Exxon Valdez Oil Spill
Restoration Project Annual Report

Genetics of Populations of Pink Salmon Inhabiting Prince William Sound

Restoration Projects 94320D and 95320D
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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Study History: This study was submitted as a preproposal in FY 1991; it was deferred until funding was approved in FY 1994 as Restoration Project 94320D. The project continued in FY 1995 as 95320D, and in subsequent years as Restoration Project 9x196.

Abstract: Allozyme and mtDNA data were collected from 27 putative populations of pink salmon spawning throughout Prince William Sound (PWS) in 1994. Sampling included two hatchery, five upstream, and 20 tidal locations distributed among five management regions (Southeast, East, North, Southwest, and Montague). Seventy-seven allozyme loci were screened in up to 100 fish per population. Thirty-eight loci had frequencies for alternate alleles ≥ 0.01 in one or more populations and were used for population analyses. Forty fish per collection were screened for haplotype variation at the ND5/ND6 region using six restriction enzymes; eight haplotypes were detected. Significant differences between upstream and tidal collections were detected within Lagoon Creek (allozymes) and within Koppen Creek (mtDNA). Significant regional heterogeneity was detected within upstream (allozymes and mtDNA) and tidal (allozymes) collections. In pair-wise tests between management regions, only the test between two best represented regions (Southwest and East) was significant for tidal populations. Armin F. Koernig Hatchery was indistinct from all regions, while there was indication that Solomon Gulch Hatchery was distinct from all regions but East. These results support managing native populations of pink salmon in PWS at the regional level, considering local subpopulation structure, rather than as a single panmictic population.

Key Words: Allozymes, *Exxon Valdez* oil spill, mtDNA, *Oncorhynchus gorbuscha*, pink salmon, Prince William Sound, stock identification.

Project Data: (will be addressed in the final report)

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EXECUTIVE SUMMARY

- Allozyme and mtDNA data were collected from 27 aggregates of pink salmon spawning in 1994 from Prince William Sound (PWS). These collections were distributed throughout PWS and included locations within each of the five major management regions (Southeast, East, North, Southwest, and Montague). Samples were collected from spawners from two hatchery, five upstream, and 20 tidal locations.

- We screened 77 allozyme loci from 92 to 100 fish per population for a total of 2686 fish. Of these loci, 38 had frequencies for alternate alleles ≥ 0.01 in at least one population and were retained for analysis.

- Haplotype data were collected from the ND5/ND6 region of mtDNA using six restriction enzymes on 40 fish per population for a total of 1080 fish. Four of these enzymes yielded a total of eight haplotypes.

- We analyzed the data for genetic structure in three steps. First, the wild collections were organized hierarchically to test for homogeneity: a) among collections within regions within elevation, b) among regions within elevation, and c) among wild collections from different elevations (tidal and upstream). The highest level of the hierarchy was a test between all the wild collections and the hatchery collections. Second, we performed pairwise log-likelihood tests within streams where we had both tidal and upstream collections. Third, we applied similar tests between collections pooled within regions to test regions against each other and to examine hatchery relationships to these regions.

- Significant differences between overall upstream and tidal collections were detected. Further examination with paired tests revealed that both Lagoon Creek (allozymes) and Koppen Creek (mtDNA) tidal and upstream collections were different. Significant regional heterogeneity was detected within upstream (allozymes and mtDNA) and tidal (allozymes) collections. In pair-wise tests between management regions after statistically accounting for multiple tests, only the test between the two best represented regions (Southwest and East) was significant for tidal populations. However, before accounting for multiple testing, 8 of the 21 tests made were significant, suggesting that we may have lacked statistical power to detect differences present among less sampled regions.

- In the region-by-region analysis of allozyme data for tidal collections, Armin F. Koernig Hatchery was indistinct from all regions, while there was indication that Solomon Gulch Hatchery was different from all regions but East. These hatchery results follow expectations based on the hatchery locations, original broodstock sources, and annual broodstock acquisition methods.

- These data support managing native populations of pink salmon in PWS at the regional level, considering local subpopulation structure, rather than as a single panmictic population.

