HATCHERY AND GENETIC MANAGEMENT PLAN:
IMPLEMENTATION REPORT FOR
FALL 2018 – SPRING 2019

Hatchery Program:	Big Creek Hatchery Chum Salmon Recovery Program
Species or Hatchery Stock:	Chum Salmon (ODFW Stock 104)
Agency/Operator:	Oregon Department of Fish and Wildlife
Watershed and Region:	Lower Columbia River/Big Creek and other tributaries/Oregon
Submitted to NOAA:	January 23, 2013
Date Last Updated:	July 25, 2016

Review of program description in HGMP

1.1) Name of hatchery or program.
Big Creek Hatchery Chum Salmon Recovery Program.

1.2) Species and population (or stock) under propagation, and ESA status.
Chum Salmon *Oncorhynchus keta* (stock 104) originated from an integrated stock of Grays River wild-origin and hatchery-origin broodstock. On arrival of eyed eggs at Big Creek Hatchery in 2010 from Grays River Hatchery, ODFW assigned stock number 104 to this Chum Salmon stock. The Columbia River Chum Salmon was listed as Threatened ESU under the federal Endangered Species Act (ESA) in March 1999. The Grays River Hatchery stock is part of the Columbia River chum ESU and is listed under the ESA. Therefore, the Big Creek Hatchery stock of Chum Salmon (stock 104) originating from the Grays River stock is considered to be an ESA listed population.

Grays River Hatchery, Washington Department of Fish and Wildlife (WDFW) – This facility/location served as the original broodstock source for the Big Creek Hatchery Chum Salmon Program. The facility provided adult capture and holding, egg incubation, and otolith marking during the startup phase of this program. Although the intent is to establish a self-sustaining broodstock source in Oregon, the continued cooperation from WDFW Grays River Hatchery will remain a potential contributor to the program if necessary, as determined annually by broodstock needs and availability.

Introduction
The populations of Chum Salmon along the Oregon side of the lower Columbia River ESU include Young's Bay, Big Creek, Clatskanie River, Scappoose Creek, Clackamas River, Sandy River, Lower Gorge, and Upper Gorge (ODFW 2010). Multiple smaller tributaries that drain directly into the Lower Columbia River are part of these Chum Salmon populations. Although there may be some remnant Chum Salmon populations in the lower Columbia River, Chum Salmon are considered to be functionally extirpated from Oregon tributaries of the Columbia River Basin (McElhany et al. 2007; ODFW 2006), which provides a strong justification to operate this recovery program. Therefore, the current program is aimed at reintroduction and reestablishment of self-sustaining populations of Chum Salmon along the Oregon side of the lower Columbia River. The Big Creek Hatchery Chum Salmon Recovery Program is part of the Lower Columbia Chum Salmon reintroduction and recovery project. The program is intended to help recover self-sustaining Chum Salmon populations along the Oregon side of the Columbia River. Currently, this is an integrated recovery program incorporating natural origin fish in the broodstock. Natural origin Chum Salmon describe those unmarked individuals that enter Big Creek Hatchery volitionally. Because the Big Creek Population is currently considered functionally extirpated, incorporation of these individuals in the broodstock does not change the status of the Big Creek Population; it remains functionally extirpated.

In this report, I describe (1) the 2018 collection of Chum Salmon broodstock at Big Creek Hatchery, egg transfers from Grays River Hatchery, and associated metrics (2) the 2019 fry

releases from Big Creek Hatchery, (3) take occurring through monitoring and research actions in fall 2018 and spring 2019, (4) remaining performance indicators, and (5) limiting factors research on *Ceratonova shasta*. No changes are proposed to the HGMP at this time.

Chum Salmon broodstock collection through fall 2018

Big Creek Hatchery operates an integrated Chum Salmon broodstock incorporating natural-origin fish with hatchery fish. Natural-origin Chum Salmon are unmarked individuals that enter Big Creek Hatchery volitionally. Marked (hatchery-origin) Chum Salmon may be marked with Coded Wire Tags (CWT), Adipose Clips (Ad-Clip), or Otolith Thermal Marks, or may be identifiable through Parentage-Based Tagging (PBT). The specific marks applied, by year, are listed in Table 1. Beginning in 2014, Chum Salmon have been spawned at Big Creek Hatchery and collected eggs have been used for the broodstock and for outplanting (Table 2). In fall 2018, a total of 102 Chum Salmon were collected at Big Creek Hatchery, including 39 individuals marked with an adipose clip. One unmarked Chum Salmon died before spawning (Table 3). Average fecundity generally decreased over the course of the spawning run (Table 4).
In anticipation of a poor return year, an additional 44,741 eggs were collected at the Grays River Hatchery and were transferred to Big Creek Hatchery at the eyed stage.

Table 1. Marks applied to the Big Creek Hatchery Chum Salmon *Oncorhynchus keta* broodstock 2010-2018, and years when marks are expected to be present in age 3-5 adult returns to the hatchery or spawning grounds. For brood years 2010- 2013 Otolith Thermal Marks were applied at the Grays River Hatchery, Coded Wire Tags (CWT) were implanted at Big Creek Hatchery, and Adipose Clips (Ad-Clip) and Parentage-Based Tagging (PBT) occurred at Big Creek Hatchery.

Brood year	Marks	Years marks observed in returns
2010	Pre-hatch thermal, CWT	2013-2015
2011	Pre-hatch thermal, CWT	2014-2016
2012	Pre-hatch thermal, CWT	2015-2017
2013	Pre-hatch thermal, CWT	2016-2018
2014	Pre and post-hatch thermal, CWT, Ad-Clip (test group)	2017-2019
2015	Pre and post-hatch thermal, Ad-Clip	2018-2020
2016	Pre and post-hatch thermal, Ad-Clip	2019-2021
2017	Pre and post-hatch thermal, PBT	2020-2022
2018	Pre and post-hatch thermal, PBT	2021-2023

Table 2. Number and origin of adult Chum Salmon *Oncorhynchus keta* collected at Big Creek Hatchery for the Big Creek Hatchery broodstock, by brood year. Totals do not include mortalities or fish collected for outplanting of adults or eyed-eggs. *It is possible that a portion of the "unmarked" fish were actually marked fish that had lost their tag. This is being investigated currently through examination of thermal marks on otoliths collected from all spawned fish.

Brood Year	Males	Females	Total	% unmarked fish	% marked fish
2014	45	40	85	0	100
2015	87	87	174	32.2	67.8
2016	28	16	44	81.8	18.2*
2017	22	38	60	86.7	13.3*
2018	45	56	101	61.3	38.7*

Table 3. Weekly spawn totals of marked and unmarked Chum Salmon *Oncorhynchus keta* at Big Creek Hatchery. Individuals marked as Mortalities died at the hatchery prior to spawning.

	Unmarked				Marked			
	Spawned		Mortality		Spawned		Mortality	
Date	Males	Females	Males	Females	Males	Females	Males	Females
11/6/2018	3	3	0	0	2	1	0	0
11/13/2018	1	1	0	0	1	1	0	0
11/19/2018	4	5	1	0	3	3	0	0
11/26/2018	17	26	0	0	9	11	0	0
11/29/2018	0	2	0	0	5	3	0	0
Totals	**25**	**37**	**1**	**0**	**20**	**19**	**0**	**0**

Table 4. Weekly fecundity estimates of Chum Salmon *Oncorhynchus keta* spawned at Big Creek Hatchery. Typically, fecundity data is collected on 33-50% of females due to time constraints during spawning. Total fecundity is presented based on expanding estimated fecundity (from pounds of eggs and eggs/ounce measurements) and based on the program average fecundity of 2,500 eggs/ female.

