HATCHERY AND GENETIC MANAGEMENT PLAN:
IMPLEMENTATION REPORT FOR
FALL 2019 – SPRING 2020

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 2019 collection of Chum Salmon broodstock at Big Creek Hatchery, egg transfers from Grays River Hatchery, and associated metrics (2) the 2020 fry releases from Big Creek Hatchery, (3) take occurring through monitoring and research actions in

fall 2019 and spring 2020, (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 2019

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 2019, a total of 64 Chum Salmon were collected at Big Creek Hatchery, including 21 individuals marked with an adipose clip. Two females arrived in October and no males were available for spawning so they were operculum punched released. Two unmarked males were held for spawning but died before any females arrived (Table 3). An additional four females arrived at the hatchery in December, but no males were available for spawning so they were released. Average fecundity varied over the course of the spawning run (Table 4). In anticipation of a poor return year, an additional 50,000 eggs were collected at the Grays River Hatchery and were transferred to Big Creek Hatchery to be fertilized.

Table 1. Marks applied to the Big Creek Hatchery Chum Salmon *Oncorhynchus keta* broodstock 2010–2019, and years when marks are expected to be present in age 3–5 adult returns to the hatchery or spawning grounds. All marks were applied at Big Creek Hatchery except for Otolith Thermal Marks that were applied at Grays River Hatchery for the 2010–2013 brood years.

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
2019	Pre and post-hatch thermal, PBT	2022–2024

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 2019, 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

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. In 2019, we examined thermal marks on otoliths collected from all fish spawned from 2014–2018. A small number of fish thought to be unmarked were found to have marks. Totals below reflect final data and may differ slightly from previous reports.

				% of Fish	
Brood Year	Males	Females	Total	Unmarked	Marked
2014	44	40	85	0	100
2015	87	87	173	27.2	72.8
2016	26	16	42	78.6	21.4
2017	22	38	60	85.0	15.0
2018	45	56	101	58.5	41.5
2019	27	28	55	65.5[a]	34.5[a]

[a] Otoliths have not yet been processed (unmarked/marked percentage may change after processing).

Table 3. Weekly spawn totals of unmarked and marked 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
11/12/2019	4	3	0	0	2	3	0	0
11/18/2019	6	6	1	0	4	5	0	0
11/22/2019	8	9	0	0	3	2	0	0
12/7/2019	0	0	2	0	0	0	0	0
Totals	**18**	**18**	**3**	**0**	**9**	**10**	**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)
11/12/2019	6	1,989	3,659	2,639	15,835	15,000
11/18/2019	11	922	2,954	2,310	25,096	27,500
11/22/2019	11	2,247	3,827	2,882	32,205	27,500
Totals	**28**	**922**	**3,827**	**2,611**	**73,136**	**70,000**

Grays River. A total of 20 females and 20 males from the Grays River were spawned at Beaver Creek Hatchery in Washington. Eggs and milt were placed on ice and transferred to Big Creek Hatchery where the eggs were fertilized. 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 2019 brood year, a total of 120,189 fed fry were released from Big Creek Hatchery using a release site located in a tidal area of Big Creek and a second site in Knappa Slough (Columbia River), near the mouth of Big Creek. This release was comprised of approximately 70,000 fry from eggs collected at Big Creek hatchery and an estimated 50,000 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					
	Method	Location	Number	Year	Date(s)	Size (fish/pound)
2010	Mesh bags	Big Cr tidewater	107,000	2011	4/7	224
2011	Liberation truck	Big Cr tidewater	110,000	2012	4/9	218
2012	Liberation truck Direct Release	Big Cr tidewater Big Cr Hatchery	108,500	2013	4/15; 4/17	168; 178
2013	Liberation truck	Big Cr tidewater	101,000	2014	4/17	185
2014	Liberation truck	Big Cr	190,188	2015	4/24; 5/15	190; 180
2015	Direct Release	Big Cr Hatchery	192,147	2016	4/25	143
2016	Liberation truck	Big Cr tidewater	37,725	2017	4/17	275
2017	Liberation truck	Big Cr tidewater	84,958	2018	3/29; 4/16	461; 401
2018[a]	Liberation truck	Big Cr tidewater	171,649	2019	3/13; 3/26; 4/2; 4/9	398; 334; 370; 449; 405
2019	Liberation truck Hand release	Big Cr tidewater Knappa Slough	120,189	2020	3/25; 4/2; 4/9	424; 412; 368

