# Puntledge River Summer Chinook Parentage-based Tagging Study
## Year 5

**FWCP Project No. COA-F19-F-2697**

*Prepared for:*

**Fish and Wildlife Compensation Program**

*Prepared by:*

**R.E. Withler[1], J. Supernault[1], M. Wetklo[1], and E. Guimond[2]**

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Fisheries and Oceans Canada, First Nations and public stakeholders.**

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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 were used in a 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 bacterial kidney disease (BKD) status on their progeny. The genetic analysis is known as 'parentage-based tagging' and 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 were 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. This project is identified in the Puntledge River Watershed Action Plan (FWCP 2017) as a Level 1 priority "Research & Information Acquisition" action (Action Table short description PUN.RLR.RI.13.01 Conduct DNA analyses…Chinook & Steelhead-P1).

Tissue sampling of Puntledge summer Chinook (SCN) for this study began in 2013 with the collection of hatchery broodstock, and in subsequent years (2014 to 2018) both the hatchery brood stock and potential spawners in the natural environment were sampled. Potential natural spawners were those captured for tissue sampling and released back to the watershed. In total, 2889 samples were successfully genotyped and included in the genetic analysis. In both 2017 and 2018, about 85% of the sampled adult returns were assigned to hatchery parents and therefore hatchery-origin. Although smaller in number, the adult progeny returns from natural spawning (≤15%) contained as much genetic diversity as the hatchery-origin fish.

For brood year (BY) 2014, hatchery male and female parents were spawned within run time group to determine if there was an influence of parental run time on progeny run time. Progeny of this BY that returned between 2016 and 2018 were also classified by run time category. There was no significant effect ($p > 0.05$) of parental return time on progeny return time.

For BYs 2013 and 2014, there was a significant difference in progeny survival from Moderate Positive (MP) females ($p < 0.05$) compared with progeny from all other maternal groups testing Negative (NEG), Low Level of Detection (LLD) or Low Positive (LP) for BKD. The lower survival observed for progeny of MP females may have been

the result of the differential treatment of this group (eggs outplanted to the river for natural incubation/rearing as opposed to hatchery incubation/rearing), but has not been verified. For both BYs, there was no significant relationship ($p > 0.05$) between maternal BKD load on the BKD load of her female offspring. The retention of eggs from BKD positive females for rearing (in the hatchery or by outplanting) could be an important factor contributing to the maintenance of genetic diversity in the hatchery population.

Additional DNA sampling on 2019 returns may provide a more complete analysis for assessing heritability of run-timing, and improve our understanding of the effects of parental Chinook return migration time and BKD status on their progeny. Included in this analysis will be an examination of Puntledge summer Chinook at the recently discovered GREBL1 gene which is associated with seasonal (spring/summer and fall) migration. This analysis will guide hatchery spawning protocols to most effectively influence the return migration time in the summer Chinook population.

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# 1 INTRODUCTION

This is year five 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 summer Chinook population is being maintained through enhancement efforts at Fisheries and Oceans Canada's (DFO) Puntledge River Hatchery, levels remain below DFO's target escapement, and recent escapements are in a downward trend. 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. This project is identified in the Puntledge River Watershed Action Plan (FWCP 2017), as a Level 1 priority "Research & Information Acquisition" action (Action Table short description PUN.RLR.RI.13.01 Conduct DNA analyses…Chinook & Steelhead-P1).

## 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. A higher proportion of the earlier returning summer Chinook are 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, this strategy 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.

## 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, better manage BKD in the summer Chinook population,

and optimize their 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 between the summer and fall Chinook salmon populations 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 through the eggs 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. Despite concerns of genetic loss arising from the practice of culling eggs from females that screen positive for the pathogen, the judicious use of males will ensure that genetic diversity is not lost (Hard et al 2006).

The fate of hatchery individuals that are disease-free (i.e. display no symptoms of the disease), but carry the *R. salmoninarum* bacterium due to vertical transmission, and their impact, or lack of, on their naturally-spawned counterparts following release is neither well understood nor simple to predict due to variable effects of environmental stressors on growth of the pathogen and probability of disease development. However, there is strong evidence that culling and segregation of eggs from higher titre females

can reduce prevalence of the disease in subsequent generations (Elliott et al. 1995, Munson et al. 2010). 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, there is value in examining the impacts of exclusion of progeny from *R. salmoninarum* positive females on genetic diversity in populations of conservation concern. Where populations are exceptionally small, the consequences of the loss of genetic diversity must be adequately balanced against the risk of inclusion of females carrying a higher pathogen load. Regardless, 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.

A secondary objective of the study is to examine the genetic diversity in the natural spawning population and determine if the genetic diversity present in the adult population is being effectively transmitted to the juvenile stage of the next generation. This will provide insight into the future success of rebuilding a sustainable Puntledge River summer Chinook population.

## 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 or weir 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 only 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 were once critical in maintaining the spatial segregation and genetic integrity of the two stocks. Today, both summer- and fall-run Chinook may access spawning habitat above these waterfalls, while only summer Chinook are permitted access to their historic spawning grounds upstream of the Puntledge diversion dam.

