Microgeographic genetic diversity of wild steelhead trout (*Oncorhynchus mykiss*) in a conservation hatchery operated coastal river: How wild are they?

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Running title: Microgeographic genetic diversity in wild and hatchery steelhead

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Abstract - Allelic variation at 10 microsatellite loci was assayed in potentially wild *Oncorhynchus mykiss* collected from 11 tributaries and three upper main stem river sites (n=547), and compared to steelhead trout from the hatchery operated in the lower main stem river (n=333). The objective was to investigate microgeographic genetic structure of wild *O. mykiss* , in the face of large-scale hatchery stocking since 1984. Measures of genetic diversity within wild samples indicated diversity were similar to what has previously been documented for wild populations. The level of microgeographic site subdivision (θ) was significant ($\theta = 0.031$), indicating moderate structure, and much higher than among sites located in the lower river where the hatchery operates ($\theta = 0.004$). Bayesian assignment clustering was used to infer genetic ancestry without relying on prior information about sampling sites of individuals, and indicated the existence of five or more, wild *O. mykiss* populations. The overall spatial pattern, however, identified no clearly separate sites, but what appeared as an overlapping mosaic of modestly genetically divergent localities. We conclude that wild *O. mykiss* populations exist in the tributaries and the upper main stem river and its tributaries. These upper river populations appear to have retained genetic diversity and differentiation in the face of extensive hatchery releases.

Introduction

Natural animal populations may be influenced negatively by introduction of domesticated individuals. Nowhere has this management practice been of the same scale as in fisheries. Releases of cultured fish and their subsequent potential interbreeding with wild populations may have complex genetic effects (e.g. Hindar et al. 1991; Utter 1998; Reisenbichler 2005), and the precautionary principle dictates caution with such management practices. If genetic effects on performance traits are documented they tend to be negative and related to the genetic introgression of non-native and/or hatchery reared fish with the wild stocks (e.g. Chilcote et al. 1986; Reisenbichler and Rubin 1999; Kostow et al. 2003). A better option is to use native brood stock, presumably genetically similar to the wild fish, and in sufficient numbers to guard against genetic drift (conservation hatcheries; e.g. Heggenes et al. 2006, Taylor et al. 2006), although hatchery production problems related to the potential for altered directional or "relaxed" selection (Lynch and O'Hely 2001, Ford 2002) and phenotypic changes (Kostow 2004; Kostow and Zhou 2006) are still potential problems. Regardless of the kind of hatchery operation, a major concern remains: the conservation of population structure and diversity of wild fish populations in watersheds influenced by hatchery operation. An understanding of under what ecological conditions and conservation schemes wild populations may be sustained in the face of hatchery operations is of considerable scientific and management interest (Brannon et al. 2004; Reisenbichler 2005, Narum et al. 2006a,b).

In North America and throughout the world where it has been widely naturalized, there is extensive hatchery production of steelhead trout (*Oncorhynchus mykiss*) a genetically variable species (Busby et al. 1996; Beacham et al. 1999, 2004; Heath et al. 2001, 2002; Hendry et al. 2002). In a previous study there was little genetic effect from twenty years of extensive conservation hatchery operation in the boreal coastal Kitimat River (Heggenes et al. 2006). This river contains one population in the lower main stem river that is directly influenced by stocking (Fig. 1). There were, however, indications in the data that the watershed might sustain additional wild populations particularly in the upper river that was not extensively

sampled by Heggenes et al. (2006). Relatively little is known about potential micro-geographic genetic differentiation in steelhead trout (Hendry et al. 2002; Narum et al. 2006a,b; Olsen et al. 2006), even though this is crucial for conservation and management programmes to maintain natural biodiversity. Other well studied salmonids tend to show extensive micro spatio-temporal genetic differentiation in particular when resident (e.g. Carlsson et al. 1999; Wofford et al. 2005; Yamamoto et al. 2004), but also for anadromous populations in small (e.g. Carlsson and Nilsson 2000; Wenburg and Bentzen 2001; Ostergaard et al. 2003) as well as larger systems (e.g. Hansen et al. 2002; see review in Youngson *et al.* 2003). These studies tended to focus on small scale spatial structure among small streams and little work has examined spatial structure within individual streams, e.g. in steelhead trout (Beacham et al. 2004; Aguilar and Garza 2006). Therefore, we addressed the conservation issue of identifying the structure and diversity of potentially wild *O. mykiss* populations remaining in the Kitimat River watershed after more than 20 years of extensive stocking in the lower main stem river. The objectives were to use DNA obtained from *O. mykiss* in all major tributaries and the upper main stem river to (1) test if wild genetic variation remained in the watershed, (2) identify microgeographic genetic structure of wild *O. mykiss* populations in the Kitimat River, and (3) compare with the previously collected hatchery data to assess a potential impact of more than 20 years of large-scale hatchery operation on natural genetic structure and molecular variation in *O. mykiss* in regions of the enhanced stream that are more distant from the main operations of the hatchery.

Methods and materials

The Kitimat River on the north coast of British Columbia (Fig. 1; watershed approximately 217 000 hectares, water discharge range 19.4 - 1 670.7 m^3s^{-1}; mean annual discharge 148.8 m^3s^{-1}) is approximately 65km long and with ten major tributaries (Fig. 1; Wedeene and Little Wedeene Rivers, Hirsch, Nalbeelah,

Humphrys, Cecil, Chist, McKay, Hunter, and Davies creeks). The fish fauna is dominated by Pacific salmon (chinook (*Oncorhynchus tshawytscha*), coho (*O. kisutch*), pink (*O. gorbuscha*), chum (*O. keta*), and sockeye salmon (*O. nerka*) and kokanee), winter and summer run steelhead including non-anadromous rainbow trout, and coastal cutthroat trout (*O. clarki clarki*). Also found are Dolly Varden char (*Salvelinus malma*), threespine stickleback (*Gasterosteus aculeatus*), prickly (*Cottus asper*) and staghorn (*Leptocottus armatus*) sculpins, eulachon (*Thaleichthys pacificus*), Rocky Mountain whitefish (*Prosopium williamsoni*), and Pacific lamprey (*Lampetra tridentata*). The annual steelhead hatchery release, since 1984, is 50 932 ±SD 8107 smolts (range 34 420 - 64 297) at seven to eight localities in the lower main stem Kitimat River (Fig. 1; see Heggenes et al. 2006 for details). It is the only hatchery in the region.

Field sampling

Samples from potentially wild steelhead trout were collected in the fall 2004 and spring 2005 by electrofishing (Smith-Root, Inc. – Model 12-B POW, Vancouver, WA., USA) one site in each of 11 tributaries where they could be accessed (Fig. 1, Appendix; Hirsch Creek, Wedeene and Little Wedeene Rivers, Nalbeelah, Humphrys, Deception, Cecil, Chist, McKay/Boulton, Tatlock, and Davies creeks), and three sites in the upper main stem Kitimat River (Fig. 1, Appendix; upstream Chist Creek confluence, downstream Hunter Creek confluence, and upstream Davies Creek confluence). Total fish length (mm) was recorded and tissue was clipped from the adipose fin and stored individually in 96% ethanol. Sampled steelhead trout were 1+ or older (9 of 14 sites), to avoid family effects, which may cause more genetic differentiation in 0+ than for older fish (Carlsson and Carlsson 2002). Exceptions where additional 0+ (<60mm) were used to obtain a sufficiently large sample size for powerful statistical analysis, were: Wedeene and Little Wedeene rivers (42% and 17%, respectively), Nalbeelah Creek (12%), Tatlock Creek (57%), and mainstem Kitimat River downstream of Hunter Creek (44%). To provide some reference for our comparisons within the Kitimat River,, wild steelhead trout population samples were also collected from the upper Kasiks River (at Huckleberry Creek, 17.5 km upstream of the Kasiks/Skeena river confluence), a tributary in the neighboring Skeena River watershed.