[a] Fry from Big Creek and Grays River origins were released on 3/13 and 3/26; fish/pound was identical between groups on 3/13 and different on 3/26.

Monitoring and reintroduction actions

In 2019, 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.	63	63		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.	6	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 2019, 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 15 – December 10, 2019. 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 2019, 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 12 Chum Salmon were observed on these surveys- 1 in the Wallooskee River (in the Youngs Bay Population), 9 in Little Creek, and 2 in Big Creek (in the Big Creek Population).

In spring 2020, rotary screw traps were operated from 28 February – 24 May on Bear Creek (Big Creek population) and 28 February – 6 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 2019, rearing and fish health parameters were monitored to ensure that fish culture standards are met. No health issues occurred for the 2019 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 or during the 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 2019.

Spawning year	Population	Survey name	Reach ID	Segment	Miles	Total Chum Observed
2019	Youngs Bay	Stavebolt Cr No.2	30042	1	0.81	0
2019	Youngs Bay	Lewis & Clark R	30045	2	0.93	0
2019	Youngs Bay	Hortill Cr	30046	1	0.32	0
2019	Youngs Bay	Lewis & Clark R	30047	1	0.57	0
2019	Youngs Bay	Lewis & Clark R	30051	1	0.90	0
2019	Youngs Bay	Loowit Cr	30052	2	0.70	0
2019	Youngs Bay	Lewis & Clark R	30055	2	1.12	0
2019	Youngs Bay	Wallooskee R	30068	2.1	1.57	1
2019	Big Creek	Mill Cr	30108	2	0.65	0
2019	Big Creek	Little Bear Cr	30126	1	1.02	0
2019	Big Creek	Little Bear Cr	30126	2	0.98	0
2019	Big Creek	Bear Cr	30127	1	1.15	0
2019	Big Creek	Bear Cr	30129	1	1.14	0
2019	Big Creek	Little Cr	30171	2	0.27	0
2019	Big Creek	Little Cr	30171	3.2	0.48	9[a]
2019	Big Creek	Big Cr	30172	3	1.11	2
2019	Big Creek	Gnat Cr	30198	1	0.81	0
2019	Clatskanie River	Plympton Cr	30239	2	1.03	0
2019	Clatskanie River	Olsen Cr	30247	1	0.67	0
2019	Clatskanie River	Conyers Cr	30276	1	0.62	0
2019	Clatskanie River	Clatskanie R	30283	1	1.12	0
2019	Clatskanie River	Clatskanie R	30285	1	0.75	0
2019	Clatskanie River	Clatskanie R	30291	1	0.85	0
2019	Clatskanie River	Beaver Cr	30336	1	1.08	0

[a] Count includes live and dead Chum Salmon and may include duplicate observations.

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 2019 – June 2020. *All intentional take of Chum Salmon fry occurred during studies on *C. shasta* as fish exposed to a pathogen cannot be released. The 210 Chum Salmon fry sacrificed for this study were from Big Creek Hatchery.