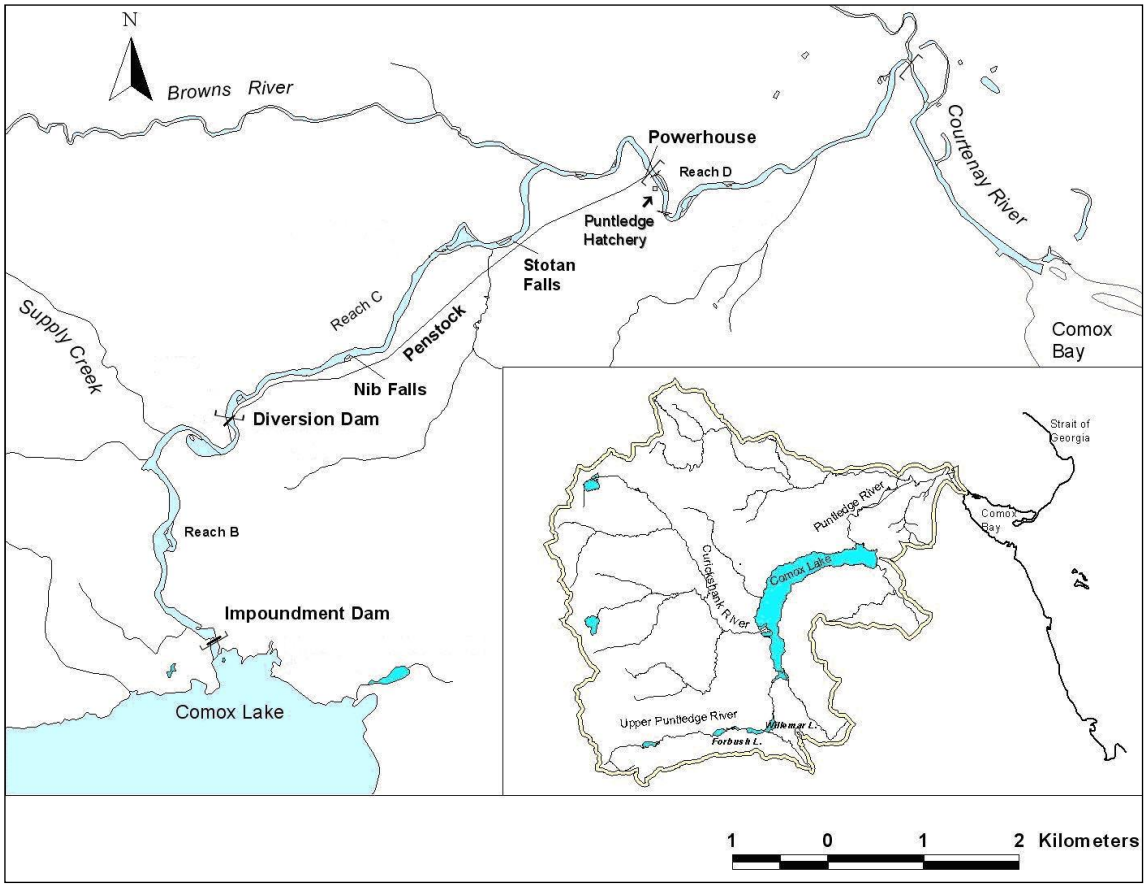


**Figure 1. Location map of the Puntledge River watershed and lower river features.**

# 3 METHODS

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

Summer Chinook broodstock collection at the lower Puntledge River Hatchery commenced in mid-June 2018. Every few days adults were loaded onto a transport tank containing chilled water (typically 4-6 $^{o}$C cooler than ambient) and transported to Rosewall Creek Hatchery. Adults may also by-pass the barrier fence or weir at the lower facility and continue to migrate upstream to the BC Hydro diversion dam. Puntledge Hatchery operates the fishway at the diversion dam to allow/restrict access to upstream migrating fish. This allows hatchery staff to monitor adult summer Chinook arrivals at the dam and either capture a portion as brood, or allow access to spawning habitat upstream of the dam. Adults that were transported and released directly into Comox Lake were sampled prior to transport for DNA analysis by collecting a tissue sample from the caudal fin using a standard paper hole punch and affixing to a Whatman tissue sample sheet. Care was taken to avoid contamination during sampling by rinsing sampling tools in water and wiping with a paper towel in between each tissue sample. A measurement of fork length from the tip of the snout to the end of the middle caudal fin rays was also collected with a waterproof measuring tape, and recorded to the nearest millimetre.

Summer Chinook broodstock transported to Rosewall hatchery were held in separate rearing tubs over the summer, based on their arrival time at Puntledge Hatchery or transport date from the diversion dam pool. Table 1 describes the collection location and migration group for all 2018 broodstock and natural spawners. Early-timing adults (Group 1) included adults arriving at the lower Puntledge Hatchery and those transported from the diversion dam pool up to July 3$^{rd}$. Late-timing fish (Group 2) were those arriving between 9 and 26 July from the two sites (Table 1).

