Genetic Assessment of summer and winter run Steelhead trout in the Somass, Ash and Stamp rivers

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Prepared For:

Hupacasath First Nation
Port Alberni B.C.
And

Fish and Wildlife Compensation Program

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EXECUTIVE SUMMARY

Genetic differentiation among samples of steelhead (*Oncorhynchus mykiss*) from the Stamp/Somass River system was investigated by means of allelic variation over a suite of 14 microsatellite genetic markers. A total of 308 individuals comprising 5 putative populations were examined after reduction of the dataset to exclude samples from hybrids and removal of unrepresentative genotype groupings through strong family structure. Both frequency-based as well as genetic distance-based analyses indicated significant population structure (F_{st} = 0.014 p=0.001). Bayes analysis, incorporating an informative prior that identified potential groupings, suggested that there is substantial introgression among genotypes. In the absence of prior information on population affiliation, Bayes clustering identified 3 groupings, one of which was dominated by Ash summer steelhead. However, there was substantial allocation of Ash samples to both hatchery and lower Stamp/Somass genotypes.

Introduction

There is great interest by Hupacasath First Nation and others in improving access of anadromous salmon such as coho and sockeye to the mid and upper Ash River system. Specifically, fish passage at Elsie Dam would allow access to suitable spawning and rearing areas above Elsie Lake Reservoir for anadromous salmon and steelhead. Hupacasath Traditional Ecological Knowledge (TEK) asserts anadromous salmon had access to Elsie Lake and above before the development of the Elsie Lake hydro power development. This claim is generally not supported by Department of Fisheries and Oceans (DFO) or Ministry of Environment (MOE) as there is no documented evidence of anadromous salmon above Dickson Lake prior to the development of the Elsie Lake Dam (Griffiths, 1993).

However, studies since 1993 have provided evidence of historical anadromous fish present above Elsie Lake based on Nitrogen 15 in lake sediments. Hatfield and Bos, 2008, found Nitrogen 15 signatures in the lake sediments of the upper Ash that indicate anadromous fish were present in Elsie, Oshinow and McLaughlin lakes prior to the dam development and disappeared just after the dam was completed. BC Conservation Foundation (BCCF) field staff have observed juvenile coho above Dickson Lake in 2009 and adult steelhead are regularly observed at the base of the Elsie Lake Dam in the late spring and early summer (Eva Wichmann, pers. comm).

Despite what appears to be a growing body of evidence that anadromous fish had access to the upper Ash in the past and some access at least to the Elsie Dam now (steelhead and coho in some years), discussions on developing fish passage objectives for the Ash with various management agencies and interested groups are constrained by concerns over the potential impacts on steelhead trout. These include the potential for hybridization between summer and winter stocks, as well as the various impacts of hatchery stocking, inter-specific competition and habitat restrictions.

Contemporary steelhead populations in the Ash River include apparently distinct summer and winter runs. Summer run fish migrate into the system in late May with a modal peak in July, moving further into the system to a point where they are possibly constrained by the Elsie Dam. Winter fish arrive later, peaking in January, and predominately utilize the Stamp/Somass rivers, with limited occurrence in the lower Ash River. However, the hatchery stocking program, which has been carried on over approximately 25 years appears to have contributed a third ecotype that enters the system over the intervening period. Escapements to the system are now multi-modal, with steelhead arriving in every month of the year (M. McCulloch Ministry of Environment pers. comm.). The shift in run timing suggests that, at least, the lower system now contains a somewhat genetically blended population. It is unknown what, if any, influence this has had on the genetics of the upper Ash summer run population.

Similarly, steelhead migration above Dickson Lake may have been improved by alteration of the configuration of Dickson Falls by blasting in 1977/78 as well as by the post impoundment flow regime. This may have engendered some greater ability for winter-run fish, or a fall-run hatchery variant to escape to the middle Ash River, as it seems to have provided for coho. Additionally, a new flow regime since 2004 (Ash WUP, 2004) has provided increased base flow requirements and pulse flows in late summer to facilitate steelhead upstream migration. It is currently assumed that the barriers in the Ash system are still providing selective access to habitats above Dickson Lake and providing a refuge for summer steelhead. Consequently, modifying access in the Ash to allow unrestricted migration of anadromous fish above Dickson Falls, could result in a loss of the distinct summer and winter steelhead populations in the Ash.
Discussions developing fish passage objectives for the Ash with various management agencies and interested groups are constrained by the inability to address questions of this nature. As a result it is

almost impossible to base decisions on data as opposed to assumption. To help address the lack of information of the status of the winter and summer steelhead populations in the Ash River, we examined the genetic variability of the summer and winter steelhead populations, to assess the degree to which these have managed to remain distinct.

MATERIALS AND METHODS

Study Area

The Ash River Watershed comprises two basins in central Vancouver Island, located approximately 40 km northwest of Port Alberni (Figure 1). The entire watershed covers 379.5 km^2 predominately lying within the Coastal Western Hemlock Bioclimatic zone (approximately 80%; Environment Canada 2005). The upper Ash sub-basin is located in the boundary region of the Ash, Campbell and Courtenay watersheds, which are separated by mountains in excess of 2,000 m high. The sub-basin is predominately old growth forest and approximately 30% of its drainage enters Oshinow Lake, at an elevation of 410 m. From Oshinow Lake, the Ash flows for 13.7 km through less steep terrain, to Elsie Lake Reservoir, at an elevation of 330.7 m. The Reservoir was created as part of the 1958 Ash River project, to divert flows to a generating station on the north shore of Great Central Lake. The Elsie Dam was constructed in 1957, increasing the lake area to 672 hectares by flooding 401 hectares of land. It averages 19 metres in height is 185 metres in length and permits a storage capacity of 77x10^6 cubic metres (BC Hydro 2000). A 4 km tunnel and a 3.4 km long penstock lead from the Elsie Lake Reservoir to the powerhouse (Lewis and Ganshorn 2005).

The Ash River flows into the Stamp River, which, in turn, joins the Somass River before discharging into Alberni Inlet. The Ash River between Elsie Dam and Dickson Lake inlet, 10.7km downstream, is considered to be the Middle Ash River (Ash River Water Use Plan Consultative Committee 2003). The Lower Ash River comprises the 12.4km section from the outlet of Dickson Lake to the confluence of the Ash and Stamp rivers.

The Stamp/Somass system produces all five species of anadromous Pacific salmon, as well as anadromous Pacific Lamprey (*Lampetra tridentata*) and steelhead (*O. mykiss*). These utilize the Ash River to varying degrees with steelhead and Pacific lamprey moving furthest into the system. The latter historically migrated into Elsie Lake prior to impoundment, possibly utilizing areas of the Upper Ash watershed (Beamish and Northcote 1989). Currently, this species cannot access Elsie Lake.

Utilization of fish habitat in the Ash River is defined by three barriers that present varying degrees of difficulty to fish passage. The first is Lanterman Falls, a 4.2m high stepped bedrock shelf, located 5.5km above the confluence with the Stamp River. This is currently considered to be a barrier to salmonids except for summer steelhead and coho (Burt and Lewis 2004), but Horncastle (1977) mistakenly indicated that coho were also unable to ascend: a report in the defunct Fish Database (FWD 2005) of chinook above the falls was not confirmed in the literature by Lewis and Ganshorn (2006).