Action	Life stage	Estimated Annual Take		
		Lower Columbia		Columbia
		Chinook	Coho	Chum
Observe or harass	Any	0	0	0
Collect for transport	Adult	0	0	0
Capture, handle, and release	Fry		116	0
	Smolt	207	1,203	
	Adult		0	
Capture, handle, tag/mark/tissue sample, and release	Fry	716	756	2
	Smolt		2,401	
	Adult	0	0	6
Capture and remove (e.g., broodstock)	Adult	0	0	58
Intentional lethal take	Fry	0		210
	Any		0	
Unintentional lethal take	Fry	9	16	0
	Smolt		3	
	Adult	0	0	0
Other take (specify)				

Limiting-factors research

Historically, Chum Salmon, *Oncorhynchus keta*, populations in the Columbia River basin were abundant, with estimated runs exceeding 1 million adults. In the 1940s and 1950s, populations declined rapidly and currently only ~ 3,000–20,000 individuals return to the basin (NWFSC 2015). In 1999, all populations of Chum Salmon in the Columbia basin were listed as threatened under the Endangered Species Act as a single Evolutionary Significant Unit (ESU; NMFS 1999). In response, Oregon and Washington implemented recovery actions including (1) curtailing harvest, (2) restoring spawning habitat, and (3) operating conservation broodstocks (LCFRB 2010; ODFW 2010; NOAA 2013). Despite significant efforts Chum Salmon populations have not rebounded and current research is focused on understanding factors that may limit survival of juvenile Chum Salmon as they migrate from natal tributaries into the Columbia River.

The myxozoan parasite *Ceratonova shasta* (Noble 1950), endemic to the Pacific Northwest, is one factor potentially limiting Chum Salmon recovery in the region. *Ceratonova shasta* infects 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 lethal (Margolis and Evelyn 1975; Zinn et al. 1977). The parasite alternates between infecting a salmon and an invertebrate host, and two waterborne spore stages during its life cycle (Bartholomew et al. 1997). The invertebrate host (*Manayunkia occidentalis)* ingests myxospores and releases actinospores into

the water column (Bartholomew et al 1997). Actinospores infect the fish host via the gills (most typical), and the parasite migrates through the circulatory system and develops into myxospores in the intestinal tissues (Bjork and Bartholomew 2010). In the fish host, this process can cause intestinal hemorrhaging and death (Johnson et al. 1979a; Bartholomew et al. 1989).

Salmonid susceptibility to *C. shasta* varies among locations, populations, and species. Different strains of *C. shasta* (genotypes O, I, and II; Atkinson and Bartholomew 2010a; 2010b) exhibit salmon host specificity and cause differential mortality (Hurst and Bartholomew 2012). Chum Salmon from outside the Columbia Basin are susceptible to *C. shasta* (Schafer 1968; Zinn et al. 1977; Johnson et al. 1979a) genotype II (Stinson et al. 2018), and there is evidence of mixed infections with genotypes I and II (Stinson et al. 2018). Assessing the susceptibility of Columbia basin Chum Salmon stocks is important for estimating risk but not necessarily predictable. Susceptibility is influenced by evolution alongside *C. shasta* (Bartholomew 1998; Bjork and Bartholomew 2009; allopatric strains highly susceptible); some sympatric stocks have resistance to infection until the dose exceeds their infectious threshold (Bartholomew 1998; Bjork and Bartholomew 2009).

Ceratonova shasta has been described from numerous tributaries within the Columbia basin (Johnson 1975; Johnson et al. 1979a; Hoffmaster et al. 1988), but fine-scale distribution data was not available to evaluate the proportion in which Chum Salmon co-occur (or used to) until recently. Consequently, in 2018 and 2019, we collected water samples in lower Columbia River and tributaries having extant and historical Chum Salmon populations during the period when Chum Salmon fry migrate downstream (Figure 1). Water sampling is an effective method for detecting *C. shasta* presence because it is transmitted to salmonid hosts through waterborne spore stages (actinospores). Samples were examined by qPCR to assess presence and abundance of *C. shasta*, and positive samples were sequenced to determine *C. shasta* genotype(s) (see above, *C. shasta* genotypes and fish host specificity). We found that *C. shasta* was detected in over 50% (23/41) and genotype II was detected at over 90% (21/23) of sites (Figure 1). The parasite was abundant in systems that do not currently support Chum Salmon populations, but at low densities or undetected from sites that overlap with extant Chum 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), and determining their susceptibility to disease is of critical importance. As such, we assessed the susceptibility of juvenile Chum Salmon from the two primary Columbian basin populations of Chum Salmon to *C. shasta* in 2019. We exposed juvenile Chum Salmon to *C. shasta in-situ* (mixed genotypes, uncontrolled dose) in the Columbia and Willamette Rivers and in laboratory challenges (pure genotype II, controlled doses). The Chum Salmon held *in-situ* in sentinel cages in the Columbia and Willamette Rivers experienced significant mortality (95% and 100%, respectively at low doses 5-10 spores/L), and those exposed in laboratory trials succumbed to parasite-induced mortality at low doses of genotype II (5 spores/ L and 5 spores/ fish). The high mortality rates were observed in Columbia River Chum Salmon post-*C. shasta* exposure were unexpected