**Table 1. Summary of brood year (BY) 2018 adult transports from the lower Puntledge Hatchery (PH) and the diversion dam to Rosewall Creek Hatchery (RSWL) and Comox Lake.**

| 2018 Transport Dates | Adult Collection Site | Adult Deposit Site | Total # SCN | Migration Timing Group |
|---|---|---|---|---|
| Jun 11 - Jul 3 | Lower PH | RSWL Tub 1 | 163 | 1 |
| Jun 26 - July 3 | Diversion Dam | RSWL Tub 1 | | 1 |
| July 9 - 16 | Lower PH | RSWL Tub 2 | 101 | 2 |
| 19-Jul | Diversion Dam | RSWL Tub 2 | 49 | 2 |
| 25-Jul | Lower PH | RSWL Tub 3 | 110 | 2 |
| July 24-26 | Diversion Dam | RSWL Tub 3 | | 2 |
| | | ***Total Broodstock Transported*** | **356** | |
| 18-Jun | Lower PH | Comox Lake | 23 | 1 |
| July 4 - Aug 2 | Lower PH and Diversion Dam | Comox Lake | 139 | 2 |
| | | ***Total Natural Spawners Transported*** | **261** | |

The broodstock in each group were spawned within their own timing group over 4 egg collection periods between 4 and 25 October 2018. During each egg-take, males and females from each spawning pair were DNA sampled, measured for length (postorbital-hypural) and scale sampled. In addition, a kidney tissue sample (for BKD analysis) was collected from each female 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 transport date), 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.

## 3.2 Microsatellite analysis

DNA for the brood year (BY) 2018 adult samples was extracted from the tissue samples using the Qiagen 96-well Dneasy® procedure or chelex based method. 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 $H_2O$. 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 5.0 (Applied Biosystems, Foster City, CA) using an internal lane sizing standard.

Parentage-based tagging (PBT) using the microsatellite genotypes was used to identify parents of surviving progeny returning to the river at ages between 2 and 5. Parentage analysis was conducted using both Cervus 3.0.3 (Kalinowski et al. 2007) and Colony 2.0.4.1 (Jones and Wang 2010) to identify the parents and year of origin for hatchery origin and natural origin fish. PBT was applied to the SCN returning adults in years 2015-2018 using parental pools of SCN hatchery broodstocks (2013-2016 BY) and natural spawners (2014-2016 BY). Effective population size ($N_e$) of adult samples was estimated using the linkage disequilibrium method in NeEstimator 2.01 under the assumption of the random mating model and excluding allele frequencies less than 0.02 (Do et al. 2014).

### 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. The plan recommends that all Chinook and Coho stocks that have a higher than average historical prevalence of BKD, be annually screened and that egg culling and progeny segregation based on female parental Enzyme Linked Immunosorbant Assay (ELISA) optical density (O.D.) readings of *R. salmoninarum* antigen levels be practiced. Other stocks are subjected to periodic prevalence assessment of 60 fish, to confirm BKD risk status. The Puntledge summer Chinook stock was identified as a high risk BKD stock during routine screening of 2009 and 2011 broodstock. As a result of the revised 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.

For the Puntledge SCN, the following hatchery rearing protocols were implemented for eggs/progeny from females with various levels of infection:

- **Negative (Neg) -** fertilized eggs/progeny from females that have a lower optical density (OD) value than those of the kidneys of the negative control fish. No restrictions on progeny rearing.
- **Low Level of Detection (LLD)** - 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. No restrictions on progeny rearing.
- **Low Positive (LP)** - OD value ≥ 0.1 but < 0.25. No restrictions on progeny rearing, but fry are not marked (CWT & adipose clip). For the 2014 BY, the LP female egg lots were divided into the **High Low Positive (HLP)** and **Low Low Positive (LLP)** groups using a median OD value of 0.14.
- **Moderately Positive (MP)** - OD value ≥ 0.25 but < 0.6. Progeny outplanted as eyed eggs.
- **High Positive (HP)** - OD ≥ 0.6. Eggs are destroyed.

### 3.4 Effect of maternal BKD load on progeny survival and BKD level

Survival of progeny was calculated for each 2013 and 2014 BY hatchery female by dividing her total number of assigned returning progeny by her fertilized egg count. BKD load was assessed in all female progeny that returned from the 2013 and 2014 BYs and were themselves used as brood fish between 2016 and 2018. One way analyses of variance (ANOVAs) were performed on progeny survival and female progeny BKD load among the 2013 and 2014 hatchery brood female BKD classifications using R.

Boxplots produced with ggplot2.2.1. in R were used to illustrate effects of maternal BKD level on offspring survival and offspring BKD load. In each plot, the median is represented by the horizontal line. The box (interquartile range, IQR) captures

50% of the data with limits from the 25th (first quartile, Q1) to the 75th (third quartile, Q3) percentiles. Vertical lines show the minimum and maximum, calculated as Q1-1.5xIQR and Q3+1.5xIQR, respectively.

