AN ABSTRACT OF THE THESIS OF

Kalyn Hubbard for the degree of Master of Science in Microbiology presented on March 1, 2019.

Title: Spatiotemporal Distribution of *Ceratonova shasta* and its Genotypes in the Deschutes River Basin.

Abstract approved: ____

Jerri L. Bartholomew

The waterborne, myxozoan parasite *Ceratonova shasta* is endemic to the Pacific Northwest and can be lethal to its secondary salmonid host, including the culturally, economically, and recreationally important spring Chinook salmon (*Oncorhynchus tshawytscha*) of the Deschutes River, OR. Previously described genotypes of *C. shasta* exhibit specificity with their salmonid hosts. As a first step toward managing *C. shasta* in the Deschutes River, the spatial and temporal distribution of this pathogen and its genotypes needed to be described. Historically, parasite prevalence was quantified by fish mortalities from sentinel surveys at 2-3 sites at different times of year on the Deschutes River mainstem. Herein I used water sampling and qPCR to provide a method for determining detailed spatiotemporal information about waterborne parasite distribution both within (Chapter 4) and between (Chapter 2) sites of the river. *Ceratonova shasta* was detected throughout the upper and lower Deschutes River basins at variable positions within sites. Though there was variation between years (average spores/L 81.0, 11.2, 46.4 in 2016, 2017, 2018, respectively), *C. shasta* density was 1.5x higher between rkm 48-82 than the average of all other spatial survey sites. Additionally, sites such as DRR (rkm 116) and DWS (rkm 155) had higher than average *C. shasta* abundance for 63% (5/8) of the surveys. Only low densities (< 1.5 spores/L) of *C. shasta* were detected in the Warm Springs River, an important spawning tributary for spring Chinook salmon. Using linear mixed effect models (LMEMs), parasite abundance at temporal monitoring sites in the

lower basin appears to be positively related to water temperature but has an inverse relationship with river discharge, supporting previously described observations in the Klamath River, CA/OR. The upper Deschutes basin exhibited different temporal dynamics than the lower basin with variable peaks in July or August. In Chapter 3, I determined that *C. shasta* genotype I dominated the lower Deschutes River basin below Round Butte Dam, type II was the main genotype present in the upper basin above Round Butte Dam, and type O was detected throughout the system at lower proportions than I or II. Type II was also identified in the lower basin, but only during August. Genotype I was not detected in the upper basin, despite the passage of spring Chinook salmon above the Round Butte Dam for over a decade. I identified that peak *C. shasta* abundance and the Chinook salmon host-specific genotype I coincide with spring Chinook adult returns and juvenile spring Chinook releases, although spring Chinook salmon are one of the least abundant salmonid species. Genotype O is associated with Steelhead trout, the most abundant species of salmonid in the upper and lower basins of the Deschutes River. However, although genotype O was found throughout both basins, supporting earlier hypotheses that the spatial and temporal patterns of genotypes may be explained by host distribution, our hypothesis that the density of genotypes in water samples would be related to salmonid abundance was not supported. Despite the high densities of *C. shasta* genotype I in water samples of the lower Deschutes, only mild to moderate pathology was observed in juvenile and adult tissue samples (Appendix A). Although *C. shasta* was the most common pathogen that we detected and caused enteronecrosis, pathology associated with *Renibacterium salmoninarum* and *Parvicapsula minibicornis* was observed also in juvenile and adult spring Chinook salmon, respectively. Therefore, while *C. shasta* may be contributing to low survival of spring Chinook salmon in the lower Deschutes River basin, it does not appear to be the sole cause.

©Copyright by Kalyn Hubbard

March 1, 2019

All Rights Reserved

Spatiotemporal Distribution of *Ceratonova shasta* and its Genotypes in the Deschutes River Basin

by

Kalyn Hubbard

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented March 1, 2019
Commencement June 2019

Master of Science thesis of Kalyn Hubbard presented on March 1, 2019.

APPROVED:

Major Professor, representing Microbiology

Head of the Department of Microbiology

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.

Kalyn Hubbard, Author

ACKNOWLEDGEMENTS

I would like to express sincere appreciation to my committee members Sascha Hallett, Jerri Bartholomew, Jonathan Armstrong, and Jovana Kovacevic. Guidance provided by my supervisors Stephen Atkinson for chemical, genetic, and bioinformatic procedures as well as Julie Alexander for statistical analyses and general professionalism coaching were invaluable to my success as a graduate student. Thank you to my lab mates, Claire Howell, Laura Taggart-Murphy, Damien Barrett, Benjamin Americus, and Sofiya Yusova for providing a welcoming, supportive, and fun atmosphere during my time in the lab. Thank you to my friends and family, with special regard towards my husband, Tim Hubbard, for his unwavering support and comic relief during this process. Funding for this research was provided by The Confederated Tribes of the Warm Springs Reservation of Oregon-Pacific Coastal Salmon Recovery Fund and an Oregon Department of Fish and Wildlife Fish Health Graduate Research Fellowship in Microbiology.

CONTRIBUTION OF AUTHORS

Jerri Bartholomew and Sascha Hallett served as major co-advisors and contributed to all papers in this thesis. Julie Alexander was instrumental to the experimental designs and statistical analyses of Chapters 2 and 4. Stephen Atkinson advised and edited all aspects of Chapter 3.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION 1

Deschutes River basin characteristics 1
Spring Chinook salmon 3
Myxozoans 5
Ceratonova shasta 5
Pre-spawn mortality 9
Detection methods 10
Control and management of *Ceratonova shasta* 11
Objectives 12
References 13

CHAPTER 2: THE SPATIAL AND TEMPORAL DISTRIBUTION OF *CERATONOVA SHASTA* IN THE DESCHUTES AND WARM SPRINGS RIVERS, OREGON, USA . 22

ABSTRACT 23
INTRODUCTION 23
METHODS 25
RESULTS 33
DISCUSSION 41
REFERENCES 46

CHAPTER 3: SPATIAL AND TEMPORAL DISTRIBUTION OF *CERATONOVA SHASTA* GENOTYPES IN THE DESCHUTES RIVER BASIN ARE LINKED TO SALMONID FISH DISTRIBUTION AND RUN TIMING 51

ABSTRACT 52
INTRODUCTION 53
METHODS 56
RESULTS 59
DISCUSSION 67
REFERENCES 73

CHAPTER 4: THE EFFECTS OF TIME AND SAMPLING LOCATION ON *CERATONOVA SHASTA* DENSITY IN WATER SAMPLES OF THE DESCHUTES RIVER 76

ABSTRACT 77
INTRODUCTION 78
METHODS 80
RESULTS 86
DISCUSSION 96
REFERENCES 100

TABLE OF CONTENTS (Continued)

CHAPTER 5: SUMMARY 102

REFERENCES 110

APPENDIX A: DETECTION OF *CERATONOVA SHASTA* IN ANAL SWABS AND SALMONID TISSUES THROUGH PCR AND HISTOLOGICAL ANALYSES AND CO-INFECTION WITH OTHER MYXOZOAN, BACTERIAL, AND VIRAL PATHOGENS 113

ABSTRACT 114
INTRODUCTION 115
METHODS 117
RESULTS 121
DISCUSSION 127
REFERENCES 130

LIST OF FIGURES

Figure Page

Figure 1.1: Map of the Deschutes River basin including major tributaries and temporal water sampling sites. Modified from 2015-2016 ODFW Fellowship Report………..…..1

Figure 1.2: *Ceratonova shasta* life cycle……………………………………………….5

Figure 2.1: Map of the Deschutes River basin including major tributaries and temporal water sampling sites……………………..…………………………………………25

Figure 2.2: Temporal water sample collection sites on the Warm Springs River and at the mouth of the Warm Springs River on the Deschutes River (DRV). Sites are represented by open circles (2017), dark grey circles (2018), and black circles filled with dark grey (2017 & 2018). The black bar represents the Warm Springs National Fish Hatchery, a migration barrier to salmonids. The Pelton Trap located below the Pelton Dam is included for reference. *Note the DRV site is on the Deschutes mainstem………………………......28

Figure 2.3: Spatial and temporal sampling sites with locality site codes that correspond to Table 2.2……………………………………………………………………….....29

Figure 2.4: Mean *Ceratonova shasta* density (y-axis) in the Deschutes River at the Oak Springs Hatchery (gray triangles). Overlays are discharge ft^3/s (blue, second y-axis) and river water temperature (red, third y-axis) over time (x-axis). Each point is the mean *C. shasta* abundance from triplicate water samples collected using an automatic sampler (ISCO). Data from 2015 and 2016 were collected and processed by Vojnovich et al., 2016……………………………………………………………………………35

Figure 2.5: Mean *Ceratonova shasta* density (y-axis) in the Deschutes River at the Pelton Trap (black circles). Overlays are discharge ft^3/s (blue, second y-axis) and river water temperature (red, third y-axis) over time (x-axis). Each point is the mean *C. shasta* abundance from triplicate water samples collected using an automatic sampler (ISCO). Data from 2015 and 2016 were collected and processed by Vojnovich et al., 2016…….36

Figure 2.6: 2017 Warm Springs River temporal water sample monitoring sites. Each data point is the mean *Ceratonova shasta* spores/L from 3 1L replicate samples. *Note that DRV is located on the Deschutes River at the mouth of the Warm Springs River. ** 2017 sites WBA, WBE, WMC, and WUP were combined as WCF in 2018. ***2017 site WLO was moved further downstream and renamed WLW in 2018……………………..…37

Figure 2.7: 2018 Warm Springs River temporal water sample monitoring sites. Each data point is the mean *Ceratonova shasta* spores/L from 3 1L replicate samples. *Note that DRV is at the confluence of the Warm Springs River and the Deschutes River mainstem…………………………………………………………………………38

LIST OF FIGURES (Continued)

Figure Page

Figure 2.8: Linear regression back transformations of temperature (x-axis) and *Ceratonova shasta* density (spores/L) (y-axis). Linear mixed effect model: $r^2 = 0.599$, $F_{1, 7} = 42.76$, $P < 0.001$……………………………………………………...39

Figure 2.9: Linear regression back transformations of discharge (x-axis) and *Ceratonova shasta* density (spores/L) (y-axis). Linear mixed effect model: $r^2 = 0.571$, $F_{1, 152} = 22.95$, $P < 0.001$…………………………………………………………………………………39

Figure 2.10: Results from all of the longitudinal water sampling surveys between 2016-2018. Size of the graduated symbols and color both represent *Ceratonova shasta* density at each site. The black bar represents the Pelton-Round Butte Dam complex, a migration barrier to all salmonids. Rkm are indicated on the first map for reference……………...41

Figure 3.1: Map of the Deschutes River basin including major tributaries and temporal water sampling sites (A) with an insert of the Pacific Northwest in North America for context (B)……………………………………………………...............................53

Figure 3.2: Spatial and temporal water sampling sites on the Deschutes River. Sites in dark blue were genotyped for *Ceratonova shasta* from 2016-2018………………………..….57

Figure 3.3: Expected distribution and density of the salmonid parasite *Ceratonova shasta* in the upper and lower Deschutes River Basin if all proposed hypotheses are supported. The black bar represents the Round Butte Dam complex, a migration barrier for anadromous fish. The relative area of each pie graph indicates the density of *Ceratonova shasta* in 1L water samples whereas the color in each graph represents the genotype proportions at each site…………………………………………………….................60

Figure 3.4: Distribution and density of the salmonid parasite *Ceratonova shasta* in the upper and lower Deschutes River Basin. The black bar represents the Round Butte Dam complex, a migration barrier for anadromous fish. The relative area of each pie graph indicates the density of *Ceratonova shasta* in 1L water samples whereas the color in each graph represents the genotype proportions at each site……..…………………….……..62

Figure 3.5: Temporal abundance of *Ceratonova shasta* in water samples collected from lower basin sites at Deschutes River near Oak Springs Hatchery at rkm 75 (top), the Warm Springs mouth on the Deschutes River at rkm 134 (middle), and the Pelton Trap at rkm 160 (bottom). Bars show total *Ceratonova shasta* detected (spores/L) (y-axis) against sample date (x-axis), with subdivisions to show genotype proportions…………….……63

Figure 3.6: Fish passage at The Dalles Dam on the Columbia River (CR) and the Pelton Trap on the Deschutes River (DR) by salmonid species. Shaded areas are estimates of when each species of salmonid is present in the lower Deschutes River……..……….............65

LIST OF FIGURES (Continued)

Figure | Page

Figure 3.7: Abundance and genotypes of the salmonid parasite *Ceratonova shasta* at the confluence of the Warm Springs and Deschutes Rivers (DRV) plotted against abundance of its salmonid hosts at the Round Butte Hatchery (PGE) in 2017 and 2018. From top to bottom: *Ceratonova shasta* density (spores/L) showing genotype proportions within bars, abundances of Sockeye salmon in red, Chinook salmon in blue, and summer Steelhead in light orange…………66

Figure 4.1: Spatial distribution of diurnal, lateral, and vertical sampling sites…………80

Figure 4.2: Big Falls site located above the Round Butte Dam. Light blue circles represent collection positions in the water…………81

Figure 4.3: Measurements between each sampling location within the Big Falls site…..81

Figure 4.4: Blue Hole site; the farthest downstream of the three sites. Light blue circles represent collection positions…………82

Figure 4.5: Measurements between each sampling location within the Big Hole site…….82

Figure 4.6: Warm Springs Bridge site. The middle site for these surveys located just downstream of the Pelton trap. Light blue circles are positions of water collection……..83

Figure 4.7: Measurements between each sampling location within the Warm Springs Bridge site…………..83

Figure 4.8: *Ceratonova shasta* spores/L (left y-axis) and temperature in degrees C (right y-axis) over the course of one day (x-axis) at the Big Falls site…………..87

Figure 4.9: *Ceratonova shasta* density (y-axis) as a function of binned time groups (x-axis) at the Big Falls site (ANOVA: F2, 18 = 13.97, P = < 0.001). Tukey's HSD values considered significant at = < 0.1…………....87

Figure 4.10: Simplified rendition of the Big Falls river cross section with the mean *Ceratonova shasta* densities from three replicates at each intersection of depth and position, represented by color coded circles…………..88

Figure 4.11: *Ceratonova shasta* density (y-axis) as a function of depth (line color) and position (x-axis) at the Big Falls site (two-way ANOVA: F3, 8 = 2.71, P = 0.12)……88

Figure 4.12: *Ceratonova shasta* spores/L (y-axis) and temperature in degrees C (second y-axis) over the course of one day (x-axis) at the Blue Hole site…………...90

LIST OF FIGURES (Continued)

Figure Page

Figure 4.13: *Ceratonova shasta* density (y-axis) as a function of binned time groups (x-axis) at the Blue Hole site (ANOVA: $F_{2, 15} = 0.092$, $P = 0.91$). Tukey's HSD values considered significant at $\alpha = < 0.1$……………………………………………………90

Figure 4.14: Simplified rendition of the Blue Hole river cross section with the mean *Ceratonova shasta* densities from three replicates at each intersection of depth and position, represented by color coded circles……………………………………………………..91

Figure 4.15: *Ceratonova shasta* density (y-axis) as a function of depth (line color) and position (x-axis) at the Blue Hole site (two-way ANOVA: F5, 12 = 2.958, P = 0.057)…...91

Figure 4.16: *Ceratonova shasta* spores/L (y-axis) and temperature in degrees C (second y-axis) over the course of one day (x-axis) at the Warm Springs bridge……………………93

Figure 4.17: *Ceratonova shasta* density (y-axis) as a function of binned time groups (x-axis) at the Warm Springs bridge (ANOVA: $F_{2, 18} = 6.607$, $P = 0.0071$). Tukey's HSD values considered significant at $\alpha = < 0.1$……………………………………………93

Figure 4.18: Simplified rendition of the Warm Springs bridge river cross section with the mean *Ceratonova shasta* densities from three replicates at each intersection of depth and position, represented by color coded circles……………………………………………..94

Figure 4.19: *Ceratonova shasta* density (y-axis) as a function of depth (line color) and position (x-axis) at the Warm Springs Bridge site (two-way ANOVA: $F_{5, 10} = 4.231$, $P = 0.025$)……………………………………………………………………………………94

Figure 4.20: *Ceratonova shasta* density (y-axis) as a function of filtration days post collection (x-axis) of pooled water from polychaete mesocosms Five replicates per treatment. (ANOVA: $F_{4, 20} = 3.095$, $P = 0.039$). Tukey's HSD values considered significant at $\alpha = < 0.1$………………………………………………………….....................95

Figure 4.21: *Ceratonova shasta* density (y-axis) as a function of filtration days post collection (x-axis) of pooled river water from the Blue Hole site. Five replicates per treatment. (ANOVA: $F_{3, 16} = 19.49$, $P = < .001$). Tukey's HSD values considered significant at $\alpha = < 0.1$……………………………………………………………...96

Figure A.1: The lower Deschutes River highlighting the Round-Butte Dam complex (bars) and Pelton Trap (star) between river kilometers 160-165………………………………117

Figure A.2: Necrotic lower intestine with presporogonic forms (black arrows) of *Ceratonova shasta* and sloughed epithelium (red oval) from an adult spring Chinook salmon collected on August 29th, 2017 from the Round Butte Hatchery (upper). Photo of a healthy adult spring Chinook salmon lower intestine (lower)…………………………...124

LIST OF FIGURES (Continued)

Figure | Page

Figure A.3: *Parvicapsula minibicornis* infected (top, middle) and uninfected (bottom) glomeruli of kidneys from adult spring Chinook salmon collected on August 29th, 2017 from the Round Butte Hatchery……………………………………………………….....125

LIST OF TABLES

Table Page

Table 2.1: Duration and frequency of monitoring water samples collected at each site by year. Sums of water samples per site are provided by year and as a total for the project. Samples from 2015 and 2016 were processed by Vojnovich et al., 2016 and included here for reference (Gray text)……………………………………………………………28

Table 2.2: River kilometer, latitude, and longitude coordinates for sites sampled during spatial surveys (grab samples) conducted from 2016-2018………………………………30

Table 2.3: Summary of maximum summer water temperatures (°C) (USGS) and maximum river discharge (m^3/s) (USGS) by water year at the Madras USGS station (14092500) and on the Deschutes River near Oak Springs Hatchery from May to September for each year included in the present study……………………………………………………………...33

Table 2.4: Summary of *Ceratonova shasta* dynamics each year at the Oak Springs and Pelton Trap sites. Absences from data include: first *C. shasta* detection was missed in 2015, sampling stopped in mid-July of 2016 at the Pelton Trap, and Oak Springs water samples were unreliable during the expected *C. shasta* season in 2017…………………………...34

Table 2.5: Comparison of maximal random effect model performances including ANOVA, r^2, and AIC results. Models used are highlighted in gray……………………………...38

Table 2.6: Mean density of *Ceratonova shasta* (spores/L) in water samples between the upper and lower basins of the Deschutes River for each month and year surveyed……..40

Table 3.1: 2016-2018 Deschutes River basin water sample localities, including longitudinal surveys, periodic monitoring sites and opportunistic ‘grab’ samples. River kilometers and latitude/longitude coordinates are given for each locality (see also Chapter 2). Sites selected for parasite genotype analysis are marked in gray and correspond to dark blue sites in Figure 3.2………………………………………………………………………………...58

Table 3.2: Summary of Deschutes River fish tissues genotyped for *Ceratonova shasta*…59

Table 3.3: Summary of fish passage timing at the selective water withdrawal tower (juveniles) and Pelton Trap (adults) by species and life stage (PGE) for 2017 and 2018….64

Table 3.4 Summary of adult fish passage timing at The Dalles Dam for 2017 and 2018….64

Table 3.5: Summary of fish abundance enumerated at the Pelton Trap (PGE) by species and year……………………………………………………………………………………..65

Table 4.1. Summary of laboratory water filtration treatment groups (n=5) with specifications for where the samples (5 1L per treatment) were held after collection and the amount of time before they were filtered………………………………………………...84

LIST OF TABLES (Continued)

Table Page

Table 4.2. Summary of river water (from Blue Hole site) filtration treatment groups with specifications for where the samples were held after collection and the amount of time before they were filtered……………………………………………………………85

Table A.1: Summary of 2017 and 2018 *C. shasta* (Cs), *P. minibicornis* (Pm), *T. bryosalmonae* (Tb), *R. salmoninarum* (Rs) and viral (Infectious hematopoietic necrosis virus (IHNV), and infectious pancreatic necrosis (IPNV)) assay results for juveniles and adult spring Chinook salmon at the Round Butte Hatchery. Results provided by ODFW in gray text………………………………………………………………………………126

Table A.2: Summary of Deschutes River fish tissues genotyped for *Ceratonova shasta*..126

CHAPTER 1: INTRODUCTION

Deschutes River basin characteristics

The Deschutes River begins at Little Lava Lake in the Cascade Mountains of central Oregon, USA. It flows south through the Crane Prairie Reservoir to the Wickiup Reservoir, then flows north until connecting with the Columbia River, at the border between Oregon and southern Washington (Figure 1.1). The mouth of the Deschutes is roughly 405 river kilometers (rkm) from its starting point (Heisler, 2012).

As a system, the Deschutes River Basin is an intriguing subject of study across disciplines, as highlighted in "*A Peculiar River: Geology, Geomorphology, and Hydrology of the Deschutes River, Oregon*". Published in 2003, this book documents the geologic history of the basin, which was formed as a result of local tectonic activity, extreme floods, and significant volcanism. The ensuing lava dams, waterfalls, and lahars formed a canyon that has made the placement of the Deschutes River quite static (Beebee et al., 2002). The Deschutes River is considered a thermally stable system due to uniform springs underneath a permeable layer of basalt (Stearns, 1930; O'Connor et. al. 2003; Zimmerman and Ratliff, 2003), which results in a maximum daily temperature range of 10 °C (USGS). The springs also minimize fluctuations in river discharge both within and between years (USDA, 1996; Gannett et al., 2001; Heisler, 2012). However, temperate river discharge is still influenced by

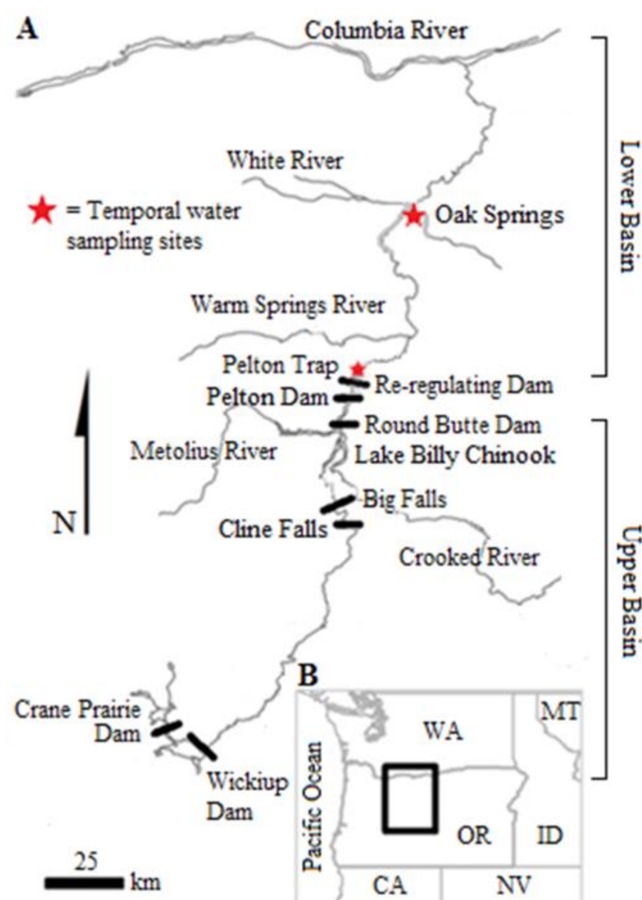

Figure 1.1: Map of the Deschutes River basin including major tributaries and temporal water sampling sites.

rainfall events (Sear et al., 1999) and the Deschutes River is no exception. Therefore, high precipitation events and large snowpack years on Mt. Hood (USDA) are reflected in the overall discharge of the Deschutes River (USGS).

Big Falls is well-established as a natural migration barrier for salmonids, located at rkm 211 (Figure 1.1; Zimmerman and Ratliff, 2003). Downstream of the Big Falls is the Pelton-Round Butte Dam Complex from rkm 160-165. Moving downstream, the dam complex currently includes the Selective Water Withdrawal (SWW) tower above the Round Butte Dam (1964), followed by the Pelton Dam (1958) and then the Reregulating Dam (1957). The Pelton Trap is below the Reregulating Dam and is where adult salmonids are collected, enumerated, and sorted for release back to the river, trucked up to the Round Butte Hatchery for spawning, or trucked above the Round Butte Dam for reintroduction. The SWW tower is i.) a collection location of juvenile Sockeye salmon, spring Chinook salmon, and Steelhead trout and ii.) responsible for regulating temperature and river discharge to emulate pre-dam conditions (Hydropower Reform Coalition and River Management Society, 2015). This dam complex separates the Deschutes River into distinct upper and lower basins.

While the Pelton-Round Butte Dam complex was built to provide geographical stability and hydropower, the Round Butte Dam was also responsible for fish passage of migrating salmonids. Unfortunately, this effort was deemed a failure in 1968 and the Round Butte Hatchery was built below the Round Butte Dam at rkm 160 in 1978 to mitigate the effects of the dam on native spring Chinook salmon and Steelhead trout (Ratliff and Schulz, 1999).

Throughout both basins, there are five resident species of trout that comprise the bulk of Deschutes River fish by abundance: the native Redband (*Oncorhynchus mykiss gairdnerii*), Bull (*Salvelinus confluentus*), Rainbow (*Oncorhynchus mykiss*), and the non-native Brown (*Salmo trutta*) and Lake (*Salvelinus namaycush*) trout. There are four salmonids that migrate through the lower basin: the native Chinook salmon (*Oncorhynchus tshawytscha*), Sockeye salmon (*Oncorhynchus nerka*), and Steelhead trout (*Oncorhynchus mykiss*), and the non-native Coho salmon (*Oncorhynchus kisutch*). Kokanee and Mountain Whitefish reside in the upper basin (*Prosopium williamsoni).*

Upper basin reintroduction efforts of Chinook salmon and Steelhead trout began in 2007, followed by Sockeye salmon beginning in 2009 (ODFW and CTWS, 2017).

Spring Chinook salmon

Chinook salmon are economically, culturally, and ecologically important species in the Pacific Northwest. Their health is crucial for many different stakeholders, including recreational, commercial, and subsistence fishermen such as the Warm Springs tribe on the Deschutes River.

Spring Chinook salmon are native to the Deschutes River basin. From 1957 to 1980, adult returns of spring Chinook salmon were lower than 500 fish per year (PGE, n.d.). With the release of hatchery fish into the system, adult returns increased to over 2,000 between 1981-1993. Since 2001, spring Chinook salmon abundance has been maintained between 1,000-5,000 adult fish. In more recent years, the population has decreased to an average 1,000 adult fish per year. Because of their high value by several interest groups, the low returns of the last few years have been of concern.

Chinook salmon start their lives upriver in "nests" called redds. Juvenile fall Chinook salmon immediately migrate to the ocean, while spring Chinook salmon spend a year in the river before migrating and then spending roughly four years maturing at sea. When they are ready to spawn, they start the arduous journey back to their natal spawning grounds in the spring and spawn in August. During this voyage, they stop eating, expend a tremendous amount of energy, and overcome a variety of physical obstacles. In-migrating salmonids also have increased stress-induced cortisol and maturation-related testosterone, both of which decrease immune system function. This can lead to higher predation rates and an increased risk of parasitic infections (Miller et. al. 2014). Like all Pacific salmon, Chinook are semelparous - they spawn once and then die.

Pathogens of Deschutes River spring Chinook salmon

Pathogenic infections of Deschutes River spring Chinook salmon may include *Renibacterium salmoninarum, Tetracapsuloides bryosalmonae*, *Parvicapsula minibicornis* and/or *Ceratonova shasta*. *Renibacterium salmoninarum* is the causative

agent of bacterial kidney disease (Fryer and Sanders, 1981). This pathogen can infect juvenile salmonids at hatcheries, and high densities of juvenile fish at these facilities exacerbate the horizontal transmission of this bacterial pathogen. Contrary to many freshwater infections, which decrease in severity or leave the host entirely as the salmonid enters the ocean, bacterial kidney disease continues to proliferate in infected salmonids after they migrate (Fryer and Sanders, 1981). Due to the challenges of effectively managing this bacterium and the severity of disease that it can inflict, the prevalence of *R. salmoninarum* is closely monitored by many state hatcheries, including the Round Butte Hatchery.

Another potential pathogen in the Deschutes River is *Tetracapsuloides bryosalmonae*, a malacosporean known to cause proliferative kidney disease (Kent et al., 2000). This parasite was responsible for a die-off of Mountain Whitefish (*Prosopium williamsoni*) in the Yellowstone River during 2017 (Carraro et al., 2018). While this parasite has not yet been detected in the Deschutes River, the timeliness, close geography, and dire population impacts suggest that screening for *T. bryosalmonae* be considered.

Similar to *T. bryosalmonae*, *P. minibicornis* is also a myxozoan that infects the kidneys of salmonids and can cause organ degradation, affecting plasma osmolality (Bradford et al., 2010). This parasite has been detected in the Sacramento, Klamath, Fraser, and Columbia River basins (Atkinson et al., 2011) and is known to affect Sockeye salmon populations (Kent et al., 1997). *P. minibicornis* is a myxozoan that shares a polychaete host (Bartholomew et al., 2006) with another confirmed Deschutes River myxozoan salmonid parasite of concern, *Ceratonova shasta*.

Viruses are also monitored in the Deschutes River. The rhabdovirus IHNV and aquatic birnavirus IPNV have nearly global distribution and can be transferred horizontally or vertically (Dixon et al., 2016). These viruses can be detected throughout the fish, depending on the mode of viral entry. Therefore, Oregon hatcheries such as the Round Butte Hatchery test the kidney, pyloric caeca, spleen, and ovarian fluid of adult salmonids for IHNV and IPNV (AFS-FHS Blue Book, 2014). IHNV has been detected at

the Round Butte Hatchery since 1975 (Mulcahy, 1976). IPNV is routinely tested for, but has not been detected at Round Butte Hatchery (ODFW communications).

Myxozoans

Myxozoans are microscopic parasites suspected to be related to cnidarians since the late 19th century (Štolc, 1899). Phylogenetic analyses of ssrDNA sequences and morphological evidence revealed that members of Myxozoa are metazoans (Smothers et al., 1994) from the phylum Cnidaria (Siddall et al., 1995; Holland et al., 2011). There are 2,200 myxozoans currently described (Fiala et al., 2015), which is an astounding 20% of Cnidaria. Like other cnidarians, they are characterized partially by their "stinging" components, called nematocysts, which manifest as polar capsules in myxozoans. These polar capsules have reduced functions as parasitic attachment (Piriatinskiy et al., 2017) and recent video documentation suggests that some myxozoans (*Myxobolus cerebralis*, in this case) might be capable of projecting toxins from their extended polar capsules (Ben-David et al., 2016). Myxozoans are unique in that they are microscopic, multicellular, and parasitic animals with a two-host life cycle. Their definitive host is an annelid or bryozoan depending on whether the organism is from the class Myxosporea or Malacosporea, respectively. Most myxozoans have a vertebrate secondary host, which is commonly a fish. In the case of *C. shasta*, that fish is a salmonid.

Ceratonova shasta

Ceratonova (syn *Ceratomyxa*) *shasta* is an endemic parasite of the Pacific Northwest of North America and has been one of the primary pathogenic contributors to mortality in salmonids (Ratliff, 1981, 1983; Fujiwara et al., 2011; Baker and Lovtang, 2016). *Ceratonova shasta* was first officially identified in the Crystal Lake Hatchery of Northern California in 1950 after a disease outbreak (Noble, 1950).

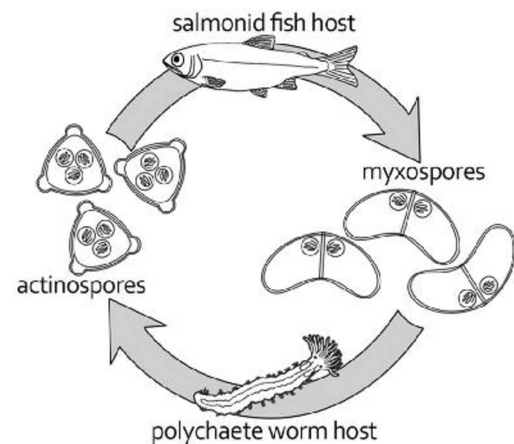

Figure 1.2: *Ceratonova shasta* life cycle. Image: S. Atkinson.

However, *C. shasta* clinical disease signs had been recorded in returning adult pacific

salmon prior to this time (Sanders, 1967). A novel genus was erected in 2014 to encompass freshwater, intestinal myxozoans after the ribosomal small subunit was sequenced and shown to be distinct from other *Ceratomyxa* species (Atkinson et al., 2014).

As a myxosporean, *C. shasta* has two spore forms; the actinospore which infects salmonids and the myxospore which infects polychaetes (Figure 1.2). These two spore forms are differentially resistant to degradation, with the actinospore being more fragile and short-lived (Bjork and Bartholomew, 2010) and the myxospore being denser and more resilient (Hallett et al., 2012). The actinosporean stage of *C. shasta* proliferates in the epidermis of *Manayunkia specios*a (Bartholomew et al., 1997). Actinospores are released into the water column, where they penetrate the gill epithelium of the salmonid secondary host. They then travel through blood vessels to the intestinal tract of the salmonid, possibly causing enteronecrosis, acute ascites, and lesions (Noble, 1950; Bjork and Bartholomew, 2010).

There are three distinct genetic types (genotypes) of *C. shasta*, which differentially infect species of salmonids (Stinson and Bartholomew, 2012; Hurst and Bartholomew, 2012; Atkinson et al., 2018, Stinson et al., 2018). These genotypes, known as types O, I, and II, were identified by a trinucleotide repeat $(ATC)_{0-3}$ in Internal Transcribed Spacer region 1 sequences (Atkinson and Bartholomew, 2010). While some genotypes are able to become pathogenic with less specificity, Chinook salmon are primarily infected and affected by genotype I.

Most of our knowledge of *C. shasta* comes from studies in the Klamath River, in Southern Oregon and Northern California. Our knowledge of how temperature and degree days (Ray et al., 2012), polychaete habitat preference and distribution (Stocking and Bartholomew, 2007), and how spatial and temporal factors affect density of the parasite (Stocking and Bartholomew, 2007; Bjork and Bartholomew, 2009) are derived from the Klamath system.

Ceratonova shasta abundance increases with temperature and decreases with river discharge (Bjork and Bartholomew, 2009; Ray et al., 2012). However, the river systems that we study this parasite in are very different from each other. While the Deschutes

River and Klamath River both have dams that affect fish migration and flow stability, their similarities stop there. Of particular regard to parameters that may affect *C. shasta* abundance, the Klamath River has higher abundance of salmonids (though fewer salmonid species) and a higher range of water temperatures (Bartholow, 2005), whereas the Deschutes River has salmonids in the system year-round and stable water temperatures (Stearns, 1930; O'Connor et. al. 2003; Zimmerman and Ratliff, 2003).

In collaboration with several government and tribal agencies, the research conducted by the Bartholomew Lab at Oregon State University has informed management strategies for the Bureau of Reclamation on the Klamath River for the reduction of fish pathogens, such as *C. shasta*. The host and water sampling techniques used in those studies have been applied to many freshwater systems in Oregon, including the Deschutes River.

***Ceratonova shasta* in the Deschutes River**

Ceratonova shasta was first identified in juvenile spring Chinook salmon and Steelhead trout of the Deschutes River in 1965 by Conrad and Decew, just one year before the first release of hatchery reared spring Chinook salmon into the lower Deschutes. *Ceratonova shasta* related research began in the Deschutes River during the 1970s after disease outbreaks at the Round Butte Hatchery prompted investigation (Zinn et al., 1977; Ratliff et al., 1981). Sentinel studies were conducted in the Deschutes mainstem and Warm Springs Rivers that confirmed the presence of *C. shasta* (Ratliff, 1981; Hoffmaster, 1985; Stinson and Bartholomew, 2012; Vojnovich et al., 2016).

Sentinel studies conducted by Don Ratliff between 1973-1979 identified a seasonal pattern of parasite levels and native spring and fall Chinook salmon mortalities with increases in Spring that continue until late Fall (Ratliff, 1981). Ratliff also determined that *C. shasta* spores degrade after 10 days in Deschutes River water by exposing native and non-native juvenile rainbow trout to infective water from 0-10 days (Ratliff, 1983).

Sentinel studies of rainbow trout from Oak Springs (native) and Roaring River (non-native) hatchery stocks in Deschutes River water at the Pelton Trap (rkm 160) were

conducted in September 1983, with 29% mortality after 23 days of exposure and in June 1984, with 45% mortality following 14 days of exposure to Deschutes River (Hoffmaster, 1985). The seasonal mortality from studies conducted by Ratliff were also observed by Hoffmaster, even with the longer exposure times in September. These studies were conducted without knowledge of the *C. shasta* lifecycle (completed in 1997 by Bartholomew) or that there are host specific genotypes.

Sentinel studies in the Deschutes River basin were not conducted again until 2011 during inter-basin *C. shasta* distribution research by Stinson and Bartholomew. Sentinel studies of spring Chinook salmon resulted in 79% mortality in the lower Deschutes basin and *C. shasta* type I was identified as the primary genotype in water samples. Type I was not detected in the upper basin and sentinel exposures of juvenile spring Chinook salmon in this area had low susceptibility (0% mortality). Passage of spring Chinook salmon above the Round Butte Dam was predicted to spread type I into the upper basin and potentially increase infection risk of spring Chinook salmon in this region (Stinson and Bartholomew, 2012). In studies with multiple salmonid species (Conrad and Decew, 1965; Stinson and Bartholomew, 2012), juvenile spring Chinook salmon appeared to be the least affected by *C. shasta* infections. Stinson and Bartholomew proposed that while genotype I may spread to the upper basin with spring Chinook salmon reintroduction, they are unlikely to be strongly affected.

Juvenile mortality

From the studies done in the Klamath River, we know that spores of *C. shasta* infective to salmonids (actinospores) begin to be released in late Winter/early Spring (Bartholomew et al., 2017). During this time in the Deschutes River, juvenile spring Chinook are held on river water at the Pelton Ladder, potentially exposing them to actinospores and increasing their risk of *C. shasta* infection. Indeed, *C. shasta* was detected (26-58%) in juvenile spring Chinook salmon through lethal sampling and PCR at the Pelton Ladder from 2016-2018 (Appendix A).

The Warm Springs National Fish Hatchery (WSNFH) reported that less than half of all juvenile spring Chinook salmon released from the hatchery passed over the

Bonneville Dam in 2016 (Connolly and McLean, 2017). While no juvenile spring Chinook salmon are positive for *C. shasta* at the WSNFH, some of these fish are infected by the time they reach the Bonneville Dam (USFWS, communications).

Ceratonova shasta has a long evolutionary history with its salmonid hosts (Stinson et al., 2018). This is reflected by the differential resistance to *C. shasta* depending on the stock of fish (Johnson et al., 1975; Zinn et al., 1977). For example, native salmonids to the Deschutes River are less likely to be strongly affected by *C. shasta* infections (Johnson et al., 1975; Zinn et al., 1977). Juvenile salmonids are also capable of clearing the infection (Holt, pers. com). However, with increasing water temperatures and decreasing water flows due to climate change (which would increase the parasite abundance and density of parasites in the water column, respectively), the amount of *C. shasta* may surpass historical, biologically tolerated levels leading to increased juvenile and pre-spawn mortality.

