DNA analysis of Puntledge River Summer Chinook - assessment of run timing inheritance and BKD (bacterial kidney disease) resistance
Year 1

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Prepared by:

R. Withler [1] and E. Guimond [2]

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[1] Fisheries and Oceans Canada
Pacific Biological Station
Molecular Genetics Section
Nanaimo, B.C. V9T 6N7

[2] 473 Leighton Ave.
Courtenay, BC
V9N 2Z5

EXECUTIVE SUMMARY

Genetic analysis methods will be used in this multi-year study to identify individual Puntledge River summer-run Chinook salmon back to parental crosses (both those that were performed in the hatchery and those that occurred in the wild) to study the effects of parental Chinook return migration time and BKD status on their progeny. The genetic analysis is known as 'parentage-based tagging' and it allows identification of an individual offspring (at any age, including adults) to its parental pair, as long as both parents have been sampled and genotyped. The genotyping of parents and offspring will be conducted with a set of fifteen microsatellite loci (genetic markers) that are analyzed in the Molecular Genetics lab (MGL) at the Pacific Biological Station. Fisheries and Oceans Canada (DFO) considers the Puntledge River summer-run Chinook salmon a population of high conservation concern. This research will provide information on the most effective strategies to implement in re-establishing successful reproduction both in the hatchery and in the wild.

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1 INTRODUCTION

This is year one of a multi-year project that will utilize genetic analysis methods to identify the Puntledge River summer-run Chinook salmon parents of first generation adult returns (the parents that were originally spawned in the hatchery and those that spawned in the wild) to investigate the heritability of migration timing and bacterial kidney disease (BKD). The genetic analysis used in this study is known as 'parentage-based tagging' (PBT) and it allows identification of an individual offspring (at any age, including adults) to its parental pair, provided that both parents were originally sampled and genotyped. The offspring will be sampled as downstream migrating juveniles or as returning adults over the next one to four years. The BKD status (negative or low-positive) of female hatchery parents and the migration timing of both male and female parents will be compared with the survival and migration timing in their offspring to determine the influence of female low level BKD infection on offspring survival and the heritability of migration time in hatchery- and naturally-spawned Puntledge summer Chinook salmon.

While the enhancement of the summer Chinook population at the Fisheries and Oceans Canada's Puntledge River Hatchery has stabilized, levels remain below DFO's target escapement. The current research will provide information on the most effective strategies to implement in re-establishing successful reproduction both in the hatchery and in the wild. The project has strong support and cooperation between DFO, the K'ómoks First Nation and BC Hydro and will provide information to guide the efforts of stakeholders in restoring the salmon populations in the Puntledge River that have been impacted by hydro development.

1.1 Background

Access and utilization of habitat above BC Hydro's diversion dam is critical to the sustainability of summer Chinook and coho salmon production in the Puntledge watershed. Past studies on summer Chinook migration in the Puntledge River have indicated that summer Chinook adults that arrive in the lower Puntledge River prior to July have a greater success migrating to the upper river (at or above the diversion dam) compared to those that arrive later in the summer (95% versus 50% success rate). The success of early arriving fish is attributed to cooler migration temperatures in the river, low recreational use, and the higher availability of spring freshet spills that aid upstream Chinook migration into Comox Lake. In contrast, later arriving Chinook must contend

with warmer river temperatures, lower flows, and a high level of disturbance from swimmers, particularly at Stotan and Nib falls, two areas that present some of the greatest challenges for migration. Furthermore, studies have also shown that Chinook that are able to hold in the cooler depths of Comox Lake throughout the summer have a spawning success rate of 95% compared to ≤ 50% for fish that hold below the diversion dam (Guimond and Taylor 2010).

This clearly demonstrates that the most productive strategy for summer Chinook adults is to migrate into Comox Lake early (i.e. before July), hold in the lake during the summer and then spawn above the diversion dam at the lake outlet (headpond) or in the two main Comox Lake tributaries (Upper Puntledge and Cruickshank rivers).

