Puntledge River Summer Chinook DNA Analyses 2007

07.Pun.01

Prepared for:

Comox Valley Project Watershed Society
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EXECUTIVE SUMMARY

The Puntledge River system is one of a few rivers on the east coast of Vancouver Island that supports both a Summer and a Fall run of chinook salmon. The two Puntledge chinook stocks likely originated from the same population, but the Summer chinook are now genetically distinct from the Fall chinook and from other chinook stocks in the Georgia Basin. This stock could potentially be classified as a unique conservation unit under the Wild Salmon Policy. It is suspected that the Summer-run stock evolved from early migrants of the Fall-run stock that were able to ascend Stotan and Nib Falls as flows increased or decreased before and after peak spring freshet between April and August. These waterfalls physically segregated the Summer and Fall chinook stocks. However, since the 1960s and 70s when fish ladders were constructed in the falls to improve fish passage, both the Summer and Fall chinook are now able to ascend the falls.

The Puntledge Hatchery staff define Puntledge River Summer chinook as fish that arrive at the hatchery before Aug 1st and only these fish are used for Summer chinook broodstock. Chinook that arrive between August 1st and August 31st are not spawned with the "True" Summers nor are they allowed to migrate past the diversion dam and spawn in the upper river. This last procedure replaces the barrier effect Stotan and Nib Falls historically had on the Fall chinook.

A study to determine the genetic composition of chinook salmon arriving between June and October was first implemented in 2006 and repeated in 2007. Genetic analysis was conducted on DNA samples of adult chinook salmon at the DFO Molecular Genetics Lab. Samples were taken from 5 discrete groups based on their time of return (arrival at the lower Puntledge hatchery) as follows: before Aug 1st, Aug 1–15, Aug 16–23, Aug 24–31 and Sept 1–15. The results illustrate that Puntledge River Summer and Fall run chinook populations are genetically distinct from each other at the twelve microsatellite loci used in this study with an FST value of 0.0170. Chinook arriving at the hatchery prior to August 1st are predominantly Summer chinook (i.e. 96.6%). However, the proportion of Summer chinook arriving in the first 2 weeks of August is also high (i.e. 80.5%). Although the current hatchery protocols used to maintain the genetic integrity of Summer chinook at the hatchery and in the river have been effective and should be continued, the hatchery broodstock population could be increased if Summer chinook females collected during the first 2 weeks of August are verified by DNA analysis and crossed with males captured before August

1st (i.e. "True" Summer chinook males). This large component of True Summer chinook found in this timing segment (80.5%) could also be utilized to increase the effective spawning population in the river. Ideally, using these fish to augment both then hatchery and river spawning population would accelerate stock rebuilding.

The assignment of an individual fish as Summer or Fall was considered reliable if the fish was assigned to one of the populations at a probability of 0.85 or greater. Chinook with lower probability values may include fish with more unusual genotypes, strays or fish with a hybrid background. These fish (termed Mixed Fish) tended to increase in proportion in the later timing groups along with fish classified as Fall chinook. Since fish with a hybrid genetic background would be expected to show an intermediate time of return, consistent with the pattern shown by 'Mixed' fish, it is possible that at least some of the 'Mixed' fish are the result of historical hybridization between Fall and Summer fish.

The proportion of hybridized fish in the overall Puntledge chinook population however is unexpectedly low. This may be attributed to the spawning protocol implemented at the hatchery or to a propensity for both Summer and Fall chinook in the river to only spawn with a mate of the same race. This latter theory will be further investigated in conjunction with the 2007/08 Puntledge Summer Chinook DNA Study. The pairing behaviour of co-existing Summer and Fall chinook spawners in an isolated section of spawning channel will be recorded and the DNA of the progeny will be analyzed to determine the incidence or level of hybridization.

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1 INTRODUCTION

Puntledge River Hatchery enhances both Summer- and Fall-run chinook salmon. The two races are genetically distinct and spatially separated at the hatchery by an "August 1st" cut-off date. Adults arriving at the hatchery prior to the 1st are treated as "True" Summer chinook", while adults arriving in the month of August are held separately as "maybes", and adults arriving after September 1st are treated and held separately as Fall chinook. In 2006, a study was implemented to characterize the transitional change in genetic composition of chinook salmon arriving between June and October (Guimond and Withler, 2007). The study found that over half of the adults arriving in the month of August were classified genetically as Summer chinook. The study was repeated in 2007 to determine whether this genetic transition is similar year-to-year.