### 3.5 Effect of parental return time on progeny return time

Return time for Puntledge SCN was estimated as the date of sampling, and effort was expended to sample each fish at the lower river fence/weir soon after river entry. However, some fish passed over the weir prior to sampling and were sampled further upstream, and some fish escaped sampling entirely. Even among fish successfully sampled at the weir, there was likely variation taken in the time taken to move upstream from the river mouth to the weir. Brood fish were transported to Rosewall Hatchery prior to tissue sampling and held in pooled run time groups so that date of entry of individual fish could not identified, whereas date of capture was recorded for fish that were DNA sampled and released back to the river for natural spawning. As a result, the broodstock of 2014 and all adult fish that returned to the watershed between 2015 and 2018 were classified to one of three return time groups consisting of early, mid and late. The date intervals for each run time group in each year are shown below. For the 2014 BY, hatchery male and female parents were spawned within run time group to determine if there was an influence of parental run time on progeny run time. Progeny of this BY that returned between 2016 and 2018 were also classified by run time category. A one way ANOVA was performed on progeny return time among the three parental return time categories.

**Table 2. Number of adult Puntledge summer Chinook sampled between 2015 and 2018 assigned to hatchery (2013-2016 BYs) and natural (2014-2016 BYs) parents.**

| Year | Run Time Group | | |
|---|---|---|---|
| | **Early** | **Mid** | **Late** |
| 2014 | May 20-June 27 | June 30 - July 7 | July 8 - August 5 |
| 2015 | May 21-July 2 | July 8-July 31 | August 1-August 7 |
| 2016 | May 3-June 30 & July 27-August 5* | July 4-July 13* | July 18-August 19 |
| 2017 | June 23-July 11** | July 4-July 10** | July 13- August 1 |
| 2018 | June 1-July 3 | July 9-July 26 | August 1 – August 2 |

*Sampling of Early fish in 2016 was done at two different river locations: Lower (May 3-June30) and Upper (July 27-August 5), representing lower hatchery and diversion dam respectively. Sampling of Mid fish was conducted only at Lower site. **Sampling of early fish in 2017 was also done at both Lower (June 23) and Upper (July 11) sites. Sampling of Mid fish was conducted only at Lower site.

# 4 RESULTS

## 4.1 Brood Year 2018 Summer Chinook Adult Sampling

Approximately 71% (433 of an estimated 838 returning adults) of the 2018 escapement were DNA sampled (Table 3) and only females were screened for BKD. Results from the ELISA screening on BY2018 females spawned at Puntledge Hatchery are summarized in Table 4.

**Table 3. Brood year (BY) 2018 summer Chinook (SCN) escapement and DNA sampling summary.**

| Escapement Count or Estimate | Number | # DNA sampled |
|---|---|---|
| Hatchery Broodstock & other removals | 433 | 433 |
| Natural Spawners above Diversion Dam (Transported to Comox Lake) | 162 | 162 |
| Natural Spawners below Diversion Dam to Hatchery fence | 243 | 0 |
| Natural Spawners below Hatchery fence | 0 | 0 |
| **Total SCN Return** | **838** | **595** |
| Proportion BY 2018 Total Return DNA sampled | | ~71% |
| Proportion BY 2018 natural spawners above the diversion dam that were DNA sampled | | ~100% |

**Table 4. BY 2018 summer Chinook broodstock BKD screening summary for early and late run-timing females only.**

| 2017 BKD Summary – Females only | | | | | | |
|---|---|---|---|---|---|---|
| | **Migration Timing** | **NEG + LLD** | **LP** | **MP** | **HP** | **Totals** |
| **Females** | **Group 1 (Early)** | 27 | 18 | 13 | 1 | 59 |
| | **Group 2 (Late)** | 36 | 34 | 16 | 2 | 88 |
| | **Grand Total** | **63** | **52** | **29** | **3** | **147** |
| | **Percent of Total** | **42.9%** | **35.4%** | **19.7%** | **2.0%** | **100%** |
| **Males** | Not BKD Sampled | | | | | |

## 4.2 Microsatellite Analysis

### 4.2.1 Assignment of summer Chinook sampled in 2015-2018

Over the four years of adult return sampling, 1458 of 2082 (70%) fish were assigned to hatchery parents. Between 2015 and 2018, the overall percentage of

returning adults assigned to hatchery parents increased because sampling of the parental brood year age classes became more complete (Table 5). For return years 2017 and 2018, the parental brood years were virtually complete because over 95% of the adult fish were from 2 to 4 years old. In each of those return years, hatchery-origin fish comprised about 85% of the return.

Assignment of adult return fish to natural-spawners sampled between 2014 and 2016 accounted for few of the natural-origin adults sampled in subsequent years. In 2017 and 2018, less than half of the natural-origin adults that returned were assigned to sampled parents that had spawned naturally in the Puntledge river system.

**Table 5. Number of adult Puntledge summer Chinook sampled between 2015 and 2018 assigned to hatchery (2013-2016 BYs) and natural (2014-2016 BYs) parents.**