The Puntledge Hatchery Salmonid Enhancement Program (SEP) has incorporated these watershed species requirements into their Production Strategy. DFO has begun to imprint and release summer Chinook hatchery smolts in Comox Lake to determine whether this will encourage the hatchery returns to migrate back to the lake where they will have the greatest chance of survival. A higher proportion of the earlier returning summer Chinook will be utilized for hatchery broodstock which is expected to re-build the earlier component of the summer Chinook returns thus improving migration success to the upper watershed. If the early returning behaviour is genetically controlled, selecting earliest returning adults for brood and mating them with each other should result in an earlier returning summer Chinook in the following generation. It is anticipated that, over time, these strategies will have the following benefits:

- increase the separation in migration timing between summer and fall Chinook,
- increase the success of summer Chinook salmon returning and migrating to the upper watershed and Comox Lake,
- increase the number of successful spawners above the diversion dam while reducing the number that remain in the lower river, and
- reduce the risk of hybridization between summer and fall Chinook.

All hatchery activities that enhance the earliest returning adults of the summer Chinook run are identified as a high priority “Species Based Actions” in the FWCP-Coastal Salmonid Action Plan for the Puntledge watershed (FWCP 2011).

1.2 Goals and Objectives

The overall goal of the study is to provide guidance for the development of appropriate hatchery protocols that will maintain the genetic distinction of the summer and fall Chinook populations, properly manage BKD in the summer Chinook

population and optimize the survival. A thorough understanding of these two factors, as described below, will be critical to the rebuilding efforts of the Puntledge River summer Chinook population.

i. **Assessment of run timing inheritance -** Migration time has been shown to be genetically controlled (and therefore heritable) in Chinook salmon (Healey 1991). Moreover, the returning progeny from early- or late-migrating parents tend to return at similar times. Therefore, we expect the early (May-June) and late (July -August) migrating adults spawned in the hatchery, and those that spawn in the wild, to produce offspring with similar adult migration timing. However, environmental factors (e.g. marine conditions, freshwater temperature and flow levels) also affect migration and introduce annual variation in migration timing (Anderson and Beer 2009). This study will enable us to calculate the degree of genetic and environmental influences on migration time in Puntledge summer Chinook salmon and the degree to which selection for early migration times may be effective in improving their survival and abundance. Selection for early migration time in the summer Chinook has the added benefit of facilitating genetic separation of the two Chinook salmon populations in hatchery and natural spawning within the Puntledge drainage; maintaining this genetic distinction is necessary for adaptation and long-term conservation of the summer run.

ii. **Assessment of BKD resistance** - *Renibacterium salmoninarum*, the causative agent of BKD, is an endemic pathogen in the Pacific Northwest. BKD is a slowly progressing, lifelong infection of salmonids. The bacterium may be horizontally transmitted between fish and vertically transmitted to the next generation. Fish infected with *R. salmoninarum* will not normally exhibit clinical signs until the fish are a year old. As such, BKD is a serious disease in salmon culture. From a husbandry perspective, good hatchery practice is to eliminate or minimize presence of the pathogen in the hatchery (and subsequently the natural) environment by culling progeny from BKD-positive female parents. However, there may be a genetic disadvantage to this practice if, in fact, the positive females that are being selected against carry genes that enable tolerance of the pathogen and the ability to survive and reproduce, even in the presence of bacterial infection.

The fate of hatchery individuals that are disease-free but carry the *R. salmoninarum* bacterium due to vertical transmission, and their impact, or lack of, on their naturally-spawned counterparts following release has not been closely studied. Good husbandry and a precautionary approach to wild interactions have been the driving factors to date in developing appropriate protocols for responding to infection in the hatchery environment. However, it is important to determine if exclusion of all

progeny from *R. salmoninarum* positive females is hindering inherent mechanisms of BKD resistance in some populations, as well as possibly contributing to an unnecessary loss of genetic diversity in populations of conservation concern. Conversely, investigating the putative role of immunotolerance and heightened BKD susceptibility following vertical transmission of the pathogen will inform future DFO SEP BKD management strategies. The ability to follow the survival and reproductive success of offspring from individual BKD positive and negative females in the Puntledge summer Chinook population will assist both in its management and in the refinement of general husbandry protocols for BKD affected hatchery populations.