	Number	Fecundity			Total eggs collected	
Date	females	Min	Max	Avg	(Est. fecundity)	(2,500 eggs/ female)
11/6/2018	4	2,486	3,024	2,804	11,214	10,000
11/13/2018	2	2,842	3,732	3,287	6,574	5,000
11/19/2018	8	1,901	3,541	2,941	23,527	20,000
11/26/2018	37	1,901	3,494	2,653	98,162	92,500
11/29/2018	5	1,997	2,771	2,447	12,385	12,500
Totals	**56**	**1,764**	**3,973**	**2,739**	**151,862**	**140,000**

As all populations of Chum Salmon on the Oregon side of the Columbia River are considered functionally extirpated, integration of variable numbers of unmarked Chum Salmon into the broodstock does not impact the current status of naturally spawning Chum Salmon (i.e., populations remain functionally extirpated). In the HGMP, it states, "Naturally produced Chum will be integrated annually as available and as needed to meet the goals of the re-introduction program as long as their removal from the naturally spawning population does not jeopardize efforts to restore self-sustaining populations." In 2018, all unmarked Chum Salmon that volitionally returned to Big Creek Hatchery were incorporated in the broodstock (Tables 2 and 3). Because returns were projected to be very low, broodstock was also collected from the Grays River. Approximately 45,000 eyed eggs were transferred from Grays River Hatchery to Big Creek Hatchery. No maximum impact levels have been established for integration of natural origin fish at this time as Oregon donor populations are considered functionally extirpated.

Big Creek Hatchery Chum Salmon fry releases

The Big Creek Hatchery Chum Salmon program is permitted to collect up to 600,000 eggs for production needs through the approved HGMP. Of these, approximately 300,000 fry can be reared and marked at Big Creek Hatchery. Currently the release is approximately 200,000 fed fry. When sufficient brood is available, the release goal may increase to 300,000 fed fry. Releases have ranged from 37,725 fed fry during a poor return year to 192,147 fry in a good return year (Table 5). For the 2018 brood year, a total of 171,649 fed fry were released from Big Creek Hatchery using a release site located in a tidal area of Big Creek. This release was comprised of 126,878 fry from eggs collected at Big Creek hatchery and 44,741 fry from eggs collected at Grays River Hatchery. The size of released fry has varied over time due to water temperature, whether fish are implanted with CWT or adipose clipped, and release strategy. In an effort to time releases more closely with wild Chum Salmon fry outmigration through the estuary, fry are now released earlier in the spring and at a smaller size (Table 5).

Table 5. Number, date, location, and size of Chum Salmon *Oncorhynchus keta* fry released from Big Creek Hatchery by brood year.

Brood year	Release location	Stage	Total number	Release dates	Release size (Fish / pound)
2010	Big Creek, tidewater	Fed-fry	107,000	4/7/2011	224
2011	Big Creek, tidewater	Fed-fry	110,000	4/9/2012	218
2012	Big Creek, tidewater	Fed-fry	108,500	4/15/2013; 4/17/2013	168; 178
2013	Big Creek, tidewater/ Big Creek Hatchery	Fed-fry	101,000	4/17/2014	185
2014	Big Creek, tidewater	Fed-fry	190,188	4/24/2015; 5/15/2015	190; 180
2015	Big Creek hatchery	Fed-fry	192,147	4/25/2016	143
2016	Big Creek, tidewater	Fed-fry	37,725	4/17/2017	275
2017	Big Creek, tidewater	Fed-fry	84,958	3/29/18; 4/16/18	461; 401
2018	Big Creek, tidewater	Fed-fry	171,649	3/13, 3/26, 4/2, 4/9	398, 370, 449, 405

Monitoring and reintroduction actions

In 2018, Chum Salmon returns to Big Creek Hatchery were insufficient to conduct any reintroduction action beyond broodstock collection. As such, no adult outplanting, eyed-egg outplanting, or fry releases in recovery populations occurred (Table 6). Once returns are consistently sufficient to maintain broodstock releases at > 200,000 fry, reintroductions will occur again. This level of fry releases is necessary to ensure that there are sufficient adults to support the broodstock and to have large reintroduction releases of adults or eyed eggs. The previous experimental reintroductions were designed to test reintroduction techniques with relatively small numbers of released adults or eggs. Larger releases are required for reintroduced populations to avoid demographic stochasticity and inbreeding, and to become self-sustaining.

Table 6. Overview of Chum Salmon *Oncorhynchus keta* adult outplanting and eyed-egg incubation in remote site incubators by brood year and location. Outplanting was done for two purposes: Supplementation (Suppl.) or Reintroduction (Reintro.)

Brood	Release				Number			Release
year	population	Release location	Stage	Purpose	Males	Females	Eggs	dates
2010	Big Creek	Above Big Cr. Canyon	Adults	Suppl.	9	17		Fall
2011	Big Creek	Above Big Cr. Canyon	Adults	Suppl.	1	3		Fall
2012	Big Creek	Above Big Cr. Canyon	Adults	Suppl.	13	24		Fall
2013	Big Creek	Above Big Cr. Canyon	Adults	Suppl.	11	4		Fall
2013	Clatskanie R.	Graham Creek	Adults	Reintro.	12	10		Fall
2013	Clatskanie R.	Stewart Creek	Adults	Reintro.	11	10		Fall
2014	Big Creek	Above Big Cr. Canyon	Adults	Suppl.	64	65		Fall
2014	Clatskanie R.	Stewart Cr.	Adults	Reintro.	6	25		Fall
2014	Clatskanie R.	Perkins Cr.	Eyed-eggs	Reintro.			47,958	January
2015	Clatskanie R.	Stewart Cr.	Adults	Reintro.	7	10		Fall
2015	Clatskanie R.	Perkins Cr.	Eyed-eggs	Reintro.			56,947	January

Monitoring for adult returns and juvenile outmigration occurred in the Big Creek and Clatskanie River populations in support of recovery and reintroduction efforts. In fall 2018, a box and panel adult trap was operated on Stewart Creek, a tributary to Beaver Creek in the Clatskanie River population. This site was used for adult outplanting from 2013-2015 and we were expecting adult returns from those efforts to occur this year. An adult trap was operated from October 16 – December 17, 2018. The trap was checked daily and spawning surveys were conducted upstream and downstream of the trap. No Chum Salmon were captured in the trap during the monitoring period.

In fall 2018, spawning ground surveys were also conducted throughout the Clatskanie River, Big Creek, and Youngs Bay populations (Table 7). Surveys were done by staff from two ODFW projects- the Chum Reintroduction Project and the Oregon Adult Salmonid Inventory Sampling

project (OASIS). A total of 16 Chum Salmon were observed on these surveys- 15 in Big Creek and one in Mill Creek (in the Big Creek Populations).

In spring 2019, rotary screw traps were operated from 25 February – 26 May on Bear Creek (Big Creek population) and 27 February – 9 June on the Clatskanie River (Clatskanie River population). Juvenile Coho Salmon, Chinook Salmon, and Chum Salmon were handled, marked, and released (Table 8), and all actions were well-within take limits in the HGMP. With the exception of the intentional lethal take of Chum Salmon fry, all individuals reported in Table 8 were unmarked. It is possible some unmarked fish could have been hatchery fish with thermal marks but no fin marks.

Hatchery performance indicators

In 2018, rearing and fish health parameters were monitored to ensure that fish culture standards are met. No health issues occurred for the 2018 brood. Fish were ponded and subsequently split out in order to maintain acceptable densities. Water flows were monitored and adjusted to maintain an appropriate flow index. ODFW pathology examined the fish regularly and prior to transfer or release. Nothing was found during the monthly exams. A total of 6 fish were examined in March during a pre liberation exam. At the hatchery, water quality parameters and results were reported to Oregon Department of Environmental Quality in March and April. No violations of the permit occurred.

