An Investigation of *Ceratomyxa shasta* sporulation following death of its Chinook salmon host

By
Krista Soderlund

A PROJECT

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Exercise Science
(Honors Scholar)

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Commencement June 2015

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Krista Soderlund for the degree of Honors Baccalaureate of Science in Exercise Science presented on November 11, 2014. Title: An Investigation of *Ceratomyxa shasta* sporulation following Chinook salmon host death.

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Abstract approved: _____

Dr. Michael Kent

As pre-spawning mortality (PSM) in Chinook salmon in the Willamette River Valley becomes a more defined and examined area of study, more focus is granted to not only preserving the health of these fish, but also expanding knowledge concerning various pathogens that utilize the salmon as hosts and may be associated with PSM. One such parasite, a myxozoan named *Ceratomyxa shasta,* has been known to be extremely detrimental to salmon health, yet little literature exists concerning what exactly happens to this parasite once the salmon die. This thesis briefly outlines the complexity regarding this parasite's relationship with the Chinook salmon, offers some context to transmission dynamics, and explores a recent investigation conducted over the course of the 2013-2014 academic year to determine the capability of *Ceratomyxa shasta* to live, continue to develop, and sporulate following the death of one of its hosts, the Chinook salmon.

Key Words: *Ceratomyxa shasta*, myxozoa, salmon

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Michael Kent, Mentor, representing Microbiology

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Toni Doolen, Dean, University Honors College

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

Krista Soderlund, Author

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Introduction

The Chinook salmon, also referred to as king salmon, is an anadromous species that rules the Pacific Ocean in waters ranging from Kotzebue, Alaska to Santa Barbara, California with the largest runs occurring in the Colombia River, Rogue River, Willamette River and Puget Sound. The males have an easily distinguishable hook shaped nose which actually serves as the premise for the scientific name of this species, which is *Oncorhynchus tshawytscha* (*Onkos* is the Greek word for "hook" and *rynchos* is the Greek word for "nose").

Maintaining the health of Chinook salmon populations is crucial in the Pacific Northwest, and research recently conducted (Keefer et al, 2010; Kent et al, 2011) has illustrated that a significant portion of the King salmon population in the Willamette River, Oregon, is suffering from pre-spawning mortality (PSM) in the summer after they return to freshwater to spawn. Factors that contribute to pre-spawning mortality include "stress, disease loads, poor energetic condition and exposure to stressful water temperatures" as noted by Kent et al (2011). High water temperature is an extremely detrimental multi-faceted component to the health of the salmon (Schreck et al, 1994) as it not only directly impacts the fish but also creates an environment that is more conducive for exposure to the infectious stage (actinospore) of *C. shasta* (Bartholomew et al. 2013).

This thesis reviews (i) the life cycle of the spring Chinook salmon, (ii) disease influence of pre-spawning mortality in Willamette valley adult Chinook salmon, (iii) the negative binomial distribution pattern of parasites, (iv) general characteristics of myxozoan parasites and how they reach their salmonid hosts, with a focus on *C. shasta*. I also present results from my

investigation of *C. shasta* prevalence and intensity in Chinook salmon hosts at the time of death compared to seven days later.

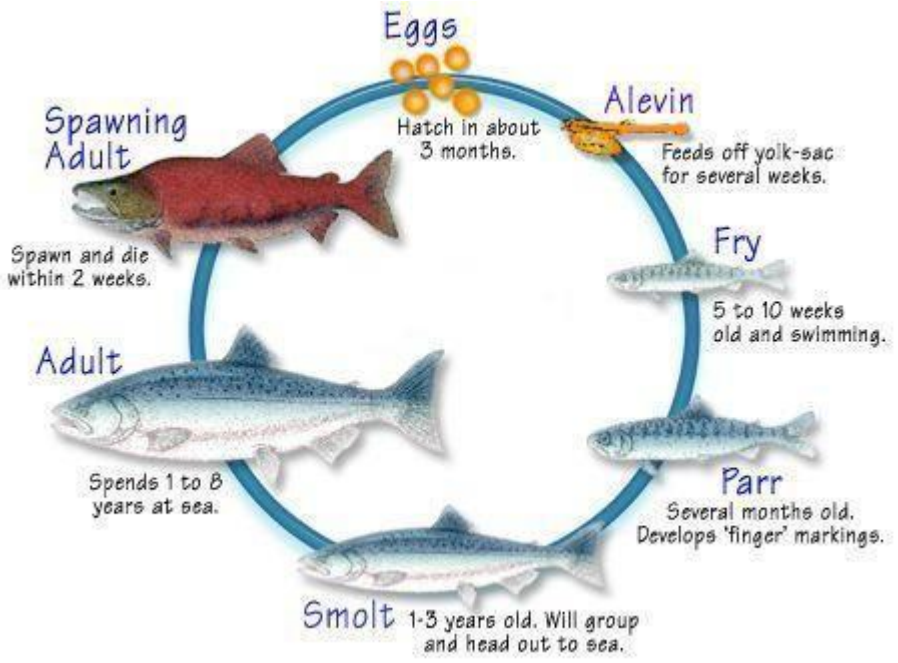
The Life Cycle of the spring Chinook salmon

Adult spring Chinook salmon usually initiate passage through the Willamette Falls in the middle of March, with a continuation of migration up until July (with a peak in April and May). The female salmon build nests in tributaries that are referred to as *redds* in August and spawning typically begins in September and continues through October (Schroeder et al, 2007). Contemporary spawn timing occurs a little later than in the past, which is believed to be related primarily to temperature elevations (Keefer et al. 2010). After the redd has been fertilized, the eggs will remain in the gravel over the course of winter while the embryos develop. In the spring, *alevins* emerge from the redd; these are small fish with the yolk sac attached to their belly, which they use as a source of nutrients over the course of several months (National Park Service, 2014). Once the yolk sac has been consumed, the fish is considered to be a *fry*. After a short time they develop into *parr,* which will spend time (typically around five months for Chinook salmon) in its natal stream before environmental cues like water temperature prompt them to head downstream to the ocean (Fig. 1).