2 STUDY AREA

The Puntledge River Watershed encompasses a 600 km^2 area west of the city of Courtenay (Figure 1). The lower Puntledge River flows from Comox Lake in a north-easterly direction for 14 km where it joins with the Tsolum River. From this point downstream the river is called the Courtenay River, and flows for another 2.9 km into the Strait of Georgia. The lower river below Comox Lake is divided into 3 major reaches. Reach B, the headpond reach, is located between the Comox impoundment dam at the outlet of Comox Lake, and the Puntledge diversion dam approximately 3.7 km downstream. Reach C, the diversion reach, extends downstream of the diversion dam for 6.3 km to the BC Hydro Puntledge Generating Station or "Powerhouse". Reach D encompasses the remaining 4 km of the Puntledge River from the Powerhouse to the Tsolum River confluence. Puntledge River Hatchery is located 400 m downstream of the Powerhouse. A barrier fence across the river directs migrating fish into a fishway where they may proceed further into concrete raceways in the facility, or continue their migration upstream in the river depending on the hatchery's broodstock collection requirements.

The Puntledge River system is one of a few rivers on the east coast of Vancouver Island that supports both a summer and fall-run of Chinook salmon. The two runs have discrete migration timings and spawning distributions in the river. Summer-run Chinook enter the river from May to August while fall-run Chinook enter from September to October. However both stocks spawn at the same time, from early October to early November.

Puntledge summer Chinook are genetically distinct from the fall Chinook stock. It is surmised that the summer-run evolved from early migrants of an ancestral fall-run stock that were able to ascend two large waterfalls in the lower river (Stotan and Nib falls) during the natural spring freshet period between April and June/July, and hold in

Comox Lake prior to spawning. The two partial obstructions have been critical in maintaining the spatial segregation and genetic integrity of the two stocks.

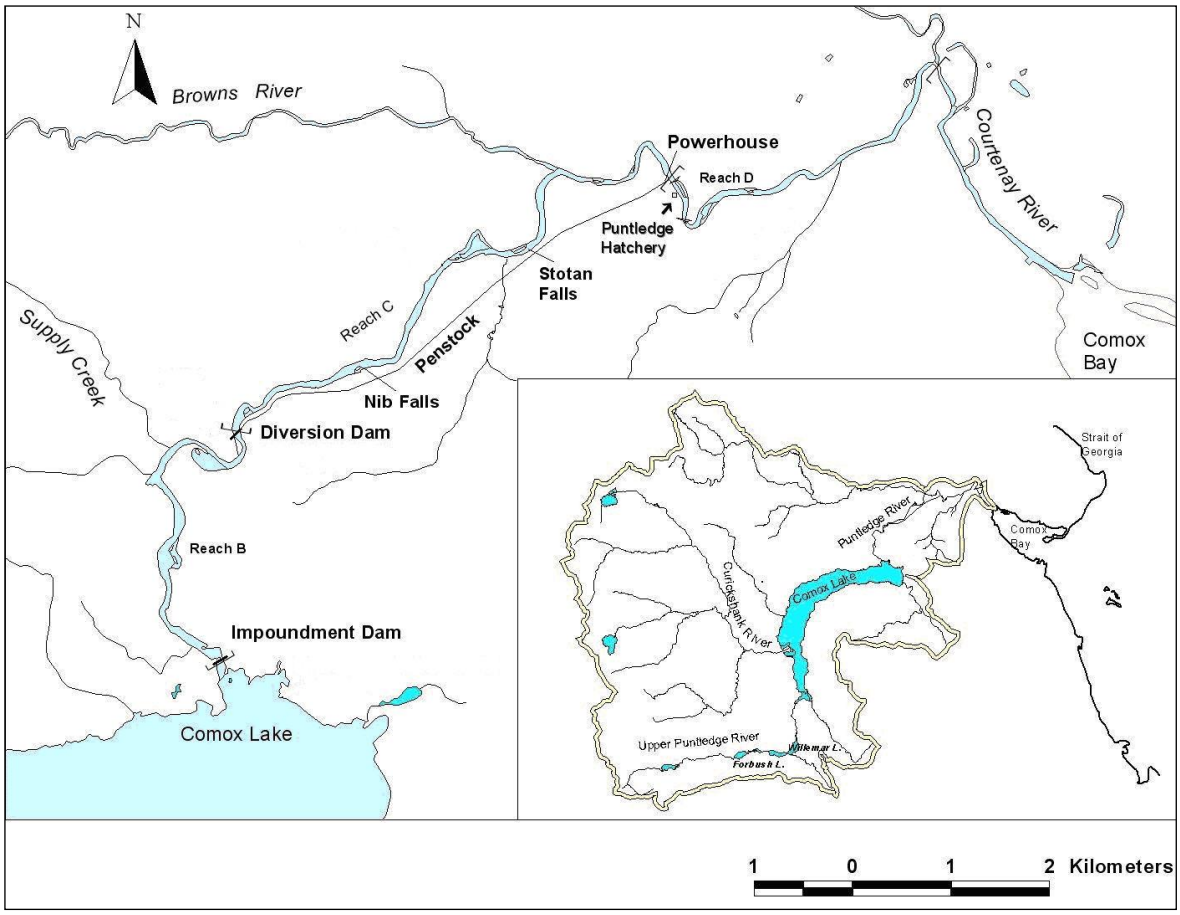

Figure 1. Location map of the Puntledge River watershed and lower river features.

