AN ABSTRACT OF THE THESIS OF

R. Adam Ray for the degree of Master of Science in Fisheries Science presented on November 24, 2009.
Title: Mortality Threshold for Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) in an Epidemiological Model of *Ceratomyxa shasta*.

Abstract approved: _____
Philippe A. Rossignol Jerri L. Bartholomew

The myxozoan parasite, *Ceratomyxa shasta*, is the most significant pathogen of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Klamath River, CA, USA. This parasite requires two hosts - a freshwater polychaete (*Manayunkia speciosa)* and a salmonid - to complete its life cycle. The complex life cycle and large geographic area where infection occurs make monitoring and managing the disease, ceratomyxosis, difficult. Epidemiological models are helpful tools to examine complex disease systems as they serve to identify parameters and rank their relative importance. A system of equations is used to derive the basic reproductive number (R_o) of the parasite. In this paper we present a model for ceratomyxosis induced mortality in Chinook salmon. The field experiments described herein quantify the mortality threshold (a critical parameter in the model), by exposing native Chinook salmon to *C. shasta* in the Klamath River. The average percent mortality that resulted from this challenge ranged from 2.5% to 98.5% over an exposure dose of 4.4 - 612 x 10^6 parasites. This study identified a non-linear mortality threshold for Iron Gate Hatchery (IGH) Chinook salmon that ranged from

5.6 - 9.9 x 10^4 total parasites. Below this threshold no mortality occurs, yet above it mortality dramatically increases. This threshold provides a target to reduce parasitism in emigrating juvenile Chinook salmon.

Mortality Threshold for Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) in an Epidemiological Model of *Ceratomyxa shasta*

by
R. Adam Ray

A THESIS

Submitted to

Oregon State University

In partial fulfillment of
the requirements for the
degree of

Master of Science

Presented November 24, 2009
Commencement June 2010

Master of Science thesis of R. Adam Ray presented on November 24, 2009.

APPROVED:

Co-Major Professor, representing Fisheries Science

Co-Major Professor, representing Fisheries Science

Head of the Department of Fisheries and Wildlife

Dean of the Graduate School

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

R. Adam Ray, Author

AKNOWLEDGEMENTS

The author expresses sincere appreciation to Dr. Jerri L. Bartholomew and Dr. Philippe A. Rossignol for the opportunity to work on this very interesting and challenging project and pursue an advance degree. I greatly appreciate the feedback, suggestions and constructive criticisms during this process of enhancing my scientific capabilities and understanding. To Rich Holt, Don Stevens, Harriet Lorz and Gerri Buckles a special thanks to all of you for sharing with me your invaluable amount of knowledge and experience in fish health, field equipment design and laboratory etiquette. I would like to also thank my fellow graduate students in the Bartholomew lab for providing me with a considerable amount of help in the field as well as the laboratory, also the willingness to talk through the numerous ideas and concepts that would cross my mind. I would like to thank my wife Katie and my parents for their constant encouragement and support throughout this process.

CONTRIBUTION OF AUTHORS

Dr. Jerri L. Bartholomew and Dr. Philippe A. Rossignol were both involved in the study design, interpretation of data and manuscript development. Dr. Philippe A. Rossignol was involved with the development and construction of the model and series of equations derived from the model.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Mortality Threshold for Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) in an Epidemiological Model of *Ceratomyxa shasta*

CHAPTER 1: Introduction

Klamath River and *Ceratomyxa shasta*

The Klamath River basin consists of over 40,000 km^2 of varied landscape from Southern Oregon to Northern California and flows for over 400 km to the Pacific Ocean (Bartholow 2005). The Klamath River was once the third largest salmon fishery in the Pacific Northwest (after the Sacramento and Columbia Rivers). In the Klamath system there are several species of anadromous fish; Chinook salmon (*Oncorhynchus tshawytscha*), steelhead (*O. mykiss*), coho salmon (*O. kisutch*), green sturgeon (*Acipenser medirostris*), eulachon (*Thaleichthys pacificus*), coastal cutthroat trout (*O. clarki clarki*), and Pacific lamprey (*Lampetra tridentata*). In the early 1900s Chinook salmon and steelhead were the two most dominate species, with populations of both estimated at about 400,000 and a total run size in the Klamath/Trinity River system estimated to be between 650,000 and 1 million fish (Leidy & Leidy 1984, Hamilton et al. 2005).

Throughout the past century, a multitude of factors led to a decrease in the number of anadromous fish in the system. Most of these factors are anthropogenic in nature and include the construction of dams, over-fishing, mining, and other landscape modifications. One of the major contributors to the decrease in fish abundance is the loss of habitat due to four dams (impassable to fish) constructed

between 1910 and 1960. These dams effectively restrict salmon from almost 970 km of viable spawning and rearing habitat in the main stem and its tributaries above (Hamilton et al. 2005). These factors impact the waters of the Klamath by limiting water flow, degrading overall water quality and increasing water temperature and runoff into the river (CAEPA 2006). Between 1978 and 1995, Chinook salmon returns averaged 58,820 with a low of 18,133; less than 20% of the historic run size (Hardy & Addley 2001). In the mid 1990s and early 2000s, as a result of improved hatchery management practices, the Chinook salmon runs in the Klamath River resembled the historic runs and reached almost 200,000 in 2001 (CADFG 2008). However, in 2002 a combination of factors led to a massive die-off of almost 34,000 returning adult salmon. Since 2002, the number of returning adults has again declined. The severity of this decline led to the closure of both the commercial and recreational harvest of Klamath River salmon in 2006. Although the reduction in habitat, altered land use and degraded water quality have effected the salmon populations on the Klamath, they are not solely to blame.

There are several pathogens in the Klamath River that are potentially detrimental to survival of both juvenile and adult salmonids. The myxozoan parasite *Ceratomyxa shasta* is recognized as the most significant pathogen of juvenile Chinook salmon (Foot et al. 2004). Annual fish health surveys conducted since 2001 have discovered that 30-60% of out-migrating juvenile salmon are infected with this myxozoan parasite (Foott et al. 2004). Ceratomyxosis is the disease caused by *C. shasta* infection and the clinical signs include lethargy, darkening of the body surface,

abdominal distension, hemorrhaging of the vent and necrosis of the intestine (Bartholomew 1989). First discovered in 1948 at the Crystal Lake Hatchery in Northern California, *C. shasta* was not described until 1950 by Noble (Noble 1950). However, it was not until 1997 that the complete life cycle, which includes an obligate polychaete host and two infective spore stages, was fully understood (Bartholomew et al. 1997). The myxospore stage is released from an infected salmonid host and infects a freshwater polychaete, *Manayunkia speciosa*. The infected polychaete sheds the actinospore stage, which in turn infects a salmonid host. The distribution of *C. shasta* is limited to certain bodies of water throughout the Pacific Northwest (Bartholomew et al. 1989) and within a given watershed it has a variable distribution and prevalence. Within the Klamath River the distribution of *C. shasta* seems to be explained by high densities of the polychaete host (Stocking & Bartholomew 2007).

The polychaete host *Manayunkia speciosa* was initially described by Leidy in 1858 and is widely distributed throughout coastal North America, from both the East and West coast and the Great Lakes region (Spencer 1976). Historically, *M. speciosa* was poorly understudied primarily due to its small size (0.2-3mm) and a lack of interest compared to larger and more economically important invertebrate species (Mackie & Qadri 1971). A recent study in the Klamath basin explored the distribution and habitat preference of *M. speciosa*. Stocking and Bartholomew (2007) discovered that *M. speciosa* is patchily distributed throughout the system and prefers slow flowing habitats in either mats of the algae *Cladophora* or sand/silt sediment.

Manayunkia speciosa requires very nutrient rich waters as they filter feed on smaller particles and utilize larger sized particles to construct their tubes (Lopez & Levinton 1987). In the Klamath River the highest prevalence of *C. shasta* infection within the polychaete populations were measured about 50 Rkm downriver from Iron Gate Dam (Stocking & Bartholomew 2007). As a result, juvenile salmon released from the hatchery must pass through this potential highly infectious area and then continue to migrate for over 250 Rkm before reaching the ocean. Although this high prevalence of infection occurs early in the migration, *M. speciosa* is patchily distributed throughout the Klamath and therefore the potential for infection exists for the entirety of the migration.

A majority of the initial research on *C. shasta* was qualitative in nature, due to limited technology and knowledge of the complex life cycle. These qualitative studies provided information on the seasonal and geographic distribution of the parasite (Schafer 1968, Sanders et al. 1970, Margolis & Evelyn 1975, Ratliff 1981, Ratliff 1983, Ching & Munday 1984a, Hoffmaster et al. 1988, Bartholomew et al. 1998). It was also demonstrated that different species and strains within a species of salmonids have varying levels of susceptibility to *C. shasta* and this depends on whether the fish originates from water where the parasite is endemic (Schaffer 1968, Sanders et al. 1970, Margolis & Evelyn 1975, Zinn et al. 1977, Buchanan et al. 1983, Ching & Munday 1984b, Ching & Parker 1989, Bartholomew et al. 1990, Bartholomew et al. 1998). These early studies helped managers better understand the

patterns of *C. shasta*, but without knowledge of the life cycle they could not implement many control strategies.

The varying degrees in susceptibility of salmonids to *C. shasta* indicated that each strain could tolerate a certain dose of parasite before succumbing to infection. A semi-quantitative study demonstrated that there are a finite number of infectious agents of *C. shasta* in a given amount of water (Ratliff 1983). From this study, Ratliff hypothesized that a single infective stage of *C. shasta* was capable of inducing ceratomyxosis in a highly susceptible rainbow trout. Recently, a controlled laboratory experiment was conducted to determine the infectious dose of *C. shasta* for both a similar strain of susceptible rainbow trout and native Klamath River Chinook salmon. This study confirmed that only one actinospore is necessary to cause mortality in the susceptible rainbow trout; however no infection or mortality was detected in Chinook salmon exposed to 5×10^3 actinospores (Bjork & Bartholomew 2009). Although no higher challenges were attempted due to difficulties in rearing and maintaining colonies of the polychaete host, this experiment supports the earlier qualitative findings that salmonids from rivers where the parasite is endemic are less susceptible to infection and parasite induced mortality.

Advances in molecular techniques led to the development of a polymerase chain reaction (PCR) assay for *C. shasta*. This assay provided a sensitive means for detecting the presence of parasite DNA in either water or tissue samples (Palenzuela 1999). A more recent advancement in molecular diagnostic tools was the establishment of a quantitative PCR assay. As the name implies, this assay provides

an estimate of the amount of parasite DNA in the water, however it cannot determine which stage of the parasite is present. Water samples have been assayed with this diagnostic tool to determine the relative parasite densities through out the Klamath River. These results reflect the pattern of polychaete distribution; in that the highest levels of parasite DNA where located in the same area as the highest densities of polychaetes (Hallett & Bartholomew 2006, Stocking & Bartholomew 2007).

The Klamath River salmonids are an important stock for establishing harvest quotas and the effect of *C. shasta* on juvenile salmonids may be a major contributor to the reduction of returning adults. A considerable amount of research has been conducted with regards to this parasite and its two obligate hosts. However, the vast geographic and temporal distribution of this parasite and hosts make implementing any management strategies difficult.

Mathematical modeling:

Mathematical models are an increasingly useful tool in the biological and ecological fields. They are used for questions that are too complex, inaccessible, numerous, diverse, unique, dangerous, expensive, big, small, slow or fast to approach by other means (McKenzie 2000). There are two core models from which most of the current ecological models are derived. The first is the Ross model which describes the malaria life cycle (Ross 1911). The second is the Lotka-Volterra model which describes inter-specific competition (Lotka 1925, Volterra 1926). Both of these models and their derivations have proven useful to the understanding of complex ecological systems and interactions.