123

124 *Microsatellite DNA*

125 Total genomic DNA was extracted using Qiagen DNeasy Tissue Kit (Qiagen Inc.), and further analysis
126 followed the same standard procedures as in Heggenes et al. (2006). Genetic variation was assayed at 10
127 polymorphic microsatellite DNA loci previously used for *Oncorhynchus mykiss* (Heggenes et al. 2006;
128 Taylor et al. 2007).

129

130 *Data analysis*

131 Descriptive statistics of microsatellite loci and populations included number of samples (N), allelic
132 richness (A_r), number of alleles (N_a), expected heterozygosity (H_e), and observed heterozygosity (H_o) and
133 which were compiled using Genepop version 3.4 (Raymond and Rousset 1995) and Fstat version 2.9.3
134 (Goudet 1995, 2002). These computer packages were also used to test for deviations from Hardy-
135 Weinberg equilibrium (HWE), genotypic linkage disequilibrium, and pair-wise population differentiation.

136

137 Genetic differentiations among sites were quantified using F_{ST} as estimated by θ (Weir and Cockerham
138 1984) and the 95% confidence intervals and partitioning of differentiation (sig w = within individuals, sig
139 a = among sites, sig b = within sites) were obtained using Fstat (version 2.9.3; Goudet 2002, with 5000
140 permutations). To guard against inflated Type I error rates in multiple comparisons, critical significance
141 levels for simultaneous tests were evaluated using the conservative standard and also sequential
142 Bonferroni adjustments (Rice 1989, but see Moran 2003). Fstat was also used to group data to compare
143 contrasts.

144

145 Genetic distances among pairs of sites were estimated with Cavalli-Sforza and Edward's (1967) chord
146 distance (C-S chord distance) calculated in the Phylip software package (Felsenstein 1993). Cavalli-
147 Sforza and Edward's chord distances were used to build an unrooted neighbor-joining tree to visualize the

148 genetic relationships among sites (1000 bootstraps). Isolation by distance was investigated by regressing
149 microgeographic distance on pairwise genetic differentiation ($F_{ST}/(1-F_{ST})$; Rousset 1997).
150
151 As an independent control, the Bayesian assignment program Structure (Pritchard et al. 2000) provided
152 inference of genetic ancestry without relying on information about sampling sites of individuals. It places
153 individuals into K clusters (representing potential populations), where K is chosen in advance but can be
154 varied across independent runs of the algorithm. Individuals can have membership in multiple clusters,
155 with membership coefficients summing to 1 across clusters. The log likelihood of our data set (ln P (X
156 | K)) was estimated given different numbers of K genetic clusters, and Bayes' theorem was used to
157 calculate the probability (α) of each K-value. We used an admixture model with uniform priors, correlated
158 allele frequencies, 100 000 burning cycles and 500 000 MCMC iterations. For each K-value we used the
159 mean log likelihood of our data set (ln P (D | K)) from several runs. To verify the most probable K value,
160 we checked the variance among runs since runs with higher K values than the true value often involve
161 higher posterior probabilities, but with a higher variance.
162
163 Using PCA-Gen (Goudet 1999), principal components analysis (PCA) was conducted on allele frequency
164 data as a comparative method to summarize genetic differentiation among all samples. Microsatellite allele
165 frequencies were also tested for evidence of recent bottlenecks in steelhead trout. The mode-shift test with
166 default values as implemented in Bottleneck (Cornuet and Luikart 1997). The TPM (Two-Phased Model
167 of Mutation) mode shift test assumes that the populations are near mutation-drift equilibrium and is
168 intermediate to mutation model (infinite alleles or stepwise mutation) for microsatellite loci (Luikart et al.
169 1998).
170
171

172 ## Results

Microsatellite variation across 477 individuals from 14 different sites in the Kitimat River watershed and at 10 microsatellite loci amplified collectable results. An additional 9 individuals were from the outlying upper Kasiks River in the Skeena watershed.

Genetic variation within populations

The number of alleles observed across all individuals in the Kitimat River watershed ranged from 2 (*Ssa*197) to 35 (*Oki*a3) with an average of 12.9 ±SD 9.3743 alleles per locus (Appendix) and 139 alleles in total. Mean allelic richness (A_r in Appendix) across sites and loci was 4.81±SD 2.52. It did not vary much among sites, from the mean 4.46±SD2.46 in the main stem Kitimat River downstream of the Hunter Creek confluence to 4.95±SD2.55 in Deception Creek (2-way ANOVA, $P = 0.2682$), but varied considerably among loci, from 1.27 for *Ots103* to 10.68 for *Okia3* (2-way ANOVA, $P < 0.0001$; Appendix). Observed heterozygosity (H_o in Appendix) averaged 0.5488±SD0.2216 across all sites and loci, with no significant differences across sites (2-way ANOVA, $P = 0.1755$; range from 0.4852±SD0.2251 at mainstem Kitimat River downstream of the Hunter Creek confluence to 0.6118±SD0.2381 at Davies Creek). Differences were, however, substantial among loci (2-way ANOVA, $P < 0.0001$; range from 0.1091±SD0.0684 for *Ots103* to 0.8803±0.1017 for *Okia3*; Appendix). The diversity measures H_o and A_r were significantly correlated with each other (simple linear Model 2 regression, $r^2 = 0.57$, $P < 0.0001$).

All ten microsatellites were in linkage equilibrium for all locus pairs across all sites ($P > 0.05$; except *Oneu8* x *Ssa197*, $P = 0.0128$) suggesting that loci segregate independently from one another. Loci and sites were generally in HWE (Appendix). Out of 150 probability tests (15 sites and 10 loci), seven significant departures from HWE were found, about as expected under a 5% Type I error (Bonferroni adjusted, sequential; 9 departures). Four of these exceptions were found at the *Oneu8* locus, while the remaining three were spread among three separate loci and sites (Appendix).

Genetic differentiation

The number and frequencies of alleles (N_a in Appendix) per locus, as a measure of genetic diversity, differed significantly among sites (G-like tests; $P < 0.0005$) for each of the ten loci independently, except for *Ots3* and *Ssa197* ($P = 0.0976$ and 0.6427, respectively), and for all loci combined across sites ($P<0.0001$, Fishers' method).

Similarly, there was genetic differentiation among sites sampled in the Kitimat River watershed, as expressed by θ (Table 1). When the 14 different Kitimat sites and the Kasiks River sample were analyzed, the overall subdivision was $\theta = 0.032$ (95% C.I. 0.009-0.070). Most of the genetic variation resided within individual fish (i.e. heterozygosity; 86.4%). Of the remaining variation, the variation among sites (3.2%) constituted about one third of the variation within sites (10.5%). More than half of the pair-wise site θ comparisons for differences in allele frequencies summed across all 10 loci were significant (55 of 105 comparisons; Table 1). Non significant results were spread among site comparisons. *O. mykiss* in the Wedeene and Little Wedeene rivers, and Nalbeelah, Cecil and Tetlock creeks, appeared to be the most distinct populations (Table 1), comparable to the Kasiks River sample which was significantly different from nine of the fourteen sites sampled in the Kitimat River watershed (Table 1). Power in the Kasiks River tests was limited due to the few fish ($n = 9$) captured. All the larger θ values, however, were associated with the Kasiks River outgroup (Table 1). Unique alleles ($n = 20$) were spread among ten sites (1 to 7 per site).