3 METHODS

3.1 Annual DNA tissue sampling of summer Chinook salmon hatchery broodstock and wild summer Chinook salmon spawners at Puntledge River Hatchery

The overall goal is to obtain high quality DNA tissue samples and accurate biological data from all broodstock spawned at the hatchery, and 50-70% of the adults that spawn in the wild.

Summer Chinook adults migrating into the Puntledge River were diverted at the barrier fence into raceways at the lower Puntledge River Hatchery beginning in mid-May 2014. Every few days the adults were crowded for sorting and inspected for coded wire tags (CWTs) using a handheld wand CWT detector (Northwest Marine Technology Inc. WA) as part of a separate study on summer Chinook homing behaviour (Guimond 2014). A portion of the identified CWT adults selected for the homing behaviour study were PIT (Passive Integrated Transponder) tagged and measured for fork length. A tissue sample (hole-punch of the tail fin) was removed and affixed to a Whatman tissue sample sheet for DNA analysis. Care was taken to avoid contamination during sampling by rinsing hole-punch tools in water and wiping with a paper towel in between each tissue sample. These fish were returned to the river at the lower hatchery and allowed to continue their migration as per the objectives of the homing behaviour study. Summer Chinook that were not required for the homing study were either loaded onto a transport tank containing chilled water (typically 4-6 ^{o}C cooler than ambient) and transported to Rosewall Creek Hatchery for hatchery broodstock, or DNA sampled, and loaded onto a separate transport tank and released directly into Comox Lake to spawn naturally.

Summer Chinook broodstock transported to Rosewall hatchery were held in separate rearing tubs over the summer, based on their arrival time at Puntledge Hatchery. Those arriving prior to July 1st were treated as "early" timing fish and those arriving between July 1st and Aug 1st as "late" timing fish. The broodstock in each group were spawned within their own timing group over 5 egg collection periods or Lots between 6 October and 5 November. During each egg-take, males and females from each spawning pair were DNA sampled, measured for length (postorbitol-hypural) and scale sampled. In addition, a kidney tissue sample (for BKD analysis) was collected from each adult spawned and placed in separate Whirl-pak® bags with corresponding ID #s. BKD sampling followed specific procedures outlined in the Puntledge River Hatchery Fish Health Management Plan.

Each tissue sample collected was labeled with a unique ID #, and used to track the samples that were DNA analyzed with other corresponding biological data including sex, date of river entry (or arrival at Puntledge Hatchery), date sampled/spawned, length, tag information, markings, and BKD screening results (hatchery broodstock only). The associated data was reviewed at the lab to ensure accurate information was recorded for every fish sampled. Any discrepancies were resolved by hatchery staff before samples were analyzed. DNA samples from broodstock whose eggs were culled were not genotyped.

3.2 Juvenile summer Chinook sample collection

A sub-sample of emergent Chinook fry captured at the Eicher evaluation facility at the Puntledge diversion dam were collected between 13 February and 31 March, 2015 for DNA analysis. The facility allows for a portion of the downstream migrating juveniles from natural spawning above the dam to be captured. Analyses of these samples will allow us to assess the spawning success and mate choice of hatchery fish that returned to spawn in the wild, and will provide insight as to whether infected adults returning from hatchery spawning were able to contribute to the next generation produced in the natural environment. The 2014 brood year (BY) fry collections will continue until the end of the migration monitoring period (August 1, 2015), so that approximately 10% of all fry captured at the evaluation facility are sampled. Chinook fry samples were collected weekly in 250 ml vials with 95% ethanol and each vial contained no more than 25% in volume of fry.