INTRODUCTION

On March 24, 1989, the supertanker *Exxon Valdez* ran aground on Bligh Reef in Prince William Sound (PWS), Alaska, spilling 41 million liters of crude oil. The oil slick, pushed by winds and currents, moved through western PWS and the western Gulf of Alaska, contaminating approximately 2000 km of coastal habitat (see overview in Wells et al. 1995), killing thousands of sea otters *Enhydra lutris* (Garrott et al. 1993; Bodkin and Udevitz 1993) and hundreds of thousands of seabirds (Ford et al. 1993), and adversely affecting many other taxa (e.g., Barber et al. 1995; Bowman et al. 1995; Bowyer et al. 1995; Duffy et al. 1994). Sublethal effects, including reproductive impairment (Ford et al. 1993) and chromosome damage (Hose 1994), were documented. Subsurface oil remains in some of the beaches in spite of the multi-billion dollar clean-up and restoration effort (Wolfe et al. 1994). Populations of some species including pink salmon *Oncorhynchus gorbuscha* may not be fully recovered (Bue et al. 1996).

Pink salmon is the most abundant North American species of the Pacific salmon (Neave 1967; Heard 1991), making it an ecological cornerstone in biological communities of the Pacific Rim and an economic mainstay for many coastal communities. Pink salmon are both anadromous and semelparous: in their natural range, they make long oceanic migrations, home to their natal streams to spawn, and die at age two. Annual catches of pink salmon ranged from 46 to 128 million fish in Alaska alone during the period from 1985-1995.

Pink salmon, of both wild and hatchery origin, was also one of the most abundant vertebrate species inhabiting the spill area. Historically, wild populations produced approximately five hundred million pink salmon fry which emerged from streams throughout PWS each year to migrate seaward. Adult returns from these juvenile migrations averaged over 10 million fish annually. These returning wild-stock adults play a critical role in the total PWS ecosystem: they convey essential nutrients and minerals from the marine ecosystem to estuaries, freshwater streams, and terrestrial ecosystems. Both juveniles and adults are important sources of food for many fishes, birds, and mammals. Wild pink salmon also play a major role in the economy of PWS because of their contribution to commercial, sport, and subsistence fisheries in the area.

Up to 75% of wild pink salmon spawning within PWS occurs in intertidal areas (Helle et al. 1964; Roys 1971). This extensive use of intertidal areas made pink salmon susceptible to adverse effects from the oil spill. Pink salmon embryos and alevins suffered increased mortality, diminished growth, and a high incidence of somatic cellular abnormalities as a result of spawning ground contamination and rearing in oiled areas. Elevated mortality of embryos in the oiled streams continued through 1993, three generations after the oiling, implicating genetic damage (Bue et al. 1996). Also in 1989, the commercial harvest of pink salmon was shifted away from the hatchery and wild stocks in the oiled areas to target the wild stocks in eastern PWS (Geiger and Savikko 1990). This resulted in over-harvest and depletion of these stocks evidenced by general run failures of eastern PWS populations of non-hatchery origin in 1991 (Geiger and Savikko 1992).

An array of conservation and restoration alternatives have been proposed for "species" impacted by the *Exxon Valdez* oil spill. But, species-based proposals often do not provide the resolution needed to sustain the conservation of genetically diverse aggregates of salmon

populations; it is essential to manage and restore these damaged pink salmon resources on a population basis in order to conserve between-population diversity (e.g., Cuenco et al. 1993; Waples 1995). Between-population diversity provides optimal production for species inhabiting diverse ecosystems such as PWS; highly diverse population mixes also provide a biological buffer to environmental change (droughts, floods, major earthquakes, major shifts in oceanic conditions, and other routine catastrophic events that occur in Pacific Rim ecosystems). Our goal was to examine naturally occurring genetic markers to delineate the population structure of PWS pink salmon and to provide a genetic basis for fish management.

Two categories of molecular markers have been used extensively to define population structure of salmonids: allozymes and mitochondrial DNA (mtDNA). Allozyme analysis remains the preferred approach for study of population genetics of salmonids because of its power to resolve populations of many species in the tetraploid-derived family by assaying many nuclear loci rapidly and at low cost (Allendorf 1994). Additional advantages of allozymes for this study include the existence of a pre-spill allozyme data set for comparison and that many laboratories cooperate on inter-institutional examinations of pink salmon using allozymes, providing a support structure including a wealth of compatible data for comparison among Pacific Rim populations (e.g., Seeb and Wishard 1977; Utter et al. 1980; Beacham et al. 1985, 1988; Gharrett et al. 1988; Shaklee et al. 1991; White and Shaklee 1991; Shaklee and Varnavskaya 1994).