Puntledge Hatchery has had difficulty reaching their target production for Summer chinook. Furthermore, there have been few adults available to naturally spawn in the river. Adults identified as "True" Summer chinook in later arrivals at the hatchery (after August 1st) could be used to supplement the hatchery target for summer chinook production and any surplus fish remaining at the hatchery could be released back into the in-river summer spawning population to increase the overall productivity in the watershed.

This project, funded by BC Hydro's Bridge Coastal Fish and Wildlife Restoration Program (BCRP) and Fisheries and Oceans Canada (DFO), is part of a long-term strategy to rebuild the Puntledge Summer run chinook stock to historical production levels. It is recognized that in order to gain confidence in the origin and timing of the later component of Summer chinook arrivals, the study will need to be repeated for three to four consecutive years. This report summarizes results obtained in 2007 and 2006.

2 BACKGROUND

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

October. Summer-run adults originally utilized spawning habitat above Stotan Falls and more predominantly, in a 4 kilometre section of river immediately below the outlet of Comox Lake. This section of river located between BC Hydro's diversion dam and the Comox Lake impoundment dam is referred to as the headpond. They also spawned to a lesser extent in the lower mainstem reaches of the Cruickshank River, a tributary to Comox Lake. Fall-run adults normally spawn downstream of the Browns River confluence.

The two Puntledge chinook stocks likely originated from the same population, but the Summer chinook are now genetically distinct from the Fall population and from other chinook stocks in the Georgia Basin. This stock could potentially be classified as a unique conservation unit under the Wild Salmon Policy and it is currently a priority for Fisheries and Oceans Canada to develop a recovery plan for this stock. It is suspected that the Summer-run stock evolved from early migrants of the Fall-run stock that were able to ascend Stotan and Nib Falls as flows increased or decreased before and after peak spring freshet between April and August. These waterfalls physically segregated the Summer and Fall chinook stocks and were a barrier to other salmon species in the watershed except steelhead. However, since the 1960s and 70s when fish ladders were constructed in the falls to improve fish passage, both the Summer and Fall chinook are now able to ascend the falls..

The Puntledge Hatchery staff define Puntledge River Summer chinook as fish that arrive at the hatchery before August 1st and only these fish are collected for Summer chinook broodstock. Chinook that arrive later are held separately at the hatchery and are not spawned with the "True" Summers. Although Fall chinook can now ascend the falls, the hatchery restricts these fish from entering the headpond by closing the fishway at the diversion dam. This procedure has been implemented to re-establish the historic nature of the falls in the lower watershed and safeguard the genetic integrity of the Summer chinook. The number of chinook that arrive at the hatchery between Aug 1st and Sept 1st can be in the hundreds. If there is a large component of "True" Summer chinook in this timing segment, not using these fish for hatchery broodstock, or not allowing them to spawn in the upper watershed, can significantly reduce Summer chinook rebuilding.

3 STUDY AREA

The Puntledge River encompasses a 600 km^2 area west of the city of Courtenay. The lower Puntledge River flows from Comox Lake in a north-easterly direction for 14 km where it joins with the Tsolum River. Downstream of this confluence, the waterway is referred to as the Courtenay River, which flows for another 2.6 km into the Strait of Georgia. BC Hydro operates a diversion dam 12.9 kilometers upstream of the estuary, and an impoundment dam a further 3.7 km upstream. The lower Puntledge Hatchery is located just downstream of the Powerhouse, approximately 6.6 km upstream of the estuary. Two major waterfalls (Nib Falls and Stotan Falls) are located in the section of river between the diversion dam and the Powerhouse (Figure 1).

4 METHODS

4.1 Summer chinook broodstock and DNA sample collection

Summer chinook arriving at the lower Puntledge hatchery were directed into hatchery raceways commencing June 18, 2007. Summer chinook arriving before that time were allowed to continue their migration upstream. Broodstock were held in separate sections of the concrete raceways at the hatchery based on their arrival dates (Table 1). For Summer chinook arriving before August 1st, one group remained at the lower hatchery (Group 1) while a second group was transported to Rosewall Creek hatchery and held in cooler ambient temperature water (Group 1.1).

Table 1. Timing of arrival at Puntledge River 'lower hatchery' for five groups of adult chinook salmon sampled in 2007. Groups 1.0 and 1.1 both arrived before Aug. 1st, with Group 1.1 being the fish that were taken to Rosewall Creek Hatchery.

