HCTF project report:

Microsatellite diversity assessment of steelhead (*Oncorhynchus mykiss*) population structure before and after hatchery operation in the Kitimat River, Skeena Region, British Columbia

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ABSTRACT

Allelic variation at 10 microsatellite loci was assayed in steelhead trout (*Oncorhynchus mykiss*) scale samples (n=333) collected in various years 1976-2003, from the lower mainstem Kitimat River, British Columbia. The objective was to investigate the genetic structure of natural steelhead populations in the Kitimat River before hatchery stocking started in 1984 (baseline samples 1976-1977, 1983-1984, n=145), and to assess potential genetic impact of in-river interbreeding of returning cultured adult fish with wild spawners over almost 20 years of large-scale hatchery operation (1987-2003, n=188). Adipose fins were clipped from all annually stocked parr, mean 50297 (±SD 8109), varying 34420-64297 among years. Annual target number of unclipped brood stock adults used for hatchery production was 40 (20 of each sex), varying 9-39 among years. The overall expected heterozygosity and average number of alleles for all Kitimat samples per locus were 0.60 and 11.5, respectively. These results are comparable with other salmonid species in British Columbia including steelhead and resident rainbow trout. The level of population subdivision (θ) among all Kitimat River samples (14 different year classes) was relatively low, 0.005, although significantly different from 0. All pairwise tests for genetic differentiation (F_{st}) were, however, non-significant, suggesting year classes did not differ much based on genetic distance. Pooled comparisons for pre and post hatchery operation θ also were not significantly different from each other. Data, however, suggested a slight, but significant reduction in allelic richness after hatchery stocking. When Kitimat River data were compared with data collected from 13 other *O. mykiss* populations from different waters in British Columbia, the total level of population subdivision was much higher, $\theta = 0.23$. Also, spatial ordination of Kitimat River steelhead and other steelhead and rainbow trout samples within British Columbia, using principal component analysis on microsatellite allele frequencies, indicated differences between coastal and inland populations. Groupings were based on geographic proximity, but we found no particular differences among the various Kitimat River samples before and after hatchery implementation. A Neighbour-joining tree, based on Cavalli-Sforza genetic distance, also demonstrated similar results. We conclude that for the current management regime there is little indication that hatchery practices of lower mainstem Kitimat River steelhead have until now had major genetic effects through genetic drift, but there is some indication that may be a reduction in allelic richness over the period of hatchery production. Substantial number of wild fish and multiple year classes in the mixed spawning population

buffer against genetic drift. Genetic data also suggested there may be more than one population inhabiting Kitimat River. Potential wild populations in the upper mainstem or in the Kitimat River tributaries, and unaffected by hatchery operations, have, however, not yet been investigated.

INTRODUCTION

Releases of cultured fish into wild populations and their subsequent interbreeding may have genetic effects on natural fish population, but the effects can be complex and unpredictable (Hindar *et al.* 1991, Kostow *et al.* 2003, Utter 1998, Williams *et al.* 1996). The ecological basis for the wide range of outcomes - from no detectable effect to complete displacement (see review by Hindar *et al.* 1991) - is not always clear. Introduced non-native fish may reproduce less successfully, presumably because they are not well adapted to the new, local environments (e.g. Adkinson 1995, Chilcote *et al.* 1986, Chilcote 2003, Kostow *et al.* 2003). In the instances where genetic effects on performance traits have been documented, they mostly appear to be negative and tied to the genetic introgression of non-native and/or hatchery reared fish with the wild stocks (e.g. Reisenbichler & Rubin 1999, Chilcote *et al.* 1986, Kostow *et al.* 2003). Therefore, the precautionary principle (e.g. Heywood 1995) implies care with respect to the management strategy of stocking fish into wild populations. Conservation-based fish hatchery programmes ensure that the brood stock comes from the local (wild) populations - i.e. they presumably have the same genetic constitution as the wild fish - and are collected annually in certain numbers to guard against genetic drift (Adkinson 1995, Caughley & Gunn 1996). For the same purpose, the cultured juveniles may be released in small numbers relative to the size of the wild population. Because of the concern to conserve the population structures and diversity of wild fish stocks, among other things, in the face of demand for hatchery production, an understanding of management regimes which do not result in genetic drift or artificial selection following introduction of animals in natural populations, under particular ecological conditions, is of considerable scientific and management interest (e.g. Brannon *et al.* 2004).

Steelhead trout (*Oncorhynchus mykiss*) is an ecologically variable species (Behnke 1992), and shows considerable genetic variation among populations (e.g. Busby *et al.* 1996, Beacham *et al.* 1999, Heath *et al.* 2001, 2002, Hendry *et al.* 2002). This is often considered to be a sign of adaptive traits that enhance survival and reproduction in the local environment (e.g. Carvalho 1993). Steelhead from the West Coast of North America have been widely stocked and naturalized throughout the world. Massive releases of hatchery-produced fish have been shown to reduce or change natural genetic diversity among wild populations in several salmonid species

(e.g. *Oncorhynchus sp.*: Nielsen *et al.* 1994, Reisenbichler & Rubin 1999; *Salmo sp.*: Fleming *et al.* 2000, Garcia-Marin *et al.* 1999, Hansen *et al.* 2000, Hansen 2002; *Salvelinus sp.*: Englbrecht *et al.* 2002). Direct genetic effects are documented through interbreeding when non-native populations of the same species of fish are introduced (e.g. Williams *et al.* 1996, Hansen 2002). Native salmonids may also be affected indirectly through overharvesting in mixed stocks, disease introductions, population fragmentation and local extinctions (Utter 1998). Natural selection may work against stocked trout and 'hybrids' (Poteaux *et al.* 1998, Hansen *et al.* 2000). It is, however, unclear what ecological conditions may cause differential natural selection. Human selection in the form of angling may perhaps also disproportionately remove stocked trout (Behnke 1992, Garcia-Marin *et al.* 1999). Cases of little or no introgression are also reported in a number of studies (e.g. Beaudeou *et al.* 1994, Arias *et al.* 1995, LeClair *et al.* 1999, Englbrecht *et al.* 2002, Hansen 2002, Kostow *et al.* 2003, Taylor 2002, Taylor & Tamkee 2001, 2003).

Considerably less, however, is known about potential genetic drift and possible genetic effects (Adkinson 1995, Palm & Ryman 1999) when local fish populations are enhanced through the use of annually collected native brood stock which are naturally reared in the wild (Blouin 2003, Kostow 2004). Theoretically, about 50 individuals in an effective parent generation is required to contain 99 % of the original genetic variation (e.g. Caughley & Gunn 1996), although maybe in different frequencies, and rare alleles may be lost. Generally larger natural populations of salmonids tend to show temporal stability in genetic structure over time (e.g. Nielsen *et al.* 1999, Heath *et al.* 2002).

Because of relatively long histories of considerable stocking through the use of wild, native brood stock, a number of watersheds in British Columbia can be regarded as long-term genetic field experiments. In the Kitimat River of the Skeena region, British Columbia, hatchery steelhead of known numbers and origin, have been released annually since 1984 (mean 50 297 ±SD 8109, Table I). Scales have been collected in various years from hatchery brood stock parents (Table I). Fortunately, steelhead scales were also collected from wild fish in 1976-1984, i.e. before hatchery stocking (Table I). Dried, preserved fish scales provide sufficient tissue for the extraction of DNA in order to look at the genetic structure of each individual fish. Thus the material collected represented a rare opportunity to test whether the natural genetic variation in

the wild populations was maintained in the presence of hatchery operation. Therefore, the objectives of this study were to use DNA obtained from archived adult fish scales to:
1) identify the genetic structure of natural steelhead populations in the Kitimat River before hatchery operation was initiated, and
2) assess the potential genetic impact on genetic structure and molecular variation, if present, of almost 20 years of large-scale hatchery operation.

STUDY AREA

The Kitimat River is located at the head of Douglas Channel on the north coast of British Columbia and flows into the Pacific Ocean approximately 230 km southeast of Prince Rupert (Figure 1, 2). The Kitimat River watershed drainage area is approximately 217 000 hectares and lies within a wide glaciated valley that has been extensively logged; almost all of the mature conifer forests have been removed. Water discharge ranges from 19.4 to 1 670.7 m^3s^{-1} and the mean annual discharge is 148.8 m^3s^{-1}. Due to the removal of much of the flow stabilising riparian vegetation within the watershed and the fact that most of the valley's soil is loose glacial till, discharge can vary dramatically over a short period of time. The climate of the Kitimat area is moderated by the Pacific Ocean; precipitation can be considerable with heavy snowfalls in winter and wet and warm summers.

The Kitimat watershed has a fish fauna which includes chinook (*Oncorhynchus tshawytscha*), coho (*O. kisutch*), pink (*O. gorbuscha*), chum (*O. keta*), kokanee (*O. nerka*) and sockeye salmon *(O. nerka*), winter and (small numbers of) summer run steelhead (including non-anadromous rainbow trout) (*O. mykiss*), and coastal cutthroat trout (*O. clarki clarki*), 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*). Sockeye, coho, pink, chum and chinook salmon escapements range from 0-8 200, 750-75 000, 750-280 000, 1 500-500 000 and 1 000-45 000, respectively.

Kitimat River steelhead hatchery operation started in 1984. The mean number of smolts released over this time is 50 932 ±SD 8107, but ranged from 34 420 (1992) to 64 297 (1998) fish. All released smolts are adipose fin clipped, average weight was 72 grams (±SD 7.2, range 52-81) at the time of release (May), and they have been consistently released at the same 7 localities in the Kitimat River (from downstream: Hatchery, Hirsch, Cablecar, Powerline, Humphries, Sawmill, 17-mile bridge, plus 1-2 additional sites in occasional years, Figure 1). Brood stock parents have been exclusively wild steelhead, which can be identified from hatchery-produced fish in the adults by their intact adipose fin (i.e. a non-clipped fish), captured by sport fishing.

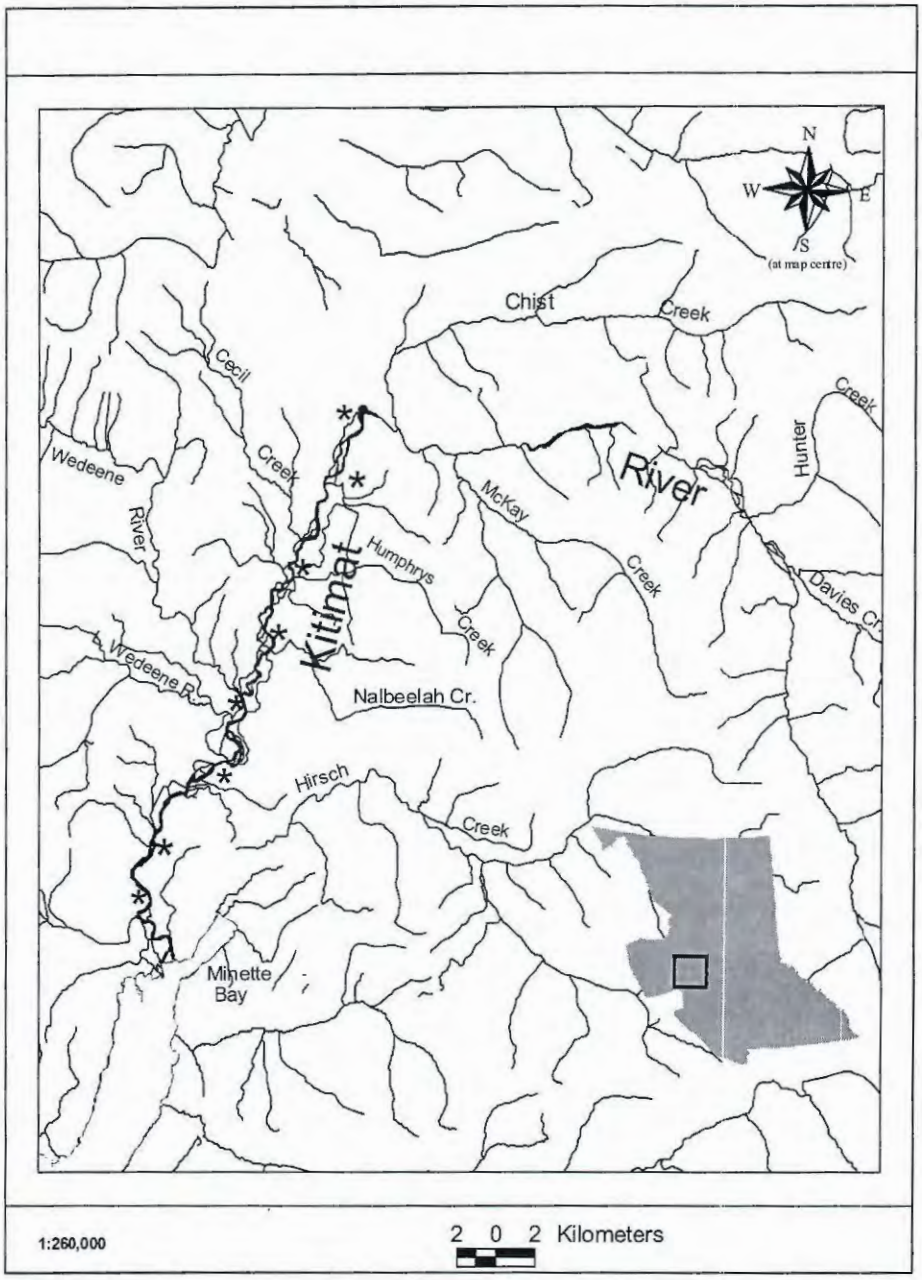

Figure 1. The Kitimat River study area. Stars indicate stocking sites in the lower mainstem of the Kitimat River. Inset location in British Columbia.