The utility of mtDNA approaches to study genetic diversity of salmonid populations is controversial for reasons such as relatively high cost and slow throughput (Allendorf 1994). Additionally, sometimes mtDNA data reveal less diversity than that detected through allozymes because mtDNA cannot recombine and is maternally inherited as a single locus so that the variation is absolutely linked (Smouse et al. 1994; contrast the lack of geographic resolution observed for mtDNA data for populations of chum salmon in Park et al. [1993] with the geographic resolution apparent for allozyme data for similar populations in Winans et al. [1994]). However, haplotype data from a pilot examination (Fetzner et al. in prep.; Appendix A) indicate a potential east-west-island and upstream-intertidal separation of populations within PWS. We believed that the complementary use of the two techniques should provide optimal resolution of the population structure for this study.

Our objective was to test for both temporal and geographical structuring among even- and odd-year classes by examining genetic differences between early- and late-season spawners, upstream and intertidal spawners, and stream-of-spawning. Additionally, genetic positioning of the local hatchery stocks within this structure was of interest because the extensive releases of pink salmon fry in PWS in recent decades may have affected the partitioning of naturally occurring genetic diversity. Some fear that hatchery production may pose a threat to native populations as or more substantial to that posed by the oil spill (see discussion in Gharrett and Smoker 1993).

Also important to this study was the fact that even- and odd-year classes have independent population structures because of the rigid two-year life cycle of pink salmon. For example, climactic, tectonic or other such events (such as the 1964 earthquake [Roys 1971] or the 1989 oil spill) may affect the population structure of one year class, cycle through subsequent generations, and leave the alternate cycle of year-classes relatively unchanged (see data in Fetzner et al. in prep; Appendix A). Therefore, population structure

and conservation strategies must be independently assessed for the even- and odd-year classes.

In this paper we report the genetic structure of even-year populations of wild pink salmon inhabiting PWS. After the assay of 2686 individuals from 27 collections for variation at 77 allozyme loci and assay of a subset of 1080 individuals from each collection for variation at the ND5/ND6 region of mtDNA, we found genetic structuring within PWS in comparisons between elevation of spawning and among regions.

OBJECTIVES

Our objective is to define the genetic structure of pink salmon stocks in the EVOS-affected area of PWS. In this multi-year project we will test for:

1. genetic differences between spawners from the five primary management regions within PWS (Southeast, East, North, Southwest, Montague).

2. genetic differences between spawners from different streams within PWS.

3. genetic differences between upstream and intertidal spawners within the same streams.

4. genetic relationships between hatcheries and native populations.

5. genetic differences between temporally isolated spawners within the same streams.

6. genetic differences between odd- and even-year pink lineages.

7. inheritance of newly detected isozyme variants and loci.

In this report, we review the results for the 1994 collections and address objectives 1, 2, 3, and 4. The study is ongoing, and objectives 5, 6, and 7 will be addressed in future years.

METHODS

Field Sampling

Tissues were collected from 92 - 100 individuals from each of 25 spawning aggregations from wild-stock streams and two hatcheries during 1994 (Table 1). Sampling incorporated a broad geographical distribution of locations within PWS; primary consideration was given to the sampling of tributaries that routinely support large runs of fish on both even and odd years.

We also distributed sampling effort among the current harvest management zones. The Sound was historically divided into subdivisions for management and conservation

purposes according to biological, geographical, and geological factors (Anonymous 1960; Randall et al. 1983; Rugolo 1984). Sampling was done to include at least one collection from each of the five major subdivisions (Southeast, East, North, Southwest, Montague; Figure 1).

Consideration was also given to the physiography of PWS. Sampling included both areas uplifted by the major 1964 earthquake, where even-year populations were reduced by up to 98%, as well as areas where populations were relatively unaffected (Roys 1971; Figure 2).

Finally, although a majority of pink salmon spawning in PWS occurs in areas of tidal influence, some larger tributaries also possess somewhat discrete aggregations that spawn in upstream areas, above the influence of tides. Samples were collected from both tidal and upstream sites from five of these creeks (Table 1; Figure 1).

Tissue samples from heart, liver, muscle, and vitreous humor from each individual were immediately frozen on liquid nitrogen and returned to Anchorage for storage at -80° C. Subsamples were shipped to the Washington Department of Fisheries and Wildlife, Olympia, Washington, on dry ice where they were also stored at -80° C prior to allozyme analysis.

Allozyme Analysis

Genetic data were collected using the techniques of allozyme electrophoresis on all samples (Aebersold et al. 1987). An extensive screening for resolution of allozyme phenotypes on 45 individuals from two collections, Erb Creek and Humpback Creek, detected 77 putative loci (Table 2). These 77 loci were screened for genetic variation in all remaining individuals. Our nomenclature followed the American Fisheries Society standard (Shaklee et al. 1990).