Table 7. Streams and reaches surveyed for Chum Salmon spawning in the Youngs Bay, Big Creek, and Clatskanie River populations, fall 2018.

Spawning year	Population	Survey name	Reach ID	Segment	Miles	Total Chum Observed
2018	Youngs Bay	Abercrombie Cr	30031	1	0.30	0
2018	Youngs Bay	Hortill Cr	30046	1	0.32	0
2018	Youngs Bay	Lewis & Clark R	30047	1	0.57	0
2018	Youngs Bay	Lewis & Clark R	30049	1	0.62	0
2018	Youngs Bay	Lewis & Clark R	30053	1	0.93	0
2018	Youngs Bay	Lewis & Clark R	30055	1	1.39	0
2018	Youngs Bay	Lewis & Clark R	30055	2	1.12	0
2018	Youngs Bay	Wallooskee R	30068	2.1	1.57	0
2018	Youngs Bay	Klaskanine R, Trib A	30080.37	2	0.32	0
2018	Youngs Bay	Klaskanine R, N Fk	30081.7	1	1.23	0
2018	Youngs Bay	Klaskanine R, S Fk	30086.3	2	1.65	0
2018	Youngs Bay	Youngs R	30089	2	0.31	0
2018	Big Creek	Mill Cr	30108	2	0.65	1
2018	Big Creek	Bear Cr #1	30125	2	0.70	0
2018	Big Creek	Little Bear Cr	30126	1	1.02	0
2018	Big Creek	Little Bear Cr	30126	2	0.98	0
2018	Big Creek	Bear Cr	30127	1	1.15	0
2018	Big Creek	Ferris Cr	30139	2	0.36	0
2018	Big Creek	Little Cr	30171	3.2	0.48	0
2018	Below Big Creek Hatchery	Big Cr	30172	3	1.11	14
2018	Below Big Creek Hatchery	Big Cr	30172	4	0.66	1
2018	Big Creek	Gnat Cr	30198	2	0.91	0
2018	Clatskanie River	Plympton Cr	30239	2	1.03	0
2018	Clatskanie River	Olsen Cr	30247	1	0.67	0
2018	Clatskanie River	Conyers Cr	30274.7	1	0.66	0
2018	Clatskanie River	Conyers Cr	30280	1	1.21	0
2018	Clatskanie River	Clatskanie R	30283	1	1.12	0
2018	Clatskanie River	Perkins Cr	30286	2	0.96	0
2018	Clatskanie River	Miller Cr	30292	1	1.02	0
2018	Clatskanie River	Clatskanie R	30298	1	1.20	0

Table 8. Actual annual take of lower Columbia River listed salmonids due to Chum Salmon recovery program through broodstock collection, adult trapping, juvenile trapping, and research on *Ceratonova shasta*, October 2018 – June 2019. *All intentional take of Chum Salmon fry occurred during studies on *C. shasta* as fish exposed to a pathogen cannot be released. Of the 540 fry sacrificed for this study, 138 were from Washougal Hatchery and 402 were from Big Creek Hatchery.

Action	**Lower Columbia Chinook**		**Columbia Chum**		**Lower Columbia Coho**	
	Life stage	**Estimated Annual take**	**Life stage**	**Estimated Annual take**	**Life stage**	**Estimated Annual take**
Observe or harass						
Collect for transport			Adult	0		
Capture, handle, and release	Fry	7	Fry	0	Fry Smolt Adult	13 8,748 0
Capture, handle, tag/mark/tissue sample, and release	Fry Adult	78 0	Fry Adult	24 0	Fry Smolt Adult	253 3,980 1
Capture and remove (e.g., broodstock)			Adult	101		
Intentional lethal take			Fry	540*		
Unintentional lethal take	Fry Adult	2 0	Fry Adult	1 1	Fry Smolt Adult	19 83 0
Other take (specify)						

Limiting-factors research

In the course of monitoring conservation broodstocks and production of remaining wild populations, it was determined that Columbia River Chum Salmon fry-to-adult (hereafter, marine) survival rates were substantially lower (range = 0.01 – 1.63%; average = 0.34%; Hillson WDFW, personal communication; Homel, ODFW unpublished data) than those typically reported for the species (range = 0.3 – 3.2%; average = 1.8%; Salo 1991). Although there are many factors that affect marine survival throughout the life cycle, it appears that the majority of mortality occurs during the time period between emergence from the gravel through shortly after ocean (Healey 1982; Bax 1983; Fukuwaka and Suzuki 2002). During these weeks to

months, Chum Salmon fry travel from natal streams through the Columbia River, into the estuary, and then into the nearshore environment of the ocean, occupying shallow water habitats along the way. Currently, assessments of limiting factors are focused on the freshwater environment, including the lower portions of tributaries and the tidal freshwater portion of the Columbia River and estuary.

One potential limiting factor is the myxozoan parasite *Ceratonova shasta* (Noble 1950). This parasite has been shown to infect Chum Salmon in British Columbia (Margolis and Evelyn 1975), Alaska (Follett et al. 1994) and Oregon (Zinn et al. 1977; Johnson 1980), and the infections may be fatal (Margolis and Evelyn 1975; Zinn et al. 1977). *Ceratonova shasta* requires two hosts to complete the life cycle (Bartholomew et al. 1997). The polycheate host (*Manayunkia speciose)* ingests myxospores and releases actinospores into the water column (Bartholomew et al 1997). Subsequently, the fish host takes up the polar filament of the actinospores through the gills, and these migrate through the circulatory system until they end up in the digestive track, where they develop into myxospores (Bjork and Bartholomew 2010). In the fish host, this process can cause intestinal hemorrhaging and death (Johnson et al. 1979; Bartholomew et al. 1989). Once the fish host dies, myxospores enter the water column where they are taken up by a polycheate host again (Bartholomew et al. 1997). In the Columbia Basin, numerous tributaries are known to contain the parasite (Johnson 1975; Johnson et al. 1979; Hoffmaster et al. 1988), but the specific distribution within habitats occupied by juvenile Chum Salmon is unknown.

In a 2018 pilot study, water samples were collected throughout the lower Columbia River and downstream portions of tributaries, with an emphasis on collections in streams with and without extant Chum Salmon populations during the time frame when Chum Salmon fry migrate downstream (Figure 1). Samples were examined for presence and density of *C. shasta* using qPCR. Subsequently, positive samples were genotyped, as different genotypes of *C. shasta* may be differentially infectious across salmon species and steelhead. Out of 30 sample sites, *C. shasta* was detected at 17, and genotype II, the one attributed to infection risk in Chum Salmon (Stinson et al. 2018) was detected at 14 sites (Figure 1). From these results, it appears the parasite was prevalent in systems that do not contain Chum Salmon, but limited or absent in locations containing extant populations. Individuals from extant populations are the source of reintroductions into other streams where *C. shasta* is present (ODFW 2010; Small et al. 2011; WDFW 2014; ODFW 2016). Therefore, it is critical to determine whether the parasite causes disease in that source population, to confirm pilot study results on the spatial and temporal distribution of the parasite, and to assess what level of exposure to the parasite (and for what amount of time) causes infection and death.

The goal of our 2019 study was to assess the potential of *C. shasta* to limit the survival of juvenile Chum Salmon. Specific objectives were to identify: (1) the spatiotemporal distribution and density of different genotypes of *C. shasta*, (2) the susceptibility of Chum Salmon fry to ambient levels of *C. shasta* in the Columbia River and tributaries, and (3) infection and mortality rates under different exposure durations and exposure levels of *C. shasta*, genotype II. To this end, we collected and filtered water samples from throughout the Columbia River estuary and tributaries during the time frame when Chum Salmon fry would co-occur to confirm the

observed distribution of the parasite. We then used qPCR to identify the presence and prevalence of *C. shasta* and genotyped any positive samples. Next, we held Chum Salmon from Big Creek and Washougal Hatchery stocks in sentinel cages at locations that tested positive for *C. shasta* in 2018 to determine infection rates at a range of ambient spore density levels. Lastly, we conducted two lab experiments. In the first, we determined infection rate at different durations of exposure to *C. shasta*, using juvenile Chum Salmon from Big Creek Hatchery, Oregon. Subsequently, we repeated experimental exposures for a much shorter duration and at a much lower dose for Big Creek and Washougal Hatchery stocks. Collectively, the results from these studies indicate the potential for *C. shasta* to function as a limiting factor for Chum Salmon.

