Puntledge River Summer Chinook
DNA Analyses 2006

06.Pun.04

Prepared for:

Comox Vallet 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 to support both a Summer and a Fall run of chinook salmon. The two Puntledge chinook stocks originated from the same population, but they are genetically distinct. It is suspected 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 after peak spring freshet between June and August. The two stocks therefore have discrete migration timings and spawning distribution in the river, although they spawn at the same time. These waterfalls which historically were barriers to most anadromous fish (except Summer runs of steelhead and chinook salmon) maintained the segregation of the early and late stocks of chinook. However, since the 1960s and 70s when fish ladders were constructed in the falls to improve fish passage, the Summer stock has been largely preserved through hatchery enhancement.

The Puntledge Hatchery staff define Puntledge River Summer chinook as fish that arrive at the hatchery before Aug 1st and only these fish are collected for Summer chinook broodstock. Chinook that arrive between Aug 1st and Sept 1st, the cut-off date for the fall chinook run, are held separately at the hatchery and are not spawned with the "True" Summers nor are they allowed to migrate past the diversion dam and spawn in the upper river. A large component of true Summer chinook in this timing segment could increase the effective spawning population, both at the hatchery and in the river, and accelerate the rebuilding of this stock to historical production levels.

A study to determine the genetic composition of chinook salmon arriving between June and October was implemented in 2006. Genetic analysis was conducted on samples of adult chinook salmon at the DFO Molecular Genetics Lab. Chinook samples were examined from 5 discrete groups based on their time of return (arrival at the lower Puntledge hatchery): before Aug 1st, Aug 1–15, Aug 16–23, Aug 24–31 and Sept 1–30. 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. 98%). However, the proportion of Summer chinook arriving in the first 2 weeks of August is also high (i.e. 85%) indicating that the current hatchery protocols used to maintain Summer chinook genetic integrity at the hatchery and in-river are effective and should be continued. The 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.

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 to only mate with other fish in the same race. This latter theory will be further investigated in conjunction with the 2007/08 Puntledge Summer Chinook DNA Analysis, whereby the pairing behaviour between Summer and Fall chinook spawners will be observed and progeny genetically analyzed to determine the presence or level of hybridization.

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1 INTRODUCTION

The Puntledge River system is one of a few rivers on the east coast of Vancouver Island to support 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, tributary to Comox Lake. Fall-run adults normally spawn downstream of the Browns River confluence.

The two Puntledge chinook stocks originated from the same population, but they are genetically distinct. It is suspected 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 after peak spring freshet between June and August. These waterfalls have maintained the segregation of the early and late stocks of chinook which historically were barriers to most anadromous fish except Summer steelhead and Summer-run chinook salmon. However, since the 1960s and 70s when fish ladders were constructed in the falls to improve fish passage, the Summer stock has been largely preserved through hatchery enhancement. It is this genetic distinctiveness of the Summer-run stock that may soon place them as a unique conservation unit by Fisheries and Oceans Canada.

The Puntledge Hatchery staff define Puntledge River Summer chinook as fish that arrive at the hatchery before Aug 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 nor are they allowed to migrate past the diversion dam and spawn in the upper river. This practice attempts to mimic the historic natural processes in the watershed and safeguard the genetic integrity of the Summer chinook. The number of chinook that arrive in the river 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 allowing them to spawn in the upper watershed, significantly reduces the stock rebuilding process.

In 2006, a study was implemented to determine the genetic composition of chinook salmon arriving between June and October. Tissue samples were collected from five groups of chinook for DNA analysis. If it is concluded that chinook arriving throughout the month of August are also genetically “True” Summer chinook, these fish could then be used to spawn with Summer chinook at the hatchery or permitted to spawn with Summer chinook above the diversion dam. Both options would increase the effective spawning population, hence accelerating the rebuilding process.

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.

2 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).

3 METHODS

3.1 Summer chinook broodstock and DNA sample collection

Summer chinook arriving at the lower Puntledge hatchery were directed into hatchery raceways commencing June 9, 2006. 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).