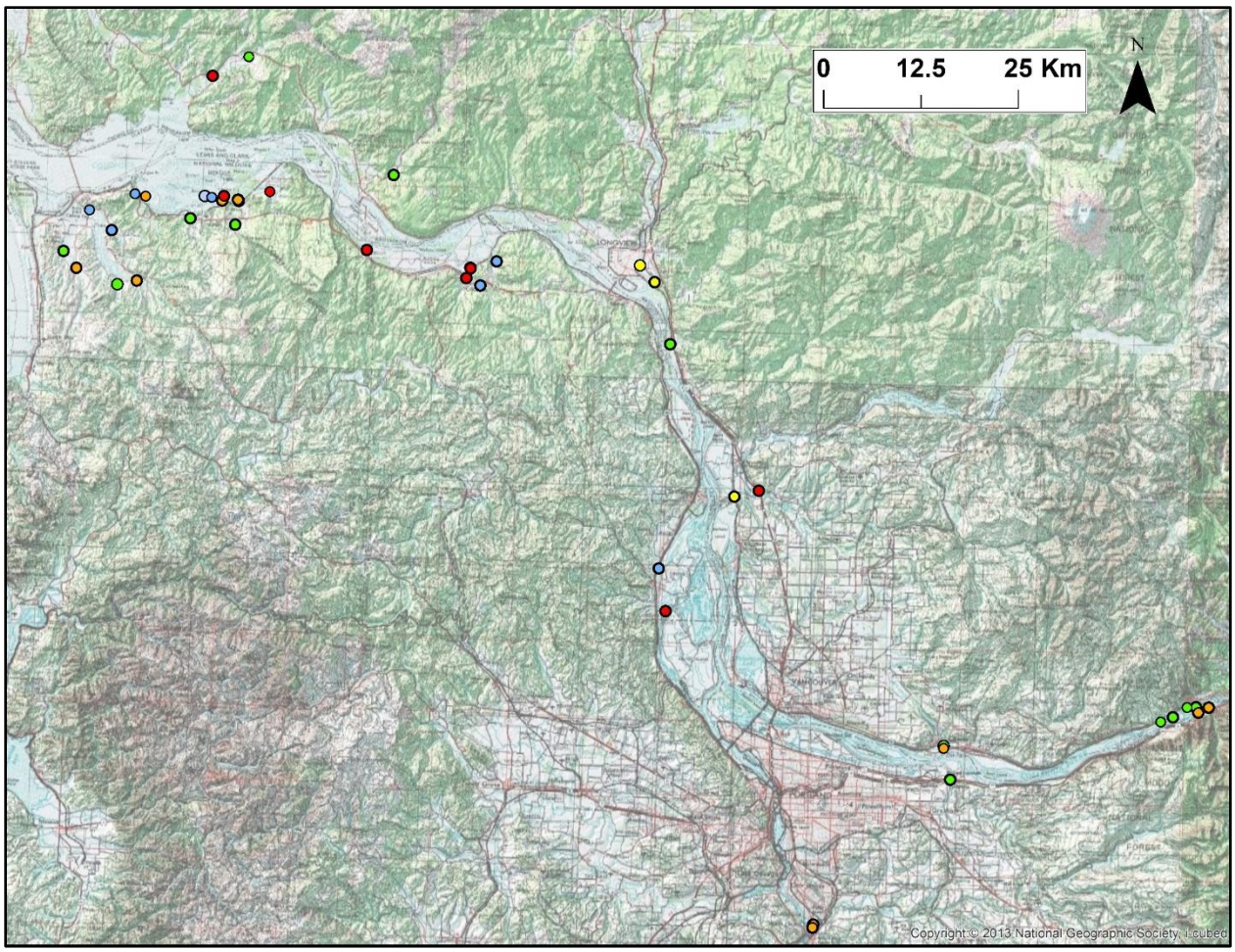

Figure 1. Sites where water samples were collected to test for presence and abundance of *Ceratonova shasta* in 2018 and 2019. Colors delineate *C. shasta* detection and genotype as follows: green = *C. shasta* not detected, yellow = genotype I, red = genotype II, orange = genotypes I and II, and blue = *C. shasta* detected but sample inhibited or genotype unavailable.

because they evolved sympatrically with *C. shasta* (other Columbia River salmonids exhibit variable levels of resistance to *C. shasta*) and we offer the following potential explanations: 1) genetic resistance to *C. shasta* may have existed previously but was lost following population bottle neck effects from 1940 – 1970s, 2) significant out-of-basin stocking from the 1960s – 1980s (Johnson et al. 1979b) could have resulted in a loss of genetic resistance as susceptible (out-of-basin) genes were introduced to the remaining small Chum Salmon populations, or 3) genetic resistance to *C. shasta* may have never existed because Chum Salmon life history timing prevented overlap with *C. shasta*. Although the mechanism is unclear, it is clear that between the widespread distribution of *C. shasta* overlapping with current and historical Chum Salmon distribution and the high susceptibility exhibited by two of the stocks being used to augment populations, we must better understand the dynamics between Chum Salmon and *C. shasta* to inform restoration and recovery opportunities in the Columbia Basin particularly in areas where other *C. shasta*-susceptible salmonid species co-occur (they may increase *C. shasta* risk for Chum Salmon).