Methods and analysis

Objective 1: spatiotemporal distribution and spore concentration of different genotypes of C. shasta

Sample sites and characteristics

The spatiotemporal distribution of *C. shasta* was examined throughout the Columbia River from Bonneville Dam to Youngs Bay, and in the downstream portion of tributaries. Sample sites included streams (1) with extant populations of Chum Salmon, (2) where *C. shasta* is known to occur, (3) that have been targeted for Chum Salmon reintroduction or restoration efforts, (4) where Chum Salmon historically occurred but are currently extirpated, and (5) where sampling occurred during the 2018 pilot study. These sites occur on both the Oregon and Washington sides of the Columbia River in freshwater and tidal freshwater.

From these candidate sites, a total of 19 temporal sites (sampled every two weeks from April 15 through May 15; three sample events) and 22 spatial sites (sampled once on 1 May) were selected (Figure 2). Both spatial and temporal sample dates were selected to correspond with the time period when Chum Salmon fry are migrating from natal streams through the lower Columbia River and are present throughout the estuary. Whereas spatial sampling allowed for finer resolution of parasite distribution, temporal sampling at a reduced number of sites was designed to characterize parasite dynamics as a function of water year, discharge, and temperature.

Field methods

To determine the presence, genotype, and concentration of *C. shasta*, water samples were collected at 41 sites, filtered, and examined for presence of *C. shasta* DNA. Following the protocol of Hallett and Bartholomew (2006), a total of 4 L of water were collected at each sample site from just below the water surface using a plastic jug. Samples were stored in a cooler on ice until they could be processed later that same day. While collecting samples, site characteristics were noted, including water temperature, weather, water height, and presence of organic matter suspended in the sample.

Water samples were filtered in 1 L increments, using a vacuum filtration set up with a MF-

Millipore filter membrane (nitrocellulose 5 µm pore size; Hallett and Bartholomew 2006). Each filter was then folded in half and then in half again and stored in a small vial in the freezer. Filter equipment was washed between each sample location, but not between each liter of water filtered from a single location and sample event.

Genetic analysis

All frozen filters were sent to the Bartholomew Lab. Subsequently, filters were processed and DNA was extracted according to the protocol described in Hallett and Bartholomew (2006). In 2018, all 4 L of the sample were processed together. In 2019, each liter of the sample was processed separately. When samples were inhibited, the samples (and positive genetic control) were diluted and processed again. The presence and concentration (spores/ L) of *C. shasta* was determined through qPCR. Any positive samples were genotyped to determine the percentage of each genotype represented in the sample. Specific genetic methods are described in Hallett and Bartholomew (2006).

Objective 2: the susceptibility of Chum Salmon fry to ambient levels of C. shasta in the Columbia River and tributaries

Field methods

Sentinel studies were conducted to test the infection rate of Chum Salmon held in ambient levels of *C. shasta*, beginning on 1 May 2019. To do so, Chum Salmon fry were collected from the Big Creek Hatchery stock (which is genetically identical and recently derived from the Grays River stock; weight = 1 – 1.5 g), and the Washougal Hatchery stock (which is derived from adults collected from mainstem spawning sites below Bonneville Dam; weight = 1 – 2.6 g). Hatchery fry were loaded into oxygenated coolers filled with river water and transported to the sentinel cage sites. Three locations were selected for sentinel cages, bracketing the range of *C. shasta* densities (of genotype II) observed in the 2018 pilot study (Willamette River = high density, Lewis and Clark River = medium density, and Tongue Point = low density; Figure 2). Hatchery stocks were held in separate cages at each sentinel site (n = 30 fish/ cage; n = 3 sites; total n = 180 fish) for seven days. Sentinel cages were constructed of mesh small enough to retain fry and prevent predation, but large enough to allow river water to flow through and provide natural food to the fish (AAHL 2016). Cages were cabled to structures on the shore or to a dock in deep enough water so to remain submerged during all tide levels (AAHL 2016).

Water samples were collected at sentinel cage sites to determine the spore densities and genotypes present during exposure. Sampling occurred on the first day, fourth day, and seventh day of the sentinel study. These samples were processed following the methodology in Objective 1, above (Hallett and Bartholomew 2006).

Lab methods

After the field exposure, fish were placed in oxygenated coolers filled with river water and transported to the lab. Water temperature was monitored during transport and ice or cold water was added to coolers as needed so temperatures did not increase more than 1 °C during transport. Once fish arrived at the lab, they were placed in separate 25 L tanks and held through July 8th. Water temperature in those tanks was approximately 14°C (similar to the

temperatures recorded at each sentinel site and within the optimum range for rearing juvenile Chum Salmon; Richter and Kolmes 2005). Fish were fed daily and examined for clinical sings of disease. When signs were observed, monitoring increased to twice daily. Mortalities were removed from the tank twice a day and were necropsied. By day 60, all remaining fish were euthanized (AAHL 2016).

During the week the sentinel study was conducted and through the remaining lab trial, a control group of Chum Salmon fry from Big Creek hatchery (n= 15) and Washougal Hatchery (n = 15) were held in separate spore-free tanks at the lab. These fish were held at 14°C and fed daily. Mortalities were recorded and examined for underlying cause (AAHL 2016).

Disease and genetic analysis

Evidence of infection was determined by taking a swab from the hind gut, preparing a wet mount on a slide, and examining it under the microscope. Spores were counted for three minutes as a measure of infection severity. During necropsy, the intestines and other organs were examined for presence of cysts or other evidence of infection and disease. When the microscopy examination did not reveal any spores, PCR was run on a sample to check for presence of *C. shasta*. All positive PCR results were genotyped.

Mortality from *C. shasta* was calculated as the percent of sentinel fish from each treatment (hatchery X location = treatment) that died, after correcting for other mortality sources. To do so, the starting number of fish was adjusted by the number that die in the sentinel cages or in the lab from causes other than *C. shasta* (with genetic confirmation that the fish was not infected). Percent mortality was compared among locations, hatcheries, and against the control group and survival curves were generated for each tank. All intestines collected for this experiment were frozen and stored for future analysis.

Objective 3: infection and mortality rates at four exposure durations using C. shasta, genotype II.

Lab methods- trial 1 (2, 7, and 13-day exposure)

Infection and mortality rates were assessed for Chum Salmon fry exposed to *C. shasta* for different durations in a lab environment. In the first trial, a total of 253 Chum Salmon fry (1 – 1.5 g) were transferred from Big Creek Hatchery to the AAHL on 1 April 2019, using transfer methods as described under Objective 3. Fry were randomly assigned to one exposure duration (2 days, 7 days, or 15 days) or to the control group (n = 30). To expose fish, a mesocosm was set up with polycheates that had been infected with *C. shasta* genotype II. Water was plumbed from this mesocosm into an exposure tank (100 L). At the time of the study, the polycheates were producing 5 spores/ L and the flow rate into the tank was 1.25 L/ min. Control fry were placed into a separate tank (25 L) on well-water. All remaining fry were held in the exposure tank together. There were three replicates per exposure duration (n = 24 or 25 fry/replicate/ duration; n = 223 fry for exposure duration trials). After 2 days, the first group was removed and transferred to three separate tanks on clean well-water. After 7 days, the next group was transferred from the exposure tank to three rearing tanks. Finally, after 13 days, the remaining fry were transferred to the last three tanks. The exposure groups were

kept in separate tanks (25 L) for up to 60 days at 14°C. All fish health, feeding, monitoring, and necropsy activities were performed as described in Objective 2.