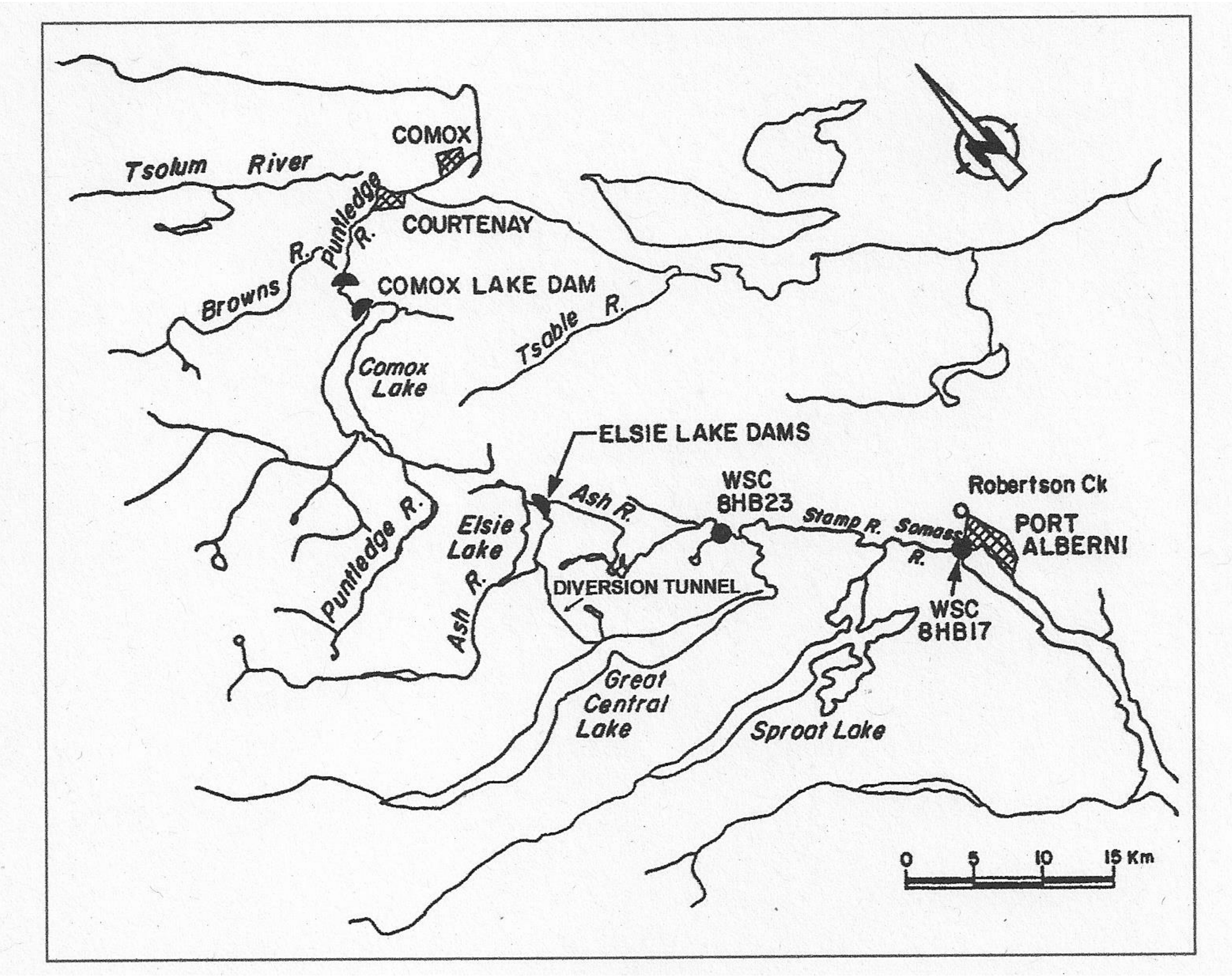

Figure 1. Location of the Ash River in relation to Somass River and Stamp River.

At ~10km above the confluence, Dickson Falls is a 9.2m high barrier, consisting of 7 discrete steps, the highest presenting a 3.2m rise (Griffith 1993). This is a more controversial area initially thought to preclude all anadromous passage (citations in Griffiths 1993 and Lewis and Ganshorn 2005). However Hryhorczuk and Silvestri (2002) noted that summer steelhead could ascend Dickson Falls. Following blasting by BC Fish and Wildlife Branch in 1977 and 1978, access was improved for summer steelhead as well as, potentially, for coho: juvenile coho were captured above Dickson in 2005. The Ash River Project Water Use Plan (BC Hydro 2004) makes provision for water releases from Elsie Reservoir to support fish habitat. Updated minimum flows agreed to in the WUP are 3.5 m^3/s from May 1 to October 31; 5 m^3/s from November 1 to April 30; and two separate pulse flows for adult steelhead migration, of 10 m^3/s for two days between August 1 and September 30, to correspond to naturally increasing flows from precipitation. The post impoundment Ash River discharge, is likely to have impacted on anadromous access to the middle watershed, however, the WUP flow regime has improved passage, at least for summer steelhead, over both Lanterman Falls and Dickson Falls.

Finally, Elsie Dam has no provision for fish access to the reservoir, due to the perceived barrier effect of Dickson Falls, and prevents all anadromous access to Elsie Lake reservoir.

An additional two barriers of lesser significance were reviewed by Lewis and Ganshorn (2006): Ash Mouth Riffle, passable to most species and Ash Island Falls, located upstream of Dickson Falls. Consensus on the latter section of river is that it can be ascended by summer steelhead.

Steelhead trout are found throughout the system, including above Lanterman and Dickson falls, to the Elsie Dam site. These are primarily summer run fish that enter the system from late May, usually peaking in abundance in July (M. McCulloch Ministry of Environment pers. comm.). Winter-run steelhead appear not utilize the Ash River past the lower 1-2 km (Griffith 1993), or potentially as far upstream as Lanterman Falls (Burt and Lewis 2004). Numbers of summer-run fish fluctuate annually, but average around 200 – 500 wild fish with a further 500-1,000 hatchery fish in the middle Ash River (M. McCulloch Ministry of Environment pers. comm.). The wild contribution to the Stamp/Somass and lower Ash from winter-run steelhead is similar, augmented by around 1,000 hatchery fish.

Samples for genetic analysis

The general methodology of the study followed that of Beacham et al. (2004), employing microsatellite genetic markers to assess variation in Ash River steelhead populations. Genetic material for analysis consisted of:

i. Tissue samples (caudal punch) from summer-run steelhead adults collected from holding and rearing locations above Dickson Falls;
ii. fresh tissue samples from adult steelhead sampled at the Robertson Creek hatchery and assessed as summer or winter fish by condition, as well as from the Stamp River; and
iii. historical tissue samples (scales) collected from winter-run fish collected prior to initiation of the hatchery program.

We constructed a dataset from 800 contemporary and historical samples from the Ash and the Stamp Somass system. The usable samples from this collection consisted of 61 Ash summer-run adults; although the sample size was lower than hoped for, we deliberately refrained from collecting juveniles to avoid non-representative sampling through sibling bias (Hansen et al. 1997). Historical scale samples also provided:

- 200 Robertson hatchery summer adults;
- 15 Stamp River late summer adults
- 29 Stamp /Ash River wild summer adults scale samples recently collected from the upper Stamp River; and
- 77 Stamp River wild winter-run adults.

The data for the first analytical run included 49 historical Robertson adult scale samples archived by Fisheries and Oceans Canada (FOC) as well as 44 Nahmint samples to act as an out-population control.

Laboratory analysis

Contemporary tissue samples preserved in 95% ethanol and historical samples consisting of dried scales or other tissue in paper envelopes were sent to the Molecular Genetics Laboratory at the Nanaimo-based Pacific Biological Station of FOC. Multilocus genotypes for each sample were determined using the current suite of 14 microsatellite genetic markers (ogo4, oke4, oki10, omm1008, omm1037, omm1276, omm5140, omy325, one111, one114, ots1, ots2, ots9 and ssa408) similar to method used by Beacham et al. (2004) to survey Steelhead nuclear DNA with an ABI 3730 capillary DNA sequencer, and genotypes scored by GeneMapper software 3.0 (Applied Biosystems, Foster City, CA).