Alleles present at frequencies above 0.01 in one or more collections were retained for data analysis. Allele observations from alleles that did not meet this criterion were excluded to reduce statistical noise associated with low frequency alleles, thereby increasing our power to detect genetic structuring (see Shaklee et al. 1994). This criteria reduced the number of loci further analyzed to 38: *sAAT-1,2*; sAAT-3*; sAAT-4*; mAAT-1*; ADA-1*; ADA-2*; sAH*; mAH-3*; mAH-4*; CK-A2*; FDHG*; bGALA*, G3PDH-1*; G3PDH-2*; G3PDH-3*; GDA-1*; GPI-B1,2*; IDDH-1*; mIDHP-1*; sIDHP-2*; LDH-A2*; LDH-B2*; sMDH-A1,2*; sMDHB-1,2*; mMEP-1*; NTP*; PEPB-1*; PEPD-2*; PEPLT*; PGDH*; PGM-2*; mSOD*; sSOD-1*; TPI-2**. Loci dropped from the population analyses included: *mAAT-2*; mAH-1*; mAH-2*; AK*; ALAT*; CK-A1*; CK-B*; CK-C1*; CK-C2*; ESTD*; FH*; GAPDH-1*; GAPDH-2*; GAPDH-3*; GAPDH-4*; GAPDH-5*; bGLUA*; GPI-A*; GR; mIDHP-2*; sIDHP-1*; LDH-A1*; LDH-B1*; LDH-C*; aMAN*; mMDH-1*; mMDH-2,3*; mMEP-2*; MPI*; PEPA*; PEPB-2*; PEPD-1*; PGK-1*; PGK-2*; sSOD-1*; sSOD-2*; TPI-1*; TPI-3*; TPI-4**.

Individual genotypic data were summarized into allelic frequencies, and tests for departure from Hardy-Weinberg were made using log-likelihood tests, α=0.05 (modified from Weir 1990) with the experimentwise significance level set at 0.05 and adjusted for multiple tests (Rice 1989). For isoloci (*sAAT-1,2*; GPI-B1,2*; sMDH-A1,2*; sMDH-B1,2**), allele frequencies were calculated using a multinomial model, assuming independence of alleles at both loci. Observed and expected heterozygosities were computed using the reduced set of loci. Paired *t*-tests were made to determine if observed heterozygosities in upstream samples

were significantly greater than tidal samples from the same system and to test for differences in heterozygosities between hatchery and wild collections.

We performed hierarchical analyses using log-likelihood ratios to test for homogeneity within and among groups of pink salmon collections (modified from Weir 1990). The wild collections were organized hierarchically to test for homogeneity: 1) among collections within regions within elevation, 2) among regions within elevation, and 3) among wild collections from different elevations (tidal and upstream). The highest level of the hierarchy was a test between all the wild collections and the hatchery collections. The log-likelihood ratio statistic is distributed approximately chi-squared with $(n - 1)(m - 1)$ degrees of freedom, where n is the number of alleles and m is number of collections in the test. If an allele was observed in a collection, we assumed that it existed within all collections, potentially at an infinitely small frequency. Therefore, the degrees of freedom and log-likelihood statistics are summable, and differences among and within collection subdivisions can be examined.

For the hierarchical analysis, comparisonwise significance levels were adjusted for multiple tests using a sequential Bonferonni adjustment (modified from Miliken and Johnson 1984 and Rice 1989) with the overall experimentwise significance level set at 0.05. The first step in the analysis was a sequentially adjusted test for differences at the first hierarchical level, i.e., between sources (hatchery and wild) and within sources. If a significant difference was found within sources, then a sequentially adjusted test was applied at the next level. Testing proceeded in this way through the hierarchy. If a test was not significant, then all remaining lower levels were combined, and a final sequentially adjusted multiple test of significance was performed.

A gene diversity analysis (Nei 1973) was performed among the wild collections to partition variation into hierarchical levels. As before, this analysis was partitioned by wild/hatchery, then by elevation, and then by region. Isoloci were excluded.

Separate hierarchical groupings were used to test for differences among paired collections within streams and to test for differences among regions. Comparison-wise significant levels were adjusted for all tests within each hierarchical grouping using a sequential Bonferonni adjustment (modified from Miliken and Johnson 1984 and Rice 1989) with the overall experimentwise significance level set at 0.05. To test for differences between tidal and upstream collections within streams, we performed log-likelihood tests between the paired collections within the five streams from which we had both. To test for differences between individual regions, we performed two groupings of pairwise log-likelihood tests after pooling tidal collections within regions and after pooling upstream collections within regions. Within the same groupings we also tested these pooled collections with individual hatchery collections.

