Assessment of the genetic relationship among sockeye salmon and kokanee populations of the lower Mainland of British Columbia: Implications for establishment of an anadromous sockeye run in the Coquitlam watershed.

Report prepared for Bridge Coastal Fish and Wildlife Restoration Program
March 30, 2007

By: R. John Nelson, SeaStar Biotech Inc., and Chris C. Wood, Fisheries and Oceans Canada,

Work carried out in partnership with:
LGL Environmental Research Associates,
BC Ministry of Environment

Executive summary

The genetic results presented here suggest that the smolts found in Coquitlam River are not unlike kokanee which inhabit Coquitlam Reservoir and are most likely volitional smolts migrating from Coquitlam Reservoir. These results are also consistent with the hypothesis that kokanee of Coquitlam Reservoir are recently derived from an anadromous sockeye run. Analysis of Coquitlam Reservoir kokanee gill raker length and number, showed that these fish had similar characteristics to sockeye-kokanee hybrids which further supports the interpretation that Coquitlam Reservoir kokanee are likely of recent descendents of sockeye (Bussanich et al 2006). Taken together these results suggest that capacity for anadromous life history exists within at least some kokanee populations, and increase our confidence in the feasibility of restoring anadromous sockeye from *O. nerka* that currently inhabit the Coquitlam watershed.

The genetic assay employed here showed no genetic variation in the samples from Alouette River, Alouette Reservoir, and Widgeon Slough and all these samples shared the same genetic type. These genetic results suggest a relationship among them, which is consistent with their geographic proximity, but due the lack of variation within samples, we cannot estimate the extent of gene flow between them. This makes it difficult to provide recommendations regarding the most effective (and genetically appropriate) route to take for re-establishment of a sockeye run the Alouette watershed.

Table of contents

Figures

Tables

Introduction

The Coquitlam hydroelectric project was completed in 1914. The dam impounded the waters of the Coquitlam River watershed, creating Coquitlam Reservoir. As a result, access to what was formerly known as Coquitlam Lake was blocked for all anadromous fish. Historically the Coquitlam system supported a spring run of anadromous sockeye salmon (hereafter called “sockeye”) that was eliminated after dam construction.

This proposal was requested by the Coquitlam Salmon Restoration Project Advisory Committee to support their goal of assessing the feasibility of restoring sockeye above the Coquitlam Dam. This study will partially address the key uncertainty of whether kokanee indigenous to the Coquitlam Reservoir can re-anadromize and form the basis of a sockeye run to the Coquitlam system. This will be done by meeting the following study objectives:

1. Complete the analysis of genetic evolution among sockeye and kokanee in the system through continued mitochondrial DNA analysis of genetic samples from the Coquitlam and Alouette systems;
2. Use the genetic analysis to assess the likelihood and feasibility of re-anadromization;
3. Recommend the most appropriate approach to re-establishing anadromous sockeye runs to the Coquitlam Reservoir