Table I. Number of adult steelhead brood stock scale samples used in analysis, timing of

brood stock capture period, number of wild brood angled (unclipped), number of incidental hatchery fish angled (clipped), and number of smolts produced by brood year. Data from the sampling period 1976-2002 in the Kitimat River, Skeena Region, British Columbia.

Year	Number of scale (DNA) samples	Brood stock capture period	Number of wild brood fish angled (female/male)	Number of hatchery fish angled (female/male)	Number of smolts produced
1976	23				
1977	102				
1983	30				
1984	11	16.04-02.05	18 / 11		65143
1985	-	15.04-19.04	22 / 13		54667
1986	-	14.04-24.04	20 / 22		54035
1987	17	13.04-27.04	18 / 8		48328
1988	15	28.03-28.04	16 / 9	0 / 3	51355
1989	-	07.04-24.04	18 / 14	14 / 22	50578
1990	22	09.09-20.04	15 / 14	34 / 46	54481
1991	22	09.04-26.04	12 / 15	51 / 29	46800
1992	9	07.04-24.04	12 / 15	20 / 32	34420
1993	18	05.04-20.04	15 / 23	27 / 45	38473
1994	-	06.04-20.04	15 / 20	29 / 42	47412
1995	-	04.04-27.04	20 / 13	8 / 22	45822
1996	27	10.04-23.04	15 / 22	3 / 10	57265
1997	-	11.04-24.04	15 / 19	3 / 4	54696
1998	-	06.04-26.04	11 / 7	11 / 23	64297
1999	-	09.04-28.04	14 / 18	11 / 9	53339
2000	-	10.04-11.05	9 / 5	13 / 5	40147
2001	11	09.04-03.05	13 / 15	12 / 4	46566
2002	39	11.04-09.05	13 / 17	15 / 14	59885
2003	39				
Total	**354**				

METHODS AND MATERIALS

FIELD SAMPLING

Brood stock steelhead adults and scale samples (Table I, II) were collected each year by sport fishing in the main stem river from the 17-mile bridge and downstream. Brood capture has commenced between March 28 (1988) and April 16 (1984), and the last day of brood capture has ranged from April 19 (1985) to May 11 (2000), respectively (Table I). For most years brood stock capture was implemented within one week. Fish were sampled along the entire reach of the

Kitimat River from the ocean to the 17-mile bridge, and thus probably spatially representative for most years. However, for some years locations were not recorded. In 1977 a more extensive survey of fishermen and spawning locations along the entire river up to Hunters Creek (about 45 km upstream 17-mile bridge) was carried out, and scale samples were collected from steelhead caught by sport anglers (Morris & Eccles 1977). Since 1988 the number of hatchery fish (adipose fin clipped) caught while fishing for brood stock was also recorded (Table I). These catch-effort data suggest spawning stock in the lower mainstem Kitimat River over the years have consisted of roughly equal numbers of wild (unclipped; mean 29 ±SD 6.5) and hatchery (clipped; mean 37 ±SD 26.9) fish, but with considerable variation between years and a predominance of hatchery fish 1990-1994 (Table I).

In most cases five fish scales were sampled from each individual, from the right side of the fish, below the adipose fin and above the lateral line. Scales were preserved in paper scale envelopes and stored in a warehouse which was neither insulated nor heated. Consequently the samples were subjected to seasonal freezing and thawing. Total length (mm) data and sex were collected for all fish. In 2002 and 2003 both scales and tissues (about 5 mm^2 of adipose fin) were sampled, and the tissue stored in ethanol-filled tubes in the field upon collection.

ACROSS-PROVINCE COMPARISONS

Thirteen additional wild steelhead and rainbow trout population samples from British Columbia were also used in the present study to compare genetic distinctiveness between populations (Figure 2, Table II). These additional *O. mykiss* samples have previously been analysed (Tamkee & Taylor, unpublished data.) and individuals from these populations were re-analyzed with the Kitimat River samples to standardize the scoring for alleles and for comparison purposes.

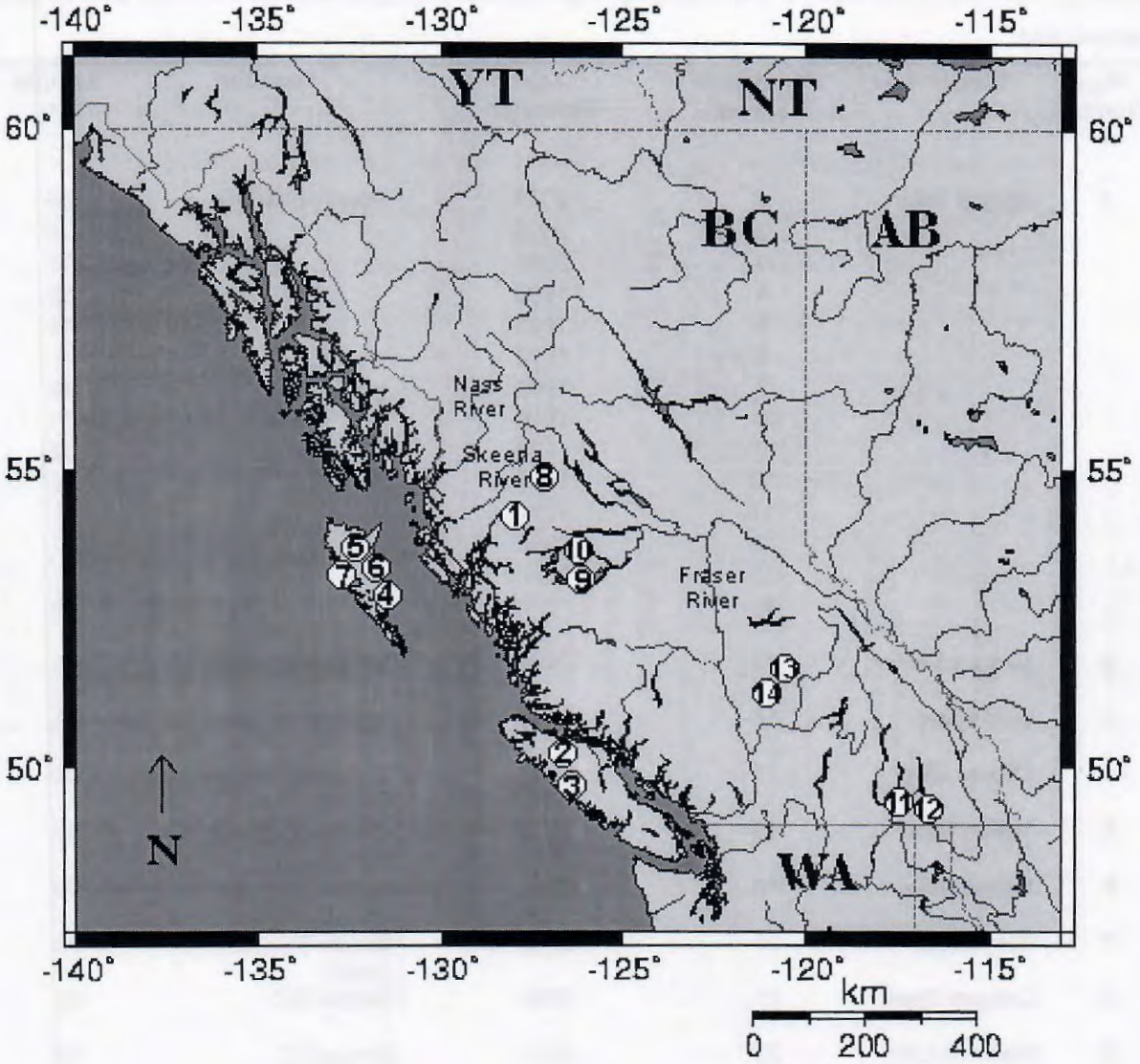

Figure 2. Location of Oncorhynchus mykiss sample sites from British Columbia, Canada, where DNA samples were obtained. All populations, save the Kitimat, are known to be wild. Sample sites are labeled as 1) Kitimat River, 2) Nimpkish River, 3) Gold River, 4) Cooper River, 5) Mamin River, 6) Yakoun River, 7) Riley Creek, 8) Canyon Creek, 9) Blanchet Lake, 10) Theletaban Lake 11) Kootenay River, 12) Salmo River, 13) 01201 LNTH Lake, and 14) 00376 DEAD Lake.

Table II. List of sample site-map numbers corresponding to Figure 1, water body the samples were collected from, population number corresponding to genetic analyses, year collected, location of sites, and sample size.

Map Number	Sample site	Population number	Year Collected	Location	Sample size
1	Kitimat River	1	2003	West Coast BC	34
		2	2002		39
		3	2001		11
		4	1996		26
		5	1993		18
		6	1992		9
		7	1991		16
		8	1990		11
		9	1988		12
		10	1987		12
		11	1984		9
		12	1983		27
		13	1977		91
		14	1976		18
2	Nimpkish River	15	2000	Vancouver Island	35
3	Gold River	16	2000	Vancouver Island	35
4	Copper River	17	2002	Queen Charlotte Island	21
5	Mamin River	18	2002	Queen Charlotte Island	31
6	Yakoun River	19	2002	Queen Charlotte Island	20
7	Riley Creek	20	2002	Queen Charlotte Island	30
8	Canyon Creek	21	2000	Central BC	32
9	Blanchet Lake	22	2000	Central BC	50
10	Theleteban Lake	23	2000	Central BC	60
11	Kootenay River	24	2000	Southern BC	32
12	Salmo River	25	2000	Southern BC	32
13	01201LNTH Lake	26	2001	South-Central BC	32
14	00376DEAD Lake	27	2001	South-Central BC	52

MICROSATELLITE DNA

Total genomic DNA was extracted from one to five unwashed scales scale(s) or from tissue samples using Qiagen DNeasy Tissue Kit (Qiagen Inc.). After extraction, the DNA was stored at -20^0C (for standard procedures details see Appendix I).

Pilot studies implemented in 2002 indicate that relatively fresh steelhead scales work well, i.e. give a DNA product 100% of the time. With 20-30 years old scales, the success rate may be reduced, due to increased 'brittleness' of scales and DNA degradation.

Genetic variation was assayed at microsatellite DNA loci. Microsatellites are short (2-6 base pair) tandemly-arranged repetitive DNA sequences that are inherited in a Mendelian fashion and which typically exhibit elevated mutation rates (10^{-4} is typical for salmonids) relative to allozymes and mitochondrial DNA, and high heterozygosities (typically 40-90%). Allelic variation at these loci was assayed using the polymerase chain reaction (PCR) with locus-specific primer pairs.

Individuals were genotyped with 10 polymorphic microsatellite markers previously used for *Oncorhynchus mykiss* within British Columbia (Tamkee and Taylor, unpublished data).
To increase efficiency and minimize cost, the PCR reactions for 6 working microsatellite markers were run in tandem (diplex). The diplexes were as follows: Oneu14 (Scribner *et al.* 1996) and Ssa197 (O'Reilly *et al.* 1996), Ssa456 (Slettan *et al.* 1995) and Omy77 (Morris *et al.* 1996), and Ots3 (Banks *et al.* 1999) and Okia3 (P. Bentzen, Dalhousie U.). Also, three microsatellite markers were run together (triplex), these included Ots100 (Nelson & Beacham, 1999), Ots103 (Nelson & Beacham, 1999), and Ssa85 (O'Reilly *et al.* 1996). Oneu8 (Scribner *et al.* 1996) was not multiplexed due to PCR incompatibilities with other markers (Table III). Multiplexes were developed based on similar individual annealing temperature for the loci, non-over lapping allele sizes, and PCR-amplification compatibility.