The Ross model is one of the earliest to quantitatively depict the dynamics of malaria transmission and is the basis for many of the current epidemiological models (Aron & May 1982). The original model is very simple and consists of only eight parameters; many of them are easily quantifiable. These parameters are used to develop two differential equations which describe the proportions of infected individuals in each population and the probability of their interactions. The simplicity of this model is one of the reasons it was able to be readily utilized and incorporated in attempts to control malaria. This model also defined the Basic Reproduction number (R_0) or the number of secondary infections that arise from the introduction of a single infective individual in a susceptible population (Ross 1911, Macdonald 1952, Smith et al. 2007).

R_0 provides a threshold value for a disease, if R_0 is < 1 the disease will not become established within the population; however if $R_0 > 1$ the disease will become endemic within that population (Macdonald 1952). The significance of R_0 in terms of disease control is that the pathogen or the vector does not need to be entirely removed, but only reduced until R_0 is < 1 and then the disease will eventually die out of the population. The equation for R_0 can also be used to determine the relative importance of the various parameters. For malaria all but one of the parameters are linear, this demonstrates that the one non-linear parameter will have a greater influence on changes in R_0 (Macdonald 1952). This basic reproduction number equation not only provides a threshold of establishment for the disease, but may highlight the importance of the parameters in the disease cycle; both of these

attributes can be utilized to more effectively reduce the transmission of pathogens in a population.

Although this initial model provides insight into the disease transmission of malaria it is also based on some very broad assumptions that limited its effectiveness in some situations. Since the initial development of the Ross model, scientists have explored many of those preliminary assumptions attempting to improve the effectiveness of the model and better understand the dynamics of disease transmission. Some of the basic assumptions from the original model that have been further studied are: that both populations are homogenous and infinite, that mosquitoes bite at a constant rate and that each bite is equally as likely to transmit an infective stage. It has been demonstrated that the values of R_0 can vary greatly depending whether a population is infinite or finite and that different control strategies may need to be adapted for varying situations (Smith et al. 2007). Another assumption of great interest is the uniform biting rate of the mosquitoes; this is also significant because this parameter was identified in the equation for R_0 as being non-linear and therefore having the greatest influence on R_0. Because of the importance of this parameter to the transmission of malaria a considerable amount of research has been conducted examining different aspects to this parameter. Different species of mosquitoes have been found to preferentially feed on different vertebrate hosts (Bruce-Chwatt et al. 1966). It has also been demonstrated that certain humans are more likely to be bitten by mosquitoes than others (Kingsolver 1987). These two revelations could result in a dilution effect in the transmission of malaria. In contrast,

the infectious stage of malaria within a mosquito constricts the intake of a blood causing the mosquito to probe more often to obtain a complete blood meal; thereby increasing the chance of transmitting the infection to more human hosts (Rossignol et al. 1986, Li et al. 1992). These are a couple examples of how, after the development of a simple initial model, research can be focused by challenging some of the basic assumptions of the original model or directly studying one of the more important parameters of the model to further understand the disease dynamics and improve management strategies to more effectively control the disease.

The second basic model used in comprehending complex ecological interactions is the Lotka-Volterra model (Lotka 1925, Volterra 1926). This model was not specifically developed for biological process, but to explain the rhythmic effects of chemical reactions. In the ecological field this model is used to describe a basic predator-prey cycle and the oscillations that arise from this interaction. This model was originally used to understand how two populations can influence the each others rate of growth through predation or competition for resources. In the late 1970s there was a paradigm shift and parasites were recognized as having similar abilities as a predator in constraining the growth of a population (Anderson & May 1978). The definition of the ecological relationship between a host and parasite is comprised of four features: *the parasite is physiologically dependent on the host, the infection process produces or tends to produce an over dispersed distribution of parasites within the host population, the parasite species has a higher reproductive potential than the host species and the parasite kills heavily infected hosts* (Crofton

1971). The Lotka-Volterra model was transformed into a generalized representation of a host-parasite relationship utilizing seven basic parameters (Anderson & May 1978). This model was not developed for a specific host parasite system, but to generally explain the process involved in the interactions. From this model, equations were developed that describe the interactions of both the host and the parasite. Several derivations were developed from the initial equations to determine what type of parasitic processes can lead to the regulation or destabilization of the host population (Anderson & May 1978, May & Anderson 1978). This general model and simple interactions therein, have been applied towards other host parasite systems and the equations can be further expanded to incorporate more complex dynamics.

In ecological context the term parasite is broadly used to include viruses, bacteria, protozoan, helminthes and arthropods (Anderson & May 1979). In this broad array of organisms a majority of them are associated with life cycles that are more complex than the model derived from the Lotka-Volterra. After the initial transition to describing host-parasite population dynamics, the model was again transformed to account for indirect transmission and parasites with environmental stages (Anderson & May 1979, May & Anderson 1979). Again, these models were generic in structure and design but were able to demonstrate patterns within these interactions and highlight the relative importance of various parameters. However, as information regarding the various methods that parasites can affect their host increased, the necessity for more complex models arose (Dobson 1988). Although these models describe more complex interactions they are generic enough to be readily adapted to

more specific systems. The equations from these complex models also help to focus research towards those parameters necessary for the continuation of the parasite. As with the Ross model, these Lotka-Volterra adapted models are based on several assumptions; however once a better understanding of the general interactions is obtained these assumptions can be challenged and allow for a more accurate representation of the host-parasite relationship.

The successful adaptation of the Lotka-Volterra model to describe the ecological implications of parasites has prompted disease ecologists to consider other ecological concepts for disease processes. One such ecological concept is the resilience, or the ability of a system to return to its original state after a perturbation (Neubert & Caswell 1997). This concept has been adapted to examine how the host parasite dynamics will react in the presence of an epidemic and what leads to a pathogen becoming endemic (Hosack et al. 2008). This model allows for short and long term predictions for various management strategies that may lead to unexpected results. For instance, after the implementation of a control strategy the parasite population may be dramatically reduced; however after several generations the parasite population may rebound to levels higher than before the implementation. While this model is based on the simple interactions of the Lotka-Volterra model it can be adjusted to predict the outcomes of management strategies for more complex host-parasites systems.

The Ross and Lotka-Volterra models provide guidelines for developing mathematical models to describe virtually any host-parasite system. Both of these

models begin simply by focusing only on the parameters necessary to the propagation of the parasite life cycle. These original models are based on very broad assumptions that can be challenged by further studies once the basic interactions are understood. Mathematical models provide a foundation to not only examine the host parasite interactions, but determine how the system will react to various management strategies.

Study Objective:

In the Klamath River over half of the out migrating juvenile Chinook salmon become infected by *C. shasta*. This high prevalence of infection may be a contributor to decreased survival of these juveniles during the ocean migration and thus to the declining returning adult population that has led to fishing closures and restrictions along the Pacific coast of the United States. In an attempt to reduce the effect of this parasite on the out-migrating juvenile salmonids and potentially improve the population of returning adults we will develop an epidemiological model of this complex life cycle. The development of this model is the foundation of this thesis and for future research. This initial model will identify the parameters necessary for the propagation and continuation of *C. shasta*. These parameters will be used to develop equations that represent the interactions between the two environmental stages and two obligate hosts of *C. shasta*. The research conducted for this thesis will attempt to quantify four of the parameters of this life cycle; three will be directly measured and one indirectly. These parameters will be quantified by exposing native Klamath River Chinook salmon (*O. tshawytscha*) from Iron Gate Hatchery in the

Klamath River for various durations. The parameters to be directly quantified in this study are the proportion of salmonids infected, the total dose of actinospores and the parasite induced mortality. The transmission rate of the actinospore stage to the salmonid will be indirectly estimated from these exposures. The quantification of these parameters will allow for the determination of a mortality threshold or lethal level of infection for the Klamath River Chinook salmon from *C. shasta*. This mortality threshold is one of the most basic and important properties in a host parasite system; yet it is also one of the most difficult to quantify (Crofton 1971). If a threshold does exist for this system it may cause the model to react differently either below or above this limit. The presence of a threshold may also provide a reduction target for management since the complete elimination of the parasite form this river is highly improbable.

CHAPTER 2: Mortality Threshold for Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) in an Epidemiological Model of *Ceratomyxa shasta*

R. Adam Ray

Department of Fisheries and Wildlife, Nash Hall, Oregon State University, Corvallis OR 97331, USA

Department of Microbiology, Nash Hall, Oregon State University, Corvallis OR 97331, USA

Philippe A. Rossignol

Department of Fisheries and Wildlife, Nash Hall, Oregon State University, Corvallis OR 97331, USA

Jerri L. Bartholomew

Department of Microbiology, Nash Hall, Oregon State University, Corvallis OR 97331, USA

Diseases of Aquatic Organisms (Submitted)
Oldendorf/Luhe, Germany

Mortality Threshold for Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) in an Epidemiological Model of *Ceratomyxa shasta*

Abstract:

The myxozoan parasite, *Ceratomyxa shasta*, is the most significant pathogen of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Klamath River, CA, USA. This parasite requires two hosts - a freshwater polychaete (*Manayunkia speciosa)* and a salmonid - to complete its life cycle. The complex life cycle and large geographic area where infection occurs make monitoring and managing the disease, ceratomyxosis, difficult. Epidemiological models are helpful tools to examine complex disease systems as they serve to identify parameters and rank their relative importance. We established a system of equations that is used to derive the basic reproductive number (R_o) of the parasite. In this paper we present a model for ceratomyxosis induced mortality in Chinook salmon. The experiments described herein quantify the mortality threshold (a critical parameter in the model), by naturally exposing native Chinook salmon to *C. shasta* in the Klamath River. The average percent mortality that resulted from this challenge ranged from 2.5% to 98.5% over an exposure dose of 4.4 - 612 x 10^6 parasites. This study identified a non-linear mortality threshold for Iron Gate Hatchery (IGH) Chinook salmon that ranged from 5.6 - 9.9 x 10^4 total parasites. Below this threshold no mortality occurs, yet above it mortality dramatically increases. This threshold provides a target to reduce parasitism in emigrating juvenile Chinook salmon.

Introduction:

Declining populations of Chinook salmon (*Oncorhynchus tshawytscha)* are a major concern ecologically, economically, and sociologically. Management of this species is complicated due to the numerous biological and environmental interactions encountered at each life stage, including loss from disease. In the Klamath River, California, USA, the myxozoan parasite, *Ceratomyxa shasta,* has been identified as the most significant pathogen of juvenile Chinook salmon (Bartholomew et al. 1988; Bartholomew et al. 1990; Foott et al. 1999; Foott et al. 2004). Prevalence of *C. shasta* in out-migrating juvenile Chinook salmon in the Klamath River has ranged from 30 to 60% since 1994 (Nichols et al. 2007).

The parasite was first characterized from an outbreak in a rainbow trout (*O. mykiss)* hatchery in California (Noble 1950). The organism is the etiological agent of ceratomyxosis, characterized by a severe inflammatory response of the intestinal tissue, which results in intestinal necrosis and mortality (Conrad & Decew 1966, Bartholomew 1989). The indirect life cycle of the parasite was not elucidated until 1997, when it was discovered that a freshwater polychaete (*Manayunkia speciosa*) was required to complete development and transmission to the salmonid host (Bartholomew et al. 1997) (Figure 1). The polychaete is patchily distributed throughout the Klamath River (Stocking & Bartholomew 2007). A potential risk of infection therefore exists throughout the main stem Klamath River for rearing and out migrating juvenile salmon.