Many developments occur in the salmon in addition to physiological changes that allow the fish to thrive in sea water; the parr begins *smolting* and acquires a silvery color to its growing scales. When the smolt has migrated all the way down the river, it reaches an estuary which provides a unique and important environment for the young salmon to acclimate to the ocean. The salmon remain in the ocean for up to eight years, and may stay primarily in coastal waters or may travel to feeding grounds, but it is more common for Chinook salmon to return to their natal

spawning grounds after four or five years (Keefer et al. 2010). This is an exhausting and debilitating journey for salmon, as they do not consume any food once they re-enter freshwater and live off of the reserves in their body. The immune system is severely compromised, especially at the end of this migration, (Mann et. al, 2010). If salmon do not die before spawning then they do shortly thereafter, which supplies nutrients to the river. It is a delicate and impressive process, and the outcome depends on factors mentioned in the introduction such as stress and temperature, and quite drastically, on diseases occurring as after they return to freshwater in the summer.

Figure 1: Life Cycle of the Chinook salmon (FishEx, 2014).



Disease influence of pre-spawning mortality in Willamette valley adult Chinook salmon

Pre-spawning mortality (PSM) in Chinook salmon in the Willamette Valley is a particularly poignant concept because the fish have made it so far only to die because of factors such as high stress, altered water temperature, or diseases before their life cycle is complete. A study conducted by Mann et al. (2010) distinctly supported the idea that parasites and lesions are responsible for PSM or play an important role at the very least. The fact that all the fish that died prespawning that were collected by Mann's research group in 2009 and 2010 had signs of infection, injury and internal diseases substantiates this idea.

This event has been extensively studied by researchers across the Pacific Northwest and some informative conclusions have been reached. It is common for salmon that spawn to be heavily infected with various pathogens as they simply have not reached a threshold that is severe enough to kill the fish prior to spawning. This can be related to the fact that salmon are semelparous, meaning that they typically only have a single reproductive episode before death, and their health rapidly deteriorates (infection or not) prior to the action of spawning. Hence, many fish with infection still die of natural causes, but it is increasingly more common (or more noticed, perhaps) for diseases to worsen quick enough to result in the demise of the fish prior to spawning.

Histopathology is often a preferred method of diagnostics because many parasites can be recognized and the pathogen impact on the host can be viewed directly by the observer, even though it is not as sensitive as other methods and can be subjective (Kent et al, 2012). This refers to the microscopic evaluation of tissue in order to determine if the organism is diseased and if so, the severity of the infection. Histopathology is also used in combination with other methods like

macroscopic observations and microbial cultures. Diseases found in Chinook salmon in the Willamette Valley include but are not limited to Digenea (flatworms, often found in gills), Myxozoa (often found in the kidney and other internal organs) and bacterial diseases (like furunculosis, which is caused by *Aeromonas salmonicida*).

Figure 2. Histological section of a Chinook salmon intestine showing numerous presporogonic forms of *Ceratomyxa shasta* (arrows).