Goals and objectives

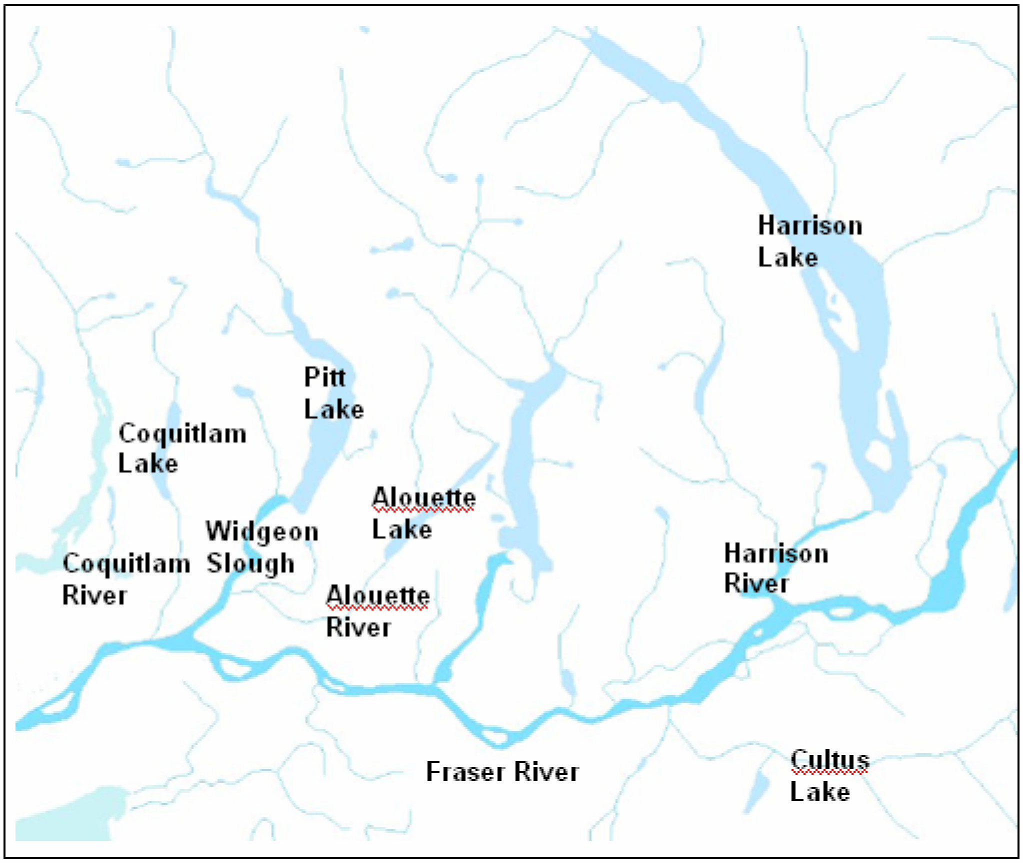
There is great interest by the general public, First Nations and governmental organization in re-establishing a sockeye run to Coquitlam Reservoir. The goal of the Coquitlam Salmon Recovery Program (CSRP) is to return Coquitlam Lake Reservoir sockeye to the status of a viable, self-sustaining and genetically robust wild population that will contribute to its ecosystem and have the potential to support sustainable use.

Prior to undertaking a re-anadromization program the feasibility of such a project must be determined and all possible options explored. Besides assessing feasibility, a key consideration for any re-anadromization project is that the resident gene pool not be put at risk. Understanding the genetic relationship among existing kokanee and sockeye populations of the region allows for informed decision making with regard to promoting re-anadromization while minimizing the risk to the existing gene pool. The genetic results reported here are aimed at addressing the feasibility of different options for re-anadromization and the likelihood of deleterious effects on the existing gene pool inherent in these different options.

Study area

The area of genetic study area ranged from Coquitlam River to Cultus and Harrison lakes (Figure 1).

Figure 1. Sample collection sites – Coquitlam Nerkid reanadromy feasibility study



Methods

Sample Collection

Tissues for genetic analysis were obtained from sockeye or kokanee (Table 1) from the locations as shown in Figure 1. Samples were stored in ethanol or frozen for storage prior to analysis. Samples from “volitional migrants” in the Coquitlam River were collected and associated fork length measurements taken from April 24th 2006 to May 12, 2006. Analysis of fork lengths showed that there were up to three different size classes of roughly 54mm to 90mm, 160mm to 170 mm and 190mm to 217mm in the sample (Figure 2.). Fish of the smaller size class were found throughout the sampling session while fish of the large classes were encountered almost entirely later in the sampling session (Figure 3). Because length data could not be assigned to individual tissue samples, tissues for genetic analysis were subsampled across the entire sampling session to better represent the entire volitional migrant population. This was accomplished by analysis of 10 samples that were collected from April 24th to April 27th and exclusively of the smaller size class, and 33 samples collected during the time when fish of all size classes were encountered (May 5th and 6th) and 6 samples collected later in the collection session also when all three size classes were encountered (May 8th and 9th).

Table 1. Samples collected and analysed by RFLP method including samples analysed previously.

Sample	**N**	**type**
Coquitlam Reservoir (CQRE)	69	kokanee
Coquitlam River (CQRI)	48	smolt
Alouette Reservoir (ALRE)	67	kokanee
Alouette River (ALRI)	55	smolt
Widgeon Slough (WS)	58	sockeye
Pitt Lake (PL)	30	sockeye
Cultus Lake (CL)	25	sockeye
Harrison River (HR)	25	sockeye
Harrison Lake (HL)	23	sockeye

Figure 2. Fork length of Nerkids sampled on Coquitlam River 2006.