We introduce an epidemiological model of ceratomyxosis. Epidemiological models are used to address the complexities of host-parasite interactions and identify the parameters necessary for the perpetuation of disease. Using these parameters one can create a system of equations and calculate the 'Basic Reproduction Number' (R_o), defined as the number of secondary infections arising from a primary case (Dietz 1993; Smith et al. 2007). These models have been successfully developed for many infectious diseases of humans, starting with the Ross model for malaria (Ross 1911, Smith et al. 2007). Some models have been developed for diseases occurring in wildlife populations. These models, however, tend to be biased towards large bodied and easily observable hosts (Dobson and Foufopoulos 2000). A few epidemiological models have been developed for diseases of cultured fish, such as infectious haematopoietic necrosis virus (IHNV) and the bacterium *Aeromonas salmonicida* (Ögüt 2003). The model and resulting system of equations that we present can be used in three primary ways: to determine the relative sensitivity of each of the parameters; to predict how different management strategies may influence the host-parasite interactions; and examine the resilience and reactivity of the system after management actions have been implemented (Neubert & Caswell 1997, Hosack et al. 2009).

An important parameter in modeling host–parasite relationships is the mortality threshold of the host, or the lethal infectious dose of the parasite (Crofton 1971). Such a threshold could provide a practical focus for management strategies of *C. shasta*. There is no known treatment for ceratomyxosis, thus the selection of an

appropriate management strategy may be the only option for decreasing morbidity and mortality in out migrating juveniles. Here we quantify a mortality threshold for juvenile Iron Gate Hatchery (IGH) Chinook salmon (*Oncorhynchus tshawytscha*) and discuss how this threshold may affect the dynamics of the model.

Methods and Materials:

Model development:

Our model, presented in Figure 1 and Table 1, and its system of equations were modified from a model describing the life cycle of a "digenean-type" parasite with two free-living stages (Dobson 1988). The goal of our experiment was to measure the parameters involved in the transmission (η_1) of actinospores (A) from the water to the salmonid (S) and the parasite induced mortality (δ) of the salmonid host (Figure 1, Table 1). The number of actinospores (A), the proportion of infection in the salmonid (S) and the parasite related mortality are all direct measurements of the experiments described below. The transmission rate (η_1) is an indirect measurement that is estimated from the number of actinospores measured divided by the total number of fish infected cage^{-1}.

Fish and Study Area:

Juvenile (first year age class, 0+) IGH fall Chinook salmon were obtained from the California Department of Fish and Game, IGH and transported to the study

site in aerated coolers. The fish were exposed to *C. shasta* in the main stem Klamath River, about 1 Rkm upriver from the confluence with Beaver Creek (Rkm 259.1). This study site is about 45 Rkm down river from Iron Gate Dam and within a reach of the river where high densities of *C. shasta* have been measured (Hallett and Bartholomew 2006; Stocking & Bartholomew 2007). Two separate exposures were conducted, in June and September 2008, to increase the range of parasite doses. The salmon exposed in June were smaller than the fish used in September at the time of their respective exposures (7.2 +/- 0.6 cm and 4.3 +/- 1.1 g; 9.8 +/- 2.0 cm and 12.0 +/- 5.9 g). Although there are differences in the size of fish used for each exposure period, it has been suggested that the size of the fish does not alter the ability of a fish to become infected (Bjork & Bartholomew 2009). During both study periods, fish were handled similarly before and after exposure. Twenty unexposed IGH Chinook salmon were held as controls to ensure there was no background infection and processed identically as the exposed fish.

Fish Exposures:

IGH Chinook salmon were exposed in the Klamath River in eight 0.28m wide by 1m long cylindrical PVC cages, placed horizontally in the water column with 0.64 cm mesh screening on each end to allow for a natural flow. In June, 40 Chinook salmon were each placed into four cages for 72 hours to determine in-river variation between cages. To assess dose effects, 20 fish were added to the remaining four cages every 24 hours for three days, for a total of 60 fish cage^{-1}. To obtain low end

estimates of the parasite dosage, this experiment was repeated, with some modifications, in September when a lower parasite challenge was expected. In September, 12 to 15 fish were added to each of the eight cages at 72, 48, 24, and 16 hours, for a total of 60 fish cage^{-1}. In June and September, each exposure group was assigned a unique fin clip to allow for separation of the groups at the end of the challenge. After exposure, each group of fish was transferred to a separate aerated cooler, transported to the Oregon State University-Salmon Disease Lab (SDL) and relocated to 25L tanks with 18°C specific pathogen free (SPF) water for the duration of the study [~90 days post exposure (dpe)]. Salmon from the four cages used to asses the dose effect, from the June study, were combined into a single aquarium for each exposure period; consequently variation between cages cannot be directly measured. Preventative treatments for bacterial infections and external parasites were administered (Stocking et al. 2006). Fish were fed and observed twice daily. Sick and moribund fish were removed, euthanized with an overdose of MS-222 and either immediately examined for infection or frozen for future examination. Fish surviving 90 dpe were euthanized and immediately examined for infection.

Actinospore Dosage:

To determine the actinospore dose, the average daily water velocity during the exposure was multiplied by the average daily density of the parasite. Water velocity through each cage was measured with a Global Water Flow Probe (Global Waters, Gold River, CA) every 2 hours for the first 24 hours and then every 4 hours for the

remaining 48 hours. These measurements were averaged for each 24 hour period and multiplied by the volume of the live cage to determine the average daily flow. To determine the parasite density, one L of water was collected every two hours by an automated water sampler (Teledyne Isco, Lincoln, NE) and pooled in a 15 L container. After 24 hours of collection, four – one L sub-samples were collected from the container. These samples were individually filtered and processed for quantification of parasite DNA by quantitative polymerase chain reaction (qPCR) as described by Hallett & Bartholomew (2006). Cycle threshold values from the qPCR assay were used to estimate the average number of actinospores liter^{-1} river water in a 24 hour period. This estimate was calculated by extrapolating from a standard curve based on the value of a known number of parasites, similar to the one developed by Hallett & Bartholomew (2006).

Determination of infection:

All dead and moribund fish and 5 randomly selected fish that survived to 90 dpe from each exposure group were microscopically examined for the myxospore stage of *C. shasta*. Material from the posterior intestine was collected with a sterilized inoculating loop, placed on a microscope slide and examined at 200x magnification for up to 3 minutes (Bartholomew 2002). If positive, the entire intestine and kidney were removed and frozen for a future study. If no spores were observed, approximately 5mm of intestinal tissue was removed and frozen for PCR analysis as described by Palenzuela et al. (1999).

Data Analysis:

Chinook salmon that died after 5 dpe were included in the analysis; earlier mortalities were ascribed to non-*Ceratomyxa shasta* causes. Percent mortality was calculated by combining fish that were positive for *C. shasta* either by microscopy or PCR analysis and divided by the total number exposed in each cage for each exposure period. The percent mortality, for each cage in an exposure period, was weighted to determine the average percent mortality. Statistical analysis was conducted using S-plus 8.0 (TIBCO software inc., Palo Alto, CA). Either a Chi-square or Fischer's exact t-test (depending on the sample size) was used to determine the difference in mortalities between cages and exposure periods. One sample and pair wise t-test and one-way ANOVA were used to determine the variations in flow between days and between cages.

Results:

Model development:

The structure of this model (Figure 1) is a flow diagram based on the life cycle of *Ceratomyxa shasta*. The parameters (Table 1) generated for this model are significant to the transmission and persistence of the parasite through its different environmental stages and its two obligate hosts. From these parameters and interactions, a series of four equations was developed that describes the transmission

and mortality rates at each stage in the life cycle. For the development of these equations we assume *C. shasta* a microparasite.

Myxospore Transmission and Mortality

$$\frac{d}{dt}M = \lambda * S - \gamma_2 * M - \eta_2 * M * (1 - P) \quad (1)$$

Actinospore Transmission and Mortality

$$\frac{d}{dt}A = \theta * P - \gamma_1 * A - \eta_1 * A * (1 - S) \quad (2)$$

Polychaete Infection and Mortality

$$\frac{d}{dt}P = \eta_2 * M * (1 - P) - (\mu + \varepsilon) * P \quad (3)$$

Salmon Infection and Mortality

$$\frac{d}{dt}S = \eta_1 * A * (1 - S) - (\pi + \delta) * S \quad (4)$$

The first two equations describe the production of spores from their respective hosts, minus the number of spores that do not find a suitable host and the transmission of spores to uninfected hosts. The latter two equations represent the transmission of the spore stages to the uninfected hosts minus the mortality rate (both natural and parasite induced) of the hosts. Equations 1-4 can be solved for the equation for R_o (5). This equation describes the transmission of myxospore to polychaete (T_{MP}) and actinospore to salmonid (T_{AS}) divided by the mortalities for both spore stages (M_M and M_A) and hosts (M_P and M_S). The experiments conducted in this study provided a

range of measurements for the parameters in equation (4): actinospore (A) dose, the proportion of salmonid population infected (S), the resulting parasite induced mortality (δ) and an indirect measurement of the transmission of the actinospore to the salmonid (η_1).

$$\mathbf{R_0} = \frac{\lambda * \eta_1 * (1 - P) * \theta * \eta_2 * (1 - S)}{\mu + (\mu + \varepsilon) * \pi * (\pi + \delta) * \eta_1 * (1 - S) + \gamma_1 * \eta_2 * (1 - P) + \gamma_2} = \frac{T_{MP} * T_{AS}}{M_P * M_S * M_M * M_A} \quad (5)$$

Fish Infection:

We evaluated the salmon mortality (δ) following different exposure durations and expect these variables to be linearly proportionate. Average percent mortality for the June 72, 48 and 24 hour exposure groups was 93.7%, 98.5% and 84.7%, respectively. The average percent mortality of fish in cages 1-4, exposed for 72 hours, was 95.1 +/- 2 %, and did not differ significantly between these four groups (Fischer's exact test, p-value = 0.87). The average percent mortality of the pooled fish exposed for 72 hours (cages 5-8) was not statistically different from that observed in cages 1-4 ($\chi^2 = 2.59$, d.f. = 4, p-value = 0.63), indicating similarly minimal variability in mortality between cages 5-8. Although the difference in mortality between all three exposure periods was statistically significant ($\chi^2 = 8.37$, d.f. = 2, p-value = 0.02) (Table 2), biologically all three periods resulted in mortality too great to identify a mortality threshold.

In September, we observed 34.9%, 17.7%, 16.7% and 2.5% mortality, for the 72, 48, 24 and 16 hour exposure periods, respectively. Differences in mortality between cages were not significantly different for the 72, 48 and 24 hour exposure periods (Fischer's exact p-value = 0.42, 0.13 and 0.92, respectively), but a statistical difference was observed in the 16 hour exposure (Fischer's exact p-value = 0.01). The cause of this difference in the 16 hour group is associated with cage 7, where 3 fish (20%) succumbed to ceratomyxosis and no other parasite related mortality was observed for that exposure. Percent mortality was significantly different between the 72 and 48 hour exposures ($\chi^2 = 8.65$, d.f. = 1, p-value = 0.003) and the 24 and 16 hour exposures ($\chi^2 = 9.08$, d.f. = 1, p-value = 0.003), but not between the 48 and 24 hour exposures ($\chi^2 = 0.02$, d.f. = 1, p-value = 0.90). Mortality from ceratomyxosis was higher in June than September; however, minimal variability occurred between cages during either exposure period. The results from these two exposure periods provide an estimate of infection proportions (S) and the resulting parasite induced mortalities (δ) for our model. We conclude that salmon mortality is not a constant, but is logarithmically proportional to the exposure duration.