Data analysis

We used a number of analyses to identify genetic population structure across all of the initial samples analysed by FOC. Data analysis was performed using both frequency-based as well as genetic distance-based analyses carried out using the Excel© add-in program GenALEx (Peakall and Smouse 2006 and Peakall and Smouse 2012). A majority of descriptive statistics, as well as testing for conformity to Hardy-Weinberg equilibrium (HWE) and population assignment outcomes were constructed with this software. Allelic richness was calculated using Fstat (Goudet 1995 version 2.9.3.2). A proximity matrix of Weir and Cockerham's (1984) θ, an approximation of F_{ST}, pairwise distances was calculated between each set of samples within the drainage using multi-locus genotypes from the microsatellites. We used F_{ST} as a distance measure because it is an unbiased estimator that has been widely used for genetic differentiation between populations (Waples & Gaggiotti 2006). The genetic distance enabled determination of statistically significantly differences among the different sample collections, indicating the level of gene flow or genetic differentiation between sites. For comparison, we also calculated Shannon's diversity index (Shannon 1984) which provides a convenient measure of genetic differentiation among populations and allows the pairwise assessment of group variability using a Mutual Information (${}^{s}H_{ua}$) measure (Sherwin et al. 2006). This relies on allele identity to identify population origin and converts to a log-likelihood G statistic for comparison with the chi-square distribution for statistical significance.

Principal Component Analysis (PCoA) was used to visualize the patterns of genetic differentiation among all samples. The procedure generates multidimensional scaling of the F_{ST} genetic distance matrix in multiple dimensions, with progressively less of the total variance incorporated in successive axes.

A Baysian approach was used to corroborate the frequency-based analyses above, using the software package Structure (Prichard et al. (2000) version 2.3.4). We used an admixture model with informative priors based on the temporal/location information associated with the historical and contemporary samples and assessed values of K from 2 to 5 with 40,000 burn in and 50,000 MCMC iterations. This provided a visual depiction of population structure including the relative contributions of our putative populations to genotype groupings. We also investigated the potential accuracy of assigning five populations by assessing the mean log likelihood of (ln P (D|K) over 2 to 7 populations (K= 2 to 7) with 20 replicates per value of K, in the absence of prior information on potential population structure.

Samples were then clustered by an agglomerative neighbor-joining algorithm (Saitou and Nei 1987) using the program TREEFIT (Kalinowski 2009). TREEVIEW (Page 1996) was used to plot the tree structure from this algorithm.

Sibling assignments over all samples were constructed using a maximum likelihood method implemented through the software program COLONY (described by Wang 2004) and delivered by FOC Molecular Genetics Laboratory. The method apportions codominant genetic markers to infer parental genotypes that may create unrepresentativeness in the genotype groupings in the same manner as potentially resulting from incorporation of juvenile steelhead sampling.

RESULTS AND DISCUSSION

Dataset construction

The first set of analyses using the seven groups of samples listed above, suggested that, while there was obvious separation among putative populations according to the PCoA analysis (Figure 1), genetic distance (F_{ST}) was not indicative of clear-cut differentiation among the groups of greatest interest: Ash Summer, Stamp Winter and Hatchery Summer. The *Fst* statistic has a range of 0 to 1, with the lower value indicative of a panmictic, or freely interbreeding, population, while a value of 1 indicates that the groups have no shared genetic variation. However, the upper bound may be arbitrarily low in highly polymorphic populations and moderate differentiation of steelhead populations can be suggested by values such as 0.066 for Skeena River steelhead populations using 13 microsatellite loci (Beacham 2004).

The seven groups generated an F*st* value of 0.022 (p=0.001) with a theoretical maximum of F*st* =0.201. However, this result was influenced by the Nahmint and Stamp Late summer groups. Selected values of paired F*st* values are shown in Table 1 to contrast these results.

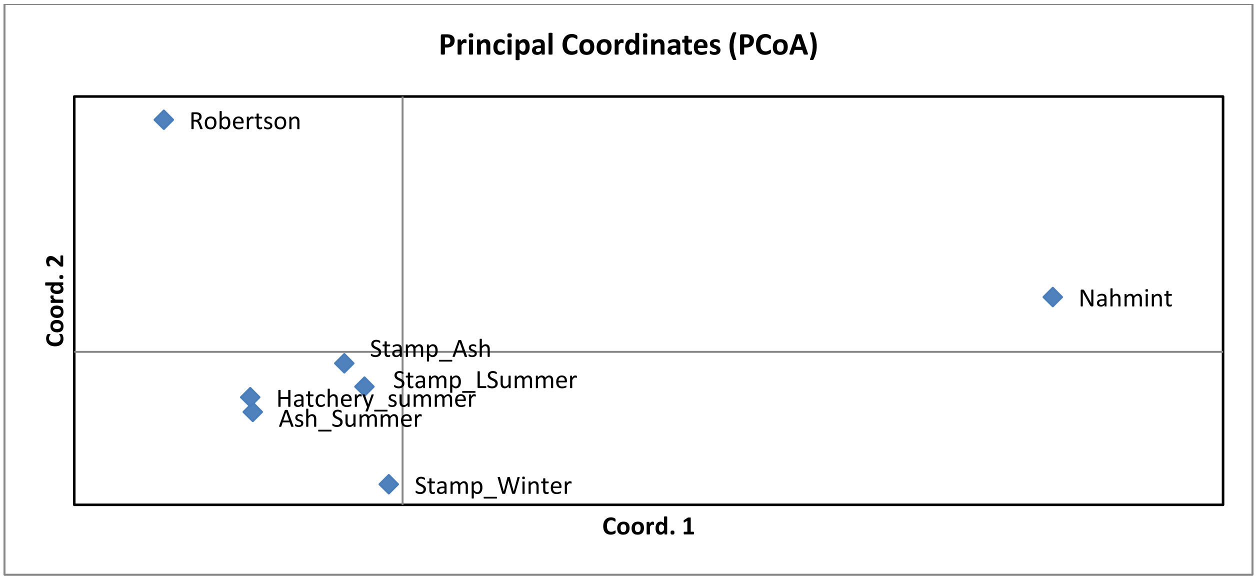

Figure 1. Principal component plot of allele frequency illustrating genetic divergence among the primary groups of Stamp Somass steelhead by migration timing and including Nahmint steelhead as an out-population control.

The distinction between a clearly separate population (Nahmint) and the various Stamp/Somass/Ash groups resulted in the consistently highest F*st* values of 0.041to 0.070 (Table 1) all of which were significant, indicating some degree of genetic differentiation based on population structure. In contrast, the Stamp Late summer group was only significantly different from the Nahmint, Robertson and Ash summer populations, suggesting that these form a single randomly mating genetic population with the Hatchery summers, Stamp Ash and Stamp winter groups.

Table 1. Pairwise values of F*st* for Nahmint and Stamp Late summer groups and probabilities. Non-significant results are indicated in bold type, highlighting lack of genetic differentiation between Stamp Late summer and most other groups.