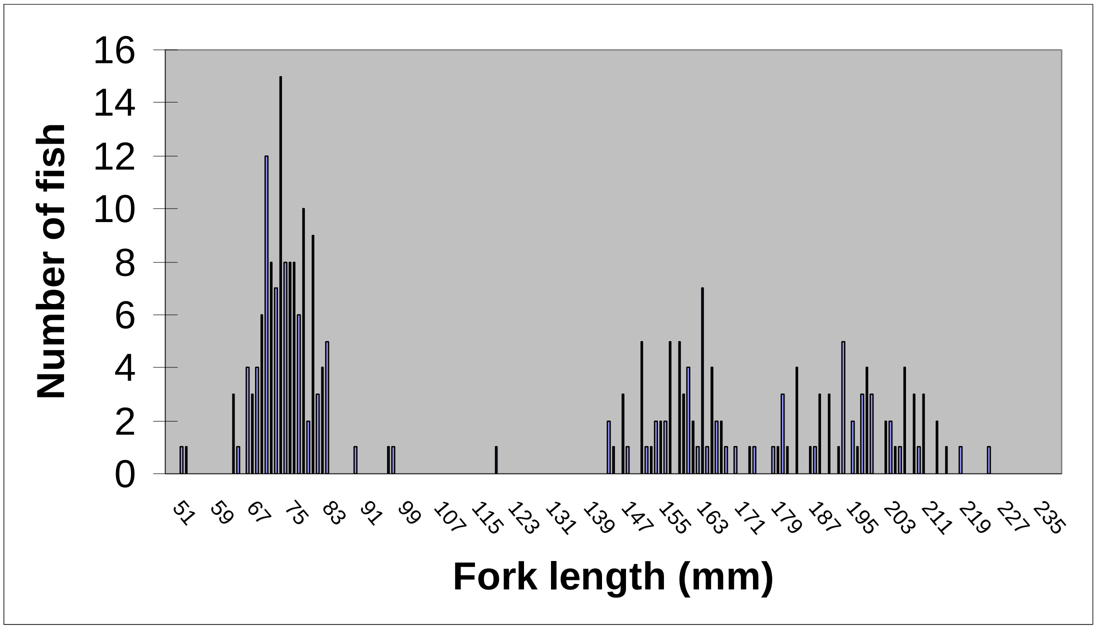
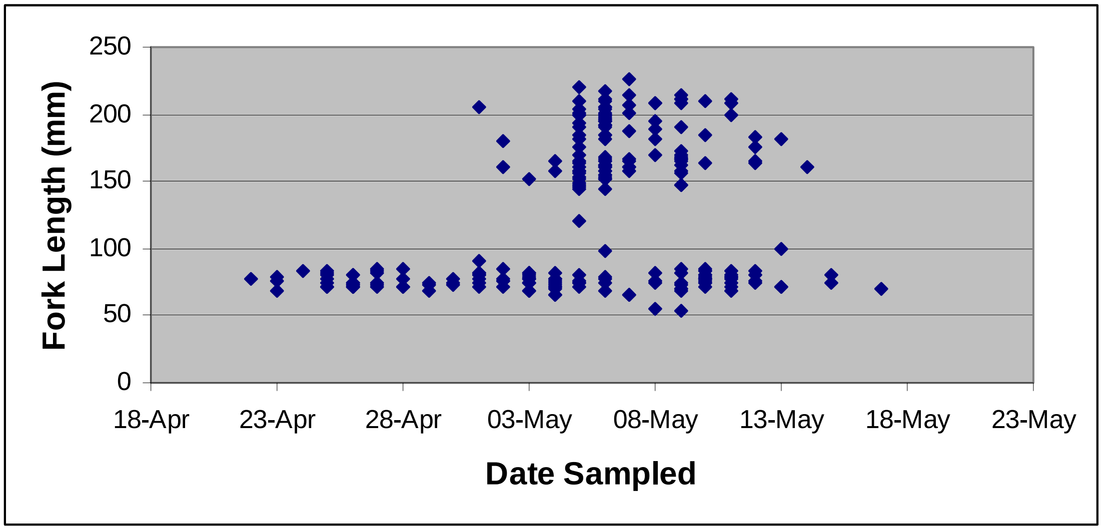