Disease and genetic analysis

Evidence of infection was determined following the same methods as in Objective 2. Mortality from *C. shasta* was calculated as the percent of fish from each replicate of each exposure level that died, after correcting for other mortality sources. To do so, the starting number of fish was adjusted by the number that died in the lab from causes other than *C. shasta* (with genetic confirmation that the fish was not infected). Percent mortality for each exposure duration was compared with the control group and survival curves were generated. All intestines collected for this experiment were frozen and stored for future analysis.

Lab methods- trial 2 (6-hour exposure)

A second lab trial was conducted to test infection and mortality rates at a much lower concentration of *C. shasta*, and to compare infection prevalence between Washougal and Big Creek Hatchery stocks. Chum Salmon fry were transferred from Big Creek Hatchery (n = 44; 1 – 1.5 g) and Washougal Hatchery (n = 33; 1 – 2.6 g) to the AAHL on 1 May 2019, using transfer methods as described under Objective 2. In addition, the control fry from the first lab trial (n = 30, Big Creek stock) were incorporated into the second trial. Fry were divided into 6 tanks (25 L, well water) and slowly acclimated to 14 °C over 7 days by increasing the tank water temperature by 1 °C/ day. The number of fry differed in each tank (Big Creek: n = 24-25/ tank, and Washougal: n = 11/ tank). To expose the fish, polycheates were infected with *C. shasta*, genotype II. A total of six polycheates were isolated and each placed in a test tube (one worm/ tube) and held in each tank for 6 hours. According to previously observed spore production rates, this single worm generated approximately 5 spores/ fish. After 6 hours, the water in each tank had recirculated and cleared *C. shasta* spores. The groups were held in these tanks for 60 days. The fish used for the sentinel control were also used for the control for this 6 hour exposure. All fish health, feeding, monitoring, and necropsy activities were performed as described in Objective 2.

Disease and genetic analysis

Evidence of infection was determined following the same methods as in Objective 2. Mortality from *C. shasta* was calculated as the percent of fish from each replicate of each hatchery stock that died, after correcting for other mortality sources. To do so, the starting number of fish was adjusted by the number that died in the lab from causes other than *C. shasta* (with genetic confirmation that the fish was not infected). Percent mortality for each hatchery stock was compared with the control group and survival curves were generated. All intestines collected for this experiment were frozen and stored for future analysis.

Results

Objective 1: spatiotemporal distribution and spore concentration of different genotypes of C. shasta

In 2018 and 2019, *C. shasta* was detected throughout streams that historically were occupied by Chum Salmon but currently are not. It was limited in streams with extant Chum Salmon populations during the timeframe Chum Salmon fry occupy or outmigrate from those streams. In sites sampled both years, the abundance of *C. shasta* was higher in 2018 than in 2019, but more widespread in 2019 (Figures 1 and 2). This difference may be due to two factors. First, the hydrograph differed between 2018 and 2019, and the floods in 2019 may have diluted or flushed out *C. shasta* spores. Second, many 2018 samples were inhibited (because we were processing the entire 4 L sample at once), and this may have resulted in false-negatives in sites where the spore load would have been near the detection limit (<1 spore/ L). In 2019, each 1 L sample (4 per site) was processed separately. No inhibition occurred in these samples and many low-level detections were observed.

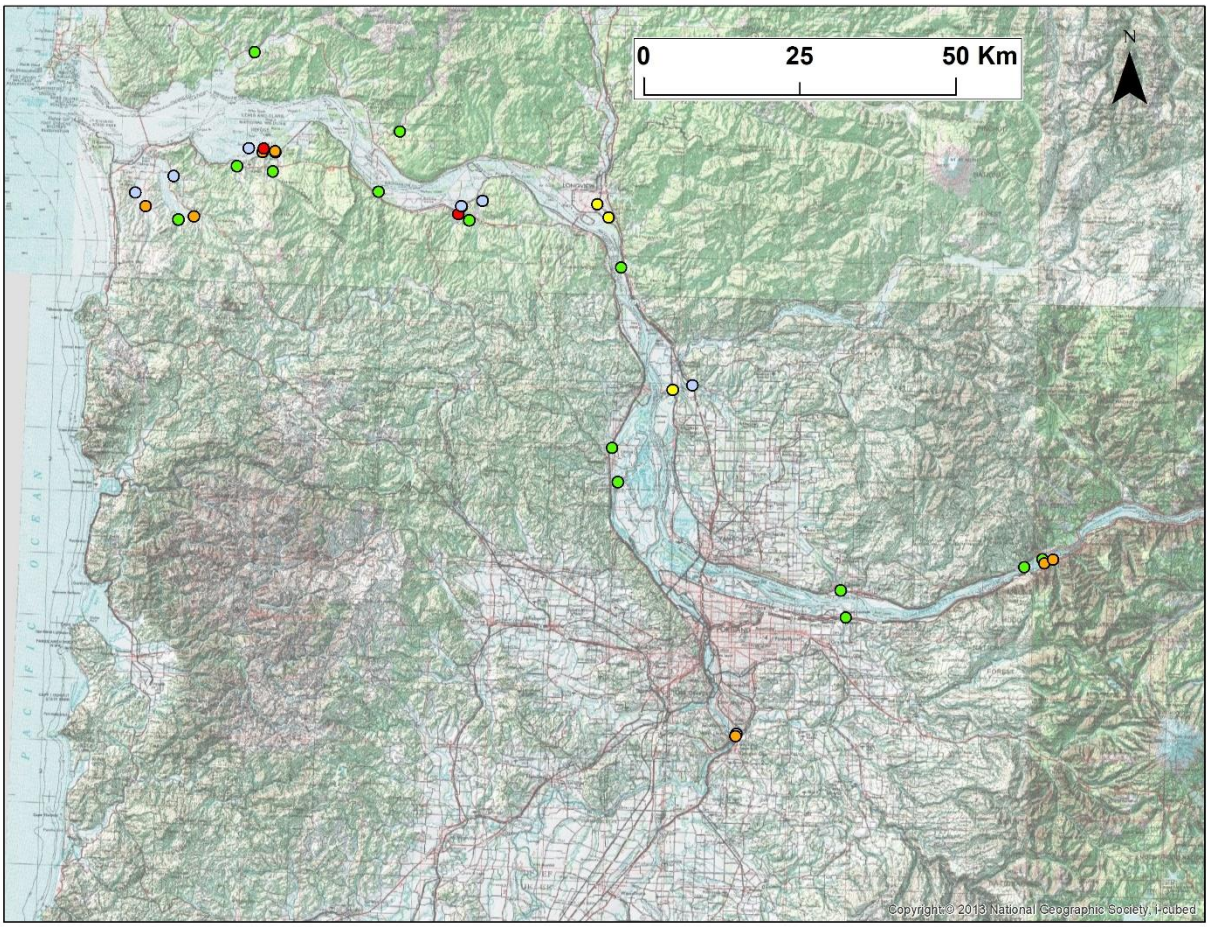

Figure 1. Sample locations for *Ceratonova shasta* in the Columbia Basin, March- May 2018. Markers indicate the genotype of *C. shasta* that was detected (green = no *C. shasta*, yellow = genotype I, red = genotype II, orange = genotypes I and II, and blue = sample inhibited.