3.3 BKD screening

The BKD specific pathogen control plan for DFO fish culture facilities has been devised to prevent clinical BKD epizootics during hatchery rearing and to reduce the risk of disease amplification through hatchery practices. It is comparable to the control strategies employed by public enhancement facilities throughout the Pacific Northwest. The plan recommends that all known BKD 'hot' Chinook and Coho stocks be annually screened and participate in egg culling and progeny segregation based on female parental Enzyme Linked Immunosorbant Assay (ELISA) optical density (O.D.) readings of *R. salmoninarum* antigen levels. Other stocks are subjected to periodic prevalence assessment of 60 fish, to confirm BKD risk status. The Puntledge summer Chinook stock was recently identified as a high risk BKD stock during routine screening of 2009 and 2011 broodstock. As a result of the altered stock BKD risk designation, the production strategy was altered to improve biosecurity and to participate in an annual BKD broodstock screening, egg segregation and culling program. Specific biosecurity measures employed include pre-spawning antibiotic administration to females prior to egg collection, iodophor egg disinfection during water hardening, incubation in individual heath trays until broodstock ELISA results are available and culling based on levels of soluble *R salmoninarum*-antigen detected using ELISA. BKD screening ratings and recommended actions include:

- **Negative** - fertilized eggs/progeny from females that have a lower optical density (OD) value than those of the kidneys of the negative control fish. These may be used for yearling programs.
- **Low Level of Detection** - OD values <0.1 but greater than the mean negative control. LLD eggs present a low enough risk of BKD to be treated as negative.
- **Low Positive** - OD value ≥ 0.1 but < 0.25. Progeny from these eggs should be released early, as unfed fry.
- **Moderately Positive** - OD value ≥ 0.25 but < 0.6. Progeny should be outplanted as eyed eggs if rearing habitat is available downstream from the water intake of the facility, or destroyed if appropriate habitat is unavailable
- **High Positive** - OD ≥ 0.6, should be destroyed.

As indicated above, progeny from females with ELISA OD values > 0.1 are normally released as unfed fry to minimize the risk of horizontal transmission of *R. salmoninarum* and to prevent BKD epizootics at SEP facilities. However, in situations where escapement numbers are low or the prevalence of BKD in the escapement is high, the prescribed culling recommendations may compromise production targets. Deviation from these recommended actions may be permitted if conservation concerns outweigh the ecological risks of propagating progeny from *R. salmoninarum*-positive fish, provided there is mutual agreement of the hatchery management, enhancement operations support staff and the DFO veterinarian. In 2012, the OD value threshold for yearling production for Puntledge summer Chinook was raised to 0.14 and progeny were reared without evidence of clinical BKD.

In 2013 and 2014, Puntledge Hatchery staff recorded all broodstock crosses, female *R. salmoninarum* antigen levels and parental DNA sample numbers. For Puntledge Hatchery, a secondary threshold of 0.14 OD was used to separate the Low Positive group into a Lower-Low Positive and a Higher-Low Positive group. Progeny from all BKD Negative and Low Level Detection females will be raised to 5 - 6 gram 0+ smolts; progeny from Lower-Low females will be released in the Puntledge River unfed, or as 0+ smolts, while progeny from Higher-Low Positive females will be reared at minimal densities to reduce stress, and also released early (unfed) when in-river conditions (food supply, flows) allow, and if population pre-release screening indicates the *R. salmoninarum* infection level is suitably low. Standard biosecurity measures will be employed at all times at the facility. Eggs from Moderate Positives were outplanted in Jack Creek as eyed-eggs, while eggs from High Positive females were culled.

3.4 Microsatellite analysis

DNA for the 2013 and 2014 adult samples was extracted from the tissue samples using the Qiagen 96-well Dneasy® procedure. Extracted DNA was used in DNA amplification of 15 microsatellite loci as follows: Ots100, Ots101, Ots104, Ots107 (Nelson and Beacham 1999); Ssa197 (O'Reilly et al. 1996); Ogo2, Ogo4 (Olsen et al. 1998); Oke4 (Buchholz et al. 2001); Omy325 (O'Connell et al. 1997); Oki100 (Beacham et al. 2008); Ots201b, Ots211, Ots213 (Grieg 2003); and Ots2, Ots9 (Banks et al. 1999). In general, PCR DNA amplifications were conducted using DNA Engine Cycler Tetrad2 (BioRad, Hercules, CA) in 6µl volumes consisting of 0.15 units of Taq polymerase, 1µl of extracted DNA, 1x PCR buffer (Qiagen, Mississauga, Ontario), 60µM each nucleotide, 0.40µM of each primer, and deionized H2O. The thermal cycling profile involved one cycle of 15 minutes at 95°C, followed by 30 – 40 cycles of 20 seconds at 94°C, 30-60 seconds at 47 - 65°C and 30-60 seconds at 68 - 72°C (depending on the locus). Specific PCR conditions for a particular locus could vary from this general outline. PCR fragments (microsatellite alleles) were size fractionated in an ABI 3730 capillary DNA sequencer, and genotypes were scored by GeneMapper software 3.0 (Applied Biosystems, Foster City, CA) using an internal lane sizing standard.