Figure 3. Date of sampling and fork length of Nerkids of Coquitlam River 2006



DNA extraction and PCR

A total of 391 specimens have now been analyzed from 9 populations. Including new (2006) results for 48 samples from each of Coquitlam Reservoir, Coquitlam River, and Alouette Reservoir and 39 samples from the Alouette River (Table 1). DNA was extracted from either fin, or muscle samples as described by Nelson et al. (1998)**.** Each polymerase chain reaction (PCR) required from between 1 to 10µls crude DNA extract depending on sample type and sample quality. PCR amplification of mitochondrial genes NADH-dehydrogenase subunit 1(*ND1)* and cytochrome b (cytb) was accomplished with primers pairs LGL 290, LGL 560 and LGL 287 and LGL 765 respectively. PCR was carried out in 96 well microtiter plates with a MJ PTC-100 thermal cycler (MJ research, Watertown, MA). 50 µl PCR reactions contained 10pmol (0.4µM) of each primer, 80µM of each nucleotide, 20mM tris-pH 8.8, 2mM $MgSO_4$, 10mM KCl, 0.1% triton X-100, 10mM $(NH_4)SO_4$, 0.1 mg/ml bovine serum albumin with the extension and denaturation at steps being 30 long at 72 C and 94 C respectively. The annealing temperature for both primers sets was 48 C. The cytb PCR product was approximately 1300 bp while the amplified segment of the ND1 gene was approximately 859 base pairs. PCR products for both genes were pooled and purified away from PCR buffer components using Qia-quick 96 PCR purification plates (Qiagen Corp.).

RFLP assay

The eluate from the Qia-quick plates was split four ways and subjected to restriction digestion with *ApaI, BfaI, BsteII* and *Rsa*I according to the enzyme supplier's instructions. After digestion the fragments were electrophoresed on a 1.5% agarose gel. All gels contained 0.5X TBE Buffer and were of a 3:1 ratio of metaphor agarose (Mandel Scientific Corp.) to standard grade agarose. Samples were electrophoresed and 100 V for three hours and stained with ethidium bromide. Band patterns from each enzyme were scored separately and band size was determined from a 1-kb ladder (Gibco-BRL Corp.). The enzymes *ApaI, BfaI, BsteII* and *RsaI* produced patterns including 7, 5, 2 and 8 different bands, respectively.

Each fish in the analysis was scored for individual enzymes and assigned one of 21 possible composite haplotypes.

Statistical Analysis

Pair-wise (between individual groups) tests of genetic homogeneity were carried out with the genetic analysis program ARLEQUIN (Schneider et al. 2000) with 10,000 steps in the Markov chain, 1000 dememorization steps and an α value of 0.05. Pair wise F_{ST} values were also calculated with ARLEQUIN with 100 permutations of the data and an α value of 0.05. Construction of a Neighbor-joining tree based on Cavalli-Sforza and Edwards genetic distances was accomplished with PHYLIP (Felsenstein 2005).

Results and interpretation

Inspection of Haplotype frequencies.

Analysis of mDNA yields a particular haplotype for each individual fish, and the haplotypes of all fish within each group is tallied up and divided by the number of individuals in each group to yield a “haplotype frequency”. These haplotype frequencies are compared statistically to judge the genetic relationship among the samples. Five different haplotypes (as defined by Wood et al. in prep) were found in the nine samples examined (h1, h2, h3, h4, h6). Two very unusual haplotypes were observed in the Coquitlam volitional migrant sample that had not been previously observed in any of the over 2000 sockeye-kokanee samples from Washington State to Alaska analysed by Wood et al. (in prep); it is unlikely that these samples are *O. nerka* but are more likely another salmonid species sampled by accident and they were not included in this analysis. The frequencies of each haplotype found in each samples are shown in Table 2. Simple inspection of the haplotype frequencies is useful to get a rough idea of the relationship among the samples examined.