Pre-spawn mortality

During migration back to spawning grounds, salmon do not consume food. In order to make the energetically expensive journey without incoming nutrients, their immune systems are compromised (Dolan et al., 2016). In addition to lack of nutrients, fish also produce stress-induced cortisol and testosterone, both of which are related to maturation and contribute to suppression of the salmonid immune system (Slater et al., 1994; Maule et al., 1996; Dolan et al., 2016; Connor et al., 2018). This depletion of the immune system, warm water temperatures during migration, and increased pathogen density due to lower flow all increase the disease risks for migrating salmon (Fenkes, 2016). The contribution of *C. shasta* to pre-spawn mortality of spring Chinook and Coho salmon of the Deschutes River has been recognized since 1967 (Sanders, 1967; Sanders et. al. 1970). Re-characterization of the temporal distribution of *C. shasta* after nearly 50 years, identification of the spatial distribution, and enumeration of the parasite in hosts are necessary to inform management strategies for *C. shasta* in the Deschutes River Basin today.

Detection methods

Sentinel fish surveys were conducted to assess the presence of *C. shasta* related mortality (AFS-FHS, 2014). These involve placing juvenile salmonids in a sentinel cage that allows flow-through of water without the escapement of fish. The caged fish are exposed to river water for 3-7 days. After exposure, fish are brought back to a laboratory and placed on pathogen-free water. *Ceratonova shasta*-associated mortality rate is determined through gross visualization of clinical signs of enteronecrosis and wet mounts of myxospores in the lower intestine. Disease can also be confirmed by observation of degradation of mucosal epithelium in the lower intestine by histology (Bartholomew, 1989). While these surveys are one of the only means of determining actinospore presence in water, they are neither time nor cost effective, require lethal sampling of fish, and cannot fully describe the temporal or spatial distribution of *C. shasta* in a river system.

The development of a DNA probe (Bartholomew et al., 1995) and polymerase chain reaction (PCR) assay for *C. shasta* (Palenzuela et al., 1999) allowed for faster and more sensitive detection of the parasite in fish tissues. Since maturation and proliferation of *C. shasta* spores occurs in the lower intestine, anal swabs were considered as a non-lethal detection method (Fox et al., 2000). Detection of *C. shasta* from anal swabs by PCR was less effective than PCR of fish tissues, but more sensitive than traditional microscopy methods.

Real-time parasite detection was made possible with the development of a quantitative polymerase chain reaction (qPCR) assay for *C. shasta* (Hallett and Bartholomew, 2006). Applying this technique to filtered, DNA extracted, 1L water samples allowed for the non-lethal detection and quantification of *C. shasta* density in spores/L. The fast collection and processing time of water samples can provide detailed temporal and spatial distribution information regarding waterborne parasites such as *C. shasta*. However, it should be noted this method cannot distinguish between actinospores and myxospores in the water sample.

Following the hypothesis that there were multiple genotypes of *C. shasta*, a sequencing protocol was developed for the delineation of these types. *Ceratonova shasta*

DNA from both water and tissue samples are first processed by PCR. The PCR products are diluted and Sanger sequenced. Sequence chromatograms are inspected by eye and characterized for genotype based on INDEL and SNP patterns. Genotypes O, I or II are called based on a respective number of ATC repeats in the ITS-1 region (Atkinson and Bartholomew, 2010). The sequencing of genotypes and subsequent discovery of genotype-host associations has been informative for assessing *C. shasta*-infection risk of salmonids.

Control and management of *Ceratonova shasta*

There are two current modes of controlling and managing *C. shasta* infections in a river system. Management can be targeted towards the parasite hosts or the water that hosts are exposed to.

i. Parasite host management

Stocking resistant strains of salmonids is an effective management strategy for *C. shasta* (Zinn et al., 1977; Buchanan et al., 1983). Resistant Deschutes River strains have been identified (Johnson et al., 1975; Zinn et al., 1977) and only these resistant, native salmonids are stocked in the Deschutes River (Northwest Power and Conservation Council, 2003). Sentinel studies conducted by Don Ratliff between 1973-1974 suggest that releasing juvenile Chinook salmon on or before May 3rd of each year decreases exposure to *C. shasta* (Ratliff, 1981). Ratliff also proposed that short exposures to *C. shasta* before their release provided resistance to the parasite and decreased mortalities.

The definitive host of *C. shasta*, *Manayunkia* sp., is a polychaete worm that inhabits tubes built from fine sediment and mucus attached to a variety of benthic substrata. In the Klamath River, high discharge in winter or spring (flow velocities that mobilize the bed) has been linked with reduced polychaete abundance (Malakauskas et al., 2013; Alexander et al., 2014, 2016) and are currently mandated in this system (United States District Court for the Northern District of California San Francisco Division, 2017). Additionally, emergency dilution flows are mandated at five *C. shasta* spores/L as this density has been quantified as the infective dose threshold that corresponds to 40% mortality for Coho salmon of the Klamath River (Hallett et al., 2012). The infection dose (density of spores and genotypes) and infective dose threshold are different for different

species (Ray et al., 2013). In order to implement emergency flows in the Deschutes River, these parameters would need to be defined through parallel sentinel fish and water sample studies to determine the infective dose for spring Chinook salmon of the Deschutes River.

ii. Water treatment

Currently, only the Cowlitz hatchery in WA treats their water for *C. shasta* where they have implemented ozone disinfection of the water since 1991 (Bartholomew et al., 2004). The original implementation of ozone disinfection involved treating the water with 2ppm of ozone for 15 minutes. Excess ozone after this time is removed using a forced-air, packed stripping unit. While this initially decreased the *C. shasta* prevalence of infection, 2ppm was determined to be too low for complete removal of the parasite from the water supply. If ozone was to be used at another hatchery to mitigate *C. shasta* infection, higher concentrations would need to be used.

Currently the only water treatment at the Round Butte Hatchery involves UV treated water for the eggs and young juveniles. With these treatments and the use of spring water at the hatchery, *C. shasta* has not been detected in juveniles while at the hatchery (ODFW, communication). However, after the juveniles are transferred to the Pelton Ladder, they are exposed to untreated Deschutes River water and are infected by *C. shasta* (Appendix A).

Objectives

The main objective of my thesis is to analyze the pathogenic contribution on the health of spring Chinook salmon in the Deschutes River basin through water sampling, fish tissue collection, and molecular techniques (PCR and qPCR) (Chapter 2). This includes monitoring waterborne *C. shasta* as it relates to abiotic factors like time, space, temperature, and water flow. The genotypes of *C. shasta* were observed and recorded in Chapter 3, contributing to our understanding of *C. shasta* host specificity and the geographical distribution of genotypes. Supporting materials that analyze the methods of water collection and filtration timing effects on *C. shasta* detection are included in Chapter 4. Funding for this research was provided by The Confederated Tribes of the Warm Springs Reservation of Oregon-Pacific Coastal Salmon Recovery Fund and an

Oregon Department of Fish and Wildlife Fish Health Graduate Research Fellowship in Microbiology.

REFERENCES

AFS-FHS (American Fisheries Society-Fish Health Section). 2014. FHS blue book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2014 edition.

Alexander, J. D., S. L. Hallett, R. W. Stocking, L. Xue, and J. L. Bartholomew. 2014. Host and parasite populations after a ten-year flood: *Manayunkia speciosa* and *Ceratonova* (syn *Ceratomyxa*) *shasta* in the Klamath River. Northwest Science 88(3):219–233.

Alexander, J. D., J. L. Bartholomew, K. A. Wright, N. A. Som, and N. J. Hetrick. 2016. Integrating models to predict distribution of the invertebrate host of myxosporean parasites. Freshwater Science 35(4):1263–1275.

Atkinson S. D. and J. L. Bartholomew. 2010. Disparate infection patterns of *Ceratomyxa shasta* (Myxozoa) in rainbow trout (*Oncorhynchus mykiss*) and Chinook salmon (*Oncorhynchus tshawytscha*) correlate with internal transcribed spacer-1 sequence variation in the parasite. Journal of Parasitology 40(5):599–604.

Atkinson, S. D., S. R. M. Jones, R. D. Adlard, and J. L. Bartholomew. 2011. Geographical and host distribution patterns of *Parvicapsula minibicornis* (Myxozoa) small subunit ribosomal RNA genetic types. Parasitology 138(8):969–977.

Atkinson, S. D., J. S. Foott, and J. L. Bartholomew. 2014. Erection of *Ceratonova* n. gen. (Myxosporea: Ceratomyxidae) to encompass freshwater species *C. gasterostea* n. sp. from threespine stickleback (*Gasterosteus aculeatus*) and *C. shasta* n. comb. from salmonid fishes. The Journal of Parasitology 100(5):640–645.

Atkinson, S. D., S. L. Hallett, and J. L. Bartholomew. 2018. Genotyping of individual *Ceratonova shasta* (Cnidaria: Myxosporea) myxospores reveals intra-spore ITS-1 variation and invalidates the distinction of genotypes II and III. Parasitology 145(12):1588–1593.

Baker, C., and J. Lovtang. 2016. Monitoring wild populations of spring Chinook salmon (*Oncorhynchus tshawytscha*) and summer Steelhead (*O. mykiss*) in tributaries of the lower Deschutes River within the boundaries of the confederated tribes of the warm springs of Oregon reservation, Natural Production Monitoring 2013-2014 to BPA, Confederated Tribes of the Warm Springs Reservation of Oregon Warm Springs, OR.

Bartholomew, J. L., C. E. Smith, J. S. Rohovec, and J. L. Fryer. 1989. Characterization of a host response to the myxosporean parasite, *Ceratomyxa shasta* (Noble), by histology, scanning electron microscopy and immunological techniques. Journal of Fish Diseases 12(5):509–522.

Bartholomew, J. L., R. J. Rodriguez, and C. K. Arakawa. 1995. Development of a DNA probe for the myxosporean parasite *Ceratomyxa shasta*, using the polymerase chain reaction with arbitrary primers. Diseases of Aquatic Organisms 21(3):215–220.

Bartholomew, J., M. Whipple, D. Stevens, and J. Fryer. 1997. The life cycle of *Ceratomyxa shasta*, a myxosporean parasite of salmonids, requires a freshwater polychaete as an alternate host. Journal of Parasitology 83(5):859-86.

Bartholomew, J. L., E. Ray, B. Torell, M. J. Whipple, and J. R. Heidel. 2004. Monitoring *Ceratomyxa shasta* infection during a hatchery rearing cycle: comparison of molecular, serological and histological methods. Diseases of Aquatic Organisms 62(1–2):85–92.

Bartholomew, J. L., S. D. Atkinson, and S. L. Hallett. 2006. Involvement of *Manayunkia speciosa* (Annelida: Polychaeta: Sabellidae) in the life cycle of *Parvicapsula minibicornis*, a Myxozoan Parasite of Pacific Salmon. The Journal of Parasitology 92(4):742–748.

Bartholomew, J., S. Hallett, R. Holt, J. Alexander, S. Atkinson, R. Craig, A. javaheri, M. Babbar-Sebens. 2017. Klamath river fish health studies: salmon disease monitoring and research. Oregon State University, BOR/USGS Interagency Agreement #R15PG00065. Annual Report.

Bartholow, J. M. 2005. Recent water temperature trends in the lower Klamath River, California. North American Journal of Fisheries Management 25(1):152–162.

Beebee, R., J. O'Connor, G. Grant. 2002. Geology and geomorphology of the lower Deschutes River canyon, Oregon Field Guide to geologic processes in Cascadia: Oregon Department of Geology and Mineral Industries Special Paper 36: 91-108.

Ben-David, J., S. Atkinson, Y. Pollak, G. Yossifon, U Shavit, J. Bartholomew, and T. Lotan. 2016. Myxozoan polar tubules display structural and functional variation. Parasites & Vectors. 9:549.

Bjork, S. J., and J. L. Bartholomew. 2009. The effects of water velocity on the *Ceratomyxa shasta* infectious cycle. Journal of fish diseases 32(2):131–142.

Bjork, S. J., and J. L. Bartholomew. 2010. Invasion of *Ceratomyxa shasta* (Myxozoa) and comparison of migration to the intestine between susceptible and resistant fish hosts. International Journal for Parasitology 40(9):1087–1095.

Bradford, M. J., J. Lovy, and D. A. Patterson. 2010. Infection of gill and kidney of Fraser River Sockeye salmon, *Oncorhynchus nerka (*Walbaum), by *Parvicapsula minibicornis* and its effect on host physiology. Journal of Fish Diseases 33(9):769–779.

Buchanan D. V., Sanders J. E., Zinn J. L., Fryer J. L. (1983) Relative susceptibility of four strains of summer Steelhead to infection by *Ceratomyxa shasta*. Transactions of the American Fisheries Society 112:541– 543

Carraro, L., L. Mari, M. Gatto, A. Rinaldo, and E. Bertuzzo. 2018. Spread of proliferative kidney disease in fish along stream networks: A spatial metacommunity framework. Freshwater Biology 63(1):114–127.

Connolly, S. and B. McLean. 2017. United State Fish & Wildlife Service (USFWS) – Fish and Aquatic Conservation: Using the power of collaboration to combat a dangerous fish parasite.

Connor, W. P., K. F. Tiffan, J. A. Chandler, D. W. Rondorf, B. D. Arnsberg, and K. C. Anderson. 2018. Upstream migration and spawning success of Chinook salmon in a highly developed, seasonally warm river system. Reviews in Fisheries Science & Aquaculture 0(0):1–50.

Conrad, J. F., and M. Decew. 1966. First report of *Ceratomyxa* in juvenile salmonids in Oregon. The Progressive Fish-Culturist 28(4):238–238.

Dixon, P., R. Paley, R. Alegria-Moran, and B. Oidtmann. 2016. Epidemiological characteristics of infectious hematopoietic necrosis virus (IHNV): a review. Veterinary Research 47(1).

Dolan, B. P., K. M. Fisher, M. E. Colvin, S. E. Benda, J. T. Peterson, M. L. Kent, and C. B. Schreck. 2016. Innate and adaptive immune responses in migrating spring-run adult Chinook salmon, *Oncorhynchus tshawytscha.* Fish & Shellfish Immunology 48:136–144.

Fenkes, M., H. A. Shiels, J. L. Fitzpatrick, and R. L. Nudds. 2016. The potential impacts of migratory difficulty, including warmer waters and altered flow conditions, on the reproductive success of salmonid fishes. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 193:11–21.

Fiala, I., M. Hlavni ková, A. Kodádková, M. A. Freeman, P. Bartošová-Sojková, and S. D. Atkinson. 2015. Evolutionary origin of *Ceratonova shasta* and phylogeny of the marine myxosporean lineage. Molecular Phylogenetics and Evolution 86:75–89.

Fox, M. D., O. Palenzuela, and J. L. Bartholomew. 2000. Strategies for the diagnosis of *Ceratomyxa shasta* using the polymerase chain reaction: Comparison of lethal and nonlethal sampling with microscopic examination. Journal of Aquatic Animal Health 12(2):100–106.

Fryer, J. L., and J. E. Sanders. 1981. Bacterial kidney disease of salmonid fish. Annual Review of Microbiology 35(1):273–298.

Fujiwara, M., M. S. Mohr, A. Greenberg, J. S. Foott, and J. L. Bartholomew. 2011. Effects of ceratomyxosis on population dynamics of Klamath fall-run Chinook salmon. Transactions of the American Fisheries Society 140(5):1380–1391.

Gannett, M.W., Lite, K.E., Morgan, D.S. and C.A. Collins. 2001. Ground-water hydrology of the upper Deschutes basin, Oregon. Water-Resources Investigations Report 00–4162. Portland, OR: USGS. 72 pp.

Hydropower Reform Coalition and River Management Society. 2015. Pelton-Round Butte hydroelectric project.

O'Connor, J., G. Grant, and T. Haluska. 2003. Overview of geology, hydrology, geomorphology, and sediment budget of the Deschutes River basin, Oregon. A Peculiar River: Geology, Geomorphology, and Hydrology of the Deschutes River, Oregon. Published by the American Geophysical Union as part of the Water Science and Application Series, Volume 7.

Hallett, S. L., and J. L. Bartholomew. 2006. Application of a real-time PCR assay to detect and quantify the myxozoan parasite *Ceratomyxa shasta* in river water samples. Diseases of Aquatic Organisms 71(2):109–118.

Hallett, S. L., R. A. Ray, C. N. Hurst, R. A. Holt, G. R. Buckles, S. D. Atkinson, and J. L. Bartholomew. 2012. Density of the waterborne parasite *Ceratomyxa shasta* and its biological effects on salmon. Applied and Environmental Microbiology 78(10):3724– 3731.

Heisler, T. 2012. Deschutes River Conservancy Deschutes Water Planning Initiative:39.

Hoffmaster, J. L. 1985. Geographic distribution of *Ceratomyxa shasta* in the Columbia River basin and susceptibility of salmonid stocks. MSc., Oregon State University, United States - Oregon.

Holland, J., B. Okamura, H. Hartikainen, and C. Secombes. 2011. A novel minicollagen gene links cnidarians and myxozoans. Proceedings of the Royal Society B: Biological Sciences. 278(1705): 546–553.

Hurst, C. N., and J. L. Bartholomew. 2012. *Ceratomyxa shasta* genotypes cause differential mortality in their salmonid hosts. Journal of Fish Diseases 35(10):725–732.

Johnson, K. A. 1975. Host susceptibility, histopathologic, and transmission studies on *Ceratomyxa shasta*, a Myxosporidian parasite of salmonid fish. PhD thesis, Oregon StateUniversity, Corvallis, OR.

Kent, M. L., D. J. Whitaker, and S. C. Dawe. 1997. *Parvicapsula minibicornis* n. sp. (Myxozoa, Myxosporea) from the kidney of Sockeye salmon (*Oncorhynchus*

nerka) from British Columbia, Canada. The Journal of Parasitology 83(6):1153–1156.

Kent, M. L., J. Khattra, R. P. Hedrick, and R. H. Devlin. 2000. *Tetracapsula renicola* n. sp. (Myxozoa:Saccosporidae); The PKX Myxozoan: The cause of proliferative kidney disease of salmonid fishes. The Journal of Parasitology 86(1):103–111.

Maule, A. G., R. Schrock, C. Slater, M. S. Fitzpatrick, and C. B. Schreck. 1996. Immune and endocrine responses of adult Chinook salmon during freshwater immigration and sexual maturation. Fish & Shellfish Immunology 6(3):221–233.

Miller, K., A. Teffer, S. Tucker, S. Li, A. Schulze, M. Trudel, F. Juanes, A. Tabata, K. Kaukinen, N. Ginther, T. Ming, S. Cooke, J. Hipfner, D. Patterson, and S. Hinch. 2014. Infectious disease, shifting climates, and opportunistic predators: cumulative factors potentially impacting wild salmon declines. Evolutionary applications. 7: 812–855.

Mulcahy, D. M. 1976. Distribution and epizootiology of infectious pancreatic necrosis and infectious hematopoietic necrosis of salmonid fishes in Oregon.

Noble, E. 1950. On a Myxosporidian (protozoan) parasite of California trout. The Journal of Parasitology. 36(5): 457-460.

Northwest Power and Conservation Council. 2003. Deschutes subbasin plan. Chapter 3: Focal species characterization and status.

Oregon Department of Fish and Wildlife (ODFW) and Confederated Tribes of the Warm Springs (CTWS). 2017. Hatchery and genetic management plan.

Palenzuela, O., G. Trobridge, and J. L. Bartholomew. 1999. Development of a polymerase chain reaction diagnostic assay for *Ceratomyxa shasta*, a myxosporean parasite of salmonid fish. Diseases of Aquatic Organisms 36(1):45–51.

Piriatinskiy, G., S. Atkinson, S. Park, D. Morgenstern, V. Brekhman, G. Yossifon, J. Bartholomew, and T. Lotan. 2017. Functional and proteomic analysis of *Ceratonova shasta* (Cnidaria: Myxozoa) polar capsules reveals adaptations to parasitism. Scientific Reports. 7: 1-10.

Portland General Electric (PGE). N.d. Deschutes daily fish counts. https://www.portlandgeneral.com/corporate-responsibility/environmental-

stewardship/water-quality-habitat-protection/fish-counts-fish-runs/deschutes-daily-fish-counts#

Ratliff, D. E. 1981. *Ceratomyxa shasta*: Epizootiology in Chinook salmon of central Oregon. Transactions of the American Fisheries Society 110(4):507–513.

Ratliff, D. E. 1983. *Ceratomyxa shasta*: Longevity, distribution, timing, and abundance of the infective stage in Central Oregon. Canadian Journal of Fisheries and Aquatic Sciences 40(10):1622–1632.

Ratliff, D.E., and E.E. Schulz. 1999. Fisheries program at the Pelton Round Butte Hydroelectric Project (Oregon), 1956–1995. Portland General Electric Company. Portland, Oregon.

Ray, R. A., R. A. Holt, and J. L. Bartholomew. 2012. Relationship between temperature and *Ceratomyxa shasta*–induced mortality in Klamath River salmonids. Journal of Parasitology 98(3):520–526.

Sanders, J. E. 1967. Occurrence of the protozoan parasite *Ceratomyxa shasta* among salmonid fishes in Oregon waters. MSc., Oregon State University, United States – Oregon.

Sanders, J. E., J. L. Fryer, and R. W. Gould. 1970. Occurrence of the myxosporidian parasite *Ceratomyxa shasta*, in salmonid fish from the Columbia River Basin and Oregon coastal streams. Pages 133-144 in S.F. Snieszko (ed.). A symposium on diseases of fishes and shellfishes. American Fisheries Society Special Publication no. 5. Washington, DC.

Siddall, M. E., D. S. Martin, D. Bridge, S. S. Desser, and D. K. Cone. 1995. The Demise of a phylum of protists: Phylogeny of myxozoa and other parasitic Cnidaria. The Journal of Parasitology 81(6):961–967.

Slater, C. H., C. B. Schreck, and P. Swanson. 1994. Plasma profiles of the sex steroids and gonadotropins in maturing female spring Chinook salmon (*Oncorhynchus tshawytscha*). Comparative Biochemistry and Physiology Part A: Physiology 109(1):167–175.

Smothers, J. F., C. D. von Dohlen, L. H. Smith Jr, and R. D. Spall. 1994. Molecular evidence that the myxozoan protists are metazoans. Science. 265(5179): 1719-1721.

Stearns, H. T. 1930. Geology and water resources of the middle Deschutes River basin, Oregon. Contributions to hydrology of united states.

Stinson, M. E. T., and J. L. Bartholomew. 2012. Predicted redistribution of *Ceratomyxa shasta* genotypes with salmonid passage in the Deschutes River, Oregon. Journal of Aquatic Animal Health 24(4):274–280.

Stinson, M. E. T., S. D. Atkinson, and J. L. Bartholomew. 2018. Widespread Distribution of *Ceratonova shasta* (Cnidaria: Myxosporea) genotypes indicates evolutionary adaptation to its salmonid fish hosts. Journal of Parasitology 104(6):645–650.

Štolc, A. 1899. Actinomyxidies, nouveau groupe de Mesozoaires parent des Myxosporidies. Bulletin International de l'Academie des Sciences de Boheme 22: 1–12.

Sear, D. A., P. D. Armitage, and F. H. Dawson. 1999. Groundwater dominated rivers. Hydrological Processes 13(3):255–276.

Stocking, R. W., and J. L. Bartholomew. 2007. Distribution and habitat characteristics of *Manayunkia speciosa* and infection prevalence with the parasite *Ceratomyxa shasta* in the Klamath River, Oregon-California. The Journal of Parasitology 93(1):78–88.

United States Department of Agriculture (USDA). 1996. Upper Deschutes wild and scenic river: Record of decision and final environmental impact statement. Bend, OR: United States Department of Agriculture Forest Service. 316 pp.

United States District Court for the Northern District of California San Francisco Division. 2017. Hoopa Valley Tribe v. U.S. Bureau of Reclamation and Klamath Water Users Association, et al. Document 111. Case No. 3:16-cv-04294-WHO.

Vojnovich, S., Hallett S., Atkinson S., Alexander J., Holt R., Craig R., and J. Bartholomew. 2016. Myxozoan disease risks in the Willamette and Deschutes Rivers, ODFW fellowship report 2015-2016. Oregon State University, Corvallis, OR.

Zimmerman, C. E. and D. E. Ratliff. 2003. Controls on the distribution and life history of fish populations in the Deschutes River: geology, hydrology, and dams. A Peculiar River: Geology, Geomorphology, and Hydrology of the Deschutes River, Oregon. Published by the American Geophysical Union as part of the Water Science and Application Series, Volume 7.

Zinn, J. L., K. A. Johnson, J. E. Sanders, and J. L. Fryer. 1977. Susceptibility of salmonid species and hatchery strains of Chinook salmon (*Oncorhynchus tshawytscha*) to Infections by *Ceratomyxa shasta*. Journal of the Fisheries Research Board of Canada 34(7):933–936.

CHAPTER 2: THE SPATIAL AND TEMPORAL DISTRIBUTION OF *CERATONOVA SHASTA* IN THE DESCHUTES AND WARM SPRINGS RIVERS, OREGON, USA

Kalyn Hubbard, Julie Alexander, Jerri Bartholomew, and Sascha Hallett

ABSTRACT

Ceratonova shasta, a waterborne myxozoan parasite endemic to the Pacific Northwest, has been a suspected contributor to mortality of juvenile and pre-spawn spring Chinook salmon (*Oncorhynchus tshawytscha*) in the Deschutes River basin since the 1970's. Sentinel fish studies have revealed spatial and seasonal variation with the highest mortalities of spring Chinook salmon occurring in June at the Oak Springs Hatchery (rkm 65) compared to further upstream at the Pelton Trap (rkm 160). Finer scale distribution information was needed to determine parasite dynamics in the Warm Springs and Deschutes Rivers. Using water sampling and qPCR, we described the spatial and temporal distribution of *C. shasta* throughout the Deschutes River basin from 2015-2018. Parasite density increased in Spring and declined until late Fall. Though there was variation between years (average spores/L 81.0, 11.2, 46.4 in 2016, 2017, 2018, respectively) *C. shasta* density was 1.5x higher between rkm 48-82 than the average of all other spatial survey sites. Additionally, sites such as the DRR (rkm 116) and DWS (rkm 155) had higher than average *C. shasta* abundance for 63% (5/8) of the surveys. Parasite density in the Warm Springs River did not exceed 1.5 spores/L in either 2017 or 2018. *Ceratonova shasta* abundance increased with water temperature and decreased with river discharge. These parameters influence the spatial and temporal variation of *C. shasta* in water samples within and between years.

INTRODUCTION

Ceratonova shasta has been implicated as a contributing factor to years of poor salmonid returns in the Deschutes River due to juvenile and pre-spawn mortality (Ratliff, 1983; Baker and Lovtang, 2016). The parasite has a complex, two host, two spore life cycle and cannot be transmitted from fish to fish. Parasite actinospores proliferate in a freshwater polychaete and are shed into the water column, where they encounter the salmonid secondary host (Bartholomew et al., 1997). Actinospores penetrate the gill epithelium and travel through the blood to the intestine, where they develop into myxospores potentially causing enteronecrosis and death of the host; severity of disease depends on fish species, life stage, parasite dose and other stressors, including pre-spawning condition (Noble, 1950; Bjork and Bartholomew, 2010).

Ceratonova shasta related research began in the Deschutes River during the 1970s after hatchery juveniles residing in Lake Simtustus (between the Pelton and Round Butte Dams, rkm 162-165; Figure 2.1) were diagnosed with ceratomyxosis (Conrad and Decew, 1966; Ratliff, 1981). Those early studies documented *C. shasta* associated mortalities in native fall (wild) and spring (hatchery) Chinook salmon (*Oncorhynchus tshawytscha*) using a combination of fish exposed in cages ("sentinel exposures") and wild fish captures for rearing in the laboratory. A 7-year study suggested *C. shasta* infections increased in spring and continued through the fall (Ratliff, 1981). This study was the first to report a host-parasite imbalance favoring *C. shasta* and to describe the variation of *C. shasta* abundance within and among years in the Deschutes River.

Sentinel studies demonstrated differences in *C. shasta* risk for juvenile spring Chinook salmon for between the upper and lower basins: Stinson and Bartholomew (2012) observed 0% *C. shasta* related mortality in juvenile spring Chinook salmon exposed in the upper basin on the Crooked River (rkm 183) and 79% *C. shasta* related mortality in juvenile spring Chinook salmon exposed in the lower basin near the Oak Springs Hatchery (rkm 75) following 3 day sentinel trials. Since then, significant juvenile and pre-spawn mortalities of spring Chinook salmon in this system have occurred (Baker and Lovtang, 2016). Hypotheses that *C. shasta* was contributing to these mortalities were explored through collaborative studies with the Oregon Department of Fish and Wildlife (ODFW), the Confederated Tribes of the Warm Springs Reservation of Oregon (CTWSRO), the United States Fish and Wildlife Service (USFWS), and Oregon State University (OSU) during 2015 and 2016. These studies examined the spatial distribution and density of *C. shasta* through molecular detection of the parasite in water samples (2015-2016) as well as prevalence of infection and mortality in sentinel fish exposed in 2015. Results of these studies supported earlier findings that *C. shasta* was abundant in the lower Deschutes River near the Oak Springs Hatchery and was associated with high mortality (48% in 2015) in sentinel juvenile spring Chinook, whereas lower abundance and associated mortality (12%) were detected at the Pelton Trap (rkm 160; Vojnovich et al., 2016).

Due to the difference in *C. shasta* density between the Oak Springs and Pelton Trap, we suspected that *C. shasta* abundance would vary throughout the Deschutes River basin as *C. shasta* density increases with temperature and decreases with river discharge (Bjork and Bartholomew, 2009; Ray et al., 2012). We predicted that peak *C. shasta* densities would vary i) inter-annually with higher parasite abundance in water years having low peak discharge, ii) intra-annually (temporally) with highest parasite densities during periods of peak water temperature, and iii) spatially with high *C. shasta* densities at spawning territories due to myxospore input from perishing adult salmonids (Kent et al., 2014). To determine the finer scale temporal and spatial distribution of *C. shasta* we quantified waterborne parasite densities in water samples collected throughout the Deschutes River basin from 2015-2018.

METHODS

Study sites

Deschutes River — The Deschutes River begins at Little Lava Lake in the Cascade Range, flows south through the Crane Prairie Reservoir, the Wickiup Reservoir, and then north where the mouth enters the Columbia River - roughly 405 river kilometers (rkm) from its starting point (Heisler, 2012). The Deschutes River is divided into upper and lower basins by the Pelton, Round Butte, and Reregulating Dams located between rkm 160-165. In addition to constructed dams, Big Falls at rkm 211 is a natural historical migration barrier to salmonids in the upper Deschutes River basin (Figure 2.1).

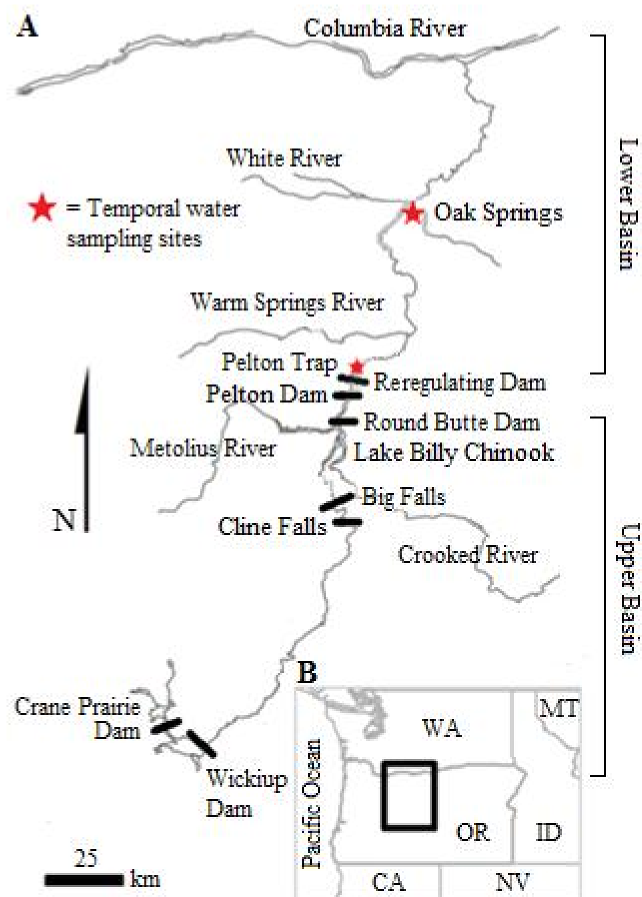

FIGURE 2.1: Map of the Deschutes River basin including major tributaries and temporal water sampling sites.

The separation of the river by the dams and natural hydrologic features could drive differences in the spatial and temporal distribution of *C. shasta* in the Deschutes River through differences in water temperatures, hydrologic disturbance regimes, and salmonid host availability.

The upper basin water temperature and hydrology are primarily driven by mountain snowpack and groundwater discharge that provides more than three-quarters of streamflow for the Deschutes River (Gannett et al., 2013) whereas water in the lower basin is controlled by the selective water withdrawal (SWW) tower (above the Round Butte Dam). The SWW tower mixes warmer surface water of Lake Billy Chinook with cooler water from the bottom of the lake. Water temperature in the lower basin is regulated by this dam to mimic pre-dam temperature and hydrologic conditions (PGE, n.d.). The Deschutes River mainstem also receives water input from large tributaries such as the Warm Springs River (rkm 134), White River (rkm 74), and Buckhollow Creek (rkm 68). Salmonid distribution and densities also vary within and between the upper and lower basin. Five species of trout comprise the largest proportion of Deschutes River salmonids and are present in both the upper and lower basins. In the lower basin alone, native anadromous salmonids including native spring and fall Chinook salmon, Sockeye salmon (*Oncorhynchus nerka*), and Steelhead trout (*O. mykiss*) and non-native Coho salmon (*O. kisutch*). However, reintroduction of spring Chinook salmon to the upper basin began in 2007 (ODFW and CTWS, 2017). While wild fish of each species exist in the Deschutes River, spring Chinook salmon, summer Steelhead, and Sockeye salmon are all reared at the Round Butte Hatchery below the Round Butte Dam and the Pelton Ladder below the Reregulating Dam (Figure 2.1). The combination of the presence of *C. shasta* and the ecological complexity inherent in the Deschutes River system warrants a clear understanding of the temporal and spatial dynamics of *C. shasta* in the context of reintroduction and conservation efforts.

Warm Springs River — The Warm Springs River is a major tributary to the Deschutes River (at Deschutes rkm 134) and provides important spawning habitat for wild spring Chinook salmon (Lindsay et al., 1989). No hatchery fish spawn above the Warm Springs National Fish Hatchery (WSNFH, Warm Springs rkm 17.2).

Temporal sampling: Deschutes and Warm Springs Rivers — To examine temporal variation in *C. shasta* densities (spores/L) in the mainstem river, index sites were established at the Pelton Trap below Pelton Dam (rkm 160) and near Oak Springs Hatchery (rkm 75) (Figure 2.1, 2.3). In general, water samples were collected every two weeks from March to October from 2015 to 2018. Exceptions include samples collected through the winter of 2015 at both sites and early termination of water sampling in July 2016 at the Pelton Trap. Data from 2015 and 2016 has been included for comparison (Vojnovich et al., 2016). 1L samples were collected every 2h for 24h by an automatic sampler (ISCO) and pooled into a composite sample (hereafter referred to as "ISCO samples"). Subsamples of three 1L replicates were collected from the composite, filtered through a 5 µm filter, frozen, and posted to Oregon State University.

In 2017-2018, temporal sampling was expanded to include sites on the Warm Springs River because of pre-spawn mortality and low returns reported for spring Chinook salmon (Baker and Lovtang, 2016). In contrast to the temporal water samples collected on the Deschutes River mainstem, samples in the Warm Springs River were collected manually (hereafter referred to as "grabs"). Three water samples were collected directly from the river at three (2018) to six (2017) sites on the Warm Springs River and one site at the confluence of the Warm Springs and Deschutes Rivers (DRV) (Figure 2.2). The samples were processed as described above, see composite samples.

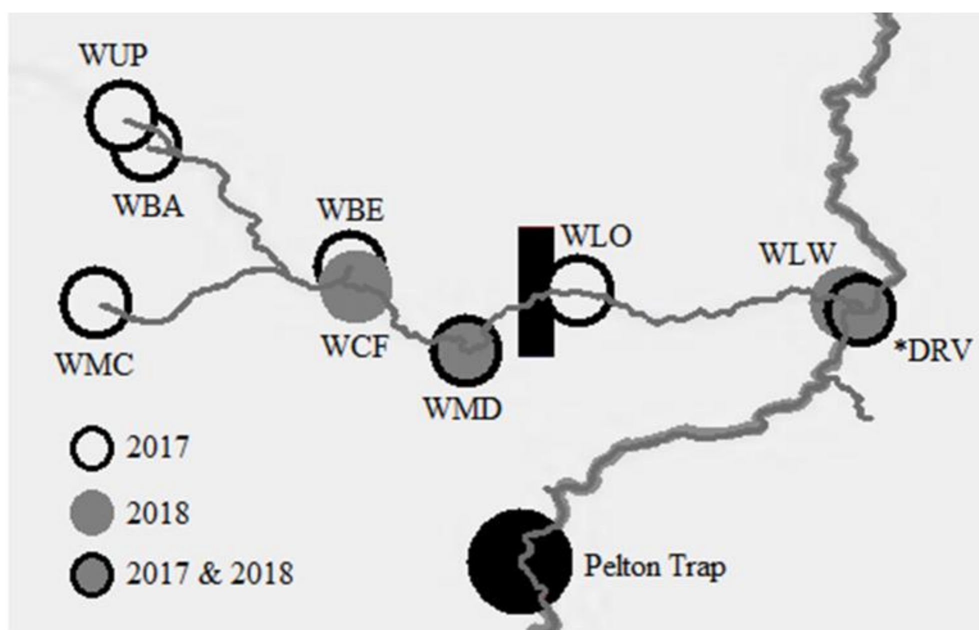

FIGURE 2.2: Temporal water sample collection sites on the Warm Springs River and at the mouth of the Warm Springs River on the Deschutes River (DRV). Sites are represented by open circles (2017), dark grey circles (2018), and black circles filled with dark grey (2017 & 2018). The black bar represents the Warm Springs National Fish Hatchery, a migration barrier to salmonids. The Pelton Trap located below the Pelton Dam is included for reference. *Note the DRV site is on the Deschutes mainstem.

TABLE 2.2: Duration and frequency of monitoring water samples collected at each site by year. Sums of water samples per site are provided by year and as a total for the project. Samples from 2015 and 2016 were processed by Vojnovich et al., 2016 and included here for reference (Gray text).