| | Return Year | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Brood Year** | **2015** | | **2016** | | **2017** | | **2018** | | **Total** | |
| | n | % | n | % | n | % | n | % | n | % |
| ***Hatchery*** | | | | | | | | | | |
| 2013 | 91 | 22.6 | 141 | 30.8 | 123 | 20.7 | 2 | 0.3 | 357 | 17.5 |
| 2014 | - | - | 197 | 43.0 | 333 | 56.0 | 266 | 45.3 | 796 | 39.0 |
| 2015 | - | - | | | 70 | 11.8 | 225 | 38.3 | 295 | 14.4 |
| 2016 | - | - | | | | | 10 | 1.7 | 10 | 0.5 |
| ***Total*** | ***91*** | ***22.6*** | ***338*** | ***73.8*** | ***526*** | ***88.4*** | ***503*** | ***85.7*** | ***1458*** | ***71.4*** |
| ***Natural*** | | | | | | | | | | |
| 2014 | - | - | 8 | 1.7 | 11 | 1.8 | 3 | 0.5 | 22 | 1.1 |
| 2015 | - | - | - | - | 15 | 2.5 | 13 | 2.2 | 28 | 1.4 |
| 2016 | - | - | - | - | - | - | 0 | 0.0 | | |
| Unassigned | 311 | 77.4 | 112 | 24.5 | 43 | 7.2 | 68 | 11.6 | 534 | 26.2 |
| ***Total*** | ***311*** | ***77.6*** | ***120*** | ***26.2*** | ***69*** | ***11.6*** | ***84*** | ***14.3*** | ***624*** | ***28.6*** |
| Total | 402 | | 458 | | 595 | | 587 | | 2042 | |

The $N_e$ of Puntledge SCN adults was slightly higher in 2017 than in 2018 (Table 6). In 2017, the genetic diversity in fish returning from hatchery and natural spawning were similar but in 2018 the diversity encompassed in the natural-origin fish was greater than that in the hatchery-origin fish.

**Table 6. Sample sizes and genetic effective population size ($N_e$) for the returning 2017 and 2018 Puntledge SCN adult returns of hatchery and natural origin.**

| | n | $N_e$ | 95% CI |
|---|---|---|---|
| **2017** | | | |
| Hatchery | 526 | 178 | 168-190 |
| Natural | 69 | 182 | 143-246 |
| Combined | 582 | 218 | 204-232 |
| | | | |
| **2018** | | | |
| Hatchery | 503 | 167 | 157-177 |
| Natural | 84 | 252 | 188-370 |
| Combined | 584 | 194 | 183-206 |

### 4.2.2 Effect of maternal BKD load on progeny survival and BKD level

Survival of Puntledge hatchery-origin SCN originating from the 2013 and 2014 broodyears was less than 0.15% (Table 7). Of 89 females spawned in 2013, 77 produced some sampled returning adults with the number of adult progeny ranging up to 12. In 2014, 124 of the 135 females spawned produced sampled adult progeny, with progeny per female ranging up to 19.

For both broodyears, there was a significant difference in progeny survival among maternal BKD levels ($P < 0.05$), due to a lower survival of progeny from MP females compared with progeny from all other maternal groups (Table 7, Figures 2 & 3). Progeny of females with LP and LLD BKD loads survived as well as progeny from females classified as negative (NEG) for BKD. The eggs from MP females were outplanted as eyed eggs compared to eggs from the other BKD groups which were reared in the hatchery under optimum conditions.

**Table 7. Number of progeny and % survival for 2013 and 2014 BY Puntledge SCN females with different BKD load.**

| 2013 BY | | | | 2014 BY | | | |
|---|---|---|---|---|---|---|---|
| **BKD level** | **Dams (n)** | **Mean (range) progeny** | **Survival (%)** | **BKD level** | **Dams (n)** | **Mean (range) progeny** | **Survival (%)** |
| MP | 21 | 1.5 (0-8) | 0.03 | MP | 6 | 0.8 (0-2) | 0.02 |
| LP | 39 | 4.7 (0-11) | 0.10 | HLP | 33 | 6.8 (0-17) | 0.15 |
| | | | | LLP | 37 | 6.6 (0-16) | 0.14 |
| LLD | 14 | 4.8 (1-12) | 0.11 | LLD | 34 | 5.8 (1-17) | 0.13 |
| NEG | 15 | 4.8 (0-12) | 0.12 | NEG | 25 | 4.8 (0-19) | 0.11 |
| **Total** | **89** | **4.0 (0-12)** | **0.09** | **Total** | **135** | **5.9 (0-19)** | **0.13** |