Cavalli-Sforza and Edwards (1967) chord distances were calculated to evaluate genetic relationships and examined with classical multidimensional scaling analysis (MDS; Lessa 1990) and with a tree constructed using unweighted pair-group method (UPGMA; Sneath and Sokal 1973). The MDS ordination technique plots genetic relationships in two dimensions so that the plotted distances between collections closely match the observed distances in multidimensional space. This technique provides a means to confirm expected structure and uncover unexpected structure by providing insight into structural demarcations. All calculations were performed using functions in *S-Plus* (Mathsoft, Inc., Seattle, WA).

Mitochondrial DNA Analysis

A subset of 40 individuals from each of the 27 collections analyzed for allozyme variation was assayed for variation at sites previously identified in the ND5/ND6 region (Fetzner et al. in prep.; Appendix A). Genomic DNA was extracted using Puregene DNA isolation kits for animal tissues (Gentra Systems, Inc. P.O. Box 13159, Research Triangle, NC 27709-13159). This process included: (1) a cell lysis solution to break down cell and nuclear membranes; (2) a Proteinase K digest to denature proteins; (3) an RNase treatment to digest RNA; (4) protein precipitation to remove Proteinase K, RNase, and denatured proteins; (5) isopropanol to precipitate DNA; (6) 70% ethanol to wash DNA; and finally (7) a hydration solution to rehydrate DNA.

After extraction, DNA was amplified using the polymerase chain reaction (PCR; Saiki et al. 1988; Kocher et al. 1989). Amplified DNA was cut with the six restriction enzymes found to detect haplotype polymorphisms (of the 30 screened in Fetzner et al. [in prep.]; *Apa I, BstU I, EcoR V, Hinf I, Rsa I, Xba I*) and electrophoresed on agarose gels. Fragments were visualized under UV light, and a photographic record was made of each gel. The restriction sites detected for each enzyme were pooled as composite haplotypes for the statistical analyses.

Nucleotide (π) and haplotype (h) diversity measures (Nei 1987) were calculated for all collections using the restriction enzyme analysis package (*REAP*; McElroy et al. 1992). These measures estimate the number of nucleotide substitutions per site between DNA sequences (i.e., sequence divergence) and the amount of DNA polymorphism within collections, respectively.

To test for heterogeneity among populations, Monte Carlo simulations with 10,000 replicates were performed (Roff and Bentzen 1989) using the REAP analysis program. Independent tests were performed to test for heterogeneity in a hierarchical manner following the levels identified in the log-likelihood analysis. However, unlike the log-likelihood analysis, the χ^2 values for individual tests are not summable. Monte Carlo tests were also performed between the paired upstream and tidal collections, and among-region tests were conducted by pooling collections within region. All significance levels were adjusted using sequential Bonferroni techniques (Rice 1989).

An analysis of the distribution of molecular variance was made using AMOVA (Excoffier et al. 1992) and utilizing a matrix of Euclidean distances between haplotypes. Pairwise Euclidean distances were calculated as the total number of site changes between haplotypes. The AMOVA analysis incorporates distance between haplotypes in the calculation of haplotypic diversity at different hierarchical levels. Haplotype correlation measures are expressed as Φ-statistics (Excoffier et al. 1992). Among regions, Φ_{CT} is defined as the correlation of random haplotypes within a group of collections relative to that of random pairs of haplotypes drawn from the entire set of collections. For the analysis among collections within regions, Φ_{SC} is the correlation of random haplotypes within collections relative to that of random pairs of haplotypes from the regions. Finally for the within-collection analysis, Φ_{ST} is the correlation of random haplotypes within collections relative to that of random pairs of haplotypes drawn from the entire set of collections. The AMOVA

analysis allows for only a two-level hierarchy, so we were unable to partition regions within elevations as in the preceding analyses. Rather, we performed two separate analyses, one based on elevation and one based on geographic regions. The significance of the observed variance components and Φ-statistics were tested using a random permutation procedure in AMOVA. The permutation approach to significance testing avoids the parametric assumptions of normality and independence that are not met by molecular distance measures (Excoffier et al. 1992). The number of permutations was set at 1000 for each analysis. Φ_{ST} between pairs of populations, a modified coancestry coefficient, were also calculated as a genetic distance and examined with MDS.

RESULTS

Allozymes

Variation was detected at 56% of the allozyme loci (43/77), although five polymorphic loci were dropped as alleles were present at a frequencies below 0.01 in all collections (Appendix B). The screening also yielded 28 rare alleles (<0.01 in each collection) which were excluded from analyses.