Actinospore Dose:

We estimated the parasite dose in relation to the exposure duration. The actinospore dose is calculated from the product of the average daily water velocities and average daily parasite density measured over the exposure duration. The average water velocity in June (5.38 +/- 0.74 liters sec^{-1}) was significantly greater than the

average velocity in September (3.93 +/- 0.97 liters sec^{-1}, t-test p-value < 0.05). The average daily parasite density in June varied greatly from day 1 to day 3 (28, 146, 1258 spores $liter^{-1}$, respectively). In September, the average daily parasite density was lower, yet more consistent between days (21, 20, 16 spores $liter^{-1}$, respectively), than in June. The total actinospore dose ranged from a maximum of 612 X 10^6 spores, during the June 72 hour exposure, to a minimum of 4.4 X 10^6 spores during the September 16 hour exposure (Table 2). These measurements provide an array of actinospore doses (A) that can be utilized in the model.

A lethal infectious dose $fish^{-1}$ can be determined by dividing the total actinospore dose by the total number of fish $cage^{-1}$, adjusting for the increasing fish density as the experiment progressed. The individual dose ranged, approximately, from 0.05 x 10^6 (September, 16 hour exposure) to 12.8 x 10^6 (June 72 hour exposure) spores $fish^{-1}$ and a non-linear relationship with percent mortality can be observed (Figure 2). The x-axis, in Figure 2, was log transformed to best display the wide ranging parasite doses. We conclude that the mortality threshold in IGH Chinook salmon (*Oncorhynchus tshawytscha*) ranges from 5.6 – 9.9 x 10^4 actinospores $fish^{-1}$.

Discussion:

We developed an epidemiological model for the *C. shasta* life cycle as a tool for identifying management strategies to improve the survival rate of out-migrating juvenile salmonids in the Klamath River. From a system of linear equations we identified the parameters necessary for transmission of the parasite and quantified

specific parameters. We estimated actinospore dose (A), proportion of salmonids infected (S) and parasite induced mortality (δ) by exposing IGH Chinook salmon to *C. shasta* in the Klamath River (Figure1). We identified and quantified a mortality threshold for IGH Chinook salmon ranges from 5.5 – 9.9 x 10^4 actinospores $fish^{-1}$, above which mortality increases non-linearly. A threshold significantly above zero is important to disease management because it provides a target for reducing the in river parasite burden and one that does not require complete eradication of the parasite to interrupt transmission.

The mortality threshold is an important parameter in disease ecology, yet this threshold has proven difficult to quantify in natural populations (Crofton 1971, Anderson & May 1978). It has been qualitatively shown that fish from waters where the parasite is endemic are less susceptible to infection and mortality from this parasite (reviewed by Bartholomew 1998). The first infectious dose was semi-quantitatively estimated by increasing the density of fish in a given volume of water, resulting in a decrease in ceratomyxosis related mortalities (Ratliff 1983). Ratliff's (1983) results suggest that a single actinospore can cause mortality in a susceptible strain of rainbow trout (*O. mykiss*). Subsequent development of laboratory challenge methods validated this prediction for susceptible rainbow trout using a quantified infectious dose (Bjork & Bartholomew 2009). In contrast, IGH Chinook salmon exposed individually to 5 x 10^3 actinospores under identical conditions failed to become infected, indicating a higher mortality threshold for this strain (Bjork & Bartholomew 2009). To achieve an infectious dose high enough to induce mortality

in less susceptible fish, Foott et al. (2007) conducted field exposures and quantified parasite density by qPCR. In their study, Trinity River Chinook salmon were exposed for 6 hours, and by measuring water velocity they calculated an exposure dose of approximately 1.4 x 10^4 actinospores $fish^{-1}$ (Foott et al. 2007). From this brief exposure, an average of 22% of the exposed Trinity River fish succumbed to ceratomyxosis, resulting in a mortality threshold about 5-fold lower than for IGH salmon. Although the Trinity River is a major tributary to the Klamath River, the levels of *C. shasta* are low in this river and the prevalence of infection in out migrating juvenile salmon is about 1 – 3 % (Nichols et al. 2008, Nichols et al. 2009). As a result of the Trinity River salmon receiving a lower exposure in the Trinity River and having a shorter migration in the main stem Klamath River, this strain is likely to be more susceptible to the parasite than IGH salmon. This increased susceptibility is reflected in the lower mortality threshold. Thus, the mortality threshold needs to be quantified for each strain in order to be used for management purposes.

The observation of a non-linear mortality threshold is important to the understanding of the epidemiology of the parasite. In general, parasitism can be divided into microparasitism and macroparasitism. For a microparasite, prevalence of infection is sufficient to evaluate the burden of the parasite in the host and in this relationship parasite dose and host mortality are not proportional. In a macroparasite relationship, parasite dose and host mortality are non-linearly related and the frequency distribution of the parasite is more important to evaluate then prevalence

with in the host (Crofton 1971, Anderson & May 1979, May & Anderson 1979, Dobson 1988). The presence of a mortality threshold indicates a macroparasitic relationship between *C. shasta* and the salmonid host. Yet the ability of *C. shasta* to replicate within the salmonid host is representative of microparasitic relationship. Recognition of when *C. shasta* behaves as a macroparasite versus a microparasite within the disease cycle is critical to further the development of this model.

Application of the *C. shasta* model is currently limited in several aspects. One limitation is a shortage of data for various parameters in this life cycle. For example, one gap is the emigration rate of juvenile salmonids, which directly relates to the potential exposure dose. A recent radio-telemetry survey recorded that juvenile IGH Chinook salmon reach the estuary in a median time of 10 days (Foott et al. 2009). Another study estimated the median travel time of coded wire tagged IGH juvenile Chinook salmon released between 1993 and 2001 to be 32 days (Wallace 2004), over a 3-fold difference in travel time. In our study we attempted to account for this difference in migration rate by using various exposure durations to reflect different exposure doses. Another limitation is that our current model does not specifically take into account environmental or genetic variables, such as water temperature, velocity, or genetic variations within the hosts or parasites. Water temperature has been demonstrated to greatly affect mortalities from ceratomyxosis (Udey et al. 1975), yet we do not know how it affects the other parameters in the model. Similarly, genotypic variations in the specificity of *C. shasta* to its salmonid host have recently been identified (Atkinson & Bartholomew 2009). These

observations open avenues to better understand ecological implications of these environmental and genetic differences and how they can be incorporated into the dynamics of this host-parasite system.

The mortality threshold, quantified in this study, was one of the critical parameters identified from the epidemiological model. The logarithmic relationship of the threshold to mortality influences the dynamics in this model. Below this threshold mortality does not occur and minimal management is needed; however, above the threshold mortality increases and the amount of management needed will be relative to the parasite dosage. For example, if 20% mortality from ceratomyxosis in juveniles is an acceptable loss, we could then approximate the dose of *C. shasta* (~2.1 x 10^5 total parasites) that results in this mortality. Then by comparing the current parasite densities in the river to the accepted numbers we can perform simulations and vary the other parameters in the system. These simulations will aid in determining an effective and efficient means for reducing levels of actinospores to achieve an acceptable level of mortality. Additionally, consideration of the macroparasitic properties of *C. shasta* will be important with respect to selecting appropriate control strategies and require monitoring the distribution of the parasite in the Klamath River to determine potential areas of high parasite burdens. Although this current model will need to be restructured to more appropriately address these macroparasitic characteristics, this study does provide useful quantification of parameters in this model and insight into some of the interactions in this disease cycle.

The primary objective of this study was to develop a model to aid in focusing management strategies on the most sensitive parameters, with the goal of reducing the effect of *C. shasta* on out-migrating juvenile Chinook salmon and potentially improving the population size of returning adults. The finding in this study of a mortality threshold will directly influence the management strategies considered to reduce the parasite burden in the Klamath River. Currently, there are several management strategies being considered to reduce the affect of *C. shasta* on out migrating juvenile salmonids. One is to establish a more dynamic flow regime. Depending on the timing, implementation of this strategy could alter the parasite life cycle either by decreasing the transmission efficiency of the myxospores released from adult carcasses to the polychaete host, thereby reducing actinospore production, or by increasing the migration rate of the out migrant juvenile salmon, resulting in reduced exposure to the actinospore. Another potential strategy is to augment the river bed with gravel and other sediment. This material could be mobilized during a flow event, disturbing polychaete populations and thus reducing actinospore density. A third strategy is removal of adult carcasses from the river to deplete the source of myxospores, thus lowering the infection prevalence in polychaete populations. Given the high mortality observed during the June exposure, it is probable that more than one strategy will need to be implemented to reduce the parasite burden on the out migrating salmonids. The presence of the mortality threshold provides a target for reduction that each of the different strategies can be compared to in order to

determine their relative effectiveness individually or in combination at reducing the parasite burden.

Acknowledgements:

We would like to thank fellow Oregon State University researchers Charlene Hurst and Zach Semerikov for their assistance in conducting the field experiments; Don Stevens at the SDL for providing guidance in cage design and production; Rich Holt for ensuring proper fish care practices; Gerri Buckles for aiding the qPCR assay; Kim Ruschton and the staff at Iron Gate Hatchery for providing Chinook salmon. Sascha Hallett, Carl Schreck (Oregon Cooperative Fishery Unit) and Josh Strange (Yurok Tribal Fisheries Program) are thanked for comments and editorial suggestions in this manuscript. We express our gratitude to the staff at Fisher's RV Park for access to the Klamath River to conduct this study. Funding for this study was provided by the Bureau of Reclamation.

Literature Cited

Anderson RM, May RM 1978 Regulation and Stability of Host-Parasite Population Interactions. *J. of Anim Ecol*. 47: 219-247.

Anderson RM, May RM 1979 Population biology of infectious diseases: Part I. Nature. 280:361-367.

Atkinson SD, Bartholomew JL. Disparate infection patterns of *Ceratomyxa shasta* (Myxozoa) in rainbow trout *Oncorhynchus mykiss* and Chinook salmon *Oncorhynchus tshawytscha* correlate with ITS-1 sequence variation in the parasite. Int J Parasitol. Accepted October 2009

Bartholomew JL, Smith CE, Rohovex JS, Fryer JL 1989 Characterization of a host response to the myxosporean parasite *Ceratomyxa shasta*, by histology, scanning electon microscopy and immunological techniques. J Fish Disease 12: 509-522

Barthomew JL 1998 Host resistance to infection by the myxosporean parasite *Ceratomyxa shasta*: a review. J Aquat Anim Health 10:112-120.

Bartholomew JL, Fryer JL, Rohovec JS 1990 Impact of the myxosporean parasite *Ceratomyxa shasta* on survival of migrating Columbia River basin salmonids. NOAA Technical Report NMFS 111: 33-41.

Bartholomew JL, Rohovec JS, Fryer JL 1989 *Ceratomyxa shasta*, a myxosporean parasite of salmonids. US Fish and Wildlife Service, Fish Disease Leaflet 80. http://www.lsc.usgs.gov/FHB/leaflets/80.asp

Bartholomew JL, Whipple MJ, Stevens DG, Fryer JL 1997 The life cycle of *Ceratomyxa shasta*, a myxosporean parasite of salmonids, requires a freshwater polychaete as an alternate host. J of Parasit 83: 859-868.

Bartholomew J.L. (2002) Salmonid ceratomyxosis. In: AFS-FHS (American Fisheries Society- Fish Health Section). FHS Blue Book: Suggested Procedures for the Detection of Certain Finfish and Shellfish Pathogens. 2007 edn. American Fisheries Society-Fish Health Section, Bethesda,Maryland.