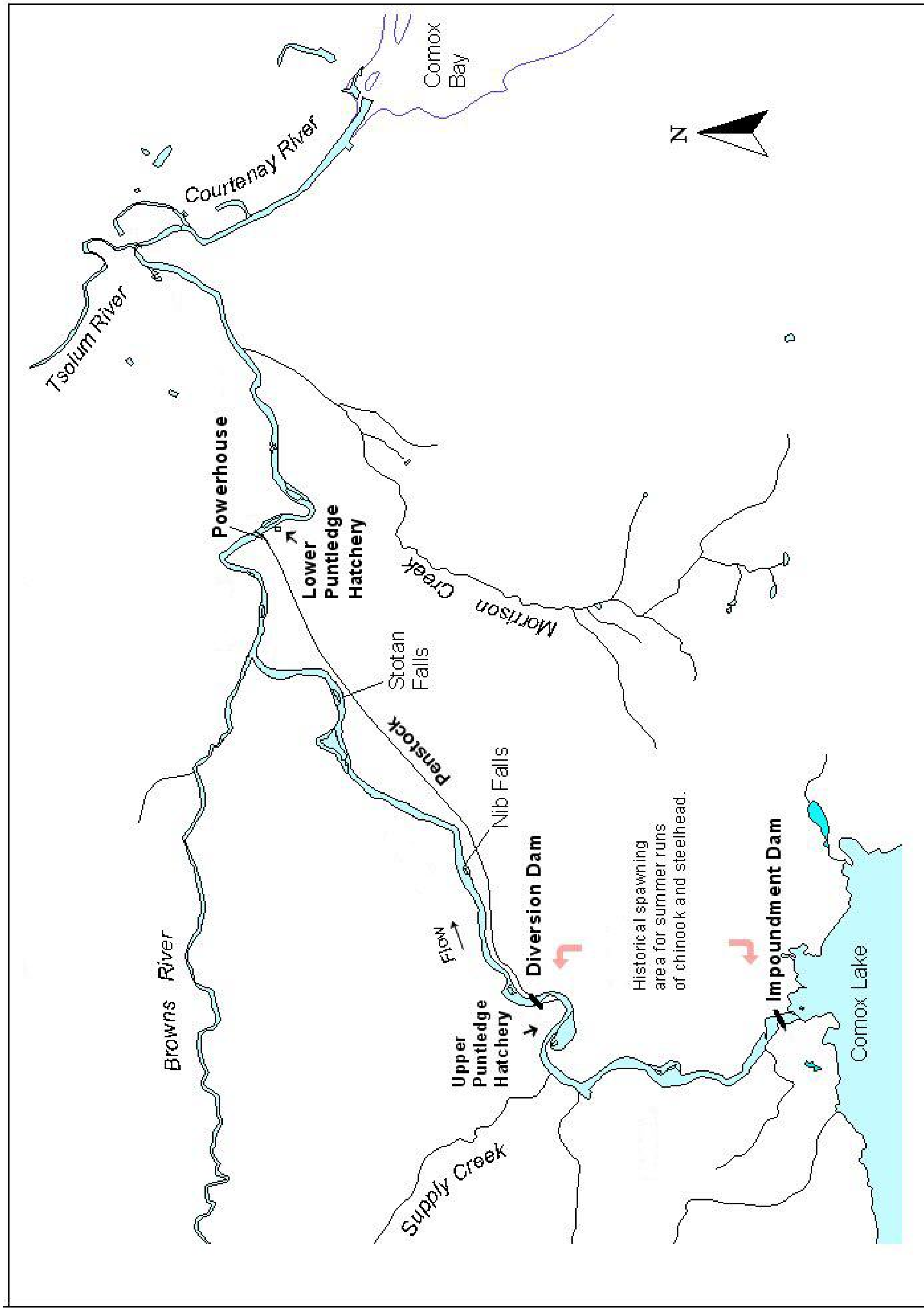


Figure 1. Puntledge River showing location of Lower and Upper Puntledge Hatchery sites and other major features.

Table 1. Timing of arrival at Puntledge River 'lower hatchery' for five groups of adult chinook salmon sampled in 2006. 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 – 30 September

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 PBS Genetics Lab in Nanaimo in batches for analysis. Chinook Jacks 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.

