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[Article]

Genetic Diversity in Steelhead before and after Conservation Hatchery Operation in a Coastal, Boreal River

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Abstract.—Allelic variation at 10 DNA microsatellite loci was assayed in scale samples of steelhead *Oncorhynchus mykiss* ($n = 333$) collected between 1976 and 2003 from the lower main stem of the Kitimat River, British Columbia. Our objectives were to (1) investigate the genetic diversity of wild steelhead populations in the river before hatchery stocking began in 1984 (baseline samples: 1976–1977, 1983–1984; $n = 145$) and (2) assess the potential genetic impacts of interbreeding of returning hatchery adult fish with wild spawners over almost 20 years of large-scale hatchery operation (1987–2003; $n = 188$). The annual target number of wild broodstock adults used for hatchery production was 40 (20 of each sex) but varied from 9 to 39 among years. The level of population subdivision (θ) among Kitimat River samples was low (0.004) and not significantly different from 0. Tests of θ between prehatchery and posthatchery operation indicated no significant changes. Similar results were obtained using other measures of genetic differentiation (principal components analysis of microsatellite allele frequencies and Cavalli-Sforza genetic distance). Our data, however, did indicate a slight but significant reduction in allelic richness after hatchery stocking. Pairwise tests for genetic differentiation among samples from different year-classes were nonsignificant. We conclude that for the current management regime there is little apparent impact of hatchery practices on either the genetic structure or variation within the lower main-stem Kitimat River steelhead, but there may be a reduction in rare alleles. The practice of using substantial numbers of wild fish and multiple year-classes in the hatchery may have minimized genetic changes via genetic drift.

Releases of cultured fish into wild populations and their subsequent interbreeding may have genetic effects on natural fish populations, but the effects can be complex and unpredictable (e.g., Hindar et al. 1991; Utter 1998; Reisenbichler 2004). 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 nonnative fish may reproduce less successfully, presumably because they are not well adapted to the new, local environments (e.g., Chilcote 2003; Kostow et al. 2003; McLean 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 nonnative fish, hatchery-reared fish, or both with the wild populations (e.g., Chilcote et al. 1986; Reisenbichler and Rubin 1999; 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 programs attempt to ensure that the broodstock comes from the local (wild) populations and are collected annually in certain numbers to guard against genetic drift (e.g., Adkison 1995; Caughley and Gunn 1996). A major concern is to conserve the population structure and diversity of wild fish populations, but there is also a demand for hatchery production with its potential for altered directional

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or ''relaxed'' selection (Lynch and O'Hely 2001; Ford 2002). Therefore, an understanding of which management regimes do not result in genetic drift or artificial selection, and under particular ecological conditions, is of considerable scientific and management interest (Brannon et al. 2004; Reisenbichler 2004).

Steelhead (anadromous rainbow trout *Oncorhynchus mykiss*) is an ecologically variable species (Behnke 1992) and shows considerable genetic variation among populations (Busby et al. 1996; Beacham et al. 1999; Heath et al. 2001, 2002; Hendry et al. 2002). Steelhead from the western 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* spp.: Nielsen et al. 1994; Reisenbichler and Rubin 1999; Reisenbichler 2004; *Salmo* spp.: Garcia-Marin et al. 1999; Fleming et al. 2000; Hansen et al. 2000; *Salvelinus* spp.: Englbrecht et al. 2002). Direct genetic effects are documented through interbreeding when nonnative 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, enhanced predator populations, population fragmentation, and local extinctions (Utter 1998; Myers et al. 2004). Natural selection may work against stocked trout and ''hybrids'' (e.g., Poteaux et al. 1998; Hansen et al. 2000; Miller et al. 2004). It is, however, unclear what ecological conditions may cause differential natural selection. Human selection in the form of angling may 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 similar studies (e.g., Hansen 2002; Kostow et al. 2003; Taylor and Tamkee 2003).

The controversy over the role of hatcheries in restoring populations (Brannon et al. 2004; Myers et al. 2004; Reisenbichler 2004) is largely based on data from releases of nonnative or hatchery fish. Considerably less is known, however, about potential genetic effects when local fish populations are enhanced through the use of annually collected native broodstock which are naturally reared in the wild (i.e., ''conservation'' hatcheries [Blouin 2003; Kostow 2004]). Theoretically, an effective population size of 50 individuals in a parental generation is required to retain 99% of the original heterozygosity (e.g., Caughley and Gunn 1996), although allele frequencies may change and rare alleles may be lost.

Because of relatively long histories of considerable stocking through the use of wild, native broodstock, a number of watersheds in British Columbia (BC) can be regarded as long-term genetic field experiments. In the coastal Kitimat River of the Skeena Region, BC (Figure 1), hatchery steelhead smolts of known numbers and origin have been released annually since 1984 (Table 1). Scales have been collected in various years from wild fish and hatchery broodstock (Table 1). 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. The objectives of our study, therefore, 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 impact, if present, on genetic structure and molecular variation of almost 20 years of conservation hatchery operation.

Study Site

The Kitimat River steelhead hatchery, on the northern coast of BC (Figure 1), began operation in 1984. The number of smolts released annually over this time was 50,932 (SD = 8,107) but ranged from 34,420 (1992) to 64,297 (1998) fish (Table 1). All released smolts (1-year-old) had their adipose fins clipped and weighed an average of 72 g (SD = 7.2; range, 52–81) at the time of release (May). All releases of smolts have been at the same seven localities in the lower Kitimat River (Figure 1), plus one to two additional sites in occasional years. Broodstock parents are considered to be wild steelhead because all have had intact adipose fins.