Microsatellite diversity was assessed at each locus using expected (H_E) and observed (H_O) heterozygosities calculated using GDA (Lewis and Zaykin 2001). The exclusion power of each microsatellite locus, and of the set of 15 loci combined, was estimated using the methods of Jamieson and Taylor (1997).

4 RESULTS AND DISCUSSION

4.1 Brood Year 2013 summer Chinook

For brood year (BY) 2013 summer Chinook adults, a total of 210 DNA samples were collected from a total escapement of 761 adults (Table 1). The majority of samples were from hatchery broodstock at Puntledge Hatchery with a small number from adults used in the summer Chinook homing behaviour study that were released back to the river to spawn naturally. Tissue samples collected from BY 2013 summer Chinook female broodstock were also screened for BKD. Results from the BKD ELISA screening are summarized in Table 2. BY2013 adults were not spawned within separate migration timing groups, therefore the DNA data will not provide information on

migration timing inheritance. Only the survival and reproductive success of offspring from individual BKD positive and negative females will be assessed.

Table 1. Brood year (BY) 2013 summer Chinook escapement and DNA sampling summary.

Group	Number	# DNA samples
Broodstock kept (Hatchery)	330	190
Transported to Comox Lake (Wild)	114	0
SCK above Diversion Dam (Wild)	229	12
SCK below Diversion Dam (Wild)	88	17
Total SCK Return	**761**	**219**
Proportion BY 2013 SCK adults DNA sampled		**29%**

SCK = Summer Chinook; * Difference due to holding mortalities and excess males that did not contribute

Table 2. BY 2013 summer Chinook broodstock BKD screening summary. Lots 1-4 represent different dates fish were spawned at the hatchery.

	BKD SUMMARY - Females Only						
Spawning Group	**NEG**	**LLD**	**NEG+LLD**	**LP**	**MP**	**HP**	**Total**
Lot 1/2	6	6	12	17	13	1	43
Lot 3	7	1	8	9	7	2	26
Lot 4	1	6	7	12	2	1	22
Lot 5	1	1	2	1	0	0	3
Grand Total	**15**	**14**	**29**	**39**	**22**	**4**	**94**
Percent of Total	**16.0%**	**14.9%**	**30.9%**	**41.5%**	**23.4%**	**4.3%**	

4.2 Brood Year 2014 summer Chinook

For BY 2014 summer Chinook, a total of 654 DNA samples were collected representing 56% of the total escapement (Table 3). Of these samples 280 were used as hatchery broodstock and both females and males were also screened for BKD. Results from the ELISA screening are summarized in Table 4. The three groups listed in Table 4 denote the three separate tanks that the adults were held in at Rosewall Hatchery based on their migration timing and arrival at the Puntledge River hatchery. Group 1 are those adults that arrived between 20 May and 27 June; Group 2 are migrants from

30 June to 7 July; and, Group 3 are migrants from 8 July to 5 August. Group 1 were considered the "early" timing fish and were spawned together, while Groups 2 and 3 were considered the "late" timing fish and the majority were also spawned within their own groups.

Table 3. Brood year (BY) 2014 summer Chinook escapement and DNA sampling summary.

Group	Number	# DNA samples
Broodstock kept (Hatchery)	443	280*
Transported to Comox Lake (Wild)	373	292
SCK above Diversion Dam (Wild)	232	35
SCK below Diversion Dam (Wild)	128	47
Total SCK Return	**1176**	**654**
Proportion BY 2014 SCK adults DNA sampled		**56%**
BY2014 SCK fry captured at the Eicher evaluation facility (13 Feb – 30 Mar 2015)	426	66

SCK = Summer Chinook; * Difference due to holding mortalities and excess males that did not contribute

Table 4. BY 2014 summer Chinook broodstock BKD screening summary for both females and males. Groups 1-3 represent separate adult holding groups based on their migration timing.