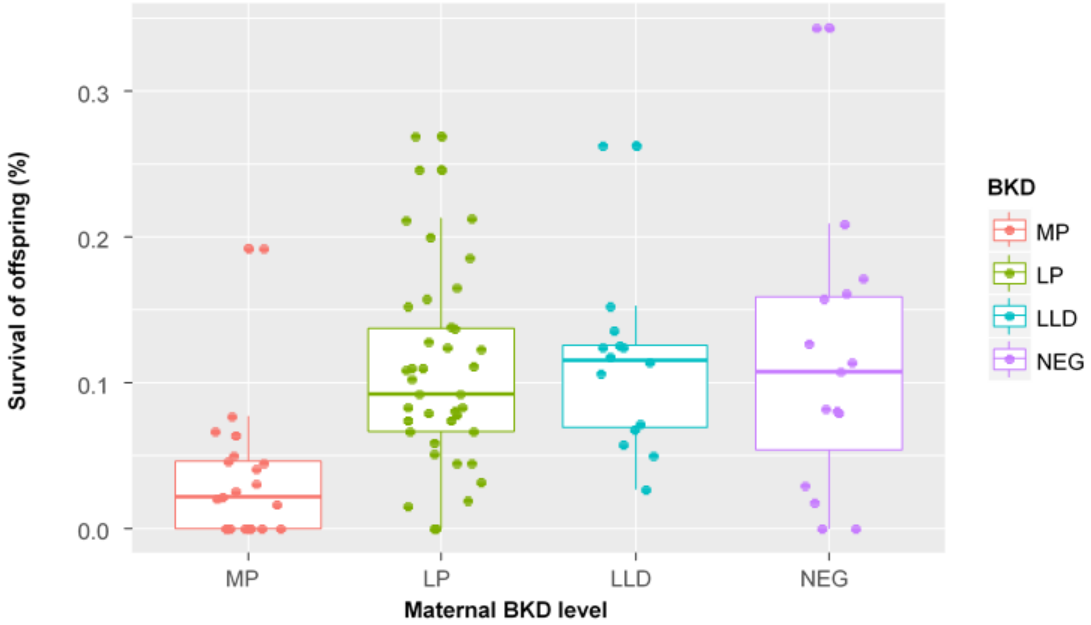


**Figure 2. Survival of 2013 BY Puntledge chinook given by maternal BKD load, ordered by decreasing BKD load.**

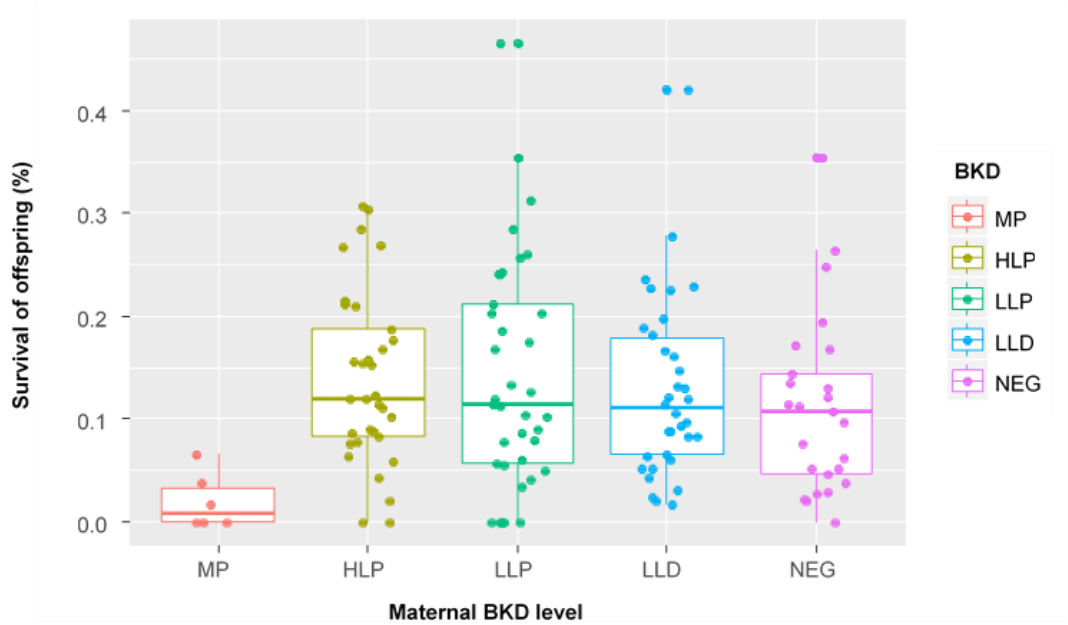


**Figure 3. Survival of 2014 BY Puntledge chinook given by maternal BKD load, ordered by decreasing BKD load.**

For the 2013 and 2014 BYs, there was no significant effect ($P > 0.05$) of maternal BKD load on the BKD load of her female offspring (Figure 4).

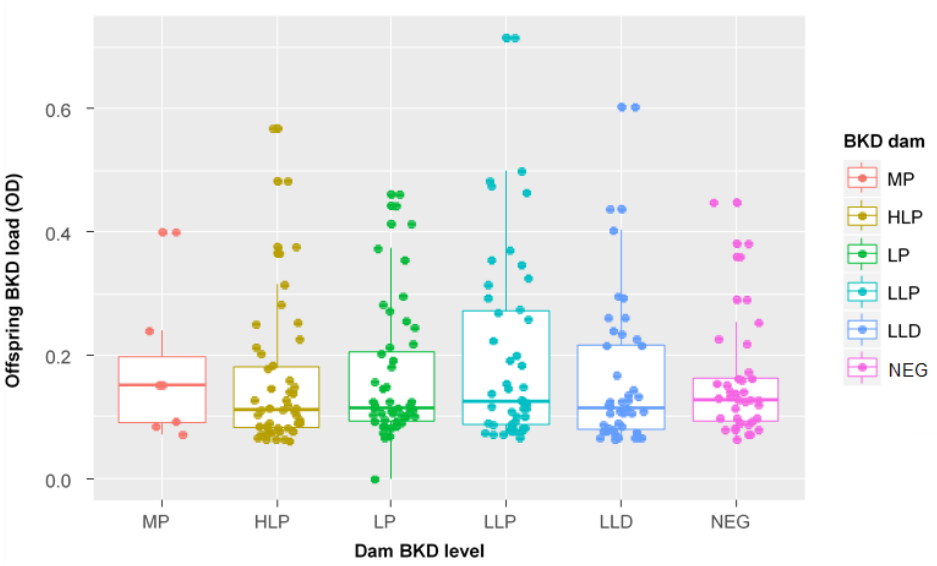


**Figure 4. BKD load of 2013 and 2104 BY progeny by maternal BKD load, ordered by decreasing BKD load.**

### 4.2.3 Effect of parental run time on progeny return time

For 2014 BY, adult progeny returned between 2016 and 2018 and were classified to run time category in a similar fashion as the 2014 brood year parents had been. There was no significant effect ($p > 0.05$) of parental return time on progeny return time (Figure 5).