The Kitimat River (Figure 1) drains approximately 217,000 ha of a wide, glaciated valley that has been extensively logged. Therefore, water discharge ranges widely from 19.4 to 1,670.7 m^3/s; mean annual discharge is 148.8 m^3/s and can vary dramatically over a short period of time. The river has a fish fauna that includes Chinook salmon *O. tshawytscha*, coho salmon *O. kisutch*, pink salmon *O. gorbuscha*, chum salmon *O. keta*, sockeye salmon *O. nerka* and kokanee (lacustrine sockeye salmon), winter and summer run steelhead including nonanadromous rainbow trout, coastal cutthroat trout *O. clarkii clarkii*, Dolly Varden *Salvelinus*

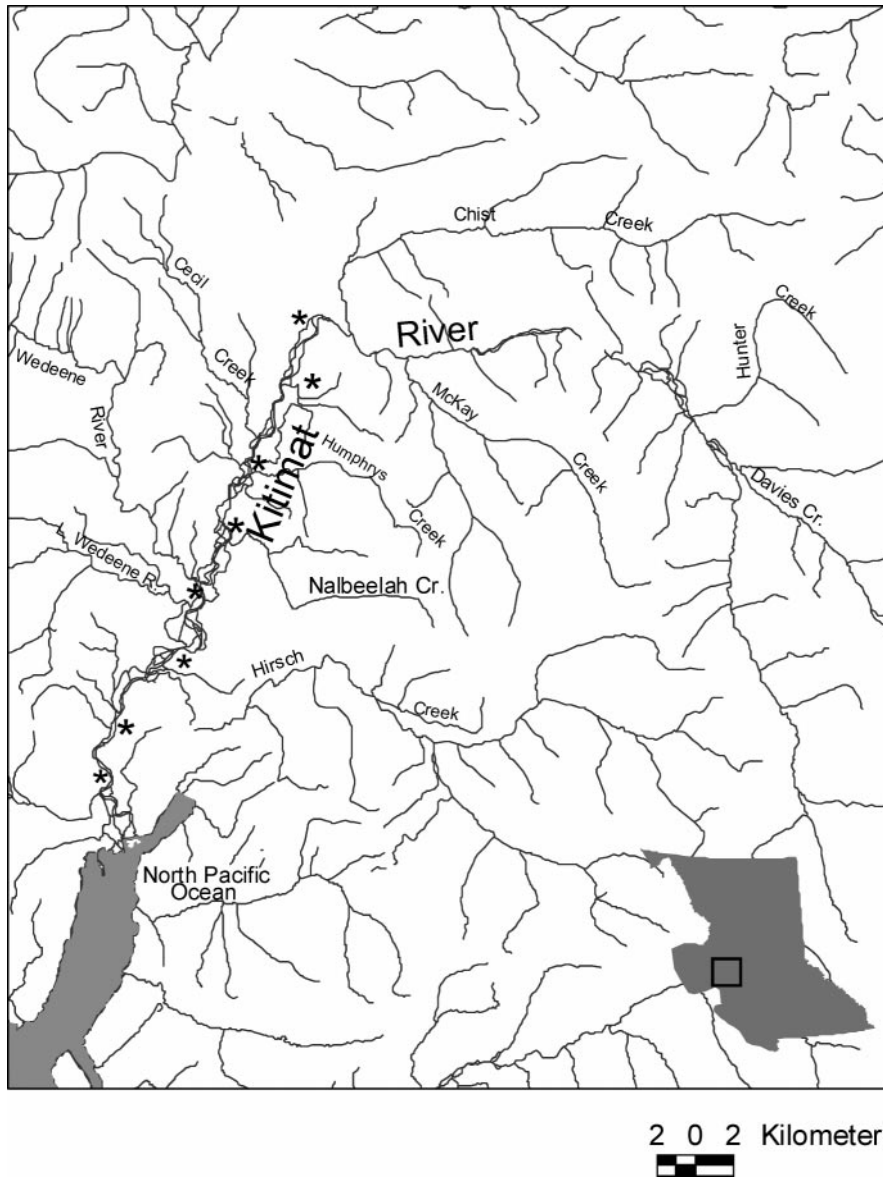

FIGURE 1.—Map of the Kitimat River study area on the northwestern Pacific coast of Canada. Stars indicate stocking sites in the lower main stem of the Kitimat River; R. = river, Cr. = creek.

malma, threespine stickleback *Gasterosteus aculeatus*, prickly sculpin *Cottus asper*, Pacific staghorn sculpin *Leptocottus armatus*, eulachon *Thaleichthys pacificus*, mountain whitefish *Prosopium williamsoni*, and Pacific lamprey *Lampetra tridentata*.

Methods

Field sampling.—The steelhead adults used in this study for scale samples and, starting in 1984, for broodstock (Table 1) were collected by sportfishing in the main stem of the river from the 17-mi bridge (37 km; Figure 1, uppermost release site) and downstream (Figure 1). Collection started between March 28, 1988, and April 16, 1984, and the last day of brood capture ranged from April 19, 1985, to May 11, 2000 (Table 1). For most years, broodstock capture was completed within one week. Fish were sampled along the entire reach of the Kitimat River from the ocean to the

TABLE 1.—Number of adult steelhead broodstock scale samples used in analysis, timing of broodstock capture period, number of wild brood angled (unclipped), number of incidental hatchery fish angled (clipped), and number of smolts produced by brood year. Data are from the sampling period 1976–2002 in the Kitimat River, British Columbia.

Year	Number of scale (DNA) samples	Broodstock capture period	Number of wild broodfish angled		Number of hatchery fish angled		Number of smolts produced
			Female	Male	Female	Male	
1976	23						
1977	102						
1983	30						
1984	11	Apr 16–May 2	18	11			65,143
1985		Apr 15–Apr 19	22	13			54,667
1986		Apr 14–Apr 24	20	22			54,035
1987	17	Apr 13–Apr 27	18	8			48,328
1988	15	Mar 28–Apr 28	16	9	0	3	51,355
1989		Apr 7–Apr 24	18	14	14	22	50,578
1990	22	Apr 9–Apr 20	15	14	34	46	54,481
1991	22	Apr 9–Apr 26	12	15	51	29	46,800
1992	9	Apr 7–Apr 24	12	15	20	32	34,420
1993	18	Apr 5–Apr 20	15	23	27	45	38,473
1994		Apr 6–Apr 20	15	20	29	42	47,412
1995		Apr 4–Apr 27	20	13	8	22	45,822
1996	27	Apr 10–Apr 23	15	22	3	10	57,265
1997		Apr 11–Apr 24	15	19	3	4	54,696
1998		Apr 6–Apr 26	11	7	11	23	64,297
1999		Apr 9–Apr 28	14	18	11	9	53,339
2000		Apr 10–May 11	9	5	13	5	40,147
2001	11	Apr 9–May 3	13	15	12	4	46,566
2002	39	Apr 11–May 9	13	17	15	14	59,885
2003	39						