The overall goal of the 2020 study was to assess the potential of *C. shasta* to limit the survival of juvenile Chum Salmon. The specific objectives were (1) to further describe the distribution of C. shasta throughout the lower Columbia River Basin, and (2) to determine the susceptibility of a persistent Oregon Coast Chum Salmon stock to different *C. shasta* genotype II doses (associated with mortality in Chum Salmon), and genotype I (infections reported in Chum Salmon, mortality risk unknown). *C. shasta* and Oregon Coastal Chum Salmon are abundant in the Nehalem River (Weber and Knispel 1977, genotypes not known). If juvenile Chum Salmon outmigrate before *C. shasta* actinospores are present in the water column, they may be susceptible to infection but their life history timing minimizes exposure to, and mortality from, *C. shasta.* Avoidance has been cited as an explanation for the variable resistance to *C. shasta* exhibited by Fraser River salmon stocks, despite co-occurrence with *C. shasta* (Ching and Munday 1984; Ching and Parker 1989). However, Nehalem Chum Salmon may exhibit genetic resistance to *C. shasta*. In 1977, Zinn and Parker held Chum Salmon from Whiskey Creek Hatchery (Netarts Bay, Oregon Coast) in the Willamette River for 66 days. 6% of fish survived (Zinn et al. 1977), in contrast to 0% following our 7-day exposure of Columbia River Chum Salmon in 2019. By characterizing the resistance of Nehalem Chum Salmon to *C. shasta*, we will be able to better inform the identification of appropriate habitat restoration or reintroduction strategies, in addition to potentially understanding how *C. shasta* resistance may be introduced into our existing conservation broodstock.