Year	*Site*	*Start date*	*End date*	*Frequency of collection*	*Total samples*
2015	Oak Springs	03/24/15	11/24/15	Weekly	102
	Pelton Trap	04/14/15	11/24/15	Bi-weekly	51
2016	Oak Springs	03/09/16	12/27/16	Weekly	126
	Pelton Trap	03/08/16	07/26/16	Bi-weekly	32
2017	Oak Springs	01/02/17	10/19/17	Bi-weekly	87
	Pelton Trap	04/04/17	10/31/17	Bi-weekly	51
	Warm Springs	04/13/17	09/14/17	Bi-weekly	207
2018	Oak Springs	03/06/18	10/30/18	Bi-weekly	54

Pelton Trap	03/06/18	10/30/18	Bi-weekly	54
Warm Springs	04/02/18	09/26/18	Bi-weekly	141
			All years	905

Deschutes River spatial surveys — To examine spatial variation in *C. shasta* density (spores/L) in the mainstem river, grab sampling was conducted at approximately 20 sites (including the two temporal index sites) on the Deschutes River mainstem as well as at the mouths of Bakeoven (tributary site with no mainstem influence) and Buckhollow creeks (mainstem site with tributary influence) (Figure 2.3, Table 2.2.). Sites were stratified every 6.4 km along rkms 1 to 230 to capture variation in hydrology, and selected based on proximity to spawning habitat and access. Spatial sampling was conducted in 2016-2018. Surveys conducted in 2016 (June 7th and August 30th, 2016) were collected and processed by Vojnovich et al., 2016. 2017 spatial surveys were conducted on roughly the same dates (June 6th and August 30th, 2017) but were collected and processed by the author. In 2018, survey dates were selected based on peak juvenile and adult spring Chinook salmon run timing. An average of the adult passage dates from the last 5 years (PGE, n.d.) indicated that spatial water samples should be collected on June 5th (25% adult spring Chinook salmon passage) and July 10th (75% adult spring Chinook salmon passage). 2017 was the first year PGE published data on juvenile passage and this

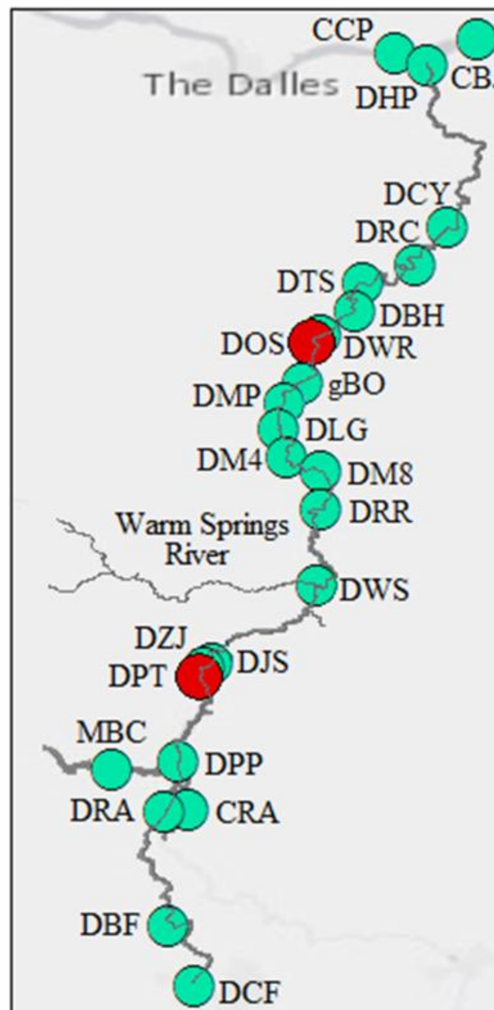

FIGURE 2.3: Spatial and temporal sampling sites with locality site codes that correspond to Table 2.2.

resulted in adding a survey on May 2nd, 2018 during peak juvenile spring Chinook salmon release.

Additional sites were also added in 2018 to obtain higher resolution of *C. shasta* densities between Round Butte Dam and Big Falls, a natural migration barrier. Lower basin sites were chosen based on their proximity to historical spawning grounds (DZJ and DJS, Figure 2.3; Grant et al., 2016) and upper basin sites were selected to assess potential parasite contribution to Lake Billy Chinook. Sites on Lake Billy Chinook (above Round Butte Dam) at the confluence of the Metolius (MBC), upper Deschutes (DRA), and Crooked rivers (CRA) were added to pinpoint sources of *C. shasta* in the upper basin. Sites located near redds were chosen from gravel augmentation project publications (Grant et al., 2016) as well as GIS visualization of areas that matched descriptions of suitable spawning habitat (Isaak et al., 2007), and reports from ODFW.

All spatial sampling events were conducted by collecting grab samples during a single day but the collection approach differed slightly each year. In 2016, the approach involved teams of collaborators collecting three 1L grab samples at 4-6 sites each, while moving upstream in the region assigned to their team. In 2017-2018, the approach involved a single team collecting 3x1L grab samples from all but 4-12 sites as they worked from the lowermost site at the mouth of the Deschutes on the Columbia River (rkm 1) to the uppermost site (rkm 230) from approximately 6:00 – 13:00. Water samples were processed as described in “Water sample DNA extraction and parasite quantification procedure.”

TABLE 2.2: River kilometer, latitude, and longitude coordinates for sites sampled during spatial surveys (grab samples) conducted from 2016-2018.

Locality site code	*River kilometer*	*Coordinates*
CCP	Columbia R – 324	45°39’02.40”N, 120°57’36.30”W
CBJ	Columbia R – 335	45°40’15.20”N, 120°50’16.90”W
DHP	0.6	45°37’55.60”N, 120°54’47.20”W
DCY	37.9	45°23’32.70”N, 120°52’51.10”W
DRC	47.7	45°20’11.20”N, 120°55’51.30”W
DTS	60.3	45°18’32.80”N, 121°00’20.00”W
DBH	67.5	45°16’03.01”N, 121°01’08.59”W
DWR	73.7	45°14’01.29”N, 121°04’01.49”W
DOS	75.5	45°13’17.71”N, 121°04’49.26”W

DMP	81.8	45°10’24.10”N, 121°04’28.60”W
gBO	81.7	45°10’26.10”N, 121°04’27.16”W
DLG	94.0	45°05’33.40”N, 121°07’49.10”W
DM4	100.5	45°03’06.81”N, 121°07’12.40”W
DM8	107.9	45°01’54.70”N, 121°04’06.05”W
DRR	115.8	44°58’26.00”N, 121°04’11.00”W
DRV	133.7	44°51’26.57”N, 121°04’04.50”W
WLW	Warm Springs R – 0.94	44°51’51.00”N, 121°04’40.00”W
WLO	Warm Springs R – 14.6	44°51’59.60”N, 121°13’05.20”W
WMD	Warm Springs R – 21.8	44°50’12.30”N, 121°16’36.00”W
WCF	Warm Springs R – 30.0	44°52’24.10”N, 121°20’33.00”W
WBE	Beaver Creek – 0.97	44°52’51.00”N, 121°20’18.30”W
WMC	Mill Creek – 9.42	44°51’41.00”N, 121°28’19.30”W
WBA	Badger Creek – 1.28	44°56’40.40”N, 121°26’45.90”W
WUP	Warm Springs R – 47.6	44°57’35.80”N, 121°27’31.90”W
DWS	154.7	44°45’31.10”N, 121°13’39.00”W
DJS	157.7	44°44’22.80”N, 121°14’27.34”W
DZJ	159.3	44°43’44.58”N, 121°14’46.32”W
DPT	159.6	44°43’32.50”N, 121°14’51.10”W
DPP	165.1	44°41’06.12”N, 121°14’22.25”W
MBC	182.6	44°35’16.21”N, 121°22’32.49"W
CRA	182.9	44°31'43.98"N, 121°15'49.37"W
DRA	186.4	44°31'31.95"N, 121°17'56.51"W
DBF	211.2	44°21'34.40"N, 121°17'39.00"W
DCF	229.6	44°16'07.60"N, 121°15'24.80"W

Water sample DNA extraction and parasite quantification procedure **—** The water filtration, DNA extraction procedure, and quantitation of *C. shasta* DNA using a quantitative polymerase chain reaction (qPCR) follow Hallett and Bartholomew, 2006 and Hallett et al., 2012 using a QIAGEN DNeasy Tissue Kit with minor modifications. Namely, the use of a drying centrifuge vacuum was implemented to decrease drying time before the acetone extraction and after the ethanol wash of the DNA extraction procedure. Additionally, we used a new qPCR machine (StepOnePlus™ Real-Time PCR System (ABI)), a duplex assay that incorporates internal positive control (IPC) for inhibition testing, and total reaction volumes per well were 10µl instead of 20µl. The 10 µl reaction volume comprised 800 nM of each primer (ABI), 400 nM *C. shasta* probe (ABI), 5 µl TaqMan Universal PCR Master Mix (contains AmpliTaq gold DNA

polymerase, dNTPs with dUTP, passive reference and optimised buffer components) (ABI), 250nM BSA (New England Biolabs), 250 nM IPC DNA (ABI) and 500nM IPC primer and probe (ABI) with either 2 µl of 1:4 diluted sample DNA or 2 µl molecular grade water.

After qPCR, the duplicate wells were averaged, resulting in one value for each sample collected. Wells that differed by more than 1 Cq value were reanalyzed. Cq values were converted to spores/L based on reference samples with known numbers of spores. Samples were considered positive for *C. shasta* if the average of the converted Cq value was higher than 0 spores/L.

Statistical analyses

Mean *C. shasta* density was calculated for each site by averaging the values from each of the 3 samples (ISCO or grab). Linear mixed-effects models (LMEMs) were used to examine relationships between mean *C. shasta* density and temperature and river discharge. Because parasite densities vary depending on time of day collected (Chapter 4), we assumed there were differences between sample sets collected by ISCO versus grab, and model data were limited to ISCO collected water samples at the two index sites (Pelton Trap and Oak Springs). Grab samples collected during spatial surveys were not included in the analysis because they were only collected in spring and summer. Additionally, Oak Springs water samples that were not processed correctly were excluded. River discharge and water temperature data were obtained from United States Geological Service (USGS) gage 14092500 near the Pelton Trap (Madras, OR) (https://waterdata.usgs.gov/usa/nwis/uv?site_no=14092500). Oak Springs water temperature data were collected using an automatic temperature data logger (HOBO, Onset Corp., MA) of which mean daily temperatures were calculated from 15-minute records. River discharge was estimated by averaging daily discharge values for the Madras and Biggs Junction USGS gages (no USGS gage is present at the Oak Springs site and Oak Springs is located in between these locations).

The linear mixed effects model included random effects for site and year and non-normally distributed data were log transformed. Model fit was assessed using random

effect adjusted r^2 and the Akaike information criterion (AIC) was used to evaluate model performance relative to competing models. Model variance was examined using Pearson's residual plots. All statistical analyses were performed using the lme4 package (Bates et al., 2015), with r^2 values with and without random effects computed using the MuMIn package (Nakagawa and Schielzeth, 2013) of R version 3.4.1 (R Development Core Team, 2008).

RESULTS

Deschutes River temporal surveys

Data collected since 2015 demonstrate *C. shasta* densities are 19x higher near Oak Springs Hatchery than at the Pelton Trap on the Deschutes River mainstem (Figure 2.1, 2.3, 2.4; Table 2.4). Despite the difference in density between the index sites, we observed similar temporal patterns at both sites; *C. shasta* increased in spring, peaked in late spring or early summer, and began to decline in the fall. However, there was significant variation among years in i) timing of increase (when density > 1 spore/L), ii) the maximum *C. shasta* density observed, and iii) in the number of peaks observed during a season. For example, 2015 *C. shasta* densities at Oak Springs were already > 1 spores/L by April 7th, peak *C. shasta* density (371.0 spores/L) was measured on April 28th, and two peaks (April and August) were observed compared to 2018 where first detection at >1 spore/L occurred on April 17th, peak (101.0 spores/L) on July 10th, and one peak at this site (Table 2.3, 2.4).

TABLE 2.3: Summary of maximum summer water temperatures (°C) (USGS) and maximum river discharge (m³/s) (USGS) by water year at the Madras USGS station (14092500) and on the Deschutes River near Oak Springs Hatchery from May to September for each year included in the present study.

	Summer water temperature (°C) Madras-Oak Springs	*River discharge (m³/s) by water year Madras-Oak Springs*
2015	**High** Max 15.4-18.6 on 7/5/15	**Moderate** Max 209-279 on 12/23/14
2016	**High** Max 14.5-17.7 on 6/9/16	**Low** Max 185-219 on 3/10/16
2017	**High** Max 14.8-17.3 on 7/3/17	**High** Max 276-328 on 3/21/17
2018	**High**	**Low**

Max 14.9-18.6 on 7/18/18	Max 179-210 on 11/24/17

TABLE 2.4: Summary of *Ceratonova shasta* dynamics each year at the Oak Springs and Pelton Trap sites. Absences from data include: first *C. shasta* detection was missed in 2015, sampling stopped in mid-July of 2016 at the Pelton Trap, and Oak Springs water samples were unreliable during the expected *C. shasta* season in 2017.

Year	*Site*	*First C. shasta detection (over 1 spore/L)*	*Temp at first C. shasta detection (°C)*	*Max C. shasta (spores/L)*	*Max C. shasta date*
2015	Oak Springs	N/A	N/A	371.7	4/28/15
	Pelton Trap	N/A	N/A	13.4	7/7/15
2016	Oak Springs	3/22/16	9.0	185.1	5/16/16
	Pelton Trap	4/5/16	10.1	10.6	7/26/16
2017	Oak Springs	4/24/17	10.5	N/A	N/A
	Pelton Trap	5/16/17	12.2	7.4	6/13/17
2018	Oak Springs	4/17/18	7.8	101.1	7/10/18
	Pelton Trap	5/1/18	10.8	13.9	6/12/18

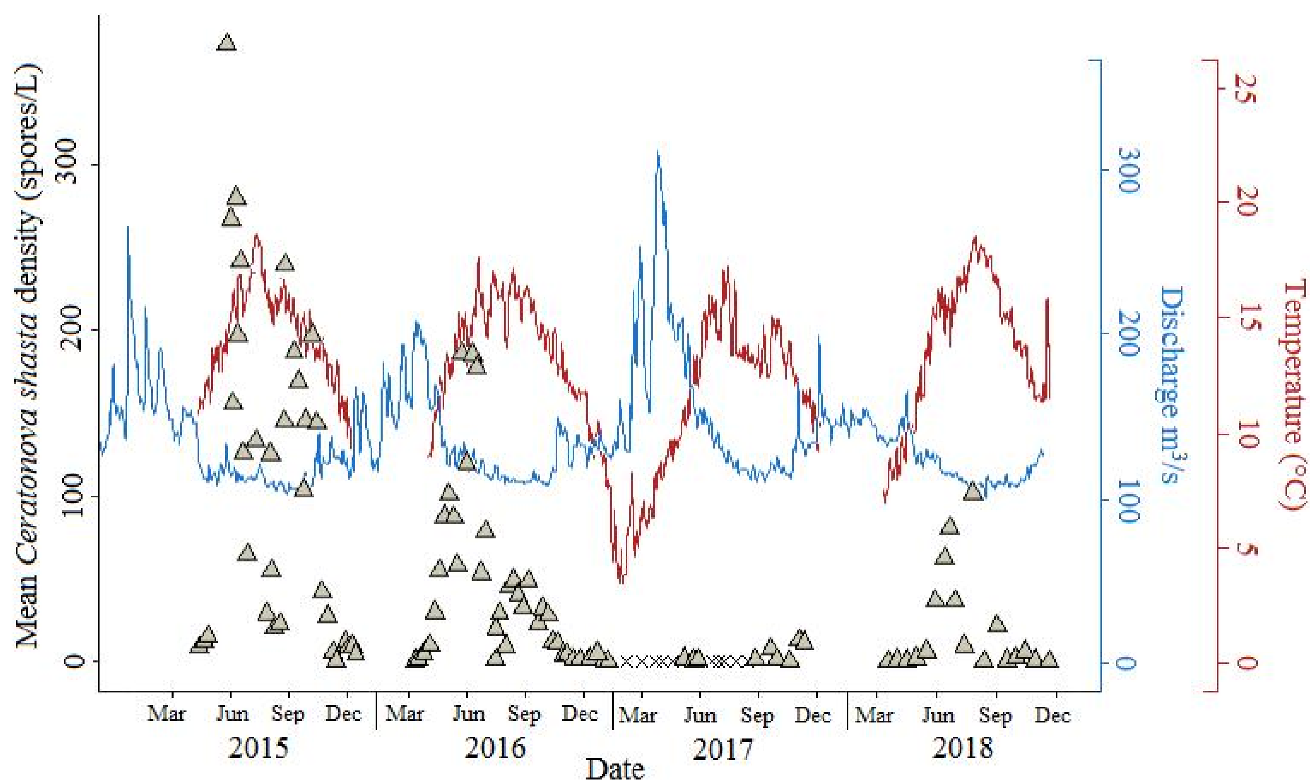

FIGURE 2.4. Mean *Ceratonova shasta* density (y-axis) in the Deschutes River at the Oak Springs Hatchery (gray triangles). Overlays are discharge m^3/s (blue, second y-axis) and river water temperature (red, third y-axis) over time (x-axis). Mishandled samples are represented by X's. Each point is the mean *C. shasta* density from triplicate water samples collected using an automatic sampler (ISCO). Data from 2015 and 2016 were collected and processed by Vojnovich et al., 2016.

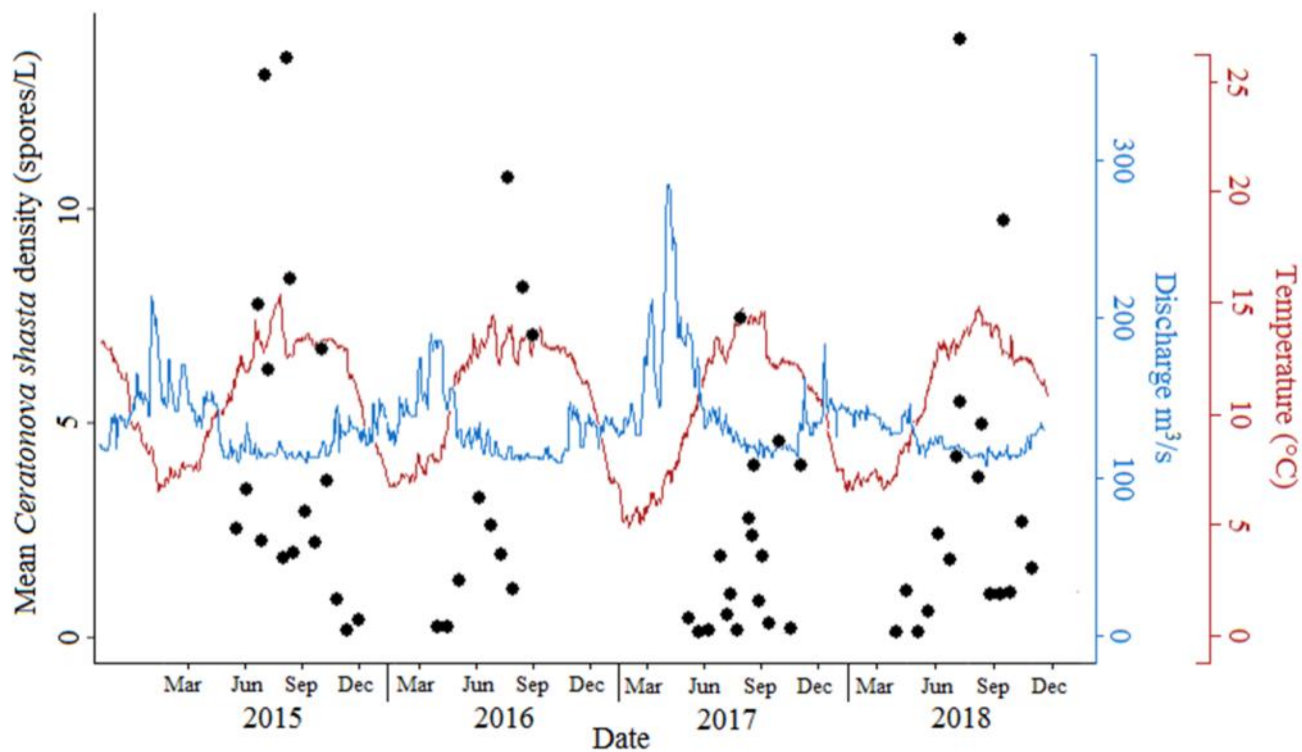

FIGURE 2.5. Mean *Ceratonova shasta* density (y-axis) in the Deschutes River at the Pelton Trap (black circles). Overlays are discharge m^3/s (blue, second y-axis) and river water temperature (red, third y-axis) over time (x-axis). Each point is the mean *C. shasta* abundance from triplicate water samples collected using an automatic sampler (ISCO). Data from 2015 and 2016 were collected and processed by Vojnovich et al., 2016.

Warm Springs River temporal surveys — Density of *C. shasta* at the mouth of the Warm Springs (on the Deschutes River mainstem downstream of the Warm Springs River (WSR) confluence) was about 20x higher than all sampling locations in the Warm Springs River, where the density of *C. shasta* was below 2.5 spores/L (Figures 2.6, 2.7). The *C. shasta* densities at the DRV site are consistent with the Deschutes River mainstem.

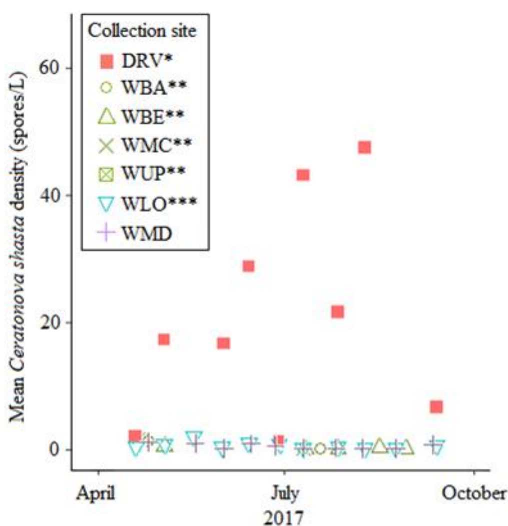

FIGURE 2.6: 2017 Warm Springs River temporal water sample monitoring sites. Each data point is the mean *Ceratonova shasta* spores/L from 3 1L replicate samples. *Note that DRV is located on the Deschutes River at the mouth of the Warm Springs River. ** 2017 sites WBA, WBE, WMC, and WUP were combined as WCF in 2018. ***2017 site WLO was moved further downstream and renamed WLW in 2018.

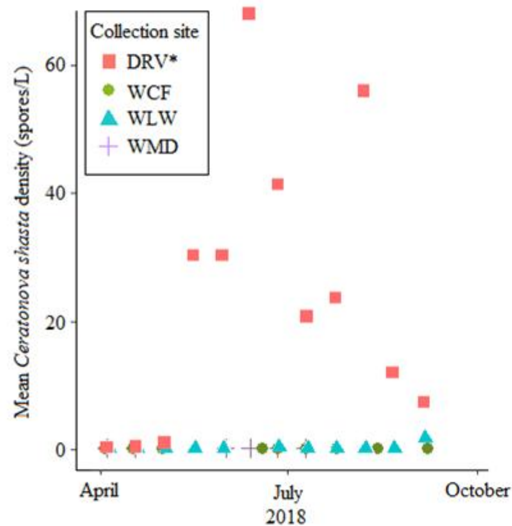

FIGURE 2.7: 2018 Warm Springs River temporal water sample monitoring sites. Each data point is the mean *Ceratonova shasta* spores/L from 3 1L replicate samples. *Note that DRV is at the confluence of the Warm Springs River and the Deschutes River mainstem

Relationships between water temperature, river discharge, and *C. shasta* density (Figures 2.8, 2.9)

Interannual variation was observed in water temperature and discharge during the study period (Figures 2.8, 2.9). Compared to the Deschutes River 10-year average peak discharge of 252 m^3/s, river discharge was low (2016, 2018) to moderate (2015) with a peak average of 213 m^3/s and a high flow event around March 20th in 2017 (peak average

302 m^3/s) (Table 2.3). *Ceratonova shasta* was detected at a wider range of temperatures and discharges at Oak Springs (~ 17.3-18 °C) than the Pelton Trap site (~ 14.5-15.4 °C) (Figure 2.4, 2.5; Table 2.3). At the Pelton Trap and Oak Springs sites, peak *C. shasta* density occurs at the same time as water temperatures between 15-16 °C (Figures 2.4, 2.5). However, as the temperature increased from ~ 16 to 18 °C in 2015 and 2016 at the Oak Springs site, *C. shasta* density decreased (Figure 2.4).

A total of 161 samples were used to examine relationships between *C. shasta* density, temperature, and discharge (n= 33, 32, 14, 18 from Oak Springs in 2015, 2016, 2017, 2018, and n= 20, 10, 17, 17 from Pelton Trap in 2015, 2016, 2017, 2018). The dataset represented samples collected from 4.1-18.5 °C and 104-236 m^3/s. Models examined ranged from single variable to 2 variables (Table 2.5). The top model (temperature ~ log(*C. shasta*), Table 2.5) explained 59.9% of the variation in *C. shasta* density, with higher densities correlated with higher temperatures. *Ceratonova shasta* density and river discharge were negatively correlated, and the second model (discharge ~ log(*C. shasta*), Table 2.5) explained 57.1% of the variation in parasite density.

TABLE 2.5: Comparison of maximal random effect model performances including ANOVA, r^2, and AIC results. Models used are highlighted in gray.

Maximal random effects models	*F stat*	*df*	r^2 *w/o random effects*	r^2 *with random effects*	*AIC score*
Temperature ~ log (mean C. shasta density)	18.8	1,2	0.188	0.599	245.44
*Temperature*Temperature^2 ~ log (mean C. shasta density)*	2.54		0.235	0.608	270.38
log (discharge) ~ log (mean C. shasta density)	22.9	1, 23	0.082	0.571	283.57
*log (discharge)*log(discharge^2 ~ log (mean C. shasta density)*	0.19		0.082	0.580	347.15

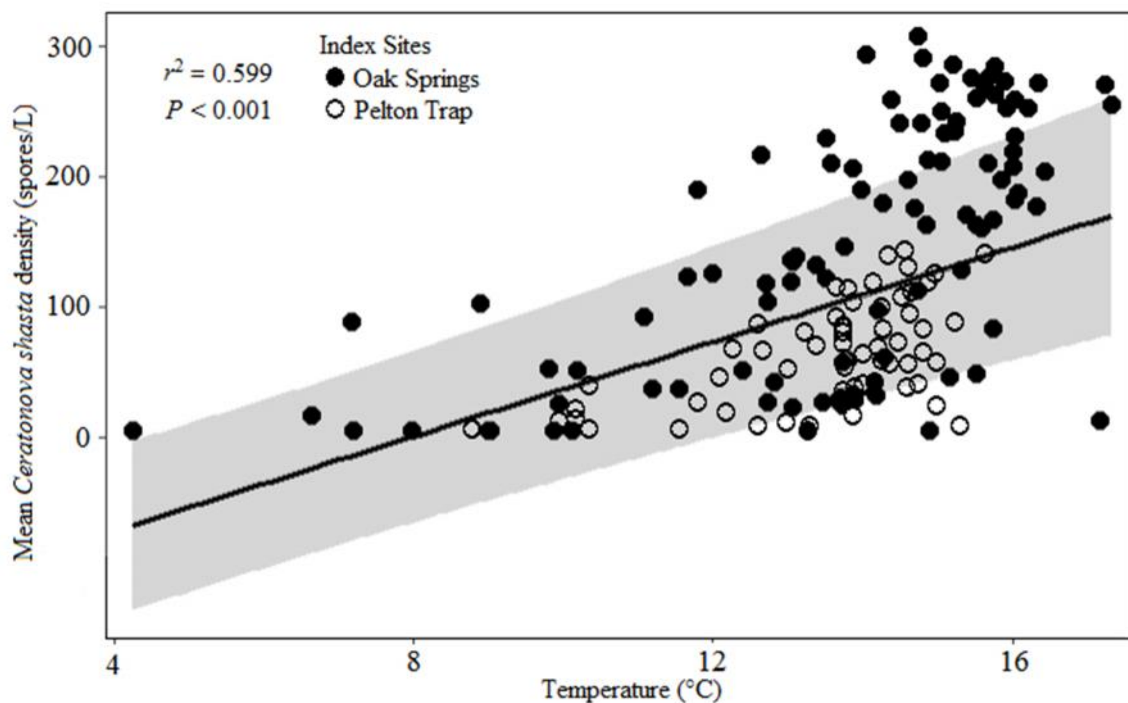

FIGURE 2.8: Linear regression back transformations of temperature (x-axis) and *Ceratonova shasta* density (spores/L) (y-axis). Linear mixed effect model: $r^2 = 0.599$, $F_{1, 7} = 42.76$, $P < 0.001$

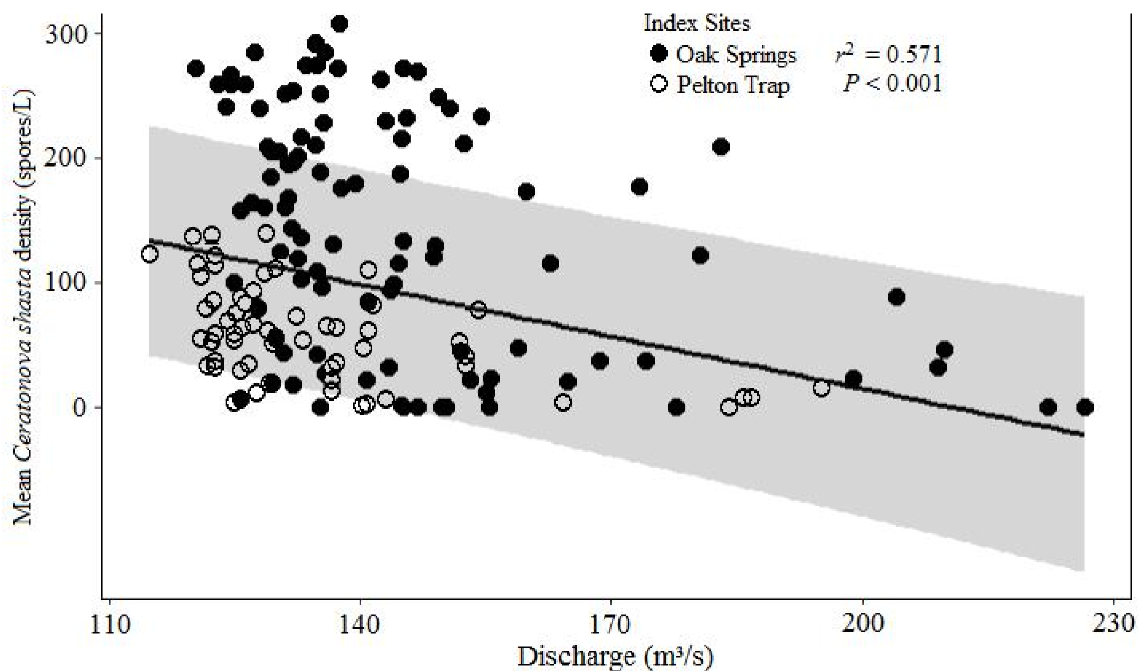

FIGURE 2.9: Linear regression back transformations of discharge (x-axis) and *Ceratonova shasta* density (spores/L) (y-axis). Linear mixed effect model: $r^2 = 0.571$, $F_{1, 152} = 22.95$, $P < 0.001$

Spatial surveys (Figures 2.3, 2.10; Table 2.6)

Higher densities of *C. shasta* (~ 11 spores/L) were observed downstream of the mouth of the Deschutes River on the Columbia River, whereas few *C. shasta* spores (maximum 2.6 spores/L in June 2016) were detected upstream of the Deschutes River confluence with the Columbia River, demonstrating *C. shasta* input from the Deschutes River. While sites vary between seasons and years, high *C. shasta* density was consistently observed in the lower basin between rkm 38 and 135 (relative to other lower basin sites during the same survey) in June. However, the Pelton Trap located below the dam remained an area with low/trace *C. shasta* abundance during our surveys despite being an area where high mortalities due to *C. shasta* during the 7-year sentinel studies conducted by Ratliff (1981). Low densities of *C. shasta* were also detected in 2018 from the upper basin sampling site in Lake Billy Chinook (MBC) as well as the Deschutes (DRA, maximum 2.3 spores/L in May) and Crooked River (CRA, maximum 2.4 spores/L in July) arm sites not affected by Lake Billy Chinook. The upper basin exhibited opposite temporal dynamics from the lower basin with *C. shasta* abundance increasing in August compared to June, except in 2018.

TABLE 2.6: Mean density of *Ceratonova shasta* (spores/L) in water samples between the upper and lower basins of the Deschutes River for each month and year surveyed.

	Year	*May*	*June*	*July*	*August*
Lower Basin	*2016*		103.3		70.0
	2017		11.4		5.0
	2018	10.0	113.2	43.5	26.1
Upper Basin	*2016*		92.3		331.6
	2017		10.3		141.6
	2018	55.2	7.3	44.6	5.2

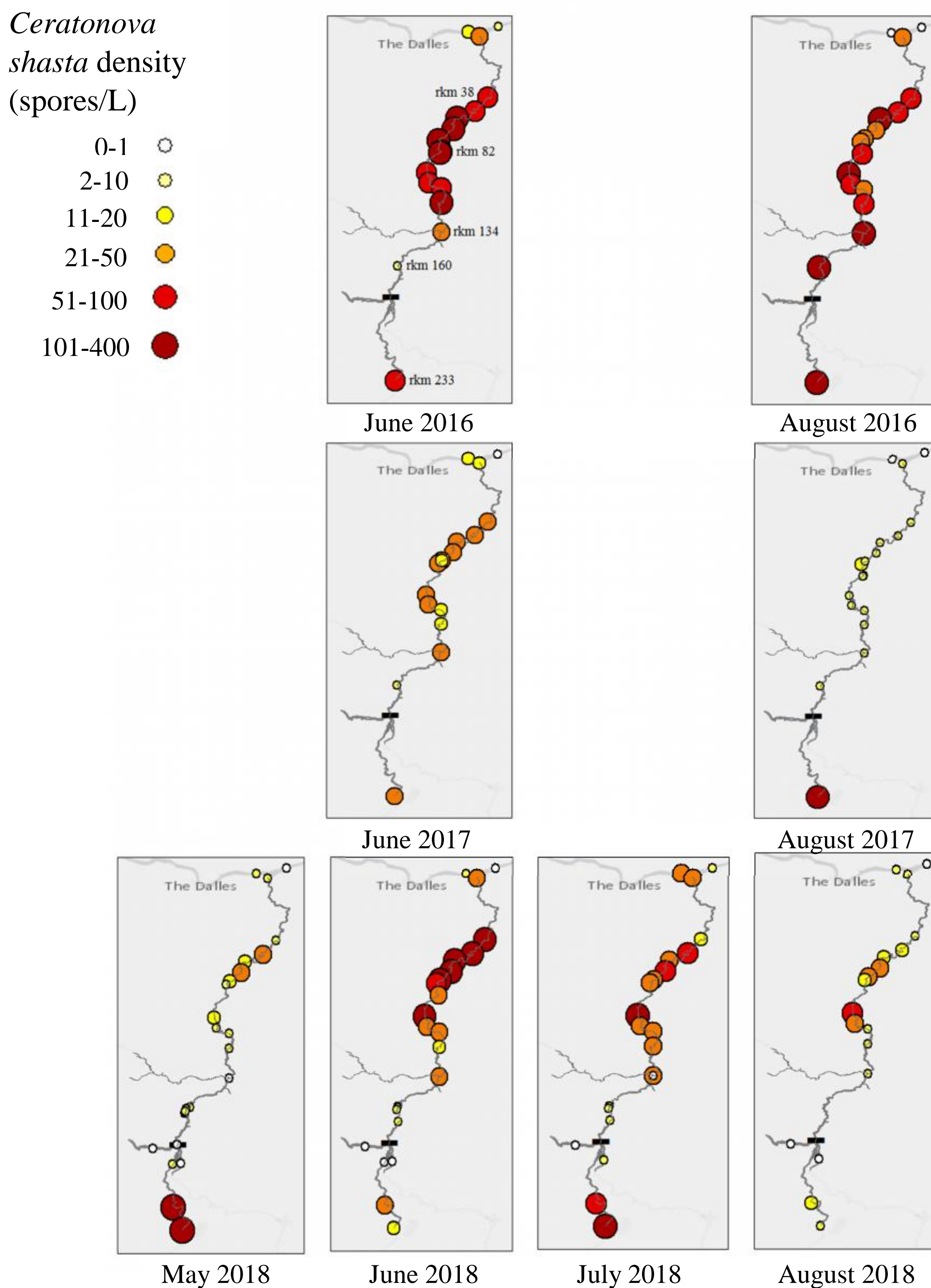

FIGURE 2.10: Results from all of the longitudinal water sampling surveys between 2016-2018. Size of the graduated symbols and color both represent *Ceratonova shasta* density at each site. The black bar represents the Pelton-Round Butte Dam complex, a migration barrier to all salmonids. Rkm are indicated on the first map for reference.

DISCUSSION

In this chapter, the spatial and temporal distribution of *C. shasta* was described in the Deschutes River basin. The only other system where this distribution has been defined is the Klamath River (Stocking et al., 2006). We also confirmed the relationships between *C. shasta* abundance, temperature, and river discharge observed in the Klamath River and laboratory research (Bjork and Bartholomew, 2009; Ray et al., 2012; Alexander et al., 2014; Alexander et al., 2016). In general, *C. shasta* abundance in the Deschutes River increases with temperature (Figure 2.8) and decreases as discharge increases (Figure 2.9).

Each year we have studied in the Deschutes River has been characterized based on the last 10 years of data from USGS with high water temperatures and low to moderate river discharge. 2017 was an exceptional year with high river discharge due to the rain induced flow event in mid-March (Table 2.3). This relatively late and high flow event may have led to reduced *C. shasta* abundance in 2017, where average parasite density in the lower basin was 19x lower than 2016 or 2018 during predicted peak abundance in June (Table 2.4, 2.6; Figure 2.10).

Water temperatures in the Klamath River have a greater range (-5-26 °C) than the Deschutes River (4.9-22.2 °C). Despite the higher temperatures, *C. shasta* was 35x higher in the Deschutes River than in the Klamath River from 2016-2018. However, 2015 was an exceptional year where the Klamath River had a maximum of ~1200 spores/L compared to the 2015 Deschutes River maximum of 372 spores/L. The differences in *C. shasta* abundance between the two rivers may be related to the temperature range and salmonid species distributions. While the Deschutes River has salmonids in the system throughout the year, the Klamath River is void of trout and only has Coho salmon in the system year-round as well as fall Chinook salmon from calendar weeks 41-50. The Klamath River also had higher average maximum discharge (751 m^3/s) than the Deschutes River (275 m^3/s) from water years 2014-2017 (Bartholomew et al., 2017). In the Klamath River, winter-spring flushing flows have been shown to reduce polychaete aggregations (Malakauskas et al., 2013; Alexander et al., 2014, 2016) whereas emergency dilution flows are mandated at 5 spores/L (United States District Court for the Northern

District of California San Francisco Division, 2017) as this density has been quantified as the infective dose threshold for 40% mortality of Coho salmon of the Klamath River (Hallett et al., 2012). The infective dose (density of spores and genotypes) and infective dose threshold are different for different species (Ray et al., 2013). In order to implement emergency flows in the Deschutes River, these parameters would need to be defined through sentinel fish and water sample studies to determine the infective dose for spring Chinook salmon of the Deschutes River. Additionally, the highest *C. shasta* densities are at least 78 rkm downstream of the Reregulating Dam. This distance might affect the ability of flushing flows to remove polychaetes in the target region of rkm 48-82.