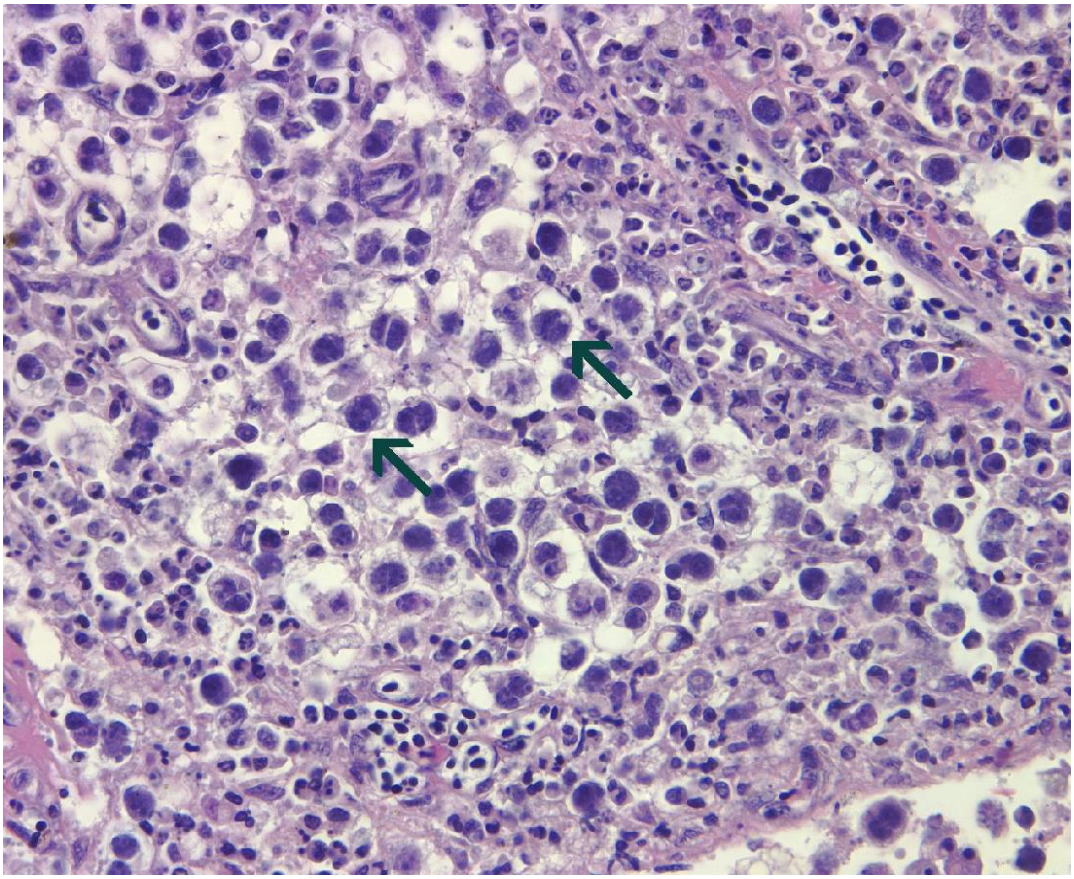


Figure 3. A *Ceratomyxa shasta* spore in a wet mount tissue preparation from the intestine, viewed with a hemocytometer.

Figure 4. An Atlantic salmon displaying skin lesions (boils) associated with a bacterial infection (Hastein, 2014).

These conditions can be seen on Chinook salmon and as research conducted by Schreck et al. (2013) illustrates, the pathogens become more common as summer progresses and the fish migrate upstream. For example, in the 2010 group of salmon observed in June at Willamette Falls, there were only two pathogens seen and only one was classified as severe (*Myxobolus* sp). In contrast, by August there were eight pathogens observed and five were classified as severe, and the samples were also taken further upstream from the Dexter and Fall Creek locations. Because the number of parasites noted was low when collected early in the summer, it indicates that the salmon either become infected with the pathogens in the basin while migrating in freshwater to their spawning locations (OSU Condition Study 2013) or that pre-existing low level infections that are not detected earlier proliferate as the summer progresses.

A similar pattern was seen in each of the observations from 2010-2013. Whereas there is great variability between the parasitic prevalence and intensity in salmon between early June and late August, there is very little variability in the disease profiles between the PSM salmon from July and August and the spawned salmon collected in September. Salmon do not consume food once they enter freshwater. Therefore, as the run season progresses they enter a catabolic state and their immune systems become increasingly compromised, thus they become more susceptible to diseases. This can also help explain why by August so many of the fish have been infected (Kent, personal communication, 2013). As noted by the OSU research group working on PSM, the diseases that are often present at the death of the fish spread too quickly in the PSM salmon. Also, the diseases can affect juvenile salmon that are outmigrating that come into contact with parasites in the fresh water before they reach the ocean (Ray & Bartholomew, 2012).

Negative Binomial Distribution of Parasites

Parasitism describes the relationship between two independent species in which one species benefits (the parasite) and the other suffers (the host). Heavily infected host individuals may die, but a simple relationship between the parasite and host does not always result in death, as can be seen with many infections exhibited in Chinook salmon. The graphical relationship between burden of infection and the host can best be represented with the *negative binomial distribution,* as first described by Crofton (1971). This graph varies from a bell-shaped curve (that tapers off on both sides) as it starts high and only tapers on one side. This is normally the distribution observed between the severity of infection (categorized as the number of parasites) and the number of hosts (Tanner et al., 1980).

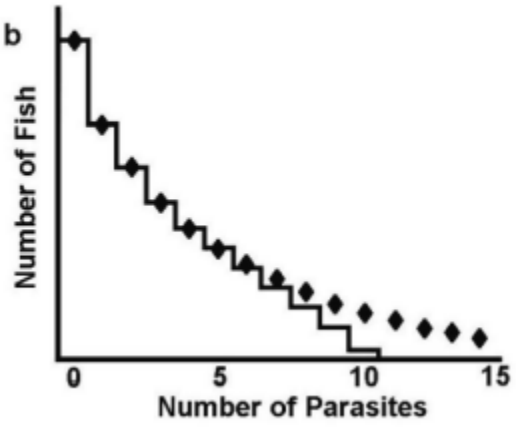
There are a few features that define this unique relationship, as noted in Crofton (1971): The parasites have a higher reproductive potential than the host species, the parasite kills *heavily* infected hosts, and the infection produces an overdispersed distribution of parasites within the host population (overdispersion is a statistical term for when there is greater variability in a data set then would be expected). The overdisperion may occur because some hosts are more susceptible to the parasites than others, or have certain behaviors that expose them to more parasites (Personal communication, Kent, 2014).