Including the previously analyzed Kitimat River lower main stem hatchery population (for details see Heggenes et al. 2006) in this analysis did not change our results substantially. The overall θ increased slightly to 0.038 (95% C.I. 0.007-0.096). As before, about half of the pair-wise site θ comparisons for differences in allele frequencies summed across all 10 loci were significant (59 of 121 comparisons). The lower main stem hatchery population itself, however, was distinct from all the other sites, except

Nalbeelah and Cecil creeks (θ = 0.006 and 0.005, respectively). Somewhat fewer alleles were found among the lower main stem population than among the wild *O. mykiss* (115 and 131, respectively).

A contrasting group comparison between wild *O. mykiss* from the upper Kitimat River and hatchery lower main stem steelhead, indicated significant differentiation for gene diversity measures (A_r = 3.99 and 3.78 P = 0.0006; θ = 0.031 and 0.004, P = 0.0044; but not H_o, P = 0.6392; one sided tests). Three alleles were only found in the lower main stem river, whereas 34 alleles were unique to the wild *O. mykiss* sites.

The Cavalli-Sforza genetic chord distances corroborated with results obtained from pair-wise sample site tests for genetic differences. There was genetic divergence among wild *O. mykiss* with overall mean genetic distance 0.0422±SD0.0201. The most divergent sites (populations) among the wild Kitimat River *O. mykiss* samples were Little Wedeene River and Tatlock Creek (C-S genetic distance 0.0696). The Neighbor-Joining (N-J) generated tree indicated a rather evenly distributed grouping of the Kitimat River wild *O. mykiss* (Fig. 2, upper panel). No striking distinctions with high bootstrap support were found to distinguish specific sites and there was no obvious pattern of isolation-by-distance (P = 0.8788) or that populations tended to get more distinct the farther away there were from hatchery stocking sites (P = 0.8403). With the lower main stem hatchery population included (Fig. 2, lower panel), it constituted a modest outgroup, and with the highest bootstrap support of all (0.658).

Clustering the wild Kitimat steelhead data without relying on information about sampling sites using the Structure algorithm corroborated these results (Fig. 3), i.e. some population structuring, but less than number and distance among sampling sites may suggest. The analysis suggested K ~ 5, i.e. partitioning of the genetic variation into five clusters was probable (Fig. 3). More clusters would improve the model fit slightly, but at the cost of increased variance, i.e. uncertainty.

Spatial ordination of samples of wild steelhead using PCA (Fig. 4) on the microsatellite allele frequencies did not indicate any clear groupings of sites. The results were not influenced when the Kitimat River lower main stem hatchery population was included in the analysis, did not influence this result.

Testing for potential bottlenecks indicated that none of the sampled sites had allele frequency class distributions consistent with having undergone recent bottlenecks (all loci fit the TPM-model, Wilcoxon two-tailed tests, $p > 0.1934$).

Discussion

Genetic variation within samples

Average observed heterozygosities of 0.49 to 0.61 (mean 0.55) for the upper Kitimat River wild *O. mykiss* is similar to that previously documented for the lower main stem hatchery influenced steelhead population (mean 0.58, range 0.51-0.62 across years; Heggenes et al. 2006), and consistent with values reported for steelhead from other regions in British Columbia (Beacham et al. 2000, 2004; Heath et al. 2001; Hendry et al. 2002; Taylor et al. 2006) and in other North American portions of the species range (e.g. Aguilar and Garza 2006; Narum et al. 2006a,b; Olsen et al. 2006). Allelic richness was higher for the upper Kitimat River wild *O. mykiss* than that previously observed for the hatchery influenced lower main stem population across loci and years (mean $A_r = 4.81$ and 3.78, respectively, Heggenes et al. 2006), but not when lower main stem steelhead were pooled as one population ($A_r = 4.56$).

Genetic differentiation among samples

Examination of microsatellite variation indicated population differentiation among the wild samples (overall subdivision $\theta = 0.032$), and much higher than previously found among year-classes for the

hatchery influenced lower Kitimat River adult steelhead (overall $\theta = 0.004$, not significantly different from 0; Heggenes et al. 2006). Including the previously analyzed lower Kitimat River hatchery population, increased subdivision only slightly (overall $\theta = 0.038$). The lower main stem hatchery population itself, however, differentiated from all the other sites, except Nalbeelah and Cecil creeks ($\theta = 0.006$ and 0.005, respectively). Therefore, the presumably wild upper Kitimat river *O. mykiss* populations show, and appear to have retained, considerable genetic differentiation, including many unique alleles, in spite of hatchery operations in the lower river. This is likely to reflect still intact natural population differentiation. Unfortunately, no temporal analysis is possible to indicate any changes in this natural population subdivision, because no tissue material has previously been collected from the upper Kitimat River.

The microgeographic within river population differentiation among wild *O. mykiss* in the upper Kitimat River, is higher than found in some other recent and more limited studies, e.g. between two adjacent sites (15km) in the Gulkana River, Alaska ($F_{ST} = 0.019$-0.028) indicating "moderate population structure" (Olsen et al. 2006). Narum et al. (2004) inferred genetic structuring based on samples from the main stem Walla Walla River, Washington, and a major tributary (Touchet River) with a calculated $F_{ST} = 0.023$, i.e. also lower than in the upper Kitimat River. In the Klickitat River in the same area, a non-native hatchery strain differentiated from native stocks ($F_{ST} = 0.078$), but differentiation among native fish was lower ($F_{ST} \leq 0.057$, except a divergent population above a waterfall; Narum et al. 2006a). Mixture analysis indicated six to seven populations. In the Grande Ronde River, Oregon, differentiation among three wild and one hatchery population was much smaller than in the Kitimat River ($F_{ST} = 0.005$-0.016; Narum et al. 2006b). Hendry et al. (2002) concluded that early and late run steelhead in the Dean River, BC, constituted separate populations with an $F_{ST} = 0.007$, i.e. much lower than we found in the upper Kitimat River.