Group	Arrival Dates
1.0	Before 01 August
1.1	Before 01 August
2	01 – 15 August
3	16 – 23 August
4	24 – 31 August
5	01 – 15 September

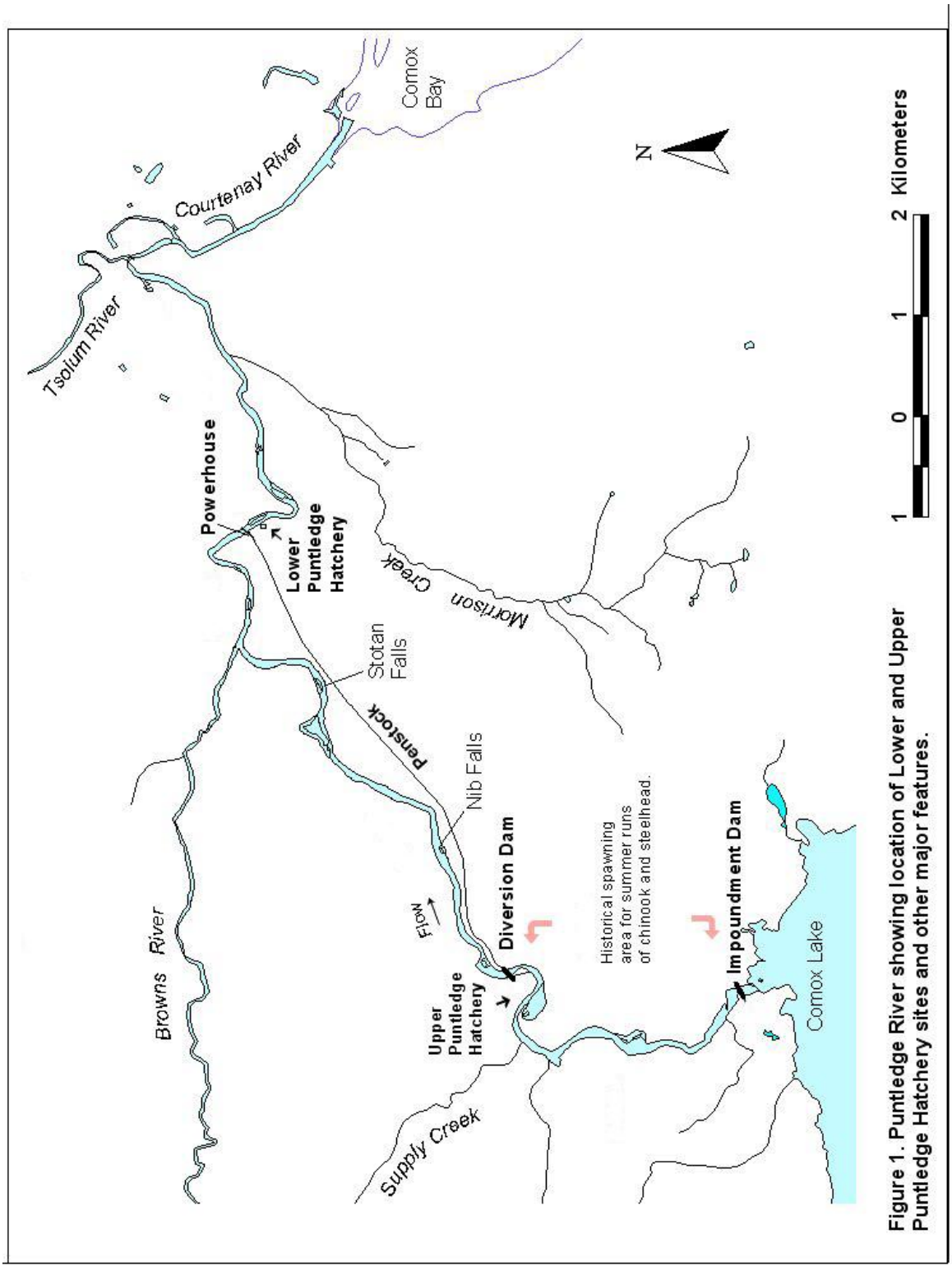


Figure 1. Puntledge River showing location of Lower and Upper Puntledge Hatchery sites and other major features.