The spatial and temporal pattern of positive *C. shasta* detections differed between years, but some sites were consistently negative and others were consistently positive. In 2018 and 2019, *C. shasta* was not detected in the Sandy River, Big Creek, Bear Creek, or the Kalama River. In

both years, it was detected in the Clackamas River, the Willamette River, Beaver Creek, the Clatskanie River (in tide water), the Lewis and Clark River (at the bridge on Lewis and Clark Rd), the Cowlitz River, the East Fork of the Lewis River, the Klaskanine River, and throughout the Columbia River at Minaker and Russian Islands, at Knappa Dock, and below Bonneville Dam (both sides of the river). Of the remaining sites sampled in both years, *C. shasta* was only detected in one year at the following sites: Hamilton Creek, the Washougal River, Stewart Creek, the Lewis and Clark River at Netul Landing, the Lewis River, the Clatskanie River just above tide, Scappoose Creek, Multnomah Channel near Sauvie Island, Westport Slough and in the Columbia River at Karlson Island. In the Grays River, *C. shasta* was not detected in 2018 and was detected in 2019 on 5/15/2019, after Chum Salmon fry had emigrated from the river.

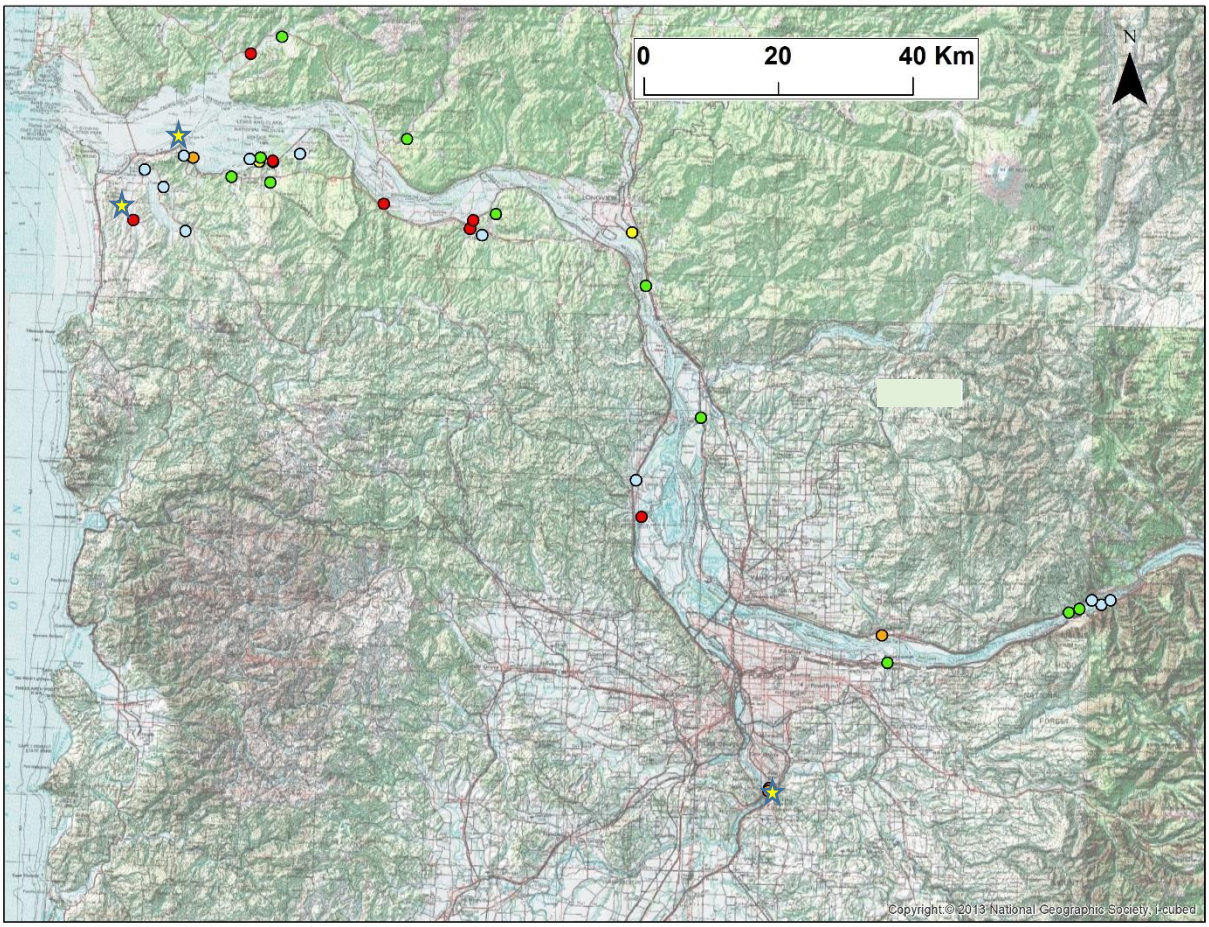

Figure 2. Sample locations for *Ceratonova shasta* in the Columbia Basin, April- May 2019. Markers indicate the genotype of *C. shasta* that was detected (green = no *C. shasta*, yellow = genotype I, red = genotype II, orange = genotypes I and II, and blue = genotype unavailable. Yellow stars indicate sites where sentinel cages were held.

Objective 2: the susceptibility of Chum Salmon fry to ambient levels of C. shasta in the Columbia River and tributaries

In general, water temperature and spore density increased at all sites over the exposure period May 1 – May 8. In water samples from sentinel sites, *C. shasta* genotype II was detected at all sites, and genotype I was detected at Tongue Point and in the Willamette River. Spore densities were highest at the Willamette River (average = 2.23 spores/ L), followed by Tongue Point (average = 1 spore/ L) and then the Lewis and Clark (average = 0.53 spores/ L; Table 9). At the Lewis and Clark, spore density was near the detection threshold. Water temperature ranged from 13 – 17.5 °C across sites (Figure 3). The warmest temperatures were recorded on the Lewis and Clark River, but that site was also associated with the lowest spore density.

The sentinel exposure was completed by 11 AM on May 8th. At this time, all sentinel cages were collected and placed into coolers filled with clean water (one cooler per site). Water temperatures in the coolers remained stable during transport. A total of two fry died during the exposure week and two died during transport to AAHL. These mortalities were subtracted from the totals observed during rearing at AAHL (and attributed to *C. shasta*).

Mortality differed significantly among locations. At the Willamette River site, 100% of Washougal Hatchery fish died (n = 30/ 30) and 100% of Big Creek Hatchery fish died (n = 29/ 29; Figure 4). All mortalities were found to be positive for *C. shasta* through either observation of spores (n = 58) or confirmation with PCR (n = 1). At the Columbia River site at Tongue Point, 96.7% of Washougal fish died (n = 30/ 31) and 100% of Big Creek fish died (n = 30/ 30; Figure 4). Of those mortalities, a total of 56 were found to be positive for *C. shasta* through either observation of spores (n = 50) or confirmation with PCR (n = 6). Clinical signs of disease were observed in all mortalities. At the Lewis and Clark River site, 30% of Washougal fish died (n = 9/ 31) and 66% of Big Creek fish died (n = 19/ 27; Figure 4). Of those mortalities, a total of 7 were found to be positive for *C. shasta* through either observation of spores (n = 5) or confirmation with PCR (n = 2). In the remaining fish, mortality was not attributed to *C. shasta*. At the time of this report, several PCR samples were being re-run and these mortality numbers may be adjusted higher.

Mean days to death differed among locations but not consistently between hatchery stocks. At the Willamette River, mortality in Washougal fish occurred days 34 – 40 (mean = 36.8; Figure 4) of the study (day 1 = the day the sentinel cage was placed in stream). For Big Creek fish, mortality occurred days 34 – 41 (mean = 38.0; Figure 4). At Tongue Point, mortality in Washougal fish occurred days 34 – 48 (mean = 42.0; Figure 4) and mortality in Big Creek fish occurred days 38 – 54 (mean = 44.0; Figure 4). At the Lewis and Clark River, mortality in Washougal fish occurred days 47 – 54 (mean = 47.0) and mortality in Big Creek fish occurred days 34 – 45 (mean = 39.7; Figure 4).