On a larger stream scale, Beacham et al. (2000, 2004) reported "moderate differentiation" among steelhead populations for nine rivers in the neighboring Skeena watershed, with overall F_{ST} 0 =.066. This is

299 comparable to differentiation found among separate river systems in coastal California (overall F_{ST} =
300 0.075; Aguilar and Garza 2006).
301
302 On a temporal scale, Heath et al. (2002) found little change in genetic diversity and structure over 40 years
303 in three wild steelhead populations from separate rivers in the Skeena River watershed, BC, but reported
304 substantial among-year variation within localities (F_{ST} ranged from 0.028 to 0.056, samples sizes 24-30;
305 Heath et al. 2002). Contrary to this, Narum et al. (2006b) reported more variation among sites than
306 between years, but in smaller spatio-temporal samples (4 sites, 2 years).
307
308 *Wild populations*
309 Previous samples from the lower Kitimat River were generally in HWE with a notable exception for the
310 year 1977, which suggested that the sample included individuals from more than one population. These
311 1977 samples were collected from steelhead caught by anglers along the entire Kitimat River up to Hunter
312 Creek (i.e. about 45 km above the lower mainstem Kitimat River; Fig. 1). Detection of unique alleles
313 found only during the year of 1977 (n = 8) also supported the idea that more than one population had been
314 sampled. Of 16 alleles previously found to be uniqe to pre-hatchery steelhead in the lower river (Heggenes
315 et al 2006), eight were found in the wild samples. The present genetic study confirms that significant
316 genetic structuring in *O. mykiss* exists in the upper Kitimat River. The lower Kitimat River steelhead
317 appeared to be quite distinct from the upper river. Pairwise site comparisons and Bayesian assignment
318 model-based clustering indicated population subdivision with the most likely population structure being
319 five distinct populations.
320
321 *Genetic variation in sympatric wild and hatchery steelhead*
322 A variety of genetic effects of releasing hatchery-reared progeny into the wild have been reported (e.g.
323 Utter 1998; Brannon et al. 2004; Reisenbichler 2005). Most such studies, however, involve release of non-
324 native stocks of fish (e.g. Chilcote 2003; Kostow et al. 2003; Narum et al. 2006a,b). By contrast, Kitimat

River steelhead brood stock was always collected annually from indigenous unclipped fish, i.e. presumably wild steelhead dating back to pre-hatchery enhancement. In a 'worst' case they could be second generation hatchery fish.

Depending on number of brood fish used, release of cultured fish from local brood stock may also have negative effects on genetic variation in wild fish populations through changes in allele frequencies and loss of rare alleles via random sampling error and genetic drift. Recent temporal studies have not detected genetic effects in steelhead (Heggenes et al. 2006; Taylor et al. 2006). These results do not, however, necessarily indicate there have been no genetic changes of any kind. Relatively few microsatellites have been studied (10), out of thousands that occur in the genome, although we may reasonably assume that they reflect genome-wide variation. However, other loci may be more variable, and thus more sensitive to genetic changes. The studied microsatellite loci represent neutral genetic loci, which may not necessarily be a relevant proxy measure for genetic variation responsible for phenotypic traits. However, a recent study of California coastal steelhead found that microsatellites and a major histocompatibility complex class II gene were correlated with each other both for allelic richness and F_{ST}, and that contemporary selection was relatively weak and difficult to detect (Aguilar and Garza 2006).

In streams where wild fish are relatively abundant and productive, and which offer areas of refuge upstream from the area of hatchery fish release and return, as in the Kitimat River, release of hatchery steelhead appear to be compatible with wild fish conservation, at least within the relatively short time scales studied (Slaney et al. 1993; Nelson et al. 2005; Narum et al. 2006a). The reproductive contribution by upper river wild spawners to total population production, in a situation with lower river hatchery spawners, may have important positive conservation effects. They may buffer against loss of rare alleles, fluctuations in genetic variation, cumulative negative effects on genetic variation, and provide the presence of multiple year classes in the spawning population.

In conclusion, the results from the upper Kitimat River indicated higher genetic differentiation than in the lower river hatchery population, reflecting retained genetic microgeographic variation among local wild *O. mykiss* populations, which appear to constitute a mosaic or patchwork of genetically distinct populations rather than follow a strict isolation-by-distance model. Such a mosaic population structure may be a common characteristic of situations where multiple localities are samples across relatively small spatial scales. Our study further emphasizes the complexity of population structure in large river systems and, in particular, that the contribution by upper river wild spawners is likely to have important positive conservation effects.

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Table 1. θ estimates for steelhead sample sites in the Kitimat River watershed. Underlined values indicates significant θ value.

	Hirsch Creek	**Wedeene River**	**Little Wedeene**	**Nalbeelah Creek**	**Humphrys Creek**	**Deception Creek**	**Cecil Creek**	**Chist Creek**	**McKay/ Boulton**	**Tatlock Creek**	**Davies Creek**	**Mainstem Chist**	**Mainstem Hunter**	**Mainstem Davies**	**Outgroup Kasiks River**
Hirsch		0.037	0.015	0.074	0.015	0.020	0.077	0.001	0.028	0.064	0.016	0.013	0.077	0.011	0.029
Wedeene			0.023	0.017	0.020	0.005	0.027	0.016	0.009	0.045	0.015	0.037	0.038	0.031	0.070
Little Wedeene				0.060	0.024	0.023	0.061	0.009	0.015	0.054	0.015	0.027	0.072	0.023	0.051
Nalbeelah					0.045	0.026	0.006	0.043	0.016	0.024	0.030	0.066	0.012	0.052	0.106
Humphrys						0.006	0.055	0.009	0.020	0.048	0.009	0.017	0.043	0.021	0.035
Deception							0.036	0.007	0.009	0.043	0.011	0.022	0.036	0.023	0.056
Cecil								0.052	0.028	0.020	0.026	0.067	0.014	0.053	0.110
Chist									0.014	0.048	0.006	0.004	0.051	0.008	0.029
McKay/ Boulton										0.023	-0.003	0.031	0.023	0.019	0.056
Tatlock											0.022	0.060	0.011	0.044	0.069
Davies												0.009	0.017	-0.001	0.028
Mainstem Chist													0.059	0.012	0.016
Mainstem Hunter														0.039	0.073
Mainstem Davies															0.031
Outgroup Kasiks															