Observed heterozygosities based on 38 loci varied over a relatively narrow range (mean 0.142, range 0.132 to 0.163; Table 3). No significant difference in heterozygosities was observed between tidal and upstream collections within the same streams (mean tidal =0.149, mean upstream = 0.147, t = 0.693, df = 8, P = 0.741). Heterozygosities of hatcheries were not different from wild collections (mean hatcheries = 0.138, mean wild = 0.147, t = 1.63, P = 0.116). No differences between hatchery and wild collections were apparent with respect to rare allele frequencies, average number of alleles, or proportion of polymorphic loci.

All polymorphic alleles were tested for departures from Hardy-Weinberg (H-W) expectations. No collection had an overall deviation from H-W. We made 743 tests, of which 15 were significant at the 0.05 level before adjusting for multiple tests, well within the range of positive results expected, and none were significant after adjusting for multiple tests. The most significant deviations were spread over nine loci, and no locus deviated in more than three collections.

Heterogeneity among wild populations

We tested for heterogeneity within regions for each elevation. For the tidal collections, Montague and Southeast regions were represented by only a single site each , Rocky and Constantine Creeks, respectively and therefore were not tested. The other three regions were represented by tidal collections from a minimum of four steams each. Heterogeneity was detected only within the Southwest region (eight collections; Table 4). No differences were detected within either the North (four collections) or East regions (seven collections).

Significant heterogeneity was detected overall among all five regions for the tidal

collections (Table 4). A closer examination of the pattern of heterogeneity was conducted through pairwise comparison of the regions (Table 5a). After statistically accounting for multiple tests, only the test between the two best represented regions (Southwest and East) was significant.

Heterogeneity tests were also made for the upstream collections. Three of the upstream collections originated from the East region, while one each originated from the North and Southeast regions (Table 1). Significant heterogeneity was detected within the East region and also in the test among all regions (Table 4).

Pairwise comparisons between pooled upstream collections within regions (Table 5a) indicated that every region was significantly different from every other region. The upstream collection from Lagoon Creek was especially divergent from the rest. This collection had the most divergent allele frequencies for: *sAAT-4*-10; ADA-2*90; mAH-4*81; G3PDH-1* -52; G3PDH-2*120; GDA-1*108,*113; sIDHP-2*125; PEPB-1*138; PEPD-2*120; PEPLT*108;* and *PGDH*86.*

The test for overall heterogeneity between the upstream and tidal collections was also highly significant (Table 4). We conducted tests between the paired upstream and tidal collections originating from Mink, Olsen, Constantine, Koppen, and Lagoon Creeks. The test between the two Lagoon Creek samples was highly significant ($P < 0.01$; Table 6).

Total diversity

A hierarchical gene diversity analysis was performed using 30 loci (isoloci were excluded). The hierarchical analysis was stratified by collection, region, and elevation. By far the majority of the variation (99.29%) occurred within collections (Table 7) and was heavily weighted by variation at *sAAT-4**, *GDA-1**, *sIDHP-2**, and *PEPD-2**. The remaining heterogeneity was divided among collections within regions (0.45%), among regions within elevation (0.19%), and between elevations (0.07%).

The UPGMA tree and the MDS including all collections confirm the uniqueness of the upstream Lagoon Creek collection (Figures 3 and 4). The tree constructed using UPGMA does not show any genetic structuring based on region or elevation (Figure 3). To better visualize the relationships among the other collections, a second MDS was generated excluding the Lagoon Creek upstream collection (Figure 5). Some regional structuring is apparent from the plot. The Southwest collections tend to occupy the left and upper portions of the plot, while the East collections occupy a lower area that extends to the extreme right of the plot. Some overlap between the Southwest and East regions occurs. The North collections tend to occupy space across both the Southwest and East regions. The hatchery collections both occur in the central positions of their respective regions, and Armin F. Koernig (AFK) Hatchery is located near the area of overlap between the Southwest and East collections.

The position of the upstream collections is particularly interesting. Upstream collections from Olsen and Koppen Creeks occupy space within the area bounded by East collections. However, upstream collections from both Mink Creek and Constantine Creek are outliers. Interestingly, the tidal collection from Mink Creek is also an outlier and shows affinity to the upstream Mink Creek collection rather than to other tidal collections from the

North region. As mentioned earlier, Lagoon Creek upstream was not included in this plot because of its highly distant position.