Tissue samples were collected from mortalities incurred during the holding period and from adults during the egg-take period at both facilities. All tissue samples (opercular punch) were preserved in individual vials containing 95% un-denatured ethanol and transported to the Pacific Biological Station (PBS) Molecular Genetics Lab in Nanaimo in batches for analysis. Chinook Jacks from Groups 2-4 were not included in DNA samples from these groups because their smaller size allows them to swim through the bar screens used to separate the groups in the raceways. Thus they could freely mix among the other groups. Jacks from Group 1 however were sampled because all early arrival summer chinook (before August 1) are kept in one discrete raceway.

All chinook sampled for DNA were sexed and measured for post-orbital hypural (POH) length. The presence/absence of coded wire tag (CWT) marks was also noted.

4.2 Analysis of DNA samples from opercular punches

Genetic analysis was conducted on samples of adult chinook salmon collected from the Puntledge River drainage in 2006. The DNA samples were screened at 12 microsatellite loci: Ots100, Ots101, Ots104, Ots107 (Nelson and Beacham 1999), Ots2, Ots9 (Banks et al. 1999), Ogo2, Ogo4 (Olsen et al. 1998), Oke4 (Buchholz et al. 2001), Oki100 (K. M. Miller, unpublished data), Omy325 (O'Connell et al. 1997), and Ssa197 (O'Reilly et al. 1996). These same twelve genetic loci have been surveyed in baseline samples of Summer and Fall run Puntledge chinook sampled in earlier years (Table 2).

Table 2. Chinook salmon baseline samples for Puntledge River Summer and Fall run chinook salmon.

Population	Years	Sample sizes	Total sample
Fall	1996, 1997, 2000, 2001	60, 127, 194, 195	576
Summer	1988, 1996, 1997, 1998, 2000	131, 196, 209, 164, 201	901

The Puntledge chinook were examined as five groups based on time of return (Table 1). A Bayesian procedure in the program cBAYES was used to assign the individual 2006 Puntledge chinook multilocus genotypes to either the Summer or Fall baseline populations. As outlined by Beacham et al. (2005a), the BAYES routine of Pella and

Masuda (2001) was modified by the PBS laboratory to a C++-based program (cBayes), which is available from the PBS laboratory website. The assignment of an individual fish as Summer or Fall was considered reliable if the fish was assigned to one of the populations at a probability of 0.85 or greater. Chinook salmon with lower probability values could simply be fish with more unusual genotypes within each group. However, one would expect that fish of hybrid background and strays from chinook salmon populations with different allele frequencies to also be classified as Puntledge fish with low probabilities.

4.3 Analysis of DNA samples from eggs

During the processing of one batch of DNA samples at the lab, approximately 21 samples were accidently mixed up. Results from the DNA analysis performed on this batch confirmed that some of these samples were allocated to Fall rather than Summer chinook. These were samples collected during an egg take at the hatchery and the eggs were being held in individual incubation trays until DNA results were received from the lab. In order to sort out the origin of these samples, approximately 10 fertilized eggs were collected from each cross of the 21 fish in question. The eggs were preserved using the same methods for regular tissue samples and screened at the 12 microsatellite loci.