Group 1	Group 2	Fst	Sample 1	Sample 2	probability
Ash summer	Nahmint	0.059	61	44	0.001
Ash summer	Robertson	0.028	61	49	0.001
Nahmint	Robertson	0.070	44	49	0.001
Ash summer	Hatchery summer	0.012	61	200	0.001
Nahmint	Hatchery summer	0.054	44	200	0.001
Robertson	Hatchery summer	0.019	49	200	0.001
Ash summer	Stamp late summer	0.012	61	15	0.006
Nahmint	Stamp late summer	0.041	44	15	0.001
Robertson	Stamp late summer	0.017	49	15	0.002
Hatchery summer	Stamp late summer	0.000	200	15	**0.469**
Ash summer	Stamp Ash	0.008	61	29	0.003
Nahmint	Stamp Ash	0.043	44	29	0.001
Robertson	Stamp Ash	0.016	49	29	0.002
Hatchery summer	Stamp Ash	0.007	200	29	0.004
Stamp late summer	Stamp Ash	0.003	15	29	**0.194**
Ash summer	Stamp winter	0.020	61	77	0.001
Nahmint	Stamp winter	0.045	44	77	0.001
Robertson	Stamp winter	0.032	49	77	0.001
Hatchery summer	Stamp winter	0.009	200	77	0.001
Stamp late summer	Stamp winter	0.001	15	77	**0.321**
Stamp Ash	Stamp winter	0.005	29	77	0.021

The lack of consistency within the main groups of interest, particularly Stamp winter and Ash summer (Table 1) suggested that a further examination of our samples for accuracy in designation was required and potentially unreliable samples were removed from the dataset. These changes, described below, are summarized in Appendix 1). The specific origins of the Stamp Late Summer samples were unclear and this group was removed. Nahmint samples were also removed, having clearly indicated separation from the Stamp/Somass groups. Additionally, wild Stamp River summer samples that had been omitted from the initial analyses were included. This resulted in five groups of samples for continued analysis. However, the Stamp summer samples included fall-run hybrids as well as late summer fish. These were removed from the main database into a hybrid category that included the Stamp late summers to investigate population assignment through the "leave one out" option in GenALEx.

Finally, the family structure of each group was assessed to determine if strong family structure was present. This is likely to cause non-representativeness for the population and usually the appearance of greater differentiation (John Candy FOC pers. comm.). The Hatchery summer group had substantially greater family structure than the other groups, with 80.5% of individuals involved in 23 inferred parental genotypes. In 8 cases, 5 or more individuals (range 5 to 11) were involved in a sibling grouping with a shared parent. Additionally, a number of family pairs were made across potential population groups, particularly between Ash summers and Stamp summers (23 pairs). Less common were Stamp summer and Stamp Winter combinations (17 pairs). This analysis was used to selectively reduce the number of genotypes, so that families were not overrepresented.

Due to these changes, we have provided summary information on, as well as detailed analyses of, only the genetic variability of the final five presumed populations, below.

Genetic Variation within populations

Summary statistics for parameters of genetic diversity, including the Shannon information index, are provided in Table 2 for means across groups and loci. Number of alleles per locus (mean 12.01 SE 3.13) varied widely between 5 (Robertson) and 24 (Stamp summer) and was significantly different between groups (ANOVA $F_{4,65}$ = 3.029 p = 0.024). Similarly, allelic richness was significantly different across groups (ANOVA $F_{4,65}$ = 2.574 p = 0.046). The Robertson samples had the lowest number of alleles as well as lowest allelic richness (Na = 9.0 Ar = 8.9) Appendix 2. The allelic pattern across groups is illustrated in Figure 2.

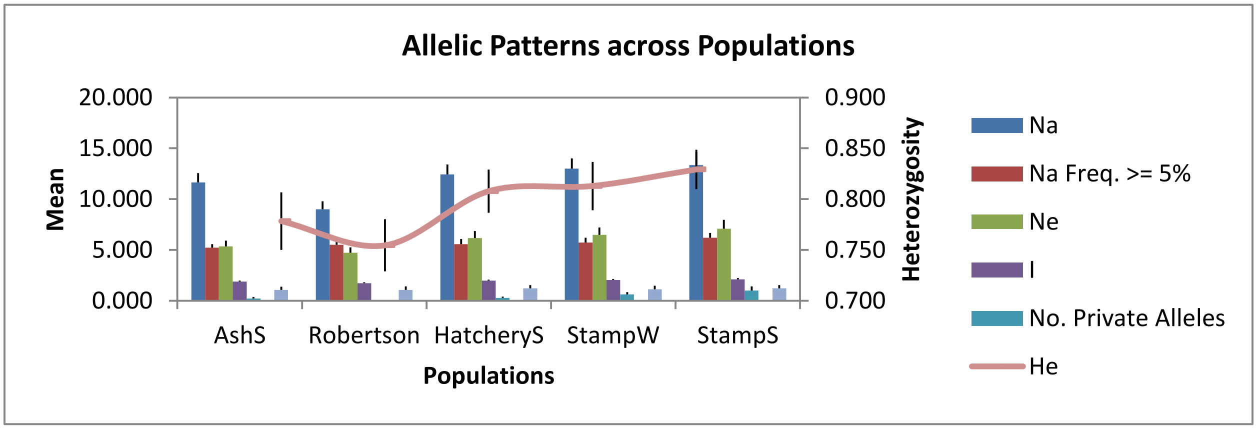

Figure 2. Allelic abundance and expected heterozygosity across presumed populations.

Over all samples statistically significant ($p<0.05$) departure from Hardy-Weinberg equilibrium was found in only 9 of 325 tests over the 5 groups (<3%): Bonferroni corrected probabilities were used to reduce the erosion of the Type I error rate (α=0.05/5 = 0.01). The exceptions occurred at 7 loci, ots1, ots2, oki10, omm1008, oke4, omm1276 and omy325, with 4 occurrences within the Stamp Summer group and 3 in the Robertson groups. We can assume that generally, the observed genotypes are consistent with the premise of random mating. However, the Stamp summer and Robertson groups may incorporate substantial inbreeding and samples may derive from highly related individuals, although this was not noted for either in the family structure analysis.

Table 2. Mean number of samples (N), number of alleles (Na), allelic richness (Ar), number of effective alleles (Ne), Shannon's Information Index (I), and observed (Ho) and expected (He) heterozygosity and standard errors across groups and loci.

Parameter	Mean	SE
N	60.243	1.571
Na	11.886	0.472
Ar	11.269	0.432
Ne	5.961	0.316
I	1.945	0.048
Ho	0.779	0.011
He	0.797	0.011

Population assignments for the five potential populations using the 'leave one out option' are summarised in Table 3. This tests the proportion of individual samples that can be assigned back to their original grouping (self), and identifies the level of contribution of misassigned individuals to other groups (other). The groups with the highest reassignment accuracy to 'self' were Robertson (83.7%) and Ash summer (71.7%), while Stamp summer individuals were allocated to 'other' in more than 88% of cases.

Table 3. Summary of Population Assignment Outcomes to 'Self' or 'Other' population (With Leave One out Option).

Group	Self	Other	% contiguous
Ash Summer	38	15	71.7%
Robertson	41	9	83.7%
Hatchery Summer	37	48	43.5%
Stamp Winter	28	32	46.7%
Stamp Summer	7	54	11.5%
Total	151	157	48.6%
Percent	49%	51%	

Hatchery summers (10.6%) Stamp summers (16.4%) and even Stamp winters (20.0%) were assigned to Ash summers (Appendix 3). Ash summers had the largest percentage of misassignments to Robertson

(11.3%) but also allocated 3 individuals (5.7% in each case) to each of Hatchery summers, Stamp summers and Stamp winters. Only Stamp summers produced fewer accurate assignments to ‘self’ (11.5%) than to another group; Robertson (34.4%) and Stamp winters (27.9%) far exceeded this level.

When the hybrid category was assessed, 28.2% were assigned to ‘self’ but 24.4% were otherwise seen as Hatchery summers and 11.5% were assigned to Ash summers. Stamp summer and winter groups received 10.3% each.