PCR reactions were carried out in 10µl volumes containing: 100ng DNA template, 10x reaction buffer (Gibco/BRL), 0.4mM DNTP, 0.25uM reverse primer, 0.025uM forward primer (Appendix

I), 1.5mM $MgCl_2$, and 0.5 units of taq polymerase, respectively. PCR amplification was performed in a PTC-100 (MJ Research) thermal-cycler (Table III). Each PCR profile (single primer or multiplex) consisted of [5X (95°C /1 min, T_A/ 1 min, 72°C /1 min), 30X (94°C / 1 min, T_A/ 1 min, 72°C / 1 min), and 1X (72°C / 5min)], where T_A is the annealing temperature(s) respectively and each primer were labeled with individual fluorescent Beckman dyes (Table III). Sample sizes varied slightly among loci due to variability in PCR amplification efficiency. Any individuals that failed to produce clear bands were reamplified under the same conditions, and if amplification was not possible in the second PCR reaction the sample(s) were removed from the study. Genotypes for all microsatellite loci were visualized using the Beckman CEQ 8000 DNA sequencer.

Table III. Multiplexed loci and the corresponding labeled Beckman dye, annealing temperature (T_A), total number of samples which amplified results (N), and range in allele size in base pairs for each locus.

Multiplex	Locus	Source species	Beckman dye	T_A (Celcius)	N	Range (bp)
1	Oneu14	*Oncorhynchus nerka*	D-4	62/60	330	145-165
	Ssa197	*Salmo salar*	D-4		333	112-116
2	Ssa456	*Salmo salar*	D-3	56/55	332	151-161
	Omy77	*Oncorhynchus mykiss*	D-3		329	94-140
3	Ots3	*Oncorhynchus tshawytscha*	D-4	52/50	326	76-96
	Okia3	*Oncorhynchus kistuch*	D-4		321	112-206
4	Ssa85	*Salmo salar*	D-3	56/55	317	97-153
	Ots103	*Oncorhynchus tshawytscha*	D-3		327	71-91
	Ots100	*Oncorhynchus tshawytscha*	D-3		305	138-218
5	Oneu8	*Oncorhynchus nerka*	D-2	58/56	333	150-184

DATA ANALYSIS

Genetic variation

Standard descriptive statistics of microsatellite loci included expected heterozygosity (H_e), observed heterozygosity (H_o), number of alleles (N_a) and average number of alleles per locus (A) which were compiled using TFPGA version 1.3 (Miller 1997). Allelic richness (Ar) was calculated using FSTAT version 2.9.3 (Goudet 1995, 2002). Allelic richness is a measure of the number of alleles independent of sample size, and hence allows comparison of the number of alleles between samples of different sizes.

Tests for deviations from Hardy-Weinberg equilibrium were performed for each locus-population combination using an exact test in which P-values were estimated using the Markov chain method in GENEPOP version 3.1 (Raymond & Rousset 1995). Tests for genotypic linkage disequilibrium for all combinations of locus pairs within populations were also made using a Markov chain method with GENEPOP default values. Tests for population differentiation between all pairs of populations was performed over all loci combined using log-likelihood (G)–based exact tests with GENEPOP default values. To guard against inflated Type I error rates in multiple comparisons, all critical significance levels for simultaneous tests were evaluated using the conservative sequential Bonferroni adjustment (Rice 1989) with an initial α level of 0.05.

To increase the power of the analysis, we also conducted analyses after year-class data were pooled to increase sample size to minimum 20 individuals (1976, 1977, 1983 pooled with 1984, 1987 with 1988, 1990 with 1991, 1992 with 1993, 1996, 2001 pooled with 2002, and 2003), and we ran the analysis for pooled pre (1976-1986) and post (1987-2003) enhancement data, where 1987 was the first year when hatchery fish were observed in substantial numbers (Anonymous 2003).

Genetic differentiation

Genetic differentiation among samples from different locations and/or years was quantified using F_{ST} as estimated by θ (Weir and Cockerham 1984) and the 95% confidence intervals were

obtained using FSTAT (version 2.9.3; Goudet 2002). F_{ST} can theoretically range from 0 (no genetic divergence) to 1 (complete fixation of alternative alleles).

Again, to increase the power of the analysis, we ran population differentiation tests using FSTAT (with 5000 permutations) for pooled year-class data (minimum 20 individuals; 1976, 1977, 1983 pooled with 1984, 1987 with 1988, 1990 with 1991, 1992 with 1993, 1996, 2001 pooled with 2002, and 2003).

Genetic distances among population pairs were estimated with Cavalli-Sforza and Edward's (1967) chord distance (C-S chord distance) calculated in the PHYLIP software package (Felsenstein 1995). Cavalli-Sforza and Edward's chord distances were used to build an unrooted neighbour-joining tree to visualize the genetic relationships among sites/year classes. Genetic distance estimates were calculated by creating a microsatellite allele frequency matrix, replicated 100 times with SEQBOOT and calculated for each replicate data set using the GENDIST program. The neighbour-joining trees were built using the program NEIGHBOUR. Reliability of tree nodes was evaluated by generating a consensus tree from 100 bootstrap replicates of the original allele frequencies using the programs SEQBOOT and CONSENSE, and the final tree was drawn in DRAWTREE.

A principal components analysis (PCA) was conducted on allele frequency data using PCA-GEN (Goudet 1999) as a comparative method to summarize genetic differentiation among all samples. The analysis summarizes all the variation across the 10 loci (154 alleles) and orients samples along major axes of variation (principal components, Pimentel 1979).

Microsatellite allele frequencies were tested for evidence of recent bottlenecks in steelhead by using the mode-shift test as implemented in *Bottleneck* (Cornuet & Luikart 1997). Populations that have undergone recent bottlenecks are expected to show a reduction in the proportion of low frequency alleles relative to alleles of moderate abundance. Recent bottlenecks are those that have occurred within 40-80 generations and the TPM (Two-Phased Model of Mutation) mode shift test assumes that the populations are near mutation-drift equilibrium and is independent of the mutation model (infinite alleles or stepwise mutation) for microsatellite loci (Luikart et al.

1998). The detection of recent bottlenecks in steelhead may be important. Bottlenecked populations may not have had time to adapt to potential problems imposed by small population sizes. It may signal populations at risk of losing heterozygosity or variation at quantitative loci affecting fitness over the longer term (Luikart et al. 1998).

RESULTS

Microsatellite variation across 354 Kitimat River individuals from 14 different years and at 10 microsatellite loci were extracted and assayed, and 333 individuals amplified collectable results, i.e. more than 90%. Considering the age of many of the scales and the relatively poor condition under which they had been stored (repeated freezing and thawing), this is a better result than might be expected (e.g. Nielsen *et al.* 1999, Meldgaard *et al.* 2003).

GENETIC VARIATION

The number of alleles observed across all usable Kitimat individuals ranged from 2 (*Ssa*197) to 31 (*Oki*3a) with an average of 11.5 ±SD 8.68 alleles per locus (Table IV). Mean allelic richness (Ar) across loci and years was 3.78 ±SE 0.04, and varied between years from 3.62 (1992; also the lowest number of samples) to 3.97 (2002). Observed heterozygosity averaged 0.58 ±SD 0.21 across all loci and years (populations), and ranged from 0.08 (*Ots*103) to 0.89 (*Oki*3a), respectively. Combined with the additional 13 wild populations of *O. mykiss* from BC, results from a total of 722 individuals at 10 microsatellite loci were obtained. The number of alleles observed across populations ranged from 2 (*Ssa*197) to 37 (*Oki*3a) with an average of 15.4 ±SD 10.75 alleles per locus. Mean allelic richness (Ar) across loci and populations was lower and more variable, 3.47 ±SE 0.14, varying between populations from 1.47 (01201LNTH Lake) to 4.15 (Gold River). Observed heterozygosity averaged 0.51 ±SD 0.18 across all loci and populations, and ranged from 0.11 (*Ots*103) to 0.78 (*Oki*3a), respectively.

There seemed to be no major loss of specific and common alleles after the implementation of the hatchery operation on the Kitimat River. In all cases where there was a loss of particular

alleles(s) from certain loci after hatchery implementation, the allele frequencies of these alleles were originally very low, i.e. rare alleles. Alleles which were lost after hatchery operation included (year documented): Oneu8*176 (1977), Ssa85*107 (1976 and 1977), Omy77*102 (1977), Omy77*122 (1977), Omy77*126 (1977), Ots100*182 (1977), Ots100*216 (1984), Ots100*218 (1984), Okia3*122 (1983), Okia3*130 (1983), Okia3*146 (1983), Okia3*154 (1983), Okia3*166 (1983), Okia3*190 (1977), Okia3*192 (1983) and Okia3*202 (1977). By contrast, in some cases new alleles were documented after the hatchery was implemented these included allele Oneu8*178 (2002), Ssa85*109 (2003), Ssa85*117 (2002), Ssa85*135 (1992 and 1993), Ots103*83 (1990), Ots3*86 (2002), Ssa456*153 (2002 and 2003), Ssa456*161 (1991), Ots100*138 (2003), Ots100*140 (1991), Ots100*192 (2002), Ots100*194 (2003), Okia3*138 (1990), Okia3*142 (1991), Okia3*180 (1996) and Okia3*196 (1988, 1992 and 2002).

Genetic variation within *O. mykiss* populations across British Columbia ranged widely (Table IV). Expected heterozygosity, averaged across the 10 loci, ranged from a low of 0.15 (00376DEAD Lake) to highs of 0.60 – 0.62 (Kitimat River 1977, 1993, Gold River, Nimpkish River, and Kootenay River). Most populations displayed relatively high levels of genetic variation with the exception of 00376DEAD Lake, were it was fixed for single alleles at 5 of the 10 loci. Within the Kitimat River year classes, there were only 3 year classes which had fixed alleles at a particular locus. These samples were the 1984, 1991, and 1992 fixed for the Ots103*079 allele (Table IV).

Virtually all samples across BC were in Hardy-Weinberg equilibrium with only 11 out of possible 270 (10 loci x 27 populations) tests showing significant heterozygote deficits, i.e. less than expected at a Type 1 error rate of 5%. These exceptions were found at several separate loci in 6 different populations and 2 different year classes among the Kitimat River samples, and do not compromise subsequent analyses. By contrast, year class 1977 had 4 loci that were not in Hardy-Weinberg equilibrium (Table IV), and results from 1977 should be interpreted with caution. Test for linkage disequilibrium resulted in only one significant departure out of possible 1052 tests, indicating that all loci were inherited independently.