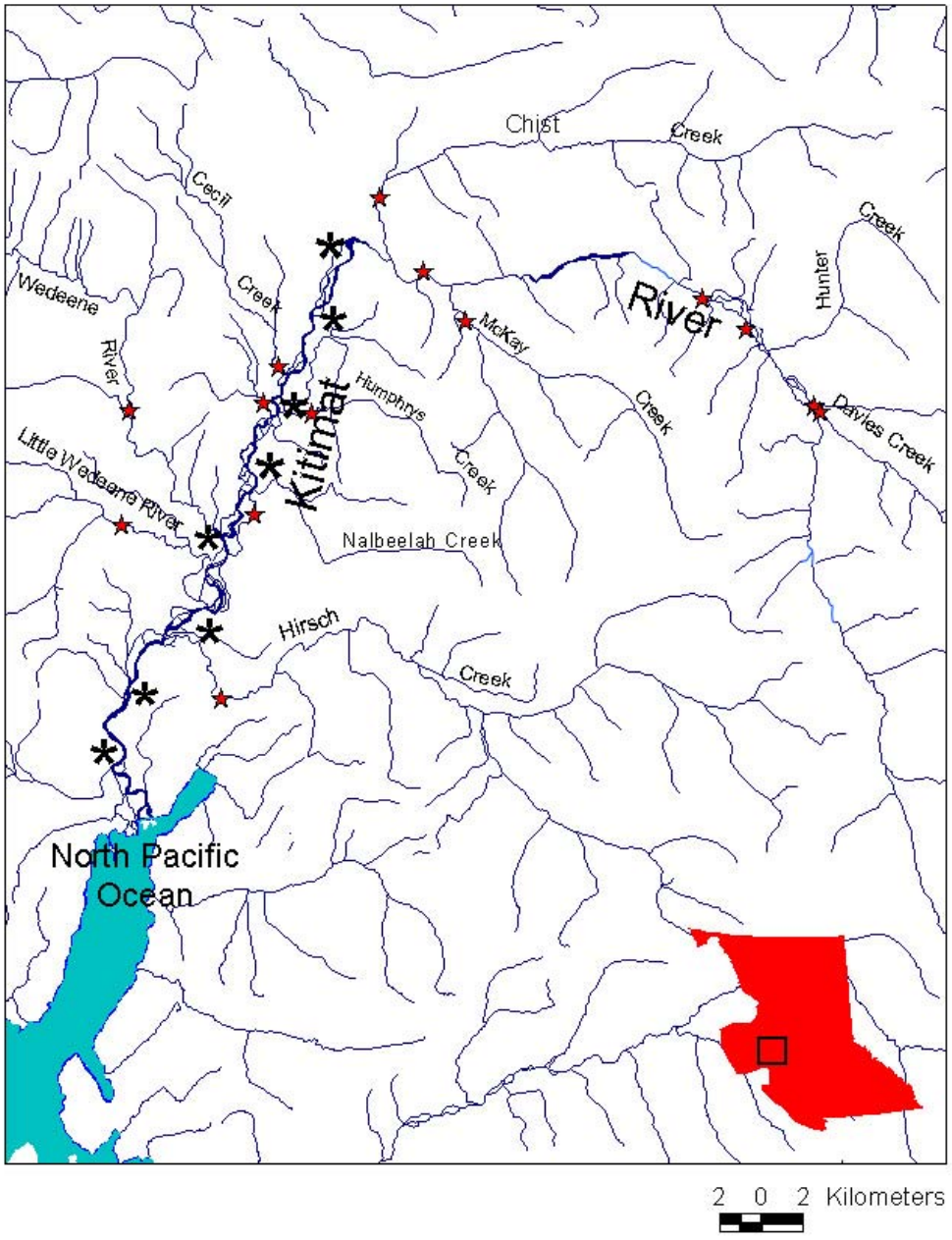
Figure legends

Figure 1. The Kitimat River study area on the northwest Pacific Coast of Canada. Small stars indicate sampling sites for wild O. mykiss in the Kitimat River watershed. Large stars indicate stocking sites for hatchery steelhead in the lower main stem of the Kitimat River. Inset location in British Columbia, western Canada.

Figure 2. Neighbour-joining tree based on Cavalli-Sforza and Edward's (1967) chord distances calculated in Phylip. Bootstrap values are labeled in lower panel. Note that the cladogram indicates clustering pattern and distances are not to scale.

Figure 3. Clustering without relying on information about sampling sites (Bayesian STRUCTURE algorithm). The simulations suggest the material may represent K = 5 populations. Although there is a slightly improved model fit with more structuring, the variation (lower panel) increases.

Figure 4. Principal component analysis based on allele frequency data to summarize genetic differentiation among all sites sampled for wild O. mykiss in the Kitimat River watershed. The analysis summarizes the variation across 10 loci (131 alleles) explaining the differentiation of individual sites at each axis. Only the first principal component was significant.



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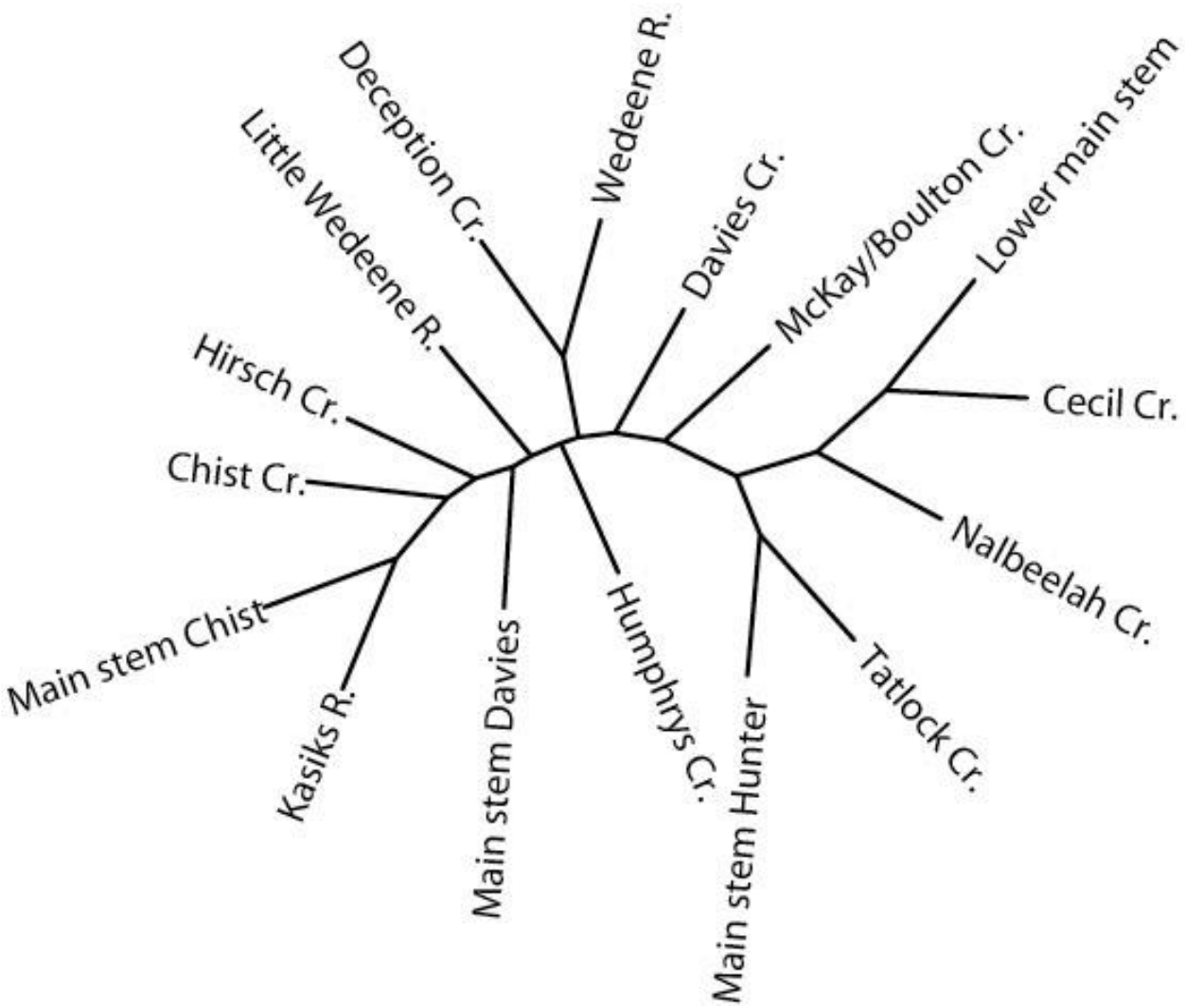
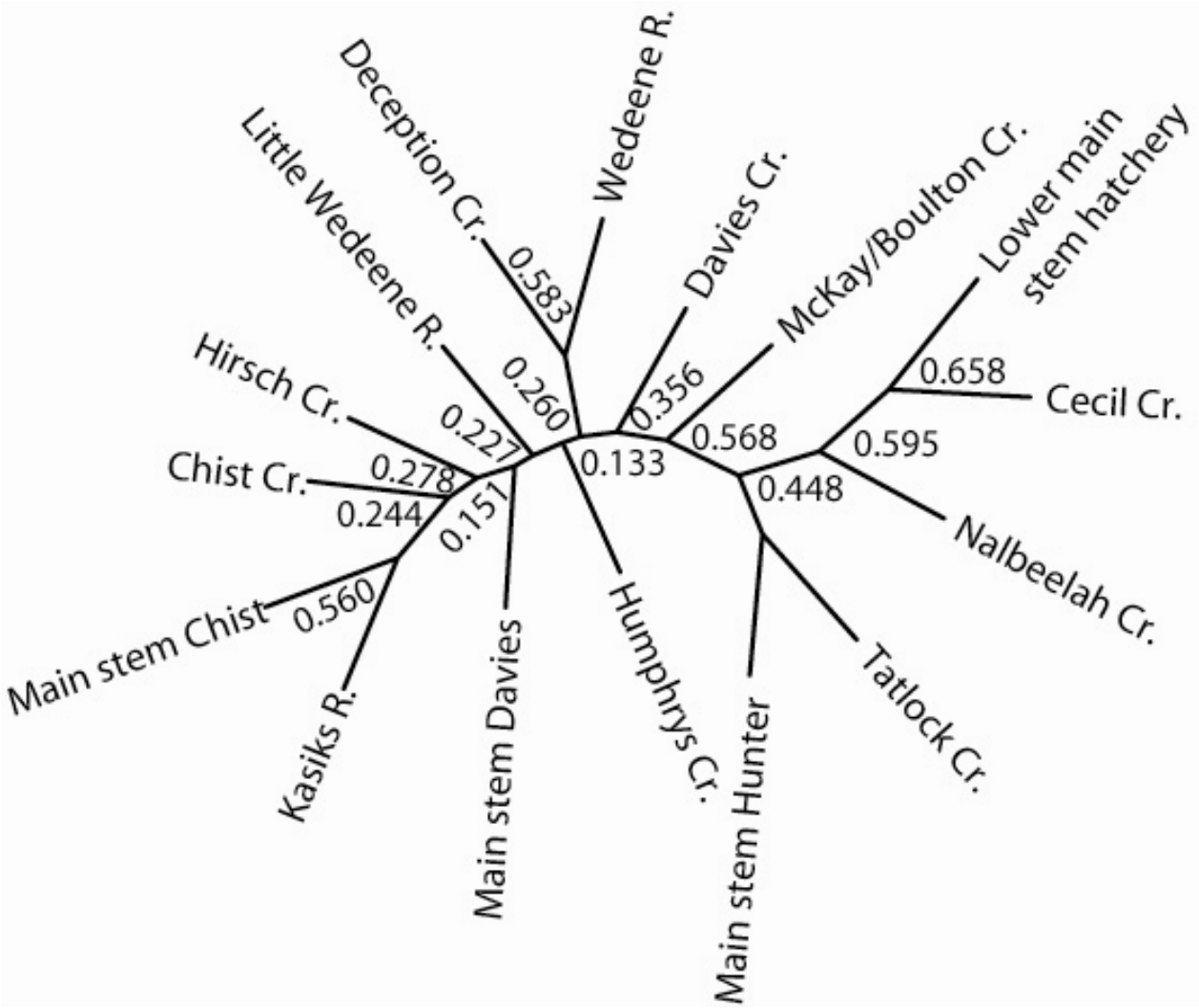