Research efforts in 2020 initiated as the Covid-19 pandemic was spreading throughout Oregon. As a consequence of public land closures and health restrictions, field sampling was substantially reduced in 2020. Fry were collected for lab exposures, but those exposures could not be initiated until May. The original budget earmarked for this project was reduced, following cuts to the budget of state agencies. We strategically identified particular samples to process using that reduced budget, but subsequent lab and university access restrictions have delayed processing of those samples. Here I report on the methods for our experiments, including portions of these methods that are incomplete, and preliminary observations following the lab trials. Final results will be summarized in a publication once sufficient funding is identified to process all genetic samples.

Methods and analysis

Objective 1: Spatiotemporal distribution of C. shasta in the Columbia River and tributaries

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. Additional sample sites were selected on the Oregon Coast, as part of our effort to understand whether Chum Salmon on the Oregon Coast exhibit any genetic resistance to *C. shasta*. Sample sites included (1) tributaries within the historical distribution of Chum Salmon, (2) sites where *C. shasta* is known to occur, (3) along the migration corridor in the Columbia River, (4) where sampling occurred during 2018 and 2019, and (5) in known Chum Salmon streams along the Oregon Coast. These sites occur on both the Oregon and Washington sides of the Columbia River in freshwater and tidal freshwater, and along the Northern coast of Oregon.

In tributaries and along the Oregon Coast, a total of 11 temporal sites (sampled April 15, May 1, and May 15) and 23 spatial sites (sampled once on 15 May) were selected (Figure 2). In the Columbia River, a total of 11 temporal sites (sampled weekly from April 15 through May 15), and 25 spatial sites (sampled May 7) were selected. 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 where they are currently being stored, pre-processing. Eventually, filters will be 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 2020, each liter of the sample will be processed separately. When samples are inhibited, the samples (and positive genetic control) will be diluted and processed again. The presence and concentration (spores/ L) of *C. shasta* will be determined through qPCR. Any positive samples will be genotyped to determine the percentage of each genotype represented in the sample. Specific genetic methods are described in Hallett and Bartholomew (2006).

Objective 2: Evaluate infectious threshold of Columbia River and Coastal Chum Salmon to C. shasta genotypes I and II

Fish Collection

Chum Salmon fry were collected from Foley Creek (n = 230), a tributary to the Nehalem River where *C. shasta* has been detected, 10 – 11 March. ODFW collected outmigrating Chum Salmon fry from Foley Creek, OR using electrofishing capture techniques. During sampling, fry were held in oxygenated coolers filled with river water and placed in a secure location on the bank. Once all fry were collected, the coolers were transported to the Aquatic Animal Health Lab

(AAHL) for acclimation and exposure.

Laboratory Challenges

Exposure challenges were conducted to test the infectious threshold of *C. shasta* genotype I and II for juvenile Chum Salmon from Oregon Coast stocks. We have established genotype specific mesocosms in which *C. shasta* actinospores are produced and available for experimental use at the Aquatic Animal Health Laboratory. Fry from Foley Creek were randomly assigned to one of three exposure treatments (genotype II at 5 spore/ fish, genotype II at 100 spores/ fish, or genotype I at 100 spores/ L) or to the control (no *C. shasta*) group (n = 15). There were three replicates per exposure treatment (treatment = genotype X spore level; n = 20 fry/replicate; n = 180 fry). Each group was exposed in separate tanks (25 L) for 6 hours. Because *C. shasta* is present in the Nehalem Basin in the late spring, it was necessary to confirm that treatment and control fry are not infected with *C. shasta* prior to beginning the lab exposures. Consequently, 15 fry were immediately euthanized examined for presence of *C. shasta* by PCR. Water samples were also collected at the time fry are collected to confirm that *C. shasta* had not yet appeared in the creek. Remaining control fry (n = 15) were held in a separate spore-free tank at the lab and treated identically to the treatment fish. After exposure, fish were reared for up to 60 days at 14°C (a temperature in 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 until the disease progressed to a point where the fish could not maintain equilibrium. At this point, the fish was removed from the tank, euthanized, and a necropsy was performed (AAHL 2016). By day 60, all remaining fish were euthanized (AAHL 2016).