Bjork SJ, Bartholomew JL 2009 Effects of *Ceratomyxa shasta* dose on a susceptible strain of rainbow trout and comparatively resistant Chinook and coho salmon. Dis Aquat Org 86:29-37.

Conrad JF, Decew M 1966 First report of Ceratomyxa in juvenile salmonids in Oregon. The Progressive Fish-Culturist 28:238-238.

Crofton HD 1971 A quantitative approach to parasitism. Parasitology 62: 179-193.

Dietz K 1993 The estimation of the basic reproduction number for infectious diseases. Statistical Methods in Medical Research 2: 1-23.

Dobson AP, Foufopoulos J 2000 Emerging infectious pathogens of wildlife. Phil Trans R Soc B 356: 1001-1012.

Dobson AP 1988 The population biology of parasite-induced changes in host behavior. Q Rev Biol 63: 139-165

Foott JS, Harmon R, Stone R 2004 Effect of water temperature on non-specific immune function and ceratomyxosis in juvenile Chinook salmon and steelhead from the Klamath River. California Fish and Game 90:71-84

Foott JS, Williamson JD, True KC 1999 Health, physiology, and migration characteristics of Iron Gate Hatchery Chinook, 1995 releases. US Fish and Wildlife Service, CA-NV Fish Health Center, Anderson CA.

Foott JS, Stutzer G, Fogerty R, Hansel HC, Juhnke SD, Beeman JW 2009 Joint USFWS-USGS Technical report: Pilot study to access the role of *Ceratomyxa shasta* infection in mortality of fall-run Chinook smolts migration through the lower Klamath River in 2008. US Fish and Wildlife Service CA-NV Fish Health Center, Anderson CA.

Hallett SL, Bartholomew JL 2006 Application of a real-time PCR assay to detect and quantify the myxozaon parasite *Ceratomyxa shasta* in river water samples. Dis Aquat Org 71:109-118.

Hosack GR, Li HW, Rossignol PA 2009 Sensitivity of system stability to model structure. Ecol Model 220: 1054-1062.

May RM, Anderson RM 1979 Population biology of infectious diseases: Part II. Nature 280: 455-461

Neubert MG, Caswell H 1997 Alternatives to resilience for measuring the responses of ecological systems to perturbations. Ecology 78:653-665

Nichols K, Ture K 2007 FY 2006 Investigational report: Monitoring incidence and severity of *Ceratomyxa shasta* and *Parvicapsula minibicornis* infections in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*O.*

kisutch) in the Klamath River. US Fish and Wildlife Service CA-NV Fish Health Center

Nichols K, True K, Fogerty R, Ratcliff L 2008 FY 2007 Investigational report: Klamath River juvenile salmonid health monitoring, April-August 2007 US Fish and Wildlife Service CA-NV Fish Health Center

Nichols K, True K, Fogerty R, Ratcliff L, Bolick A 20098 Myxosporean parasite (*Ceratomyxa shasta* and *Parvicapsula minibicornis*) prevalence and severity in Klamath River Basin juvenile Chinook (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*), April-August 2008. US Fish and Wildlife Service CA-NV Fish Health Center

Noble ER 1950 On a myxosporidian (Protozoan) parasite of California trout. J Parasit 36: 457-460

Ogüt H 2003 Modeling of fish disease dynamics: A new approach to an old problem. Turkish J Fish Aquat Sci 3: 67-74

Ratliff DE 1983 *Ceratomyxa shasta*: Longevity, distribution, timing, and abundance of the infective stage in central Oregon. Can J Fish Aquat Sci 40:1622-1632

Ross R 1911 The prevention of malaria. London: John Murray. 669p

Smith DL, McKenzie FE, Snow RW, Hay SI 2007 Revisiting the basic reproductive number for malaria and its implications for malaria control. PLoS Biol 5: 631-642.

Stocking RW, Bartholomew JL 2007 Distribution and habitat characteristics of *Manayunkia speciosa* and infection prevalence with the parasite *Ceratomyxa shasta* in the Klamath River, Oregon-California. J Parasit 93:78-88

Stocking RW, Holt RA, Foott JS, Bartholomew JL 2006 Spatial and temporal occurrence of the salmonid parasite *Ceratomyxa shasta* in the Oregon-California Klamath River Basin. J Aquat Anim Health 18:194-202

Udey LR, Fryer JL, Pilcher KS 1975 Relation of water temperature to ceratomyxosis in rainbow trou (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). Fish Res. Board Can 32: 1545-1551

Wallace M (2004) Natural vs. hatchery proportions of juvenile salmonids migrating through the Klamath River Estuary and monitor natural and hatchery juvenile salmonid emigration from the Klamath River Basin: July 1, 1998 through June

30, 2003. California Department of Fish and Game Inland and Anadromous Sport Fish Management and Research, Klamath River Basin Juvenile Salmonid Investigations. Final performance report - Federal Aid in Sport Fish Restoration Act - Project No. F-51-R-6, Arcata, CA. 50 p.

Tables & Figures:

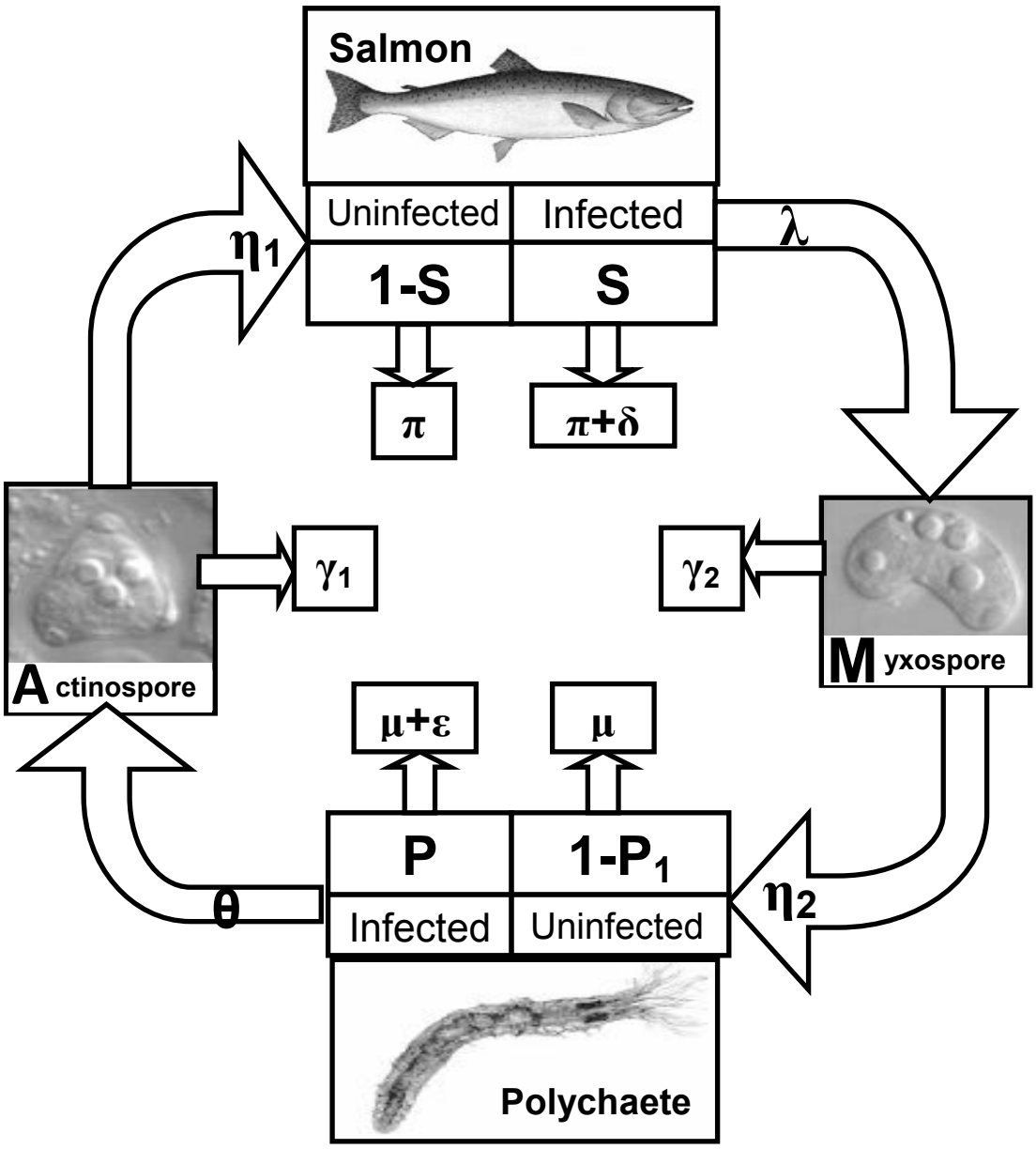

Figure 1: A flow chart for the life cycle of *Ceratomyxa shasta* and its hosts. The myxospore stage (M) infects a proportion of the polychaete host (P), which produces the actinospore stage (A) that infects a proportion of the salmonid host (S). The experiments in this study provide a series of values for the transmission (η_1) of actinospores (A) to the salmonid host (S) and the resulting mortality (δ). Parameters are described in Table 1. The differential equations (1-5) are detailed in the results section.

Table 1: Definition of parameters in the epidemiological model for *Ceratomyxa shasta*

Symbol	Parameter
P	Proportion of infected polychaete
S	Proportion of infected salmon
A	Number of Actinospores
M	Number of Myxospores
η_1	Transmission rate to salmon
η_2	Transmission rate to polychaete
λ	Myxospore production
θ	Actinospore production
π	Natural mortality of salmon
δ	Parasite induced mortality
μ	Natural mortality of polychaete
ε	Parasite induced mortality
γ_1	Loss of actinospores
γ_2	Loss of myxospores

Table 2: Comparison of total *Ceratomyxa shasta* dosage and resulting mortality of Iron Gate Hatchery Chinook salmon (*Oncorhynchus tshawytscha*) exposed in the Klamath River for different durations in June and September 2008.
* pooled into one sample in the laboratory

Exposure	June		September	
Duration (hours)	**Actinospore Dose (x 10^6)**	**% Mortality**	**Actinospore Dose (x 10^6)**	**% Mortality**
72	612.0 (+/- 51.0)	94.2 % (+/- 2.7)	153.2 (+/- 4.3)	34.9 % (+/- 12.6)
48	594.5 (+/- 48.2)	98.5 % *	13.2 (+/- 3.7)	17.7% (+/- 13.3)
24	535.4 (+/- 41.5)	84.7 % *	6.6 (+/- 2.0)	16.7% (+/- 6.6)
16	---	---	4.4 (+/- 1.3)	2.5% (+/- 7.1)