In addition to tissue samples, other morphological measurements were collected on each DNA sampled fish to identify possible external features that may assist with distinguishing the two stocks. All chinook sampled for DNA were sexed, measured for post-orbital hypural (POH) and fork length, peduncle girth and dorsal girth (circumference). The presence/absence of coded wire tag (CWT) marks was also noted.

3.2 Analysis of DNA samples

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 our laboratory to a C++-based program (cBayes), which is available from our 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.

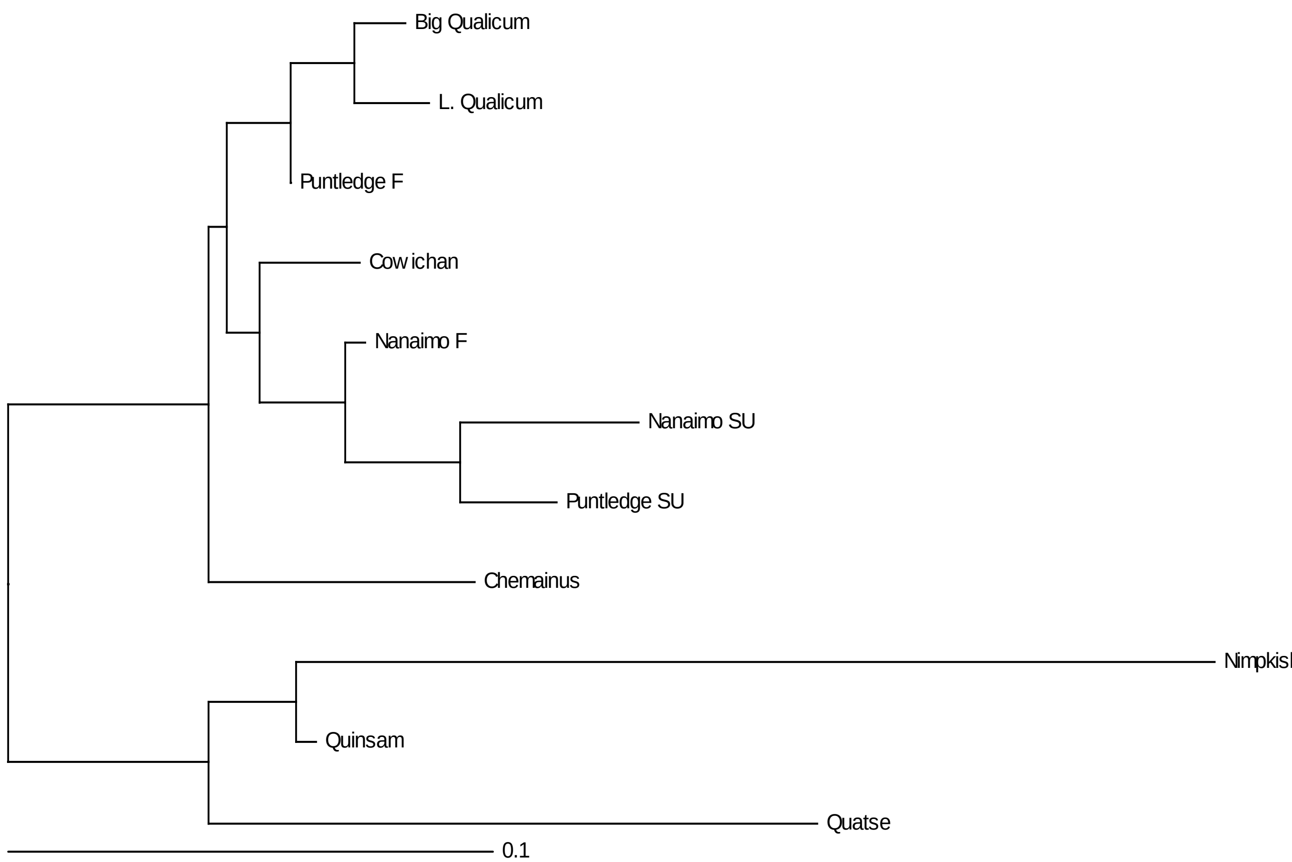
3.3 Communications

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 notices in local newspapers, displays at a BC Rivers Day Open House Event at Puntledge Hatchery, 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.