Disease and genetic analysis

Disease and genetic analysis have been delayed, but samples are preserved and will be analyzed at a later date. Evidence of infection will be determined by collecting a hind gut swab to assess the presence of parasite spores. Spore density will be determined from a 3 minute count of spores. In addition, during necropsy, the intestines and other organs will be examined for presence of cysts or other evidence of infection and disease. If spores ate not observed during microscopy, tissue samples will be collected to assay by PCR to check for presence of *C. shasta*. PCR will also be performed on 25% of fish that were positive for *C. shasta* spores to confirm results. Genotyping will be conducted to confirm results of exposure experiments and doses. PCR (and genotyping as necessary) will also be performed for pre-experiment Foley Creek samples to test for any background exposure/infection.

Mortality from *C. shasta* will be calculated as the percent of fish from each treatment that died, after correcting for other mortality sources. To do so, the starting number of fish will be adjusted by the number that die from causes other than *C. shasta* (with genetic confirmation that the fish was not infected). Percent mortality will be compared among locations, density levels, replicates, and against the control group; survival curves will be generated for each tank.

Results

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

In 2020, *C. shasta* sampling was modified by the Covid-19 pandemic. Many samples sites (e.g., in Washington and on public lands) could not be accessed and sampling effort was concentrated in Oregon sites (Figure 2). Currently, samples have been extracted and are being stored in a freezer at OSU until funds are available to process them.