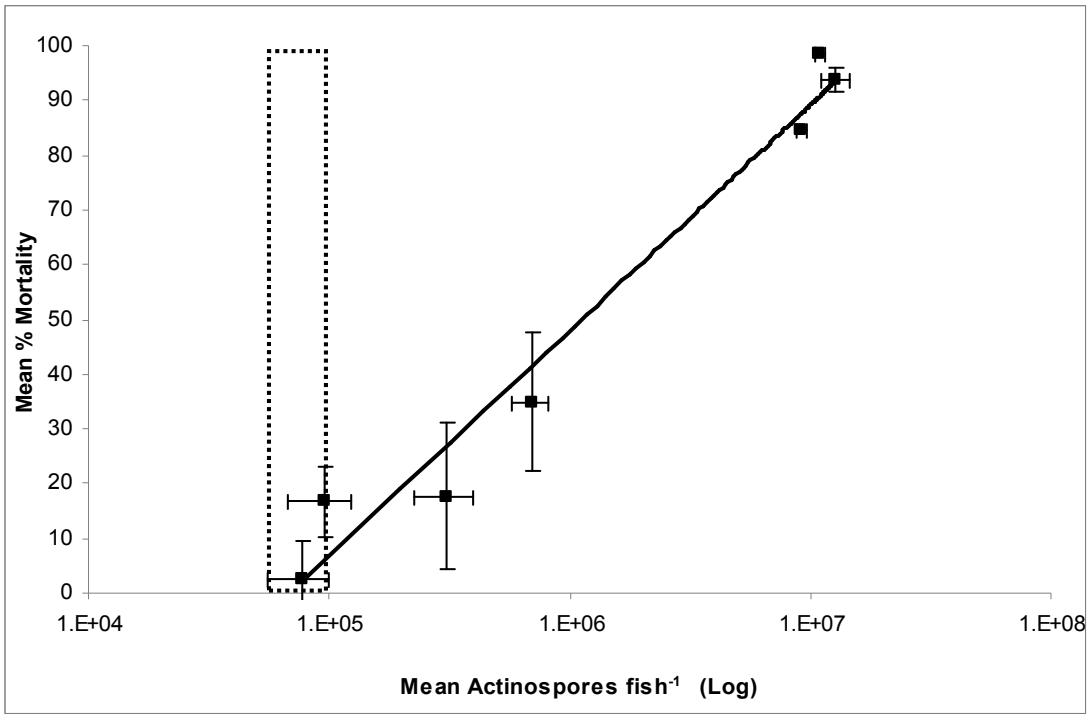

Figure 2: Relationship between *Ceratomyxa shasta* induced mortality in Iron Gate Hatchery Chinook salmon (*Oncorhynchus tshawytscha*) and estimated actinospore dose fish^{-1}. The bars represent the standard deviations on the percent mortality and actinospore dose. The x-axis is log transformed to better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

CHAPTER 3: Summary

Development of this epidemiological model is the foundation of this thesis. This model can be used to provide a framework for integrating the data that has been collected and signify where new and further research is required. A series of four differential equations were developed from this model and four specific parameters were quantified by in-river exposures. The quantification of these parameters led to the determination of a mortality threshold. IGH Chinook salmon were shown to have a mortality threshold to *C. shasta* that ranges from 5.5-9.9 x 10^4 actinospores per fish; above this threshold there is a logarithmic increase in mortality with increasing parasite dose.

Management implications:

- This mortality threshold provides a target for reducing the number of actinospores in the Klamath River and decreasing the effect of *C. shasta* on out-migrating juvenile salmonids. This threshold also indicates that complete eradication of the parasite is not necessary to decrease the effect of the parasite on the juvenile survival rates.
- Given the high parasite densities measured in this and previous studies within the Klamath River, several different management strategies may be required to successfully achieve the desired reduction.

- Given the different susceptibilities of different salmonid species and strains to *C. shasta*, the mortality threshold will need to be quantified for each in watersheds where management of the parasite may be necessary.

Future Research:

- In this study the transmission rate of actinospores to the salmonid host was indirectly estimated from the other parameters directly quantified. However this may be one of the more important parameters in the disease cycle and therefore a more directly quantified study is required. This may be achieved by exposing IGH Chinook salmon in the Klamath River, quantifying the parasite dose, immediately removing the gills and analyze the gill tissue with qPCR. This parasite dose and attachment of spores on the gill can potentially relate the transmission efficiency to mortality induced by the parasite. This experiment could be extended to examine the effects of different water velocities on the transmission rates, if it is deemed important in relation to the model.
- Except for the previously mentioned experiment, the remaining parameters in the life cycle consist of the interactions between the myxospore stage and the polychaete host. Parameters that will need to be quantified are the prevalence of infection among returning adults, production of myxospores from infected adults, the transmission of the myxospore to the polychaete and the proportion of infected polychaetes. However, quantification of these parameters is not

the only component necessary for complete understanding of this portion of the model; it also requires information on the timing and duration of the myxospore release and how that overlaps with population demographics of the polychaete host.

- This model does not directly address environmental or genetic variables that may effect the transmission or dissemination of *C. shasta*. Water temperature has been demonstrated to affect the parasite induced mortality rate of the salmonid host, but it is not well understood in relation to incubation, release or transmission to either host. Water velocity has been shown to affect the prevalence of infection in polychaete host; however this study was conduct as a run feature with two different velocities. The influence of different river features, such as eddies, has yet to be examined. The circular flow of eddies may cause the myxospores the settle out in higher densities and also in closer proximity to the polychaete host thereby increasing the probability of infection within this patchily distributed host. Recent findings indicate that different strains of the parasite are specific to a salmonid host. However it is not yet known how these different strains may alter the infection rate or virulence within a salmonid. These strains may cooperatively infect the host, dramatically reducing its immune function allowing for fewer parasites to induce infection and mortality. On the other hand, the strains could compete to infect the host thereby further increasing the number of parasites required

for infection. The dynamics of the strain interaction need to be further understood before this research can be incorporated into the model

BIBLIOGRAPHY:

Aron JL, May RM 1982 The population dynamics of malaria.In: Population dynamics and infectious disease. London: Chapman and Hall. 139-179

Anderson RM, May RM 1978 Regulation and Stability of Host-Parasite Population Interactions. Journal of Animal Ecololgy 47: 219-247.

Anderson RM, May RM 1979 Population biology of infectious diseases: Part I. Nature 280:361-367.

Atkinson SD, Bartholomew JL. Disparate infection patterns of *Ceratomyxa shasta* (Myxozoa) in rainbow trout *Oncorhynchus mykiss* and Chinook salmon *Oncorhynchus tshawytscha* correlate with ITS-1 sequence variation in the parasite. International Journal of Parasitology Accepted October 2009

Bartholomew JL, Smith CE, Rohovex JS, Fryer JL 1989 Characterization of a host response to the myxosporean parasite *Ceratomyxa shasta*, by histology, scanning electon microscopy and immunological techniques. Journal of Fish Diseases 12: 509-522

Barthomew JL 1998 Host resistance to infection by the myxosporean parasite *Ceratomyxa shasta*: a review. Journal of Aquatic Animal Health 10:112-120.

Bartholomew JL, Fryer JL, Rohovec JS 1990 Impact of the myxosporean parasite *Ceratomyxa shasta* on survival of migrating Columbia River basin salmonids. NOAA Technical Report NMFS 111: 33-41.

Bartholomew JL, Rohovec JS, Fryer JL 1989 *Ceratomyxa shasta*, a myxosporean parasite of salmonids. US Fish and Wildlife Service, Fish Disease Leaflet 80. http://www.lsc.usgs.gov/FHB/leaflets/80.asp

Bartholomew JL, Whipple MJ, Stevens DG, Fryer JL 1997 The life cycle of *Ceratomyxa shasta*, a myxosporean parasite of salmonids, requires a freshwater polychaete as an alternate host. Journal of Parasitology 83: 859-868.

Bartholomew JL 2002 Salmonid ceratomyxosis. In: AFS-FHS (American Fisheries Society- Fish Health Section). FHS Blue Book: Suggested procedures for the detection of certain finfish and shellfish pathogens. 2007 edn. AFS-FHS, Bethesda,Maryland.

Bartholow JM 2005 Recent water temperature trends in the lower Klamath River, California. North American Journal of Fisheries Management 25: 152-162.

Bjork SJ, Bartholomew JL 2009 Effects of *Ceratomyxa shasta* dose on a susceptible strain of rainbow trout and comparatively resistant Chinook and coho salmon. Diseases of Aquatic Organisms 86:29-37.

Bruce-Chwatt LJ, Garrett-Jones C, Weitz B 1966. Ten years study (1955-1964) of host selection by anopheline mosquitoes. Bulletin World Heath Oragnization 35: 405-439

Buchanan DV, Sanders JE, Zinn JL, Fryer JL 1983j Relative susceptibility of four strains of summer steelhead to infection by *Ceratomyxa shasta*. Transactions of the American Fisheries Society 112: 541-543

CADFG (California Department of Fish and Game) 2008 Klamath River Fall Chinook Salmon Age-specific escapement, river harvest and run-size estimates, 2008 run. http://www.dfg.ca.gov/marine/pdfs/salmon2009handouts.pdf

CAEPA (California Environmental Protection Agency) 2006 Clean water act section 303(d) list of water quality limited segments requiring TMDLs. http://www.swrcb.ca.gov/water_issues/programs/tmdl/docs/303dlists2006/epa/r1_06_303d_reqtmdls.pdf

Ching HL, Munday DR 1984a Geographic and seasonal distribution of the infectious stage of *Ceratomyxa shasta* Noble, 1950, a myxozoan salmonid pathogen in the Fraser River system. Canadian Journal of Zoology 62: 1075-1080

Ching HL, Munday DR 1984b Susceptibility of six Fraser Chinook salmon stocks to *Ceratomyxa shasta* and the effects of salinity on ceratomyxosis. Canadian Journal of Zoology 62: 1081-1083

Ching HL, Parker L 1989 Experimental exposure of trout and salmon from 12 British Columbian stocks to the myxozoan parasite *Ceratomyxa shasta*. Journal of Aquatic Animal Health 1: 205-208

Conrad JF, Decew M 1966 First report of Ceratomyxa in juvenile salmonids in Oregon. The Progressive Fish-Culturist 28:238-238.

Crofton HD 1971 A quantitative approach to parasitism. Parasitology 62: 179-193.

Dietz K 1993 The estimation of the basic reproduction number for infectious diseases. Statistical Methods in Medical Research 2: 1-23.

Dobson AP, Foufopoulos J 2000 Emerging infectious pathogens of wildlife. Philosophical Transactions of the Royal Society of London. Series B. Biological Sciences 356: 1001-1012.

Dobson AP 1988 The population biology of parasite-induced changes in host behavior. Quarterly Review of Biology: 139-165

Foott JS, Harmon R, Stone R 2004 Effect of water temperature on non-specific immune function and ceratomyxosis in juvenile Chinook salmon and steelhead from the Klamath River. California Fish and Game 90:71-84

Foott JS, Williamson JD, True KC 1999 Health, physiology, and migration characteristics of Iron Gate Hatchery Chinook, 1995 releases. US Fish and Wildlife Service, CA-NV Fish Health Center, Anderson CA.

Foott JS, Stutzer G, Fogerty R, Hansel HC, Juhnke SD, Beeman JW 2009 Joint USFWS-USGS Technical report: Pilot study to access the role of *Ceratomyxa shasta* infection in mortality of fall-run Chinook smolts migration through the lower Klamath River in 2008. US Fish and Wildlife Service CA-NV Fish Health Center, Anderson CA.

Hallett SL, Bartholomew JL 2006 Application of a real-time PCR assay to detect and quantify the myxozaon parasite *Ceratomyxa shasta* in river water samples. Diseases of Aquatic Organisms 71:109-118.

Hamilton JB, Curtis GL, Snedaker SM, White DK 2005 Distribution of anadromous fishes in the Upper Klamath River watershed prior to hydropower dams—A synthesis of the historical evidence. Fisheries 30:10-20

Hardy TB, Addley RC 2001 Evaluation of interim instream flow needs in the Klamath River, Phase II final report. Draft report prepared for US Dept of the Interior, byt Insititute for Natural Systems Engineering. Utah. Nov 2001. 304 p.

Hosack GR, Li HW, Rossignol PA 2009 Sensitivity of system stability to model structure. Ecological Modeling 220: 1054-1062.