Truncation of the Negative Binomial Distribution

Occasionally, there is a reduction in the expected number of hosts with heavy parasitic infections, which can be described as *truncation.* This often occurs when the distribution graph is generated using only lightly-infected host data. In a truncated model, the threshold for parasite

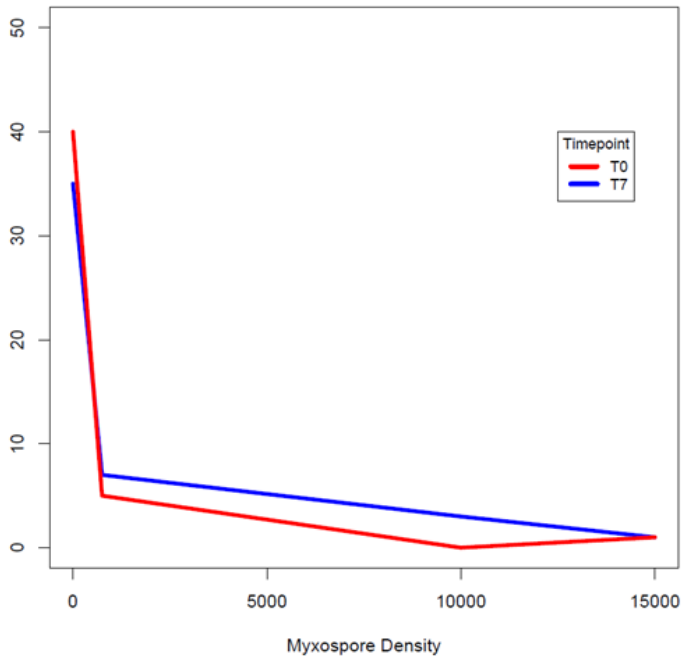
associated mortality is lower than the predicted value for the negative binomial distribution (Kent, 2011). This causes a loss of representation in the negative binomial distribution because heavily infected hosts have died (Crofton, 1971). As Rossignol and Royce (1990) described, if the mortality of hosts is density dependent on the parasites, then the predicted distribution pattern will show a higher number of hosts with heavy parasitic infections, compared to the actual data.

Figure 5. Predicted (diamonds) and actual (lines) of the binomial distribution of parasites. Taken from Kent (2011).



In this graph, the actual distribution of parasites is truncated, and the threshold for parasite associated mortality is lower than the predicted value.

Figure 6. Number of Salmon vs. *Ceratomyxa shasta* Spore Density: Negative Binomial Distribution where T_0 = time zero and T_7 = 7 days following death in the same fish.



In Figure 6, the y-axis represents the number of salmon and x-axis represents myxospore density (severity of infection). This illustrates the negative binomial distribution with the actual values for infected fish from this study. Note that at T_7 there are more myxospores.

Statistical Description of the Negative Binomial Distribution

First, a binomial distribution will be summarized. A binomial random variable with *n* independent Bernoulli trials must have the same probability of success *p* for each trial. A Bernoulli trial is when there are only two choices, essentially a "yes" or a "no" to answer the question. In order for the probability of success *p* to be the same for each trial, there needs to be a large enough population where taking some samples from it does not affect the rest of the

population. The point of a binomial distribution is to describe the probability of getting *k* successes out of *n* trials. In this situation, the number of trials is fixed and the number of *k* successes is random. The random variable is the number of *k* successes out of *n* trials (Journal of Statistics Education, 2013).

A negative binomial distribution is similar but instead of having a concrete number designated as the *n* number of trials, there is a fixed number of *k* successes and the question is related to how many trials must be conducted until the predetermined number of *k* successes has been reached (Bittner, 2013). This is just the opposite of a binomial distribution. The random variable is the number of *n* trials to find *k* successes. For example, if the interest is in how many trials need to be conducted until 10 infected fish are found, and there were 20 trials conducted to find the correct number of infected fish, then the random variable is the number of trials conducted (20) and a negative binomial distribution would be used.

Crofton (1971) first presented the negative binomial distribution as a tool to indicate parasite prevalence and since then it has been common practice to use it in most cases related to parasite distribution (Poulin, 2007). As is common in parasite distribution, there is a high number of hosts that have a low infection rate, a medium number of hosts that have a medium infection rate, and a low number of hosts that have a high infection rate. This can be partially attributed to the fact that many of the hosts that had high infection rates have already perished, thus there are fewer samples in this category to collect. If the host population size increases, then the transmission rate of the parasites will also increase and more hosts will end up infected, though the pattern with the negative binomial distribution will typically remain (Crofton, 1971). The negative binomial distribution is thus an effective tool in estimating mortality associated with

parasitism (Ferguson et al. 2011), and it is a favored approximation tool for host-parasite relationships that may be affected by a number of different independent variables (Tanner et al. 1980).

The binomial formula is as follows:

$P(x) = ({}_{n-1}C_{k-1})p^{k}\,(1-p)^{n-k}$

n= number of trials

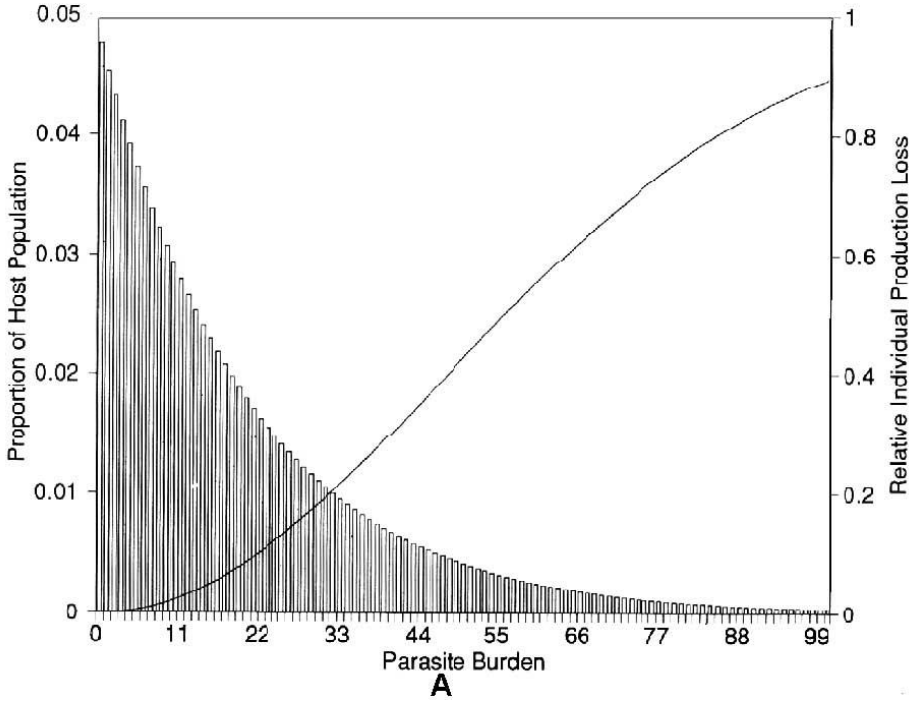
k= number of successes

p= probability of success on a single trial

C= mathematical expression "choose":

$[(n-1)!/\ \{(n-1)-(k-1)\}!\ ]\ /\ (k-1)!$

Figure 7. Common example of Parasite Burden vs. Proportion of Host Population Infected with a negative binomial distribution



Myxozoa Parasites

Myxozoa are microscopic, aquatic, spore-forming metazoan parasites that typically involve two hosts (a fish and an annelid worm). There are over 2,000 species in the phylum and many of them, particularly *Myxobolus cerebralis* and *Ceratomyxa shasta* have proven to be quite detrimental to salmonid populations (Hallet and Bartholomew, 2011). Infections are first established in fish by actinospores released into the water from the annelid host. Chemical signals occur to allow the parasite to discharge polar filaments and anchor to the fish, after which the myxozoan can enter the fish epidermis through a mucous cell. The trophozoite is the name for the parasite at the point in the life cycle when it is growing, multiplying, and actively absorbing nutrients from the fish host (Kent, personal communication, 2013). The myxozoan spreads throughout the body to various organs, depending on the species. Pathogenic species eventually elicit an inflammatory response or cause severe tissue damage. Various species of myxozoans prefer different organs; for example, C. shasta is usually found in the digestive tract (intestines), liver and he kidney, whereas *M. cerebralis* prefers neural and cartilaginous tissue (Hallett and Bartholomew, 2012). Then, as with all myxozoan, at some point the parasite sporulates within the fish host. These spores eventually are released from the fish host. Depending on their location, spores may be released in feces or urine, or from other deep tissue sites after the fish is either eaten by a predator or dies in the environment. Spores survive for many months if not years depending on the species, and ultimately infect the invertebrate hosts. In other words, the general dogma is that only the spores survive after the death of the fish host.

Abiotic Factors in C. shasta *Transmission Dynamics*

C. shasta has been known to infect trout and salmon ranging from Alaska to California, however, salmonids from enzootic waters are more likely to be resistant to infection than salmonids from non-enzootic waters (the native salmon demonstrate better resistance) (Bartholomew, 2002). As noted by Ray & Bartholomew (2013), the transmission of the *C. shasta* actinospore to the host depends on multiple factors including water temperature, the density of the parasite in the water and exposure length of fish to the water. As was predicted, there was higher transmission of *C. shasta* to the salmon when they were exposed for longer time periods, and there was also a particular range of water temperature that was conducive to transmission (around 18℃). Interestingly enough, the 14 ℃ and 22 ℃ groups had very similar transmission data when other variables were controlled. This indicates that *C. shasta* prefers a specific water temperature range, although it is not necessary for the water to be 18 ℃ for any transmission to occur (it seemingly happens less). Additionally, it was predicted that the water velocity would also affect the rate of transmission (slower velocity was predicted to correlate to higher transmission). The data did not support this from the 2013 paper published by Ray & Bartholomew but there was a very limited range of velocities used in the study which could have accounted for this lack of support to the prediction.

Multiple Strategies of C. shasta *in Infection*

The *C. shasta* parasite has possibly coevolved with the Chinook salmon, which allows for the different developmental strategies proposed by Kent et al. (2014). In young salmon that are still growing in freshwater, the parasite seems to develop more expeditiously and rapidly goes into sporulation following infection (Zinn et al., 1977). This contrasts the infection mechanism

that was the primary focus of my study. My hypothesis is that with adult Chinook salmon the infection occurs as they are entering freshwater after being in the ocean, but that in contrast to infections in juveniles, sporulation is delayed and the fish could possibly be infected for long periods of time with only presporogonic stages proliferating in the fish. Our hypothesis in general is that the parasite goes through its life stages rapidly when exposed to juvenile salmon, but progresses slower when in contact with adult salmon (Kent. Et al. 2014).

Physiological Signs of Infection

Once the parasite is within the fish host, there are a number of physiological signs that can be associated with the infection. *Ceratomyxa shasta* primarily infects the gastrointestinal tract, so there is usually damage to the stomach, pyloric caeca and lower intestine (Kent et al, 2013). The salmon exhibit lethargy and emaciation and upon closer examination they often have lesions on the kidneys and necrosis of internal organs like the liver and gallbladder (Bartholomew, 2002). In microscopic samples all stages, including spores (though rarely seen in adult fish) can be seen in infected fish within the intestinal epithelial tissue. The rate of progression of the disease depends on factors mentioned above and some fish infected early on may survive all the way to spawning while others die prematurely.