Deception Cr.
Wedeene R.
Little Wedeene R.
Davies Cr.
McKay/Boulton Cr.
Lower main stem
Hirsch Cr.
Cecil Cr.
Chist Cr.
Nalbeelah Cr.
Main stem Chist
Kasiks R.
Main stem Davies
Humphrys Cr.
Main stem Hunter
Tatlock Cr.



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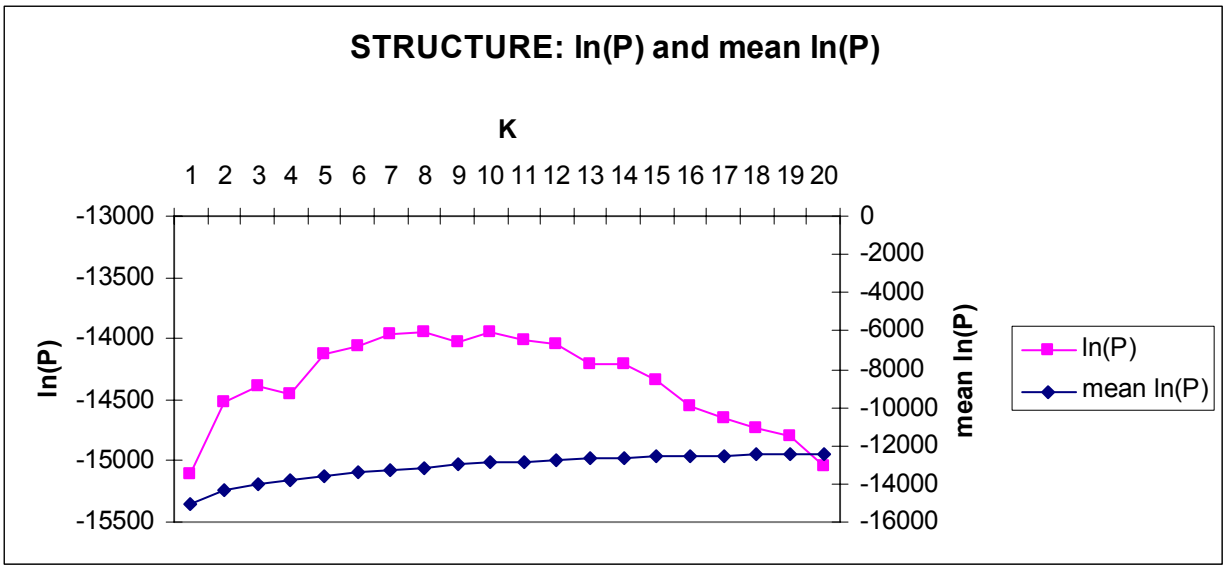