Hoffmaster JL, Sanders JE, Rohovec JS, Fryer JL, Stevens DG 1988 Geographic distribution of the myxosporean parasite, *Ceratomyxa shasta* Noble, 1950, in the Columbia River Basin, USA. Journal of Fish Disease 11: 97-100

Kingsolver JG 1987 Mosquito host choice and the epidemiology of malaria. The American Naturalist. 130: 811-827

Leidy RA, Leidy GR 1984 Life stage periodicities of anadromous salmonids in the Klamath River Basin, Northwestern California. US Fish and Wildlife Service, Division of Ecological Services. Sacramento CA. 39 .p

Li X, Sina B, Rossignol PA 1992 Probing behavior and sporozoite delivery by *Anaopheles stephensi* infected with *Plasmodium berghei* Medical Veterinarian Entomology. 6: 57-61

Lopez GR, Levinton 1987 Ecology of deposit-feeding animals in marine sediments. The Quarterly Review of Biology 62: 235-260

Lotka AJ 1920 Analytical note on certain rhythmic relations in organic systems. Proceeding of the National Academy of Sciences. 6: 410-415.

Mackie GL, Qadri SU 1971 A polychaete *Manayunkia speciosa*, from the Ottoawa River, and its North American distribution. Canadian Journal of Zoology. 49:780-782

Margolis L, Evelyn TPT 1975 *Ceratomyxa shasta* (Myxosporidia) disease in chum salmon (*Oncorhynchus keta*) in British Columbia. Journal of Fisheries Research Board Canada. 32: 1640-1643

May RM, Anderson RM 1978 Regulation and Stability of Host-Parasite Population Interactions. Journal of Animal Ecology 47: 249-267.

May RM, Anderson RM 1979 Population biology of infectious diseases: Part II. Nature 280: 455-461

Macdonald G 1952 The analysis of equilibrium in malaria. Tropical Disease Bulletin 49: 813-829.

McKenzie FE 2000 Why model malaria? Parasitology Today 16: 511-516

Neubert MG, Caswell H 1997 Alternatives to resilience for measuring the responses of ecological systems to perturbations. Ecology 78:653-665

Nichols K, Ture K 2007 FY 2006 Investigational report: Monitoring incidence and severity of *Ceratomyxa shasta* and *Parvicapsula minibicornis* infections in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*) in the Klamath River. US Fish and Wildlife Service CA-NV Fish Health Center

Nichols K, True K, Fogerty R, Ratcliff L 2008 FY 2007 Investigational report: Klamath River juvenile salmonid health monitoring, April-August 2007 US Fish and Wildlife Service CA-NV Fish Health Center

Nichols K, True K, Fogerty R, Ratcliff L, Bolick A 20098 Myxosporean parasite (*Ceratomyxa shasta* and *Parvicapsula minibicornis*) prevalence and severity in Klamath River Basin juvenile Chinook (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*), April-August 2008. US Fish and Wildlife Service CA-NV Fish Health Center

Noble ER 1950 On a myxosporidian (Protozoan) parasite of California trout. Journal of Parasitology 36: 457-460

Palenzeula O, Trobridge G, Bartholomew JL 1999 Development of a polymerase chain reaction diagnostic assay of *Ceratomyxa shasta*, a myxosporean parasite of salmonid fish. Diseases of Aquatic Organisms. 36:45-51

Ogüt H 2003 Modeling of fish disease dynamics: A new approach to an old problem. Turkish Journal of Fisheries and Aquatic Sciences 3: 67-74

Ratliff DE 1981 *Ceratomyxa shasta*: Epizootiology in Chinook salmon of central Oregon. Transaction of the American Fisheries Society 110: 507-513

Ratliff DE 1983 *Ceratomyxa shasta*: Longevity, distribution, timing, and abundance of the infective stage in central Oregon. Canadian Journal of Fisheries and Aquatic Sciences 40:1622-1632

Ross R 1911 The Prevention of Malaria. London: John Murray. 669p

Rossignol PA, Riberio JMC, Spielman A 1986 Increased biting rate and reduced fecundity in sporozoite-infected mosquitoes. American Journal of Tropical Medical Hygiene. 35: 277-279.

Sanders JE, Fryer JL, Gould RW 1970 Occurrence of the myxosporidian parasite *Ceratomyxa shasta*, in salmonid fish from the Columbia River Basin and Oregon coastal streams. A Symposium on disease of fish and shellfishes. American Fisheries Society Special Publication. 5: 131-141

Schafer WE 1968 Studites on the epizootiology of the myxosporidan *Ceratomyxa shasta* Noble. California Fish and Game 54: 90-99

Smith DL, McKenzie FE, Snow RW, Hay SI 2007 Revisiting the basic reproductive number for malaria and its implications for malaria control. Public Library of Science Biology 5: 631-642.

Spencer DR, 1976 Occurrence of *Manayunkia speciosa* (Polychaeta: Sabellidae) in Cayuga Lake, New York, with additional notes on its North American distribution. Transactions of the American Microscopical Society. 95:127-128

Stocking RW, Bartholomew JL 2007 Distribution and habitat characteristics of *Manayunkia speciosa* and infection prevalence with the parasite *Ceratomyxa shasta* in the Klamath River, Oregon-California. Journal of Parasitology 93:78-88

Stocking RW, Holt RA, Foott JS, Bartholomew JL 2006 Spatial and temporal occurrence of the salmonid parasite *Ceratomyxa shasta* in the Oregon-California Klamath River Basin. Journal of Aquatic Animal Health 18:194-202

Udey LR, Fryer JL, Pilcher KS 1975 Relation of water temperature to ceratomyxosis in rainbow trou (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). Journal of Fisheries Research Board Canada 32: 1545-1551

Volterra V 1926 Fluctuations in the abundance of a species considered mathematically. Nature 118: 558-560

Wallace M (2004) Natural vs. hatchery proportions of juvenile salmonids migrating through the Klamath River Estuary and monitor natural and hatchery juvenile salmonid emigration from the Klamath River Basin: July 1, 1998 through June 30, 2003. California Department of Fish and Game Inland and Anadromous Sport Fish Management and Research, Klamath River Basin Juvenile Salmonid Investigations. Final performance report - Federal Aid in Sport Fish Restoration Act - Project No. F-51-R-6, Arcata, CA. 50 p.

Zinn JL, Johnson KA, Sanders JE, Fryer JL 1977 Susceptibility of salmonid species and hatchery strains of Chinook salmon (*Oncorhynchus tshawytscha*) to infections by *Ceratomyxa shasta*. Journal of Fisheries Research Board of Canada 34: 933-936.

APPENDICIES

APPENDIX A: *Ceratomyxa shasta* myxospores released by juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and potential implications for ceratomyxosis management

Introduction:

The myxozoan parasite *Ceratomyxa shasta* has a complex life cycle involving two environmental stages and two obligate hosts (Bartholomew et al. 1997). The actinospore stage is released from the polychaete host (*Manyunkia speciosa)* into the water column and infects the salmonid host. The myxospore stage then develops within the fish and is released back into the water column to infect the polychaete. The polychaete host has been identified as the sole contributor of actinospores. However, there are two life stages of the anadromous salmon (*Oncorhynchus* sp) host that can be a source of the myxospore stage. Juveniles, although small, are very numerous in the system and can become heavily infected. On the other hand, there are fewer but much larger returning adult salmon whose depleted immune system allows for relatively easy infections.

It is currently assumed that adult salmon are the main source of myxospore contribution to the disease cycle. In the Klamath, these infected adults cannot migrate above Iron Gate Dam; therefore a high number of infected adults spawn and die in the river directly below the dam and adjacent tributary, Bogus Creek. As the carcasses decompose, myxospores are released and disperse down river. This highly concentrated source overlaps with dense polychaete populations, resulting in a high incidence of infection in polychaete hosts (Stocking & Bartholomew 2007). As part of a carcass removal study in Bogus Creek, the production of myxospores from

returning adult Chinook salmon (*O. tshawytscha*) was estimated. Between October and November 2008, 100 returning adult salmon were sampled in Bogus Creek. The prevalence of infection in these fish was 30% and the average myxospore production was 1.28×10^6 per fish (Foott et. al. 2009). These numbers were then used to extrapolate an approximate production of 4.6×10^9 myxospores from adult salmon in the stretch of river between Iron Gate Dam to the Scott River confluence (~ 75 Rkm) (Foott et al. 2009).

The goal of this study was to examine the potential production of myxospores by emigrating juvenile Chinook salmon. Although these fish are leaving the system they can become heavily infected. This intense infection may result in a decreased migration rate, as lethargy is one of the clinical signs of disease (Schafer 1968, Bartholomew et al. 1989). This prolonged residence time may increase the probability of infected juveniles succumbing to the parasite and result in the release of myxospores, further contributing to the disease cycle. This study also provides insight into the reproductive dynamics of *C. shasta* within the salmonid host.

Methods and Materials:

Iron Gate Hatchery Chinook salmon (0+ age class) were held in live cages in the Klamath River for three different lengths of time in June 2008 (72, 48 and 24 hrs) and four durations in September 2008 (72, 48, 24 and 16 hours) as part of a study to estimate the lethal infection dose (Ray et al. 2009). Dead and moribund fish were microscopically examined for the myxospore stages of *C. shasta* as established by

American Fisheries Society blue-book protocol (Bartholomew 2002). Days post exposure was recorded for each dead and moribund fish to determine the mean day to death (MDD) for each exposure period. Intestine and kidney material was removed from visually positive fish. The post-mortem weight of each fish was measured before and after the intestine and kidney was removed to obtain an estimate of the tissue weight. The tissues were placed into stomacher bags with 1mL of water and the contents were then masticated in a stomacher for 2 minutes. The resulting suspension was aspirated into a 2 ml micro-centrifuge tube. If the volume of liquid was greater than the tube, multiple tubes were used then concentrated into one tube through a series of centrifugation steps at 8944 g for 5 minutes. If after this step the total volume was still greater than 1mL, it was noted and adjustments were made to account for the increased volume. If the total volume was less, water was added to 1 ml. The sample was then vortexed and approximately 10ul aliquots were dispensed onto each side of a hemocytometer. Four aliquots were counted for half of the fish from each exposure period and two aliquots were counted for the remaining fish. The total number of myxospores per fish was calculated as (myxospores counted x 10^4 x ml sample x dilution factor)/ grids counted. To account for the difference in sizes between June fish (average size at exposure 7.2 +/- .6 cm, 4.3 +/- 1.1 g) and September fish (average size at exposure 9.0 +/- 2.0 cm, 12.0 +/- 5.9 g) the total number of spores was divided by the total weight of the tissue to obtain an estimate of spores per gram.

Calculations similar to those conducted by Foott et al. (2009) were used to estimate the potential myxospore contribution from juvenile Chinook salmon. An initial population of 5 x 10^6 fish was based on the number of juvenile Chinook released from Iron Gate Hatchery between May and June. The different myxospore per fish values were actual minimum, average and maximum values recorded from the fish exposed in June. The prevalence of infection (POI) values selected represents the range observed from the juvenile health monitoring surveys (Nichols 2009). The myxospore production values are products of all three parameters.

Results:

In June, 92.4% of all the fish exposed succumbed to *C. shasta.* Conversely, in September only 18.9% fish died over the four exposure durations. No significant difference in myxospore production between any of the exposure groups in June (ANOVA p-value = 0.18) or September (ANOVA p-value = 0.36) was detected. There was no statistical difference in myxospore production between June and September exposed fish (p-value = 0.08); however, there appears to be an inverse relationship between the exposure dose and the production of myxospores per fish (Figure 1). Considering the actinospore dose in June was over 10 fold greater, the average myxospore production per fish was almost half that of the fish exposed in September (4.99 x10^5 & 9.08 x 10^5, respectively). Yet when adjusted for the differences in fish size, the myxospore production values are similar between June and September (5.7 x 10^5 & 4.3 x 10^5).