Investigation of *C. shasta* in deceased adult Chinook salmon hosts

It is very common to observe numerous prespore stages (trophozoites) in adult Chinook salmon right after spawning, even though they potentially could have been infected for an extended length of time and it would make sense based on the progression of the disease in juveniles to see more myxospores. The current belief held was that the spores do not continue

after the death of the Chinook salmon (Hendrickson & Campsie, 2013) because it had never been confirmed in *C. shasta* or other myxozoan species. In contrast, Dr. Scott Footte has suggested that post-mortem sporulation may occur with *C. shasta* as his surveys have revealed high numbers of spores in Chinook salmon carcasses in the fall after spawning (Footte et al., 2013). From an evolutionary standpoint it would make sense for the trophozoites to continue sporulating after the death of the salmon (as they are semelparous and have a predetermined time of death). The parasite otherwise would have invested a lot of time and effort into trophozoites that would never turn into myxospores, which is the stage that perpetuates the parasite. My study investigated whether there were more *C. shasta* myxospores present in intestinal samples after a week compared to immediately following the death of the salmon host under laboratory conditions that simulated the Willamette River.

Methods

A total of 30 adult Spring Chinook salmon carcasses were collected immediately after spawning at the Willamette Hatchery, Oregon and the Oregon State University Fish Performance Lab. The intestines were removed from the fish and transported to the Kent Lab at Oregon State University at about 16℃ to be processed for histology. This involved taking small cross sections (10 mm) pieces of both the anterior lower intestine and the posterior lower intestine, which were preserved in Dietrich's solution and then cut into smaller pieces at the histology lab (0.5 cm). At the OSU Veterinary Diagnostic Laboratory (histology lab) the samples were processed using standard techniques and were stained with Giemsa to better allow visualizations of trophozoites and spores within the lower intestine. They were viewed with a magnification of 200 X and in the severity values spores were seen in the lamina proporina as well as the intestinal epithelium.

The histology results served as a basis on which to compare severity of infection with the spore counts obtained from wet mount preparations. In total, there were 60 Giemsa stained samples (30 fish x 2 intestinal portions per fish) and they were each assigned a value that categorized the noted severity of infection, as follows: 0 (absent), no parasites observed; 1 (mild), isolated trophozoites occasionally seen, restricted to epithelium; 2, parasites observed in most fields of view, extending into lamina propria; 3 (severe), parasites observed in all fields of view, often replacing epithelium and lamina propria. A total of 23 fish were scored as positive based on histology (Table 1).

In addition, the remaining lower intestine was divided into four pieces (two anterior and two posterior sections). They were placed individually in Whirl-Pak® bags, one piece of each part of the intestine was placed in a freezer at -20 ℃ which represented T_0. One anterior and one posterior section of intestine were processed the same as noted above except that they were placed in an incubator refrigerator at 17 ℃ for 7 days and were marked as "T_7." After the 7 day period, the samples from the incubator were frozen. The rationale for this protocol was to halt the sporulation of myxospores in the T_0 samples, and to allow sporulation in an environment that was simulatory of a river for one week and then to halt it in the T_7 samples. Each sample was removed and a lengthwise measurement of the intestinal piece was taken. The intestine was then cut sagittally and one milliliter of phosphate buffered saline solution was added to the sample. A scalpel blade was used to scrape off the epithelial lining of the intestine (which is where most myxospores lie) and the solution of saline and tissue was homogenized with a pipette. The spores were observed in four separate subsamples by placing 0.9 nanoliters of the homogenized solution in a hemocytometer and viewing them at 200X magnification. The density of the samples using

spores/cm of intestine were calculated by multiplying the counts by 1,111 per cm of the sample. Additionally, fold increases were evaluated by comparing the T_7 samples to the T_0 samples. The results of the spore counts can be found with the histology samples in Table 1 following the discussion.

Results

A total of 23 fish were scored as positive based on histology (Table 1). For fish that were classified by positive for *C. shasta* by histology, there was an increase in spores in the liquid mixture of saline/intestinal tissue from the T_0 samples to the T_7 samples in 11 of the salmon for at least one intestinal sample (i.e., just the anterior portion, just the posterior portion, or both portions). There was a strong statistical positive correlation ($p < 0.004$) between the fold increase between the T_7 and T_0 samples and the severity of infection as determined by histopathology before any of the samples were examined under a microscope in the Kent laboratory (Kent et al., 2014). There was a Pearson "r" value of 0.41 illustrating a 41% correlation between these two variables.

There were 6 salmon out of the 23 histologically-positive fish that illustrated a meaningful increase in spores between the T_7 and T_0 samples ($p < 0.05$) when analyzing the entire intestine (which involved combining the anterior and posterior sections). This was when the data from the anterior intestines was combined with the data from the posterior intestines to really get a grasp on how many fish had total increases in spore counts regardless of the exact location. Upon a more conservative approach using the False Discovery Rate (q-value) there were three fish that were found to have moderate increases (Fish 1,3,18) with a q-value under 0.10 and one fish that was found to have significant increases in spore counts between the T_7 and

T_0 counts (Fish 19) with a q-value under 0.05. A false positive occurs when we get significant results with a p value under 0.05 when in reality none exists; the False Discovery Rate (q-value) is a mechanism to avoid inflation of positive results and make sure that we see the most accurate representation of data (Personal communication, Sharpton, 2014). This explains why there are multiple statistical analysis utilized to comprehend the data.