17-mi bridge, and thus was probably spatially representative for most years. For some years, however, capture locations were not recorded. In 1977 a more extensive survey of fishermen and spawning locations along the entire river up to Hunter Creek (62 km; Figure 1) was carried out, and scale samples were collected from steelhead caught by sport anglers (Morris and Eccles 1977). Since 1988 the number of hatchery fish (adipose fin clipped) caught while fishing for broodstock was also recorded (Table 1). These catch-per-effort data suggest that spawning steelhead 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 among years and a predominance of hatchery fish from 1990 to 1994 (Table 1).

In most cases five scales were sampled from each fish, placed 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) and sex were recorded for all fish. In 2002 and 2003 both scales and tissues (about 5 mm^2 of adipose fin) were sampled, and the tissue was stored in ethanol-filled tubes in the field upon collection.

Additional wild steelhead and rainbow trout population samples from BC were used as outgroups in the present study to compare some aspects of genetic distinctiveness among populations (coastal: Nimpkish and Copper Rivers; interior: Kootenay River and Blanchet Lake; see Heggenes et al. 2004 for details).

Microsatellite DNA.—Total genomic DNA was extracted using the Qiagen DNeasy Tissue Kit (Qiagen, Inc.). After extraction, the DNA was stored at −20°C (see Heggenes et al. 2004 for standard procedure details).

Genetic variation was assayed at microsatellite DNA loci using polymerase chain reaction (PCR) with locus-specific primer pairs. Individuals were genotyped with 10 polymorphic microsatellite markers previously used for steelhead within BC (P. Tamkee and E. B. Taylor, unpublished data). To increase efficiency and minimize cost, the PCR reactions for six working microsatellite markers were run in tandem (diplex; Appendix Table A.1). The diplexes were as follows: *One*μ*14* (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 University, unpublished data; Table A.1). Also, three microsatellite markers were run together (triplex), these included *Ots100* (Nelson and Beacham 1999), *Ots103* (Nelson and Beacham 1999), and *Ssa85* (O'Reilly et al. 1996). *One*μ*8* (Scribner et al. 1996) was not multiplexed because of PCR incompatibilities with other markers. Multiplexes were created based on similar individual annealing temperatures for the loci, nonoverlapping allele sizes, and PCR amplification compatibility.

Polymerase chain reactions were carried out in 10 μL volumes containing 100 ng DNA template, 10× reaction buffer (Gibco/BRL), 0.4 mM deoxynucleotide triphosphates, 0.25 μM reverse primer, 0.025 μM forward primer, 1.5 mM $MgCl_2$, and 0.5 units of *Taq* polymerase. PCR amplification was performed in a PTC-100 (MJ Research) thermal cycler. Each PCR profile (single primer or multiplex) consisted of 5× [95°C/1 min, T_A/1 min, 72°C/1 min], 30× [94°C/1 min, T_A/1 min, 72°C/1 min], and 1× [72°C/5 min], where T_A is the annealing temperature, and each primer was labeled with individual fluorescent Beckman-Coulter dyes (Table A.1). 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 (or samples) was (or were) removed from the study. Genotypes for all microsatellite loci were visualized using the Beckman CEQ 8000 DNA analysis system.

Data analysis.—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 version 1.3 of TFPGA (Miller 1997). Allelic richness (A_r) was calculated using version 2.9.3 of FSTAT (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. It is more sensitive than heterozygosity to loss of rare alleles (Hartl and Clark 1989).

Tests for deviations from Hardy–Weinberg equilibrium, genotypic linkage disequilibrium, and pairwise population differentiation were performed using version 3.1 of GENEPOP (Raymond and Rousset 1995).

To have the greatest possible power in the analysis (Waples 1998), we tested contrasts between pooled preenhancement (1976–1986) and postenhancement (1987–2003) data, where 1987 was the first year when hatchery fish were observed in substantial numbers (Anonymous 2003). We also conducted analyses on year-classes where year-class data were pooled to increase sample size (i.e., power of tests, to a minimum of 18 individuals; 1976, 1977, 1983 pooled with 1984; 1987 pooled with 1988; 1990 pooled with 1991; 1992 pooled with 1993; 1996 and 2001 pooled with 2002; and 2003). Although some variation may occur between some of the pooled year-classes, Heath et al. (2002) reported little genetic differentiation among years in three wild steelhead populations in the adjacent watershed. Microsatellite variation was partitioned in a hierarchical fashion (analysis of molecular variance [ANOVA]; prehatchery and posthatchery, among years and within years) as implemented in version 2.0 of ARLEQUIN (Schneider et al. 2000).

Genetic differentiation among contrasts (i.e., samples from different years) were 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, with 5,000 permutations; Goudet 2002). To guard against inflated type I error rates in multiple comparisons, all critical significance levels for simultaneous tests were evaluated using the standard Bonferroni adjustment (Rice 1989) with an initial α level of 0.05.

Genetic distances among population pairs were estimated with Cavalli-Sforza and Edwards's (1967) chord distance (C-S chord distance) calculated in the Phylip software package (Felsenstein 1993). Cavalli-Sforza and Edwards's chord distances were used to build an unrooted neighbor-joining tree to visualize the genetic relationships among sites and year-classes.

A principal components analysis (PCA) was conducted on allele frequency data using PCAGEN (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 caused by low numbers of returning adults. We used the mode shift test as implemented in Bottleneck (Cornuet and Luikart 1997). The TPM (two-phased model of mutation) mode shift test assumes that the populations are near mutation drift equilibrium and are independent of the mutation model (infi-

nite alleles or stepwise mutation) for microsatellite loci (Luikart et al. 1998).