In the Klamath River *C. shasta* increases in spring of each year. Sampling of wild fish during spring, years of sentinel studies, and year-round water sample monitoring all inform the assumption that actinospores are released in late winter/early spring of each year in the Klamath River. Release timing of actinospores from polychaetes needs to be defined in the Deschutes River to identify when juvenile spring Chinook salmon are becoming infected. However, due to fish being in the system throughout the year in the Deschutes River, it is possible that infection of fish by actinospores and ingestion of myxospores by polychaetes are also occurring year-round. Therefore, polychaetes and spores may be capable of overwintering in the Deschutes River with moderate river temperatures, moderate discharge, and salmonid hosts in the system year-round relative to the Klamath River.

While there was temporal variability between sites and years, the *C. shasta* densities were an average of 1.5x higher between rkm 48-82 relative to other sites sampled during each spatial survey. Additionally, DRR (rkm 116) and DWS (rkm 155) had higher than average *C. shasta* abundance for 63% (5/8) surveys. Overall, spatial distribution of *C. shasta* in the lower basin did not vary between years (Figure 2.10, Table 2.6). *Ceratonova shasta* density at the monitoring sites informed us that the seasonality of *C. shasta* is highest in June/July and low the rest of the year; these data were supported by the spatial surveys. The monitoring sites also informed the spatial surveys by providing evidence that there was spatial variation between sites. Parasite density was higher (average 58.6 spores/L) at the Oak Springs monitoring site, which is

situated in the *C. shasta* hotspot between rkm 48-82, than the Pelton Trap (average 11.6 spores/L) upstream at rkm 160. Low *C. shasta* density was also observed at the three sites added between the Round Butte Dam and Big Falls on the Deschutes River arm (DRA), Crooked River (CRA), and in Lake Billy Chinook (MBC) during 2018. *Ceratonova shasta* was not detected at the site in Lake Billy Chinook and was only detected at low densities at CRA (2.4 spores/L in July) and DRA (2.3 spores/L in May). These results are consistent with the spatial distribution described by Ratliff (1983) where no *C. shasta* related mortalities were observed from fish exposed in Lake Billy Chinook.

Spatial data also supported temporal data in that high *C. shasta* abundance was observed in both data sets in years where there were low winter flow events and high river water temperatures, such as 2015 (Tables 2.3, 2.4). Peak discharge during our study years occurred in November/December of 2018 and 2015, respectively, whereas peak discharge was in mid-March in 2016 and 2017. Later peak discharge may cause a decrease in summer *C. shasta* abundance; while the late river discharge in 2016 was likely too low to dislodge polychaetes, the late and high flow event in early Spring of 2017 may have decreased *C. shasta* abundance by reducing the density of the polychaete hosts and *C. shasta* spores (Malakauskas et al., 2013; Alexander et al., 2014). 2016 and 2018 were moderate temperature and discharge years (Table 2.3), which is reflected by the moderate *C. shasta* abundance compared to other years with different abiotic parameters (Figures 2.4, 2.5).

Due to the high water temperatures of 2015, the days expected for *C. shasta* to complete its life cycle may have been decreased leading to a secondary influx of parasite DNA in August from the release of myxospores by salmonid hosts. Secondary parasite peaks were not observed during other years or at other sites.

The unequal variability of mean *C. shasta* abundance across the range of values of temperature and discharge was observed in residual plots of our linear mixed effect models. While we had strong correlations between our variables, the residual plot results indicate that additional data should be collected from monitoring sites over several years before a predictive model can be developed for the effects of water temperature and river discharge on *C. shasta* abundance in the Deschutes River basin.

Ceratonova shasta was observed in higher densities below the mouth of the Deschutes River than at or above the confluence with the Columbia River. However, the Deschutes River is not the sole contributor to *C. shasta* on the Columbia River (Sanders, 1967; Hoffmaster, 1985). Therefore, migrating juvenile Chinook salmon will continue to be exposed to the parasite after they leave the Deschutes River and adult spring Chinook salmon will be exposed before they reach the Deschutes River (Appendix A). Low abundance of *C. shasta* (< 1.5 spores/L) was detected in the Warm Springs River, which suggests that this tributary does not contribute this parasite to the Deschutes River. Therefore, salmon that succumb to *C. shasta*-related pre-spawn mortalities in the Warm Springs River likely contracted the infection during their time in the Columbia and Deschutes Rivers. The Warm Springs National Fish Hatchery is where most of the natural spawning occurs in the Deschutes River basin (Lindsay et al., 1989). Natural spawning also occurs at sites chosen for gravel augmentation by PGE from rkm 157 to 159 (Grant et al., 2016). However, few *C. shasta* spores (3.0 spores/L) were detected at these locations. These data contradict our spatial distribution hypothesis that high *C. shasta* abundance would be related to high myxospore input from adults perishing at spawning areas (Kent et al., 2014).

The upper basin above Big Falls exhibited opposite temporal dynamics from the lower basin with *C. shasta* abundance increasing in August compared to June. However, this did not occur in 2018 supporting our hypothesis that the upper basin does not have the same spatial parasite dynamics as the lower basin due to the physical separation by the Pelton-Round Butte dam complex. The separation of the basins contributes to the differences in salmonid host abundance, salmonid species distribution (Chapter 3), and water temperature. Water temperature was collected at each site during spatial surveys. On average, the upper basin was 2.65°C higher than the lower basin. The higher temperatures of the upper basin may partially explain the variance in spatial parasite dynamics given the positive correlation between water temperature and *C. shasta* abundance (Figure 2.8). Within years, the temperature threshold of 20 °C described by Bjork (2010) may explain the variable parasite density observed in 2018 in the upper basin. Additionally, the salmonid species abundance and distribution between the basins

may be related to *C. shasta* abundance and distribution. This assumption is combined with host genotype specificity and explored further in Chapter 3.

Acknowledgements

Ken Lujan (USFWS), Stacy Strickland (ODFW), and Graham Boostrom (CTWSRO) for collecting, filtering and sending us monitoring water samples every two weeks for three years; without their collaboration, this project would not have been possible. I would also like to thank the J. L. Fryer Aquatic Animal Health Laboratory for providing supplies for field surveys and fellow graduate students, Claire Howell, Benjamin Americus, and Laura Taggart-Murphy for participating in the sampling. Funding for this research was provided by The Confederated Tribes of the Warm Springs Reservation of Oregon-Pacific Coastal Salmon Recovery Fund and an Oregon Department of Fish and Wildlife Fish Health Graduate Research Fellowship in Microbiology.

REFERENCES

Alexander, J. D., S. L. Hallett, R. W. Stocking, L. Xue, and J. L. Bartholomew. 2014. Host and parasite populations after a ten year-flood: *Manayunkia speciosa* and *Ceratonov*a (syn *Ceratomyxa*) *shasta* in the Klamath River. Northwest Science 88(3):219–233.

Alexander, J. D., J. L. Bartholomew, K. A. Wright, N. A. Som, and N. J. Hetrick. 2016. Integrating models to predict distribution of the invertebrate host of myxosporean parasites. Freshwater Science 35(4):1263–1275.

Atkinson, S. D., S. L. Hallett, and J. L. Bartholomew. 2018. Genotyping of individual *Ceratonova shasta* (Cnidaria: Myxosporea) myxospores reveals intra-spore ITS-1 variation and invalidates the distinction of genotypes II and III. Parasitology 145(12):1588–1593.

Baker, C., and J. Lovtang. 2016. Monitoring wild populations of spring Chinook salmon (*Oncorhynchus tshawytscha*) and summer Steelhead (*O. mykiss*) in tributaries of the lower Deschutes River within the boundaries of the confederated tribes of the warm springs of Oregon reservation, Natural Production Monitoring 2013-2014 to

BPA, Confederated Tribes of the Warm Springs Reservation of Oregon Warm Springs, OR.

Bartholomew, J. L., C. E. Smith, J. S. Rohovec, and J. L. Fryer. 1989. Characterization of a host response to the myxosporean parasite, *Ceratomyxa shasta* (Noble), by histology, scanning electron microscopy and immunological techniques. Journal of Fish Diseases 12(5):509–522.

Bartholomew, J. L., M. J. Whipple, D. G. Stevens, and J. L. Fryer. 1997. The Life Cycle of *Ceratomyxa shasta*, a Myxosporean parasite of salmonids, requires a freshwater polychaete as an alternate host. The Journal of Parasitology 83(5):859–868.

Bartholomew, J. L., E. Ray, B. Torrell, M. J. Whipple, and J. R. Heidel. 2004. Monitoring *Ceratomyxa shasta* infection during a hatchery rearing cycle: comparison of molecular, serological, and histological methods. Diseases of Aquatic Organisms 62: 85-92.

Bartholomew, J., S. Hallett, R. Holt, J. Alexander, S. Atkinson, R. Craig, A. javaheri, M. Babbar-Sebens. 2017. Klamath river fish health studies: salmon disease monitoring and research. Oregon State University, BOR/USGS Interagency Agreement #R15PG00065. Annual Report.

Barton, B. A., C. B. Schreck, and L. A. Sigismondi. 1986. Multiple acute disturbances evoke cumulative physiological stress responses in juvenile Chinook salmon. Transactions of the American Fisheries Society 115(2):245–251.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67(1).

Bjork, S. J., and J. L. Bartholomew. 2009. The effects of water velocity on the *Ceratomyxa shasta* infectious cycle. Journal of fish diseases 32(2):131–142.

Bjork, S. J. 2010. Factors affecting the *Ceratomyxa shasta* infectious cycle and transmission between polychaete and salmonid hosts. Ph.D., Oregon State University, United States -- Oregon.

Bjork, S. J., and J. L. Bartholomew. 2010. Invasion of *Ceratomyxa shasta* (Myxozoa) and comparison of migration to the intestine between susceptible and resistant fish hosts. International Journal for Parasitology 40(9):1087–1095.

Conrad, J. F., and M. Decew. 1966. First Report of *Ceratomyxa* in juvenile salmonids in Oregon. The Progressive Fish-Culturist 28(4):238–238.

Gannett, M. W., M. Manga, and K. E. Lite. 2013. Groundwater hydrology of the Upper Deschutes basin and its influence on streamflow. Pages 31–49 *in* Water Science and Application. American Geophysical Union, Washington, D. C.

Grant, D. G., S. J. Way, C. Huntington, S. Cove, S. McBain, and M. Associates. 2016. Lower Deschutes River gravel study review. Portland General Electric 121 SW Salmon Street Portland, OR 97204:46.

Hallett, S. L., and J. L. Bartholomew. 2006. Application of a real-time PCR assay to detect and quantify the myxozoan parasite *Ceratomyxa shasta* in river water samples. Diseases of Aquatic Organisms 71(2):109–118.

Hallett, S. L., R. A. Ray, C. N. Hurst, R. A. Holt, G. R. Buckles, S. D. Atkinson, and J. L. Bartholomew. 2012. Density of the waterborne parasite *Ceratomyxa shasta* and its biological effects on salmon. Applied Environmental Microbiology 78(10): 3724-3731.

Heisler, T. (2012). Deschutes River Conservancy Deschutes water planning initiative:39.

Hoffmaster, J. L. 1985. Geographic distribution of *Ceratomyxa shasta* in the Columbia River basin and susceptibility of salmonid stocks. Oregon State University, Master’s Thesis.

Isaak, D. J., R. F. Thurow, B. E. Rieman, and J. B. Dunham. 2007. Chinook salmon use of spawning patches: Relative roles of habitat quality, size, and connectivity. Ecological Applications 17(2):352–364.

Kent, M. L., K. Soderlund, E. Thomann, C. B. Schreck, and T. J. Sharpton. 2014. Post-mortem sporulation of *Ceratomyxa shasta* (Myxozoa) after death in adult Chinook salmon. The Journal of Parasitology 100(5):679–683.

Lindsay, R. B., B. C. Jonasson, R. K. Schroeder, and B. C. Cates. 1989. Spring Chinook Salmon in the Deschutes River, Oregon. Oregon Department of Fish and Wildlife Information Report: 89-4.

Malakauskas, D. M., S. J. Willson, M. A. Wilzbach, and N. A. Som. 2013. Flow variation and substrate type affect dislodgement of the freshwater polychaete, *Manayunkia speciosa*. Freshwater Science 32(3):862–873.

Nakagawa, S., and H. Schielzeth. 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods in Ecology and Evolution 4(2):133–142.

Noble, E. R. 1950. On a myxosporidian (protozoan) parasite of California trout. The Journal of Parasitology 36(5):457–460.

Oregon Department of Fish and Wildlife (ODFW) and Confederated Tribes of the Warm Springs (CTWS). 2017. Hatchery and Genetic Management Plan.

Portland General Electric (PGE). N.d. Deschutes daily fish counts. https://www.portlandgeneral.com/corporate-responsibility/environmental-stewardship/water-quality-habitat-protection/fish-counts-fish-runs/deschutes-daily-fish-counts#

Portland General Electric (PGE) N.d. Restoring fish passage on the Deschutes. Informational pamphlet.

R Development Core Team. 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, http://www.R-project.org.

Ratliff, D. E. 1981. *Ceratomyxa shasta*: Epizootiology in Chinook Salmon of Central Oregon. Transactions of the American Fisheries Society 110(4):507–513.

Ratliff, D. E. 1983. *Ceratomyxa shasta*: Longevity, distribution, timing, and abundance of the infective stage in Central Oregon. Canadian Journal of Fisheries and Aquatic Sciences 40(10):1622–1632.

Ray, R. A., R. A. Holt, and J. L. Bartholomew. 2012. Relationship between temperature and *Ceratomyxa shasta*–induced mortality in Klamath River salmonids. Journal of Parasitology 98(3):520–526.

Ray, R. A., and J. L. Bartholomew. 2013. Estimation of transmission dynamics of the *Ceratomyxa shasta* actinospore to the salmonid host. Parasitology; Cambridge 140(7):907–16.

Sanders, J. E. 1967. Occurrence of the protozoan parasite *Ceratomyxa shasta* among salmonid fishes in Oregon waters.

Stinson, M. E. T., and J. L. Bartholomew. 2012. Predicted redistribution of *Ceratomyxa shasta* genotypes with salmonid passage in the Deschutes River, Oregon. Journal of Aquatic Animal Health 24(4):274–280.

Stocking, R. W., R. A. Holt, J. S. Foott, and J. L. Bartholomew. 2006. Spatial and temporal occurrence of the salmonid parasite *Ceratomyxa shasta* in the Oregon–California Klamath River basin. Journal of Aquatic Animal Health 18(3):194–202.

United States Department of Agriculture (USDA). Natural Resources Conservation Service of Oregon. https://www.nrcs.usda.gov/wps/portal/nrcs/detail/or/home/?cid=nrcs142p2_046290

Vojnovich, S., Hallett S., Atkinson S., Alexander J., Holt R., Craig R., and J. Bartholomew. 2016. Myxozoan disease risks in the Willamette and Deschutes Rivers, ODFW fellowship report 2015-2016. Oregon State University, Corvallis, OR.

CHAPTER 3: SPATIAL AND TEMPORAL DISTRIBUTION OF *CERATONOVA SHASTA* GENOTYPES IN THE DESCHUTES RIVER BASIN ARE LINKED TO SALMONID FISH DISTRIBUTION AND RUN TIMING

Kalyn Hubbard, Stephen Atkinson, Jerri Bartholomew, and Sascha Hallett

ABSTRACT

Ceratonova shasta is a waterborne, myxozoan parasite suspected to be a primary contributor to juvenile and pre-spawn mortality of the culturally, economically, and recreationally important spring Chinook salmon (*Oncorhynchus tshawytscha*) of the Deschutes River basin. There are three primary ITS-1 genotypes of the myxozoan parasite *Ceratonova shasta* (Cnidaria: Myxozoa), each of which infects and causes different levels of disease in particular species of salmonid fishes. Genotype O is associated with Steelhead trout, genotype I with Chinook salmon, and genotype II is a generalist variant that infects Steelhead and resident trout as well as Chinook salmon. While genotype specificity and salmonid host associations were previously described in the Deschutes River basin, we described the likely infection risk for salmonids of the Deschutes River by analyzing the co-presence of *C. shasta* abundance and genotypes with their target host species. Total *C. shasta* abundance (quantified with qPCR; Chapter 2) and specific genotypes (PCR and Sanger sequencing) in the Deschutes River, OR, were determined for 116 water samples and 15 salmonid lower intestines and kidneys in 2017 and 2018 (Chapter 3; Appendix A). As proposed by earlier work, the spatial and temporal distribution of genotypes was related to the spatiotemporal distribution of corresponding fish hosts. We determined type I dominated the lower Deschutes River basin below Round Butte Dam, type II was the main genotype present in the upper basin above Round Butte Dam, and type O was detected throughout the system at lower proportions than I or II. Type II was also identified in the lower basin, but only during August of both years. Presence of type II at that time may be related to the occurrence of Coho and/or Sockeye salmon in the lower basin, depending on the year. Despite Steelhead trout being the most abundant fish species, the associated genotype O was detected at the lowest proportion. Similarly, juvenile and adult Chinook salmon are one of the least abundant salmonid species yet genotype I was detected at the highest proportion. Therefore, the host-parasite imbalance described previously in the Deschutes River appears to be genotype specific.

INTRODUCTION

The Deschutes River (OR) begins at Little Lava Lake in the Cascade Range, flows through the Crane Prairie Reservoir, the Wickiup Reservoir, and then north to the Columbia River - roughly 405 river kilometers (rkm) from its starting point (Heisler, 2012). The Deschutes River is separated into upper and lower basins divided by the complex of Pelton, Round Butte, and Reregulating dams from rkm 160-165. In addition to modern dams, Big Falls at rkm 211 is a historical, natural migration barrier to salmonids in the upper Deschutes River basin (Figure 3.1).

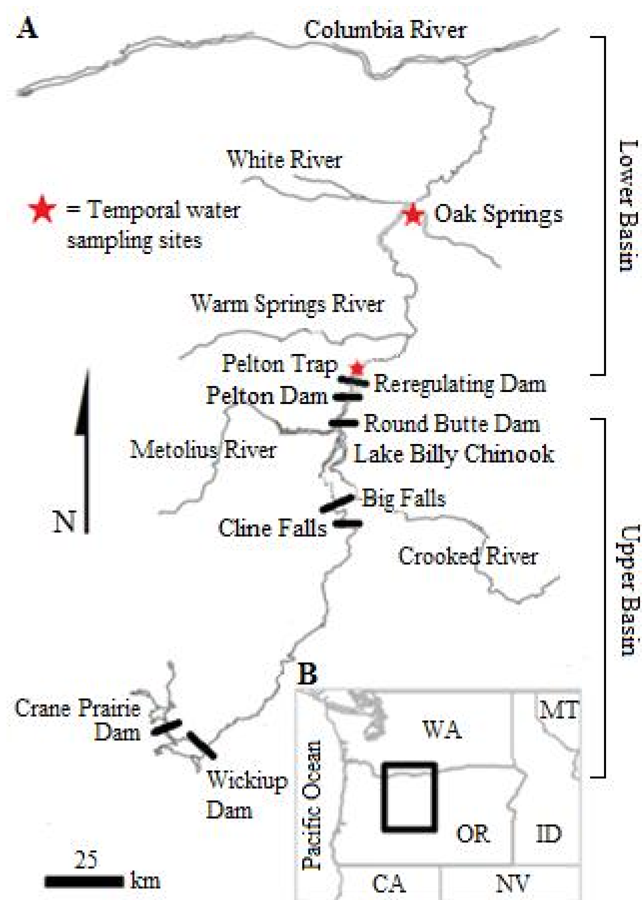

FIGURE 3.1: Map of the Deschutes River basin including major tributaries and temporal water sampling sites (A) with an insert of the Pacific Northwest in North America for context (B).

Spring and fall Chinook (*Oncorhynchus tshawytscha*), Sockeye (*Oncorhynchus nerka*), and non-native Coho (*Oncorhynchus kisutch*) salmon as well as Steelhead (*Oncorhynchus mykiss*) trout are found in the lower Deschutes basin. Upper basin reintroduction efforts of spring Chinook salmon and Steelhead trout began in 2007 and in 2009 for Sockeye salmon (ODFW and CTWS, 2017). Mountain Whitefish (*Prosopium williamsoni*) as well as Rainbow (*Oncorhynchus mykiss*), Cutthroat (*Oncorhynchus clarkia*), Brook (*Salvelinus fontinalis*), Bull (*Salvelinus confluentus*) and Brown (*Salmo trutta*) trout are located in both basins whereas Kokanee salmon (*Oncorhynchus nerka*) and Lake Trout (*Salvelinus namaycush*) are restricted to Lake Billy Chinook above the Round Butte Dam. These fish can all be infected by the myxozoan parasite *Ceratonova shasta* (Conrad

and Decew, 1966; Gould, 1970; Ratliff, 1981; Hoffmaster, 1985; Stinson and Bartholomew, 2012).

Ceratonova shasta has been implicated as a contributing factor to years of poor salmonid returns in the Deschutes River due to juvenile and pre-spawn mortality (Ratliff, 1983; Baker and Lovtang, 2016). The parasite has a complex, two host, two spore life cycle and cannot be transmitted from fish to fish. Parasite actinospores proliferate in a freshwater polychaete and are shed into the water column, where they encounter the salmonid secondary host (Bartholomew et al., 1997). Actinospores penetrate the gill epithelium and travel through the blood to the intestine, where they develop into myxospores potentially causing enteronecrosis and death of the host; severity of disease depends on fish species, life stage, parasite dose and other stressors, including pre-spawning condition (Noble, 1950; Bjork and Bartholomew, 2010).

The parasite has been characterized into three primary genotypes (types), each of which have different host specificity and virulence in particular species of salmonids (Atkinson and Bartholomew, 2010a; Hurst and Bartholomew, 2012; Atkinson et al., 2018). In the Deschutes River, type O is carried by Steelhead and trout, type I infects Chinook salmon, and type II is a generalist genotype that infects Steelhead, rainbow, cutthroat, brook and brown trout, and Coho, kokanee and Chinook salmon (Stinson and Bartholomew, 2012).

Studies on *C. shasta* in the Deschutes River basin have been conducted since the early 1970s to define the fine-scale spatial and temporal abundance of the parasite, and more recently its genotype profile. Some of these studies involved caged ‘sentinel’ fish surveys at up to three locations in the upper and lower basins (Ratliff, 1981; Ratliff, 1983; Hoffmaster, 1985; Stinson and Bartholomew, 2012; Vojnovich et al., 2016). Genetic analyses of abundance and genotype of *C. shasta* in river water samples showed type I as the primary genotype in the lower Deschutes basin, which was confirmed by infection of caged juvenile spring Chinook salmon (Stinson and Bartholomew, 2012). Type I was not detected in upper basin water samples or by sentinel exposures of Chinook salmon. This suggested that spring Chinook salmon were at an increased infection risk only in the lower basin. The studies conducted by Stinson and

Bartholomew, 2012 also provide evidence that type II can develop in kokanee salmon. While the genotype of *C. shasta* was sequenced from the lower intestines of eight salmonid species (Stinson and Bartholomew, 2012), Sockeye and Coho salmon were not available at that time.

We hypothesized that *C. shasta* genotypes have an abundance and distribution similar to that of their respective salmonid hosts, as has been shown in the Klamath River (Atkinson and Bartholomew, 2010b; Hurst and Bartholomew, 2012). We also wanted to test the hypothesis proposed by Stinson and Bartholomew (2012) that type I would become established in the upper basin with the reintroduction of spring Chinook salmon. While Stinson and Bartholomew (2012) examined the distribution of *C. shasta* genotypes through sentinel and water sampling surveys in the lower Deschutes and Crooked Rivers, the goal of this study was to provide detailed temporal and spatial distribution information at many sites along the Deschutes River. Building upon the work done in 2012, we predicted that type I would be dominant in the lower basin; type II would be dominant in the upper basin where resident kokanee, anadromous Sockeye, and spring Chinook salmon passed above the dam might become infected; and type O should be detectable throughout the system associated with infections in Steelhead, in both the upper and lower basins. We tested this hypothesis by determining the genotype of parasite DNA from both water samples and fish tissues. We collected water samples at 28 sites over three years, and DNA extracted fish tissue samples were provided from USFWS collaborator (Ken Lujan, Lower Columbia River Fish Health Center). These samples were then analyzed for parasite DNA with PCR (fish tissue), qPCR (water samples), and Sanger sequencing. We used these results in combination with fish passage data from the Round Butte Dam, which separates the upper and lower basins, to test our hypothesis of parasite genotype and host presence.

METHODS

Fish tissues

67 DNA extracted and *C. shasta* positive (PCR) lower intestine samples from Coho salmon were collected opportunistically from 2014-2018 and sent to us by Ken Lujan (USFWS). We genotyped the parasite from 10 lower intestines of Coho salmon using a specific PCR assay and DNA sequencing (see below).

Fish passage data

Portland General Electric (PGE) provides total in-migrating adult and out-migrating juvenile fish per day at the Pelton Trap below the Round Butte Dam through their daily fish count monitoring webpage (Portland General Electric, n.d.). Species of fish enumerated include summer Steelhead, spring and fall Chinook salmon, Sockeye salmon, bull trout, and rainbow trout. We calculated sums of anadromous fish per day per species and plotted these over time (Figure 3.6).

Coho salmon stray from the Columbia River and while they are found in the Deschutes River, they are not native to this system and are therefore not enumerated. However, Coho salmon passed above the Dalles Dam are quantified each year and this data is provided by the Columbia River Data Access in Real Time (DART) program (University of Washington, n.d.).

Juvenile fish passage data was only available in 2017 and 2018. In an effort to have the most consistency between juvenile and adult fish passage data and lower Deschutes River genotypes we used only the site at the mouth of the Warm Springs River on the Deschutes River for comparison.

Water samples

Spatial and temporal surveys of waterborne *C. shasta* abundance were conducted at multiple localities in 2016, 2017 and 2018 (Table 3.1; Figure 3.2; Chapter 2). Sites were sub-selected for genotyping of water samples based on regions of high *C. shasta* density (rkm 48-82, DRR, DRV) as well as temporal survey sites including Oak Springs (DOS), Pelton Trap (DPT), and the mouth of the Warm Springs River (DRV). Sites were also selected from the mouth of the Deschutes (DHP), between the Round Butte Dam and Big Falls (DRA, CRA), and the upper basin (DCF) to cover all regions of the study area. Sites with genotyped water samples are marked in gray in Table 3.1 and Figure 3.2.

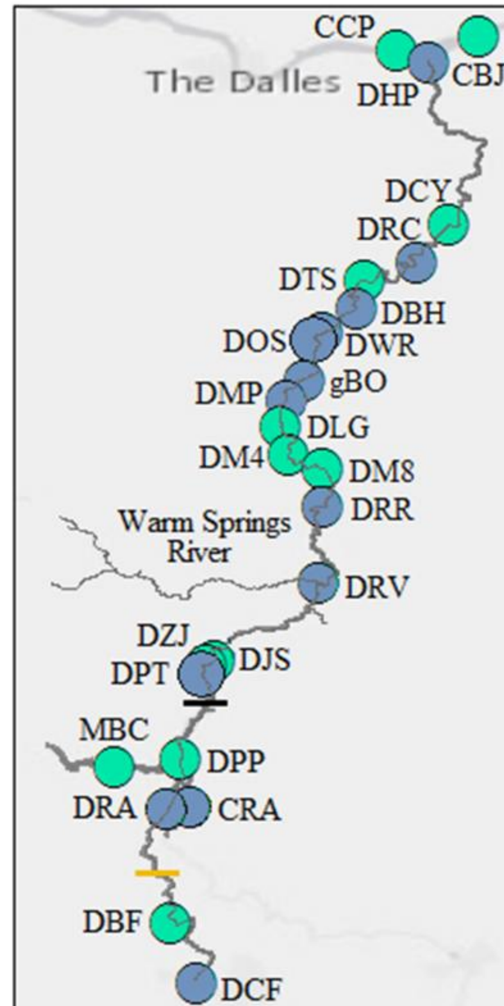

FIGURE 3.2: Spatial and temporal water sampling sites on the Deschutes River. Sites in dark blue were genotyped for *Ceratonova shasta* from 2016-2018.

TABLE 3.1: 2016-2018 Deschutes River basin water sample localities, including longitudinal surveys, periodic monitoring sites and opportunistic ‘grab’ samples. River kilometers and latitude/longitude coordinates are given for each locality (see also Chapter 2). Sites selected for parasite genotype analysis are marked in gray and correspond to dark blue sites in Figure 3.2.

Locality (site code)	*River kilometer*	*Coordinates*
CCP	Columbia R – 324	45°39'02.40"N, 120°57'36.30"W
CBJ	Columbia R – 335	45°40'15.20"N, 120°50'16.90"W
DHP	0.6	45°37'55.60"N, 120°54'47.20"W
DCY	37.9	45°23'32.70"N, 120°52'51.10"W
DRC	47.7	45°20'11.20"N, 120°55'51.30"W
DTS	60.3	45°18'32.80"N, 121°00'20.00"W
DBH	67.5	45°16'03.01"N, 121°01'08.59"W
DWR	73.7	45°14'01.29"N, 121°04'01.49"W
Oak Springs (DOS)	75.5	45°13'17.71"N, 121°04'49.26"W
DMP	81.8	45°10'24.10"N, 121°04'28.60"W
gBO	81.7	45°10'26.10"N, 121°04'27.16"W
DLG	94.0	45°05'33.40"N, 121°07'49.10"W
DM4	100.5	45°03'06.81"N, 121°07'12.40"W
DM8	107.9	45°01'54.70"N, 121°04'06.05"W
DRR	115.8	44°58'26.00"N, 121°04'11.00"W
Warm Springs R. mouth (DRV)	133.7	44°51'26.57"N, 121°04'04.50"W
DJS	157.7	44°44'22.80"N, 121°14'27.34"W
DZJ	159.3	44°43'44.58"N, 121°14'46.32"W
Pelton Trap (DPT)	159.6	44°43'32.50"N, 121°14'51.10"W
DPP	165.1	44°41'06.12"N, 121°14'22.25"W
MBC	182.6	44°35'16.21"N, 121°22'32.49"W
CRA	182.9	44°31'43.98"N, 121°15'49.37"W
DRA	186.4	44°31'31.95"N, 121°17'56.51"W
DBF	211.2	44°21'34.40"N, 121°17'39.00"W
DCF	229.6	44°16'07.60"N, 121°15'24.80"W

Genotype determination

After quantitation, parasite genotype was determined with one sample from each set of three. We determined the *C. shasta* genotype from DNA extracted from both water and tissue samples (complete sample list given in Appendix A). We used a published PCR assay and sequencing protocol (Atkinson et al., 2018). Briefly, primers Cs1479F and Cs2067R were used to target the parasite’s ITS-1 rDNA, with Titanium Taq

(Clontech) and modified PCR thermocycling program: 95 °C for 3 min, 95 °C for 20 sec, 68 °C for 45 sec. We estimated DNA yield based on the brightness of band fluorescence in gel electrophoresis, and used simple dilution of this product for sequencing. Samples were Sanger sequenced with forward primer Cs1479F at the Center for Genome Research and Biocomputing (CGRB) at Oregon State University. Sequence chromatograms were inspected by eye and characterized for genotype based on insertion/deletion patterns and any polymorphisms using BioEdit (Hall, 1999; Atkinson and Bartholomew, 2010a). Genotypes O, I or II were determined based on the number of ATC repeats (Atkinson and Bartholomew, 2010a). Proportions of different genotypes in mixed chromatograms were calculated by measuring the average heights of the component co-incident peaks over 5-10 positions, adding these together to determine the total area under the curve, then dividing the average height of each component by this total to give the respective genotype proportions in the sample. Chromatograms with three or more coincident peaks were regarded as unreadable.

RESULTS

Fish tissues

All Coho salmon lower intestines sequenced for *C. shasta* were genotype II (Table 3.2).

TABLE 3.2: Summary of Deschutes River fish tissues genotyped for *Ceratonova shasta*

Species	*Collection Date*	*Location*	*Life stage*	*Condition*	*Tissue*	*Samples genotyped*	*Genotype*
Coho salmon	10/21/15	Warm Springs R	Wild Adult	Survey Capture	Lower Intestine	3/5	Type II
Coho salmon	12/08/15	Buckhollow Creek	Hatchery Adult	Survey Capture	Lower Intestine	2/5	Type II
Coho salmon	12/10/15	Buckhollow Creek	Hatchery Adult	Survey Capture	Lower Intestine	1/1	Type II
Coho salmon	11/08/17	Warm Springs R	Wild Adult	Survey Capture	Lower Intestine	1/1	Type II
Coho salmon	11/14/17	Warm Springs R	Wild Adult	Survey Capture	Lower Intestine	3/8	Type II

Water samples (Figures 3.3, 3.4, 3.5, 3.7)

Our hypotheses for this chapter were that *C. shasta* genotype abundance and corresponding salmonid host abundance would be correlated. We also explored the hypotheses from Stinson and Bartholomew (2012) that there would be a correlation between the spatiotemporal distribution of genotypes and salmonid hosts with type I in the upper basin (between the Round Butte Dam and DBF; Figure 3.2) with the reintroduction of Chinook salmon. Using fish passage data provided by PGE (Table 3.5) in combination with genotyped fish tissue data from Stinson and Bartholomew (2012), the expected results of these hypotheses are displayed graphically in Figure 3.3. This figure does not account for resident trout populations (which carry type O and II) as they have not been quantified in the Deschutes River.

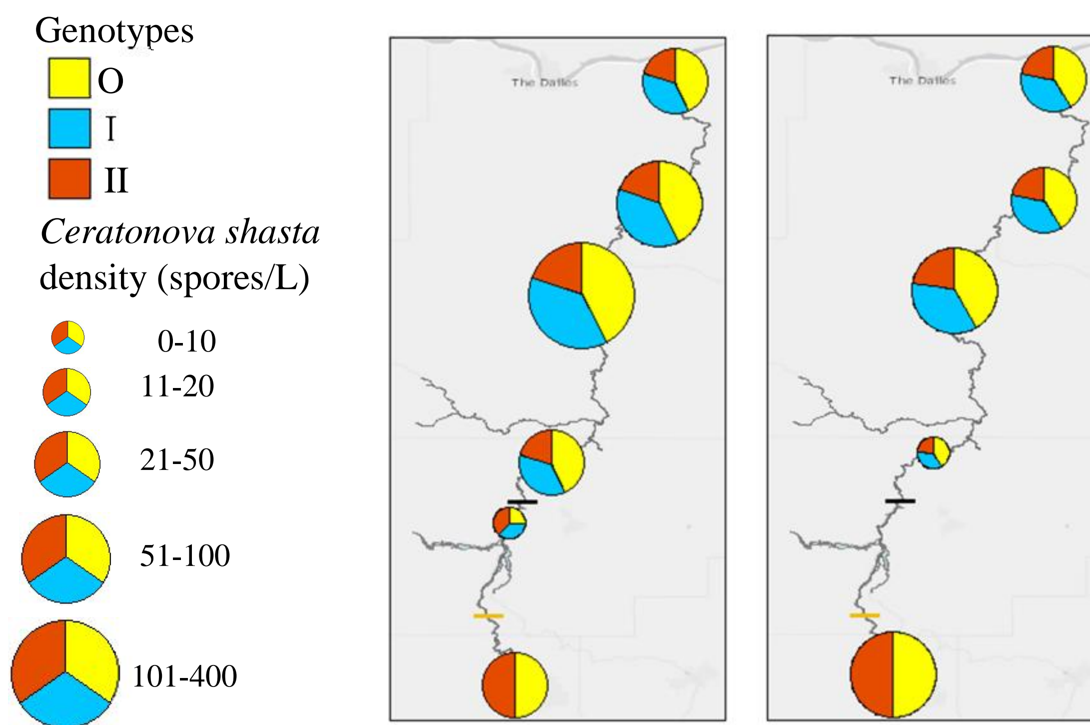

FIGURE 3.3: Expected distribution and density of the salmonid parasite *Ceratonova shasta* in the upper and lower Deschutes River Basin if all proposed hypotheses are supported. The black bar represents the Round Butte Dam complex, a migration barrier for anadromous fish. The relative area of each pie graph indicates the density of *Ceratonova shasta* in 1L water samples whereas the color in each graph represents the genotype proportions at each site.

We detected the three primary *C. shasta* genotypes in water samples from the lower Deschutes River basin, but only types O and II in the upper basin. Independent of time, the lower Deschutes River was dominated by genotype I and the upper basin was dominated by genotype II. Genotype O was detected throughout the system at lower proportion than either type I or II.

We observed a different genotype pattern in August: while the dominant genotypes in the upper and lower basins were still type II and I, respectively, the proportion of type O increased in the lower basin but decreased in the upper basin, and type II was observed in the lower basin in August only. When type II was detected in the lower basin, the proportion of type II in the water sample was low (3.8 spores/L in August 2017 to 10.6 spores/L in August 2018) relative to other genotypes in samples from the lower basin during the same survey (e.g. type I proportion was 5.4-22 spores/L in August 2017; 23-90.7 spores/L in August 2018).

There were often multiple genotypes present in water samples, visible as either polymorphic loci or offset, overlapping sequence traces. In practice, the minimum height proportion for resolving a genotype from a mixture was around 10%. Noise in chromatograms was around 5% and peaks below this threshold were not considered reliable, as outlined in Atkinson and Bartholomew, 2010a. Chromatograms of 10.4% (11/105) of the water samples spread across both basins, had three overlapping signals and were not readable. We observed that one of these complicating signals was from a novel *C. shasta*-like sequence that could not be ascribed to O, I, or II; and that this unknown signal was different in upper and lower basin samples.