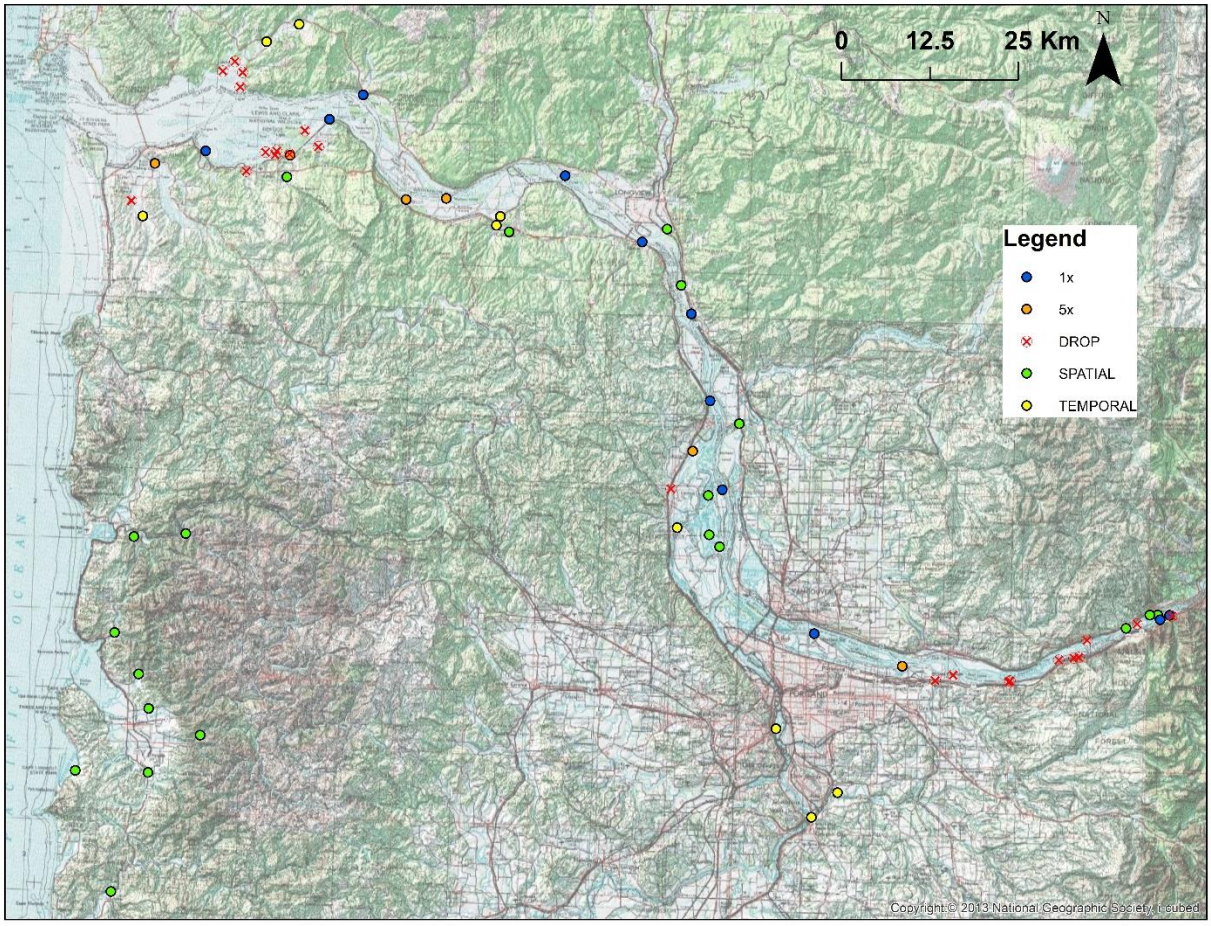

Figure 2. Columbia River and tributaries where we planned to collect water samples to test for *Ceratonova shasta* in 2020. Green dots = tributary sites sampled on May 15, yellow dots = tributary sites sampled every two weeks from April 15 – May 15, blue dots = Columbia River sites sampled on May 7, and orange dots = Columbia River sites sampled every week weeks from April 15 – May 15. Sites that could not be accessed are shown here as a red circle with an X in the middle.

Objective 2: Evaluate infectious threshold of Columbia River and Coastal Chum Salmon to C. shasta genotypes I and II

<u>Trails I and 2: genotype II- high dose and low dose</u>

The exposure for lab trials 1 and 2 occurred on 14 April 2020 and subsequently fish were reared on well-water until 14 June 2020. 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.

Early in both genotype II exposures, fry from Foley Creek died of natural causes in treatments and in the controls. Treatment mortality rates have not yet been adjusted as the control

mortality exceeded treatment mortality during the first two weeks of the experiment. Some mortality also occurred in the Big Creek controls, but that occurred beginning in the third week.

As data were only recently available and have not been error checked, I provide only general observations here. Moreover, mortalities are currently being examined for the presence of *C. shasta* spores, so tallies of negative and positive fish are not available. Both Big Creek and Foley Creek fry experienced higher mortality after exposure to the High dose, relative to the Low dose. Without having statistically corrected for mortality in controls there does appear to be a pattern of slightly lower mortality in the Foley Creek fish than the Big Creek fish, for both High and Low doses. It also appears that mortality in the Foley Creek fish began earlier than in Big Creek fish. Of those fish that have been examined for spores, most are positive.

Trial 3: genotype I

The exposure for lab trial 3 began in early June, 2020 and concluded in August. Water temperatures during the exposure were held at 13 °C. Infections with genotype I were not observed when looking at intestinal swabs, but these results are awaiting confirmation with PCR. Natural mortality has been observed in some treatments.

Summary

Lab trials demonstrated that juvenile Chum Salmon are highly susceptible to lethal infection by low doses of C. shasta genotype II. Both Coastal and Columbia River stocks exhibited high mortality rates at low and high exposure doses. Interestingly, neither stock appeared susceptible to genotype I. Mixed infections with genotype I and II have been observed in Chum Salmon adults, but this was not observed in our trial. Based on lab trials and previous research on the distribution and density of *C. shasta* in habitat occupied by Chum Salmon in the Columbia River, it appears that *C. shasta* functions as a mortality factor. However, the degree to which it causes population-level effects requires further research.

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