Population differentiation

Overall genetic differentiation assessed through AMOVA is suggestive of relatively low, but significant differentiation among groups (F*st* = 0.014 p=0.001 F*st* maximum 0.187). Molecular variance was almost exclusively contained within individual samples (93%), with only 6% due to among individuals and 1% among groups. Significant genetic differentiation was found at all but one of the 14 loci sampled (Table 4), however, F*st* values were considerably smaller than recorded for Skeena and Nass steelhead by Beacham et al. (2000): mean 0.026 Skeena and 0.024 Nass compared with 0.014 in this study.

Table 4. Mean number of alleles (Na) per locus over 5 groups with the standard error, genetic differentiation (F*st*) and significance level. Non-significant results are indicated in bold type.

Locus	mean Na	SE Na	Fst	Probability
ogo4	9.0	1.049	0.012	0.006
oke4	8.4	0.510	0.016	0.001
oki10	11.6	1.568	0.012	0.001
omm1008	12.4	0.927	0.028	0.001
omm1037	16.0	0.837	0.018	0.001
omm1276	8.8	0.860	0.015	0.001
omm5140	6.8	0.200	0.008	0.011
omy325	17.4	2.249	0.010	0.001
one111	8.0	0.775	0.002	**0.231**
one114	15.6	0.678	0.012	0.001
ots1	10.8	0.490	0.013	0.002
ots2	15.6	1.122	0.008	0.004
ots9	11.6	0.400	0.025	0.001
ssa408	14.4	1.208	0.011	0.001
Total			0.014	0.001

Similarly, pairwise comparisons suggest that none of the groups are genetically similar. The comparison of Robertson with Ash summer and with Stamp winter groups (Table 5) indicated the greatest likelihood of distinct populations (F*st* = 0.029 and 0.032 p=0.001). In contrast Stamp summer and Hatchery summer approach the conventional probability level (α =0.05) for significance. These results complement the original findings in Table 1, excluding the Stamp Late summer group, where population structure can be assumed among the five selected groups from the Stamp/Somass system.

Table 5. Pairwise matrix of F*st* values over all loci (below diagonal) and level of significance (above diagonal).

Group	Ash summer	Robertson	Hatchery summer	Stamp winter	Stamp summer
Ash summer	-	0.001	0.001	0.001	0.001
Robertson	0.029	-	0.001	0.001	0.001
Hatchery summer	0.014	0.016	-	0.001	0.042
Stamp winter	0.021	0.032	0.007	-	0.027
Stamp summer	0.012	0.018	0.001	0.002	-

We compared the degree of between group variability above, with that found using Shannon's Mutual Information (${}^{s}H_{ua}$) measure (Table 6). The primary difference was the higher degree of separation Hatchery summer and Stamp summer groups (p=0.003) unlike the F_{st} value above. The pairing of Robertson and Stamp winter consistently showed the most genetic differentiation (${}^{s}H_{ua}$ =0.104) followed by the comparison of Robertson with Ash summer (${}^{s}H_{ua}$ =0.086). However, the Shannon method places proportionally greater weight on the degree of separation between the Stamp summer and winter groups, as well as the Hatchery summers with both of those two groups (F*st* range = 0.001 to 0.007, ${}^{s}H_{ua}$ range = 0.035 to 0.045), versus the maximum values for these parameters (0.032 and 0.104 respectively).

Table 6. Pairwise matrix of ${}^{S}H_{ua}$ values over all loci (below diagonal) and level of significance (above diagonal).

Group	Ash summer	Robertson	Hatchery summer	Stamp winter	Stamp summer
Ash summer	-	0.001	0.001	0.001	0.001
Robertson	0.086	-	0.001	0.001	0.001
Hatchery summer	0.057	0.056	-	0.001	0.003
Stamp winter	0.070	0.104	0.045	-	0.002
Stamp summer	0.058	0.078	0.035	0.043	-

The above results are more clearly visualized through PCoA (Figure 3). The greatest degree of genetic differentiation belongs to the Ash summer and Robertson groups, with more variation between the other three groups than was displayed in Figure 1 for the original dataset. .

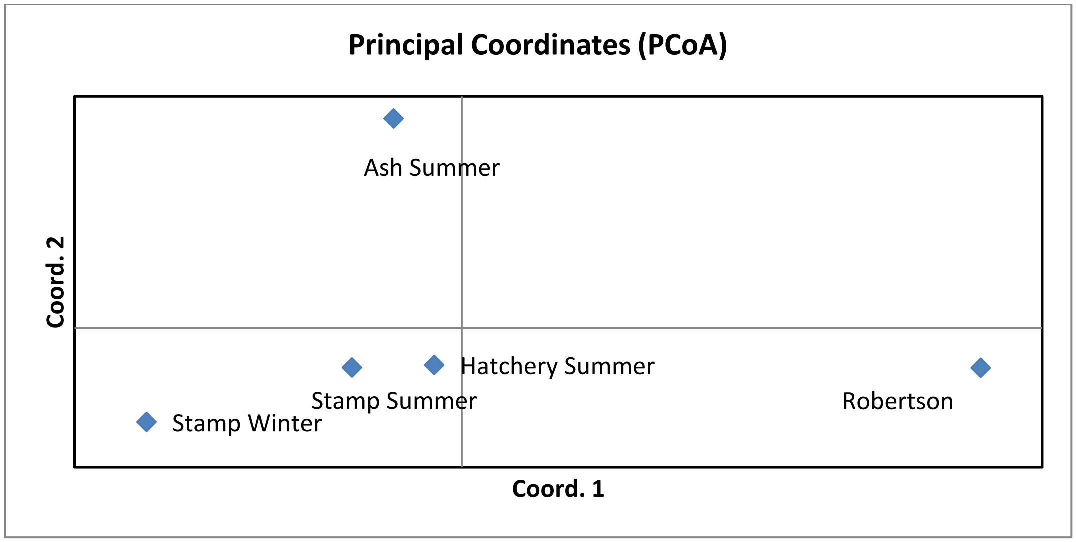

Figure 3. Principal component plot of allele frequency illustrating genetic divergence among the primary groups of Stamp Somass steelhead.

The constructed neighbour-joining tree is shown in Figure 4 with a visual depiction of the composition of population groupings (clusters) based on genotype origin. While the fit to the observed genetic distances was good ($R^2 = 0.955$), branch distances were very small. Interior branch length separating the Ash summer and Robertson groups from the Stamp summer, winter and Hatchery summer groups was .0024. That between the Ash, Robertson and Hatchery summer groups and the other two was 0.0036. Bootstrap support (1,000 iterations) for the former was lower (0.56) than the latter (0.95), suggesting that Hatchery summers group more closely with Ash summers than the population structure in Figure 4 depicts.

The apportioning of populations into inferred clusters using Structure, illustrates the lack of cohesion within the five putative populations, shown earlier through the 'leave one out' analysis. However, differences were found between the Bayes estimate of proportional membership within the clusters (Table 7) and that found in the earlier allocation analysis detailed in Appendix 2. The Robertson group was unchanged (84%), while the Ash summer group was less well represented within the cluster that it dominated (cluster 5, 52%) than suggested in Table 3 (72%). Stamp winters increases in assignment to 'self' from 44% to 54%, and were proportionally better represented in the Robertson dominated cluster (cluster 3, 33%) than shown in Appendix 3 (17.6%). In contrast to the earlier assignment of Stamp summers to Robertson (34% Appendix 3) the Bayes assignments reduced this to 22% while increasing the contribution to Stamp winters (cluster 1) to 51%.

It should be noted that the clusters illustrated in the Figure 4 inset do not reflect the numerical composition of the groups, so that a small percentage of movement from a specific genotype can appear as a large block of colour if the receiving cluster has few individual members. For example, cluster 4 (yellow) has only 16 members, and is dominated by Hatchery summers (12 individuals); the number of individuals involved in genotype allocations across clusters is provided in Appendix 4 for reference. None of the other populations contributed more than 4% to this cluster.