		BKD Summary – Females and Males							
	Migration Timing	NEG	LLD	NEG+LLD	LP	HLP	MP	HP	Totals
Females	**Group 1**	10	7	17	17	9	3	0	46
	Group 2	8	11	19	5	10	2	2	38
	Group 3	9	16	25	15	14	1	1	56
	Grand Total	**27**	**34**	**61**	**37**	**33**	**6**	**3**	**140**
	Percent of Total	**19.3%**	**24.3%**	**43.6%**	**26.4%**	**23.6%**	**4.3%**	**2.1%**	
Males	**Grp 1**	19	8	27	10	5	2	0	44
	Grp 2	7	9	16	16	4	1	1	38
	Grp 3	10	18	28	15	12	1	0	56
	Grand Total	**36**	**35**	**71**	**41**	**21**	**4**	**1**	**138***
	Percent of Total	**26.1%**	**25.4%**	**51.4%**	**29.7%**	**15.2%**	**2.9%**	**0.7%**	

* No BKD results for two males

4.3 Microsatellite analysis

High quality DNA was extracted from the 2013 and 2014 adult summer Chinook salmon sampled from the Puntledge River. DNA amplification of the 2014 samples is complete and of the 2013 samples is underway. All 15 microsatellite loci amplified well in the 2014 samples and were polymorphic (possessed several different alleles) in the hatchery and natural spawners (Table 5). Between 6 and 38 alleles were observed at the fifteen loci. This high level of polymorphism lead to expected heterozygosity levels that ranged from 0.39 to 0.93 among loci, reflecting significant variation within the population. The fact that observed heterozygosity levels were very similar to the H_E values indicates that all loci are in Hardy Weinberg equilibrium (HWE) within the population, as would be expected in a random breeding population.

Table 5. Microsatellite data for BY 2014 Puntledge River summer Chinook salmon hatchery broodstock and natural spawners combined. For each locus, the total number of alleles (N), expected heterozygosity (H_E), observed heterozygosity (H_O) and exclusion power is given.

Microsatellite Data for 2014 Puntledge River Chinook Adult Spawners					
Microsatellite Locus	**N**	**H_E**	**H_O**	**HWE**	**Exclusion**
Ogo2	9	0.73	0.72	yes	**0.692**
Ogo4	14	0.67	0.64	yes	**0.694**
Oke4	7	0.63	0.67	yes	**0.545**
Oki100	27	0.90	0.89	yes	**0.936**
Omy325	12	0.73	0.75	yes	**0.667**
Ots100	38	0.93	0.90	yes	**0.970**
Ots101	24	0.85	0.86	yes	**0.512**
Ots104	26	0.92	0.91	yes	**0.954**
Ots107	25	0.83	0.84	yes	**0.863**
Ots2	16	0.82	0.83	yes	**0.824**
Ots201b	24	0.87	0.88	yes	**0.905**
Ots211	24	0.89	0.87	yes	**0.927**
Ots213	24	0.88	0.85	yes	**0.918**
Ots9	6	0.39	0.40	yes	**0.357**
Ssa197	23	0.90	0.90	yes	**0.941**
Mean	19.9	**0.80**	**0.79**	-	-
Total	**299**	-	-	**yes**	**1.0**

The high level of polymorphism also resulted in each locus having an 'exclusion power' of between 0.357 and 0.970. Exclusion power is the likelihood that a progeny will be matched to the 'right' parents (i.e. will **not** be assigned to a parental pair of genotypes in which one or both are not the true parents). A low exclusion value

indicates that many wrong parents cannot be excluded as non-parents. A high exclusion value indicates that most wrong parents can be excluded (a value of 1.0 indicates that all wrong parents can be excluded and the progeny will be assigned to the correct parents). When multiple loci are used to conduct parentage assignment, the exclusion power is the cumulative ability of all loci used to exclude wrong parents. For the 15 loci of this study, the cumulative exclusion power is 1.0. This indicates that this set of loci has enough power to successfully assign Puntledge River summer Chinook hatchery and natural progeny to the correct parents.