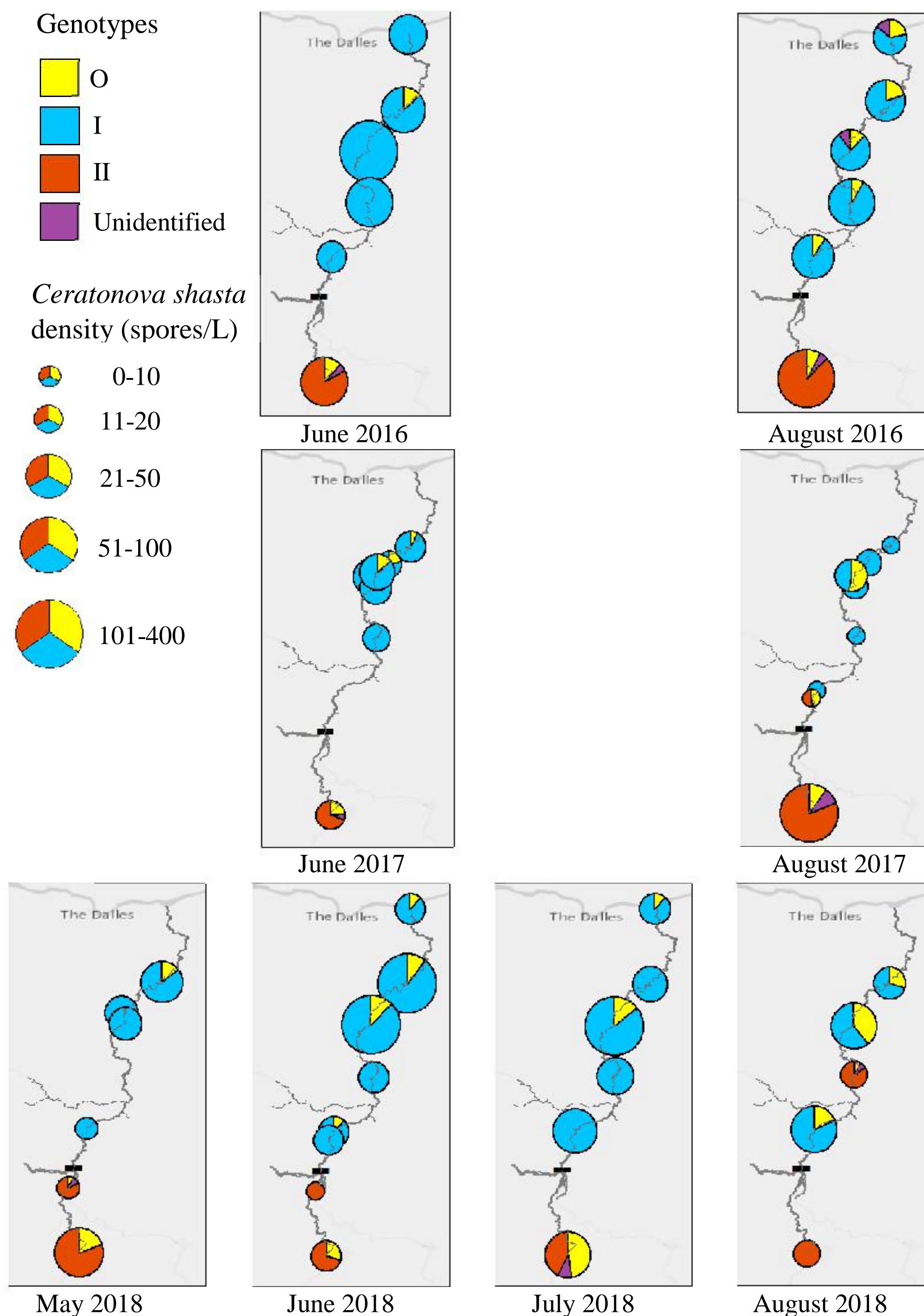

FIGURE 3.4: Distribution and density of the salmonid parasite *Ceratonova shasta* in the upper and lower Deschutes River Basin. The black bar represents the Round Butte Dam complex, a migration barrier for anadromous fish. The relative area of each pie graph indicates the density of *Ceratonova shasta* in 1L water samples whereas the color in each graph represents the genotype proportions at each site.

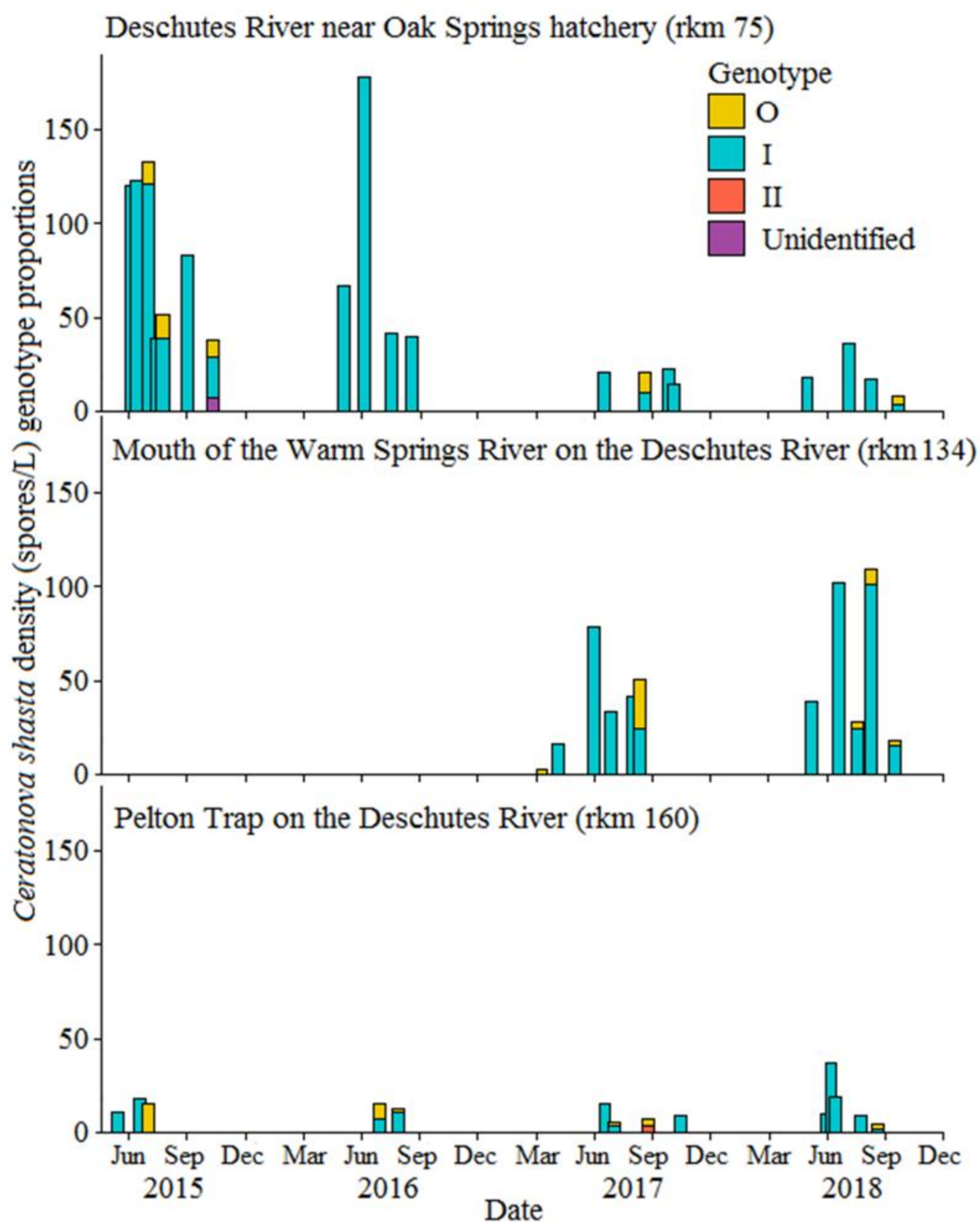

FIGURE 3.5: Temporal abundance of *Ceratonova shasta* in water samples collected from lower basin sites at Deschutes River near Oak Springs Hatchery at rkm 75 (top), the Warm Springs mouth on the Deschutes River at rkm 134 (middle), and the Pelton Trap at rkm 160 (bottom). Bars show total *Ceratonova shasta* detected (spores/L) (y-axis) against sample date (x-axis), with subdivisions to show genotype proportions.

Fish passage

According to data from PGE, returning adult migration differs between species. Summer Steelhead return to the Pelton Trap year-round with peak returns in early February. Spring Chinook salmon return from May to September with peak in-migration in early July. Fall Chinook salmon return from August to late February and peak in late November. Sockeye salmon in-migrate from July to October with a peak in mid-August. Release of juvenile spring Chinook salmon, Sockeye salmon, and Steelhead trout occurs from March to mid-June with peak releases in May. Coho salmon stray from the Columbia River in to the Deschutes River. On average, they pass through the Dalles Dam from late August to late November each year. Fish passage timing at the Pelton Trap (Table 3.3) and the Dalles Dam (Table 3.4) is summarized below and represented graphically (Figure 3.6).

TABLE 3.3: Summary of fish passage timing at the selective water withdrawal tower (juveniles) and Pelton Trap (adults) by species and life stage (PGE) for 2017 and 2018.

Species	*Life stage*	*Average migration start date*	*Average peak migration date*	*Average migration end date*
Spring Chinook salmon	Juvenile	March 1st	May 19th	June 15th
Summer Steelhead	Juvenile	March 2nd	May 24th	June 15th
Sockeye salmon	Juvenile	March 3rd	May 6th	June 14th
Spring Chinook salmon	Adult	May 7th	July 5th	September 17th
Summer Steelhead	Adult	Year-round	February 5th	Year-round
Sockeye salmon	Adult	July 17th	August 20th	October 5th
Fall Chinook salmon	Adult	August 1st	November 30th	February 26th

TABLE 3.4 Summary of adult fish passage timing at the Dalles Dam for 2017 and 2018.

Species	*Average migration start date*	*Average peak migration date*	*Average migration end date*
Chinook salmon	April 7th	September 3rd	December 1st
Steelhead	July 17th	September 15th	March 15th
Sockeye salmon	May 28th	June 27th	October 9th
Coho salmon	August 25th	September 19th	November 27th

TABLE 3.5: Summary of fish abundance enumerated at the Pelton Trap (PGE) by species and year.

Species	*Life stage*	*2015*	*2016*	*2017*	*2018*
Summer Steelhead trout	Adults	3199	2457	2292	1873
Summer Steelhead trout	Juveniles			8100	8864
Spring Chinook salmon	Adults	1138	841	2620	1003
Spring Chinook salmon	Juveniles			26724	19408
Sockeye salmon	Adults	36	527	57	48
Sockeye salmon	Juveniles			419747	46379
Fall Chinook salmon	Adults	1383	778	196	479

Summer Steelhead make up the bulk of anadromous salmonid abundance in the Deschutes River followed by spring Chinook, fall Chinook, and then Sockeye salmon (Table 3.5). However, anecdotal reports suggest resident species of trout may dominate the lower Deschutes River (John Shearholtz, local fly fisherman, pers. comm.). Despite juvenile Sockeye salmon releases from the Round Butte Hatchery between 46,000 - 420,000, adult returns were only 36-57 fish per year with an exceptional return year in 2016 of 527 fish. Fall Chinook salmon abundance declined overall for the years observed.

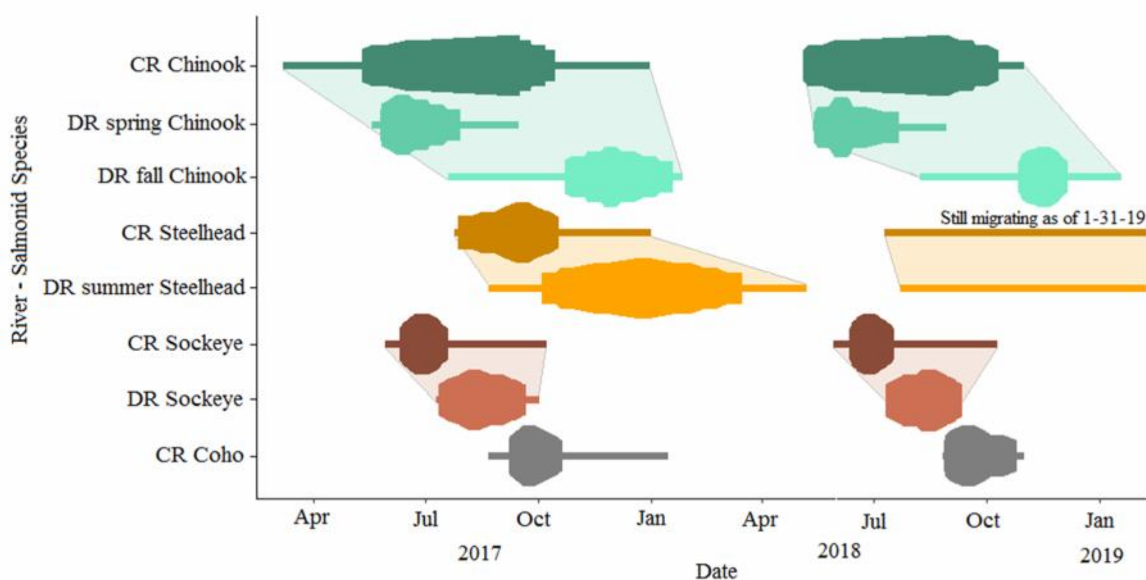

FIGURE 3.6: Fish passage at the Dalles Dam on the Columbia River (CR) and the Pelton Trap on the Deschutes River (DR) by salmonid species. Shaded areas are estimates of when each species of salmonid is present in the lower Deschutes River.

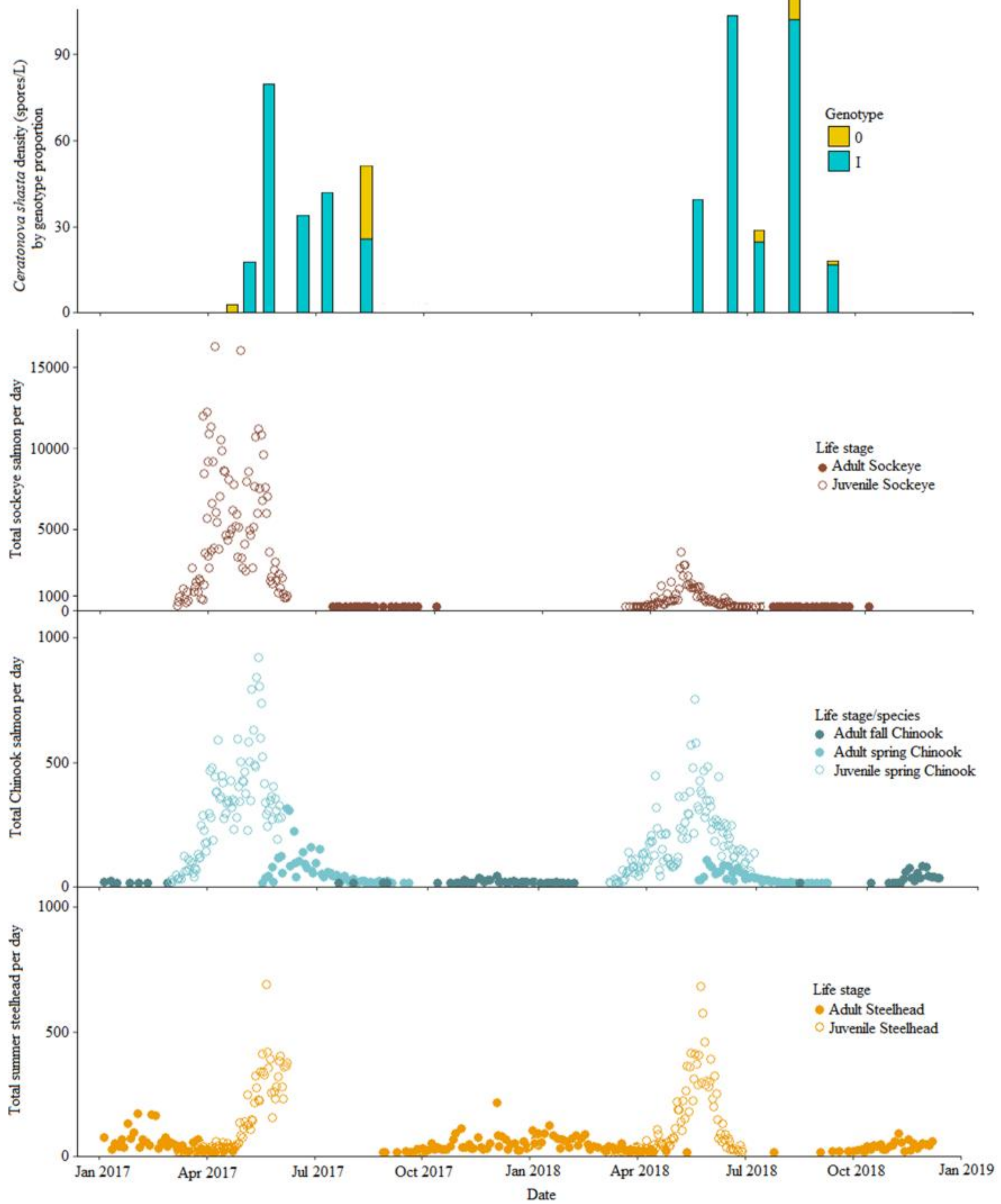

FIGURE 3.7: Abundance and genotypes of the salmonid parasite *Ceratonova shasta* at the confluence of the Warm Springs and Deschutes Rivers (DRV) plotted against abundance of its salmonid hosts at the Round Butte Hatchery (PGE) in 2017 and 2018. From top to bottom: *Ceratonova shasta* density (spores/L) showing genotype proportions within bars, abundances of Sockeye salmon in red, Chinook salmon in blue, and summer Steelhead in light orange.

DISCUSSION

We determined *C. shasta* abundance in 184 fish (Appendix A) and 1084 water samples from the Deschutes River basin, and the parasite genotype from subsets of 15 fish (Table 3.2; Appendix A) and 116 water samples. These data confirmed relationships of parasite genotypes with specific fish hosts (Hurst and Bartholomew, 2012; Stinson and Bartholomew, 2012; Stinson et al., 2018). Genotype O was detected at low proportion in water samples throughout the Deschutes River Basin. Genotype I was detected only in the lower basin, and in Chinook salmon. The distribution of genotype II was limited to the upper basin, with the exception of low abundances (3.8 to 10.6 spores/L) detected in the lower basin in August only. We compared these parasite genotype and abundance data with fish passage data, and as predicted, the presence of the parasite genotypes in the water samples were consistent with the presence of the specific host fish corresponding to each genotype; and this was true in different places/times within the basin. Specifically, genotype I was detected in the lower basin during juvenile and adult spring Chinook migration, with type II detected in the upper basin, to which resident kokanee and trout might be contributing. Temporal data also revealed genotype II in the lower basin in August during adult Sockeye in-migration.

Our research provided higher spatial and temporal resolution to the patterns of *C. shasta* genotype, basin locality and time of year seen previously (Ratliff, 1981; Hoffmaster, 1985; Stinson and Bartholomew, 2012). We combined fish passage data with our temporal and spatial water data to develop a representation of *C. shasta* genotypes and their salmonid hosts in the Deschutes River basin.

Genotype O and its primary host O. mykiss (Steelhead trout) — Juvenile Steelhead out-migration in the Deschutes River occurred from March to July and overlaps with total peak *C. shasta* abundance in the lower basin (Figure 3.7) and suggested that these juveniles are either exposed to or contributing to the parasite abundance during this migration. While Steelhead and trout are infected by genotype O, this type of *C. shasta* is not known to cause disease in this species (Stinson and Bartholomew, 2012; Hurst and Bartholomew, 2012; Stinson et al., 2018). However, both resident and anadromous forms of this species are considered carriers of this genotype and this might explain the

presence of genotype O throughout the upper and lower basins at different times. Summer Steelhead are the most abundant anadromous salmonid in the Deschutes River. Stinson (2012) hypothesized that the spatial and temporal distribution of salmonid hosts would be related to the spatiotemporal distribution of the corresponding genotype. While the distribution of type O and summer Steelhead appear to coincide, the abundance of type O was lower than expected given the abundance of this host species. This supports Stinson's hypothesis that spatial distribution would be correlated, but our hypothesis that the abundance of genotypes would correspond with abundance of fish hosts was not supported.

However, this led us to a new hypothesis that modifies Ratliff's (1981) proposal that there is a parasite-host imbalance favoring *C. shasta*. I propose that the parasite-host imbalance is genotype specific. In the Deschutes river, genotype O appears to be in balance with few observed effects on Steelhead and trout health or abundance (Table 3.5; Stinson and Bartholomew, 2012). However, genotype I has been found in high densities (>150 spores/L, Figure 3.5) and may contribute to the decrease in spring Chinook salmon abundance in the Deschutes River (Table 3.5; Appendix A).

Genotype I and its primary host O. tshawytscha (Chinook salmon) — Lower intestines of Deschutes River sentinel juvenile salmonids were genotyped in 2012 by Stinson and Bartholomew, which confirmed *C. shasta* genotype host specificity observed in the Klamath River (Atkinson and Bartholomew, 2010b; Hurst and Bartholomew, 2012). We observed a high abundance (~80-90 spores/L) of waterborne *C. shasta* type I in the lower basin during peak juvenile spring Chinook salmon out-migration in 2017 and 2018 (Figure 3.6) and *C. shasta* type I was detected in the 58% of the lower intestine samples of this species and life stage a day before their release from the Pelton Ladder on April 4th, 2018 (Appendix A). *C. shasta* type I densities were also high during peak in-migration of adult spring Chinook salmon (Figure 3.7), with type I detected in kidneys and lower intestines in 2017 and 2018 (Appendix A).

The hypothesis proposed by Stinson and Bartholomew (2012) that genotype I would become established in the upper basin due to reintroduction of spring Chinook

salmon was not supported (Figure 3.3, 3.4). This conclusion was surprising as the distribution of genotypes coincided with the presence of salmonid hosts in all other reaches. Since 100% of the adult hatchery spring Chinook salmon lower intestines sampled in 2017 and 2018 were positive for *C. shasta*, we would expect that fish passed above the Round Butte Dam would also be infected and contributing myxospores. The area between the Round Butte Dam (rkm 165) and the natural migration barrier, Big Falls (rkm 211), is where we would expect to see reintroduced spring Chinook salmon and genotype I. Surprisingly, this is a region where low total *C. shasta* abundance was detected (< 3 spores/L). It is possible that the specific locations that we sampled on Lake Billy Chinook (MBC), Deschutes River Arm (DRA), and Crooked River (CRA) are not representative of parasite abundance in this reach. Particularly for the Lake Billy Chinook site, where the depth and volume may also contribute to underestimation of the parasite due to sinking myxospores and dilution of both spore types, respectively. I propose that *C. shasta* has not become established at the sites we sampled and therefore neither has genotype I. Moving forward, sites should be added further upstream on the Metolius, Crooked, and Deschutes Rivers at published spawning locations to deduce the presence of genotype I in the upper basin between the Round Butte Dam and Big Falls.

In the lower basin, we propose that the coincidence of spring Chinook salmon and high genotype I spore levels in water, leads to increased infection risk for juvenile and adult spring Chinook salmon. It is exactly this concern that has led to management actions in the Klamath River, CA/OR. Studies in that system showed that the infective dose that corresponds to a 40% mortality threshold for fall Chinook in the Klamath River is 10 spores/L of genotype I (Hallett et al., 2012; United States District Court for the Northern District of California San Francisco Division, 2017).

Genotype II and its multiple host species —Kokanee salmon are likely to be infected and affected by and contributing *C. shasta* in the upper basin (Stinson and Bartholomew, 2012; Figure 3.4). While anadromous Deschutes River Sockeye salmon have not been assessed for *C. shasta* genotype, we assume that they are infected by genotype II, which has been shown for Kokanee. If this is true, adult in-migrating Sockeye salmon are likely to be infected by genotype II in the lower basin during August (Figure 3.4, 3.5). Given

there are currently efforts to re-introduce Sockeye into the upper basin and the concern for low populations of migrating Sockeye salmon despite reintroduction (Deschutes River Conservancy, 2018), we propose that Sockeye salmon tissues should be sampled in 2019 to confirm which *C. shasta* genotype can infect this species.

Given our single detection of genotype II in the lower basin, we considered several possible sources and reasons for this detection – both biological and related to our methodology. We sequenced lower intestines from ten infected Coho salmon and detected genotype II in all ten fish. While there are no published records of Coho salmon run numbers or timing in the Deschutes River, adult Coho salmon reached The Dalles Dam below the mouth of the Deschutes River on August 20th, 2017 and August 30th, 2018 (University of Washington, N.d.). Given that Coho salmon are only detected passing the Dalles Dam in mid-late August (Figure 3.6), they are unlikely to contribute any type II signal to the August Deschutes water samples (Figures 3.4, 3.5). In-migrating Sockeye salmon are more likely to be contributing to the presence of type II in the lower basin during August, as they are present in the system from mid-July to early October (Figures 3.4-3.7; Tables 3.5, 3.6).

Alternatively, underlying parasite biology might be a factor in the lower basin, with *C. shasta* production partially dependent on the relationship between temperature and parasite genotype due to coevolution with salmonids that migrate at different times of the year (Stinson et al., 2018). This hypothesis could be tested in the laboratory by quantifying actinospore production from polychaetes infected with genotype O, I, and II at different temperatures.

An alternate hypothesis that we considered is that the increase in genotype II in August was due to a general decrease in *C. shasta* genotype I contribution from adult Chinook salmon, which fish passage data show were present in lower relative abundance to other salmonids in August (Figure 3.7; Table 3.5); this decrease in genotype I might have ‘unmasked’ the genotype II signal in our sequence chromatogram data. It is possible that genotype II was present in water samples from many times of the year, but that it was not visible on the chromatogram because it was covered by a higher density of genotype I. However, we did not observe genotype II at other times of the year when *C. shasta*

abundance was low (Figures 3.4, 3.5, 3.7). This could be tested by assaying water samples with a genotype-II specific assay that is unaffected by the level of genotype I. This assay is under development in our laboratory and could be used in 2019 (Atkinson pers. comm.).

The presence of multiple genotypes in samples was a more general problem – 10.4% (11/105) of water samples genotyped had at least three genotypes present, which made visual delineation between types impossible with our current methods. We suggest several possible approaches for more accurately determining *C. shasta* genotypes in these complex environmental samples. DNA could be amplified using novel genotype-specific PCR primers, although this would require that we correctly understand the range of genetic variation within each "genotype" so that primers accurately detect each one. Alternatively, we could use different detection technologies with the current primers to resolve components of complex genotype mixtures. For example, we could use cloning to generate multiple amplicons from the PCR products, and therefore sequence multiple reads from the sample. Commercial kits exist for this process, for example, the Invitrogen™ TOPO™ TA Cloning™ Kit with pCR™ 4-TOPO™ Vector and One Shot™ TOP10 chemically competent *E. coli* cells, with post cloning purification using a PureLink™ Quick Plasmid Miniprep Kit before sequencing. This approach would create separate colonies that contain only a single genotype, which could then be extracted and sequenced. However, there would be a proportional increase in labor and sequencing costs with this method, and we suggest that a subset of mixed samples be trialed using this method to assess whether the extra information obtained is worth the effort, and if it would inform the higher questions of the study.

An alternate method for determining components of complex parasite metapopulations is to use a MiSeq or HiSeq approach to sequence thousands to millions of parasite sequences from the environmental sample. While this would save time, it would cost several thousand dollars per run, although samples could be multiplexed and cost some fraction of this individually. This deep sequencing would help us describe the unidentified genotype and complex genotype mixtures, but the specificity of this technique might also identify many more genotypes, which could complicate

interpretation. Until more subtle patterns of sub-genotypes and host infections are determined (if they exist), we consider it sufficient to bin sequences by types O, I, II, and hence direct sequencing is effective, with a 10% loss of unreadable assays using current methods.

Conclusions

Our spatial and temporal data supported our hypothesis that *C. shasta* genotype distribution is mediated primarily by the presence of specific fish hosts: We showed that genotype I was dominant in the lower Deschutes River basin, where there are high numbers of spring Chinook salmon, and genotype II was dominant in the upper basin where resident kokanee and trout predominate. These results are consistent with the host-specific relationships described by Stinson and Bartholomew (2012) in the Deschutes River. However, the 2012 hypothesis that genotype I would move into the upper basin with the passage of spring Chinook salmon above the Round Butte Dam (since 2007) is not supported by our water sample data. We identified genotype O in both upper and lower basins, which corresponded to the presence of resident trout and summer Steelhead. Low densities of genotype O compared to Steelhead and trout abundance and mortality (Stinson and Bartholomew, 2012) may imply that the parasite-host imbalance favoring *C. shasta* proposed by Ratliff (1981) is genotype specific and does not apply to genotype O in the Deschutes River. One exception to the spatiotemporal pattern was in August 2017 and 2018 when we observed type II in the lower basin, which may have been related to increased Sockeye salmon returns or temperature preference of genotypes, or simply due to type II becoming visible in chromatogram measurements once type I abundance had decreased. We observed unidentifiable genotypes and mixtures in 10.4% (11/105) of the water samples spread across both basins, but with different sequences in each basin. Additional work, such as novel primer design or cloning, is needed to determine if these sequences represent previously un-sequenced genotypes of *C. shasta* or a new *Ceratonova* species.

ACKNOWLEDGEMENTS

Stacy Strickland (Oregon Department of Fish and Wildlife) and Ken Lujan (United States Fish and Wildlife Service) for providing tissue samples, allowing us to collect tissue samples during spawns and juvenile releases, and for their continued collaboration with the Bartholomew laboratory. Funding for this research was provided by The Confederated Tribes of the Warm Springs Reservation of Oregon-Pacific Coastal Salmon Recovery Fund and an Oregon Department of Fish and Wildlife Fish Health Graduate Research Fellowship in Microbiology.

REFERENCES

Atkinson, S. D., and J. L. Bartholomew. 2010a. Disparate infection patterns of *Ceratomyxa shasta* (Myxozoa) in rainbow trout (*Oncorhynchus mykiss*) and Chinook salmon (*Oncorhynchus tshawytscha*) correlate with internal transcribed spacer-1 sequence variation in the parasite. International Journal for Parasitology 40(5):599–604.

Atkinson, S. D., and J. L. Bartholomew. 2010b. Spatial, temporal and host factors structure the *Ceratomyxa shasta* (Myxozoa) population in the Klamath River basin. Infection, Genetics and Evolution 10(7):1019–1026.

Atkinson, S. D., S. L. Hallett, and J. L. Bartholomew. 2018. Genotyping of individual *Ceratonova shasta* (Cnidaria: Myxosporea) myxospores reveals intra-spore ITS-1 variation and invalidates the distinction of genotypes II and III. Parasitology 145(12):1588–1593.

Baker, C., and J. Lovtang. 2016. Monitoring wild populations of spring Chinook salmon (*Oncorhynchus tshawytscha*) and summer Steelhead (*O. mykiss*) in tributaries of the lower Deschutes River within the boundaries of the confederated tribes of the warm springs of Oregon reservation, Natural Production Monitoring 2013-2014 to BPA, Confederated Tribes of the Warm Springs Reservation of Oregon Warm Springs, OR.

Bartholomew, J. L., M. J. Whipple, D. G. Stevens, and J. L. Fryer. 1997. The Life Cycle of *Ceratomyxa shasta*, a Myxosporean Parasite of Salmonids, Requires a

Freshwater Polychaete as an Alternate Host. The Journal of Parasitology 83(5):859–868.

Bjork, S. J., and J. L. Bartholomew. 2010. Invasion of *Ceratomyxa shasta* (Myxozoa) and comparison of migration to the intestine between susceptible and resistant fish hosts. International Journal for Parasitology 40(9):1087–1095.

Conrad, J. F., and M. Decew. 1966. First Report of *Ceratomyxa* in Juvenile Salmonids in Oregon. The Progressive Fish-Culturist 28(4):238–238.

Deschutes River Conservancy. 2018. By the numbers: Trapping, transporting salmonids in reintroduction efforts in blocked upper Deschutes. https://www.deschutesriver.org/news-and-resources/news-&-media/news/by-the-numberstrappingtransporting-salmonids-in-reintroduction-efforts-in-blocked-upper-deschutes

Gould, R. W. 1970. *Ceratomyxa shasta* in the Deschutes River basin and the ultrastructure of the spore. MSc, Oregon State University, United States -- Oregon.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acid Symposium Series 41: 95-98.

Heisler, T. 2012. Deschutes River Conservancy Deschutes Water Planning Initiative:39.

Hoffmaster, J. L. 1985. Geographic distribution of *Ceratomyxa shasta* in the Columbia River basin and susceptibility of salmonid stocks. MSc, Oregon State University, United States -- Oregon.

Hurst, C. N., and J. L. Bartholomew. 2012. *Ceratomyxa shasta* genotypes cause differential mortality in their salmonid hosts. Journal of Fish Diseases 35(10):725–732.

Noble, E. R. 1950. On a Myxosporidian (Protozoan) Parasite of California Trout. The Journal of Parasitology 36(5):457–460.

Oregon Department of Fish and Wildlife (ODFW) and Confederated Tribes of the Warm Springs (CTWS). 2017. Hatchery and Genetic Management Plan.

Portland General Electric (PGE). N.d. Deschutes daily fish counts. https://www.portlandgeneral.com/corporate-responsibility/environmental-stewardship/water-quality-habitat-protection/fish-counts-fish-runs/deschutes-daily-fish-counts#

Ratliff, D. E. 1981. *Ceratomyxa shasta*: Epizootiology in Chinook Salmon of Central Oregon. Transactions of the American Fisheries Society 110(4):507–513.

Ratliff, D. E. 1983. *Ceratomyxa shasta*: Longevity, Distribution, Timing, and Abundance of the Infective Stage in Central Oregon. Canadian Journal of Fisheries and Aquatic Sciences 40(10):1622–1632.

Stinson, M. E. T., and J. L. Bartholomew. 2012. Predicted redistribution of *Ceratomyxa shasta* genotypes with salmonid passage in the Deschutes River, Oregon. Journal of Aquatic Animal Health 24(4):274–280.

Stinson, M. E. T., S. D. Atkinson, and J. L. Bartholomew. 2018. Widespread distribution of *Ceratonova shasta* (Cnidiaria: Myxosporea) genotypes indicates evolutionary adaptation to its salmonid fish hosts. Journal of Parasitology 104(6): 645-650.

United States District Court for the Northern District of California San Francisco Division. 2017. Hoopa Valley Tribe v. U.S. Bureau of Reclamation and Klamath Water Users Association, et al. Document 111. Case No. 3:16-cv-04294-WHO.

University of Washington - School of Aquatic & Fishery Sciences. Columbia Basin Research. N.d. http://www.cbr.washington.edu/dart

Vojnovich, S., Hallett S., Atkinson S., Alexander J., Holt R., Craig R., and J. Bartholomew. 2016. Myxozoan disease risks in the Willamette and Deschutes Rivers, ODFW fellowship report 2015-2016. Oregon State University, Corvallis, OR.

CHAPTER 4: THE EFFECTS OF TIME AND SAMPLING LOCATION ON *CERATONOVA SHASTA* DENSITY IN WATER SAMPLES OF THE DESCHUTES RIVER

Kalyn M Hubbard, Julie D Alexander, Jerri L Bartholomew and Sascha L Hallett

ABSTRACT

We examined research questions related to the logistics associated with measuring density of the salmonid parasite, *Ceratanova shasta*, in Deschutes River water samples. Although these methods have been validated and broadly applied in other systems, sampling constraints unique to the Deschutes River warranted additional research on the effects of site selection/position of sample collection and processing time on *C. shasta* densities. We employed field and laboratory experiments to examine the influence(s) of (i) depth, distance from shore, and water temperature, and (ii) sample processing time on *C. shasta* densities in environmental water samples. We tested the first set of potential influences in three field experiments in which water samples were collected at varying locations in the water column. Position and depth of water sample collection had a significant effect on the density of *C. shasta* (spores/L) at two of the three sites tested (Warm Springs Bridge and Blue Hole), with the highest densities observed in the deepest locations sampled. Water samples were collected every two hours from 6:00 to 18:00 to test the effects of time of day of sample collection on *C. shasta* density. Time of day had a significant effect at two of the three sites (Warm Springs Bridge and Big Falls), with the highest *C. shasta* densities observed in the late group (16:00-18:00). We also tested the effects of elapsed processing time following sample collection on *C. shasta* density in water samples filtered at different intervals, from 1-14 days post collection. We expected *C. shasta* densities would be highest in samples processed within 24 hrs. However, results differed between Deschutes River and laboratory (mesocosm) water samples; *C. shasta* densities were highest in Deschutes River water samples 7 days after collection and lowest 14 days after collection, but highest in mesocosm samples 5 days after collection and lowest 3 days after collection. Variability between replicates increased with time. This result was used to set 7 days as a threshold of time before water samples were considered unreliable (Chapter 2); however, 24 hours appears to provide the most reliable results with lowest variation between replicates.

INTRODUCTION

The effects of time, depth, and position (distance from the shore) of water collection as well as timing of filtration after collection have not previously been described for *Ceratonova shasta.* Knowledge of temporal and/or spatial distributions of parasites is critical to effective collection and management protocols. For example, the distribution of waterborne protozoa and *Escherichia coli* infectious to humans in the Upper Sûre Lake (Northwestern Luxembourg) is influenced by reservoir hydrodynamics, with densities of both increasing with depth and proximity to the reservoir inlet (Burnet et al., 2015). Clear spatial and temporal trends have also been observed for the parasitic amoeba *Neoparamoeba pemaquidensis*, which causes amoebic gill disease and is problematic in Tasmanian salmonid aquaculture (Douglas-Helders et al., 2003). Regular monitoring employed to manage infection risk has shown densities to be highest in summer at depths of 5.5 meters. Additionally, the distribution of the fish skin fluke *Neobenedenia girellae* in sun vs. shade in marine net pens indicates significantly fewer *N. girellae* on fish in shaded pens vs. unshaded pens (Agawa et al., 2016). Thus, knowledge of spatiotemporal dynamics within specific river sampling sites is an important component of a well-designed sampling approach. Without this knowledge, our results run the risk of sampling bias and inaccuracies because of unknown cross-sectional and/or diurnal spatiotemporal trends. In an effort to inform management strategies with the most accurate water sampling results from the Deschutes River, timed experiments were conducted at controlled depths and cross-sectional positions at three longitudinal sampling sites along the Deschutes River mainstem.

Effects of sample collection on *C. shasta* density in Deschutes River water samples

Ceratonova shasta is a non-motile waterborne parasite with two spore stages measured in water samples as spores/L. The actinospore is the infective stage to salmonids and has neutral buoyancy in the water column (Bjork and Bartholomew, 2010). Myxospores excreted by the salmonid, are denser, and fall toward the bottom (Hallett et al., 2012). While qPCR provides quantification of *C. shasta* DNA, it cannot distinguish between the two spore forms, which exhibit different phenotypes but share the same genotype. We expected that the river cross-sectional position (distance from the

bank) and depth influenced the density of *C. shasta* DNA, with the highest abundance being found in the deepest, most centered point in the river due mixing of actinospores and myxospores in the water samples at greater depths.

The seasonal increase in water temperature has been linked to increased densities of *C. shasta* in the Klamath (Hendrickson, 1989; Bjork and Bartholomew, 2010; Ray et al., 2012) and Deschutes (Chapter 2) Rivers. Therefore, we hypothesized that as the time of day (and temperature) increased, so would the density (spores/L) of *C. shasta*. In particular, we expected that peak *C. shasta* densities would occur around 16:00, when water temperature was predicted to peak at that time of year (USGS).

Because *C. shasta* abundance has been linked with seasonal increase in water temperatures in other systems (Ray et al., 2012; Chapter 2) and occurs simultaneously with predicted host fish passage timing (Chapter 3), three surveys were conducted from late May to early July to test the effects of sample collection on *C. shasta* density in Deschutes River water samples.

Water sample integrity experiments

The second half of this chapter describes the relationship between density of spores and time in refrigerated, unfiltered samples. Laboratory protocol is to filter water samples within 24 hours of collection to maintain sample integrity and minimize impacts, such as spore degradation, from co-sampled microbes.

Two experiments were conducted to quantify the relationship between time post-collection and density of *C. shasta* in water samples when water samples were filtered at different times (1 day, 3 days, 5 days, 7 days, and 14 days) post-collection. We expected that the density of *C. shasta* in the 1L water samples would be inversely proportional to time: density would decrease as the filtration days post-collection increased. Laboratory samples were collected from pooled mesocosms on August 14th, 2017 at the J. L. Fryer Aquatic Animal Health Laboratory (AAHL) in Corvallis, OR. Field samples were collected on July 3rd, 2018 at the Blue Hole site on the Deschutes River. The aim of this experiment was to observe how *C. shasta* density may change over time in filtered and unfiltered river water samples. Combined, these studies provide information on proper post-collection handling of water samples in both laboratory and field settings.