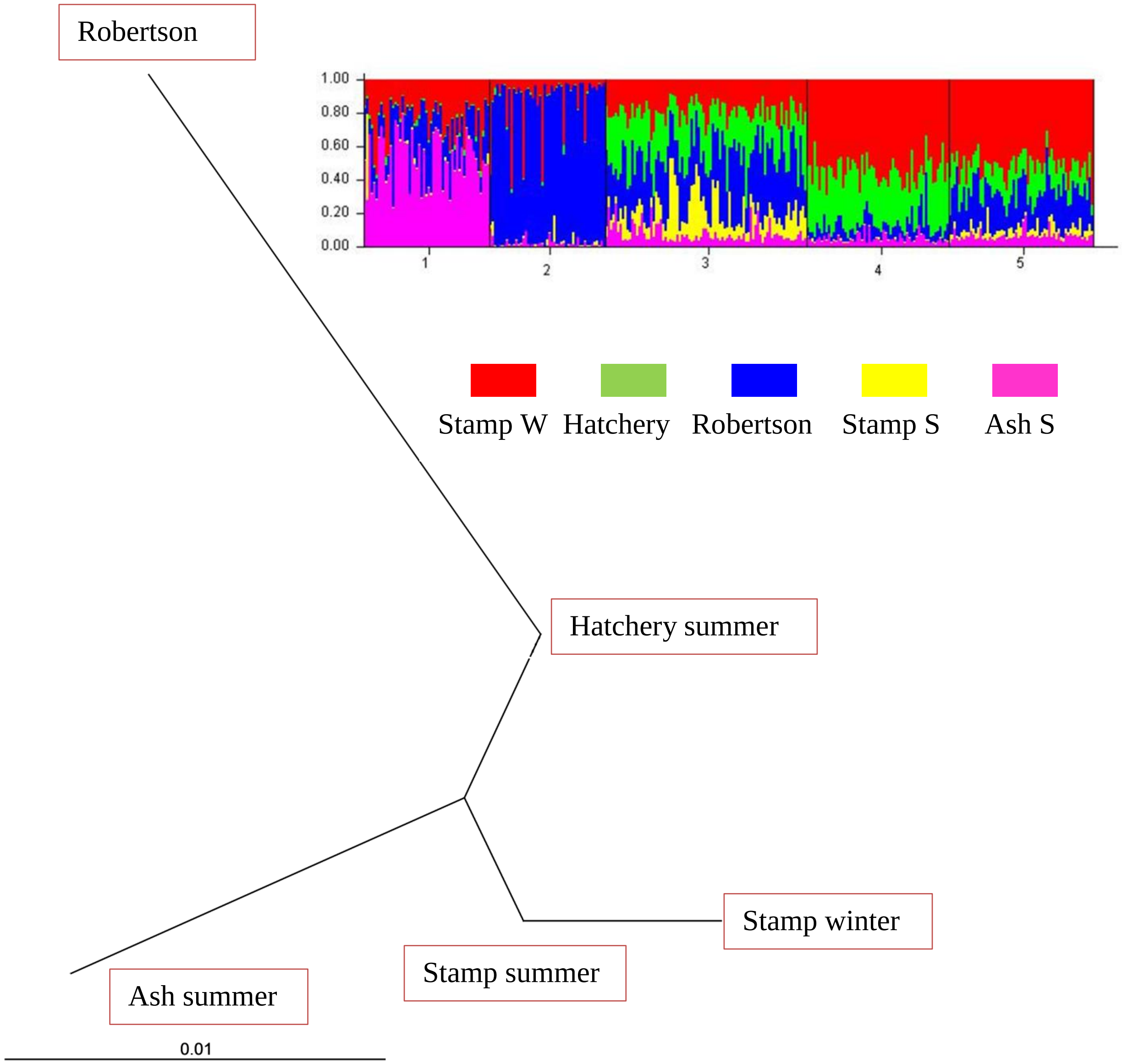

Figure 4. Neighbour-joining tree structure of five Stamp/Somass steelhead groups based on Weir and Cockerham's (1984) theta. Branch length is approximately to scale. The colour inset illustrates the percentages of each genetic identity originating from each of the populations, labelled 1 through 5, to form inferred clusters. The colour blocks identify the order of five clusters from left to right and the relative contributions to each from the five populations.

Table 7. Proportional composition of each cluster derived from the 5 putative populations.

	CLUSTER					
	1	2	3	4	5	# samples
Ash summer	0.236	0.015	0.209	0.019	0.521	53
Robertson	0.117	0.006	0.842	0.012	0.023	49
Hatchery summer	0.191	0.258	0.331	0.139	0.081	85
Stamp winter	0.544	0.322	0.078	0.005	0.051	60
Stamp summer	0.511	0.156	0.224	0.042	0.068	61

If the five potential populations demonstrated no discernable geneflow, then the inset in Figure 4 would show solid blocks of individual colours and each cluster would correspond to a single population. The Ash summer and Robertson groups provide greatest stability in terms of geneflow, but the overall contributions from other groups is substantial: although Ash summers dominate cluster 5 (magenta) they represent 64% of the total individuals, while Hatchery summers contribute 16% of the Ash cluster (Appendix 4).

Generally, however the tree population structure and proportional geneflow among clusters reflect greater genetic differentiation in the Ash and Robertson samples based on incorporation of an informative prior (identified population groupings) in the Bayesian algorithm. In the absence of an integrated model statement on genetic structure, likelihood estimation points towards only three probable populations within the data (Figure 5).

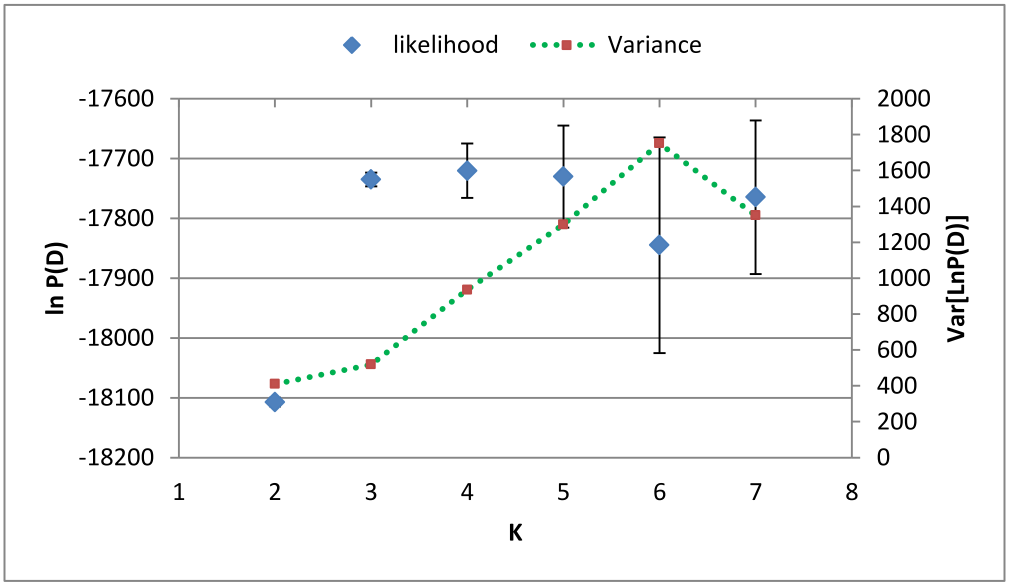

Figure 5. Mean log likelihoods, with standard deviations, from 20 runs of each of 6 population level values (K) between 2 and 7. Associated uncertainty is depicted by the likelihood variances.