Table IV. Summary of allelic variation at 10 microsatellite loci for 27 Oncorhynchus mykiss populations and year classes included in this study. Number of samples which amplified results (N), allelic richness (Ar), number of alleles per locus (Na), expected heterozygosity (He), and observed heterozygosity (Ho) are given for each population. Significant departures from Hardy Weinberg Equilibrium are denoted by "" (using Bonferroni corrections for 27 populations; p= 0.05/27=0.00185).*

	Oneu8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Results over all loci
Kitimat 2003											
N	34	33	34	34	34	34	34	34	31	34	
Ar	3.198	4.482	1.447	3.436	2.892	4.893	3.615	1.999	4.555	7.018	
Na	5	7	2	5	4	8	5	2	8	11	
He	0.4537	0.7062	0.0843	0.6012	0.5649	0.7405	0.6916	0.4892	0.7242	0.8741	0.5920
Ho	0.4706	0.5152	0.0882	0.5588	0.4118	0.6471	0.5000	0.5000	0.7097	0.8529	0.5254
Kitimat 2002											
N	39	38	37	38	39	39	39	39	36	38	
Ar	3.527	3.952	1.162	3.401	2.439	5.349	4.169	2	4.486	7.497	
Na	7	9	2	7	4	9	7	2	8	15	
He	0.4931	0.5405	0.0267	0.5637	0.5302	0.7659	0.7048	0.4947	0.6894	0.8930	0.5702
Ho	0.5385	0.5789	0.0270	0.5263	0.4872	0.6667	0.6154	0.6923	0.6944	0.9211	0.5748
Kitimat 2001											
N	11	10	9	9	11	11	11	11	10	11	
Ar	1.922	4.15	1.667	4.681	2.968	5.169	3.507	2	3.438	7.977	
Na	2	5	2	5	3	7	4	2	4	11	
He	0.2355	0.6800	0.1049	0.6852	0.5950	0.6612	0.6157	0.4835	0.5250	0.8760	0.5462
Ho	0.0909	0.7000	0.1111	0.6667	0.6364	0.4545	0.6364	0.2727	0.7000	0.9091	0.5178
Kitimat 1996											
N	26	24	26	25	26	25	26	26	25	26	
Ar	3.689	3.482	1.231	4.033	2.41	4.855	3.957	2	4.64	8.261	
Na	6	4	2	5	3	7	5	2	7	15	
He	0.4970	0.5773	0.0377	0.6600	0.5096	0.7536	0.6790	0.4882	0.7432	0.9105	0.5856
Ho	0.5000	0.5417	0.0385	0.5200	0.5385	0.5600	0.6154	0.4615	0.6800	0.9231	0.5379
Kitimat 1993											
N	18	16	18	18	18	18	18	18	17	18	
Ar	3.374	4.653	1.562	3.883	2.562	4.964	3.867	2	4.016	7.17	
Na	5	7	2	6	3	7	5	2	5	11	
He	0.4846	0.7148	0.1049	0.6620	0.5509	0.7623	0.6975	0.4938	0.6799	0.8688	0.6020
Ho	0.5556	0.7500	0.1111	0.7222	0.6111	0.6111	0.7222	0.5556	0.6471	0.9444	0.6230
Kitimat 1992											
N	9	6	9	9	9	9	9	9	9	9	
Ar	1.667	5	1	3.618	2	3.309	3.877	2	4.544	6.985	
Na	2	5	1	4	2	4	4	2	5	8	
He	0.1049	0.7639	0.0000	0.5679	0.5000	0.4444	0.6852	0.4938	0.7346	0.8519	0.5147

Ho	0.1111	0.8333	0.0000	0.5556	0.5556	0.3333	0.4444	0.4444	0.8889	0.8889	0.5056
Kitimat 1991											
N	16	16	16	16	16	16	16	16	16	16	
Ar	3.102	4.102	1	3.981	2.992	5.392	3.289	1.999	5.126	7.939	
Na	5	5	1	6	4	9	4	2	8	12	
He	0.4766	0.7051	0.0000	0.6426	0.5801	0.7539	0.5762	0.4512	0.7441	0.8926	0.5822
Ho	0.5625	0.5000	0.0000	0.7500	0.6875	0.6875	0.5000	0.6875	0.7500	0.8750	0.6000
Kitimat 1990											
N	11	11	11	11	11	10	11	11	11	11	
Ar	2.896	4.221	1.545	3.516	1.997	4.509	3.533	2	6.02	8.549	
Na	4	5	2	4	2	5	4	2	8	12	
He	0.3182	0.5620	0.0868	0.6570	0.3967	0.6450	0.6653	0.4628	0.8058	0.8884	0.5488
Ho	0.3636	0.3636	0.0909	0.5455	0.5455	0.8000	0.4545	0.3636	0.8182	1.0000	0.5345
Kitimat 1988											
N	12	12	12	12	12	12	12	12	12	12	
Ar	2.761	4.214	1.5	2.873	2	4.929	3.753	2	5.586	6.54	
Na	4	6	2	3	2	6	4	2	7	8	
He	0.2951	0.5694	0.07799	0.4965	0.4688	0.7188	0.7083	0.4688	0.7917	0.8403	0.5437
Ho	0.2500	0.6667	0.0833	0.4167	0.4167	0.5000	0.5833	0.4167	00.9167	0.9167	0.5167
Kitimat 1987											
N	12	12	12	12	12	12	12	12	12	12	
Ar	3.838	4.668	1.761	3.714	2	4.503	3.932	2	3.391	7.381	
Na	4	6	2	5	2	6	7	2	4	10	
He	0.6840	0.6840	0.1528	0.5174	0.4861	0.6458	0.7326	0.4965	0.6354	0.8611	0.5896
Ho	0.5833	0.5833	0.1667	0.5000	0.5000	0.5000	0.5833	0.7500	0.8333	1.0000	0.6000
Kitimat 1984											
N	9	9	9	9	9	9	9	9	9	9	
Ar	3.558	5.087	1	2.902	1.999	2.95	4.655	2	3.796	8.694	
Na	4	6	1	3	2	3	5	2	5	12	
He	0.4506	0.7778	0.0000	0.4383	0.4012	0.564	0.7284	0.4444	0.5679	0.9012	0.5296
Ho	0.1111	0.6667	0.0000	0.5556	0.5556	0.4444	0.6667	0.6667	0.6667	1.0000	0.5333
Kitimat 1983											
N	27	27	27	27	26	26	27	27	27	27	
Ar	3.895	4.009	1.412	3.598	1.995	4.917	3.476	2	4.399	8.985	
Na	6	6	2	6	2	7	6	2	8	20	
He	0.5302	0.6728	0.0713	0.6056	0.4401	0.7337	0.6180	0.4890	0.6934	0.9246	0.5779
Ho	0.5556	0.8148	0.0741	0.6296	0.5769	0.60538	0.5556	0.5556	0.7407	0.8889	0.6046
Kitimat 1977											
N	91	87	90*	90	91*	90*	88	91	74	84*	
Ar	3.461	4.275	1.763	4.048	2.127	4.995	3.919	1.999	4.49	7.933	
Na	7	11	3	6	3	11	8	2	10	20	
He	0.4944	0.6556	0.1637	0.6901	0.5108	0.7760	0.7085	0.4951	0.7084	0.9093	0.6112
Ho	0.3956	0.6437	0.1111	0.6444	0.9560	0.9222	0.5682	0.5275	0.7027	0.8214	0.6293

Kitimat 1976											
N	18	17	16	16	18	18	18*	18	16	14	
Ar	3.306	5.31	1.992	3.739	2.319	4.435	3.704	2	4.755	8.167	
Na	5	8	3	5	3	7	4	2	8	13	
He	0.4522	0.7059	0.1738	0.5859	0.4151	0.6898	0.6836	0.4938	0.6855	0.8878	0.5774
Ho	0.3889	0.4706	0.1875	0.5625	0.3333	0.7778	0.2222	0.5556	0.7500	0.9286	0.5177
Nimpkish R.											
N	34	32	33	35	33	33	34	34	31	30	
Ar	4.694	5.134	1	2.901	3.659	4.269	4.024	1.999	4.498	6.265	
Na	7	8	1	4	5	7	6	2	6	9	
He	0.7535	0.7935	0.0000	0.5286	0.5684	0.7342	0.6830	0.4844	0.7430	0.8556	0.6144
Ho	0.6471	0.7188	0.0000	0.6571	0.6061	0.7273	0.5294	0.4118	0.7419	0.9000	0.5939
Gold R.											
N	35	33*	35	34	35	35	32	33	27	32	
Ar	3.908	5.416	1	3.01	3.894	5.8	4.467	2	5.061	6.898	
Na	6	9	1	4	5	11	6	2	9	10	
He	0.5265	0.7943	0.0000	0.5753	0.5959	0.8143	0.7217	0.5000	0.7620	0.8774	0.6169
Ho	0.6286	0.5455	0.0000	0.5588	0.5429	0.8286	0.5938	0.4545	0.5926	0.7813	0.5526
Copper R.											
N	21	21	16	21	20	20	21	21	20	20	
Ar	2.612	4.823	1.75	3.604	3.075	5.695	4.861	1.992	3.936	6.456	
Na	5	7	2	6	4	10	7	2	6	11	
He	0.2948	0.7370	0.1172	0.6077	0.5987	0.7675	0.7608	0.4082	0.5675	0.8288	0.5690
Ho	0.2381	0.8095	0.1250	0.7143	0.6500	0.7000	0.8571	0.4762	0.5000	0.8500	0.5920
Mamin R.											
N	31	31	31	31	31	30	30*	31	31	31	
Ar	1.904	5.294	1.352	2.93	2.793	5.128	3.834	2	4.091	7.243	
Na	2	9	2	3	3	8	5	2	5	12	
He	0.2706	0.7622	0.0624	0.6041	0.5718	0.7711	0.5939	0.4953	0.7196	0.8809	0.5739
Ho	0.2581	0.9032	0.0645	0.4839	0.6129	0.8000	0.3000	0.5806	0.7742	0.8710	0.5648
Yakoun R.											
N	20	20	15	19	20	20	20	20	19	19	
Ar	1.968	4.312	1.4	2.957	2.298	6.169	3.726	2	3.358	6.989	
Na	3	7	2	3	3	9	4	2	4	12	
He	0.1838	0.7025	0.0644	0.6094	0.4862	0.8275	0.6937	0.4988	0.4972	0.8587	0.5422
Ho	0.1000	0.5500	0.0667	0.6316	0.3500	0.6500	0.5000	0.3500	0.5789	1.0000	0.4777
Riley Creek											
N	30	30	29	30	24	24	28	30	29	29	
Ar	1	4.118	1	3.526	2.25	5.61	4	1.999	3.795	6.422	
Na	1	5	1	5	3	9	5	2	6	10	
He	0.0000	0.7328	0.0000	0.6806	0.5148	0.7977	0.7175	0.4800	0.6326	0.8478	0.5354
Ho	0.0000	0.7333	0.0000	0.7667	0.4167	0.6250	0.7500	0.5333	0.6207	0.8276	0.5204

Canyon Creek											
N	32	31	32	32	32	32	32	32	32	30	
Ar	1	2.587	1.188	1.375	1.83	2.187	1.963	1.83	1.342	1.763	
Na	1	3	2	2	2	3	2	2	2	4	
He	0.0000	0.5578	0.0308	0.0605	0.2188	0.5005	0.3418	0.2188	0.0605	0.1272	0.2117
Ho	0.0000	0.5484	0.0313	0.0625	0.1250	0.3750	0.3750	0.2500	0.0625	0.1333	0.1963
Blanchet Lake											
N	50	49	50	44	49	49	47	48	50	46	
Ar	2.938	1.997	1.548	3.059	1.782	2	1	2	1	5.068	
Na	3	2	2	4	2	2	1	2	1	10	
He	0.6136	0.4592	0.0950	0.4703	0.1993	0.4998	0.0000	0.4991	0.0000	0.7346	0.3559
Ho	0.5200	0.4286	0.1000	0.4545	0.1837	0.6122	0.0000	0.4583	0.0000	0.7391	0.3474
Salmo R.											
N	59	60*	56	47	60	60*	59	59	60	57	
Ar	5.974	5.093	1	2.99	2.73	6.663	3.194	1.972	5.112	6.116	
Na	11	9	2	5	4	13	5	2	12	15	
He	0.8443	0.7818	0.0177	0.5620	0.4186	0.8692	0.6192	0.3707	0.7878	0.8369	0.6090
Ho	0.9322	0.7833	0.0179	0.4681	0.3167	0.7667	0.6271	0.3898	0.6833	0.7018	0.5669
Theleteban L.											
N	31	31	32	27	32	32	32	32	32	28*	
Ar	3.858	3.191	1	3.344	1	3.126	1	1.866	1	5.185	
Na	6	4	1	5	1	4	1	2	1	9	
He	0.6202	0.5416	0.0000	0.5905	0.0000	0.6167	0.0000	0.2417	0.0000	0.7487	0.3362
Ho	0.5484	0.6129	0.0000	0.6667	0.0000	0.7813	0.0000	0.2188	0.0000	0.4286	0.3257
01202 LNTH L.											
N	27	28	27	32	30	26	32	32	24	28	
Ar	2.868	1.522	3.948	2	1.686	3.195	1.988	1.785	3.227	2.561	
Na	4	2	5	2	2	5	2	2	4	3	
He	0.5823	0.1014	0.7106	0.4980	0.1528	0.6036	0.4043	0.1948	0.6658	0.4005	0.4311
Ho	0.4444	0.1071	0.5926	0.5625	0.1667	0.6538	0.3125	0.2188	0.7917	0.2857	0.4136
00376 DEAD L.											
N	32	32	30	28	32	32	32	32	32	32	
Ar	1.984	1	1.963	1	1.47	1.342	1	1	1	2.984	
Na	2	1	2	1	2	2	1	1	1	3	
He	0.3901	0.0000	0.3394	0.0000	0.0894	0.0605	0.0000	0.0000	0.0000	0.6616	0.1541
Ho	0.2813	0.0000	0.3667	0.0000	0.0938	0.0625	0.0000	0.0000	0.0000	0.7500	0.1554
Kootenay R.											
N	52	52	45	49	46	44	52*	52	49	50	
Ar	4.855	3.999	2.474	2.972	2.507	5.556	3.323	1.999	5.319	5.799	
Na	9	8	4	5	3	12	6	2	12	11	
He	0.7219	0.5939	0.4649	0.4196	0.5187	0.7859	0.6084	0.4933	0.7793	0.8130	0.6205
Ho	0.6923	0.5962	0.3556	0.4286	0.5000	0.6364	0.4038	0.4231	0.6531	0.8400	0.5529

GENETIC DIFFERENTIATION AMONG YEARS AND POPULATIONS

There was little detectable genetic differentiation among sampled years in the Kitimat River, as expressed by θ (Table V). When the 14 different year classes of steelhead were analyzed, the overall subdivision was low at $\theta = 0.005$, but significantly different from 0 (95% C.I. 0.001-0.01, $p<0.005$). This means that approximately 5 % of the total microsatellite DNA variation resolved may be ascribed to differences among year classes. The remaining 95 % of variation resides within year classes (i.e. among individuals) or within individual fish (i.e. heterozygosity).