Discussion

Based on statistical analysis, it is apparent that some fish exhibited post-mortem sporulation. The False Discovery Rate test is very strict and the fact that 11 of the salmon had increases over time in some portion of the intestine is relevant and valid.

Additionally, there was never a decrease in the number of spores observed in the T_7 samples as compared to the T_0; there was either an increase or no difference observed. The reasons for why the *C. shasta* does not continue sporulation in all of the salmon observed remain unknown, but it may have to to do with the physiological changes that occur within the body of the salmon after death in conjunction with the severity of the infection right before the salmon died. There are multiple factors that have the potential to vary greatly among fish, which could contribute to the ability of the *C. shasta* to sporulate (like oxygen levels and alterations in the bacteria present within the fish).

The evolutionary goal of the salmon is to make it to spawning, and the evolutionary goal of *C. shasta* is to infect organisms and continue the cycle, which ultimately involves sporulation in the fish host. When the adult Chinook salmon enters freshwater in spring or early summer, it ceases to eat which results in an extremely compromised immune system for the fish. This

provides an excellent opportunity for *C. shasta* to infect and proliferate in the salmon, thus increasing the parasites chances of evolutionary success. It is evident from studies conducted before this research that there are multiple scenarios that may transpire regarding this unique relationship between adult Chinook salmon and the *C. shasta* parasite: Sometimes, the salmon "wins," ie the salmon does not get infected with a parasite and spawns successfully. Other times, the parasite "wins" ie the *C. shasta* infects the salmon, sporulates, and the salmon dies before spawning. However, after this research, we can see that sometimes, according to this definition of winning (which is continuing the survival of the species), both the salmon *and* the *C. shasta* "win" i.e., the salmon successfully spawns, and after its death the parasite sporulates and may then be transmitted into the fresh water.

Hendrickson and Campsie (2013) noted that there were no differences observed in myxospores in the gut of a deceased Chinook salmon, but we saw in my research that sporulation does not occur in every fish following death. Nevertheless, it does occur, particularly in fish that die with heavy presporogonic (trophozoite) infections. It had been proposed previously that *C. shasta* post-mortem sporulation was happening because naturally spawned deceased Chinook salmon often exhibit high myxospore counts, but it was mostly educated speculation (True et al. 2012; Foott et al. 2013). With this new knowledge from my study, we have a better understanding of the relationship between the *C. shasta* parasite and its host, the Chinook salmon.

Table 1. Taken from Kent. Et al (2014) Research Note

Ceratomyxa shasta in Chinook salmon (*Oncorhynchus tshwaytscha*) diagnosed as positive by histology. A = anterior intestine, P = Posterior intestine. Histology; 0 = absent, 1 = mild, 2 = moderate, 3 = prominent/severe, S = spores present. Size = length of intestine sample. Counts represent four separate 9 µl samples evaluated for each intestinal sample. Fold increase = change from T_0 to T_7 based on myxospores/cm of intestine, and samples with a mean of zero are assigned 0.25 for mean counts.

Fish #	Location	Histology	Size (cm)	Counts	Mean Counts	Mean Spore/cm	Fold Increase
1[§, #]	A_{T0}	0,0	3.9	0,0,0,0	0	0	
	A_{T7}[†]		6.7	1,5,3,3	3	497	7
	P_{T0}	1,1	3.5	0,0,5,1	1.5	476	
	P_{T7}		2.6	3,3,3,5	3.75	1,602	3.4
2	A_{T0}	1,1	3.5	0,0,0,0	0	0	
	A_{T7}		3.4	0,0,0,0	0	0	0
	P_{T0}	1,1	5.2	0,0,0,0	0	0	
	P_{T7}		5.7	0,0,0,0	0	0	0
3	A_{T0}	1,0	2.3	0,0,0,0	0	0	
	A_{T7}		3.1	0,0,0,0	0	0	0
	P_{T0}	0,0	3.5	0,0,0,0	0	0	
	P_{T7}		5.0	0,0,0,0	0	0	0
4	A_{T0}	1,0	3.1	0,0,0,0	0	0	
	A_{T7}		4.1	0,0,0,0	0	0	0

	P_{T0}	2,2	4.5	0,0,0,0	0	0	
	P_{T7}		2.8	9,0,0,0	2.25	893	14.5
5[§]	A_{T0}	2,3	2.9	0,0,0,0,	0	0	
	A_{T7}		2.8	0,0,0,0	0	0	0
	P_{T0}	0,2	3.1	0,0,0,0	0	0	
	P_{T7}[†]		4.7	4,2,3,7	4	946	10.6
6	A_{T0}	0,1	2.1	0,0,0,0	0	0	
	A_{T7}		4.2	0,0,0,0	0	0	0
	P_{T0}	0,1	3.6	0,0,0,0	0	0	
	P_{T7}		4.7	0,0,0,0	0	0	0
7	A_{T0}	0,2	2.2	0,0,0,0	0	0	
	A_{T7}		5.7	1,0,0,0	0.25	49	0
	P_{T0}	2,3	2.5	0,0,0,0	0	0	
	P_{T7}		5.2	0,0,0,0	0	0	0
8[§,#]	A_{T0}	3,1 S	2.8	2,4,6,4	4	1,587	
	A_{T7}		3.2	3,7,5,9	6	2,083	1.3
	P_{T0}	3,1	3.7	6,3,4,2	3.75	1,126	
	P_{T7}[†, ‡]		3.0	35,25,33,39	33	12,221	10.9