5 RECOMMENDATIONS

1. Age class analysis on summer Chinook returns to the river was only completed from scale samples collected on broodstock spawned at the hatchery. No scale samples were collected on any adults that were released back to the river or to Comox Lake to spawn naturally. This was to reduce handling stress on adults before their release back to the river, especially during warm river temperatures (18-21 ^{o}C), and the risk of potentially influencing migration of adults used in the homing behaviour study. Consideration must be given to these factors if scale samples from wild spawners are required.
2. BC Hydro will be conducting their annual spring maintenance of the Puntledge generating facility from 30 March to 24 April. During this period, operation of the collection facility at the diversion dam will be interrupted for up to 3 weeks. From past years of monitoring of summer Chinook juvenile migration at this facility, this timing generally coincides with peak migration, although the migration period extends into late July. If DNA sample collection during this peak migration period is critical, we will need to investigate other options of fry collection in the river when the evaluation facility is not available.

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APPENDICES

APPENDIX A - Confirmation of FWCP Recognition

Press release submitted to Comox Valley Record and Comox Valley Echo for publication.

"DNA investigation sheds light on Puntledge Summer Chinook Salmon"

Scientists are investigating adult Puntledge River summer Chinook migration timing, spawning behavior, and interaction between the natural and hatchery spawners in the river using the same techniques employed on CSI and TV talk shows. This is being done to increase overall productivity of Chinook in the Puntledge River.

In order to find out 'who mated with whom' in the Puntledge River, fish paternity tests are being conducted on mature summer Chinook returning to the Puntledge River Hatchery through a partnership involving the K'ómoks First Nation and Fisheries and Oceans Canada (DFO).

Puntledge Summer Chinook faced near extinction in the early 1990s. Darcy Miller, the Manager at the Puntledge River Hatchery, credits an intensive DFO hatchery program for rebuilding the population which is now stable at approximately 1000 adults. This has mainly been successful through the hatchery staff's effort to get more of the earliest returning summer Chinook back to the river. However, this is only a third of DFO's target escapement for the watershed. A key problem is that this population migrates into the Puntledge River between May and mid-August, when river temperatures later in the summer can increase to over 20^{o}C. This is very stressful for salmon and can lead to mortalities. Esther Guimond, a consultant who coordinated four years of radio-telemetry tracking of these Chinook, found that "adults that migrate before July are successful in reaching Comox Lake. These fish can descend deep in the lake where it is much cooler. Adults that reach and hold in the lake are less stressed and therefore achieve twice the spawning survival as fish holding in the river all summer."

The migration timing of some salmon populations has been linked to genetic control. An investigation is underway to see if this is true for Puntledge summer Chinook. Hatchery staff can potentially rebuild the early component of the migration period by collecting and spawning a higher proportion of early migrating fish and thereby increase the survival and productivity of this population. To determine if this strategy works, staff are recording the genetic identity (genotyping) of all male and female parents by taking a tissue sample and analyzing the DNA of all adults spawned at the hatchery. Other information being recorded for each fish sampled includes size, age, arrival at the hatchery (migration timing), and disease profile of each fish. In addition, adults that are captured at the hatchery and returned to the river to spawn naturally are also DNA sampled.

Once the parents are genotyped, all of their offspring can be linked back to their parents by analyzing their DNA. The genetic technology used in this study is known as 'parentage-based tagging' and is currently being perfected for Chinook at DFO's Molecular Genetics Lab at the Pacific Biological Station in Nanaimo.

The progeny from the hatchery, and those from the natural environment, migrate to the ocean and rear for 1 to 4 years. When they mature and return to the Puntledge River, the adults will be captured and information will be collected from their DNA. This information will be cross referenced back to the parents and then analyzed to determine how migration timing and the other measured traits are inherited.

Miller expects that the results of this DNA study will help DFO develop additional hatchery strategies to maximize survival and abundance of Puntledge River summer-run Chinook. This population represents a significant cultural food fish for K'ómoks First Nation and has significant value for the Georgia Basin recreational and commercial fisheries.

This investigation is being funded by BC Hydro's Fish and Wildlife Compensation Program (FWCP) and the study partners are thankful for their generous support. Since 1999, this program has been used to offset the impacts resulting from construction of BC Hydro dams by annually providing grants which support recovery efforts for fish and wildlife. The FWCP is sponsored through a partnership among BC Hydro, the Province, Fisheries and Oceans Canada, First Nations and public stakeholders.