Neither comparison among all year-classes before and after hatchery operation nor between pooled years classes pre and post hatchery indicated any significant genetic differentiation among before and after hatchery operation as expressed by θ (0.009 and 0.004, $p = 0.4156$, and 0.007 and 0.003, $p = 0.3640$, respectively), heterozygosity (H_O: 0.606 and 0.555, $p = 0.2140$, and 0.606 and 0.555, $p = 0.2024$; H_S: 0.605 and 0.591, $p = 0.3872$, and 0.606 and 0.592, $p = 0.3692$) or by allelic richness (3.890 and 3.748, $p = 0.0810$, and 5.352 and 5.038, $p= 0.0764$, respectively). Given, however, that our results for allelic richness were close to significant with the standard two-sided test, our data suggest a potential weak negative trend in allelic richness. This may be expected if brood stock numbers are less than 50 (Table I). Therefore, a one-sided test was also applied to investigate a potential loss of (rare) alleles. The results suggested a weakly significant reduction in allelic richness after hatchery operation for all year classes and pooled year classes pre and post hatchery ($p = 0.0188$ and $p = 0.0448$, respectively). Excluding the 1977 year-class from the data did not change these results (two sided tests: Ar = 3.884 and 3.748 with $p = 0.1440$ for all year classes, and 5.410 and 5.038 with $p = 0.0772$ for pooled year classes; one sided tests: $p = 0.0398$ and 0.0366, respectively). The trend is, however, weak, as indicated by the allele numbers before and after enhancement (Table V).

Table V. Number of alleles detected among Kitimat River steelhead before and after hatchery operation, and in total.

Microsatellite	*Pre-hatchery # of alleles*	*Post-hatchery # of alleles*	*Total # of alleles*
Oneu8	9	9	10
Ssa85	12	14	15
Ots103	3	4	4
Ots3	6	7	7
Ssa456	3	5	5
Omy77	14	10	14
Oneu14	8	8	8
Ssa197	2	2	2
Ots100	14	15	19
Okia3	26	23	31
Mean ±SD	9.6 ± 7.01	9.7 ± 6.22	11.5 ± 8.68

A functional relationship between mean allelic richness (Ar) and years was calculated and the resulting regression was not significant (Figure 3; $F = 0.743$, $p = 0.40$). However, there were indications that Ar was to some extent influenced by lower sample sizes, in particular by the low sample sizes in post hatchery year 1992 (Figure 3, n=9).

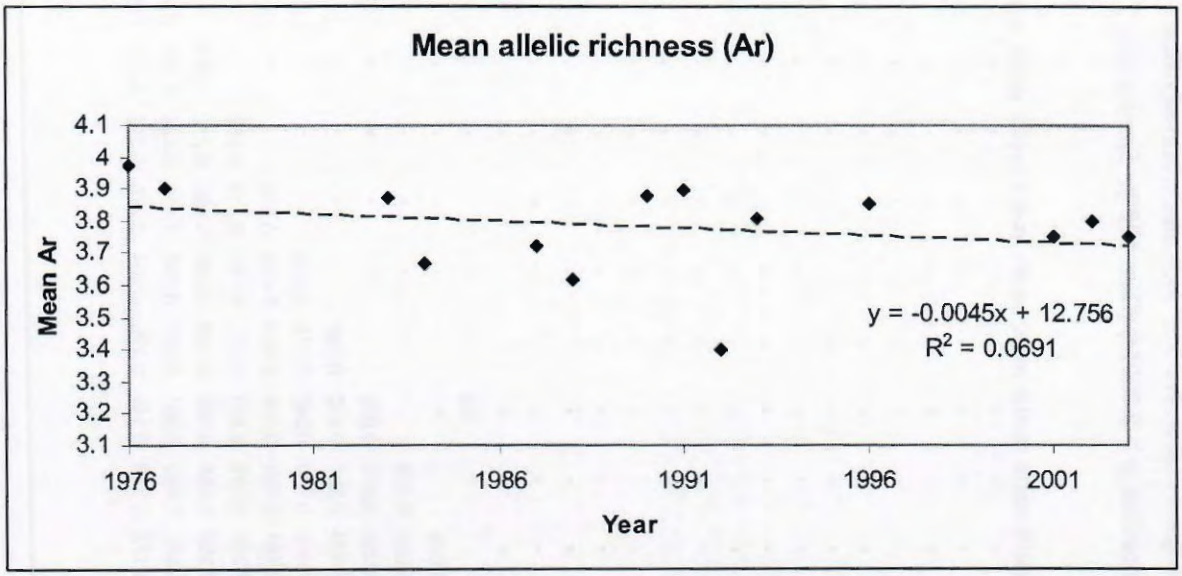

Figure 3. Regression of year-class versus allelic richness in the Kitimat River (F = 0.743, p = 0.40).

In comparison, when data were analyzed for all *O. mykiss* populations in the Kitimat River and other regions of BC, the overall subdivision was high with $\theta = 0.23$ (95% C.I. 0.19-0.28) and significantly greater than 0 ($p<0.005$). This indicates that much more of the total microsatellite DNA variation, i.e. approximately 23 %, is due to differences among populations.

More detailed analysis revealed extensive variation among populations in this survey. There were 351 (27 pops: 26+25+24…+1=378) pairwise comparisons made between populations for differences in allele frequencies summed across all 10 loci. Fewer than one-third (92) of these comparisons were not significant ($p \geq 0.00014$ after having adjusted for multiple comparisons; Table V). The non-significant results included all 91 (13+12+11….+1=91, Table V) pair-wise comparisons among the Kitimat River year classes and the comparison between the Copper and Yakoun rivers from the Queen Charlotte Islands.

Table V. Fst estimates for individual regions by locus overall in the lower triangular matrix. In the upper triangular matrix, the "NS" indicates a non-significant Fst value or monomorphic locus. Overall values were judged to be significant based on the Bonferroni procedure ($p \geq 0.00014$) (Rice 1989). Sample sites correspond to Population number in Table II.

	pop1	pop2	pop3	pop4	pop5	pop6	pop7	pop8	Pop9	Pop10	Pop11	pop12	Pop13	Pop14	pop15	pop16	pop17	pop18	pop19	pop20	pop21	pop22	pop23	pop24	pop25	pop26	pop27
pop1		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*	*	*	*	*	*	*	*	*	*	*	*
pop2	0.002		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*	*	*	*	*	*	*	*	*	*	*	*
pop3	0.008	0.010		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*	*	*	*	*	*	*	*	*	*	*	*
pop4	0.005	0.002	0.008		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*	*	*	*	*	*	*	*	*	*	*	*
pop5	-0.010	-0.004	0.002	-0.001		NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*	*	*	*	*	*	*	*	*	*	*	*
pop6	-0.002	0.020	0.019	0.018	0.006		NS	NS	NS	NS	NS	NS	NS	NS	*	*	*	*	*	*	*	*	*	*	*	*	*
pop7	-0.009	0.006	0.012	0.005	-0.011	0.015		NS	NS	NS	NS	NS	NS	NS	*	*	*	*	*	*	*	*	*	*	*	*	*
pop8	-0.007	0.001	-0.002	-0.005	0.001	0.003	-0.001		NS	NS	NS	NS	NS	NS	*	*	*	*	*	*	*	*	*	*	*	*	*
pop9	-0.005	0.007	0.029	0.014	0.002	-0.001	0.008	0.006		NS	NS	NS	NS	NS	*	*	*	*	*	*	*	*	*	*	*	*	*
pop10	0.004	0.013	0.031	0.008	0.002	0.012	0.019	0.034	0.003		NS	NS	NS	NS	*	*	*	*	*	*	*	*	*	*	*	*	*
pop11	0.002	0.033	0.033	0.031	0.016	-0.012	0.021	0.010	0.016	0.021		NS	NS	NS	*	*	*	*	*	*	*	*	*	*	*	*	*
pop12	-0.003	0.000	0.002	0.002	-0.007	0.021	-0.006	-0.005	0.018	0.016	0.014		NS	NS	*	*	*	*	*	*	*	*	*	*	*	*	*
pop13	0.002	0.005	0.018	0.006	-0.008	0.018	0.001	0.003	0.007	0.013	0.023	0.004		NS	*	*	*	*	*	*	*	*	*	*	*	*	*
pop14	0.006	0.002	-0.001	-0.010	-0.001	0.009	0.010	-0.007	0.024	0.015	0.017	-0.003	0.011		*	*	*	*	*	*	*	*	*	*	*	*	*
pop15	0.079	0.081	0.081	0.083	0.075	0.091	0.084	0.089	0.108	0.074	0.081	0.073	0.090	0.074		*	*	*	*	*	*	*	*	*	*	*	*
pop16	0.056	0.061	0.048	0.061	0.052	0.062	0.058	0.057	0.079	0.066	0.064	0.053	0.070	0.048	0.016		*	*	*	*	*	*	*	*	*	*	*
pop17	0.113	0.130	0.141	0.129	0.116	0.136	0.121	0.117	0.128	0.146	0.126	0.124	0.128	0.129	0.126	0.081		*	NS	*	*	*	*	*	*	*	*
pop18	0.096	0.111	0.112	0.102	0.093	0.124	0.097	0.106	0.123	0.119	0.119	0.094	0.109	0.101	0.087	0.053	0.042		*	*	*	*	*	*	*	*	*
pop19	0.117	0.133	0.130	0.116	0.112	0.132	0.127	0.115	0.146	0.140	0.131	0.121	0.131	0.108	0.120	0.068	0.021	0.033		*	*	*	*	*	*	*	*
pop20	0.138	0.149	0.144	0.135	0.128	0.142	0.149	0.139	0.166	0.165	0.161	0.146	0.145	0.131	0.137	0.081	0.056	0.062	0.023		*	*	*	*	*	*	*
pop21	0.405	0.418	0.482	0.442	0.445	0.524	0.449	0.496	0.482	0.502	0.530	0.430	0.368	0.477	0.419	0.370	0.409	0.381	0.432	0.389		*	*	*	*	*	*
pop22	0.338	0.331	0.346	0.327	0.335	0.424	0.337	0.376	0.421	0.367	0.414	0.319	0.306	0.327	0.298	0.290	0.405	0.336	0.380	0.375	0.530		*	*	*	*	*
pop23	0.159	0.169	0.181	0.158	0.146	0.183	0.166	0.159	0.194	0.162	0.178	0.163	0.158	0.146	0.141	0.128	0.183	0.156	0.156	0.153	0.422	0.292		*	*	*	*
pop24	0.368	0.363	0.389	0.377	0.370	0.460	0.369	0.426	0.441	0.402	0.457	0.366	0.336	0.391	0.346	0.326	0.394	0.349	0.401	0.375	0.495	0.219	0.359		*	*	*
pop25	0.374	0.383	0.417	0.386	0.374	0.444	0.391	0.418	0.428	0.397	0.441	0.391	0.352	0.389	0.359	0.348	0.365	0.336	0.360	0.332	0.596	0.496	0.279	0.468		*	*
pop26	0.517	0.517	0.620	0.559	0.555	0.666	0.578	0.613	0.626	0.624	0.654	0.548	0.448	0.589	0.527	0.503	0.553	0.545	0.591	0.561	0.758	0.651	0.465	0.664	0.566		*
pop27	0.174	0.184	0.174	0.179	0.158	0.187	0.179	0.179	0.197	0.172	0.177	0.179	0.174	0.168	0.151	0.126	0.172	0.148	0.149	0.125	0.364	0.279	0.092	0.313	0.232	0.436	

Calculation of Cavalli-Sforza genetic chord distances corroborated with results obtained from tests for pair-wise genetic differences. The most genetically divergent populations were Canyon Creek and 01201LNTH Lake with a genetic distance of 0.758. In contrast, the least genetically divergent populations were those of Kitimat River year classes where genetic distances ranged from –0.001 to 0.031. The most divergent years classes (populations) among the Kitimat River samples were 1987 and 2001 (both post-hatchery years) with a genetic distance of 0.031, but they were not found to be significantly different from another (see above).