Results

Microsatellite variation across 385 Kitimat River individuals from 14 different years and at 10 microsatellite loci was assayed, and 333 individuals amplified collectable results.

Genetic Variation within Populations

The number of alleles observed across all usable Kitimat individuals ranged from 2 (*Ssa197*) to 31 (*Oki3a*) with an average of 11.5 (SD, 8.68) alleles per locus (Table A.2). Mean allelic richness (A_r) across loci and years was 3.78 (SE, 0.04) and varied among 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 (*Ots103*) to 0.89 (*Oki3a*), respectively.

There was no major loss of 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) at certain loci after hatchery implementation (16 alleles, documented 1976–1984; eight in 1977), the allele frequencies of these alleles were originally very low (i.e., rare alleles; median frequency, 0.019; 95% C.I., 0.006–0.019; range, 0.005–0.059). By contrast, new alleles were resolved after the hatchery was implemented (16 alleles, documented 1988–2003).

Expected heterozygosities (averaged across the 10 loci) in the Kitimat River (range, 0.51–0.61) are among the highest found across BC (range, 0.15–0.62). Most populations displayed relatively high levels of genetic variation. Within the Kitimat River year-classes, there were only three year-classes which had fixed alleles at a particular locus. These samples were the 1984, 1991, and 1992 fixed for the *Ots103*079* allele.

Virtually all samples in the Kitimat River were in Hardy–Weinberg equilibrium. Exceptions were found at separate loci in two different year-classes among the Kitimat River samples. Year-class 1977 in the Kitimat River, however, had four loci that were not in Hardy–Weinberg equilibrium (Table A.2), and results from 1977 should be interpreted with caution. Test for linkage disequilibrium indicated that all loci were inherited independently.

Genetic Differentiation

There was little detectable genetic differentiation among sampled years in the Kitimat River, as expressed by θ. When the nine different (pooled) year-classes of steelhead were analyzed, the overall subdivision was low at θ equal to 0.004 and not significant (95% CI, 0.000–0.009). Almost all the genetic variation resided within year-classes (i.e., among individuals) or within individual fish (i.e., heterozygosity, 99.36%; AMOVA, $P < 0.001$; among year-classes: 0.70%, $P < 0.001$; between prehatchery and posthatchery: -0.06, $P = 0.6676$). When all 14 year-classes were analyzed separately (mean, 24; SD, 21.6; range, 9–91), the results were similar, but with a slightly higher θ (0.005) that was marginally significant (95% CI, 0.001–0.01).

Neither comparison between pooled year-class data prehatchery ($n = 145$) and posthatchery ($n = 188$) nor between separate year-classes prehatchery and posthatchery indicated any significant genetic differentiation between before and after hatchery operation as expressed by θ ($P \geq 0.3640$), heterozygosity (H_O: $P \geq 0.2024$; H_S: $p \geq 0.3692$), or by allelic richness ($P \geq 0.0764$). No differentiation between prehatchery and posthatchery was consistent across 9 of 10 loci (the exception was *Omy77*; $P = 0.002$). 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. A loss of rare alleles may be expected if broodstock numbers are few (Table 1). Therefore, a one-sided test was used to investigate a potential loss of (rare) alleles. The results suggested a weakly significant reduction in allelic richness after hatchery operation for pooled year-classes and all year-classes prehatchery and posthatchery ($P = 0.0188$ and $P = 0.0448$, respectively). Excluding the 1977 year-class from the data did not change these results. A regression between mean allelic richness (A_r) and years was, however, not significant ($F = 0.743$; $P = 0.40$).

Pairwise comparisons made between pooled year-classes in the Kitimat River for differences in allele frequencies summed across all 10 loci were all nonsignificant (Table 2). The θ values were generally low (mean θ, 0.003; SD, 0.0066; range, -0.0086 to 0.021; Table 2). Pairwise testing of all separate year-classes also gave no significant differences (mean θ, 0.007; SD, 0.0109; range, -0.011 to 0.034), but the power of the tests was low in some cases because of few individual samples in some years (Table 1).

Calculation of Cavalli-Sforza genetic chord distances corroborated with results obtained from tests for genetic differences. There was little genetic divergence among Kitimat River year-clas-

TABLE 2.—Estimates of population differentiation (θ) for pooled year-classes (minimum sample size = 18) in the Kitimat River by locus overall are given in the lower triangular matrix. In the upper triangular matrix, NS indicates a nonsignificant θ-value.

Year-class	1976	1977	1983–1984	1987–1988	1990–1991	1992–1993	1996	2001–2002	2003
1976		NS	NS	NS	NS	NS	NS	NS	NS
1977	0.0117		NS	NS	NS	NS	NS	NS	NS
1983–1984	0.0002	0.0063		NS	NS	NS	NS	NS	NS
1987–1988	0.0210	0.0093	0.0141		NS	NS	NS	NS	NS
1990–1991	0.0045	0.0014	−0.0032	0.0159		NS	NS	NS	NS
1992–1993	0.0023	−0.0008	−0.0008	0.0015	−0.0021		NS	NS	NS
1996	−0.0086	0.0078	0.0048	0.0159	0.0010	0.0026		NS	NS
2001–2002	0.0024	0.0055	0.0053	0.0095	0.0038	0.0020	0.0018		NS
2003	0.0055	−0.0022	−0.0038	−0.0002	−0.0070	−0.0075	0.0041	0.0017	

ses, and genetic distances ranged from −0.001 to 0.031. The most divergent year-classes (populations) among the Kitimat River samples were 1987 and 2001 (both posthatchery years) with a genetic distance of 0.031, but they were not found to be significantly different from another (above). The neighbor-joining (N-J) generated tree demonstrated the grouping of the Kitimat River year-classes (Figure 2). No striking distinctions with high bootstrap support, however, were found to distinguish a potential influence of hatchery supplementation (e.g., more genetic drift-induced differentiation in hatchery versus nonhatchery years). All Kitimat River populations grouped closely with one another, and with relatively low bootstrap support for separation (mean, 33.6; SD, 14.95; range, 16–58), except 1990 and 2001, and 1984 and 1992