Table 9. Spore density (spores/ L) and genotypes of *Ceratonova shasta* at sentinel sites on the Willamette River, Tongue Point (Columbia River) and the Lewis and Clark River in the Columbia Basin, May 1- May 8, 2019.

Site	Spores/ L (avg)	Spores/ L (range)	Genotypes present
Willamette R.	2.23	1.35 − 3.05	I, II
Columbia R. at Tongue Point	1	0.59 − 1.3	I, II
Lewis and Clark R.	0.53	0 − 0.89	II

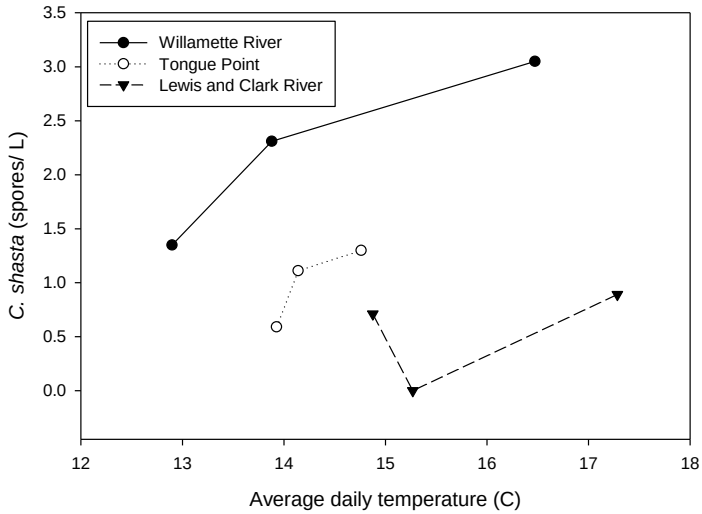

Figure 3. Average daily temperature at sentinel sites on the Willamette River, Columbia River at Tongue Point, and Lewis and Clark River, Oregon, May 1 – 8, 2019.

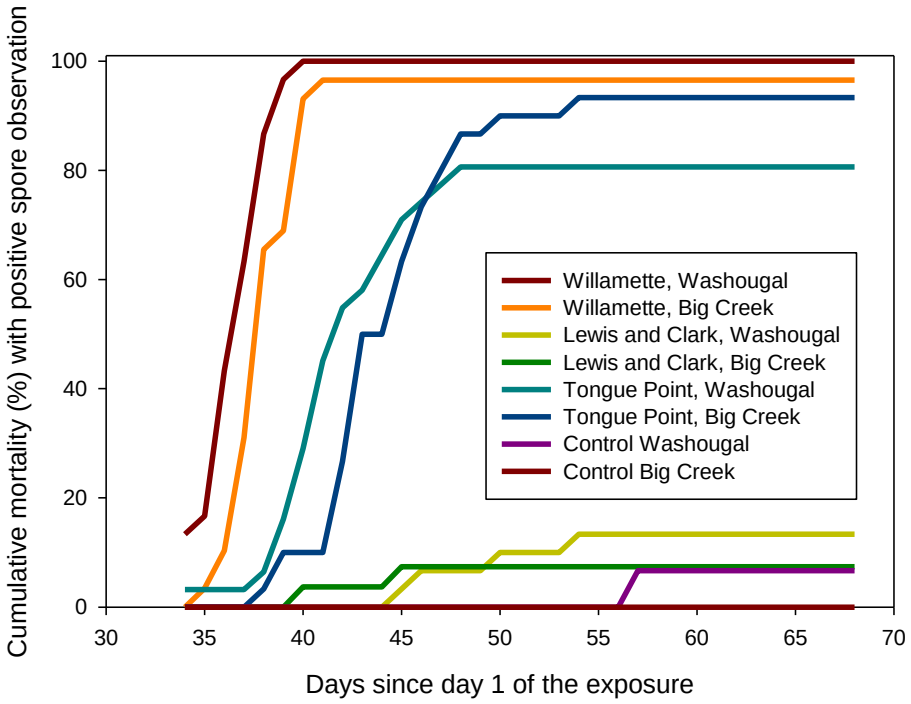

Figure 4. Cumulative percent mortality of Chum Salmon *Oncorhynchus keta* fry from Washougal Hatchery and Big Creek Hatchery exposed to *Ceratonova shasta* at sentinel sites on the Willamette River, Columbia River at Tongue Point, and Lewis and Clark River. Mortalities are only included if *C. shasta* spores were observed or if infection was confirmed through PCR. Mortality in hatchery control groups was from natural causes (n=1).

Objective 3: infection and mortality rates at four exposure durations using C. shasta, genotype II.

Trial 1 (2, 7, and 13-day exposure)

Exposures for lab trial 1 occurred 26 March to 8 April, 2019. During this time frame, water temperatures ranged from 13.5 to 14.1 °C. On day 12 of the study, the nearby Willamette River entered flood stage. Turbidity increased in the water flowing into the exposure tanks. Although the plan had been to expose fish for 15 days, the increase in turbidity posed a health risk to the fish. As such, all fish remaining in the exposure tanks were transferred to rearing tanks (25 L) on day 13. Those tanks were on well-water. No fish were lost as a result of the flood. After each group of fish completed their exposure duration (2, 7, or 13 days), they were removed from the tank and split into three rearing tanks. Lengths and batch-weight were measured for 5 fish from each rearing tank (Table 10).

Table 10. Length (mm fork length, FL) and weight (g) measurements of Chum Salmon *Oncorhynchus keta* recorded after each exposure to *Ceratonova shasta* concluded in 2019.

Exposure	Measurement Date	Length (mm FL) range	Mean length (mm FL)	Weight (g) range	Mean weight (g)
2- day	3/28/19	45 – 58	53.1	1.23 – 1.78	1.47
7- day	4/2/2019	48 – 63	57.9	1.73 – 1.94	1.81
13-day	4/10/2019	48 – 68	59.8	1.91 – 2.42	2.13
6-hour Washougal	5/8/2019	60 – 70	63.6	2.5 – 2.6	2.57
6- hour Big Creek	5/8/2019	60 – 82	67.8	2.18 – 4.16	3.07

For the 2, 7, and 13-day exposures, all treatment fish died and all control fish lived (Figure 5). Mortalities began on day 22 since the first exposure day and all fish had died by day 28. Fish in the 13-day exposure died before the 7-day and 2-day exposure fish (Figure 5). Mean days to death differed among exposure duration treatments. For 2-day exposures (all three tanks), mortality occurred days 23 – 28 (mean = 26.4; Figure 5) of the study (day 1 = the day of the exposure). For 7-day exposures (all three tanks), mortality occurred days 22 – 28 (mean = 26.0; Figure 5) of the study. For 13-day exposures (all three tanks), mortality occurred days 22 – 27 (mean = 24.6; Figure 5) of the study.

All treatment mortalities exhibited clinical signs of disease including pale gills, a hemorrhaged intestine, jaundice, or fluid in the stomach. When swabs from the intestines of these fish were examined under the microscope, *C. shasta* myxospores were observed in every fish. The rapid rate of mortality observed in these fish at 5 spores/ L suggests this is a high exposure dose for Chum Salmon fry.