Of particular interest for this report is the genetic relationship between fish in Coquitlam Reservoir and Coquitlam River. The predominant haplotype in both of these samples was h3 and they share all the same haplotypes with the exception that a single fish bearing h4 was observed in the Coquitlam River sample. Even so the samples from the Coquitlam Watershed appear by this simple inspection, to be closely related to each other. No novel sockeye-kokanee haplotypes were observed in Coquitlam Reservoir or River, and all haplotypes seen in these samples are present nearby in the region. These results suggest that the Coquitlam nerkids have not been isolated from the other populations for an extensive period of time. Further supporting this suggestion, h3 is the most prevalent haplotype found in Coquitlam Reservoir and River (77% and 74% respectively) and this haplotype is also the most prevalent haplotype found in the nearby populations of Widgeon Slough, Pitt Lake, Alouette River and Alouette Reservoir.

The samples from Alouette River and Alouette Reservoir and Widgeon Slough were genetically identical, containing only h3 even though relatively large numbers of samples from these locales were analysed. This suggests a relationship among them which is consistent with their geographic proximity, but due the lack of variation within samples, we cannot estimate the extent of gene flow between them. The samples from Harrison River and Cultus Lake stand out as the only samples not predominated by h3 (but rather h2 and h4, respectively).

Table 2. Haplotype frequencies

		Haplotype					
Sample	**N**	**1**	**2**	**3**	**4**	**5**	**6**
Coquitlam Reservoir (CQRE)	69	0.14	0.06	0.77	0.00	0.00	0.03
Coquitlam River (CQRI)	48	0.04	0.06	0.74	0.02	0.00	0.11
Alouette Reservoir (ALRE)	48	0.00	0.00	1.00	0.00	0.00	0.00
Alouette River (ALRI)	74	0.00	0.00	1.00	0.00	0.00	0.00
Widgeon Slough (WS)	58	0.00	0.00	1.00	0.00	0.00	0.00
Pitt Lake (PL)	30	0.17	0.17	0.67	0.00	0.00	0.00
Cultus Lake (CL)	25	0.00	0.20	0.24	0.56	0.00	0.00
Harrison River (HR)	25	0.08	0.52	0.40	0.00	0.00	0.00
Harrison Lake (HL)	23	0.13	0.13	0.52	0.13	0.00	0.09

Tests of genetic homogeneity.

The test of genetic homogeneity determines whether the null hypothesis of genetic homogeneity (random breeding) between population samples can be rejected and can be used to statistically explore the relationships suggested by simple inspection of the haplotype frequencies. A rejection of the null hypothesis of random breeding indicates significant differentiation of the populations the samples represent; however an inability to reject the hypothesis does not imply that the populations are the same, just that they cannot be shown to be different with this test. For example, Type II errors (accept null hypothesis when false) typically occur when small sample sizes are small resulting in low statistical power. Any inconsistencies in statistical results between this report and the last (Bussanich et al. 2006) likely arise from small sample sizes (particularly for Coquitlam Reservoir for which the sample size was 21 individuals in the work reported in spring 2006) The present results should be regarded as more definitive due to greater sample size.

Pair-wise tests of genetic homogeneity (Table 3.) show that the null hypothesis of genetic homogeneity could not be rejected between Coquitlam Reservoir and Coquitlam River. Pair-wise tests of genetic homogeneity between Coquitlam Reservoir and all other samples were rejected with the exception of Pitt Lake ($p = 0.28$); however, Pitt Lake was (marginally) significantly different from Coquitlam River ($p=0.041$). Tests of genetic homogeneity of Coquitlam River with all other samples were rejected with the exception of Harrison Lake ($p = 0.11$); however, Harrison Lake was significantly different from Coquitlam Reservoir ($p= 0.008$). The pair-wise test of genetic homogeneity could not be rejected for Pitt Lake and Harrison Lake. These pair-wise tests corroborate our earlier conclusions based on an inspection of the overall pattern in the haplotype frequencies, that the Coquitlam watershed samples are very closely related, and together, they are most genetically similar to those from Pitt and Harrison lakes. The inability to reject genetic homogeneity in pair-wise comparisons of Harrison Lake with Pitt Lake, and Harrison Lake with Coquitlam River might be caused by the low sample size for Harrison Lake ($N=23$), and we caution that these populations should not yet be regarded as genetically homogeneous. The Harrison River and Cultus Lake populations were not genetically homogeneous with any other populations examined. Not surprisingly, genetic homogeneity cannot be rejected for the Widgeon Slough and Alouette populations given the invariant, identical samples.