4.4 Communications

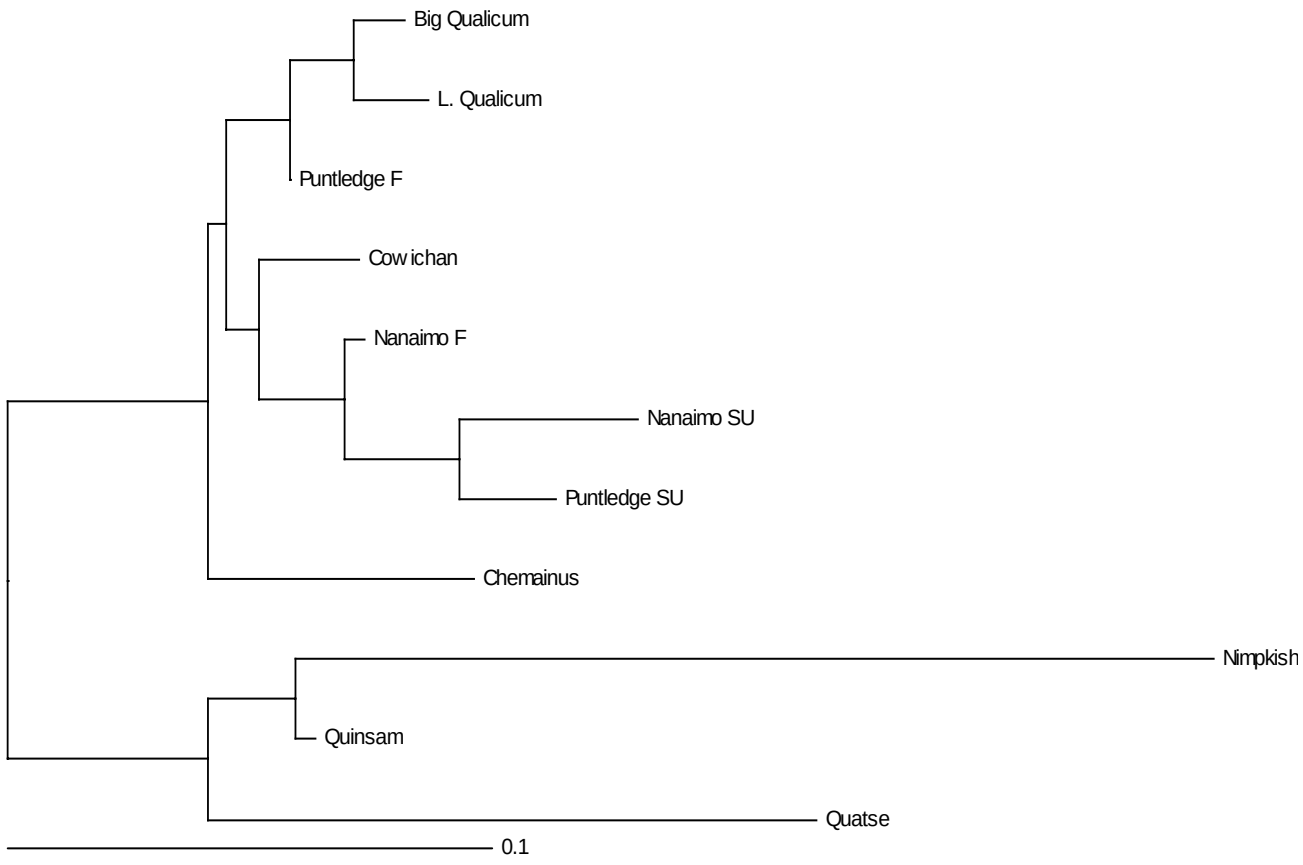
A Communications Plan conducted by staff of Comox Valley Project Watershed Society informed the public about the Puntledge River Summer chinook DNA analysis project through displays at BC Rivers Day and an article in the *Watershed News* (Appendix B). More detailed reporting of the Community Outreach Program associated with this and three other BCRP projects in the Puntledge River watershed is summarized in a separate report.

5 RESULTS

The Puntledge River Summer and Fall run chinook populations are genetically distinct from each other at the twelve microsatellite loci used in this study with an FST value of 0.0170. In comparison, the Puntledge Fall chinook are more closely related to other southeastern Vancouver Island Fall run populations such as Big Qualicum (FST =

0.002). The Puntledge Summer run fish are most closely related to the Nanaimo Summer run population (FST = 0.0136). The following dendrogram depicts the relationships among chinook salmon populations on the east coast of Vancouver Island (Figure 2).

Figure 2. Neighbour-joining dendrogram showing genetic relationships among chinook salmon populations on the east coast of Vancouver Island based on pairwise measurement of Nei's 1972 genetic distance.



Both the percentage of Puntledge chinook salmon fish classified as Fall and the percentage of fish classified with low probability (<0.85) as either Fall or Summer (termed Mixed fish) tended to increase in proportion in the later timing groups (Table 3). These results are consistent with expectations that fish that arrive before 01 August are primarily or entirely from the Summer population, whereas later-arriving fish contain increasing proportions of fish from the Fall population. Fish with a hybrid genetic background would be expected to show an intermediate time of return, consistent with the pattern shown by 'Mixed' fish (those classified as either Summer or Fall with a probability < 0.85). Thus, it is possible that at least some of the Mixed fish are the result of historical hybridization between Fall and Summer fish, either through natural pairing on the spawning grounds or unintentional pairing at the hatchery.

Table 3. Classification of Puntledge chinook salmon sampled in 2007. N is the total sample size. Fall (% Fall) gives the number (percentage) of fish classified as Fall, regardless of the probability level of classification. Mixed (% Mixed) gives the number (percentage) of fish classified as Summer or Fall at a probability of less than 0.85. The final column shows the percentage classified as Fall (any probability) plus those classified as Mixed Summer.