The likelihood estimates produce an early, rapid, approach to a plateau, with notable increases in variance associated with increases in the potential number of populations (K). Assessing the potential for 3 or 4 populations the fit to the model increases only slightly, but with substantial increase in variance and hence uncertainty.

Similarly, using the Evanno et al. (2005) delta method (mean (|L”(K)|)/sd(L(K))) the large peak in Figure 6 also suggests that three populations provide the best fit to the data. The proportional geneflow contributions to three clusters derived from the original five putative populations is shown in Table 8, with the accompanying numerical structure of each cluster.

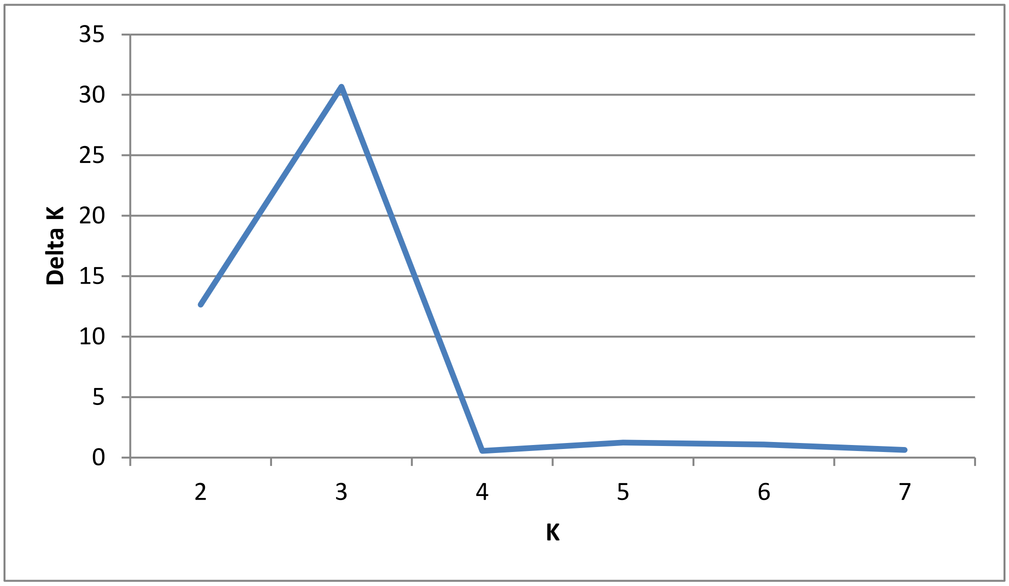

Figure 6. The magnitude of the second order rate of change of the likelihood ΔK as a function of mean K (± sd) over 20 replicates.

Cluster 1 now contains 171 individuals of which 88% derive from Hatchery and Stamp summers and Stamp winters. Cluster 2 is primarily Ash summers (77% of a total of 35 individuals) due to the minor contributions from the other populations. However, almost 50% of our original Ash summer population has now been split into the other 2 clusters. Finally, cluster 3 is 42% Robertson individuals, representing 85% of our original Robertson population, but with a significant contribution from hatchery summers (31%, representing 37% of the original sample designations).

Table 8. Proportional composition of 3 inferred clusters derived from the 5 putative populations. The numerical distribution of individuals to each cluster is shown below the associated percentage division.

	CLUSTER			
	1	2	3	# samples
Ash summer %	25.0	51.4	23.6	53
individuals	13	27	13	
Robertson %	14.0	1.10	84.8	49
individuals	7	1	42	
Hatchery summer %	59.2	3.40	37.3	85
individuals	50	3	32	
Stamp winter %	92.6	1.70	5.70	60
individuals	56	1	3	
Stamp summer %	73.3	5.00	21.7	61
individuals	45	3	13	
Totals	171	35	102	

CONCLUSIONS

We found reasonable agreement between the frequency based (F*st*, Shannon) results and the distance based (AMPOVA, PCoA) depictions of genetic differentiation between the five groups, suggesting some degree of population structure. However, while statistically significant, the level at which groups can be separated among our data suffers from genotype integration across almost all potential populations. It may not be unreasonable to suggest that the Stamp groups, summer and winter, are less divergent from each other than the other groups and may have some genetic association with hatchery releases, since Stamp fish are likely to be routinely included in hatchery brood stocks. However, at least in larger drainages, hatchery operations do not necessarily have a noticeable effect on wild populations as indicated by the genetic differentiation between upper (wild) and lower (hatchery) mainstem Kitimat River populations (Heggenes et al. 2007). In the case of the Stamp/Somass, if we resolve the identified genotypes into 3 clusters, only 16 of 61 samples of the Stamp summer group are grouped outside of the predominately hatchery dominated cluster. Similarly, 56 out of 60 Stamp winters (93%) were clustered with Hatchery summers and Stamp summers. The Robertson samples represent a historical record that appears to be contemporary with early hatchery operations. As such they group with Hatchery summers and have a lower affiliation with the Stamp and Ash summers, and even less with Stamp winters.

While geographical differentiation of steelhead populations within stocks in British Columbia and the West Coast of the United States has been successfully demonstrated in a number of studies (Beacham et al. 2004,), little success has been achieved in attempting to find consistent genetic differences between run-types within a region *(*NOAA-NWFSC Tech Memo-19). Kassler et al. (2011) found that natural origin steelhead populations on the Olympic Peninsula were genetically indistinguishable, while Reisenbichler and Phelps (1989) ascribed non-significant genetic variation within and among drainages in northwestern Washington to gene flow from historical hatchery stocks. Exceptions to these studies include the Nass River where summer and winter run steelhead were found to be genetically distinct (Beacham et al. 2000): it should be noted that in comparison to the Stamp/Somass, the Nass is 380km long and drains approximately 21,000km^2. Similarly, within a smaller Columbia River system, Van Doornik et al. (2015) were able to separate Willamette steelhead between winter and summer runs on the basis of tributary location. They also found a lack of genetic introgression from non-native run-types although the potential appeared to exist to allow for gene flow.

It appears that summer-run and other temporally distinct escapement classes may be genetically indistinguishable in some systems, particularly in situations lacking clear evidence of reproductive isolation. Possibly, access to the middle Ash River by other than summer run steelhead has occurred. Similarly, the degree of geographical separation between the Ash and the adjacent lower Stamp/Somass, in comparison with the Nass degree of population separation, suggests that strong genetic divergence between behaviourally distinguishable groups is lacking.

The most probable division of our data into 3 populations suggests that almost 50% of the designated Ash summers cluster with other, hatchery influenced, groups. Sample integrity may play a role in the relatively poor level of discrimination among potential populations in our data. In particular, the degree to which Ash summer steelhead samples are apportioned to the Stamp winter and Robertson clusters suggest that our sampling may not reflect exclusively summer-run steelhead. Persistent warm dry conditions delayed the initiation of sampling until 2 October 2014 and sampling was not concluded until 6 January 2015. It is possible that some fall/winter fish as well as hatchery stock were included in the DNA samples, since we know that these fish utilize the Ash River as far upstream as Lanterman Falls and potentially have the ability to move further into the system due to the flow regime under the Ash River Project Water Use Plan.

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APPENDIX **1.** Summary of removal of samples from the final dataset for analysis.