Table 3. Test of genetic homogeneity

	CQRE	CQRI	ALRE	ALRI	WS	PL	CL	HRI	HL
CQRE	/								
CQRI	nd	/							
ALRE	d	d	/						
ALRI	d	d	nd	/					
WS	d	d	nd	nd	/				
PL	nd	d	d	d	d	/			
CL	d	d	d	d	d	d	/		
HRI	d	d	d	d	d	d	d	/	
HL	d	nd	d	d	d	nd	d	d	/

“d” indicates a rejection of the test of the null hypothesis of genetic homogeneity, “nd” indicates that the null hypothesis could not be rejected (α = 0.05). “/” indicates sample identity.

Calculation of F_{ST}

The pair wise homogeneity test can confirm that genetic differentiation exists, but it does not provide a quantitative estimate of the degree of differentiation between samples as does the widely used estimator of population sub-division, F_{ST}. The values for F_{ST} can range from zero to one. An F_{ST} value of zero indicates no genetic differentiation while an F_{ST} value of one indicates very strong genetic differentiation. Examination of pair wise F_{ST} (Table 4) shows that Coquitlam Reservoir is closest genetically to Coquitlam River and Pitt Lake (F_{ST} = 0.0 for both samples) and Coquitlam River is closest to Coquitlam Reservoir (F_{ST} = 0.0) and Pitt Lake (F_{ST} 0.02, but not statistically different than 0). The F_{ST} for Coquitlam River and Harrison Lake is higher (0.05) but also non-significant, whereas the F_{ST} for Harrison Lake and Coquitlam Reservoir is 0.06 and almost significant (p = 0.06). The high but statistically non-significant F_{ST} values between the Coquitlam samples and Harrison Lake suggest that the power of this analysis is limited by the low samples number for

Harrison Lake. These results again suggest that the Coquitlam River and Reservoir populations are closely related to each other, and to the Pitt Lake (and possibly the Harrison Lake) population.