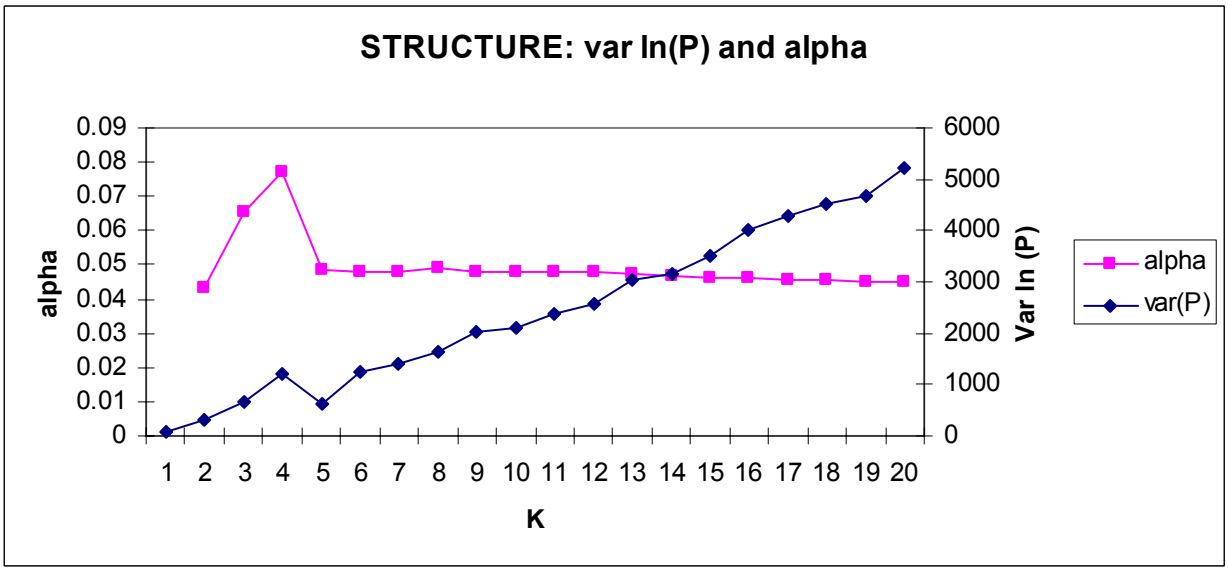

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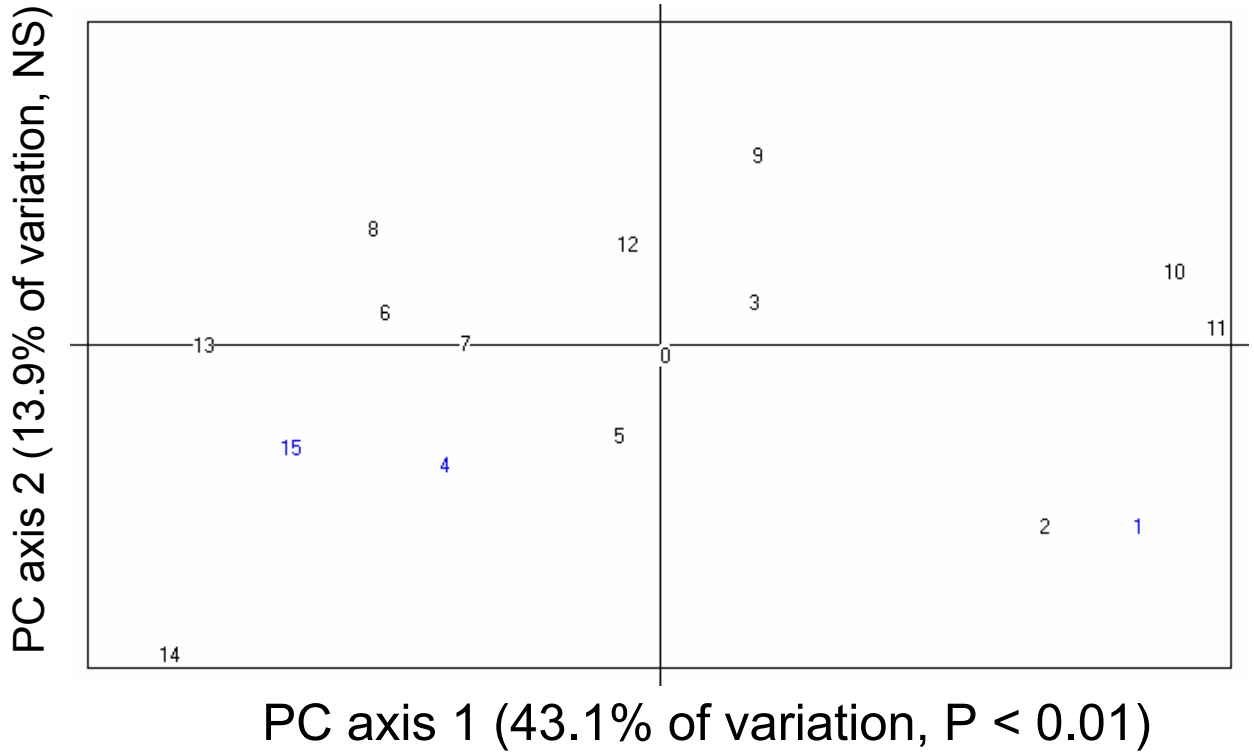

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71 Appendix

72 Summary of allelic variation at 10 microsatellite loci for 15 sites sampled for Oncorhynchus mykiss in the Kitimat River watershed. Number of samples
73 which amplified results (N), allelic richness ($A_{r)}$, number of alleles per locus (N_a), and observed heterozygosity (H_o) are given for each population.
74 Significant departures from Hardy Weinberg Equilibrium are denoted by asterisk "*" (using Bonferroni correction for 15 sites and 10 loci; p=
75 0.05/150=0.0003).

	Oneu8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Results over all loci, mean±SD
Hirsch Creek											
N	40	38	40	38	39	39	40	40	37	35	38.6±1.6466
Ar	3.559	5.992	-	4.148	2.821	5.947	4.592	2.000	5.599	10.947	4.6605±2.7723
Na	6	8	1	5	4	9	6	2	8	19	6.8±5.0067
He	0.3538	0.7561	-	0.6101	0.5475	0.7876	0.7336	0.5060	0.7856	0.9321	0.6680±0.1778
Ho	0.2750	0.5526	-	0.6579	0.4359	0.6667	0.7500	0.4250	0.8649	0.8286	0.6063±0.1993
Weedene River											
N	30	31	32	32	32	30	31	31	31	31	31.1±0.7379
Ar	2.963	5.278	2.792	4.420	3.307	4.887	3.975	2	5.383	12.361	4.7366±2.9060
Na	4	8	4	6	4	6	4	2	8	22	6.8±5.6726
He	0.5689	0.6938	0.2813	0.6285	0.6047	0.7147	0.7356	0.5034	0.7287	0.9514	0.6411±0.1752
Ho	0.2333*	0.6774	0.2500	0.5625	0.4688	0.7000	0.6129	0.5161	0.7419	1.0000	0.5763±0.2293
Little Weedene River											
N	31	31	31	30	30	29	31	31	31	31	30.6±0.6992
Ar	5.205	5.785	1.650	4.447	2.513	5.870	4.362	2	6.005	9.940	4.7777±2.4371
Na	7	8	2	4	3	7	6	2	9	16	6.4±4.1952
He	0.6822	0.7631	0.0936	0.6802	0.4842	0.7683	0.6811	0.4892	0.6854	0.9175	0.6245±0.2258
Ho	0.5807	0.6129	0.0968	0.6000	0.4667	0.5172	0.4194	0.4839	0.7419	0.8710	0.5391±0.2057