Sample type	No. analysed	No. retained	Sample attrition due to
Ash summer	61	53	Class1, Class 3, Class 6
Robertson	49	49	historical samples DFO
Nahmint	44	0	historical samples DFO Class 2
Hatchery summer	200	85	Class 4
Stamp winter	77	60	Class 5
Stamp summer	84	61	Class 1, Class 6
Stamp Ash	29	0	Class 5
Stamp late summer	15	0	Class 5
Fall hybrids	33	0	Class 6
Totals	592	308	

Class1	no condition recorded
CLass2	control dataset geographically distinct
Class3	some family structure
Class4	strong family structure
Class5	no origin recorded
Class6	possible CTT hybrid

APPENDIX 2. Summary of various estimates of genetic diversity incorporating 14 microsatellite loci over 5 putative populations of steelhead from the Stamp/Somass river system: number of samples (N), number of alleles (Na), allelic richness (Ar), number of effective alleles (Ne), Shannon information index (I), observed heterozygosity (Ho) and expected heterozygosity (He).

Population		ogo4	oke4	oki10	omm1008	omm1037	omm1276	omm5140	omy325	one111	one114	ots1	ots2	ots9	ssa408	Mean
Ash Summer	**N**	53	53	53	53	53	53	53	52	52	53	52	53	53	53	52.8
	Na	7	9	11	12	18	10	7	14	9	14	10	17	11	14	11.6
	Ar	6.49	8.803	10.315	11.628	16.948	9.486	7	13.644	8.516	13.797	9.381	15.725	10.147	13.431	11.094
	Ne	2.095	6.120	2.625	5.057	8.751	4.994	4.761	5.333	3.694	8.974	3.191	6.793	5.103	7.531	5.359
	I	1.066	1.951	1.473	1.967	2.439	1.817	1.718	2.125	1.563	2.383	1.476	2.216	1.819	2.230	1.875
	Ho	0.604	0.792	0.642	0.698	0.849	0.811	0.755	0.827	0.769	0.962	0.673	0.811	0.774	0.906	0.777
	He	0.523	0.837	0.619	0.802	0.886	0.800	0.790	0.813	0.729	0.889	0.687	0.853	0.804	0.867	0.778
Robertson	**N**	47	49	49	45	48	48	44	45	49	46	48	45	47	45	46.8
	Na	6	7	6	9	13	6	6	11	5	14	10	12	11	10	9.0
	Ar	5.936	6.991	5.796	8.978	12.749	5.917	6	10.978	5	13.952	9.904	11.933	10.798	9.956	8.921
	Ne	2.765	4.318	2.375	4.897	6.669	3.423	3.872	6.853	3.717	9.200	3.962	5.688	2.529	5.853	4.723
	I	1.247	1.650	1.129	1.840	2.138	1.402	1.531	2.140	1.450	2.379	1.720	1.970	1.440	1.971	1.715
	Ho	0.638	0.735	0.653	0.733	0.833	0.729	0.795	0.800	0.776	0.826	0.604	0.644	0.617	0.911	0.735
	He	0.638	0.768	0.579	0.796	0.850	0.708	0.742	0.854	0.731	0.891	0.748	0.824	0.605	0.829	0.754
Hatchery Summer	**N**	85	85	85	84	85	84	85	85	84	84	84	85	85	84	84.6
	Na	11	8	12	13	16	9	7	19	8	17	12	14	13	15	12.4
	Ar	9.134	7.923	10.406	12.547	14.413	8.024	6.495	15.862	7.247	16.072	11.519	12.584	11.838	13.506	11.255
	Ne	3.418	5.562	2.965	8.486	9.122	5.062	3.581	8.196	3.484	11.969	4.654	5.739	5.862	8.050	6.154
	I	1.543	1.842	1.596	2.299	2.415	1.753	1.472	2.410	1.497	2.611	1.884	2.071	2.076	2.294	1.983
	Ho	0.706	0.788	0.506	0.881	0.941	0.750	0.824	0.812	0.726	0.940	0.774	0.835	0.824	0.893	0.800
	He	0.707	0.820	0.663	0.882	0.890	0.802	0.721	0.878	0.713	0.916	0.785	0.826	0.829	0.876	0.808

APPENDIX 2 Cont'd

Population		ogo4	oke4	oki10	omm1008	omm1037	omm1276	omm5140	omy325	one111	one114	ots1	ots2	ots9	ssa408	Mean
Stamp	**N**	60	58	60	58	58	50	55	60	60	60	55	59	56	56	57.5
Winter	**Na**	10	8	14	14	17	11	7	19	9	17	12	17	11	16	13.0
	Ar	9.121	7.753	12.753	13.447	16.61	10.625	6.761	18.072	8.679	15.951	11.762	16.019	10.766	15.256	12.398
	Ne	3.568	4.512	2.512	9.489	11.084	5.741	4.017	8.571	3.850	9.613	4.501	8.191	6.089	8.973	6.479
	I	1.560	1.697	1.547	2.388	2.587	1.939	1.566	2.487	1.638	2.461	1.895	2.390	2.033	2.410	2.043
	Ho	0.683	0.759	0.633	0.724	0.897	0.820	0.745	0.850	0.700	0.917	0.764	0.864	0.839	0.911	0.793
	He	0.720	0.778	0.602	0.895	0.910	0.826	0.751	0.883	0.740	0.896	0.778	0.878	0.836	0.889	0.813
Stamp	**N**	61	61	61	61	61	60	57	61	61	61	55	60	57	57	59.6
Summer	**Na**	11	10	15	14	16	8	7	24	9	16	10	18	12	17	13.4
	Ar	9.936	9.772	13.941	13.599	15.269	7.646	7	22.079	8.436	15.579	9.92	16.469	11.52	16.33	12.678
	Ne	3.836	5.778	3.599	9.154	10.896	4.423	4.500	11.984	3.944	12.003	4.384	8.295	6.045	10.447	7.092
	I	1.658	1.944	1.878	2.388	2.526	1.645	1.698	2.790	1.652	2.588	1.801	2.391	2.073	2.528	2.111
	Ho	0.738	0.738	0.705	0.869	0.918	0.767	0.754	0.902	0.656	0.852	0.655	0.867	0.789	0.860	0.791
	He	0.739	0.827	0.722	0.891	0.908	0.774	0.778	0.917	0.746	0.917	0.772	0.879	0.835	0.904	0.829

APPENDIX 3. Proportion of assignments to self or other among putative populations based on 'leave one out'

Sample	Assigned to	Count	% of assignments
Ash summer	Ash summer	38	71.7%
Ash summer	Robertson	6	11.3%
Ash summer	Hatchery summer	3	5.7%
Ash summer	Stamp summer	3	5.7%
Ash summer	Stamp winter	3	5.7%
Robertson	Robertson	41	83.7%
Robertson	Ash summer	1	2.0%
Robertson	Hatchery summer	4	8.2%
Robertson	Stamp summer	1	2.0%
Robertson	Stamp winter	2	4.1%
Hatchery summer	Hatchery summer	37	43.5%
Hatchery summer	Ash summer	9	10.6%
Hatchery summer	Robertson	15	17.6%
Hatchery summer	Stamp summer	13	15.3%
Hatchery summer	Stamp winter	11	12.9%
Stamp winter	Stamp winter	28	46.7%
Stamp winter	Hatchery summer	5	8.3%
Stamp winter	Robertson	6	10.0%
Stamp winter	Stamp summer	9	15.0%
Stamp winter	Ash summer	12	20.0%
Stamp summer	Stamp summer	7	11.5%
Stamp summer	Ash summer	10	16.4%
Stamp summer	Hatchery summer	6	9.8%
Stamp summer	Robertson	21	34.4%
Stamp summer	Stamp winter	17	27.9%

APPENDIX 4. Numbers of individuals derived from each group that contribute to the constructed clusters.

	CLUSTER					
	1	2	3	4	5	# samples
Ash summer	13	1	11	1	28	53
Robertson	6	0	41	1	1	49
Hatchery summer	16	22	28	12	7	85
Stamp winter	33	19	5	0	3	60
Stamp summer	31	10	14	3	4	61
Totals	98	52	99	16	43	