The Neighbour-Joining (N-J) generated tree demonstrated groupings of populations that corresponded to geographic proximity, i.e. four main groups were resolved; Kitimat River year classes, Vancouver Island, Queen Charlotte Islands, and Central-Interior (Figure 4). No striking distinctions with high bootstrap support, however, were found to distinguish a potential influence of hatchery supplementation, e.g. more genetic drift in hatchery versus non-hatchery years. All Kitimat River populations grouped closely with one another, particularly 1993 and 1983, and the 1984 and 1992 year classes. Samples within these tight clusters included before and after hatchery supplementation years, where 96 and 84 times out of 100 the paired year classes clustered together, respectively (Figure 4). There was variation among years, but there was no discernible pattern that would indicate an effect of hatchery stocking.

Testing for potential bottlenecks indicated that neither pooled year-classes nor pre-post-hatchery populations had undergone recent bottleneck (all loci fit the TPM-model (=Two-Phased Model of mutation) mutation drift equilibrium, Wilcoxon tests, $p > 0.0654$).

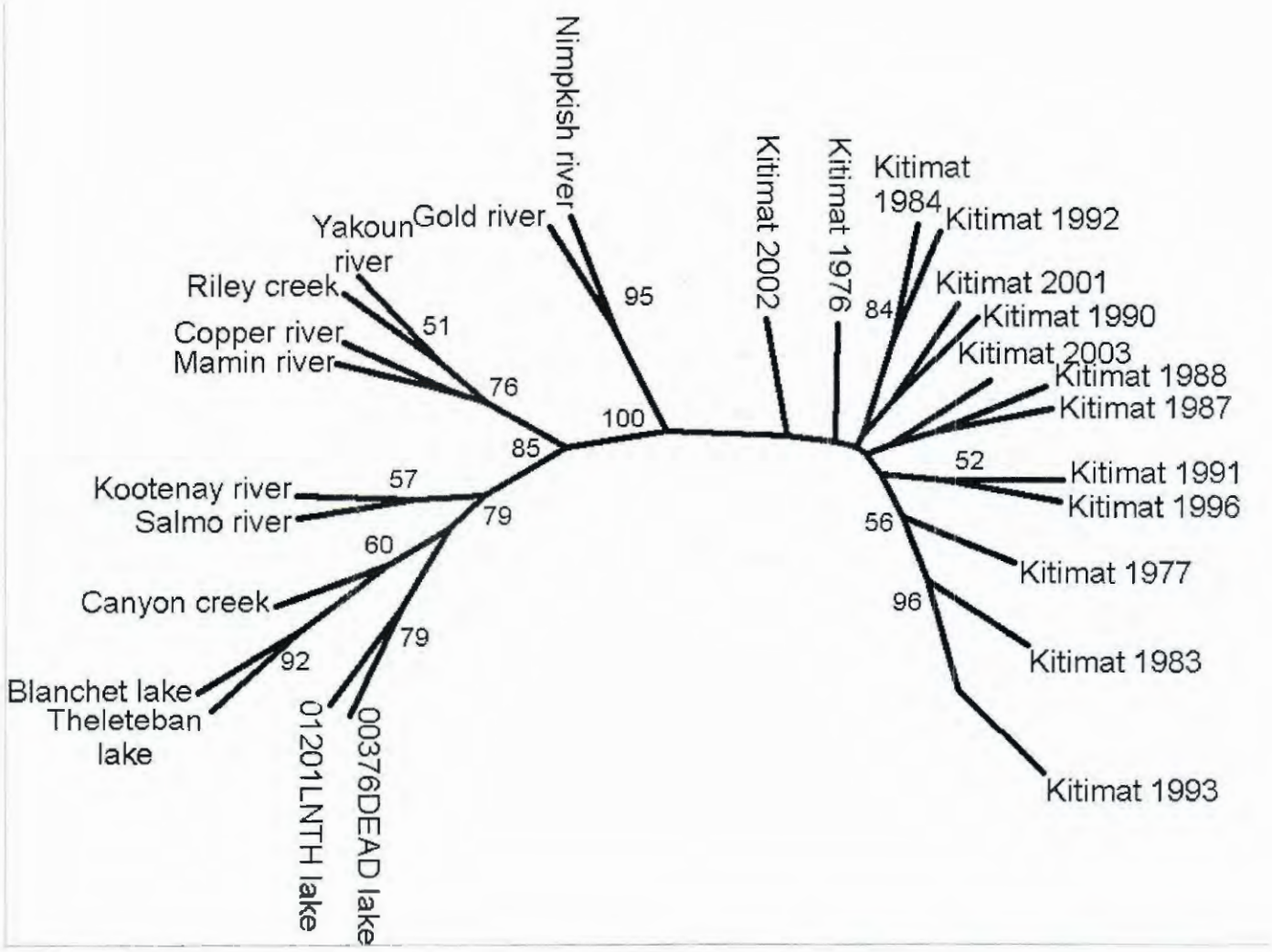

Figure 4. Neighbour-joining tree based on Cavalli-Sforza and Edward's (1967) chord distances calculated in PHYLIP. Bootstrap values greater than 50% are labeled. Note that the cladogram indicates clustering pattern and distances are not to scale.

Spatial ordination of samples using PCA (Figure 5) on the microsatellite allele frequencies also indicated similar groupings of populations, and did not indicate any striking differences between samples collected before and after hatchery supplementation. Similar to data presented in Figure 4, all Kitimat River year classes grouped closely together (Figure 5).

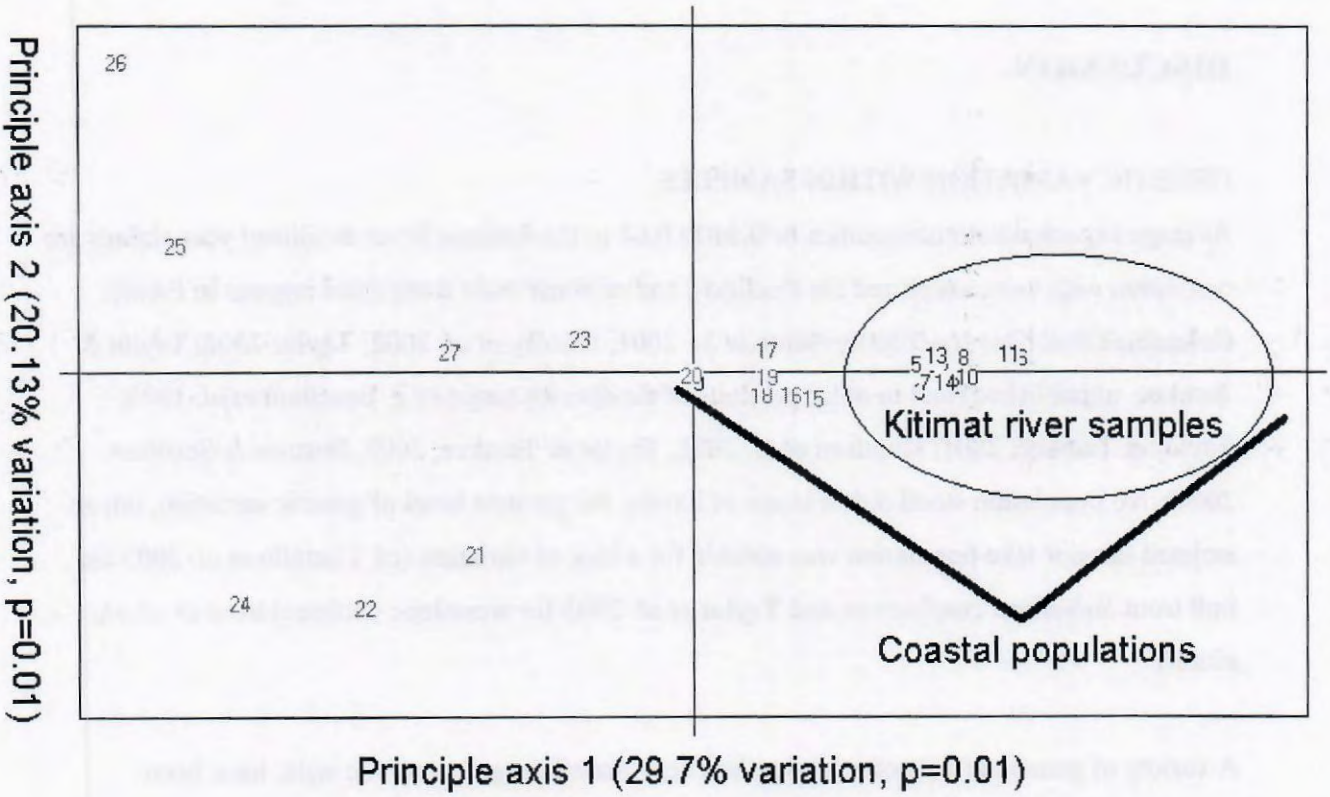

Figure 5. Principal component analysis based on allele frequency data to summarize genetic differentiation among all samples. The analysis summarizes the variation across 10 loci (154 alleles) explaining the differentiation of individual populations at each axis. Sample site corresponds to Population number in Table II. Distances are to scale.

Both neighbor-joining tree and PCA suggested an interior and a coastal group of *O. mykiss*. This is clearly seen in the PCA analysis where samples along PC axis 1, which alone accounts for

almost 30% of the allele frequency variation, were divided into these two groups (Figure 5). The Kitimat River populations grouped closely with the other coastal populations (Queen Charlotte Islands and Vancouver Island), and were separated from the interior samples (Kootenay River, 000376DEAD Lake, 01201LNTH Lake, Theleteban Lake, Salmo River, Blanchet Lake, and Canyon Creek; Figures 4 and 5).

DISCUSSION

GENETIC VARIATION WITHIN SAMPLES

Average expected heterozygosities of 0.51 to 0.61 in the Kitimat River steelhead year classes are consistent with values reported for steelhead and rainbow trout from other regions in British Columbia (Beacham *et al.* 2000; Heath *et al.* 2001; Hendry *et al.* 2002; Taylor 2002, Taylor & Tamkee, unpublished) and in other portions of the species range (e.g. Beacham *et al.* 1999; Taylor & Tamkee, 2001; Knudsen *et al.* 2002; Taylor & Tamkee, 2003, Bartron & Scribner 2004). No population stood out in terms of having the greatest level of genetic variation, but an isolated interior lake population was notable for a lack of variation (cf. Costello *et al.* 2003 for bull trout *Salvelinus confluentus* and Taylor *et al.* 2003 for westslope cutthroat trout *O. clarki clarki*).

A variety of genetic effects of releasing hatchery-reared progeny into the wild, have been reported (e.g. Utter 1998, Brannon *et al.* 2004). Most such studies, however, involve release of non-native stocks of fish (e.g. Chilcot 2003, Kostow *et al.* 2003). For example, in a previous study, Taylor & Tamkee (2003) demonstrated that hatchery samples of *O. mykiss* portrayed higher levels of genetic variation than stocked/indigenous populations possibly due to the mixing of different donor populations as a source for hatchery brood stock (see also Bartron & Scribner 2004). Similar results have been reported for other *Oncorhynchus* species (Nielsen *et al.* 1994, Brannon *et al.* 2004). By contrast, the unusual situation in the present study is that from our stocking records, Kitimat River steelhead brood stock was always collected annually from indigenous unclipped fish, i.e. presumably wild steelhead from a lineage dating back to pre-

hatchery enhancement, or in the 'worst' case second generation hatchery fish, either a mixture from both hatchery and wild fish spawning, or possibly F1 feral off-spring from hatchery X hatchery matings that had naturalized in the stream environment, smolted and returned as adults. Therefore one would expect that after hatchery supplementation no new genetic variation, e.g. non-native alleles, would be present. In concordance with this, we detected little change in genetic variation in Kitimat River steelhead over the years, or before and after enhancement started.