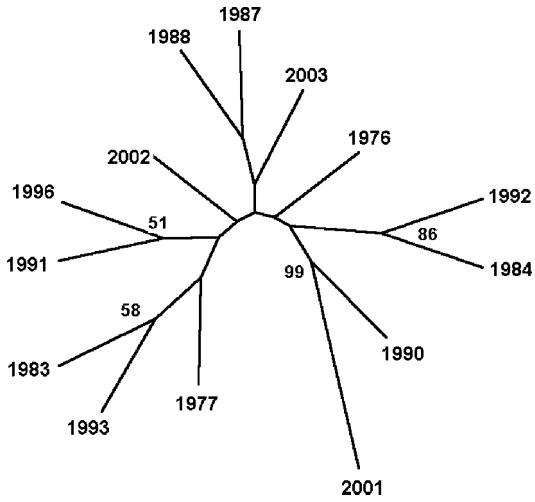

FIGURE 2.—Neighbor-joining tree based on Cavalli-Sforza and Edwards's (1967) chord distances calculated in Phylip. Bootstrap values greater than 50% are labeled. Note that the cladogram indicates a clustering pattern and that distances are not to scale.

which, however, clustered together. Samples within these tight clusters included before and after hatchery supplementation years (e.g., 1993 and 1983, and the 1984 and 1992 year-classes; Figure 2). There was variation among years, but there was no discernible pattern that would indicate an effect of hatchery stocking.

Spatial ordination of samples using PCA (Figure 3) on the microsatellite allele frequencies and, for comparison, also including 14 other *O. mykiss* populations across BC (Tamkee and Taylor, unpublished), resulted in groupings of populations that corresponded to geographic proximity. The Kitimat River year-classes grouped closely together, and there were no striking differences between samples collected before and after hatchery supplementation (Figure 3).

Testing for potential bottlenecks indicated that neither pooled year-classes nor prehatchery or posthatchery populations had allele frequency class distributions consistent with having undergone recent bottlenecks (all loci fit the TPM model; Wilcoxon two-tailed tests: $p > 0.1308$).

Discussion

Genetic Variation within Samples

Average expected heterozygosities of 0.51–0.61 in the Kitimat River steelhead year-classes are consistent with values reported for steelhead and rainbow trout from other regions in BC (Beacham et al. 2000; Heath et al. 2001; Hendry et al. 2002; Taylor and Tamkee, unpublished) and in other portions of the species range (e.g., Beacham et al. 1999; Knudsen et al. 2002; Taylor and Tamkee, unpublished).

A variety of genetic effects of releasing hatchery-reared progeny into the wild have been reported (e.g., Utter 1998; Brannon et al. 2004; Reisenbichler et al. 2004). Most such studies, however, involve the release of nonnative stocks of fish (Chil-

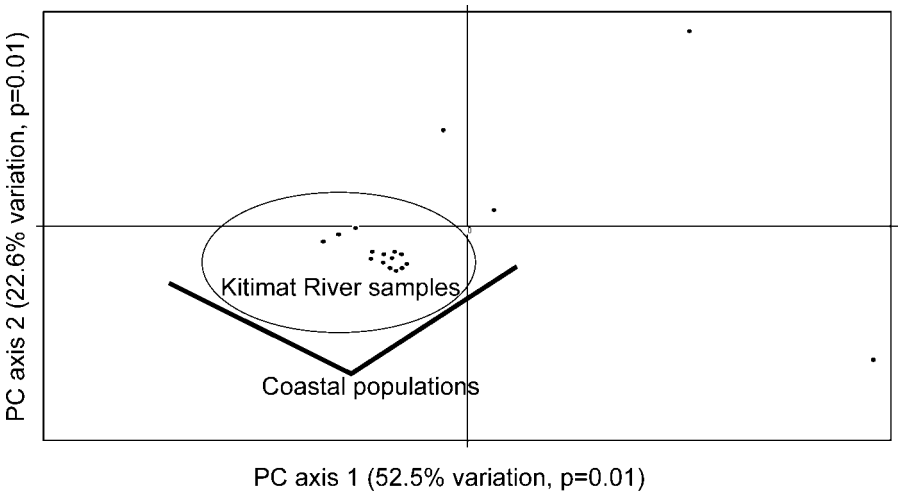

FIGURE 3.—Principal components analysis based on allele frequency data to summarize genetic differentiation among all Kitimat River samples. The analysis summarizes the variation across 10 loci (154 alleles) explaining the differentiation of individual populations at each axis. To illustrate the range of differentiation, two other coastal (Nimpkish and Copper rivers) and interior (Kootenay River and Blanchet Lake) populations in British Columbia are shown to the right of the circled samples.

cote 2003; Kostow et al. 2003; Reisenbichler 2004). By contrast, the unusual situation in the present study is that from our stocking records, Kitimat River steelhead broodstock was always collected annually from indigenous unclipped fish (i.e., presumably wild steelhead dating back to pre-hatchery enhancement). In a ''worst'' case they could be second generation hatchery fish, either a mixture from both hatchery and wild fish spawning or possibly F_1 feral offspring from hatchery $\times$ hatchery matings that had naturalized in the stream environment, migrated to the sea, and returned as adults. In accordance with this, we detected little change in genetic variation in Kitimat River steelhead before and after enhancement started or over the intervening years.

Depending on the number of broodfish used, however, the artificial spawning and release of cultured fish into the stream from local broodstock may have negative effects on genetic variation in natural fish populations through changes in allele frequencies and the loss of rare alleles via random sampling error and genetic drift. Careful planning and implementation of broodstock collection (i.e., enough individuals [minimum effective population size, N_e per generation $\geq$ 50; e.g., Caughley and Gunn 1996; Waples 2004] and representative of local natural population structure) is therefore crucial. Also, in a mixed wild and hatchery spawning population like in the Kitimat River, the wild multiple year-classes of fish spawning in nature will buffer such potential negative effects.