4 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 2006. 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		
1.0	131	3	2.3	1	0	0.8	3.1
1.1	168	2	1.2	3	0	1.8	3.0
combined	299	5	1.7	4	0	1.3	3.0
2	61	9	14.8	2	2	6.6	18.0
3	46	23	50	4	7	23.9	58.7
4	51	29	56.9	5	6	21.6	66.7
5	57	47	82.5	3	5	14.0	87.7

Results from morphological sampling (measurements of external features) did not show a significant enough difference between individuals from the two stocks (Table 4). With more sampling a statistical analysis of morphological data may possibly show significant differences between the two stocks as a whole, but differentiating individuals with any confidence that would be acceptable to maintain genetic integrity at the hatchery or in the river does not appear to be possible.

Table 4. Summary of morphological measurements collected on DNA sampled chinook classified as "True" Summer (Groups 1 and 1.1) and Fall (Group 5).

		POH Length (mm)	NF Length (mm)	Peduncle Girth (mm)	Dorsal Girth (mm)
Summer CN	**AVG**	609.2	750.9	177.9	369.0
	StD	105.0	122.9	30.6	88.9
	Max	836	1061	250	564
	Min	307	401	100	173
Fall CN	**AVG**	669.9	824.9	202.8	463.0
	StD	87.1	112.9	30.5	68.8
	Max	837	1073	273	590
	Min	427	524	120	293

5 DISCUSSION

The level of genetic differentiation between Puntledge Summer and Fall run fish in the genetic baseline allows accurate classification of the current (2006) 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 river for individual Fall run fish may not be accurate (Beacham et al. 2006). Therefore, the baseline used in this study was restricted to the Puntledge Fall and Summer populations, with the understanding that this provided no ability to recognize stray fish from other populations. 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.

Results clearly show that fish arriving at the hatchery prior to August 1st are predominantly Summer chinook (i.e. 98%). The proportion of Summer chinook arriving in the first 2 weeks of August is also high (i.e. 85%). 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 a week). Females determined to be of Fall origin would be destroyed to avoid hybridization.

The proportion of hybridized fish in the overall Puntledge chinook population 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. Although the morphometric measures collected during this study were not useful in identifying whether an individual chinook was a Summer or Fall, there are likely overall differences between to two groups and more subtle differences, not recognizable to man but recognizable to fish, that allows a fish to mate with another fish in same the 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, the lower hatchery fence should remain closed after August 1st until a series of scientific studies on mating selection first verifies high selectivity in a mixed population.

6 RECOMMENDATIONS

It was identified in the 2006 BCRP application for the DNA Analysis project that the study would likely need to be repeated for two to three 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 2007. Based on the results obtained in 2006, the project will follow the same study design with the following recommendations:

1. Maintain the same group timing segregation (Groups 1-5) but fewer samples from Group 1 will be required.

2. No morphological measurements will be collected due to the unliklihood of identifying visual differences between individuals from the two stocks from the data.

3. Spawn all August Group females with Group 1 (early Summer) males and keep separate until DNA results are available. They can be discarded if hybrid crosses occur.

4. Continue to close diversion dam fishway August 1st to preserve the headpond area for Summer-run chinook and maintain the same protocol for fishway operation as in 2006.

5. 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 2007 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 10 fish per redd should be collected for DNA analysis.

    ii. A study should 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.

Results from (i) are presently being compiled and will be available in the ***2007/08 Summer Chinook DNA Analysis*** report (BCRP). In-kind assistance for completing the field component in (ii) would be provided by DFO.

7 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 collecting DNA samples and measurements from broodstock; and to the molecular biology technicians for providing timely results from the PBS Genetics Lab.

