natural field conditions transmission is most likely frequency-dependent, but density-dependent transmission can occur at extremely high parasite densities (>100 parasites/L.)

Chapter 4: Using an epidemiological model to quantify sensitivity of myxozoan disease dynamics to demographic parameters: application to *Ceratomyxa shasta* in the Klamath River.

- The epidemiological model constructed in this chapter can be adapted for other myxozoan parasite life cycles. It also provides a framework for the parameters of interest and data required to evaluate potential management actions.
- Developmental delay proved to be the most influential parameter on R_0 for the *C. shasta* life cycle, but also one of the most difficult to affect.

- The next most influential parameter was transmission of the myxospore from the adult carcass to the winter polychaete populations. This parameter could be affected by releasing water from Iron Gate Dam in the winter, after peak spawning.
- Almost complete removal of either hosts and/or spore parasite stages was required for $R_0 < 1$, indicating that these alone were not ideal parameters to target with management actions.

Chapter 5: Using cure models for analyzing the influence of pathogens on salmon survival.

- Cure models provide an alternative survival analysis method for pathogens of wildlife populations.
- Different parameters were retained between the Chinook and coho cure models highlighting varying responses and interactions between these species, *C. shasta*, and the environment. This supports findings from Chapter 2 that demonstrated Chinook and coho respond differently to *C. shasta* infections.
- Genotype I, specific to Chinook, may be a more virulent strain than genotype II (coho). However, coho may be more susceptible to infection and disease under increasing water temperatures. This supports findings from Chapter 2, where in 2007 parasite-induced mortality was greater in coho than Chinook at higher water temperatures although total parasite densities were low (<10 parasite/L).

MANAGEMENT IMPLICATIONS:

- Disrupting the spatial and/or temporal overlap between *C. shasta* and its host, should be the goal of any management action. In many systems this may be nearly impossible; however the Klamath River system provides a couple unique opportunities to perturb the interactions between the salmon population and the parasite
    - Currently the salmon populations are supplemented by hatchery production. These hatchery salmon are released once they reach a specific size (≈ 90 fish/lb) and this generally results in a release date of mid-May through late June, often during peak *C. shasta* densities. It may be possible to essentially "remove" the juvenile salmon population during this period of high parasite production by releasing the hatchery fish either a month earlier or two months later, thereby disrupting the parasite lifecycle.
    - Alternatively, a series of four dams that prohibit upstream adult salmon migration are scheduled to be removed starting in 2020. The removal of these dams will provide more spawning habitat for returning adult salmon and allow for lower densities of adult salmon. Lower densities may lead to lower transmission rates of myxospores to the polychaete and reduce the severity of disease in the Klamath River.
- Another characteristic of the Klamath River system that may allow for disease management is the ability to alter and manage the water released from Iron Gate Dam.

- Influencing winter discharge is the most likely management scenario as there is generally an excess of water during this time that can be utilized. Increasing the discharge during the winter may affect two stages of the life cycle: 1) It may decrease the transmission rate of the myxospore to the polychaete host and 2) as this will be cold water, it may cause a delay in the development of either the myxospore stage in the adult salmon carcass or the actinospore stage in polychaete host. Both could reduce the disease severity in the juvenile salmon population.
- An alternative strategy may be to decrease discharge from Iron Gate Dam during the summer (July/August). This could increase the water temperature resulting in an faster developmental rate and earlier release time of the actinospore stage when no salmonid hosts are present in the system.

- Restoring a natural flow regime may also help alleviate the severity of disease in the Klamath River. Currently, there is a mandate to maintain a minimum flow during the summer months. This mandated flow may actually promote the growth and spread of polychaete populations as it provides a consistent and stable flow regime; whereas the polychaetes are highly susceptible to flow events. Historical flows may have been much lower than the mandated and could influence the disease dynamics in a few ways:
  - Reduce the amount of available polychaete habitat, thereby decreasing the overall population.

  - Increased water temperatures could cause the polychaete to expel the parasite before it is completely developed, decreasing the potential exposure dose for the returning adult salmon.
  - Increase the migration rate of juvenile salmon, again decreasing their exposure duration to *C. shasta*.

- Historically, adult Chinook salmon returned to the Klamath River during the spring (April and May) as the water levels increased due to snow melt. Dam construction altered the timing and occurrence of these spring flows and the hatchery production shifted to fall-run Chinook where the adults return to the Klamath in late August through November. I hypothesize that *C. shasta* evolved with the spring run adults and it is these fish that are the intended host. Under current conditions the parasite is able to complete its life cycle, but the heavily infected and affected juvenile Chinook salmon are incidental hosts. To reduce the effects of this parasite on the juvenile population, management should attempt to return the Klamath to a more natural flow regime and also re-introduce spring-run Chinook.

FUTURE RESEARCH:

- Data gaps remain with respect to transmission myxospores from the adult salmon to the polychaete host. Adult salmon are uninfected as they enter the Klamath River and during their upstream migration they become infected. I hypothesize that the adult carcasses are the main source of myxospores into the Klamath River and it is those carcasses that settle out near polychaete populations that contribute

to the life cycle. To address this hypothesis I suggest radio-tagging fresh carcasses then follow their downstream "migration" downstream, identify pools or eddies where they stop, and then asses the prevalence of those polychaete populations.

- Data gaps also exist with respect to the environmental effects on infection and production of *C. shasta* in the polychaete host. This is primarily due to difficulties rearing polychaete populations in a laboratory setting. However, interesting relationships between temperature and infection have been observed for *Myxobolus cerebralis*, especially the fact that the oligochaete host purges the parasite at high water temperatures (~25°C). For disease management, especially in the Klamath River, it may be important to identify an upper thermal limit for *C. shasta* in the polychaete host.
- Although different strains of *C. shasta* were identified, it is unclear how these strains interact within the hosts. It is possible that the strains could compete with each other thereby decreasing their virulence, or they could act synergistically within the host resulting in a higher than normal virulence. Quantifying these interactions could be important for modeling disease dynamics as management actions could vary depending on the genotypes present in a river system.
- If reintroducing a spring-run Chinook population becomes a viable management option, studies should be conducted to assess their susceptibility to *C. shasta* and also their response to infection, in terms of mortality rate. These spring-run specific parameters could also be used in the epidemiological model to evaluate

the effectiveness of this management action. Instead two temporally separate interactions (juvenile and adult) as there is currently, there would be three overlapping host populations. I hypothesize that adults returning in the spring will act as an actinospore filter, reducing the exposure dose, and also the infection severity for the out-migrating juvenile salmon.

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