Group	N	Fall	% Fall	Mixed		% Mixed	% Fall & SU Mixed
				SU	F		
1	250	9	3.6	14	6	8	9.2
1.1	100	3	3	3	2	5	6.0
combined	350	12	3.4	17	8	7.1	8.3
2	82	16	19.5	4	7	13.4	24.4
3	72	38	52.8	8	9	23.6	63.9
4	161	96	59.6	16	15	19.3	69.6
5	90	78	86.7	7	11	20	94.4

Table 4 compares results for the percentage of Fall Chinook and the percentage of Mixed obtained in 2006 and 2007.

Table 4. Comparison of % Fall, % Mixed and % Fall and SU Mixed as described above in Table 3, for 2006 and 2007.

Group	% Fall		% Mixed		% Fall & SU Mixed	
	2006	2007	2006	2007	2006	2007
1	2.3	3.6	0.8	8	3.1	9.2
1.1	1.2	3	1.8	5	3	6.0
combined	1.7	3.4	1.3	7.1	3	8.3
2	14.8	19.5	6.6	13.4	18	24.4
3	50	52.8	23.9	23.6	58.7	63.9
4	56.9	59.6	21.6	19.3	66.7	69.6
5	82.5	86.7	14	20	87.7	94.4

6 DISCUSSION

The level of genetic differentiation between Puntledge Summer and Fall run fish in the genetic baseline allows accurate classification of the current (2007) fish as Summer or Fall run. However, the Puntledge Fall run is genetically similar to other east coast Vancouver Island Fall run populations and assignment to a specific river for an individual Fall run fish may not be accurate (Beacham et al. 2006). Therefore, the baseline used in this study was restricted to segregating the Puntledge Fall from Summer populations, with the understanding that this provided no ability to recognize stray fish from other populations. In 2006, one fish identified by CWT as originating from the Skagit River was indeed classified as a Skagit River chinook salmon when the baseline was expanded to include other chinook salmon populations from Vancouver Island and Washington.

This study assumes that fish entering the river throughout the migration period take approximately the same amount of time migrating to the lower hatchery fence and into the hatchery raceway. However, it is recognized that Summer chinook migration upstream and/or access into the hatchery can be influenced by several factors. The physical presence of the barrier fence, the number and variety of predators in the river (i.e. seals and otters), and recreational activity may all have some influence on chinook migration, potentially delaying or accelerating upstream passage. In the past, chinook migration timing has been assessed using snorkel surveys by hatchery personnel in the pool below the barrier fence. However, the frequency of these surveys has diminished due to budget and staff cutbacks.

Another factor influencing summer chinook migration is river discharge. BC Hydro currently releases ~85 m^3/s into Reach C for a kayak pulse flow event, typically around the beginning of June. It is also worth noting that during high inflow and snow melt events in the Spring season, BC Hydro also spills water for flood-routing. Based on video surveillance footage of chinook migration through the lower hatchery and upper hatchery fishways in the past 2 years, it has been documented that summer chinook are able to migrate above the barrier fence in the lower river during these flows without using the fishway. In both 2006 and 2007, over 200 summer chinook had bypassed the lower fence undetected, likely during several high flow events that occurred prior to broodstock collection at the hatchery. Presently, Summer chinook broodstock collection at the hatchery does not commence until mid June because juveniles are still being held until the beginning of June, and cleaning/maintenance of raceways must be completed prior to broodstock holding. In the last 2 years, these early arriving fish have been given access to habitat above the diversion dam. No adults were collected for broodstock at the Upper hatchery. Therefore a large proportion of the "early" run has not been utilized for broodstock in the last 2 years. Although all of these early fish had the benefit of spawning naturally, a portion of this group should also collected for enhancement, to ensure genetic material from the entire run is represented.

Results for 2007 are comparable to 2006, and clearly show that fish arriving at the hatchery prior to August 1st are predominantly Summer chinook (i.e. 96.6%). The proportion of Summer chinook arriving in the first 2 weeks of August is also high (i.e. 81.5%). Based on these results, it appears that the current hatchery protocols used to maintain Summer chinook genetic integrity at the hatchery and in-river are appropriate and should be continued. However, the effective hatchery broodstock population could be increased if females collected during the first 2 weeks of August are tissue sampled and spawned with males captured before August 1st. Once identified through DNA analysis as a Summer chinook, the eggs could be pooled with the hatchery Summer chinook production (results from DNA analysis can be available within 2-3 days). Females determined to be of Fall origin would be destroyed to avoid hybridization.