METHODS

Study sites

Three sites were chosen for their accessibility, proximity to the Round Butte Dam (upstream, downstream, far downstream) which separates the upper and lower basins of the Deschutes River, and *C. shasta* abundance (Figure 4.1). Historically, Blue Hole was characterized by high parasite densities and is the furthest downstream. Big Falls is the upstream site with moderate *C. shasta* density and Warm Springs Bridge is located downstream of the Round Butte Dam with the lowest abundance of *C. shasta* (Chapter 2).

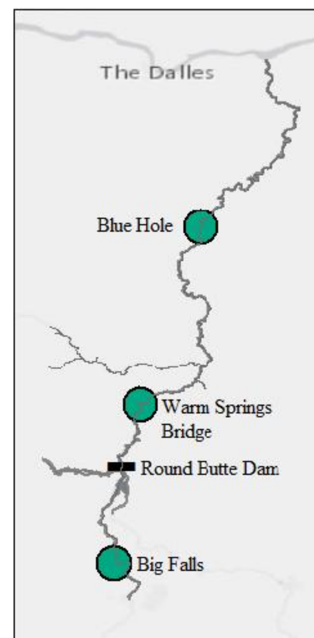

FIGURE 4.1. Spatial distribution of diurnal, lateral, and vertical sampling sites.

Position is defined as distance from the bank, depth is the depth in the water from the surface, and location is defined as the intersection of position and depth. Locations and number of samples taken for the position and depth surveys were dependent on the bathymetry, depth, and width of the river at each site and are explained below. Since the highest water temperatures occurred around 16:00 (USGS) depth and position surveys were performed at that time at each site to determine the distribution of *C. shasta* across the river and at multiple depths.

For all experimental sites, three 1L water samples were collected manually every two hours from 6:00 to 18:00 from each of the three sites (Figure 4.1) and stored on ice until filtration, within 24 hours of collection. The sampling location for diurnal surveys was the same as where water samples would be collected during spatial, longitudinal surveys (Chapter 2). Times of day were binned as “early” (6:00 – 10:00), “mid-day” (12:00-14:00), or “late” (16:00-18:00). These bins were selected to capture the different water temperatures throughout the day. Temperature was recorded with a digital thermometer in degrees Celsius.

Big Falls – May 29th, 2018 (44.359565, -121.294156) — The Big Falls site is shallow, with a maximum depth of 0.61 meters. The width of the river is approximately 21 meters, and sampling positions were spaced 10.5 meters apart (Figure 4.2, 4.3). This site is the only one upstream of the Round Butte Dam and was chosen for its moderate density of *C. shasta* (Chapter 2). The flow was assumed to be relatively uniform across the reach because the water was clear and devoid of obstructions that might create riffles. Samples were collected simultaneously from the North bank and center of the river at two depths, and in triplicate (Figure 4.3).

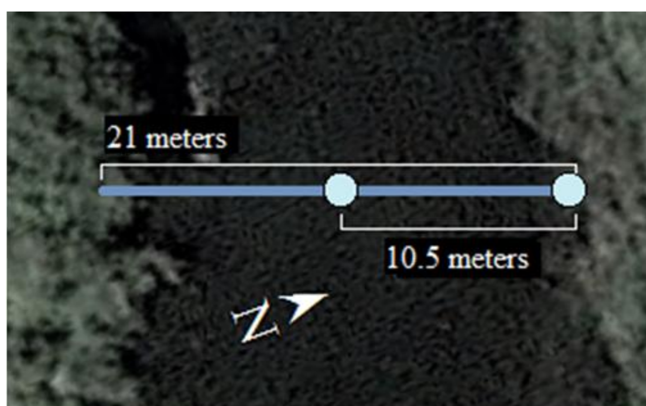

FIGURE 4.2. Big Falls site located above the Round Butte Dam. Light blue circles represent collection positions in the water.

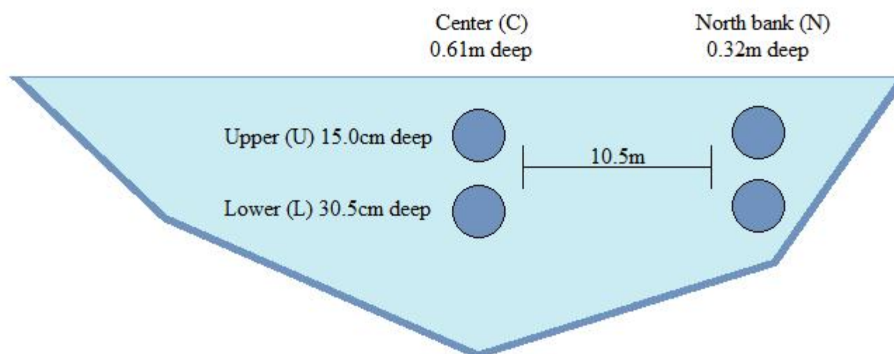

FIGURE 4.3. Measurements between each sampling location within the Big Falls site

Blue Hole – July 3rd, 2018 (45.212999, -121.073384) — The width of the river at this site is 47 meters (Figure 4.4). While the river proved to be flowing too fast to sample its entire width, a subsection was sampled to test sampling location variation within a safe distance from the bank (9.8 m). Since the lower 82 rkm has similar hydrology, any cross

section in that stretch of the river would have had similar safety issues. Due to safety concerns samples were not collected simultaneously at this site. To sample the lower depths, the volunteer dived with the sample bottle upside down and then inverted the bottle to collect the sample at the appropriate depth; the bottle was capped at the surface. Samples were collected in triplicate at three different positions and two depths (Figure 4.5).

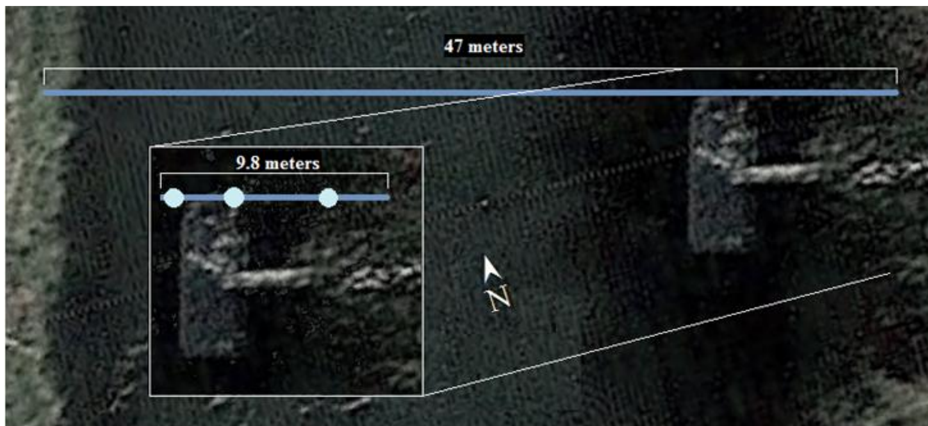

FIGURE 4.4. Blue Hole site; the farthest downstream of the three sites. Light blue circles represent collection positions.

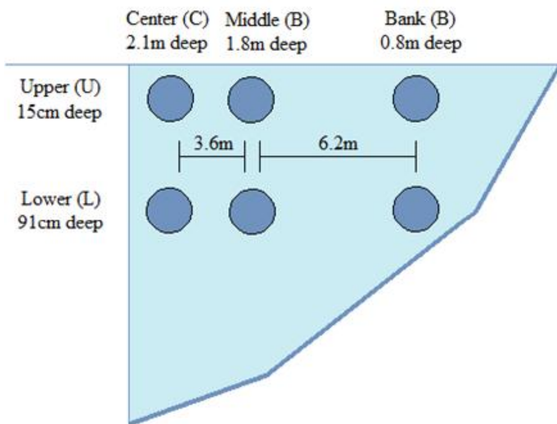

FIGURE 4.5. Measurements between each sampling location within the Big Hole site

Warm Springs Bridge – July 11th, 2018 (44.761198, -121.228669) — The Warm Springs Bridge site is approximately 91 meters across (Figure 4.6). We were able to sample the entire cross section by using the bridge as an anchor point for a harnessed volunteer, although due to the fast moving, deep water, we needed two people on the bridge to move the rope and re-tie it safely. All location (depth and position) samples were collected in triplicate (Figure 4.7).

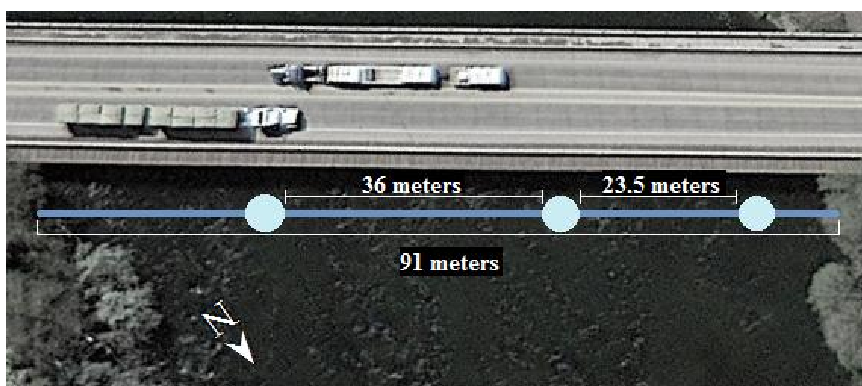

FIGURE 4.6. Warm Springs Bridge site. The middle site for these surveys located just downstream of the Pelton trap. Light blue circles are positions of water collection.

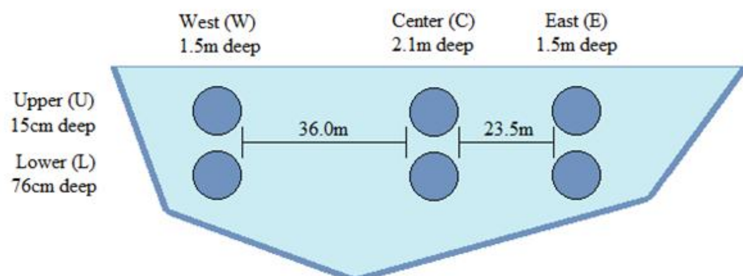

FIGURE 4.7. Measurements between each sampling location within the Warm Springs Bridge site

Effect of water filtration timing post-collection on *Ceratonova shasta* density detection in water samples

Laboratory water filtration — Polychaete mesocosms at the AAHL were plumbed into a 100L tank with an air stone for oxygenation and turbulence. On the day of sampling, the river water being pumped through the mesocosms was 16°C. During this time, myxospore dosing was withheld from the mesocosms to ensure that I collected only the infective stage to salmonids, the actinospore. In order to ensure even dispersal and suspension of the spores, I used a pole to swirl the water at four evenly spaced intervals during water collection. There were five treatments with five 1L-replicates each: samples were filtered within 24 hours of collection as a control and at 3, 5, 7, and 14 days post-collection (Table 4.1). Samples were collected and randomly assigned to each treatment. Five of the samples were labeled to be filtered within 24 hours. Twenty 1L-samples were placed in their collection bottle in the refrigerator for subsequent sampling periods.

TABLE 4.1. Summary of laboratory water filtration treatment groups (n=5) with specifications for where the samples (5 1L per treatment) were held after collection and the amount of time before they were filtered.

Treatment	***Time of filtration***
Filtered within 24 hours and stored frozen (control)	< 1 day
Collected and refrigerated for 3 days before filtration	3 days
Collected and refrigerated for 5 days before filtration	5 days
Collected and refrigerated for 7 days before being filtered	7 days
Collected and refrigerated for 14 days before being filtered	14 days

River water filtration — The study conducted on August 14th, 2017 from the mesocosms in the laboratory allowed us to refine our procedure for collection of river water samples. The second iteration of this project was performed on the Deschutes at the Blue Hole dock on July 3rd, 2018, the same day and site as the 2nd DLV experiment. River water was at 17.3°C and collected into one cooler. Twenty-five 1L samples were taken from this same cooler. These samples were randomized and then labeled with the five different

treatments – filtration within 1 day, 3 days, 7 days, and 14 days post-collection (Table 4.2).

Ceratonova shasta quantitation from water samples

Water samples were transported back to the laboratory on ice, filtered through a 5 µm filter, and the retentate frozen. DNA from the retentate was extracted and *C. shasta* enumerated using quantitative polymerase chain reaction (qPCR) (Hallett and Bartholomew, 2006; Hallett et al., 2012). Diurnal, depth, and position samples were filtered within 24 hours of collection whereas filtration experiment samples were filtered according to their specific treatment (Table 4.1, 4.2).

TABLE 4.2. Summary of river water (from Blue Hole site) filtration treatment groups with specifications for where the samples were held after collection and the amount of time before they were filtered

Treatment	***Time of filtration***
Filtered within 1 day and put in freezer (control)	<24 hours
Collected and refrigerated for 3 days before being filtered	3 days
Collected and refrigerated for 7 days before being filtered	7 days
Collected and refrigerated for 14 days before being filtered	14 days

Statistical analyses

To test the effect of time of collection or time of filtration on *C. shasta* density, a one-way ANOVA was applied. Significant differences were followed with a post-hoc Tukey's Honest Significant Difference (HSD) test. Values were considered significant at $\alpha = < 0.1$. The effect of location of collection on *C. shasta* density was analyzed using two-way ANOVAs. In the case of significant differences, a post-hoc Tukey's honest significant difference (HSD) test was used. Values were considered significant at $\alpha = < 0.1$.

RESULTS

Effects of sample collection on *Ceratonova shasta* density detection in Deschutes River water samples

Big Falls — At the Big Falls site, parasite densities ranged from 11 to 122 spores/L over the course of the day (Figure 4.8). Densities of *C. shasta* increased with time of day (Figure 4.9; ANOVA: $F_{2, 18} = 13.97$, $P = 0.0002$).

Moving across the river, *C. shasta* density did not vary significantly from bank to center (Figure 4.10). An increase in *C. shasta* spores/L was observed with the highest concentrations in the thalweg. However, after comparing the position in the water with the depth at which the samples were taken, we did not detect a significant difference (Figure 4.11; two-way ANOVA: $F_{3, 8} = 2.71$, $P = 0.12$).

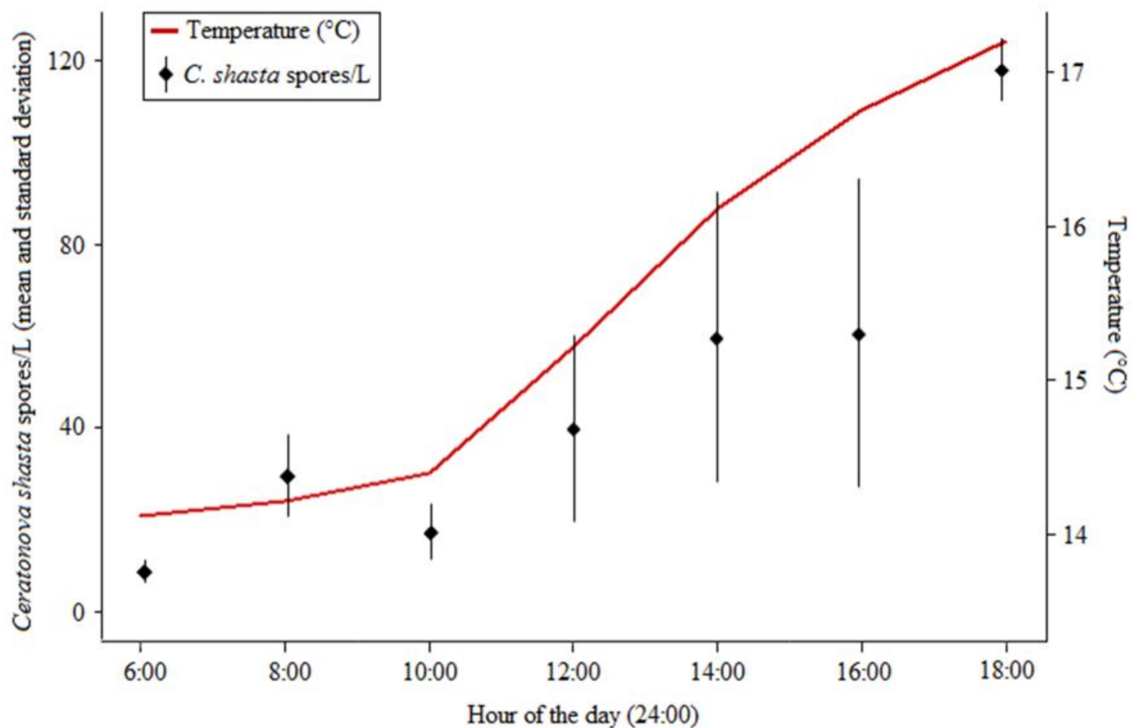

FIGURE 4.8. *Ceratonova shasta* spores/L (left y-axis) and temperature in degrees C (right y-axis) over the course of one day (x-axis) at the Big Falls site.

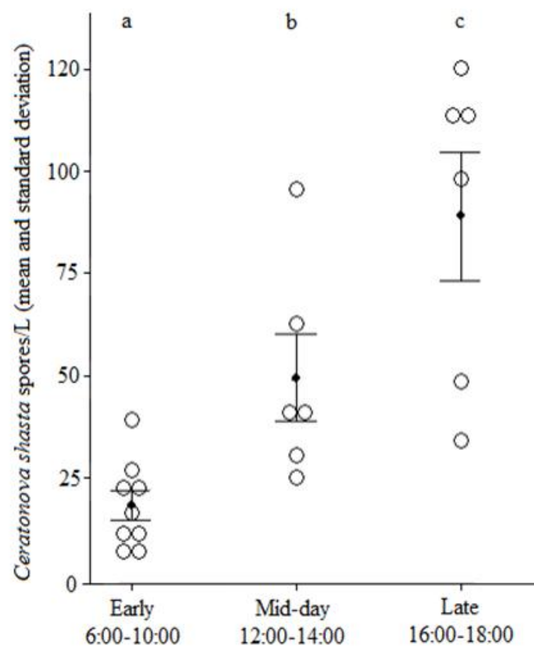

FIGURE 4.9. *Ceratonova shasta* density (y-axis) as a function of binned time groups (x-axis) at the Big Falls site (ANOVA: $F_{2, 18} = 13.97$, $P = < 0.001$). Tukey's HSD values considered significant at $\alpha = < 0.1$

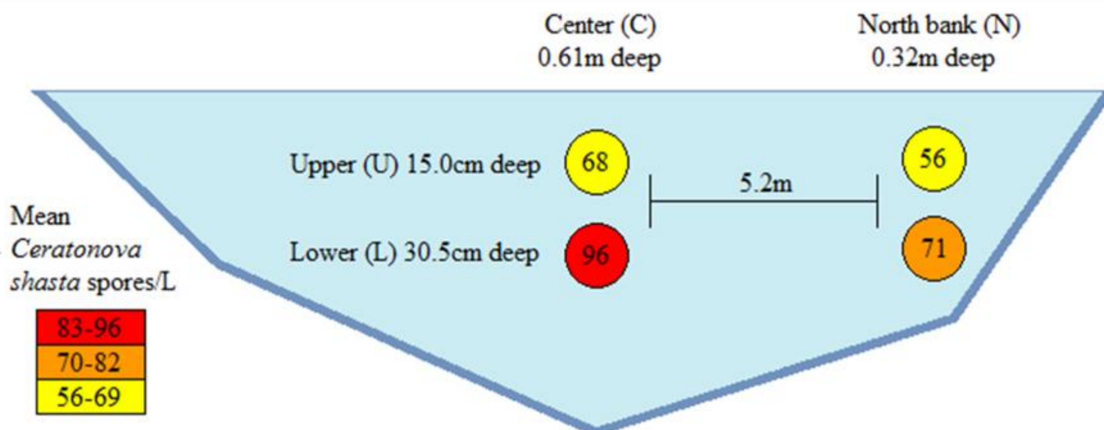

FIGURE 4.10. Simplified rendition of the Big Falls river cross section with the mean *Ceratonova shasta* densities from three replicates at each intersection of depth and position, represented by color coded circles signifying high (red), medium (orange), or low (yellow) *Ceratonova shasta* density at this site.

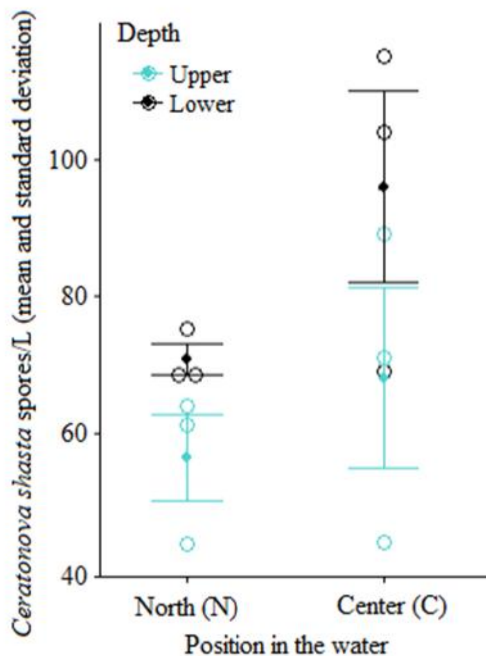

FIGURE 4.11. *Ceratonova shasta* density (y-axis) as a function of depth (line color) and position (x-axis) at the Big Falls site (two-way ANOVA: $F_{3,8} = 2.71$, $P = 0.12$).

Blue Hole — Ceratonova shasta density at this site was not influenced by time of day (binned time-of-day groups; ANOVA: $F_{2,\,15} = 0.092$, $P = 0.91$) (Figure 4.13)).

Average density of *C. shasta* within the water column ranged from 18 spores/L to 38 spores/L (Figure 4.14). Depth and position had an effect on *C. shasta* density at the Blue Hole site (two-way ANOVA: $F_{5,\,12} = 2.958$, $P = 0.057$(Figure 4.15)). This result was explained by the difference in *C. shasta* density between depths (Tukey's HSD: $P = 0.086$) with the largest discrepancy between the upper and lower depths in the center of the river.

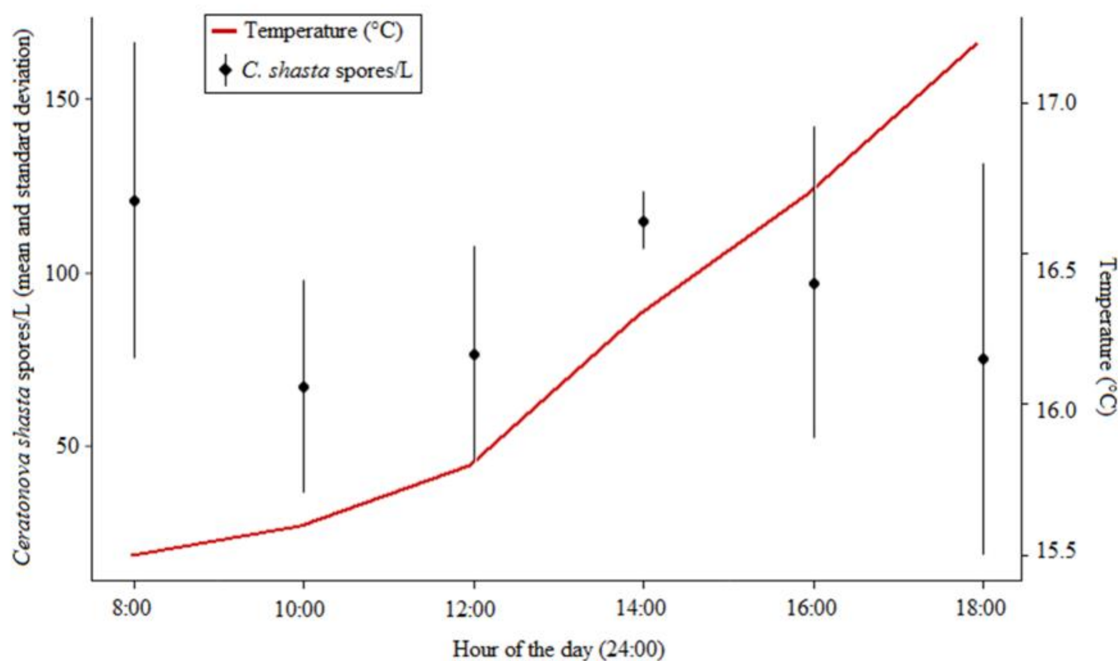

FIGURE 4.12. *Ceratonova shasta* spores/L (y-axis) and temperature in degrees C (second y-axis) over the course of one day (x-axis) at the Blue Hole site

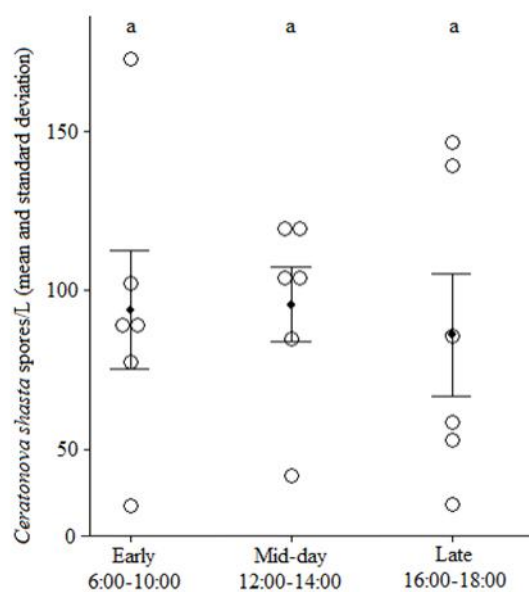

FIGURE 4.13. *Ceratonova shasta* density (y-axis) as a function of binned time groups (x-axis) at the Blue Hole site (ANOVA: $F_{2, 15} = 0.092$, $P = 0.91$). Tukey's HSD values considered significant at $\alpha = < 0.1$

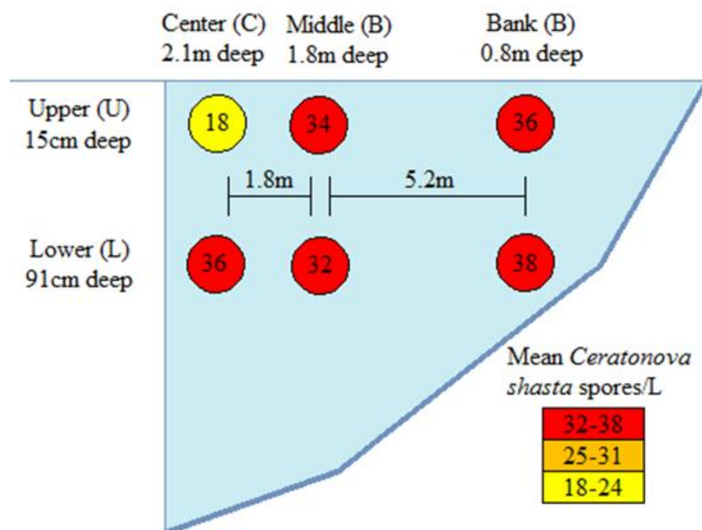

FIGURE 4.14. Simplified rendition of the Blue Hole river cross section with the mean *Ceratonova shasta* densities from three replicates at each intersection of depth and position, represented by color coded circles signifying high (red), medium (orange), or low (yellow) *Ceratonova shasta* density at this site.

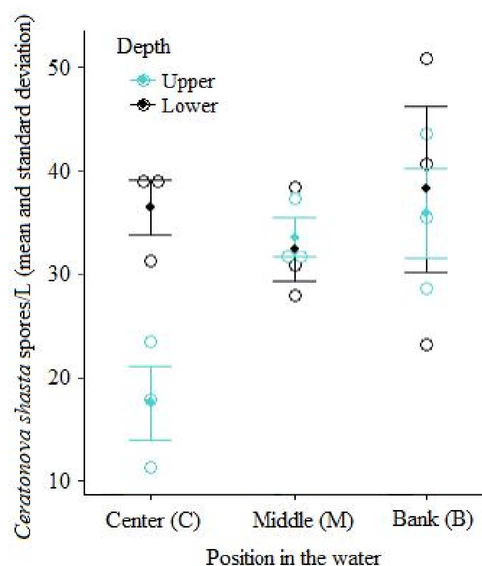

FIGURE 4.15. *Ceratonova shasta* density (y-axis) as a function of depth (line color) and position (x-axis) at the Blue Hole site (two-way ANOVA: $F_{5, 12} = 2.958$, $P = 0.057$)

Warm Springs Bridge —The density of *C. shasta* at the Warm Springs Bridge site was highest at 6:00 and 16:00 hours, although the water temperature steadily increased from 13.6 to 17.7 °C over the course of the 12-hour day (Figure 4.16). The lowest densities were detected during the middle of the day (Figure 4.17; ANOVA: $F_{2, 18} = 6.607$, $P = 0.0071$) with the largest difference between mid-day and late groups (Tukey's HSD: $P = 0.038$) and the highest densities observed in the late group.

Average *C. shasta* density was highest near the banks and lowest in the center (Figure 4.18). On the East bank, *C. shasta* density was higher in the upper depths compared to the lower depth locations. Higher densities were detected at lower depths on the West bank, closer to the thalweg in that cross section of the river (Figure 4.19; two-way ANOVA: $F_{5, 10} = 4.231$, $P = 0.025$). However, the main difference was observed between positions (Tukey's HSD: $P = 0.01$) as opposed to depths.

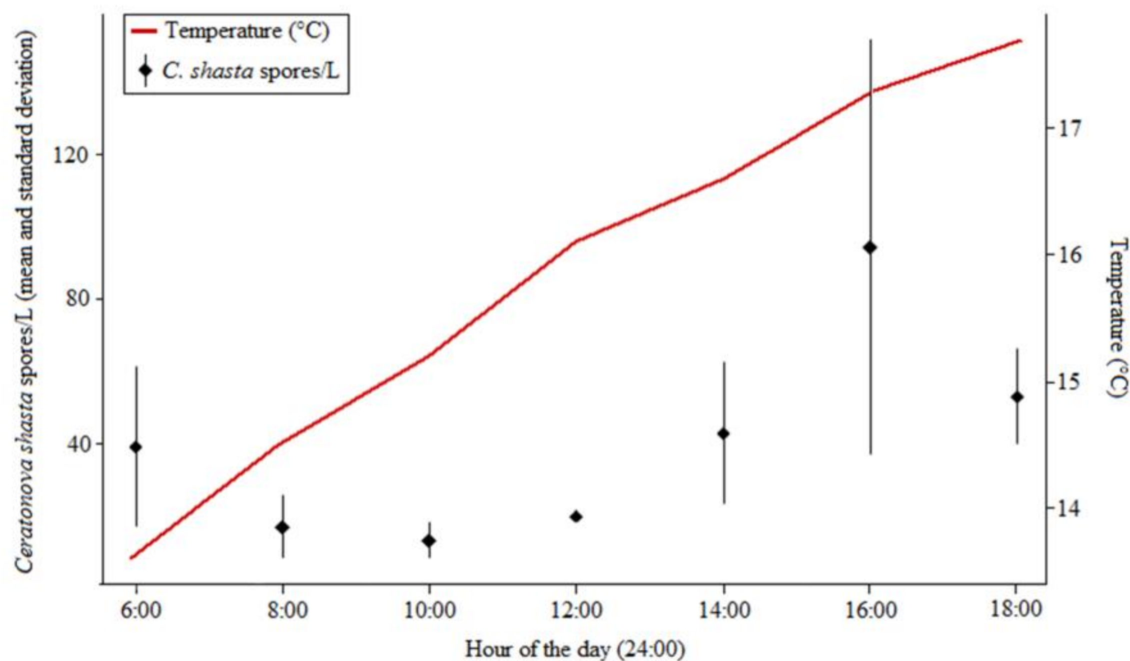

FIGURE 4.16. *Ceratonova shasta* spores/L (y-axis) and temperature in degrees C (second y-axis) over the course of one day (x-axis) at the Warm Springs bridge.

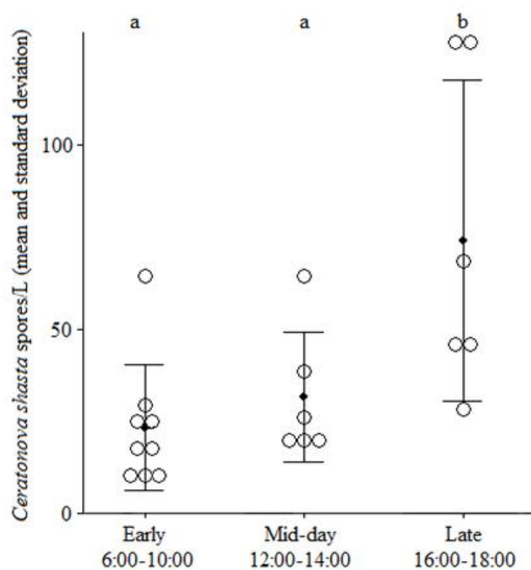

FIGURE 4.17. *Ceratonova shasta* density (y-axis) as a function of binned time groups (x-axis) at the Warm Springs bridge (ANOVA: $F_{2, 18} = 6.607$, $P = 0.0071$). Tukey's HSD values considered significant at $\alpha = < 0.1$

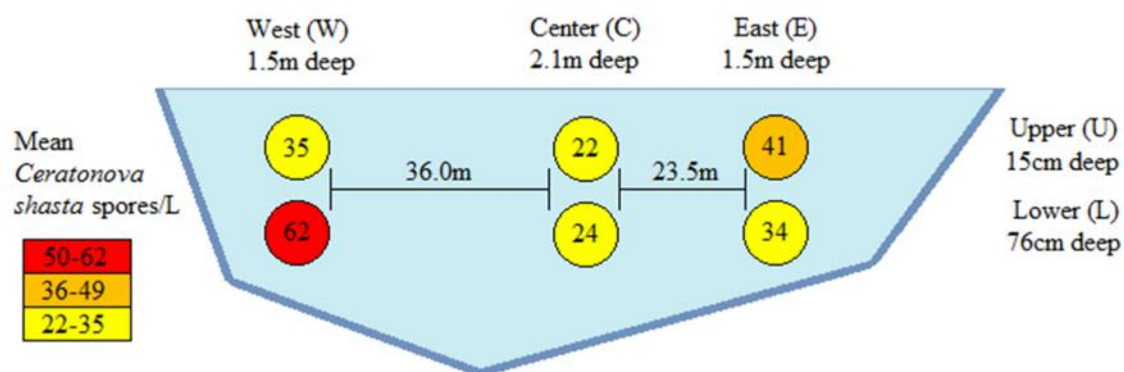

FIGURE 4.18. Simplified rendition of the Warm Springs bridge river cross section with the mean *Ceratonova shasta* densities from three replicates at each intersection of depth and position, represented by color coded circles signifying high (red), medium (orange), or low (yellow) *Ceratonova shasta* density at this site.

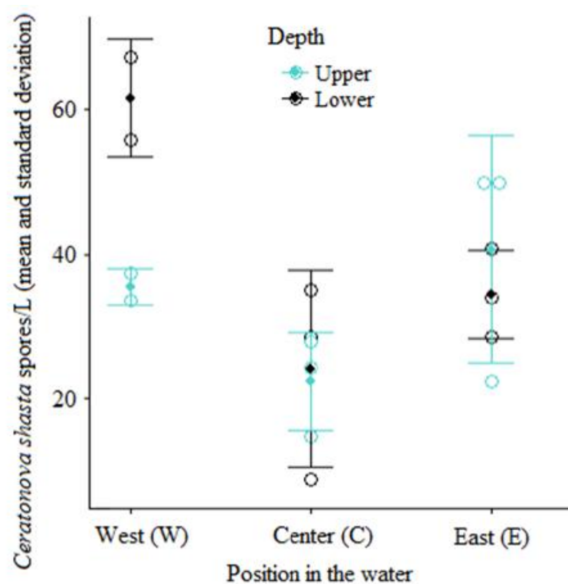

FIGURE 4.19. *Ceratonova shasta* density (y-axis) as a function of depth (line color) and position (x-axis) at the Warm Springs Bridge site (two-way ANOVA: $F_{5, 10} = 4.231$, $P = 0.025$)

Effect of water filtration timing post-collection

Laboratory water filtration — *Ceratonova shasta* densities differed among sample time periods (ANOVA: $F_{4, 20} = 3.095$, $P = 0.039$) and decreased after day five. The greatest difference in *C. shasta* density occurred between post-filtration days five and seven (Figure 4.20; $P = 0.045$). Variation between replicate samples increased over time.

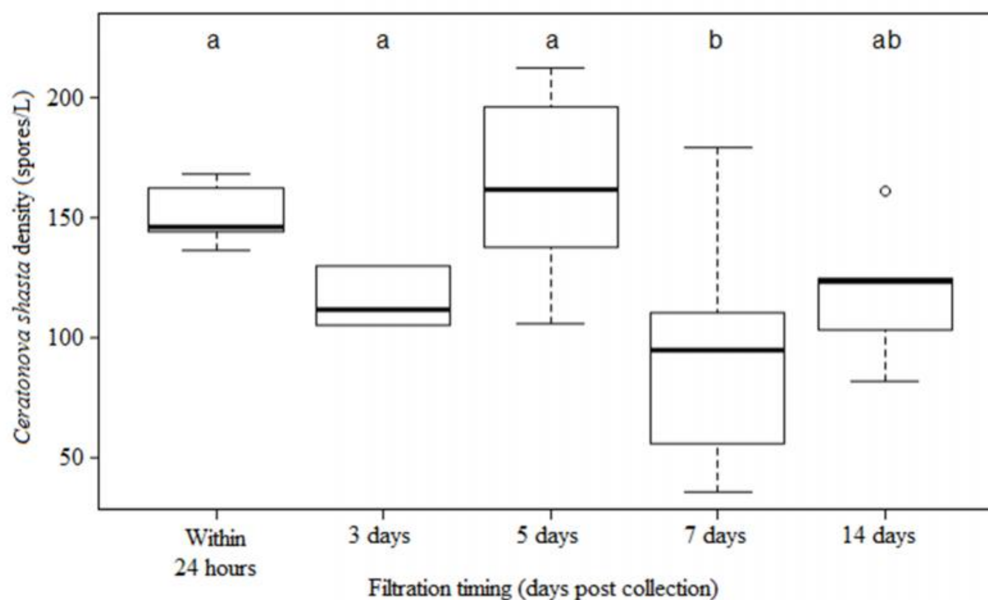

FIGURE 4.20. *Ceratonova shasta* density (y-axis) as a function of filtration days post collection (x-axis) of pooled water from polychaete mesocosms Five replicates per treatment. (ANOVA: $F_{4, 20} = 3.095$, $P = 0.039$). Tukey's HSD values considered significant at $\alpha = < 0.1$.

River water filtration — In contrast to mesocosm water samples, we detected an increase in *C. shasta* density in river water samples at seven days post-collection (Figure 4.21: ANOVA: $F_{3, 16} = 19.49$, $P = < .001$). Variation between replicate samples increased over time.