Table 4. Pair wise F_{ST}

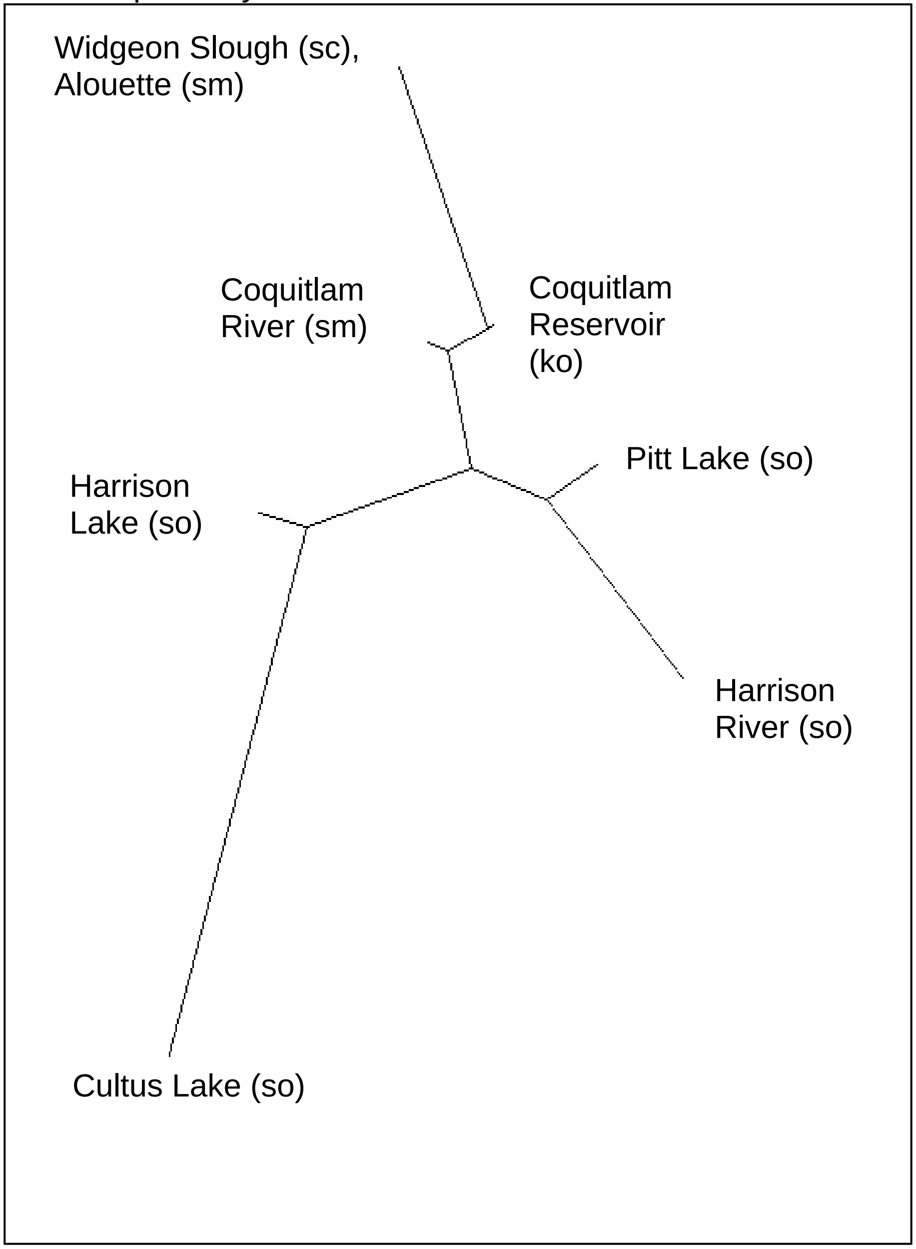
	CQRE	CQRI	ALRE	ALRI	WS	PL	CL	HRI	HL
CQRE	/								
CQRI	0.00	/							
ALRE	**0.16**	**0.17**	/						
ALRI	**0.16**	**0.18**	0.00	/					
WS	**0.15**	**0.16**	0.00	0.00	/				
PL	0.00	0.02	**0.33**	**0.35**	**0.31**	/			
CL	**0.40**	**0.37**	**0.74**	**0.75**	**0.72**	**0.30**	/		
HRI	**0.27**	**0.25**	**0.67**	**0.68**	**0.64**	**0.13**	**0.25**	/	
HL	**0.06**	0.05	**0.44**	**0.46**	**0.41**	0.00	**0.15**	**0.10**	/

Values emboldened are significant with $\alpha = 0.05$.

Neighbour-joining tree

Neighbour-joining tree was created to visually represent the overall relationship among the sample groups (Figure 4). The length of each branch corresponds to the estimated evolutionary difference between samples. Coquitlam Reservoir and River and located closest to each other on the tree, consistent with the pair wise genetic homogeneity test, and analysis of F_{ST}. Closest to Coquitlam is Pitt Lake followed by Harrison Lake all of which is consistent with previous results.

Figure 4. Neighbour-joining tree of Sockeye and Kokanee samples from lower Mainland British Columbia. "sc", "ko", and "sm" indicated sockeye, kokanee and smolt respectively.



Discussion

The genetic results presented here suggest that the volitional smolts found in Coquitlam River are not unlike kokanee which inhabit Coquitlam Reservoir and are most likely volitional smolts migrating from Coquitlam Reservoir. These results are also consistent with the hypothesis that kokanee of Coquitlam Reservoir are recently derived from an anadromous sockeye run. Analysis of Coquitlam Reservoir kokanee gill raker length and number, showed that these fish had similar characteristics to sockeye-kokanee hybrids which further supports the interpretation that Coquitlam Reservoir kokanee are likely of recent descendents of sockeye (Bussanich et al 2006). Recently, an acoustic tracking study has confirmed that kokanee and sockeye from Sakinaw Lake exhibited similar marine migratory behaviour during the first few months following their (forcible) release from a sea cage near the estuary of Sakinaw River (CCW, unpubl, data). Taken together these results suggest that capacity for anadromous life history exists within at least some kokanee populations, and increase our confidence in the feasibility of restoring anadromous sockeye from *O. nerka* that currently inhabit the Coquitlam watershed.