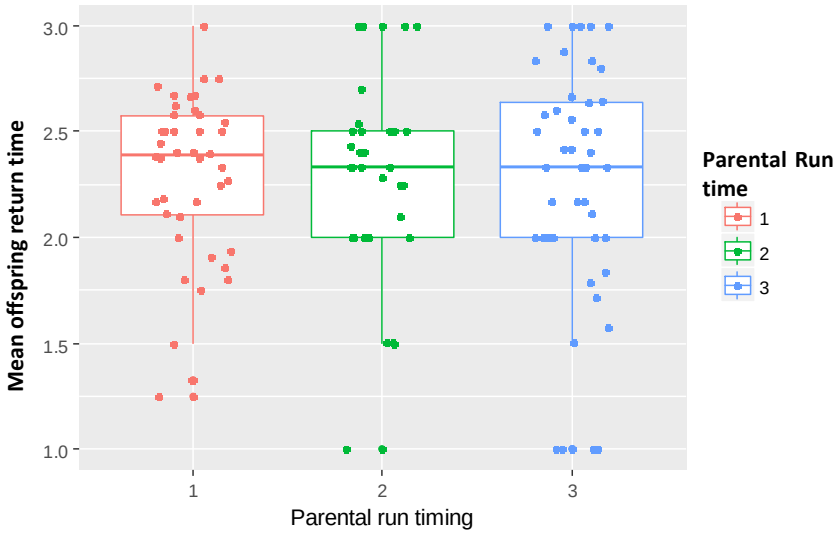


**Figure 5. Return time of 2014BY progeny by parental return time group. Parental and progeny groups 1, 2 and 3 were the Early, Mid and Late returning fish, respectively.**

# 5 DISCUSSION

Parentage analysis in the Puntledge SCN population indicated that hatchery production plays a significant role in maintaining genetic diversity in the population, although survival from hatchery release to adult return remains low (<1%). Adults return between ages 2 and 5 yrs, so the 2018 return year was the first year for which all parental BYs had been sampled. In both 2017 (for which 4 parental BYS were present) and 2018, about 85% of the sampled adult returns were assigned to hatchery parents and therefore hatchery-origin. Hatchery production was therefore responsible for the majority of returning Puntledge SCN in 2017 and 2018.

A small number of the fish in the study that were not assigned to previously sampled parents may have been hatchery-origin, with parents for which a genotype was not obtained (i.e. unsampled BY 2012 parents). However, unassigned fish were predominantly natural-origin fish for which parental fish had not been sampled. Sampling of fish that had the potential to spawn in the watershed began in the 2014 BY, with the result that the majority of natural-origin fish that returned in 2018 should have been assigned to parents if genetic sampling on these potential parents was complete. Therefore ≤15% of the returning adults in 2017 and 2018 were from natural spawners.

Fewer than half of the natural-origin fish that returned in 2017 and 2018 were assigned to sampled fish that spawned naturally in the Puntledge watershed between 2014 and 2016. This suggests that, despite significant sampling effort, many adult spawners in the watershed were not sampled each year. We know that in some years, many of these fish enter the river early in the spring migration season when water flow is relatively high and fish can pass over the hatchery fence, escaping collection and sampling. More recent observations indicate that adults can by-pass the fence

Although smaller in number, the adult progeny returns from natural spawning contained as much genetic diversity as the hatchery-origin fish. The relatively successful, but poorly sampled adult fish, believed to be early-migrating, were the impetus for the study of return timing. If parental return time is heritable in the population, the possibility exists to select early-returning fish as hatchery brood in order to increase the proportion of fish that return early in spring when migratory conditions are favourable. However, we found that parental return time category had no effect on the return time category of progeny for BY2014 parents.

It is possible that that the apparent lack of parental influence on progeny return time reflects a low level of genetic variation for return time within the Puntledge population, but this is not necessarily the case. There is no impassable weir on the lower

river system at which time of river entry can be reliably established. Instead, time of entry for this study was the time of first sampling of the fish at one of several different sampling locations. Time of entry was likely not reliably established for fish that evaded capture and/or spent considerable time in the lower river prior to moving upstream. The hatchery is located 6.6 km upstream of the estuary and the barrier fence in the river, used to divert adults into the hatchery for brood collection, has recently become more accessible, even at low river discharges. These factors affect the reliability of establishing river entry, particularly on 'late' migrants, and maintaining consistently distinct run timing groups throughout the study. Additionally, due to limited capacity at Rosewall Creek Hatchery, brood fish were held in pooled groups; these fish were not sampled at the time of capture in the spring but at time of spawning in the fall for parentage-based tagging. Therefore, brood fish were categorized by early, mid, and late rather than assigned a specific date of return.