Genetic Differentiation among Samples

Examination of microsatellite variation demonstrated little pooled year-class contrasts and among-year-class variability in Kitimat River adult steelhead (overall $\theta = 0.004$, not significantly different from 0). In comparison, when data were analyzed for all steelhead populations in the Kitimat River and 14 other populations across BC (Tamkee and Taylor, unpublished), the overall subdivision was high with θ equal to 0.23 (95% CI, 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 because of differences among populations. Pairwise comparisons among Kitimat River year-classes were all nonsignificant. When the analysis included 14 other *O. mykiss* populations in BC (351 comparisons), less than one-third (92) were not significant. These, however, included all 91 pairwise comparisons among the Kitimat River year-classes.

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 neighboring BC watershed. They reported, however, considerably more among-year variation than we found in the

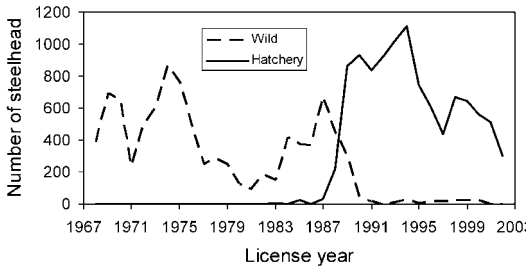

FIGURE 4.—Kitimat River steelhead harvest based on steelhead harvest analysis (SHA) data, 1968–2002.

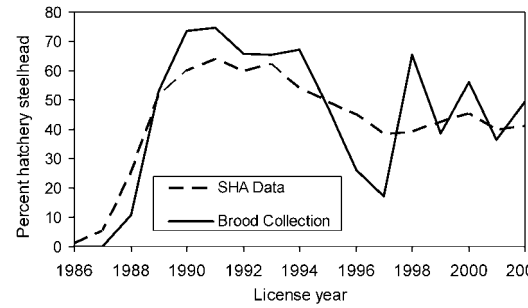

FIGURE 5.—Percent of total steelhead (wild and hatchery) captured in the Kitimat River that were hatchery fish, as determined by steelhead harvest analysis (SHA) data and brood collection, 1986–2002.

Kitimat River (F_{ST} ranged from 0.028 to 0.056, samples sizes 24–30; Heath et al. 2002). Garant et al. (2000) reported a substantial temporal component to genetic variation in Atlantic salmon *Salmo salar*, and found the component of genetic variance attributable to either temporal instability, random sampling errors, or both 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 substructuring in situations of weak genetic differentiation may occur (Garant et al. 2000). For the overall prehatchery and posthatchery comparisons in the Kitimat River, our sample sizes are substantial and our results consistent. For the more detailed pairwise year-class comparisons, mean sample sizes in the Kitimat material may be sufficient, but they were variable (mean *n*, 24 ; SD, 21.6), and for some year-classes, small. According to Waples (1998), intralocus sampling error may be expected to introduce noise on estimates of population divergence of magnitude $1/(2S)$, where S is sample size. For the year with the smallest samples size (nine in 1992; i.e., posthatchery), this translates into noise of about 0.056, indicating limited power for some of the detailed pairwise differentiation tests. We therefore pooled year-classes to achieve a minimum sample size (and intralocus noise) of 18 (0.028). All calculated θ values, however, were considerably less than this (overall mean, 0.003; SD, 0.0066; range, -0.0086 to 0.021), but should still be interpreted with caution.

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 population is small relative to the numbers of hatchery fish being stocked. On the other hand, if hatchery fish have lower survival (Smith and Ward 2000; Kostow 2004), higher harvest mortalities, or both compared with wild fish (Anonymous 2003), 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 populations (Ryman and Laikre 1991; Waples 2004). There are no direct estimates of number of wild compared with number of hatchery spawners in the Kitimat River. Catch statistics (Figures 4, 5), however, clearly indicate a high number of returning hatchery steelhead. Furthermore, the catch-per-effort data from the broodstock fishery indicate about equal proportions of wild and hatchery fish in the spawning population in the lower main stem (Table 1; Figures 4, 5). 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. Estimates of ocean survival for steelhead in BC vary in space and time, from 1% to 24% (Keogh River; Smith and Ward 2000; Ward 2000; B. Ward, University of British Columbia, personal communication). With a survival set at 3–5%, the run of hatchery fish may be about 2,000 fish, of which perhaps 500 are harvested (Figure 4), indicating a substantial contribution of hatchery fish to an estimated total spawning population of perhaps about 3,000 steelhead.

Samples from the Kitimat River were generally in Hardy–Weinberg equilibrium (with the notable exception of the year 1977), suggesting that the sample included individuals from more than one population. According to Morris and Eccles (1977), samples in 1977 were collected from steel-

head caught by anglers along the entire Kitimat River up to Hunter Creek, which is above the lower main-stem Kitimat River (Figure 1). It appears that the 1977 data cover a larger area and, to some extent, 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. The genetic results therefore indicate that there are possibly more wild populations in the upper Kitimat River system. These populations may be unaffected by the hatchery stocking of steelhead in the lower main stem.

Waples (1990) suggested an N_e on 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. In the Kitimat River 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 in terms of reproduction, for example, because of high harvest rates or reduced survival (e.g., Chilcote 2003; McLean et al. 2003; Kostow 2004). 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 posthatchery 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 broodstock. Even though there may not be significant genetic differentiation due to genetic drift, allelic richness may slowly erode in years to come, unless the number of broodstock is increased. However, the contribution by wild spawners has buffered—and will continue to buffer—against this, as does the presence of multiple year-classes in the spawning population. In fact, although most wild steelhead in Kitimat River are age 3.2 fish (27.3%), other year-classes—such as 4.2 (16.4%), 4.3 (13.6%), 3.2S_1 (14.5%; S_1 = one spawning event), and 3.3 (10.0%)—comprise significant proportions of the 14 year-classes present (Chudyk et al. 1977).