The proportion of hybridized fish in the overall Puntledge chinook population, although higher in 2007 than 2006, is surprisingly low. This may be attributed to the spawning protocol implemented at the hatchery. It may also be due to a propensity for both Summer and Fall chinook to only mate with other fish in the same race. If there is a high fidelity for Summers to only spawn with Summers, the need to artificially barricade post-July arrivals into the river could be delayed until late August or even

eliminated. This change in operation could potentially increase the effective Summer spawning population in the river. However, until a series of scientific studies on mating selection verifies high selectivity in a mixed population, the lower and upper hatchery bypass should continue to be operated to maintain a spawning refuge for the summer stock.

At this time, it is not clear why there is a slightly higher prevalence of "Mixed" fish appearing in Group 1 and Group 2 in 2007 (7.1% and 13.4% respectively) versus 2006 (1.3% and 6.6% respectively), or whether this is a significant increase. The practices employed at the hatchery during the 2003 and 2004 eggtake (i.e. fish from August arrivals potentially being used as broodstock to boost egg targets) or operation of the lower fence could have contributed to this higher incidence, but have not been verified. The incidence of "Mixed" fish will be closely followed over the next 2 years to see if there is a trend.

7 RECOMMENDATIONS

It was identified in the initial BCRP DNA Analysis application (2006) that the study will likely need to be repeated for three or four consecutive years in order to develop confidence in the results on the genetic composition of the August group. BCRP has recently approved the continuation of the ***Puntledge River Summer Chinook DNA Analyses*** project for 2008. Based on the results obtained in 2007, the project will follow the same study design, with the following recommendations:

1. Maintain the same group timing segregation (Groups 1-5).

2. Pre-screen all Summer chinook males and jacks from Group 1 prior to spawning. Spawn all August Group females with Group 1 (early Summer) males and keep individual crosses in separate incubation trays until DNA results are confirmed. Hybridized crosses (i.e. a Fall female crossed with a Summer male) will be discarded because there is typically an abundance of Summer chinook males, and a strong Fall chinook population.

3. If pre-screening is possible, the hatchery should use a 1:2 (female:male) mating ratio for True crosses. This will allow more Summer chinook male genetic material to be included in the hatchery egg-take, since there is typically a high male sex ratio (i.e. 5 males:1 female). If the hatchery is unable to pre-screen all

males, then a 1:1 mating has been suggested since sperm viability appears to be high.

4. Jacks should be used at the same proportion that exists in the wild. Therefore, methods to include jacks from Groups 2-4 that are currently not DNA sampled, into the enhancement program should be explored.

5. Operate the upper hatchery (diversion dam) fishway such that a portion of the early summer chinook migrants that are able to bypass the lower hatchery prior to broodstock collection in early June can be utilized. These fish will be collected in the upper hatchery raceways and then transported to Rosewall Creek hatchery rather than holding at this site until spawning. The proportion taken for enhancement and those allowed to spawn naturally will be determined during the migration period. The diversion dam fishway will be closed in early September (or earlier depending on when the lower hatchery fishway is open) to prevent Fall chinook from entering the headpond area. This upper reach will be used exclusively by Summer-run chinook.

6. Summer Chinook that are classified with low probability (<0.85) should not be used as broodstock but can be released above the hatchery barrier fence. Similarly, True Fall chinook (if not kept for hatchery broodstock) and FCN <0.85 can be released above the hatchery fence. True SCN not used as broodstock must be released in the headpond or Comox Lake.

7. Investigate the pairing behaviour between Summer and Fall chinook spawners in a natural spawning environment. This will be investigated as follows:

    i. DNA samples from alevins/fry collected by hydraulic sampling in February 2008 at Bull Island, a location utilized by both Fall and Summer chinook can be analyzed to determine whether hybridization between SU and F fish is occurring (i.e. mating between SU and F parents). A minimum of 10 redds and 20 fish per redd should be collected for DNA analysis. Results from sampling in 2007 indicated that not enough eggs/alevins were collected to determine phenotypes.

    ii. A study will be set up in Jack Creek, a small side-channel at the lower hatchery, or at the upper hatchery spawning channel where tagged Fall chinook and untagged Summer chinook spawners can be released in an

enclosed spawning area where pairing behaviour can be observed and recorded. Adults will be DNA sampled before release to ensure pure Puntledge Fall and Summer stocks are used (not hybrids or strays). DNA analysis would be conducted on samples of progeny from the study to determine the presence or level of hybridization. The numbers of adults used in the study will be determined closer to the spawning period. This study was not conducted in 2007 due to the lower than expected returns.