Nalbeelah Creek											
N	42	33	33	33	42	41	42	42	37	43	38.8±4.3153
Ar	4.577	5.537	-	3.881	3.139	5.078	3.955	2.000	5.201	10.264	4.8480±2.3136
Na	7	9	1	5	5	8	4	2	9	21	7.1±5.6065
He	0.5083	0.6791	-	0.5535	0.5829	0.6899	0.7301	0.4773	0.7383	0.9201	0.6533±0.1384
Ho	0.1905*	0.5581	-	0.5116	0.6190	0.5610	0.4286*	0.4286	0.8108	0.9767	0.5650±0.2273
Humphrys Creek											
N	40	40	39	37	28	25	37	40	38	40	36.4±5.3996
Ar	3.647	5.913	1.961	4.422	2.865	5.601	3.747	2.000	4.870	9.635	4.4661±2.2699
Na	5	10	3	5	4	8	4	2	8	18	6.7±4.6916
He	0.5775	0.7658	0.1235	0.6890	0.5589	0.7657	0.6594	0.5035	0.6449	0.8968	0.6185±0.2086
Ho	0.4750	0.6250	0.0769	0.6487	0.5357	0.6400	0.6756	0.5250	0.6842	0.8750	0.5761±0.2072
Deception Creek											
N	17	22	23	23	23	21	22	21	23	21	21.6±1.8379
Ar	3.845	5.498	2.419	5.007	3.419	5.771	4.470	2.000	5.949	11.069	4.9447±2.5456
Na	5	8	3	7	4	9	6	2	8	16	6.8±3.9665
He	0.6168	0.6892	0.2039	0.7034	0.5691	0.6702	0.7167	0.5110	0.7478	0.9373	0.6365±0.1902
Ho	0.2941	0.5909	0.1304	0.6957	0.3913	0.6667	0.6364	0.4762	0.6087	0.9524	0.5443±0.2312
Cecil Creek											
N	35	34	35	36	35	36	36	36	20	34	33.7±4.8774
Ar	4.728	5.459	1.451	4.665	3.697	6.004	4.022	2.000	5.698	10.630	4.8354±2.5291
Na	8	9	2	6	5	11	5	2	7	20	7.5±5.2334
He	0.4816	0.6422	0.0563	0.6624	0.5909	0.7977	0.7121	0.4930	0.8013	0.9267	0.6164±0.2411
Ho	0.2571*	0.5588	0.0571	0.5833	0.5429	0.7222	0.5556	0.5000	0.7500	0.8824	0.5409±0.2385

Chist Creek											
N	39	40	38	34	38	38	38	39	39	39	38.2±1.6193
Ar	4.167	5.567	1.657	3.993	2.989	5.599	4.280	2	4.681	11.167	4.6100±2.6612
Na	4	8	3	5	4	9	5	2	7	21	6.8±5.4528
He	0.5545	0.7044	0.0775	0.5975	0.5733	0.7421	0.7407	0.5035	0.7033	0.9297	0.6127±0.2245
Ho	0.4615	0.5750	0.0790	0.5588	0.5000	0.6842	0.6053	0.5128	0.6923	0.8718	0.5541±0.2052
McKay/Boulton Creek											
N	34	33	33	27	33	33	27	32	33	31	31.6±2.5473
Ar	5.118	5.200	1.273	4.264	3.081	4.893	5.250	2	5.871	10.513	4.7463±2.5331
Na	7	7	2	6	4	8	6	2	10	18	7.0±4.6188
He	0.7353	0.7380	0.0303	0.5933	0.5730	0.5706	0.7848	0.4955	0.7553	0.9281	0.6204±0.2441
Ho	0.5588	0.4546*	0.0303	0.5556	0.6364	0.4849	0.5556	0.4688	0. 6364	0.8710	0.5129±0.2203
Tatlock Creek											
N	29	29	29	27	28	28	23	27	28	27	27.5±1.7795
Ar	4.756	5.010	-	4.619	2.861	4.115	3.970	2.000	5.651	9.151	4.6814±2.0114
Na	6	7	1	7	3	5	4	2	8	16	5.9±4.2282
He	0.7235	0.7139	-	0.5381	0.4513	0.7110	0.7082	0.5066	0.7312	0.8595	0.6604±0.1317
Ho	0.4138	0.6897	-	0.4444	0.3571	0.6071	0.6522	0.6296	0.8572	0.6296	0.5867±0.1561
Davies Creek											
N	24	24	24	20	24	24	23	24	24	22	23.3±1.3375
Ar	3.974	6.191	1.859	5.171	3.905	4.831	4.418	2	5.725	9.427	4.7501±2.1713
Na	5	9	2	6	5	6	5	2	9	13	6.2±3.3599
He	0.6764	0.7819	0.1560	0.7192	0.5966	0.7057	0.7411	0.5027	0.7766	0.9123	0.6569±0.2074
Ho	0.4583	0.6250	0.1667	0.8500	0.4583	0.6667	0.6087	0.4583	0.9167	0.9091	0.6118±0.2381

Main stem upstream Chist confluence											
N	37	40	38	36	33	36	36	38	22	36	35.2±4.9844
Ar	4.146	5.148	2.230	4.630	3.227	4.588	4.515	2	5.516	8.683	4.4683±1.8890
Na	6	7	3	6	4	7	7	2	7	16	6.5±3.8079
He	0.5183	0.7114	0.1737	0.6953	0.5930	0.7046	0.7085	0.5067	0.7347	0.8858	0.6232±0.1932
Ho	0.4865	0.7000	0.1842	0.5556	0.4546	0.6389	0.6111	0.5790	0.5455	0.9722	0.5728±0.1986
Main stem downstream Hunter confluence											
N	36	34	35	34	35	34	25	32	34	33	33.2±3.0840
Ar	3.846	5.601	1.596	3.937	2.704	4.464	5.009	2.000	5.060	10.338	4.4555±2.4609
Na	6	8	2	6	3	6	7	2	7	18	6.5±4.5765
He	0.5227	0.7634	0.0832	0.5224	0.5105	0.6769	0.6482	0.5060	0.7261	0.9224	0.5882±0.2234
Ho	0.1944*	0.5588	0.0287	0.5588	0.3714	0.6471	0.5600	0.5000	0.7059	0.7273*	0.4852±0.2251
Main stem upstream Davies confluence											
N	30	29	30	29	30	30	30	30	30	29	29.7±0.4831
Ar	4.284	5.831	2.178	5.036	2.514	5.171	4.324	2.000	5.331	11.142	4.7811±2.6287
Na	5	8	3	7	3	8	6	2	8	19	6.9±4.8178
He	0.6011	0.7647	0.1588	0.6534	0.5130	0.7452	0.6898	0.4520	0.7701	0.9310	0.6279±0.2145
Ho	0.4667	0.6552	0.1000	0.6552	0.4637	0.7000	0.5667	0.5333	0.7333	0.9655	0.5839±0.2252
Results over all populations											
N											477
Ar	4.503	5.910	1.751	4.442	3.116	5.647	4.529	2.000	5.516	10.682	4.8096±2.5237

Na	10	16	8	9	6	15	8	2	20	35	12.9±9.3743
He	0.5801 ±0.1038	0.7262 ±0.0416	0.1307 ±0.0731	0.6319 ±0.0644	0.5535 ±0.0465	0.7179 ±0.0583	0.7136 ±0.0358	0.4969 ±0.0157	0.7378 ±.0.0412	0.9224 ±0.0170	0.63131±0.1947
Ho	0.3818 ±0.1357	0.6024 ±0.0667	0.1091 ±0.0684	0.6027 ±0.0966	0.4787 ±0.0830	0.6359 ±0.0703	0.5884 ±0.0875	0.5026 ±0.0550	0.7426 ±0.1030	0.8809 ±0.1017	0.5488±0.2216
Kasiks River, outgroup											
N	9	9	9	9	9	9	9	9	9	9	9±0
Ar	3.000	8.000	-	4.000	3.000	4.000	5.000	2.000	6.000	8.000	4.4±2.3664
Na	3	8	1	4	3	4	5	2	6	8	4.4±2.3664
He	0.2157	0.8758	-	0.6471	0.5229	0.6994	0.7255	0.5229	0.6994	0.8693	0.6420±0.2031
Ho	0.2222	0.8889	-	0.7778	0.6667	0.7778	0.4444	0.4444	0.6667	1.0000	0.6543±0.2450