Progeny survival varied among individual female parents but was not related to egg BKD levels for females classified as NEG, LLD and LP (including HLP and LLP in 2014). Lower survival was observed for progeny of MP females, but eggs from these females were outplanted to Jack Creek for natural incubation and juvenile rearing (as opposed to hatchery incubation and rearing for the other groups). Some of the reduced survival of progeny from MP females may be the result of outplanting rather than BKD load, although this has not been tested. Outplanting of MP eggs is a cautious approach that provides some benefit of return compared to programs where eggs would normally be destroyed.

These results are consistent with analyses from earlier years that showed the hatchery program was necessary for both maintaining abundance and genetic diversity in the Puntledge SCN population (Withler et al. 2018, Wetklo et al. 2017). In contrast to results obtained from genetic sampling on out-migrating naturally-spawned juvenile fry (Wetklo et al. 2017, Withler and Guimond 2016), the sampling of known natural-origin adult fish in 2017 and 2018 revealed that the genetic diversity, measured by $N_e$ values, was as great as that of hatchery-origin fish. This may reflect the non-representative nature of the juvenile sampling as it was restricted to periods in which the BC Hydro diversion dam penstock sampling facility was operating. The facility was shut-down for 2-3 weeks during the juvenile out-migration period for annual maintenance. In addition, the adult natural spawners comprise fish from several parental BYs, whereas the sampling of juveniles each year included fish from a single BY of fish that spawned in the wild.

DFO is concerned with the low level of success of naturally spawning SCN in the Puntledge River. Restoration of a self-sustaining natural population will require both a higher number and survival rate of fish spawning in the natural environment. The retention of eggs from low BKD positive females, and outplanting of eggs from MP screened fish, may contribute to the maintenance of genetic diversity in the hatchery population, particularly when returns are exceptionally small.

# 6 CONCLUSION AND RECOMMENDATIONS

The genetic analysis of the Puntledge summer Chinook population indicates that genetic diversity is being retained in fish originating from both hatchery and natural spawning, but survival of fish is extremely low. This study demonstrated that, under current conditions, low BKD positive females (HLP, LP, LLD) whose eggs were reared in the hatchery, produced as many adult progeny as did BKD negative females, and that female progeny from BKD positive mothers did not have increased BKD loads at return. Although lower survival was observed for progeny of MP females, the differential treatment of this group (eggs outplanted to the river for natural incubation/rearing versus hatchery incubation/rearing) may account for some of that reduced survival. This affirms the utility of retaining eggs from low BKD positive females for in-hatchery rearing and outplanting of eggs from MP females while abundance and survival are low.

The failure to detect a significant influence of parental return time on progeny return time in the BY2014 indicates that either the genetic influence on return time in the population is too low to be detected, or that measurement error in the classification of return time was sufficiently large to obscure genetic effects. As the study progressed, the ability of fish to enter and ascend the watershed undetected (and unsampled) in the early spring became more apparent.

This was consistent with the fact many parents of natural-origin fish that returned in 2018 were not identified among potential natural spawners sampled in 2014-2016, likely because they had been missed in sampling. The fact that a high proportion of successful natural spawners were not sampled supports the suggestion that early-returning fish may be disproportionately successful in ascending the watershed to Comox Lake in the spring and surviving to spawn in the fall.

The inability to detect and sample all fish at a single location shortly after river entry, the lack of standardization in fish sampling over the return time among years, and

the pooling of hatchery brood fish, likely led to substantial error in the classification or return time for both the 2014 adults and their returning progeny in subsequent years. Therefore, it is possible that substantial additive genetic variation for return time exists in the population that was not detected in this study. Additional DNA analysis on 2019 returns may provide a more complete analysis for assessing heritability of run-timing, improve our understanding of the effects of parental Chinook return migration time and BKD status on their progeny, and better support management actions focused on improving wild and hatchery summer Chinook productivity and preservation of the genetic integrity of the two stocks.

Since the study began, a new genomic region (termed GREBL1) has been identified in Chinook salmon to be associated with seasonal migration time. Genetic variants (alleles) in this region differ between spring/summer and fall migrating populations in many coastal river systems and are necessary for early or late seasonal migration (Prince et al. 2017, Thompson et al. 2018). We examined this genomic region in the Puntledge SCN and found that the variant associated with spring/summer migration is present. Over the next year, we will complete a more comprehensive screening of the Puntledge SCN population and determine the frequency of the spring/summer variant in the population.

Additionally, a collaborative study has been initiated with researchers at the University of Victoria (UVic) to try and identify additional genetic regions within the Chinook salmon genome that may be associated with return time in the Puntledge SCN. The UVic researchers are performing a GWAS (Genome Wide Association Study) on early and late Puntledge SCN that arose from the hatchery 2014 BY and returned in 2017 and 2018. For each sex, 40 fish each that returned early and late are being used in the study in an effort to identify other genes that might influence time of return within the seasonal return time associated with the GREBL1 genotype. The identification of such molecular markers of return time would enable selection for early return time in the hatchery to be carried out with molecular screening (prior to spawning) of brood fish rather than being simply based on observed return time.

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