The only previous studies we are aware of which directly investigated the genetic effects of conservation hatchery practices relevant to the Kitimat River results were undertaken on the Hood River, Oregon. 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 populations (i.e., only wild broodstock 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. Our study did not set out to evaluate potential hatchery effects related to change in life history and productivity or its components (e.g., reproductive success differences between hatchery and wild fish induced each generation through domestication in the hatchery; Lynch and O'Hely 2001; Ford 2002), which cannot be directly evaluated with our approach. Such effects may, however, in turn lead to modified selection and potential genetic changes (Reisenbichler 2004). Based on results in Blouin (2003), an ongoing project in the Hood River is currently testing the prediction that using wild broodstock for hatchery production will not have negative genetic effects on the wild population. The results from the Kitimat River, where new conservation-based hatchery populations have been practiced since its start in 1984, appear to be consistent with this prediction.

In conclusion, the results from the Kitimat River indicate little genetic differentiation among the studied year-classes or between prehatchery and posthatchery populations. Likewise, pairwise testing did not indicate any significant trends or changes in our measures of genetic variation. Compared with 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 substantial numbers of wild fish and multiple year-classes in the mixed spawning population probably 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. Furthermore, as a precautionary step, it is recommended that the annual hatchery broodstock should not be less than 25 wild males and 25 wild females, and preferably more. Our data also suggest there is (as yet) unresolved population sub-

structure 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.

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Appendix: Results of Genetic Analysis and Allelic Variation

TABLE A.1.—Multiplexed loci and the corresponding labeled Beckman dye, annealing temperature, total number of samples which amplified results (*N*), and range in allele size in base pairs (bp) for each locus.

Multiplex	Locus	Beckman dye	T_A (C°)[a]	*N*	Range (bp)
1	*Oneμ14*	D-4	62/60	330	145–165
	Ssa197	D-4		333	112–116
2	*Ssa456*	D-3	56/55	332	151–161
	Omy77	D-3		329	94–140
3	*Ots3*	D-4	52/50	326	76–96
	Okia3	D-4		321	112–206
4	*Ssa85*	D-3	56/55	317	97–153
	Ots103	D-3		327	71–91
	Ots100	D-3		305	138–218
5	*Oneμ8*	D-2	58/56	333	150–184

[a] The numerator is the annealing temperature during the first 5 cycles of the amplification, the denominator is the annealing temperature during the subsequent 30 cycles. See text for details.

TABLE A.2.—Summary of allelic variation at 10 microsatellite loci for 27 steelhead populations and year-classes included in this study. Number of samples which amplified results (N), allelic richness (A_r), number of alleles per locus (N_a), expected heterozygosity (H_e), and observed heterozygosity (H_o) are given for each population. Significant departures from Hardy–Weinberg equilibrium are denoted by an asterisk (using standard Bonferroni corrections for 27 populations; $P = 0.05/27 = 0.00185$).

Data item	*Oneμ8*	*Ssa85*	*Ots103*	*Ots3*	*Ssa456*
			Kitimat 2003		
N	34	33	34	34	34
A_r	3.198	4.482	1.447	3.436	2.892
N_a	5	7	2	5	4
H_e	0.4537	0.7062	0.0843	0.6012	0.5649
H_o	0.4706	0.5152	0.0882	0.5588	0.4118
			Kitimat 2002		
N	39	38	37	38	39
A_r	3.527	3.952	1.162	3.401	2.439
N_a	7	9	2	7	4
H_e	0.4931	0.5405	0.0267	0.5637	0.5302
H_o	0.5385	0.5789	0.0270	0.5263	0.4872
			Kitimat 2001		
N	11	10	9	9	11
A_r	1.922	4.15	1.667	4.681	2.968
N_a	2	5	2	5	3
H_e	0.2355	0.6800	0.1049	0.6852	0.5950
H_o	0.0909	0.7000	0.1111	0.6667	0.6364
			Kitimat 1996		
N	26	24	26	25	26
A_r	3.689	3.482	1.231	4.033	2.41
N_a	6	4	2	5	3
H_e	0.4970	0.5773	0.0377	0.6600	0.5096
H_o	0.5000	0.5417	0.0385	0.5200	0.5385
			Kitimat 1993		
N	18	18	18	18	18
A_r	3.374	4.653	1.562	3.883	2.562
N_a	5	7	2	6	3
H_e	0.4846	0.7148	0.1049	0.6620	0.5509
H_o	0.5556	0.7500	0.1111	0.7222	0.6111
			Kitimat 1992		
N	9	6	9	9	9
A_r	1.667	5	1	3.618	2
N_a	2	5	1	4	2
H_e	0.1049	0.7639	0.0000	0.5679	0.5000
H_o	0.1111	0.8333	0.0000	0.5556	0.5556
			Kitimat 1991		
N	16	16	16	16	16
A_r	3.102	4.102	1	3.981	2.992
N_a	5	5	1	6	4
H_e	0.4766	0.7051	0.0000	0.6426	0.5801
H_o	0.5625	0.5000	0.0000	0.7500	0.6875
			Kitimat 1990		
N	11	11	11	11	11
A_r	2.896	4.221	1.545	3.516	1.997
N_a	4	5	2	4	2
H_e	0.3182	0.5620	0.0868	0.6570	0.3967
H_o	0.3636	0.3636	0.0909	0.5455	0.5455
			Kitimat 1988		
N	12	12	12	12	12
A_r	2.761	4.214	1.5	2.873	2
N_a	4	6	2	3	2
H_e	0.2951	0.5694	0.07799	0.4965	0.4688
H_o	0.2500	0.6667	0.0833	0.4167	0.4167

TABLE A.2.—Extended.