Hatchery collections

No significant difference was detected in the heterogeneity test between the two hatchery collections (Table 5a). The log-likelihood test for homogeneity between the wild and hatchery groups at the highest level of the hierarchy was also not significant (Table 4). However paired log-likelihood tests between each hatchery and pooled collections within regions the test between Solomon Gulch Hatchery and upstream Southeast collections was significant. AFK Hatchery was not different from any of the regions for tidal collections.

In the MDS analysis, although both hatcheries clustered into their respective regions, AFK Hatchery clustered near the area overlapped by the East region collections (Figures 4-5). AFK Hatchery also clustered closely with Duck River, an eastern PWS site from which gametes were collected to found its even-year hatchery stock in 1976. Again the tree constructed using UPGMA does not show any apparent genetic relationship between the hatcheries and streams within regions (Figure 3).

Mitochondrial DNA

Forty individuals from each of the 27 collections were examined for variation at ND5/ND6 using six restriction enzymes previously identified to reveal polymorphisms in pink salmon (Fetzner et al. in prep.; Table 8). Eight unique haplotypes were defined from 1080 individuals detected with four of the six restriction enzymes tested (Table 9). No polymorphic sites were detected with two enzymes, *Rsa I* or *Xba I* . Four of the haplotypes (V, VI, VII, XV) were rare with seven or fewer individuals observed and frequencies less than 0.01. The two rarest haplotypes, VII and XV, were observed only once each.

Haplotype and nucleotide diversity

Haplotype diversity (h) ranged from 0.144 in Hartney Creek to 0.543 in Cathead Creek and averaged 0.381 (Table 9). Corresponding nucleotide diversity values (π) ranged from 0.0012 in Hartney Creek to 0.0050 in Cathead Creek and averaged 0.0039. No regional or elevational patterns in diversities were observed. Nucleotide and haplotype diversities were high for both hatchery collections, Solomon Gulch Hatchery (h = 0.504, π = 0.0047) and AFK Hatchery (h = 0.470, π = 0.0042), although neither of the hatchery values were the largest observed. No significant difference in the nucleotide diversities between the paired upstream and tidal collections were detected (paired t-test; $P > 0.80$).

Heterogeneity detected by Monte Carlo tests

A Monte Carlo test of all collections (hatchery, upstream, and tidal) yielded a significant test statistic (Table 10). Tidal collections were tested for homogeneity within each region, among regions, and among all tidal collections. No test was significant indicating

overall homogeneity among tidal collections. The upstream collections were evaluated in a similar manner; however unlike the tidal tests, all upstream tests were significant ($P < 0.01$, Table 10). Hatchery collections were tested and were not significantly different from each other (Table 10). We also performed an analysis on a region-by-region basis with hatcheries, pooled tidal, and pooled upstream collections similar to that performed with allozymes (Table 5a). For the comparisons between tidal regions, none were significantly different after adjusting for multiple tests (Table 5a). However, in the upstream comparisons, North and East were significantly different from each other after adjusting for multiple tests. For comparisons with hatcheries, no differences between Solomon Gulch Hatchery and tidal collections within any regions were found after adjusting for multiple tests (Table 5a). AFK Hatchery was significantly different from the North upstream region after adjusting for multiple tests. All other tests were not significant.

We also performed a series of Monte Carlo simulations between paired tidal and upstream collections. Only the test for Koppen Creek was significant after adjusting for multiple tests (Table 6). This within-stream difference was quite apparent in the haplotype counts and distribution of haplotypes. For example, haplotype II occurred at a frequency of 0.200 in the Koppen Creek tidal collection, but was absent from the upstream collection.

AMOVA analyses

An AMOVA analysis that partitioned the molecular variation by elevation was also performed. The majority of the variation (98.4%) was within collections ($\Phi_{ST} = 0.016$, Table 11). Most of the variation among collections was within elevation ($\Phi_{SC} = 0.011$). Both Φ_{ST} and Φ_{SC} were significant based on the permutation analysis (Table 11). The between-elevation component, Φ_{CT} , was not significant. A second AMOVA analysis was performed with the partitioning by region (Table 11). The results were quite similar to that obtained for the elevation analysis, indicating that much of the among-collection variation was among collections within regions.

An MDS plot was generated using distances computed from Φ-statistics (Figure 7). The plot resembles that of the allozyme data with Lagoon Creek as the most divergent collection. Other divergent collections include Koppen Creek upstream, Swanson Creek, Hartney Creek, and Constantine Creek upstream.

DISCUSSION

Understanding genetic structure of Pacific salmon populations is critical to their management and conservation. For example, managing on too fine a scale may adversely affect the fishing industry and waste management resources, while managing on too large a scale may result in loss of genetic adaptations and diversity (see Mundy et al. 1993). Here we report our initial findings in an examination of the even-year lineage of commercially important populations of pink salmon that inhabit PWS, Alaska.