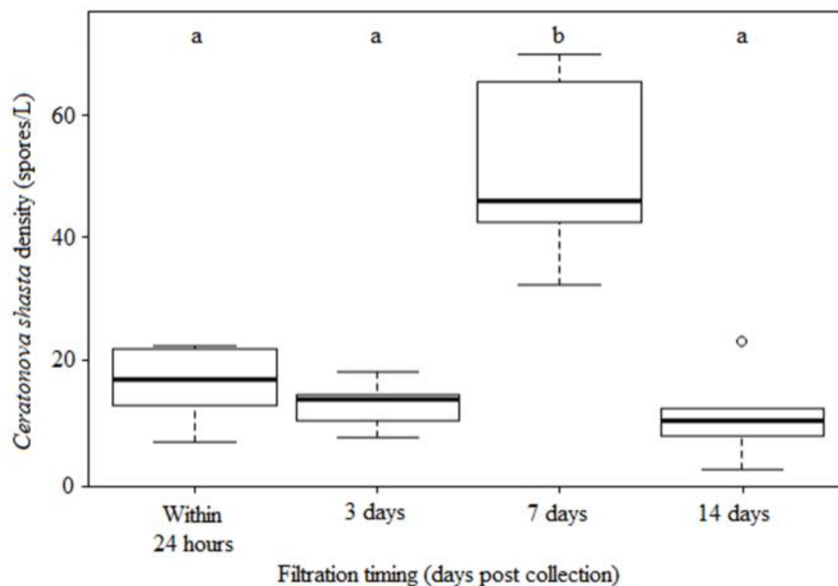

FIGURE 4.21. *Ceratonova shasta* density (y-axis) as a function of filtration days post collection (x-axis) of pooled river water from the Blue Hole site. Five replicates per treatment. (ANOVA: $F_{3, 16} = 19.49$, $P = < .001$). Tukey's HSD values considered significant at $\alpha = < 0.1$.

DISCUSSION

Detection and quantification of pathogens in water samples within sites can provide detailed distribution information. These methods are becoming more widely used to determine parasite distribution, leading to more precise and effective management strategies (Douglas-Helders et al., 2003; Burnet et al., 2015; Agawa et al., 2016).

To better understand the effects of time, depth, and position of water collection as well as timing of filtration after collection, we conducted a series of field and laboratory experiments. To determine the distribution of *C. shasta* within the water column and throughout the course of one day, we conducted water sampling experiments at three sites on the Deschutes River. In addition, environmental water samples from the river and

laboratory mesocosms were held for 1 – 14 days after collection to examine the effects time on water sample integrity.

Effects of sample collection on *Ceratonova shasta* density detection in Deschutes River water samples

Our hypothesis that *C. shasta* density is dependent on the depth and position of collection in the water column was supported at two of the three sites that were tested (Blue Hole and Warm Springs Bridge). At the Big Falls site, there was no effect of depth or position on *C. shasta* density (Figure 4.11). At the Blue Hole site, depth was the influential parameter with higher densities at deeper depths (Figure 4.15). In contrast, the significant parameter at the Warm Springs Bridge site was position with higher *C. shasta* densities detected near the banks of the river as opposed to the center (Figure 4.19).

Similar to the study conducted by Burnet et al. (2015) assessing *E. coli* densities at different depths and positions in a large Northwestern Luxembourg reservoir, our conflicting results suggest that *C. shasta* density in the water column is influenced by differing hydrodynamics at each of the three sites. Based on our results, further research about the effects of depth and position on *C. shasta* density in the Deschutes River should include several sites that have similar hydrodynamics. Some of these similarity parameters may include Froude number (Fr), local bed slope, water depth, and unit stream power (*QS*) (Daraio et al., 2010). To calculate *QS*, it would be necessary to first quantify the river discharge at that site using, for example, an acoustic doppler instrument. Similar sites can be binned to provide more confidence in statistical analyses. Using a hydrodynamic model, results can then be extrapolated for more accurate reporting of density at sites that fit one of the pre-determined categories (Bergstein Ben-Dan et al., 2001). To avoid safety issues on collection day, as observed with the Blue Hole, only sites with stationary structures spanning the width of the river should be selected.

Our second hypothesis was that the density of *C. shasta* increases as the temperature and time of day increase. This was true for two out of the three sites investigated (Big Falls and Warm Springs Bridge). The time of day and *C. shasta* densities appear to be closely related at the Big Falls site (Figure 4.5) with densities

differing between all three binned groups (early, mid-day, and late). Spore density also differed between the times of collection at the Warm Springs Bridge between the early and late groups as well as the mid-day and late groups. In contrast, parasite density did not differ throughout the day at the Blue Hole site.

Our results indicate that the time of day and *C. shasta* density are not directly proportional at every site on the Deschutes River. This suggests that spatial surveys estimating the density of *C. shasta* over the course of one day on the Deschutes River may have either no effect or may be underestimating the daily density at a particular site (Chapter 2). For the most accurate snapshot of *C. shasta* density on the Deschutes River, more collaborative teams should be employed to collect spatial water samples within a 2-hour time period, as specified by the binned time intervals (Figure 4.9, 4.13, 4.17).

Should these experiments be improved upon and reimplemented, an ISCO automatic sampler set to collect water every 2 hours and a temperature data logger at both of the monitoring sites (Oak Springs and Pelton Trap) over the course of one week would provide more accurate information on how *C. shasta* corresponds to diurnal temperature variation. These samples would need to be collected at the end of each day and filtered within a week (Figure 4.21). Results from this study would be informative for better understanding of *C. shasta* temperature dynamics and this knowledge may contribute to the development of a predictive model for *C. shasta* on the Deschutes River.

Effect of water filtration timing post-collection

Ceratonova shasta-DNA increased at around 5 or 7 days in both the laboratory and river water filtration experiments, respectively. The reason for the sharp increase is unclear since all samples were randomly collected from one container, randomly assigned a treatment group, and all samples were run on the same qPCR plate (reducing variation in reported values). Since the parasite does not replicate outside its host, we would expect the amount of *C. shasta* DNA to stay the same or decrease, but not increase.

One explanation is that as the spores started to degrade, DNA was more readily accessible during the extraction process leading to a higher perceived concentration of *C. shasta* DNA. The water filtration protocol developed in 2006 used 5µm filters in order to

catch intact, viable spores as opposed to spore fragments (Hallett and Bartholomew, 2006). However, it is possible that spore fragments and DNA are caught on the filters due to the adherent properties of DNA and/or accumulation of material on filter papers that decrease pore size during filtration. We recommend that this hypothesis be explored in future research by quantifying DNA from water samples with intact spores versus water samples in which the spores have degraded.

The differences in seven-day timepoints between the river and laboratory experiments may be related to whether or not the water samples were primarily myxospores or actinospores. Degradation of myxospores and actinospores has been examined in previous, laboratory-based research. Actinospores remain viable up to 18 days at 4°C (Bjork, 2010) whereas 6% of myxospores remain viable up to 175 days at an average temperature of 7.4°C (Chiaramonte, 2013). The abundance of both spore types decreased linearly while the myxospore variation between samples increased over time. Within the riverine system, there is no way for us to know the exact proportion of myxospores and actinospores and contribution of myxospores to the river water samples may overestimate *C. shasta* DNA in the water sample.

While the results of the water filtration experiments did not completely support our hypothesis that days post-filtration and *C. shasta* density were inversely related, we did observe an increase in variation between samples over time. The increase in DNA around 5 or 7 days also suggests that filtration during that window of time may overestimate *C. shasta* abundance in water samples, but this conclusion should be explored further with the small experiment mentioned above.

Conclusions

For the most consistent water sampling during spatial surveys of *C. shasta* in the Deschutes River, we recommend that samples are collected at the same depth and position at each site and within a two-hour time-frame. The collection of spatial samples for the 230-river kilometer stretch of the Deschutes River within two hours will require teams of collaborators. Note that while samples collected at the banks of the river are the safest, most accessible option for water sample collection, the parasite density at the

banks are likely to be underestimations of *C. shasta* in that river cross section as parasite abundance was higher in the thalweg of the river at two of the three sites (Figure 4.15, 4.19). While samples can be refrigerated for up to seven days before filtration, they should optimally be filtered within 24 hours for the least amount of variation between replicate samples.

Acknowledgements

Many volunteers assisted with the successful collection of water samples for the diurnal, depth, and position surveys including Dustin DeGeorge, Olivia Crowley, Tim Hubbard, Sophia Jadzak, Milan Sengthep, and Benjamin Americus. Funding for this research was provided by The Confederated Tribes of the Warm Springs Reservation of Oregon-Pacific Coastal Salmon Recovery Fund and an Oregon Department of Fish and Wildlife Fish Health Graduate Research Fellowship in Microbiology.

REFERENCES

Agawa, Y., K. Tani, S. Yamamoto, C. Hirano, S. Shirakashi. 2016. Development of a quantitative polymerase chain reaction assay for the detection of skin fluke *Neobenedenia girellae* larvae from environmental water. Fisheries Science 82(5): 827-833.

Bergstein Ben-Dan, T., B. Shteinman, Y. Kamenir, O. Itzhak, and A. Hochman. 2001. Hydrodynamical effects on spatial distribution of enteric bacteria in the Jordan River—Lake Kinneret contact zone. Water Research 35(1):311–314.

Bjork, S. J. 2010. Factors affecting the *Ceratomyxa shasta* infectious cycle and transmission between polychaete and salmonid hosts. Ph.D., Oregon State University, United States -- Oregon.

Bjork, S. J., and J. L. Bartholomew. 2010. Invasion of *Ceratomyxa shasta* (Myxozoa) and comparison of migration to the intestine between susceptible and resistant fish hosts. International Journal for Parasitology 40(9):1087–1095.

Burnet, J.-B., L. Ogorzaly, C. Penny, H.-M. Cauchie, J.-B. Burnet, L. Ogorzaly, C. Penny, and H.-M. Cauchie. 2015. Fine-scale spatial heterogeneity in the

distribution of waterborne protozoa in a drinking water reservoir. International Journal of Environmental Research and Public Health 12(9):11910–11928.

Chiaramonte, L. V. 2013. Climate warming effects on the life cycle of the parasite *Ceratomyxa shasta* in salmon of the Pacific Northwest. MSc., Oregon State University, United States -- Oregon.

Daraio, J. A., L. J. Weber, and T. J. Newton. 2010. Hydrodynamic modeling of juvenile mussel dispersal in a large river: the potential effects of bed shear stress and other parameters. Journal of the North American Benthological Society 29(3):838–851.

Douglas-Helders, G. M., D. P. O'Brien, B. E. McCorkell, D. Zilberg, A. Gross, J. Carson, and B. F. Nowak. 2003. Temporal and spatial distribution of paramoebae in the water column – a pilot study. Journal of Fish Diseases 26(4):231–240.

Hallett, S. L., and J. L. Bartholomew. 2006. Application of a real-time PCR assay to detect and quantify the myxozoan parasite *Ceratomyxa shasta* in river water samples. Diseases of Aquatic Organisms 71(2):109–118.

Hallett, S. L., R. A. Ray, C. N. Hurst, R. A. Holt, G. R. Buckles, S. D. Atkinson, and J. L. Bartholomew. 2012. Density of the waterborne parasite *Ceratomyxa shasta* and its biological effects on salmon. Applied and Environmental Microbiology 78(10):3724–3731.

Hendrickson, G., A. Carleton, and D. Manzer. 1989. Geographic and seasonal distribution of the infective stage of *Ceratomyxa shasta* (Myxozoa) in Northern California. Diseases of Aquatic Organisms 7:165–169.

Ray, R. A., R. A. Holt, and J. L. Bartholomew. 2012. Relationship between temperature and *Ceratomyxa shasta*–induced mortality in Klamath River salmonids. Journal of Parasitology 98(3):520–526.

CHAPTER 5: SUMMARY

CONCLUSIONS

The driving objective of this project was to identify potential causes of juvenile and pre-spawn mortality in spring Chinook salmon of the Deschutes River. The waterborne, myxozoan parasite, *Ceratonova shasta*, has been suspected as a primary contributor to these afflictions since the 1970's. As a first step toward evaluating this hypothesis, we needed to determine the spatial and temporal distribution of the parasite (Chapter 2) and its genotypes (Chapter 3) as well as the prevalence of infection and associated pathology within the host (Appendix A).

Ceratonova shasta was targeted during spring Chinook salmon tissue collections at the Round Butte Hatchery, but the bacterium *Renibacterium salmoninarum*, two myxozoans (*Tetracapsuloides bryosalmonae*, *Parvicapsula minibicornis*), and two viruses (infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic necrosis virus (IPNV)) were also assayed from the kidneys and lower intestines of juvenile and adult spring Chinook salmon. *Tetracapsuloides bryosalmonae* and IPNV were not detected from any cohort sampled at the Round Butte Hatchery, though IHNV was identified in 13% of adult spring Chinook salmon in 2018. *Ceratonova shasta* was detected in 58% of juvenile spring Chinook salmon one day before their release from the Pelton Ladder and 100% of spawning adult spring Chinook salmon in 2017 and 2018 at the Round Butte Hatchery. Mild to moderate pathology was associated with these infections in all groups. Although *C. shasta* was the most common pathogen that we detected, pathology associated with *Renibacterium salmoninarum* and *Parvicapsula minibicornis* was observed in juveniles and adult spring Chinook salmon, respectively. With the evidence of co-infections, we cannot conclude that *C. shasta* is the sole contributor to juvenile and pre-spawn mortality in the Deschutes River.

There are three known primary genetic types (genotypes) of *C. shasta* identified through ATC repeats in the ITS-1 region of ribosomal DNA and they have previously described, specific salmonid host associations (Hurst and Bartholomew, 2012; Stinson and Bartholomew, 2012; Stinson et al., 2018). We confirmed that genotype I infects the

lower intestines of Deschutes River spring Chinook salmon and that genotype II infects Coho salmon found in the Deschutes River (Chapter 3, Appendix A).

While we were able to take fish tissue samples from the Round Butte Hatchery and the SWW and Pelton Ladder, different detection methods needed to be explored for the Warm Springs National Fish Hatchery (WSNFH) where lethal sampling of spring Chinook salmon is not permitted (Appendix A). Since *C. shasta* proliferates in the lower intestine, we confirmed the use of a previously developed anal swab sampling method (Fox et al., 2000) for implementation at the WSNFH. Anal swabs were an effective detection method for *C. shasta* (100%) when the swabs had a wooden applicator and were submerged in 100% ethanol immediately after collection.

The spatial and temporal distribution of *C. shasta* and its genotypes was also determined non-lethally through water sampling and qPCR (Hallett and Bartholomew, 2006; Hallett et al., 2012) (Chapter 2). At two temporal index sites (Oak Springs and the Pelton Trap), parasite density increased in spring and declined until late fall from 2015-2018. Though there was variation between years (average spores/L 81.0, 11.2, 46.4 in 2016, 2017, 2018, respectively), *C. shasta* density was 1.5x higher between rkm 48-82 than the average of all other spatial survey sites. Additionally, DRR (rkm 116) and DWS (rkm 155) had higher than average *C. shasta* abundance for 63% (5/8) surveys.

Parasite density was low in the Warm Springs River (< 1.5 spores/L), above the mouth of the Deschutes River on the Columbia River (< 3 spores/L) compared to below the mouth (< 6 spores/L), and at sites between the Round Butte Dam and the natural migration barrier, Big Falls (< 2.5 spores/L). The latter results are consistent with the spatial distribution described by Ratliff (1983) where no *C. shasta* related mortalities were observed from fish exposed in Lake Billy Chinook. The Deschutes River is contributing spores to the Columbia River, but *C. shasta* has also been identified in the Columbia River independent of that contribution (Sanders, 1967; Hoffmaster, 1985). Therefore, migrating juvenile Chinook salmon will continue to be exposed to the parasite after they leave the Deschutes River and adult spring Chinook salmon will be exposed before they reach the Deschutes River (Appendix A). The low abundance of *C. shasta* in

the Warm Springs River suggests that this tributary does not contribute this parasite to the Deschutes River. Therefore, salmon that succumb to *C. shasta*-related pre-spawn mortalities in the Warm Springs River likely contracted the infection during their time in the Columbia and Deschutes Rivers.

Ceratonova shasta genotype proportions were determined from spatial and temporal water samples collected for Chapter 2. As proposed by earlier work, the spatial and temporal distribution of genotypes was related to the spatiotemporal distribution of corresponding fish hosts. We determined type I dominated the lower Deschutes River basin below Round Butte Dam, type II was the main genotype present in the upper basin above Round Butte Dam, and type O was detected throughout the system at lower proportions than I or II. Type II was also identified in the lower basin, but only during August of both years. Presence of type II at that time may be related to the occurrence of Coho and/or Sockeye salmon in the lower basin, depending on the year. The hypothesis proposed by Stinson and Bartholomew (2012) that genotype I would move into the upper basin with the passage of spring Chinook salmon above the Round Butte Dam (since 2007) was not supported by our water sample data. Though this might change with continued reintroduction efforts. Despite Steelhead trout being the most abundant fish species throughout the basin, the associated genotype O was detected at the lowest proportion. Similarly, juvenile and adult Chinook salmon are one of the least abundant salmonid species yet genotype I was detected at the highest proportion. These results do not support our hypothesis that genotype distribution is related to salmonid host abundance, but they do suggest that the host-parasite imbalance described previously in the Deschutes River is genotype specific.

Linear mixed effect models that compared *C. shasta* density with temperature and discharge on the Deschutes River indicated that *C. shasta* abundance increases with water temperature and decreases with river discharge. These parameters influenced the spatial and temporal variation of *C. shasta* in water samples both within and between years. 2015 was the hottest year of our data set with low snowpack and river discharge, leading to the highest observed *C. shasta* densities at our temporal index sites. Conversely, there was high snowpack and a high flow event in 2017 which may have decreased the amount

of polychaetes and spores in the system as densities were lowest this year compared to others. 2016 and 2018 were moderate temperature and discharge years, which is reflected by the moderate *C. shasta* densities compared to other years with different abiotic parameters (Chapter 2).

To optimize our water sample collection and handling techniques, laboratory and field experiments were conducted to test the influence of time of day, location of sampling, and sample integrity over time on *C. shasta* density in water samples (Chapter 4). We found that samples should be collected at the same depth and position at each site and within a two-hour time-frame for the most consistent spatial water sample results. In order to accomplish this for the 230-river kilometer stretch of the Deschutes River within two hours, teams of collaborators will be required. While samples collected at the banks of the river are the safest, most accessible option for water sample collection, the parasite density at the banks are likely to be underestimations of *C. shasta* in that river cross section as parasite abundance was higher in the thalweg of the river at two of the three sites we assessed. Additionally, while samples can be refrigerated for up to seven days before filtration, they should optimally be filtered within 24 hours for the least amount of variation between replicate samples.

FUTURE STUDIES

Sentinel fish studies

While Chapter 3 described the likely infection risk for salmonids of the Deschutes River by analyzing copresence of *C. shasta* abundance and genotypes with their target host species, sentinel studies need to be conducted to specifically test the disease risk to the target species/age group at particular times and localities as informed by previous work and this thesis. Sentinel studies also allow us to determine if the infective stage of *C. shasta* (the actinospore) is present in water samples.

We suggest that there is a particular need to assess risk to Sockeye salmon. The reintroduction program for Sockeye salmon began in 2009 (ODFW and CTWS, 2017). Results of this program have been mixed: despite maximum daily releases around 15,000 juvenile Sockeye salmon from the Selective Water Withdrawal tower, adult returns have averaged only 36-57 per year. While the exceptional Sockeye run of 536 returning adults

in 2016 and a record 440,000 out-migrating juveniles in 2017 suggests that this program might be working (Deschutes River Conservancy, 2018), the Round Butte Hatchery rearing of Sockeye salmon will be discontinued in 2019 (ODFW, pers. comm.). It remains to be seen if the population can sustain itself without hatchery supplementation. The success of the population will partially depend on disease risk which will need to be assessed through sentinel studies. Timing and placement of these should be informed by our results from this Chapter 2 and 3. The genotype associated with infection in Sockeye salmon also needs to be determined. To test our hypothesis that Deschutes River Sockeye salmon are infected with type II, as observed in Kokanee (Stinson and Bartholomew, 2012), lower intestines from juvenile Sockeye salmon will be sent to OSU from the Selective Water Withdrawal tower in March 2019 and adult Sockeye salmon in August. *C. shasta*-positive samples (by PCR) will be genotyped. Additionally, kokanee will continue to be surveyed in Lake Billy Chinook in Spring and Fall, and tissue subsamples will be sent to us for *C. shasta* detection and genotyping from ODFW collaborators at the Round Butte Hatchery.

Hatchery management suggestions

Juvenile spring Chinook salmon become infected with *C. shasta* during their time in the Pelton Ladder below the Reregulating Dam (Appendix A). Sentinel studies conducted by Don Ratliff between 1973-1974 suggest that releasing juvenile Chinook salmon on or before May 3rd of each year decreases exposure to *C. shasta* (Ratliff, 1981). The seasonal patterns of *C. shasta* in the Deschutes River have not changed drastically since these reports nearly 40 years ago, suggesting that the May 3rd cut-off date might still apply. Ratliff also proposed that short exposures to *C. shasta* before their release provided resistance to the parasite and decreased mortalities (Ratliff, 1983). Short exposure of juvenile spring Chinook salmon to a genotype they are less susceptible to (genotype II) followed by exposure to the host specific genotype I did not reveal increased resistance to the parasite (Hurst and Bartholomew, 2015). I propose an experiment where juvenile spring Chinook salmon are exposed to a low dose of genotype I, given time to pass the infection in pathogen free water, and then re-exposed to genotype I at the same dose which would be expected in the Deschutes River (Chapter 3).

Several treatments were considered by Sanders et al. (1972) for the Round Butte Hatchery on the Deschutes River. In that study, 500 juvenile Steelhead were subjected to Deschutes River water that was either filtered using MicroFLOC, a combination of MicroFLOC and chlorination, or water that was UV irradiated. The MicroFLOC alone was not enough to completely combat *C. shasta* (mortality rate of 6% due to *C. shasta*), but the other two treatments were effective (up to 1% mortality rate, but *not* due to *C. shasta*). Currently, the only water treatment at the Round Butte Hatchery involves UV irradiation for the eggs and young juveniles. After the juveniles are transferred to outdoor raceways at the Pelton Ladder, they are exposed to untreated Deschutes River water (ODFW, pers. comm.).

Since the water treatments are not feasible on a large scale, I suggest a combination of the strategies proposed by Ratliff, Sanders, and Fryer. A controlled amount of *C. shasta* could be administered to juveniles at the hatchery followed by exposure to water treated by MicroFLOC filtration-chlorination and UV irradiation. Those individuals in the population which have acquired resistance might have a better chance of clearing the infection and building immunity to *C. shasta* if they are in pathogen free water prior to being released in the Deschutes River. This hypothesis would require further testing before being implemented.

Field management suggestions

Copepods have been observed ingesting actinospores of the myxozoan parasite, *Myxobolus pseudodispar* without becoming infected (Rácz et al., 2006). Interestingly, copepods are the most common zooplankton in the Lake Billy Chinook reservoir (Thiesfeld, 1999), where *C. shasta* abundance was low throughout the summer of 2018 (Figure 2.9). Copepods only comprise 0.3-0.4% of the macroinvertebrate composition in the lower Deschutes River below the reregulating dam and above Shitike creek, respectively (R2 Resource Consultants, 2000). Those percentages decrease the farther downstream they were surveyed for with copepod abundance reaching 0% below the Warm Springs River and beyond, where *C. shasta* abundance is highest (Figure 2.9). I propose that native calanoid and/or cyclopoid copepods be examined as a biological filter for *C. shasta*. To test this idea in a laboratory setting, a filtering system could be

developed where *C. shasta*-laden water could be either held in or passed through a tank with copepods before fish are introduced to the water.

Warm Springs River water quality

It was revealed in April 2018 that pollution from the Warm Springs waste water treatment facility, Kah-Nee-Ta resort, and seepage from the dump have been accumulating in the Warm Springs River for an undocumented amount of time (Cyndi Baker, CTWSRO). USGS will be conducting soil quality surveys to assess the extent of the damage using juvenile Lamprey as a proxy for the effects these pollutants may have on other freshwater species in the area.

Water quality issues in the Deschutes River have been noted as a potential contributor to juvenile and pre-spawn mortality since 1989 (Lindsay, 1989). According to that report, pre-spawn mortality was between 34-75% from 1977-1986, even with inoculations for the suspected contributor to mortality, *Renibacterium salmoninarum* from 1982-1986. Since the Warm Springs National Fish Hatchery (WSNFH) is supplied with water from the Warm Springs River, the juvenile spring Chinook salmon reared at that hatchery have likely been affected by the poor water quality. With the trace amounts of *C. shasta* observed in the Warm Springs River and juveniles reared at the WSNFH we suggest that the water quality issues should be investigated further as an important contributor to juvenile and pre-spawn mortality in the Warm Springs River.

Polychaete management

The determinate host of *C. shasta*, *Manayunkia* sp., is a polychaete worm which forms its habitat out of detritus casings in and on benthic substrate and sediment. In the Klamath River, winter-spring flushing flows have been shown to reduce polychaete aggregations (Malakauskas et al., 2013; Alexander et al., 2014, 2016) and are currently mandated in this system (United States District Court for the Northern District of California San Francisco Division, 2017). Additionally, emergency dilution flows are mandated at 5 *C. shasta* spores/L as this density has been quantified as the infective dose threshold that corresponds to 40% mortality for Coho salmon of the Klamath River (Hallett et al., 2012). The infection dose (density of spores and genotypes) and infection

dose threshold are different for different species (Ray et al., 2013). In order to implement emergency flows in the Deschutes River, these parameters would need to be defined through sentinel fish and water sample studies to determine the infective dose for spring Chinook salmon of the Deschutes River. Additionally, the highest *C. shasta* densities are at least 78 rkm downstream of the Reregulating Dam. This distance might affect the ability of flushing flows to remove polychaetes in the target region of rkm 48-82.

The spatial and temporal distribution of the polychaete host, *Manayunkia* sp., in the Deschutes River is not well described but preliminary investigations are underway (J. Alexander, pers. comm.). Because this host releases the *C. shasta* spore stages that infect the salmonid host (actinospores), these data are important for understanding *C. shasta* dynamics in the Deschutes River system. I recommend including such data in models to describe salmonid risk of enteronecrosis as a function of variation in parasite spatial (data available from 2015-2018), and temporal distribution (data available for parts of 2015-2018), winter snowpack (USDA; available from 1901-2018), river discharge (USGS; available from 1928-2018), water temperatures (USGS available from 1950-2018) fish passage data from PGE (data available from 2014-2018). Following the successful development and validation of correlative statistical models, models could then be developed for predicting the risk of *C. shasta* infection for the salmonid species of the Deschutes River in future water years.

The effects of temperature and discharge on the temporal and spatial distribution of Ceratonova shasta

The unequal variability of mean *C. shasta* abundance across the range of values of temperature and discharge was observed in residual plots of our linear mixed effect models. While we had strong correlations between our variables, the residual plot results indicate that additional data should be collected from monitoring sites over several years before a predictive model can be developed for the effects of water temperature and river discharge on *C. shasta* abundance in the Deschutes River basin.

Unidentified genotype determination

We observed unidentifiable genotypes and mixtures in 10.4% (11/105) of the water samples spread across both basins, but with different sequences in each basin. Additional work, such as novel primer design or cloning, is needed to determine if these sequences represent previously un-sequenced genotypes of *C. shasta* or a new *Ceratonova* species.

REFERENCES

Alexander, J. D., S. L. Hallett, R. W. Stocking, L. Xue, and J. L. Bartholomew. 2014. Host and parasite populations after a ten-year flood: *Manayunkia speciosa* and *Ceratonova* (syn *Ceratomyxa*) shasta in the Klamath River. Northwest Science 88(3):219–233.

Alexander, J. D., J. L. Bartholomew, K. A. Wright, N. A. Som, and N. J. Hetrick. 2016. Integrating models to predict distribution of the invertebrate host of myxosporean parasites. Freshwater Science 35(4):1263–1275.

Bartholomew, J. L., E. Ray, B. Torell, M. J. Whipple, and J. R. Heidel. 2004. Monitoring *Ceratomyxa shasta* infection during a hatchery rearing cycle: comparison of molecular, serological and histological methods. Diseases of Aquatic Organisms 62(1–2):85–92.

Deschutes River Conservancy. 2018. By the numbers: Trapping, transporting salmonids in reintroduction efforts in blocked upper Deschutes. https://www.deschutesriver.org/news-and-resources/news-&-media/news/by-the-numberstrappingtransporting-salmonids-in-reintroduction-efforts-in-blocked-upper-deschutes

Hallett, S. L., and J. L. Bartholomew. 2006. Application of a real-time PCR assay to detect and quantify the myxozoan parasite *Ceratomyxa shasta in* river water samples. Diseases of Aquatic Organisms 71(2):109–118.

Hallett, S. L., R. A. Ray, C. N. Hurst, R. A. Holt, G. R. Buckles, S. D. Atkinson, and J. L. Bartholomew. 2012. Density of the waterborne parasite *Ceratomyxa shasta* and its biological effects on salmon. Applied and Environmental Microbiology 78(10):3724–3731.

Hoffmaster, J. L. 1985. Geographic distribution of *Ceratomyxa shasta* in the Columbia River basin and susceptibility of salmonid stocks.

Hurst, C. N., and J. L. Bartholomew. 2012. *Ceratomyxa shasta* genotypes cause differential mortality in their salmonid hosts. Journal of Fish Diseases 35(10):725–732.

Hurst, C. N., and J. L. Bartholomew. 2015. Lack of protection following re-exposure of Chinook salmon to *Ceratonova shasta* (Myxozoa). Journal of Aquatic Animal Health 27(1):20–24.

Lindsay, R. B., B. C. Jonasson, R. K. Schroeder, and B. C. Cates. 1989. Spring Chinook salmon in the Deschutes River, Oregon. Oregon Department of Fish and Wildlife Information Report: 89-4.

Malakauskas, D. M., S. J. Willson, M. A. Wilzbach, and N. A. Som. 2013. Flow variation and substrate type affect dislodgement of the freshwater polychaete, *Manayunkia speciosa*. Freshwater Science 32(3):862–873.

Oregon Department of Fish and Wildlife (ODFW) and Confederated Tribes of the Warm Springs (CTWS). 2017. Hatchery and Genetic Management Plan

R2 Resource Consultants. 2000. Lower Deschutes River Macroinvertebrate Monitoring Report.

Rácz, O., C. Székely, and K. Molnár. 2006. The role of copepods (*Cyclops* spp.) in eliminating the actinospore stages of fish-parasitic myxozoans. Acta Veterinaria Hungarica 54(1):61–70.

Ratliff, D. E. 1981. *Ceratomyxa shasta*: Epizootiology in Chinook salmon of Central Oregon. Transactions of the American Fisheries Society 110(4):507–513.

Ratliff, D. E. 1983. *Ceratomyxa shasta*: Longevity, distribution, timing, and abundance of the infective stage in Central Oregon. Canadian Journal of Fisheries and Aquatic Sciences 40(10):1622–1632.

Ray, R. A., and J. L. Bartholomew. 2013. Estimation of transmission dynamics of the *Ceratomyxa shasta* actinospore to the salmonid host. Parasitology; Cambridge 140(7):907–16.

Sanders, J. E. 1967. Occurrence of the protozoan parasite *Ceratomyxa shasta* among salmonid fishes in Oregon waters.

Sanders, J. E., J. L. Fryer, D. A. Leith, and K. D. Moore. 1972. Control of the infectious protozoan *Ceratomyxa shasta* by treating hatchery water supplies. The Progressive Fish-Culturist 34(1):13–17.

Stinson, M. E. T., and J. L. Bartholomew. 2012. Predicted redistribution of *Ceratomyxa shasta* genotypes with salmonid passage in the Deschutes River, Oregon. Journal of Aquatic Animal Health 24(4):274–280.

Stinson, M. E. T., S. D. Atkinson, and J. L. Bartholomew. 2018. Widespread distribution of *Ceratonova shasta* (Cnidaria: Myxosporea) genotypes indicates evolutionary adaptation to its salmonid fish hosts. Journal of Parasitology 104(6):645–650.

Thiesfeld S. L., J. C. Kern, A. R. Dale, M. W. Chilcote, and M. A. Buckman. 1999. Lake Billy Chinook, Sockeye salmon, and kokanee research study 1996-1998 contract completion report. Pelton round butte hydroelectric project. Ferc no. 2030.

United States District Court for the Northern District of California San Francisco Division. 2017. Hoopa Valley Tribe v. U.S. Bureau of Reclamation and Klamath Water Users Association, et al. Document 111. Case No. 3:16-cv-04294-WHO.

APPENDIX A: DETECTION OF *CERATONOVA SHASTA* IN ANAL SWABS AND SALMONID TISSUES THROUGH PCR AND HISTOLOGICAL ANALYSES AND CO-INFECTION WITH OTHER MYXOZOAN, BACTERIAL, AND VIRAL PATHOGENS

Kalyn Hubbard, Jerri Bartholomew, and Sascha Hallett

ABSTRACT

Ceratonova shasta has been a suspected contributor to juvenile and pre-spawn mortality in spring Chinook salmon of the Deschutes River since the 1970's. qPCR of water samples and histology are an effective paired mechanism for detecting pathogens related to pre-spawn mortality in adult Chinook salmon of the Willamette River. We applied these methods in the Deschutes River to indicate contribution of *C. shasta* and non-target pathogens to juvenile and pre-spawn mortality in spring Chinook salmon. While the spatial and temporal distribution of *Ceratonova shasta* in the Deschutes River was defined through parasite quantification (qPCR) in water samples (Chapters 2 and 3), fish tissue sampling at the Round Butte Hatchery and Pelton Ladder was necessary to ascertain pathogen related disease in the salmonid host. In addition to *C. shasta*, pathology associated with *Renibacterium salmoninarum* and *Parvicapsula minibicornis* was observed in juveniles and adult spring Chinook salmon, respectively. Lethal sampling of salmonids is not always permitted, as is the case at the Warm Springs National Fish Hatchery (WSNFH). During *C. shasta* and non-target pathogen surveys conducted at the Round Butte Hatchery in 2017 and 2018, the use of anal swabs as an effective sampling technique for *C. shasta* in adult spring Chinook salmon was tested to inform potential implementation at the WSNFH. Anal swabs were an effective detection method for *C. shasta* (100%) when the swabs had a wooden applicator and were submerged in 100% ethanol immediately after collection. Although *C. shasta* was the most common pathogen that we detected and infections were pathogenic in spring Chinook salmon, co-infection and associated pathology with other pathogens suggests that *C. shasta* is not the sole contributor to juvenile and pre-spawn mortality in the Deschutes River. However, we recommend continued monitoring of the pathogen through opportunistic lethal sampling as well as non-lethal detection through anal swabs.

INTRODUCTION

The myxozoan parasite *Ceratonova shasta* was first identified as a lethal pathogen to salmonids in the Deschutes River basin in 1965 (Conrad and Decew, 1966). Since then, significant juvenile and pre-spawn mortalities of Chinook salmon in this system have occurred (Baker and Lovtang, 2016). Hypotheses that *C. shasta* was contributing to these mortalities were assessed through collaborative studies with the Oregon Department of Fish and Wildlife (ODFW), the Confederated Tribes of the Warm Springs Reservation of Oregon, and the United States Fish and Wildlife Service (USFWS) involving water sampling surveys and sentinel fish exposures during 2015 and 2016 to assess prevalence of *C. shasta*. These initial studies indicated that *C. shasta* was abundant in certain locations in the basin and was associated with high mortality in sentinel juvenile spring Chinook (Vojnovich et al., 2016). These studies also revealed that coinfections were common (up to 91%) with pathogens such as *C. shasta*, *Renibacterium salmoninarum*, and fungi in juveniles from the Warm Springs Hatchery and the Round Butte Hatchery exposed at the Pelton Trap and Oak Springs. In addition to *R. salmoninarum*, non-target pathogens to our study might include *Tetracapsuloides bryosalmonae*, *Parvicapsula minibicornis*, infectious hematopoietic necrosis virus (IHNV), and infectious pancreatic necrosis virus (IPNV).

Potential non-target pathogens in the Deschutes River

Renibacterium salmoninarum is the causative agent of bacterial kidney disease. This pathogen can infect juvenile salmonids at hatcheries. High densities of juvenile fish at these facilities exacerbates the horizontal transmission of this bacterial pathogen. While many freshwater pathogenic infections decrease in severity or leave the host entirely as the salmonid enters the ocean, bacterial kidney disease continues to proliferate in infected salmonids (Sanders et al., 1992). Due to challenges of effectively managing this bacterium and the severity of disease that it can inflict, the prevalence of *R. salmoninarum* is closely monitored by many state hatcheries including the Round Butte Hatchery on the Deschutes River.

Tetracapsuloides bryosalmonae is a malacosporean parasite known to cause proliferative kidney disease (Kent et al., 2000). This pathogen was responsible for a die-

off of mountain whitefish (*Prosopium williamsoni*) in the Yellowstone River during 2017 (Carraro et al., 2018). Due to concern that this parasite could be in the Deschutes River, spring Chinook salmon kidneys were assayed for this parasite.

Parvicapsula minibicornis infects the kidneys of salmonids and can cause organ degradation (Kent et al., 1997). Within infected kidney tissues, *Parvicapsula minibicornis* is located in the renal interstitium (Jones et al., 2003), glomeruli, and renal tubules (Bradford et al., 2010b). Clinical signs include breach of glomerular membranes, eosinophic material in glomeruli (Bradford et al., 2010a), and glomerulonephritis (Foott et al., 2006). This myxozoan parasite has been detected in the Sacramento, Klamath, Fraser, and Columbia River basins (Atkinson et al., 2011) and is known to affect Sockeye salmon populations (Jones et al., 2003; Bradford et al., 2010). Since *P. minibicornis* shares a polychaete host with *C. shasta*, we tested for this parasite in kidneys of Deschutes River spring Chinook salmon.

The rhabdovirus IHNV and aquatic birnavirus IPNV have nearly global distribution and can be transferred horizontally or vertically (Dixon et al., 2016). These viruses can be detected throughout the fish, depending on the mode of viral entry. Therefore, Oregon hatcheries such as the Round Butte Hatchery test the kidney, pyloric caeca, spleen, and ovarian fluid of adult salmonids for IHNV and IPNV (AFS-FHS Blue Book, 2014). IHNV has been detected at the Round Butte Hatchery since 1975 (Mulcahy, 1976). IPNV is routinely tested for, but has not been detected at Round Butte Hatchery (ODFW communications).

Anal swabs as a non-lethal detection method in the Deschutes River

The current method for detection of *C. shasta* in fish involves the collection of lower intestine from deceased and/or culled fish. While such samples can be obtained from adult spring Chinook salmon at the Round Butte Hatchery, these methods cannot be applied at the Warm Springs National Fish Hatchery. Since *C. shasta* accumulates in the lower intestine, we hypothesized that the non-lethal anal swabbing method described by Fox et al. (2000) could be tested at the Round Butte Hatchery during spawning surveys in late August of 2017 and 2018 for potential implementation at the Warm Springs National Fish Hatchery.

***Ceratonova shasta* genotype in salmonid tissue samples**

While abundance of *C. shasta* in water samples as well as prevalence of infection in tissue samples are important indicators that this parasite is affecting salmonids, the other consideration is which genotype of *C. shasta* the salmonid is infected with. There are three known primary genotypes of *C. shasta*: type O, I, and II. While the genotype-salmonid host specificity has been determined in the Deschutes River for multiple species during sentinel surveys in 2010 (Stinson and Bartholomew, 2012; Stinson et al., 2018), we sought to reaffirm the presence of *C. shasta* genotype I in spring Chinook salmon and describe the genotype that infects Coho salmon of the lower Deschutes River.