Data item	*Omy77*	*Oneμ14*	*Ssa197*	*Ots100*	*Okia3*	Results over all loci
			Kitimat 2003			
N	34	34	34	31	34	
A_r	4.893	3.615	1.999	4.555	7.018	
N_a	8	5	2	8	11	
H_e	0.7405	0.6916	0.4892	0.7242	0.8741	0.5920
H_o	0.6471	0.5000	0.5000	0.7097	0.8529	0.5254
			Kitimat 2002			
N	39	39	39	36	38	
A_r	5.349	4.169	2	4.486	7.497	
N_a	9	7	2	8	15	
H_e	0.7659	0.7048	0.4947	0.6894	0.8930	0.5702
H_o	0.6667	0.6154	0.6923	0.6944	0.9211	0.5748
			Kitimat 2001			
N	11	11	11	10	11	
A_r	5.169	3.507	2	3.438	7.977	
N_a	7	4	2	4	11	
H_e	0.6612	0.6157	0.4835	0.5250	0.8760	0.5462
H_o	0.4545	0.6364	0.2727	0.7000	0.9091	0.5178
			Kitimat 1996			
N	25	26	26	25	26	
A_r	4.855	3.957	2	4.64	8.261	
N_a	7	5	2	7	15	
H_e	0.7536	0.6790	0.4882	0.7432	0.9105	0.5856
H_o	0.5600	0.6154	0.4615	0.6800	0.9231	0.5379
			Kitimat 1993			
N	18	18	18	17	18	
A_r	4.964	3.867	2	4.016	7.17	
N_a	7	5	2	5	11	
H_e	0.7623	0.6975	0.4938	0.6799	0.8688	0.6020
H_o	0.6111	0.7222	0.5556	0.6471	0.9444	0.6230
			Kitimat 1992			
N	9	9	9	9	9	
A_r	3.309	3.877	2	4.544	6.985	
N_a	4	4	2	5	8	
H_e	0.4444	0.6852	0.4938	0.7346	0.8519	0.5147
H_o	0.3333	0.4444	0.4444	0.8889	0.8889	0.5056
			Kitimat 1991			
N	16	16	16	16	16	
A_r	5.392	3.289	1.999	5.126	7.939	
N_a	9	4	2	8	12	
H_e	0.7539	0.5762	0.4512	0.7441	0.8926	0.5822
H_o	0.6875	0.5000	0.6875	0.7500	0.8750	0.6000
			Kitimat 1990			
N	10	11	11	11	11	
A_r	4.509	3.533	2	6.02	8.549	
N_a	5	4	2	8	12	
H_e	0.6450	0.6653	0.4628	0.8058	0.8884	0.5488
H_o	0.8000	0.4545	0.3636	0.8182	1.0000	0.5345
			Kitimat 1988			
N	12	12	12	12	12	
A_r	4.929	3.753	2	5.586	6.54	
N_a	6	4	2	7	8	
H_e	0.7188	0.7083	0.4688	0.7917	0.8403	0.5437
H_o	0.5000	0.5833	0.4167	0.9167	0.9167	0.5167

TABLE A.2.—Continued.

Data item	*Oneμ8*	*Ssa85*	*Ots103*	*Ots3*	*Ssa456*
			Kitimat 1987		
N	12	12	12	12	12
A_r	3.838	4.668	1.761	3.714	2
N_a	4	6	2	5	2
H_e	0.6840	0.6840	0.1528	0.5174	0.4861
H_o	0.5833	0.5833	0.1667	0.5000	0.5000
			Kitimat 1984		
N	9	9	9	9	9
A_r	3.558	5.087	1	2.902	1.999
N_a	4	6	1	3	2
H_e	0.4506	0.7778	0.0000	0.4383	0.4012
H_o	0.1111	0.6667	0.0000	0.5556	0.5556
			Kitimat 1983		
N	27	27	27	27	26
A_r	3.895	4.009	1.412	3.598	1.995
N_a	6	6	2	6	2
H_e	0.5302	0.6728	0.0713	0.6056	0.4401
H_o	0.5556	0.8148	0.0741	0.6296	0.5769
			Kitimat 1977		
N	91	87	90*	90	91*
A_r	3.461	4.275	1.763	4.048	2.127
N_a	7	11	3	6	3
H_e	0.4944	0.6556	0.1637	0.6901	0.5108
H_o	0.3956	0.6437	0.1111	0.6444	0.9560
			Kitimat 1976		
N	18	17	16	16	18
A_r	3.306	5.31	1.992	3.739	2.319
N_a	5	8	3	5	3
H_e	0.4522	0.7059	0.1738	0.5859	0.4151
H_o	0.3889	0.4706	0.1875	0.5625	0.3333

TABLE A.2.—Extended Continued.

Data item	*Omy77*	*Oneμ14*	*Ssa197*	*Ots100*	*Okia3*	Results over all loci
			Kitimat 1987			
N	12	12	12	12	12	
A_r	4.503	3.932	2	3.391	7.381	
N_a	6	7	2	4	10	
H_e	0.6458	0.7326	0.4965	0.6354	0.8611	0.5896
H_o	0.5000	0.5833	0.7500	0.8333	1.0000	0.6000
			Kitimat 1984			
N	9	9	9	9	9	
A_r	2.95	4.655	2	3.796	8.694	
N_a	3	5	2	5	12	
H_e	0.564	0.7284	0.4444	0.5679	0.9012	0.5296
H_o	0.4444	0.6667	0.6667	0.6667	1.0000	0.5333
			Kitimat 1983			
N	26	27	27	27	27	
A_r	4.917	3.476	2	4.399	8.985	
N_a	7	6	2	8	20	
H_e	0.7337	0.6180	0.4890	0.6934	0.9246	0.5779
H_o	0.60538	0.5556	0.5556	0.7407	0.8889	0.6046
			Kitimat 1977			
N	90*	88	91	74	84*	
A_r	4.995	3.919	1.999	4.49	7.933	
N_a	11	8	2	10	20	
H_e	0.7760	0.7085	0.4951	0.7084	0.9093	0.6112
H_o	0.9222	0.5682	0.5275	0.7027	0.8214	0.6293
			Kitimat 1976			
N	18	18*	18	16	14	
A_r	4.435	3.704	2	4.755	8.167	
N_a	7	4	2	8	13	
H_e	0.6898	0.6836	0.4938	0.6855	0.8878	0.5774
H_o	0.7778	0.2222	0.5556	0.7500	0.9286	0.5177