8 ACKNOWLEDGEMENTS

We are grateful for the financial support for this study from BC Hydro Bridge Coastal Fish and Wildlife Restoration Program (BCRP), and technical support from Fisheries and Oceans Canada. Special thanks go to Puntledge Hatchery staff for managing chinook arrival groups and collecting DNA samples from broodstock; and to the molecular biology technicians for providing timely results from the PBS Molecular Genetics Lab.

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APPENDICES

Appendix A - Financial Statement Form **Project #: 07.Pun.01**

INCOME	BUDGET			ACTUAL		
	BCRP	Other (Cash)	Other (in-kind)	BCRP	Other (cash)	Other (in-kind)
Total by Source	$17,330.50		$11,220.00	$17,330.50		$11,220.00
Grand Total Income (BCRP + Other)	**$28,550.50**			**$28,550.50**		
EXPENSES						
Project Personnel						
Biologist (contractor)	$4,320.00			$4,613.40		
Technician (contractor)	$1,800.00			$1,175.00		
Communications Technician	$1,125.00			$1,125.00		
DFO Biologist			$4,800.00			$4,800.00
DFO Technicians			$5,400.00			$5,400.00
Honoraria						
Material and Equipment						
Small Tools/supplies & equipment rental	$150.00					
DNA Analysis	$8,000.00			$8,000.00		
Travel	$360.00			$304.40		
Adiministration						
Office Supplies						
10%	$1,575.00		$1,020.00	$1,521.78		$1,020.00
Total Expenses	**$17,330.00**	**$0.00**	**$11,220.00**	**$16,739.58**	**$0.00**	**$11,220.00**
Grand Total Expenses (BCRP + others)	**$28,550.00**			**$27,959.58**		
Balance (Grand Total Income - Grand Total Expenses	**$0.00**			**$590.92**		
BCRP Balance (surplus)	**($590.92)**					

* Any unspent BCRP financial contribution to be returned to: BC Hydro, BCRP
6911 Southpoint Drive (E14)
Burnaby, B.C. V3N 4X8

APPENDIX B: Confirmation of BCRP Recognition

Article on the BCRP Summer Chinook DNA Study, appearing in the ***Comox Valley Project Watershed News***, March 2008.

Two Stocks of Puntledge Chinook

The Puntledge is one of a few rivers on the east coast of Vancouver Island to support both a summer and fall run of Chinook salmon. Although both stocks spawn at the same time (early October to early November), the two runs have separate spawning distributions in the river. Summer-run adults historically have utilized spawning habitat above Stotan Falls with fall-run adults spawning downstream of the Browns River confluence.

Due to the genetic distinctiveness of the summer-run Chinook, Fisheries and Oceans Canada has designated them as unique conservation unit. DNA analysis has confirmed that the two Puntledge chinook stocks are genetically distinct, but they likely originated from the same population. Biologists suspect that the summer-run stock evolved from early migrants of the fall-run stock that were able to negotiate Stotan and Nib Falls as flows decreased between June and August.

A long-term strategy by Fisheries and Oceans Canada (DFO) is attempting to rebuild the Puntledge summer-run chinook stock to historical production levels. In a project funded by BC Hydro's Bridge Coastal Fish and Wildlife Restoration Program (BCRP) and DFO, fish arriving at the hatchery from August 1-31 are being genetically analyzed to determine whether they are summer or fall Chinook. Fish that are identified as summer Chinook will be used as broodstock at the hatchery or given access to habitat above the diversion dam where they can spawn with the earlier arriving summer chinook. Fall Chinook are excluded from the river upstream of the diversion dam because historically they did not utilize this habitat. Furthermore, since the installation of fish ladders at the falls and creation of hatcheries, barrier fences are used to control which fish has access to the upper watershed. These measures will increase the effective spawning population of the summer-run Chinook, which will safeguard the genetic integrity of, and help to rebuild, the stock.

www.projectwatershed.bc.ca/index.htm

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