METHODS

Study sites (Figure A.1)

The Pelton Ladder (near Pelton Trap) at river kilometer (rkm) 160 is located downstream of the Reregulating dam (rkm 162) and is where juvenile spring Chinook salmon are held from early November until their release in April of each year (PGE).

Adult spring Chinook salmon are captured at the Pelton Trap where the fish are sorted by origin and species. Hatchery fish are trucked upstream to be spawned at the Round Butte Hatchery below the Round Butte Dam (rkm 165).

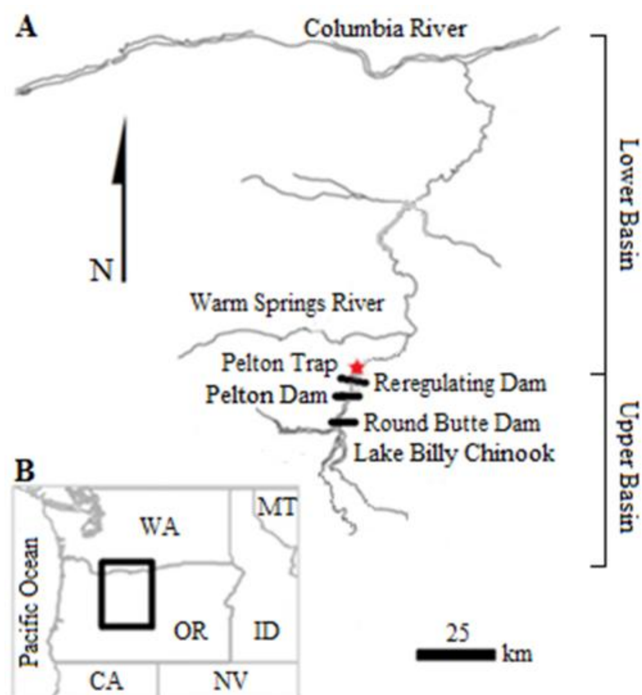

FIGURE A.1: The lower Deschutes River highlighting the Round Butte Dam complex (bars) and Pelton Trap (star) between river kilometers 160-165.

Fish Tissues

Tissue collections — In the collection of both juvenile and adult fish tissues from 2017-2018, utensils were sterilized by submersion in 10% bleach for one minute and gloved hands were rinsed in iodine solution between fish (AFS-FHS, 2014).

Kidney and lower intestine samples were collected for histological analysis (29 fish) and molecular analysis (59 fish) as a subsample of the spring Chinook salmon stock spawned at the Round Butte Hatchery on August 29th, 2017. Kidney and lower intestinal samples were also collected from 60 adults during a spawn at Round Butte Hatchery on August 28th, 2018 for molecular analysis. An anal swab (see below for details) was collected from each fish at this time to compare the non-lethal *C. shasta* assay developed by Fox et al. (2000) with direct tissue sampling. Fish were incised from cloaca to pectoral fin during the spawning process, their snouts were collected, and the fish were placed in a large tub. The most recently deceased salmon was selected for sampling.

Intestines and kidneys were also collected for histology and molecular analysis from 60 juvenile Chinook at the Pelton Trap on January 23rd, 2018 by ODFW and April 4th, 2018 by me. On April 4th, juvenile fish were bled, inspected, weighed, measured, and split ventrally by a crew from the National Oceanic and Atmospheric Association (NOAA) before tissue samples were taken.

For histology samples, each kidney and lower intestine sample was less than 3 mm thick and fixed in 45 ml of Dietrich's solution at a minimum 1:10 ratio of tissue to solution (Fournie et al., 2000). After 72 hours, the fixed tissues were cut to fit cassettes and suspended in a jar of formalin. Sectioning and staining with hematoxylin and eosin (H&E) were performed by the Oregon Veterinary Diagnostic Laboratory. Additional 3 mm sections of lower intestine and kidney were excised and preserved in 200 µl of 100% ethanol in a 2 ml tube, then stored at -20 °C for molecular analysis.

In addition to spring Chinook salmon tissues, 61 DNA-extracted and *C. shasta*-positive (PCR) lower intestine and kidney samples from Coho salmon were collected opportunistically from 2014-2018 and sent to us by Ken Lujan (USFWS).

Molecular analysis of fish tissues — Each 3 mm section of lower intestines and kidney sample collected at the spawns in late August of 2017 and 2018 as well as the juvenile

fish tissues collected in April 2018 received 180 µl of buffer ATL and 20 µl proteinase K to break down the tissues for DNA extraction. The samples were vortexed after 10 minutes and placed in a rocking incubator set to 37°C overnight (Palenzuela et. al., 1999).

Kidneys have more RNA than other tissues that can increase smearing during gel electrophoresis. Therefore, an RNase treatment was applied to the kidney samples after overnight incubation. 10 µl of diluted RNase was added to each crude kidney sample (AFS-FHS, 2014). Either after the RNase treatment or after sitting overnight, all samples were vortexed and placed in a dry heat block set to 85 °C for at least 15 minutes. At this point, the residual proteinase K had denatured leaving the tubes with extracted DNA. After DNA extraction, parasite genotype was determined for a subsample of tissues (see below).

PCR assay for *C. shasta* was performed with Cs1479F and Cs2067R primers to amplify ribosomal DNA of *C. shasta* (Atkinson et al., 2018). The thermocycling program involved 35 cycles of 95 °C for 3 min, 95 °C for 1 min, 66 °C for 1 min, 72 °C for 1 min.

The kidneys of both juvenile and adult spring Chinook salmon were PCR-tested for *Tetracapsuloides bryosalmonae* and *Parvicapsula minibicornis*. The *T. bryosalmonae* assay utilized the primers MALATK5F and ERIB10 (unpublished, provided by Stephen Atkinson). The PCR thermocycling program ran for 35 cycles at 95 °C for 2 min, 94 °C for 20 sec, 58 °C for 1 min, 72 °C for 1:30 min (unpublished, provided by Stephen Atkinson). The *P. minibicornis* PCR assay was performed using the PMATK1f and PMATK2r primers according to Atkinson et al. (2011).

4 µl of each amplified sample was pipetted into a 1% agarose gel well for electrophoresis. The gels were run at 160 mV for 15 minutes then photographed using a GelDoc-It Imaging System (Analytik Jena). Presence of a ~588 base pair (bp) amplicon is considered a positive indication of *Ceratonova shasta*. Whereas positive detection of *T. bryosalmonae* and *P. minibicornis* was a ~950 and ~934 bp amplicon, respectively.

As part of their monitoring program, ODFW uses sandwich enzyme-linked immunosorbent assay (ELISA) to detect *R. salmoninarum* from salmonid kidneys (AFS-FHS, 2014). They also take samples of pyloric caecum, kidney, and spleen as well as

ovarian fluid to culture for infectious hematopoietic necrosis virus (IHNV) and/or infectious pancreatic necrosis virus (IPNV) (AFS-FHS, 2014). Results were combined for more comprehensive screening of pathogens in spring Chinook salmon of the Deschutes River.

Genotype determination — Ceratonova shasta-DNA from both tissue sample types was genotyped from 10 spring Chinook salmon and 10 Coho salmon using the PCR process described above. We estimated DNA yield based on the brightness of band fluorescence in gel electrophoresis, and used simple dilution of this product for sequencing. Samples were Sanger sequenced with forward primer Cs1479F at the Center for Genome Research and Biocomputing (CGRB) at Oregon State University. Sequence chromatograms were inspected by eye and characterized for genotype based on INDEL and SNP patterns using BioEdit (Hall, 1999; Atkinson and Bartholomew, 2010). Genotypes O, I or II were determined based on a respective number of ATC repeats in the ITS-1 region (Atkinson and Bartholomew, 2010; Atkinson et al., 2018).

Round Butte Hatchery anal swabs

Anal swabs were collected during tissue collections at spawning surveys at the Round Butte Hatchery in late August of 2017 and 2018 from 30 and 48 adult spring Chinook salmon, respectively. Cotton swabs with a wooden applicator were used to collect mucus from the anus of each fish. During the 2017 collection, fish were split ventrally and swabs were inserted into the vent. The swab was briefly swiped on a clean, pre-labeled microscope slide. This technique was abandoned in 2018 due to an inability to decipher *C. shasta* spores from sperm and blood swab contaminants. In an effort to mimic the collection of anal swabs from live fish, the collection method was revised in 2018 and the anal swab from each adult was taken after the fish was culled but before the fish was opened and spawned. A numbered tag was placed on the fish, the fish was spawned according to standard hatchery protocol, and the anal swab samples were linked to their corresponding tissue samples by the paper tags and labeled tubes, respectively.

Unlike the method developed by Fox et al. (2000) the tips of swabs were broken off and submerged in a 2 ml tube with 200 µl of 100% ethanol. In the lab, the swab tubes were opened, placed in a heated vacuum centrifuge, and the ethanol evaporated. Using a

crude extraction protocol (Palenzuela et al., 1999), 180 µl of ATL buffer and 20 µl of proteinase K was added to each tube. These tubes were vortexed, and incubated overnight at 37°C. For full degradation of proteinase K, the tubes were incubated for an additional 25 minutes at 85°C. The samples were assayed for *C. shasta* by PCR directly after this step or stored at -20°C.

Warm Springs National Fish Hatchery anal swabs

The Warm Springs National Fish Hatchery staff collected 47 anal swabs from spring Chinook salmon in the Warm Springs River during 2017 and 17 anal swabs in 2018. These samples were posted to OSU. In 2017, the swabs used had cotton applicators. In 2018, cotton swabs with wooden applicator tips were used. The swabs were not submerged in ethanol either year.

RESULTS

All 59 adult hatchery fish sampled at Round Butte Hatchery on August 30th, 2017 tested positive for *C. shasta* by PCR of tissue samples (Table A.1.). However, only 78% (46/59) of the anal swabs taken from the same fish were PCR-positive. Myxospores were not detected visually because the samples were dominated by blood and sperm. In 2018, 100% of the 60 adult hatchery spring Chinook salmon were positive for *C. shasta* by PCR of tissue samples. With the modified system of collecting anal swabs post-cull but pre-spawn, 100% of the 48 anal swabs detected *C. shasta*. Despite extracting anal swabs using the same process outlined for the Round Butte Hatchery, amplification of *C. shasta* from swabs collected at the Warm Springs National Fish Hatchery did not occur in 2017 or 2018.

Histological examination of 10/29 of the adult spring Chinook salmon collected in 2017, revealed myxospores and presporogonic forms of *C. shasta* contributing to deterioration of the lower intestine. The mucosal epithelium was completely sloughed off, leaving an internal negative space between the mucosal epithelium and the basal layer of the lower intestine (Figure A.2). Presporogonic forms of *C. shasta* were also observed in the 8/10 kidney slides from this cohort.

Kidneys and lower intestines of five juvenile spring Chinook salmon (sampled on April 4th, 2018) that were PCR-positive for *C. shasta* in both organs were selected for histology. Presporogonic forms of *C. shasta* were observed in histological sections of the lower intestines of the five juvenile spring Chinook salmon collected in 2018. Juvenile samples ranged in severity from mild (grade 2) to moderate (grade 3) (Bartholomew et al., 2004). Mild signs of pathology included inflammatory foci surrounding parasites but not mucosal damage or necrosis. Moderate signs are observed as inflammatory and necrotic foci, parasites and necrotic cells in the lumen, and slight sloughing of the mucosal epithelium.

Sequence analysis of *C. shasta* infections in the intestines of juvenile spring Chinook salmon (3) as well as the kidneys (3) and lower intestines (4) revealed only genotype I. In contrast, only type II was detected in the lower intestines of the ten Coho salmon (Table A.2).

Parvicapsula minibicornis was detected by PCR in 76% (45/59) of kidney samples collected from adult spring Chinook salmon at the Round Butte Hatchery on August 30th, 2017. Of the 29 histology subsamples, 8 of those were positive for *P. minibicornis* by PCR and inspected for pathology related to the parasite (Figure A.3). Presence of the parasite in glomeruli and mild glomerulonephritis was observed in all eight tissue samples. Using the scoring system outlined by Dolan et al. (2016) these clinical signs translated to moderate pathology.

Tetracapsuloides bryosalmonae was not detected by PCR in kidney tissues of adult or juvenile spring Chinook salmon in 2017 or 2018. Prevalence of infection with viruses and *R. salmoninarum* were provided by collaborators at ODFW. *Renibacterium salmoninarum* was detected at a low prevalence in 2017 and 2018 adults (0.1-1%, respectively), however, in 2018, 16.7% of juveniles tested positive. Infection of *R. salmoninarum* in juveniles was also detected by visualization of gross pathology including granulomatous-like lesions on the kidney (Richards et al., 2017). Viruses were not detected in adult tissues in 2017, but 13% tested positive in 2018 for IHNV. Fish were not assigned a specific identifier for the *R. salmoninarum* or IHNV assays. Therefore, we do not have information on which fish had dual infections with these

pathogens and *C. shasta*. While the Round Butte Hatchery routinely tests for IPNV, it has not been detected. Summaries of these pathogen assay results and tissue collection dates are provided in Table A.1.

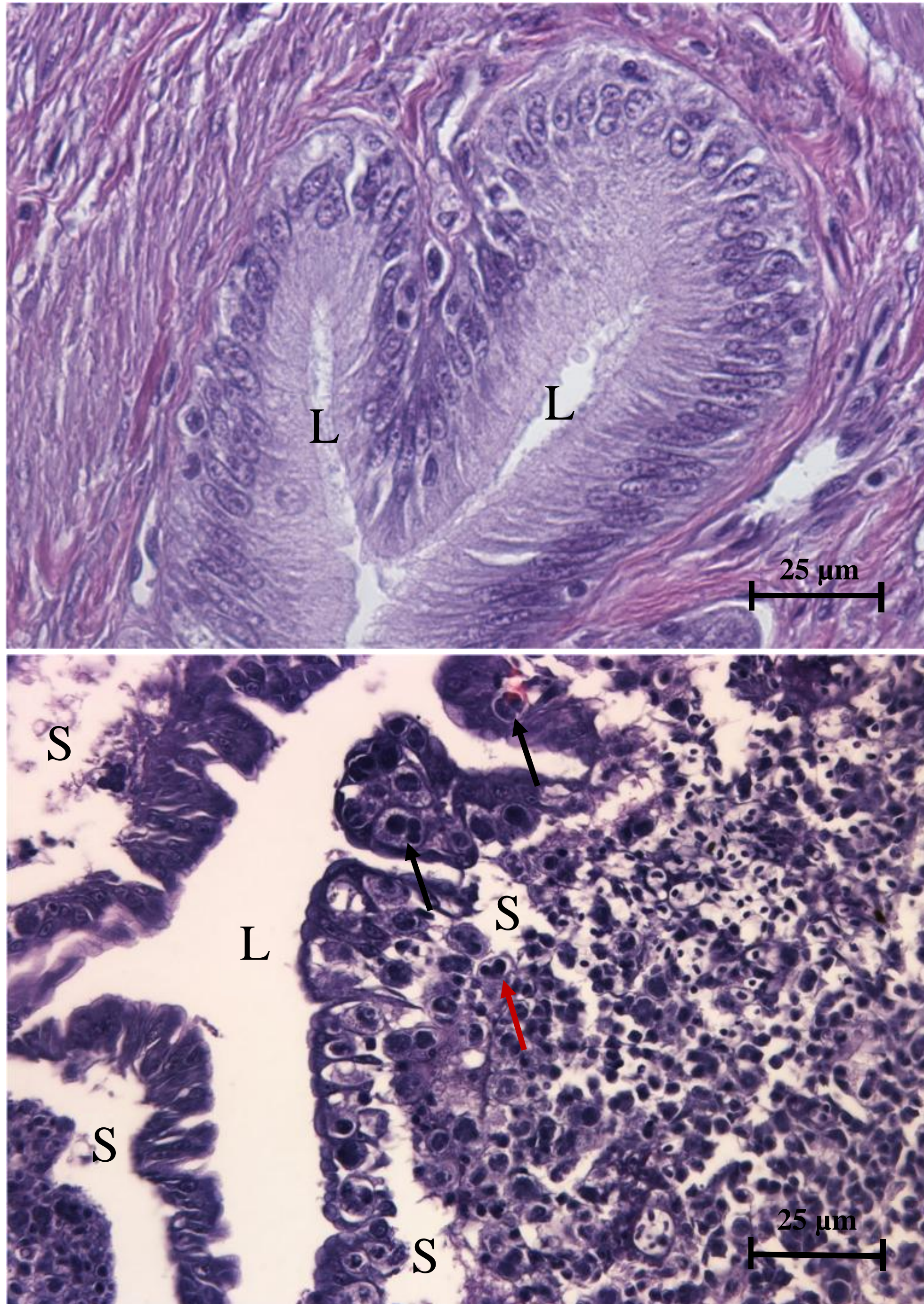

FIGURE A.2: Lower intestines fixed with Dietrich's and stained with hematoxylin and eosin, and visualized at 400x collected from adult spring Chinook salmon on August 29th, 2017 from the Round Butte Hatchery. Healthy adult spring Chinook salmon (upper). Necrotic lower intestine with presporogonic forms (black arrows) and myxospores (red arrow) of *Ceratonova shasta*. Lumen (L) and areas of sloughed epithelium (S) have been highlighted.

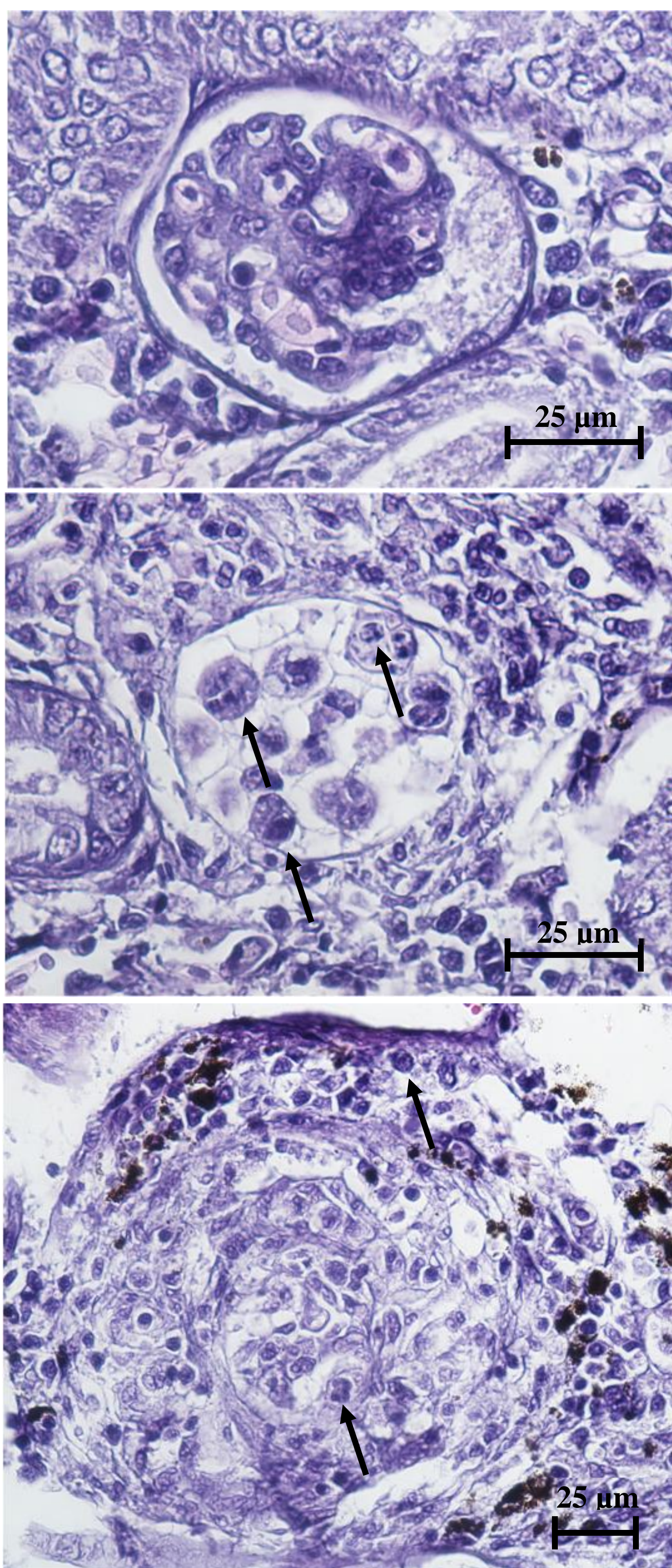

FIGURE A.3: Kidneys fixed with Dietrich's fixative, stained with hematoxylin and eosin, and visualized at 400x collected from adult spring Chinook salmon on August 29th, 2017 from the Round Butte Hatchery. Healthy adult spring Chinook salmon glomeruli (upper). Glomerulus infected with *Parvicapsula minibicornis* (center) and inflamed glomerulus (glomerulonephritis; lower) with parasites highlighted by black arrows.

TABLE A.1: Summary of 2017 and 2018 *C. shasta* (Cs), *P. minibicornis* (Pm), *T. bryosalmonae* (Tb), *R. salmoninarum* (Rs) and viral (Infectious hematopoietic necrosis virus (IHNV), and infectious pancreatic necrosis (IPNV)) assay results for juveniles and adult spring Chinook salmon at the Round Butte Hatchery. Results provided by ODFW in gray text.

	Cs	***Cs***	***Pm***	***Tb***	***Rs***	***IHNV, IPNV***
Tissue	Lower intestines	Anal swabs	Kidneys	Kidneys	Kidneys	Kidneys, spleen, pyloric caeca
Method	PCR	PCR	PCR	PCR	ELISA	Culture
Juveniles						
1/23/18	27.5% (8/28)	ND	ND	ND	ND	ND
4/4/18	58% (35/60)	ND	0% (0/60)	0% (0/60)	16.7% (10/60)	ND
Adults						
8/30/17	100% (59/59)	78% (46/59)	76% (45/59)	0% (0/59)	0.01% (5/468)	0% (0/210)
8/28/18	100% (60/60)	100% (48/48)	70% (42/60)	0% (0/60)	1% (4/412)	13% IHNV (20/154) 0% IPNV

Table A.2: Summary of Deschutes River fish tissues genotyped for *Ceratonova shasta*

Species	*Collection Date*	*Location*	*Life stage*	*Condition*	*Tissue*	*Samples genotyped*	*Genotype*
spring Chinook salmon	08/30/17	Round Butte Hatchery	Hatchery Adult	Spawned	Kidney	3/56	Type I
spring Chinook salmon	7/13/18-08/27/18	Round Butte Hatchery	Hatchery Adult	Pre-spawn mortality	Lower Intestine	4/8	Type I
spring Chinook salmon	4/4/18	Round Butte Hatchery	Hatchery Juvenile	Pre-release	Lower Intestine	3/60	Type I
Coho salmon	10/21/15	Warm Springs R	Wild Adult	Survey Capture	Lower Intestine	3/5	Type II
Coho salmon	12/08/15	Buckhollow Creek	Hatchery Adult	Survey capture	Lower Intestine	2/5	Type II
Coho salmon	12/10/15	Buckhollow Creek	Hatchery Adult	Survey Capture	Lower Intestine	1/1	Type II
Coho salmon	11/08/17	Warm Springs R	Wild Adult	Survey capture	Lower Intestine	1/1	Type II
Coho salmon	11/14/17	Warm Springs R	Wild Adult	Survey capture	Lower Intestine	3/8	Type II

DISCUSSION

Of the six salmonid pathogens that were tested for in juvenile and adult spring Chinook salmon of the Deschutes River, we detected four. These included the myxozoan parasites *C. shasta* and *P. minibicornis*, the bacterium *R. salmoninarum* and the virus IHNV. However, IPNV and the myxozoan *T. bryosalmonae* were not detected. *Ceratonova shasta* was most prevalent, infecting all fish groups tested (at 27.5 – 100%). Myxospores were observed in the lower intestines of adult spring Chinook salmon and presporogonic forms were detected in the lower intestines of juvenile spring Chinook salmon. In either life stage, sloughing of the mucosal epithelium was observed by histology and these infections were confirmed to be genotype I (Table A.2).

Infection of juvenile spring Chinook salmon by *C. shasta* as early as January 23rd, 2018 is concerning, but not surprising, as these juveniles are reared at the Round Butte Hatchery and the Pelton Ladder for a year before their release in late March/early April. The beginning of the infective period of *C. shasta* in the Deschutes River has been observed between 6.9 – 8.6 °C (Ratliff, 1981). The temperature was 7.1 °C on January 23rd and the range of water temperatures at the Pelton Trap (below the Round Butte Hatchery) during the winter of 2017 were between 6.6 and 9.0 °C. In addition to water temperatures conducive to parasite proliferation during winter, juvenile Sockeye salmon, adult Steelhead, adult Fall Chinook salmon, and resident trout also over winter in the Deschutes River (Chapter 3) and potentially contribute myxospores during this time. From what we know about degree days in the Klamath River (Ray et al., 2012), it is possible that *C. shasta* could be completing its life cycle during the winter months in the Deschutes River.

The use of anal swabs as a non-lethal sampling technique for *C. shasta* is less effective than PCR of lower intestines, but more effective than microscopy alone (Fox et al., 2000). With the modification of submerging anal swabs in ethanol, this method was also successful at detecting *C. shasta* from 100% (48/48) of adult spring Chinook salmon that were positive for *C. shasta* by PCR of lower intestines at the Round Butte Hatchery on August 28th, 2018. During anal swab collection at the Warm Springs Hatchery, the

swabs used had cotton applicators that soaked up extractable liquid in 2017. In 2018, cotton swabs with wooden applicator tips were used. Swabs were not submerged in ethanol during 2017 or 2018 collections. Despite extracting anal swabs using the same process outlined for the Round Butte Hatchery, amplification of *C. shasta* from swabs collected at the Warm Springs National Fish Hatchery (WSNFH) did not occur in 2017 or 2018. The addition of 200 µl of 100% ethanol to the anal swab protocol at the WSNFH will likely bolster the efficacy of the assay.

The lower intestine samples of Coho salmon sent to us by USFWS were not candidates for histology because they were in the form of extracted DNA. Therefore, severity of infection could not be determined, but we were able to sequence genotype II. This result is complimentary to the previously observed genotype-host specificity of *C. shasta* and Coho salmon (Hurst and Bartholomew, 2012; Stinson et al., 2018).

Severe *P. minibicornis* infections associated with pre-spawn mortality of Sockeye salmon in the Fraser River include glomerulonephritis and presence of the parasite in renal tubules (Jones et al., 2003). These results were observed in all eight of our histology samples from spring Chinook salmon collected at the Round Butte Hatchery on August 30th, 2017. Co-infection with *C. shasta*, another suspected contributor to pre-spawn mortality was observed in all histology samples infected with *P. minibicornis*.

Parvicapsula minibicornis and its effects on Sockeye salmon have been well studied in the Fraser River where the prevalence of infection is between 47-100% (St-Hilaire et al., 2002). Klamath River studies of this parasite revealed clinical disease signs in juvenile Chinook salmon as well as a similar spatial and temporal distribution pattern to *C. shasta* (Bartholomew et al., 2007; Foott et al., 2007). While detections in the Klamath River were not associated with mortality, fifteen different genotypes of *P. minibicornis* have been identified among five basins along the west coast of North America from British Columbia to Northern California which exhibit geographic and salmonid host specificity (Atkinson et al., 2011). In the Deschutes River, *P. minibicornis* was detected by PCR but not observed by histology in glomeruli of four Deschutes River Sockeye salmon in 2002 (Jones et al., 2004). We observed *Parvicapsula minibicornis* and

associated glomerulonephritis in the glomeruli of all adult spring Chinook salmon inspected by histology (8/29) at the Round Butte hatchery in 2017. The severity of infection observed may warrant further exploration of this parasite in the Deschutes River.

Co-infections of *C. shasta* and *R. salmoninarum* of juvenile spring Chinook salmon (Table A.1; Vojnovich et al., 2016), the stress associated with hatchery rearing, (Barton et al., 1986; Olsen et al., 2005) and high winter temperatures at the hatchery may all be contributing to low survival of juveniles before they even leave the hatchery. The immune systems of returning adult spring Chinook salmon are compromised due to energetics associated with migration which, among other things, are associated with the degradation of the mucosal epithelium (Dolan et al., 2016). Co-infections of *C. shasta* and *P. minibicornis* may exacerbate the physiological stress associated with migration leading to an increase in pre-spawn mortality. Based on our observance of co-infections through molecular and histological analyses, we cannot conclude that *C. shasta* is the sole pathogenic contributor to low returns of Deschutes River spring Chinook salmon.

Acknowledgements

Stacy Strickland from ODFW for allowing us to collect samples at Round Butte Hatchery as well as providing virology and bacteriology diagnostic results. Fellow graduate students, Claire Howell, Benjamin Americus, and Laura Taggart-Murphy for participating in tissue sampling. PhD candidate Drew Janik for helping cut and cassette histology samples. Dr. Michael Kent (OSU) for direction and advice on histology collection, fixation, and analyses. Funding for this research was provided by The Confederated Tribes of the Warm Springs Reservation of Oregon-Pacific Coastal Salmon Recovery Fund and an Oregon Department of Fish and Wildlife Fish Health Graduate Research Fellowship in Microbiology.

REFERENCES

AFS-FHS (American Fisheries Society-Fish Health Section). 2014. FHS blue book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2014 edition.

Atkinson, S. D., and J. L. Bartholomew. 2010. Disparate infection patterns of *Ceratomyxa shasta* (Myxozoa) in rainbow trout (*Oncorhynchus mykiss*) and Chinook salmon (*Oncorhynchus tshawytscha*) correlate with internal transcribed spacer-1 sequence variation in the parasite. International Journal for Parasitology 40(5):599–604.

Atkinson, S. D., S. R. M. Jones, R. D. Adlard, and J. L. Bartholomew. 2011. Geographical and host distribution patterns of *Parvicapsula minibicornis* (Myxozoa) small subunit ribosomal RNA genetic types. Parasitology 138(8):969–977.

Atkinson, S. D., S. L. Hallett, and J. L. Bartholomew. 2018. Genotyping of individual *Ceratonova shasta* (Cnidaria: Myxosporea) myxospores reveals intra-spore ITS-1 variation and invalidates the distinction of genotypes II and III. Parasitology 145(12):1588–1593.

Baker, C., and J. Lovtang. 2016. Monitoring wild populations of spring Chinook salmon (*Oncorhynchus tshawytscha*) and summer Steelhead (*O. mykiss*) in tributaries of the lower Deschutes River within the boundaries of the confederated tribes of the warm springs of Oregon reservation, Natural Production Monitoring 2013-2014 to BPA, Confederated Tribes of the Warm Springs Reservation of Oregon Warm Springs, OR.

Bartholomew, J. L., E. Ray, B. Torrell, M. J. Whipple, and J. R. Heidel. 2004. Monitoring *Ceratomyxa shasta* infection during a hatchery rearing cycle: comparison of molecular, serological, and histological methods. Dis. Aquat. Org. 62: 85-92.

Bartholomew, J. L., S. D. Atkinson, S. L. Hallett, C. M. Zielinski, and J. S. Foott. 2007. Distribution and abundance of the salmonid parasite *Parvicapsula minibicornis*

(Myxozoa) in the Klamath River basin (Oregon-California, USA). Diseases of Aquatic Organisms 78(2):137–146.

Barton, B. A., C. B. Schreck, and L. A. Sigismondi. 1986. Multiple acute disturbances evoke cumulative physiological stress responses in juvenile Chinook Salmon. Transactions of the American Fisheries Society 115(2):245–251

Bradford, M. J., J. Lovy, D. A. Patterson, D. J. Speare, W. R. Bennett, A. R. Stobbart, and C. P. Tovey. 2010a. *Parvicapsula minibicornis* infections in gill and kidney and the premature mortality of adult Sockeye salmon (*Oncorhynchus nerka*) from Cultus Lake, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 67(4):673–683.

Bradford, M. J., J. Lovy, and D. A. Patterson. 2010b. Infection of gill and kidney of Fraser River Sockeye salmon, *Oncorhynchus nerka* (Walbaum), by *Parvicapsula minibicornis* and its effect on host physiology. Journal of Fish Diseases 33(9):769–779.

Carraro, L., L. Mari, M. Gatto, A. Rinaldo, and E. Bertuzzo. 2018. Spread of proliferative kidney disease in fish along stream networks: A spatial metacommunity framework. Freshwater Biology 63(1):114–127.

Conrad, J. F., and M. Decew. 1966. First Report of *Ceratomyxa* in Juvenile Salmonids in Oregon. The Progressive Fish-Culturist 28(4):238–238.

Dixon, P., R. Paley, R. Alegria-Moran, and B. Oidtmann. 2016. Epidemiological characteristics of infectious hematopoietic necrosis virus (IHNV): a review. Veterinary Research 47(1).

Dolan, BP, Fisher, KM, Colvin, ME, Benda, SE, Peterson, JT, Kent, ML, Schreck, CB. 2016. Innate and adaptive immune responses in migrating spring-run adult Chinook salmon, *Oncorhynchus tshawytscha*. Fish & Shellfish Immunology, (48), 136-144.

Foott, J. S., R. Stone, and K. True. 2006. Relationship between *Ceratomyxa shasta* and *Parvicapsula minibicornis* actinospore exposure in the Klamath River and infection in juvenile Chinook salmon.:19.

Foott, J. S., R. Stone, E. Wiseman, K. True, and K. Nichols. 2007. Longevity of *Ceratomyxa shasta* and *Parvicapsula minibicornis* actinospore infectivity in the Klamath River. Journal of Aquatic Animal Health 19(2):77–83.

Fournie, J. W., R. M. Krol, and W. E. Hawkins. 2000. Chapter 34 - Fixation of Fish Tissues. Pages 569–578 *in* G. K. Ostrander, editor. The Laboratory Fish. Academic Press, London.

Fox, M. D., O. Palenzuela, and J. L. Bartholomew. 2000. Strategies for the diagnosis of *Ceratomyxa shasta* using the polymerase chain reaction: Comparison of lethal and nonlethal sampling with microscopic examination. Journal of Aquatic Animal Health 12(2):100–106.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acid Symposium Series 41: 95-98.

Hurst, C. N., and J. L. Bartholomew. 2012. *Ceratomyxa shasta* genotypes cause differential mortality in their salmonid hosts. Journal of Fish Diseases 35(10):725–732.

Jones, S. R. M., G. Prosperi-Porta, S. C. Dawe, and D. P. Barnes. 2003. Distribution, prevalence and severity of *Parvicapsula minibicornis* infections among anadromous salmonids in the Fraser River, British Columbia, Canada. Diseases of Aquatic Organisms 54(1):49–54.

Jones, S., G. Prosperi-Porta, S. Dawe, K. Taylor, and B. Goh. 2004. *Parvicapsula minibicornis* in Anadromous Sockeye (*Oncorhynchus nerka*) and Coho (Oncorhynchus kisutch) salmon from tributaries of the Columbia River. The Journal of Parasitology 90(4):882–885.

Kent, M. L., D. J. Whitaker, and S. C. Dawe. 1997. *Parvicapsula minibicornis* n. sp. (Myxozoa, Myxosporea) from the kidney of Sockeye Salmon (*Oncorhynchus nerka*) from British Columbia, Canada. The Journal of Parasitology 83(6):1153–1156.

Kent, M. L., J. Khattra, R. P. Hedrick, and R. H. Devlin. 2000. *Tetracapsula renicola* n. sp. (Myxozoa: Saccosporidae); The PKX Myxozoan: The cause of proliferative kidney disease of salmonid fishes. The Journal of Parasitology 86(1):103–111.

Kent M. L., S. Benda, S. St-Hilaire, and C. B. Schreck. 2013. Sensitivity and specificity of histology for diagnoses of four common pathogens in adult Chinook salmon (*Oncorhynchus tshawytscha*) in fresh water. J Vet Diagn Invest 25(3):341-351

Lindsay, R. B., B. C. Jonasson, R. K. Schroeder, and B. C. Cates. 1989. Spring Chinook salmon in the Deschutes River, Oregon. Oregon Department of Fish and Wildlife Information Report: 89-4.

Miller, K. M., Teffer, A., Tucker, S., Li, S., Schulze, A. D., Trudel, M., Juanes, F., Tabata, A., Kaukinen, K. H., Ginther, N. G., Ming, T. J., Cooke, S. J., Hipfner, J. M., Patterson, D. A. and Hinch, S. G. 2014. Infectious disease, shifting climates, and opportunistic predators: cumulative factors potentially impacting wild salmon declines. Evol Appl, 7: 812–855.

Mulcahy, D. M. 1976. Distribution and epizootiology of infectious pancreatic necrosis and infectious hematopoietic necrosis of salmonid fishes in Oregon. Ph.D., Oregon State University, United States -- Oregon.

Olsen, R. E., K. Sundell, T. M. Mayhew, R. Myklebust, and E. Ringø. 2005. Acute stress alters intestinal function of rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture 250(1):480–495.

Palenzuela, O., G. Trobridge, and J. L. Bartholomew. 1999. Development of a polymerase chain reaction diagnostic assay for *Ceratomyxa shasta*, a myxosporean parasite of salmonid fish. Diseases of Aquatic Organisms 36(1):45–51.

Portland General Electric (PGE). N.d. Deschutes daily fish counts. https://www.portlandgeneral.com/corporate-responsibility/environmental-stewardship/water-quality-habitat-protection/fish-counts-fish-runs/deschutes-daily-fish-counts#

Ratliff, D. E. 1983. *Ceratomyxa shasta*: Longevity, distribution, timing, and abundance of the infective stage in Central Oregon. Canadian Journal of Fisheries and Aquatic Sciences 40(10):1622–1632.

Ray, R. A., R. A. Holt, and J. L. Bartholomew. 2012. Relationship between temperature and *Ceratomyxa shasta*–induced mortality in Klamath River Salmonids. Journal of Parasitology 98(3):520–526.

Richards, C. A., C. A. Murphy, T. O. Brenden, T. P. Loch, and M. Faisal. 2017. Detection accuracy of *Renibacterium salmoninarum* in Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum) from non-lethally collected samples: Effects of exposure route and disease severity. Preventive Veterinary Medicine 145:110–120.

Sanders, J. E., J. J. Long, C. K. Arakawa, and J. L. Bartholomew. 1992. Prevalence of *Renibacterium salmoninarum* among downstream migrating salmonids in the Columbia River. Journal of Aquatic Animal Health 4: 72-75.

St-Hilaire, S., M. Boichuk, D. Barnes, M. Higgins, R. Devlin, R. Withler, J. Khattra, S. Jones, and D. Kieser. 2002. Epizootiology of *Parvicapsula minibicornis* in Fraser River Sockeye salmon, *Oncorhynchus nerka* (Walbaum). Journal of Fish Diseases 25(2):107–120.

Stinson, M. E. T., and J. L. Bartholomew. 2012. Predicted redistribution of *Ceratomyxa shasta* genotypes with salmonid passage in the Deschutes River, Oregon. Journal of Aquatic Animal Health 24(4):274–280.

Stinson, M. E. T., S. D. Atkinson, and J. L. Bartholomew. 2018. Widespread distribution of *Ceratonova shasta* (Cnidaria: Myxosporea) genotypes indicates evolutionary adaptation to its salmonid fish hosts. Journal of Parasitology 104(6):645–650.

Vojnovich, S., Hallett S., Atkinson S., Alexander J., Holt R., Craig R., and J. Bartholomew. 2016. Myxozoan disease risks in the Willamette and Deschutes Rivers, ODFW fellowship report 2015-2016. Oregon State University, Corvallis, OR.