Depending on number of brood fish used, however, the artificial spawning and release of cultured fish into the stream from local brood stock may have negative effects on genetic variation in natural fish populations through changes in allele frequencies and loss of rare alleles via random sampling error and genetic drift due to a large number of returning progeny from a small number of parents interbreeding with the wild population.. Careful planning and implementation of brood stock collection, i.e. enough individuals (minimum effective population size N_e equal to or larger than 50; e.g. Caughley & Gunn 1996, Waples 2004) and representative of local natural population structure, is therefore crucial, as is the number of effective hatchery spawners. In a mixed wild and hatchery spawning population like in the Kitimat River, the wild spawners will buffer such potential negative effects.

GENETIC DIFFERETIATION AMONG SAMPLES IN TIME

Examination of microsatellite variation demonstrated little among-year-class variability in Kitimat River adult steelhead, as compared to considerable divergence among the total British Columbia population samples analyzed. The relatively few studies that have addressed this question, tend to indicate that natural salmonid populations appear to be genetically stable when sampled over time, for example in brown trout (Hansen 2002) and Atlantic salmon (Nielsen *et al.* 1999, Garant *et al.* 2000). Paralleling our results from Kitimat River, Heath *et al.* (2002) found little change in genetic diversity and structure over 40 years in three wild steelhead populations from a neighbouring British Columbia watershed, the Skeena River. They reported, however, considerably more among-year variation than we found in the Kitimat River (F_{ST} ranged from

0.028 to 0.056; Heath *et al.* 2002). Bartron & Scribner (2004) found an increase in genetic variation with time for Michigan Lake steelhead, but this was caused by recent introductions of additional hatchery strains. Garant *et al.* (2000) also reported a substantial temporal component to genetic variation in Atlantic salmon, and found the component of genetic variance attributable to either temporal instability and/or random sampling errors to be almost three times more important than the pure spatial component. Consequently, if the strength of the signal (population structure) is not considered in relation to the background 'noise' (e.g. small sample sizes, different age-classes of spawners returning from multiple years of reproduction), overestimation of genetic sub-structuring in situations of weak genetic differentiation may occur (Garant *et al.* 2000); this suggests that some caution is required, when interpreting results like we have for the Kitimat River. This corroborates the inference that there is little effect of hatchery operation on genetic structuring of steelhead in the river.

Hendry *et al.* (2002) collected samples from steelhead trout through one migratory season (July 2 – September 30) in the Dean River, British Columbia, and reported highly significant genetic differences between early and late run fish. This suggested genetically differentiated and isolated populations, although morphological data that they also collected from the same fish at the same time did not show any differences. Our data from Kitimat River were collected within a much shorter time period, during the month of April. One interesting aspect of the Dean River study, is the small magnitude of the genetic differences (F_{ST} = 0.007), comparable to what was observed among years in Kitimat River results. The Dean River study also showed differences much smaller than reported in most other studies of *O. mykiss* (see Hendry *et al.* 2002 and references therein, and Appendix II). This may in part be explained by the smaller spatial scale studied, and Hendry *et al.*'s results (2002) were mainly based on results from one microsatellite loci (*Sfo8*), which was not used in our study or in similar ones (Beacham 2004, Heath *et al.* 2001, 2002). Bartron & Scribner similarly found small inter-population genetic differences in Michigan Lake steelhead (F_{ST} = 0.002-0.006) which originate from one or few hatchery (since 1983) populations.

The level of genetic differentiation among Kitimat River samples is not likely to differ greatly over time (e.g. among years and populations before and after hatchery implementation), unless N_e of the existing wild stock is small relative to the numbers of hatchery fish being stocked. This

would cause a situation which could result in genetic drift if relatively large numbers of returning hatchery-produced siblings of similar genotypes interbreed with the few wild fish. In most years hatchery brood stock numbers have been close to or less than 20 of each sex and in all years N_e of fish spawned in the hatchery is less than 50, i.e. the theoretical minimum number recommended to maintain 99% of genetic variation per generation (e.g. Caughley & Gunn 1996). In the mixed wild-hatchery spawning population, however, wild fish also contribute to increase the effective population, and thus buffer potential loss of genetic diversity.

Although we did not detect any substantial changes in genetic variation over the time period sampled, the annual number of hatchery brood stock collected appear to be small based on theoretical assumptions. For our study there were some indications that allelic richness has been reduced over time, which might be expected under the current hatchery management regime, i.e. relatively few brood stock fish. We suggest the number of brood stock never be less than 25 males and 25 females of wild fish, and preferably higher. Waples (1990) suggested a N_e in the order of 100 for minimum viable populations in Pacific salmon, but such estimates are complex (Ford 2004, Waples 2004). We have less knowledge about rare alleles, which are prone to be lost in small populations over time through stochastic events. There may also be a delayed cumulative negative effect on genetic variation, if gradually more and more of the presumed wild fish (unclipped) are second generation hatchery fish, aggravating initial potential random sampling error. A potential cumulative effect may be further delayed if hatchery fish contribute less relative to the reproduction for example due to high harvest rates or reduced survival (e.g. Blouin 2003, Chilcote *et al.* 1986, Chilcote 2003, Fleming *et al.* 2000, Kostow *et al.* 2003). Because we sampled returning adult fish, it may appear likely that such potential future changes would have been reflected in our results over the 15-year post-hatchery period that this study covered. Nevertheless, it must also be recognized that this covers only about three generation intervals for wild fish (the majority of Kitimat River steelhead are age 3-2 fish, Chudyk *et al.* 1977), and unclipped fish were always selected for brood stock. We therefore suspect that even though there may not be significant genetic differentiation due to genetic drift, allelic richness will be slowly reduced in years to come, unless the number of brood stock is increased. The contribution by wild spawners has and will buffer against this, as does the presence of multiple years classes in the spawning population. Although most wild steelhead in Kitimat River are age 3.2 fish

(27.3%), 4.2 (16.4%), 4.3 (13.6%), 3.2S1 (14.5%) and 3.3 (10.0%) comprise significant proportions of the fourteen year classes present (Chudyk *et al.* 1977).

The only previous studies we are aware of which directly investigated the questions relevant to the Kitimat River results were undertaken on the Hood River, Washington, USA. Based on DNA studies, Blouin (2003) reported that traditional 'old' domesticated hatchery stocks (multiple generations in the hatchery, out-of-basin origin) of steelhead had shown much lower total fitness than wild fish, while 'new' conservation based hatchery stocks (i.e. only wild brood stock were used each year as for the Kitimat River, had fitness similar to that of wild fish. This contrasts with results in Kostow (2004), where juvenile local hatchery steelhead exhibited poorer survival than wild fish, probably for environmental reasons. This will, however, lead to modified selection and potential genetic changes. Based on results in Blouin (2003), an ongoing project in the Hood River is currently testing the prediction that using wild brood stock for hatchery production will not have negative genetic effects on the wild population. The results from the Kitimat River, where 'new' conservation based hatchery stocks have always been practiced, since its start in 1984, appear to confirm this prediction.

If hatchery fish have low ocean survival and/or high harvest mortalities compared to wild fish, few hatchery fish will contribute to reproduction. Any genetic effect of hatchery fish on a combined captive-wild population is a function of the effective sizes of the hatchery and wild breeding phases (Ryman & Laikre 1991, Waples 2004). There are no direct estimates of number of wild compared to number of hatchery spawners in the Kitimat River. Catch statistics (Figure 6, 7), however, clearly indicate a high number of returning hatchery steelhead. Furthermore, the catch-effort data from the brood stock fishery indicate about equal numbers of wild and hatchery fish in the spawning population in the lower mainstem (Table I, Figure 6, 7). It appears likely, therefore, that any detectable substantial genetic effects of hatchery operation would be reflected in the material analyzed in this study, but small cumulative effects may not.

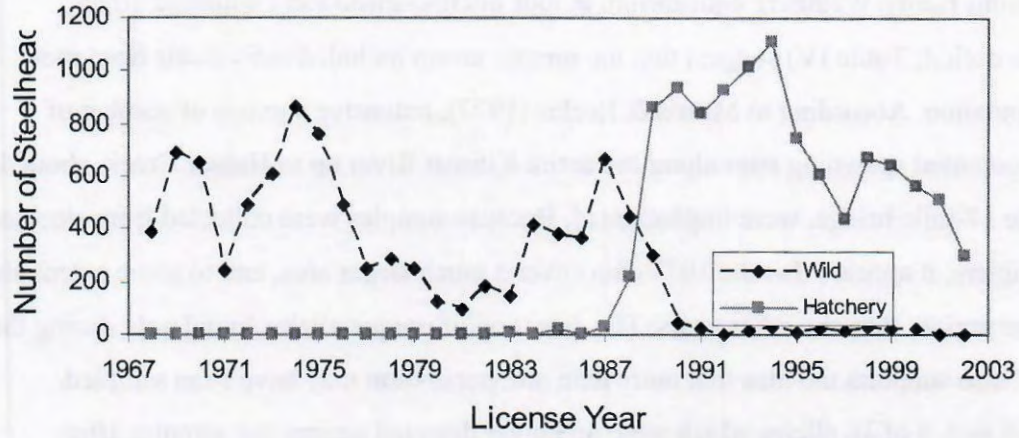

Figure 6. Kitimat River steelhead harvest based on steelhead harvest analysis (SHA) data 1968-2002.

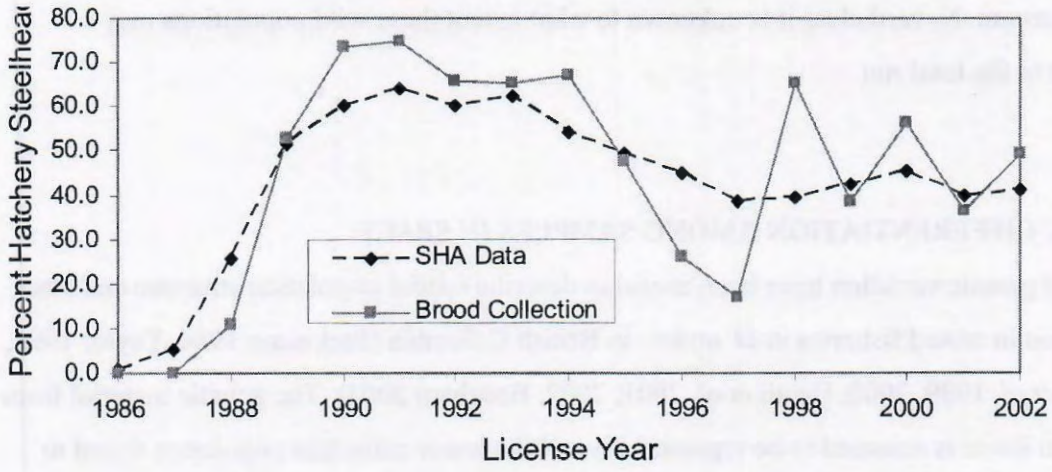

Figure 7. Percent hatchery of total steelhead (wild and hatchery) captured in the Kitimat River as reported by the steelhead harvest analysis (SHA) and brood collection 1986-2002. (SHA = steelhead harvest analysis, see Figure 6)

How much total natural recruitment contributes in relation to hatchery fish recruitment in the Kitimat River is unknown, but further studies of upstream and tributary populations may give an indication. Samples from Kitimat River were generally in Hardy-Weinberg equilibrium with the

notable exception for the year 1977. This was also the largest sample year for the natural population before hatchery operation, with 91 analyzed samples, i.e. more than in any other year. Deviations from Hardy-Weinberg equilibrium at four microsatellite loci (Wahlund effect, heterozygote deficit; Table IV) suggest that the sample group included individuals from more than one population. According to Morris & Eccles (1977), extensive surveys of number of anglers and potential spawning sites along the entire Kitimat River up to Hunter Creek, about 45 km above the 17-mile bridge, were implemented. Because samples were collected from steelhead caught by anglers, it appears that the 1977 data cover a much larger area, and to some extent also a greater time period, than the other years. The detection of unique alleles found only during the year of 1977 also supports the idea that more than one population may have been sampled. Among all 10 loci, 8 of 16 alleles which were no longer detected among our samples after hatchery belonged to some individuals from 1977. Indeed, 7 of the 8 alleles undetected later on were from 1977 alone. Following 1977, the absence of these unique alleles may be explained by the reduction of sampling area to specific sites below the 17-mile bridge. The genetic results therefore indicate that there are possibly more wild populations in the Kitimat River system. Furthermore, these stocks most likely are unaffected by the hatchery stocking of steelhead in the lower mainstem. Nevertheless it is unknown to what extent these wild populations may contribute to the total run.