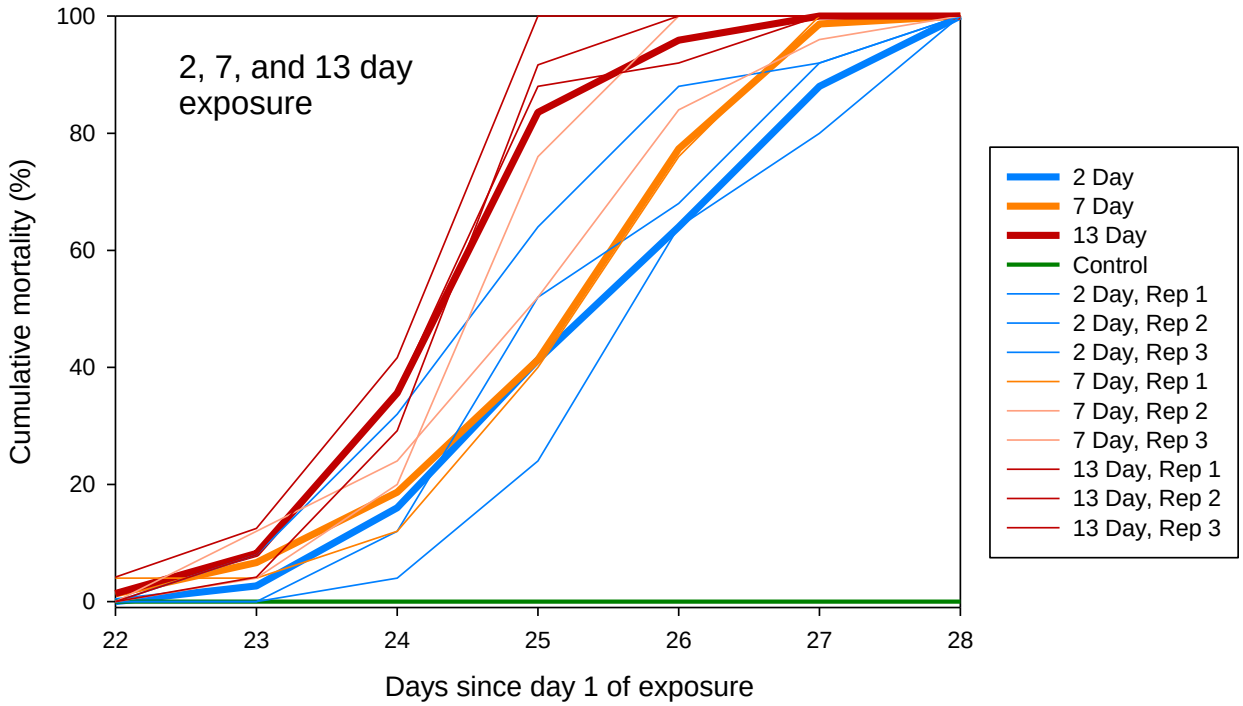

Figure 5. Cumulative percent mortality of Chum Salmon *Oncorhynchus keta* fry from Big Creek Hatchery exposed to *Ceratonova shasta* genotype II at a dose of 5 spores/ L. Mortalities are only included if *C. shasta* spores were observed or if infection was confirmed through PCR.

Trial 2 (6-hour exposure)

The exposure for lab trial 2 occurred on 8 May 2019 and subsequently fish were reared on well-water until 8 July 2019. At this time, all fish that were still alive were euthanized. Water temperatures during the exposure and post-exposure rearing were held at 13 °C. Lengths and batch-weight were measured for 5 fish from each rearing tank (Table 10).

In the 6-hour exposure, 84.7% of Chum Salmon fry from Big Creek Hatchery died and 67% of fry from Washougal Hatchery died. However, mortality varied substantially among rearing tanks (18.2 – 100% mortality), likely due to low exposure dose; at low doses, some fish may not actually be infected at the desired level (5 spores). Considering this variation in mortality among tanks, one replicate tank of fish from Washougal Hatchery was considered an outlier and was excluded from calculations of average mortality. Of the 84.7% of Big Creek fish that died (n = 61/ 72), a total of 58 were found to be positive for *C. shasta* through observation of spores (n = 55) or confirmation with PCR (n = 3). Of the 91.6% of Washougal Hatchery fish that died (n = 22/24; updated total), a total of 20 were found to be positive for *C. shasta* through observation of spores (n = 18) or confirmation with PCR (n = 2). At the time of this report, several PCR samples were being re-run and these mortality numbers may be adjusted higher.

Mean days to death differed among replicates but not consistently between hatchery stocks. For Washougal fish (all three tanks), mortality occurred days 38 – 48 (mean = 42.7; Figure 6) of the study (day 1 = the day of the exposure). For Big Creek fish, mortality occurred days 35 – 55 (mean = 40.2; Figure 6).

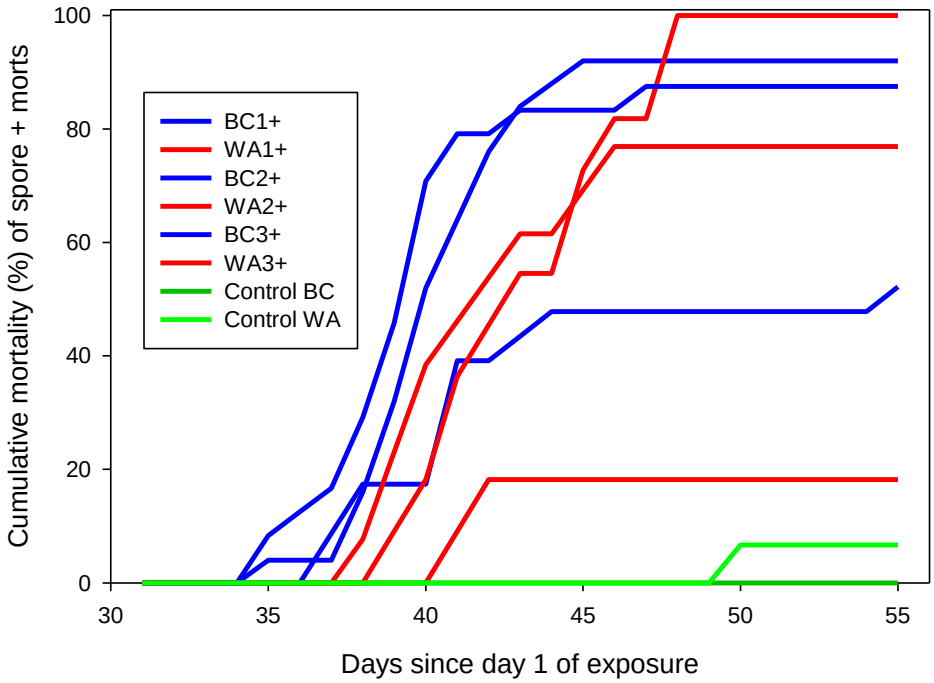

Figure 6. Cumulative percent mortality of Chum Salmon *Oncorhynchus keta* fry from Washougal Hatchery and Big Creek Hatchery exposed to *Ceratonova shasta*, genotype II at a dose of 5 spores/ fish. Mortalities are only included if *C. shasta* spores were observed or if infection was confirmed through PCR. Mortality in hatchery control groups was from natural causes (n=1).

Summary

Collectively, field and lab experiments on *C. shasta* demonstrated that the parasite is present in tributaries throughout the lower Columbia River and in the Columbia River estuary (freshwater portion) during the timeframe that Chum Salmon juveniles are outmigrating. Sentinel studies demonstrated that very low levels of *C. shasta* were lethal to Chum Salmon, and this was further confirmed in lab trials. Additional analysis will be completed to relate the abundance of *C. shasta* spores to cumulative temperature. This relationship will be used to estimate mortality rates in wild populations of Chum Salmon based on the temperature regime and probable abundance of *C. shasta*. Based on these early studies, it appears that *C. shasta* functions as a mortality factor for Chum Salmon in the Columbia Basin, although the degree to which it causes population-level effects requires further research.

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