Estimates of the potential myxospore contribution from juvenile salmon for a range of infection prevalence and myxospore production values are displayed in Table 1. The myxospore production values are based on the non-weight adjusted, minimum, mean and maximum measurements of the fish exposed in June (6.3×10^2, 4.99×10^5 & 4.17×10^6, respectively). The potential contribution ranges from 9.38×10^8 (at 30% POI & minimum myxospore production) to 1.77×10^{13} (at 85% POI & maximum myxospore production). The estimate of myxospore production based solely on the minimum values is about 20% of the value estimated for the adults (Foott et al 2009); whereas the maximum value is almost 4000 fold greater. The average MDD for all fish that died from *C. shasta* infection did not vary between the different exposure doses. The average MDD between the fish exposed in June and September do not differ greatly (16 & 19 days, respectively).

Discussion:

Reduction of myxospores is one of the proposed strategies for controlling *C. shasta* in the Klamath, with the assumption that adult salmon are the primary contributors of myxospores. However, this study shows that infected juvenile Chinook salmon are capable of releasing a large number of myxospores; which may support the continued presence of ceratomyxosis in the Klamath River. This contribution from juveniles is not currently considered in this control strategy and may need to be further incorporated to reduce the effect of this parasite.

Based on the calculation of myxospore production (Table 1) the potential contribution of myxospores from emigrating juvenile Chinook salmon could be considerably greater than the contribution from returning adults. However, before integrating this information into our epidemiological model we need to better understand the spatial and temporal overlap with the polychaete host. Unlike adult salmon that become infected as they migrate up the river, and above the larger polychaete populations, juveniles are infected while migrating to the ocean, and below these polychaete populations. Given the MDD in June of 16 days, one could assume that these fish would be far down river when myxospore release occurs. However, the effect of infection on behavior during migration is unknown. One possibility is that these fish increase their migration rate and move into salt water; however, the transition to salt water is extremely taxing and cannot usually be accomplished when the fish is in a weakened or stressed state (McCormick et al. 1998). Another possibility is that the infected juveniles will experience a decrease in swimming capabilities and therefore be unable to proceed down river, as this has been demonstrated for juvenile and adult salmon for many different pathogens (Butler & Millemann 1971, Tierney & Farrell 2004, Barber 2007). Therefore a majority of the infected juveniles may remain in the fresh water habitat and eventually succumb to the infection, resulting in the release their myxospores into the main stem Klamath River.

If juvenile fish release myxospores during migration, the transmission efficiency of these myxospores will likely be much lower than that of the adult fish as

the juveniles will most die and release spores down river of the high density polychaete populations (Stocking & Bartholomew 2007). It is also likely that the demographics of the polychaete population may not be as receptive to the infective stage during the summer as adult polychaete numbers seem to decline and the young polychaetes may not yet be of suitable size to feed upon the myxospore (Josh Strange, Yurok Tribal Fisheries Biologist Pers. Comm.). Despite these constraints, the juvenile contributed myxospores may result in more polychaete populations having higher infection prevalence farther down river; thereby extending the infective area through which the adults must traverse.

This study also demonstrates the prolific replication of *C. shasta*, regardless of dose. Although the exposure doses varied, the total myxospores produced were relatively similar between each of the groups, when adjusted for the different sizes of fish. Conversely, it has been demonstrated for *Myxobolus cerebralis*, a myxozoan parasite similar to *C. shasta*, that myxospore production may be linked to the triactinomyxon dose. Rainbow trout of similar size were exposed to four different doses of triactinomyxons, and as the dose increased, so too did the number of myxospores produced (Hedrick et al. 1999). Our study supports a previous suggestion that proliferation of *C. shasta* in the salmonid host is only limited by the amount of available intestinal tissue (Bjork & Bartholomew 2009). Although the total myxospore production values differed between the June and September exposures, it found to be a result of the difference in body size and not related to the dose received, as demonstrated for *M. cerebralis*.

In this study we demonstrate that, although physically small, large numbers of infected juvenile salmonids could contribute a significant numbers of myxospores To fully appreciate the implications of these findings further studies are needed to determine the probability and transmission efficiency between this myxospore source and the polychaete host.

Literature Cited:

Barber I 2007 Parasites, behaviour and welfare in fish. Applied Animal Behaviour Science 104: 251-264

Bartholomew JL, Whipple MJ, Stevens DG, Fryer JL 1997 The life cycle of *Ceratomyxa shasta*, a myxosporean parasite of salmonids, requires a freshwater polychaete as an alternate host. Journal of Parasitology 83: 859-868.

Bartholomew JL, Smith CE, Rohovex JS, Fryer JL 1989 Characterization of a host response to the myxosporean parasite *Ceratomyxa shasta*, by histology, scanning electon microscopy and immunological techniques. Journal of Fish Diseases 12: 509-522

Bartholomew JL 2002 Salmonid ceratomyxosis. In: AFS-FHS (American Fisheries Society- Fish Health Section). FHS Blue Book: Suggested Procedures for the Detection of Certain Finfish and Shellfish Pathogens. 2007 edn. AFS-FHS, Bethesda,Maryland.

Bjork SJ, Bartholomew JL 2009 Effects of *Ceratomyxa shasta* dose on a susceptible strain of rainbow trout and comparatively resistant Chinook and coho salmon. Diseases of Aquatic Organisms 86:29-37.

Butler JA, Millemann RE 1971 Effect of the "salmon poisoning" trematode, *Nanophyetus salmincola*, on the swimming ability of juvenile salmonid fishes. The Journal of Parasitology 57: 860-865

Foott JS, Stone R, Fogerty R, True K 2009 FY2008 Technical Report: *Ceratomyxa shasta* myxospore survey of Fall-run Chinook salmon carcasses and sentinel trout exposures in Bogus Creek: Component of joint OSU-Yurok Fisheries-CDFG pilot project testing the effect of carcass removal on *C.shasta* levels in Bogus Creek, 2008. .U.S. Fish & Wildlife Service California – Nevada Fish Health Center, Anderson, CA.

Hedrick RP, McDowell TS, Gay M, Marty GD, Georgiadias MP, MacConnell E 1999 Comparative susceptibility of rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) to *Myxobolus cerebralis*, the cause of salmonid whirling disease. Diseases of Aquatic Organisms 37: 173-183

McCormick SD, Hansen LP, Quinn TP, Saunders RL 1998 Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 55: 77-92

Nichols K, True K, Fogerty R, Ratcliff L, Bolick A 2009 Myxosporean parasite (*Ceratomyxa shasta* and *Parvicapsula minibicornis*) prevalence and severity in Klamath River Basin juvenile Chinook (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*), April-August 2008. US Fish and Wildlife Service CA-NV Fish Health Center

Ray RA, Rossignol PA, Bartholomew JB Mortality Threshold for Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) in an Epidemiological Model of *Ceratomyxa shasta*. Diseases of Aquatic Organisms. Submitted Nov 2009.

Schafer WE 1968 Studites on the epizootiology of the myxosporidan *Ceratomyxa shasta* Noble. California Fish and Game 54: 90-99

Stocking RW, Bartholomew JL 2007 Distribution and habitat characteristics of *Manayunkia speciosa* and infection prevalence with the parasite *Ceratomyxa shasta* in the Klamath River, Oregon-California. Journal of Parasitology 93:78-88

Tierney KB, Farrell AP 2004 The relationships between fish health, metabolic rate, swimming performance and recovery in return-run sockeye salmon, *Oncorhynchus nerka* (Walbaum). Journal of Fish Disease 27: 663-671

Figure and Table

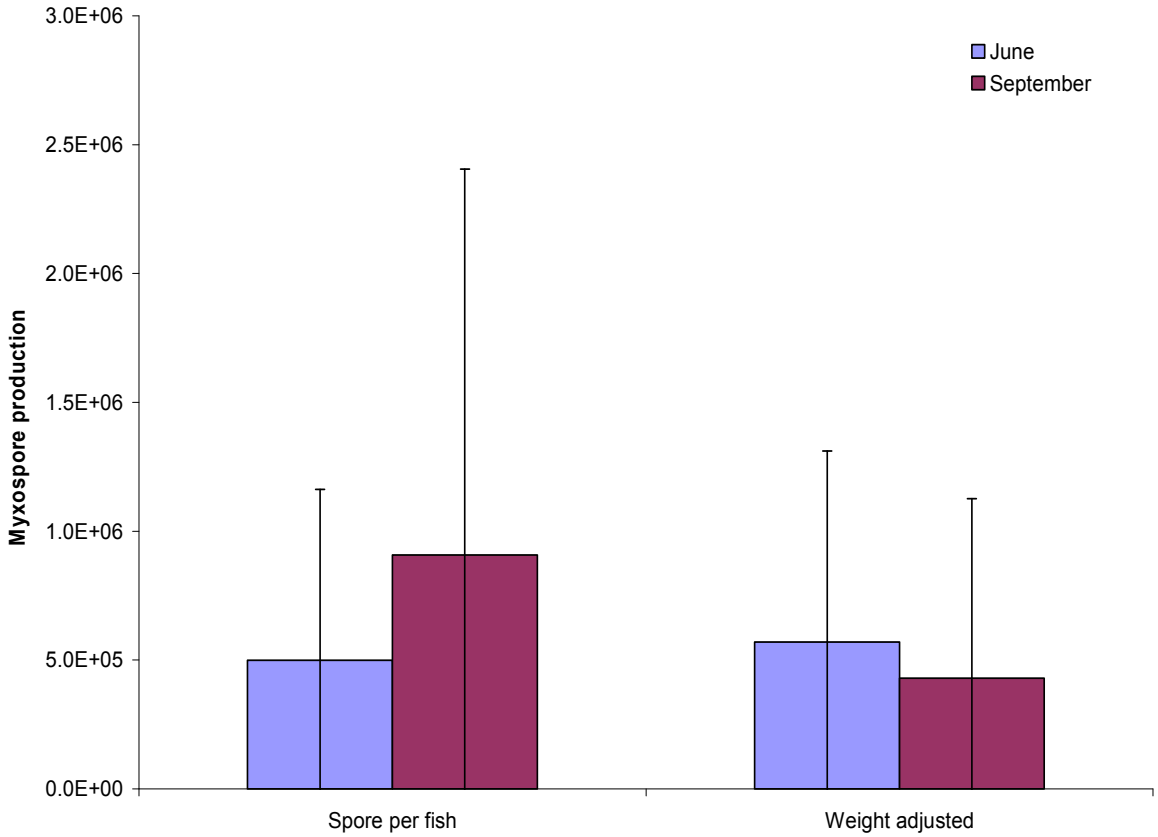

Figure 1 Average total myxospores produced per fish (left) and adjusted for the difference in weight, from Iron Gate Hatchery Chinook salmon that were exposed in June and September in the Klamath River.

Table 1: Calculations of potential myxospore contributions from 5 x 10^6 Iron Gate Hatchery released Chinook salmon between May and June for a variety of infection and myxospore production values.

	Myxospore Production		
Prevalence	Minimum	Mean	Maximum
of Infection	6.25 x 10^2	4.99 x 10^5	4.17 x 10^6
30 %	9.38 x 10^8	7.49 x 10^{11}	6.26 x 10^{12}
50 %	1.56 x 10^9	1.25 x 10^{12}	1.04 x 10^{13}
85 %	2.66 x 10^9	2.12 x 10^{12}	1.77 x 10^{13}