Fish found in the Coquitlam Reservoir and River have similar genetic characteristics to others found in the region suggesting that this system was connected by gene flow to other systems in the area historically, probably through a common colonizing population, and perhaps subsequently, through straying. The small extent of differentiation between samples from the Coquitlam watershed and from anadromous sockeye in Pitt lake supports the possibility that the historical anadromous character of the Coquitlam run has been retained in present day Coquitlam nerkids.

Recommendations

The observation of volitional migrants in the Coquitlam system interpreted along with the genetic results reported here and morphological (gill raker) studies suggest that re-anadromization strategies that utilize kokanee or volitional migrants from the Coquitlam watershed have the potential to be effective in establishing an anadromous run in the Coquitlam watershed. The use of the Pitt Lake population to establish an anadromous run in the Coquitlam watershed is a secondary possibility. However, further genetic and life history study is warranted before this route is taken. For example haplotype h6 was never observed in the Pitt lake sample but was observed in both Coquitlam samples. This difference might be an artifact of the low sample size from Pitt Lake (N=30) but the current state of our understanding of this system suggests that there is no population sample that is genetically *identical* to the nerkids found in Coquitlam watershed.

The samples from Alouette River and Alouette Reservoir and Widgeon Slough were genetically identical, containing only h3 even though relatively large numbers of samples from these locales were analysed. This suggests a relationship among them which is consistent with their geographic proximity, but due the lack of variation within samples, we cannot estimate the extent of gene flow between them. This makes it difficult to provide recommendations regarding the most effective (and genetically appropriate) route to take for re-establishment of a sockeye run the Alouette watershed. Further studies on the relationship between these populations should employ microsatellite DNA markers which would very likely have a higher degree of genetic variation.

Acknowledgements

The cooperation of many people was essential in meeting the objectives of this study. Special thanks to the Greater Vancouver Regional District, University of Victoria, Kwikwetlem First Nation, Chief and Elders, Fisheries and Oceans Canada, and to all whom assisted in the collection, processing, and analysis of the field data for this study. Technical assistance was provided by: Matt Foy, Doug Lofthouse, Stu Barneston, Al Stobart, Les Clint, Greg Bonnell, Wayne Krause, Maurice Coulter-Boisvert, Don MacKinley, Carol Cross, and Dorothee Keiser of Fisheries and Oceans Canada, Dr. Chris Foote (Malaspina University College), Geoff Clayton and Jennifer Ljunggren (ARMS). From GVRD, Dave Dunkley, Ken Juvik, Heidi Walsh, and several Coquitlam Reservoir security personnel offered technical assistance in the field. Thanks to George Chaffee, Nancy Joe (Kwikwetlem First Nation) for their support. From LGL (Richard Bussanich and Bob Bocking and field crews for collection of samples in Coquitlam Reservoir and Alouette River. Thanks to Shannon Harris (MOE) for collection of samples from Alouette Reservoir and Alf Leake (BC Hydro) and crew for providing samples from the Coquitlam River. Funding for this project was provided by the BC Hydro-Bridge Coastal Fish & Wildlife Restoration Program and Fisheries Oceans Canada.

References

R. Bussanich, R. Bocking, R. J. Nelson and C. Wood (2006). Feasibility Assessment of kokanee re-anadromization, And Planning of FISH Propagation FOR RE-Introduction of Sockeye Salmon in Coquitlam Reservoir. Bridge Coastal Fish and Wildlife Restoration Program Report No. 05.Co.08

Felsenstein, J. 1995. PHYLIP 3.5c. University of Washington, Department Genetics, Seattle, Washington 98195-7360.

Nelson, R.J., T. D. Beacham and M. P. Small. 1998. Microsatellite analysis of the population structure of a Vancouver Island sockeye salmon (*Oncorhynchus nerka*) stock complex using non-denaturing gel electrophoresis. Molecular Marine Biology and Biotechnology 7, 312-319.

Schneider, S., D. Roessli, and L. Excoffier. 1999. Arlequin ver. 2.0: A software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.