9	A_{T0}	1,1	2.3	0,0,0,0	0	0	
	A_{T7}		2.1	0,0,0,0	0	0	0
	P_{T0}	1,1	4.8	0,0,0,0	0	0	
	P_{T7}		1.3	0,0,0,0	0	0	0
10	A_{T0}	0,0	2.1	0,0,0,0	0	0	
	A_{T7}		3.9	0,0,0,0	0	0	0
	P_{T0}	0,1	4.3	0,0,0,0	0	0	
	P_{T7}		6.5	0,0,0,0	0	0	0
11	A_{T0}	0,1	3.5	0,0,0,0	0	0	
	A_{T7}		2.1	0,0,0,0	0	0	0
	P_{T0}	0,1	4.1	0,0,0,0	0	0	
	P_{T7}		4.7	0,0,0,0	0	0	0
12[§]	A_{T0}	0,1	3.8	3,5,2,6	4	1169	
	A_{T7}		2.0	31,7,4,33	18.75	10415	8.9
	P_{T0}	3,3	2.8	23,15,34,17	22.25	8828	
	P_{T7}		2.3	19,18,32,16	21.25	10264	1.2
13[§, #]	A_{T0}	0,1	4.5	0,0,0,0	0	0	
	A_{T7} [†]		4.5	1,0,1,1	0.75	185	3.0

	P_{T0}	0,1	1.5	0,1,0,0	0.25	185	
	P_{T7}†		2.4	2,3,1,2	2	925	5.0
14	A_{T0}	1,1	3.5	0,0,0,0	0	0	
	A_{T7}		3.6	0,0,0,0,	0	0	0
	P_{T0}	1,0	2.3	0,0,0,0,	0	0	
	P_{T7}		3.2	0,0,0,0	0	0	0
15	A_{T0}	1,0	3.0	0,0,0,0	0	0	
	A_{T7}		3.2	0,0,0,0	0	0	0
	P_{T0}	1,1	2.5	0,0,1,1	0.5	222	
	P_{T7}		2.7	0,0,0,0	0	0	0.46
16	A_{T0}	0,1	2.2	0,0.0,0	0		
	A_{T7}		3.2	0,0,0,0	0	0	0
	P_{T0}	0,0	2.5	0,0,0.0	0		
	P_{T7}		4.7	0,0,0,0	0	0	0
17	A_{T0}	0,0	3.3	0,0,0,0	0	0	
	A_{T7}		3.7	0,0,0,0	0	0	0
	P_{T0}	0,0	4.5	0,0,0,0	0	0	
	P_{T7}		3.5	1,1,0,0	0.5	159	2.6

18	A_{T0}	0,1	3.5	0,0,0,0	0	0	
	A_{T7}		3.4	0,0,0,0	0	0	0
	P_{T0}	1,2	3.4	0,0,0,0	0	0	
	P_{T7}		3.3	0,0,0,0	0	0	0
19 [§, ‖]	A_{T0}	3,3	2.3	0,0,0,0	0	0	
	A_{T7}[†]		2.8	1,6,4,3	3.5	1388	11.5
	P_{T0}	3,2	2.4	0,0,0,0	0	0	
	P_{T7}		2.4	0,3,1,1	1.25	579	5.0
20	A_{T0}	3,3	4.2	99,116,104,102	105.25	27,841	
	A_{T7}[†, ‡]		2.8	305,328,317,354	326	129352	4.6
	P_{T0}	3,3	2.9	3,1,2,2	2.0	766	
	P_{T7}[†]		4.5	23,18,8,14	15.75	3889	5.1
21	A_{T0}	2,1	2.5	0,0,0,0	0	0	
	A_{T7}		5.6	0,0,0,0	0	0	0
	P_{T0}	2,2	1.3	0,0,0,0	0	0	
	P_{T7}		5.5	0,0,0,0	0	0	0
22	A_{T0}	1,1	3.2	0,0,0,0	0	0	
	A_{T7}		2.0	0,0,0,0	0	0	0

	P_{T0}	1,1	3.0	0,0,0,0	0	0	
	P_{T7}		2.1	0,0,0,0	0	0	0
23	A_{T0}	0,1	1.9	0,0,0,0	0	0	
	A_{T7}		3.5	0,0,0,0	0	0	0
	P_{T0}	1,2	3.8	0,0,0,0	0	0	
	P_{T7}		2.3	0,0,0,0	0	0	0

Footnotes
* The lowest possible mean spore count (0.25) was added to T0 values that where zero to perform a calculation of fold change.
† = statistically different from T_0 as independent sample ($p < 0.05$),
‡ = statistically different from T0 as independent sample with multi-test correction ($q < 0.05$)
§ = statistically different from T_0 with combined anterior and posterior data for each fish ($p < 0.05$).
|| = statistically different from T_0 with combined anterior and posterior data and with multi-test correction ($q < 0.05$).
= marginally significant from T_0 with combined anterior and posterior data with multi-test correction ($q < 0.1$).

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