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Appendix A - Financial Statement Form **Project #: 06.Pun.04**

	BUDGET			ACTUAL		
INCOME	**BCRP**	**Other (Cash)**	**Other (in-kind)**	**BCRP**	**Other (cash)**	**Other (in-kind)**
Total by Source	$16,225.00		$11,580.00	$16,225.00		$11,330.00
Grand Total Income (BCRP + Other)	**$27,805.00**			**$27,555.00**		
EXPENSES						
Project Personnel						
Biologist (contractor)	$3,010.00			$2,976.80		
Technician (contractor)	$600.00			$500.00		
Communications Technician	$1,800.00			$1,800.00		
DFO Biologist			$4,800.00			$4,800.00
DFO Technicians			$5,400.00			$5,400.00
Honoraria			$200.00			
Material and Equipment						
Small Tools/supplies & equipment rental	$115.00					
DNA Analysis	$9,000.00			$9,000.00		
Travel	$225.00			$214.20		$100.00
Adiministration						
Office Supplies	$75.00					
10%	$1,400.00		$1,180.00	$1,449.08		$1,030.00
Total Expenses	**$16,225.00**	**$0.00**	**$11,580.00**	**$15,940.08**	**$0.00**	**$11,330.00**
Grand Total Expenses (BCRP + others)	**$27,805.00**			**$27,270.08**		
Balance (Grand Total Income - Grand Total Expenses	**$0.00**			**$284.92**		
BCRP Balance (surplus)	**($284.92)**					

* 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 in the Comox Valley Record announcing the Puntledge River Summer Chinook DNA Analysis Project, October 25, 2006.

COMOX VALLEY RECORD Wednesday, October 25, 2006 A9

Summer-run chinook spawning in Puntledge

Watershed walk Sunday at river with biologist Esther Guimond

Despite this fall's low river levels, two dozen summer-run chinook are now spawning at a new habitat restoration project on the Puntledge River, Project Watershed says in a news release.

The project is approximately three kilometres downstream from BC Hydro's impoundment dam at Comox Lake in a section of the river known as the Headpond.

In 2005, 6,700 tonnes of gravel was added in an area of the Headpond, creating a new spawning bed measuring approximately 4,750 square metres, says Project Watershed. This year the site is being closely monitored and assessed for its ability to provide effective habitat for spawning and incubation.

"We seem to be off to a good start," says project biologist Esther Guimond. "The gravel was spread out over a 100-metre stretch, and spans across the entire river. Although we have only seen 12 pairs spawning this year, there is enough habitat there to eventually support over 400 pairs."

The Headpond section was historically the most important spawning area for summer-runs of chinook salmon and steelhead, notes Project Watershed. Following expansion of the hydro facilities in the 1950s, this habitat was severely impacted through a combination of flooding, reduced velocities and altered hydrology, the group adds.

The Puntledge River Hatchery, built in 1977, has been instrumental in conserving and maintaining the summer-run chinook stock over the past four decades.

According to Chris Beggs, manager at Puntledge Hatchery, "A key element in the successful re-establishment of the summer-run chinook salmon stock will be restoration of the historical spawning habitats.

"Since 2001 the hatchery has enabled a greater portion of the returning summer chinooks access above the Puntledge Diversion Dam into the Headpond and Comox Lake, after we acquire broodstock. This year, over 200 passed through the fish ladder at the Diversion Dam," he adds.

> *"Since 2001 the hatchery has enabled a greater portion of the returning summer chinooks access above the Puntledge Diversion Dam into the Headpond and Comox Lake, after we acquire broodstock. This year, over 200 passed through the fish ladder at the Diversion Dam."* Chris Beggs

Running simultaneously to the Headpond project is a genetic analysis study of summer and fall-run chinook. This information will help researchers and hatchery staff better understand the migration timing of these two distinct species in order to safeguard and rebuild the summer-run stock, says Project Watershed.

Both projects are funded through BC Hydro Bridge Coastal Fish and Wildlife Restoration Program (BCRP) and Fisheries and Oceans Canada.

The Puntledge River system is one of a few rivers on the east coast of Vancouver Island to support both a summer and a fall run of chinook salmon, states Project Watershed.

The Comox Valley Project Watershed Society says it is a non-profit society established in 1993 and run by a volunteer board of directors.

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Project Watershed is having a Watershed Walk in the upper Puntledge River from the upper hatchery to a new spawning site in the Headpond.

You can join biologist Esther Guimond on Sunday, Oct. 29 at 1 p.m. to learn more about the history of the BC Hydro dam and the importance of a healthy stream habitat for the survival of salmon populations. The walk will require good walking shoes and last for one to two hours. People will meet at the entrance to Barber's Pool on Forbidden Plateau Road.

For more information call Heather Beckett of Comox Valley Project Watershed at 703-2871.