GENETIC DIFFERENTIATION AMONG SAMPLES IN SPACE

Surveys of genetic variation have been useful to describe spatial population structure and stock composition in mixed fisheries in *O. mykiss* in British Columbia (Parkinson 1984, Taylor 1995, Beacham *et al.* 1999, 2000, Heath *et al.* 2001, 2002, Beacham 2004). The genetic material from the Kitimat River is assumed to be representative of the lower mainstem population found to occur there in April. Whether there is greater population structuring related to natural populations in the different tributaries and further upstream, we do not know, as samples have not yet been collected. The more extensive data from 1977 indicate that there is more complex population structuring in the Kitimat River and tributaries (above). Further research should focus on discriminating other possible wild populations in the tributaries and upper watershed

Pooled across loci, all the sample populations examined in this study (n= 27) of *O. mykiss* were highly divergent from one other with a level of subdivision (θ) averaged among all populations at about 0.23. This is higher than found in previous steelhead studies (0.007 – 0.07; Hendry *et al.* 2002 and references therein, Beacham 2004), but slightly lower than those found in a previous study of rainbow trout in Alberta (0.31; Taylor & Tamkee 2003), where divergence among populations was considerably higher than reported for rainbow trout introduced into Lake Ontario (θ = 0.012; O'Connell *et al.* 1997). Stronger isolation (lakes, watersheds) is one likely reason for higher divergence in rainbow trout compared to steelhead (marine phase, highly migratory and having the opportunity to (re)colonize watersheds). Also, our study covered almost the entire geographic range of *O. mykiss* in BC, whereas the other studies were geographically more localized.

Our estimates of θ, however, were also lower than those of other native salmonid species throughout British Columbia including rainbow trout, bull trout (*Salvelinus confluentus*), and westslope cutthroat trout (*Oncorhynchus clarki lewisi*) where θ was 0.39, 0.33 and 0.32, respectively (Costello *et al.* 2003; Taylor *et al.* 2003). This may in part be explained by the influence of analyzing each individual Kitimat River year class as individual populations rather than pooling them into one population. The level of subdivision among the Kitimat River steelhead year classes was lower at 0.005 (95% C.I. 0.001-0.011) and consequently downwardly biased the overall level of subdivision among all populations. After pooling the different year classes into one Kitimat River population, and omitting the 1977 year class, the level of population subdivision increased to θ = 0.269 (95% CF 0.228 – 0.314), i.e. more comparable to other native salmonids in British Columbia.

In conclusion, the results from the Kitimat River indicate little genetic differentiation among the studied year classes, or between pre and post hatchery populations. Likewise, pairwise testing did not indicate any significant trends or changes. Compared to other relevant studies, there is little indication to date that hatchery stocking of steelhead trout in the Kitimat River until now has had any substantial genetic effects, at least as assayed using microsatellite DNA variation. The presence of a substantial number of wild fish and multiple year classes in the mixed spawning population tend to buffer potential negative effects. Nevertheless, as a cautionary note our

observations suggest a small reduction in genetic variation expressed as allelic richness. This may increase with time and should be monitored in the future now that a baseline characterization of the population structure has been completed. Furthermore, as a precautionary step, it is recommended that the annual hatchery brood stock should never be less than 25 wild males and 25 wild females, and preferably more. Our data also suggest there is as yet unresolved population substructure within the Kitimat River that may represent distinct wild steelhead populations that may show different responses to hatchery stocking. Further studies are needed to investigate this in order to conserve these potentially unique genotypes.

Acknowledgments

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APPENDIX I

Microsatellite loci included in the Kitimat Steelhead study: annealing temperatures, primer concentrations and volumes, and PCR conditions.

SINGLE AND MULTIPLEX PCR

There were a total of 10 microsatellite loci used in this study. To conduct microsatellite DNA detection, the Beckman CEQ 8000 was used. DNA from individual samples were amplified by Polymerase Chain Reaction (PCR) with microsatellite forward primers labelled with Beckman dye (D2, D3 or D4). There were some microsatellite loci that were amplified simultaneously with two other loci (triplex), with one other locus (diplex), or by itself (single). PCR annealing temperatures (Ta) also varied depending on the loci combinations (multiplexes).

	Beckman dye	Loci	Source species	Reference	Ta (C)
Triplex	D3	Ots100	*Oncorhynchus tshawytscha*	Nelson and Beacham (1999)	
		Ots103	*Oncorhynchus tshawytscha*	Nelson and Beacham (1999)	56/55
		Ssa85	*Salmo salar*	O'Reilly et al. (1996)	
Diplex	D3	Ssa456	*Salmo salar*	Slettan et al. (1995)	56/55
		Omy77	*Oncorhynchus mykiss*	Morris et al. (1996)	
	D4	Ots3	*Oncorhynchus tshawytscha*	Banks et al. (1999)	52/50
		Okia3	*Oncorhynchus kistuch*	P. Bentzen, Dalhousie U.	
	D4	Ssa197	*Salmo salar*	O'Reilly et al. (1996)	62/60
		Oneu14	*Oncorhynchus nerka*	Scribner et al. (1996)	
Single	D2	Oneu8	*Oncorhynchus nerka*	Scribner et al. (1996)	58/56

PCR amplification was performed using PTC-100 (MJ Research) thermal-cycler under optimal annealing conditions for each single or multiplex PCR (above). Each PCR profile consisted of [5X (95°C /1 min, Ta/ 1 min, 72°C /1 min), 30X (94°C / 1 min, Ta/ 1 min, 72°C / 1 min), and 1X (72°C / 5min)], where Ta are the annealing temperatures respectively (above).

Single and Multiplex PCR Primer Concentrations

For each multiplex or single PCR reaction, the concentrations and volumes of labeled forward primer also varied.

	Beckman dye	Loci	Concentration of labelled forward primer	Concentration of reverse primer
Triplex				
	D3	Ots100	2uM	5uM
		Ots103	0.5uM	5uM
		Ssa85	0.5um	5uM
Diplex				
	D3	Ssa456	2uM	5uM
		Omy77	2uM	5uM
	D4	Ots3	0.5uM	5uM
		Okia3	0.5uM	5uM
	D4	Ssa197	0.5uM	5uM
		Oneu14	0.5uM	
Single				
	D2	Oneu8	2uM	5uM

Each PCR reaction solution was in 10µl volumes containing 100ng DNA template, 10x reaction buffer (Gibco/BRL), 0.4mM DNTP, **XX** reverse primer, **XX** forward primer (volumes of primers are listed below corresponding with the above concentrations), 1.5mM $MgCl_2$, and 0.5 units of taq polymerase.

	Loci	Volume of labelled forward primer for each PCR reaction	Volume of reverse primer for each PCR reaction
Triplex			
	Ots100	0.4ul	0.4ul
	Ots103	0.4ul	0.4ul
	Ssa85	0.4ul	0.4ul
Diplex			
	Ssa456	0.5ul	0.5ul
	Omy77	0.6ul	0.6ul
	Ots3	0.5ul	0.5ul
	Okia3	0.6ul	0.6ul
	Ssa197	0.3ul	0.3ul
	Oneu14	0.5ul	0.5ul
Single			
	Oneu8	0.5ul	0.5ul

Microsatellite Gel separation

Following PCR, the products were diluted with autoclaved-distilled water (1:10), mixed with other PCR products from different loci and ran on separation gel to identify microsatellite size fragments. To identify microsatellite size fragments in all 10 loci, 2 different runs through separation gels were required (5 loci per separation gel). The first gel consisted of specific volumes of diluted PCR product from Omy77/Ssa456 (diplex), Ssa197/Onue14 (diplex), and Oneu8 (single). The second gel consisted of specific diluted volumes of PCR product from Ots3/Okia3 (diplex) and Ots100/Ots103/Ssa85 (triplex). The volumes of each diluted single or multiplexed PCR used to run on separation gel are listed below:

	PCR product	Volume of diluted (1:10) PCR product used in separation gel
Gel 1	Ssa456 Omy77	4ul
	Ssa197 Oneu14	2.5ul
	Oneu8	6ul
Gel2	Ots100 Ots103 Ssa85	5ul
	Ots3 Okia3	5ul

Following dilution and pooling of specific PCR products, 0.3ul of Beckman 400 base pair ladder and 40ul of Beckman Sample Loading Solution was also added for each sample. Samples were then placed in the Beckman CEQ 8000 and results were collected following product analysis.

APPENDIX II

Summary of F_{ST} values for nuclear markers in steelhead. Spatial scale: range in fluvial distance between study populations. After Hendry et al. 2002, with additional recent data.

Reference	Fst (range among loci)	Temporal/spatial sampling	# populations	Spatial scale	Genetic markers
Present study	0.005	Different years in a single river; Kitimat River, BC	1	~0-30 km	10 microsatellites
Bartron & Scribner (2004)	0.006 (0.000.0.032) 0.002 (0.000-0.007)	Tributaries in different years, hatchery populations; Michigan Lake	6, and before and after supplementation with additional hatchery populations	~30-200 km	6 microsatellites
Beacham (2004)	0.066 (0.033-0.113)	Tributaries (different years in six); Upper Skeena, BC	9	~5-540 km	13 microsatellites
Beacham *et al.* (2000)	0.026 (0.008-0.039)	Tributaries; Skeena River, BC	7	~20-300 km	8 microsatellites
Beacham et al. (2000)	0.024 (0.011-0.033)	Trbitaries, Nass River, BC	10	~20-150 km	8 microsatellites
Beacham et al. (1999)	0.076 (0.063-0.143)	Rivers into ocean with tributaries; BC & WA	22	~50-1600 km	8 microsatellites
Heath et al. (2002)	0.028-0.059	Different years in tributaries; Upper Skeena	3	~150-335 km	7 microsatellites
Heath et al. (2001)	0.039	Three watersheds with tributaries; Northern BC	10	~80-850 km	6 microsatellites
Hendry et al. (2002)	0.007 (0.0.034)	Different times in a single run; Dean River, BC	2	~20-60 km	10 microsatellites
Nielsen & Fountain (1999)	0.01 (0.00-0.33)	Seasonal races in one river; Middle Fork Eel River, CA	2	<100 km (?)	16 microsatellites
*Reisenbichler et al. (1992)	0.015	Watersheds with tributaries; CA, WA, OR	19	~20-420 km	8 polymorphic allozymes
*Reisenbichler & Phelps (1989)	0.015	Watersheds with tributaries; WA	27	~10-450 km	23 poloymorphic allozymes

*different technique, not directly comparable

APPENDIX 3

Tests for genetic diversity among groups of steelhead samples in the Kitimat River.

	Two-sided tests											
	All year classes			All year classes – 1977 year class			Pooled year classes			Pooled year classes – 1977 year class		
	Pre	Post	p	Pre	Post	P	Pre	Post	p	Pre	Post	p
Ar	3.890	3.748	0.0810	3.884	3.748	0.1440	5.352	5.038	0.0764	5.410	5.038	0.0772
H_O	0.606	0.555	0.2104	0.566	0.555	0.8380	0.606	0.555	0.2024	0.566	0.555	0.7994
H_S	0.605	0.591	0.3872	0.588	0.591	0.8598	0.606	0.592	0.3692	0.589	0.592	0.8604
θ	0.009	0.004	0.4156	0.004	0.004	0.9394	0.007	0.003	0.3640	0.000	0.003	0.6656
	One-sided tests											
Ar	3.890	3.748	0.0188	3.884	3.748	0.0398	5.352	5.038	0.0448	5.410	5.038	0.0366
H_O	0.606	0.555	0.1116	0.566	0.555	0.3050	0.606	0.555	0.1308	0.566	0.555	0.2888
H_S	0.605	0.591	0.1704	0.588	0.591	0.4790	0.606	0.592	0.1954	0.589	0.592	0.4216
θ	0.009	0.004	0.2308	0.004	0.004	0.4852	0.007	0.003	0.1968	0.000	0.003	0.6154