Inferences from studies showing genetic homogeneity for allozymes over vast geographic distances (e.g., Shaklee and Varnavskaya 1994) lead some to suggest that pink salmon populations within PWS, spanning only 100 kilometers, should be genetically

homogenous. In contrast, implications from other allozyme studies (Lane 1990) suggest that pink salmon populations in PWS might be substantially heterogenous. Our objective was to generate molecular genetic data to support or reject these alternatives.

Three recent and major factors have impacted these populations. The *Exxon Valdez* oil spill of 1989 adversely affected pink salmon through a combination of direct lethal effects, sublethal effects, and alterations in fishing pressure (Bue et al. 1996); study of effects of the oil spill instigated our study. Further, the major tectonic upheaval of 1964 produced bottlenecks in some populations. However, arguably one of the most serious factors influencing population structure may be deleterious effects of hatchery/wild-stock interactions and the potential erosion of locally adapted genotypes (Gharrett and Smoker 1993). Prince William Sound is the center of one of the world's largest aquacultural industries. Six-hundred million pink salmon fry of hatchery origin are released annually. Alaska Department of Fish and Game has been grappling with management of the wild populations in face of intractable hatchery/wild-stock interactions for nearly a decade. The *Exxon Valdez* oil spill-related damages to wild populations, coupled with full-scale hatchery egg takes, exacerbated wild-stock conservation concerns.

Our analysis of the 1994 collections showed significant substructuring of pink salmon in PWS based upon both allozyme and mtDNA data sets. The heterogeneity analysis, a conservative analysis because all alleles observed are assumed to exist in all collections thereby inflating the degrees of freedom, showed significant allele frequency differences occurring between stream elevations and among and within regions. In the allozyme data, pairwise homogeneity tests among regions indicate that, for tidally spawning aggregates, the Southwest and East regions are distinct from each other after adjusting for multiple tests. However, these were also the two most heavily sampled regions. Other regions may have also been different from eachother had there been more sampling. Evidence of this is found in the number of tests that were significant before accounting for multiple tests. Before adjusting critical values for multiple tests, 4 of the 10 regional tests were significant (more than would be expected by chance if no heterogeneity among regions existed), suggesting that we may have lacked statistical power to detect differences present among less sampled regions (Table 5b). For upstream spawners, pairwise comparisons show genetic differences occurring among all regions where upstream spawners were sampled.

These data provided insight not only into the structure of the wild fish within PWS, but also into the genetic relationships between hatchery fish and these wild fish. Allozyme data did not distinguish AFK Hatchery from any of the regions when tidal fish within region were pooled. The even-year lineage for AFK Hatchery was founded originally with gametes from Duck River, a site across PWS in the East region. Annual propagation at the hatchery comes from broodstock seined from fish milling in front of the hatchery, and evidence from coded-wire-tag recoveries suggests that these milling fish include some wild fish headed for other areas (Sharr et al. 1995). AFK Hatchery is located adjacent to the strait through which most pink salmon enter PWS on their way to their spawning streams (Templin et al. 1996); it is possible that wild fish included in the hatchery broodstock may come from anywhere throughout PWS. Therefore the inability to distinguish AFK Hatchery fish from other regions is not surprising. Conversely, Solomon Gulch Hatchery is located at the end on the Valdez Arm in eastern PWS. Few pink salmon bound for other regions of PWS are likely to be

milling near this hatchery when broodstock are seined for Solomon Gulch Hatchery. In addition, founding broodstock for this hatchery was locally obtained. Although the Solomon Gulch Hatchery collection was not different from any other region after accounting for multiple tests, there is again evidence that this inability to detect differences may be due to a lack of statistical power. Before adjusting the critical values for multiple tests, significant differences were found between Solomon Gulch Hatchery and all regions except the East tidal region (Table 5b). These differences disappear after multiple test adjustments are made, indicating that statistical power may not be adequate to test the hypothesis until regions are better represented.

The fact that the mtDNA data and allozyme data provided generally concordant results strengthens our interpretations. Concordance is not always observed (c., Ward et al. 1989; Adams et al. 1994), but in this study both approaches demonstrate similar heterogeneity among the spawning aggregates. Interestingly, in contrast to expectations generated by mtDNA differences observed in the pilot study (Fetzner et al. in prep), the allozyme data tend to provide comparatively better resolution of regional population structure within PWS. However, both show significant differences between tidal and upstream spawning aggregates and substantial structuring among upstream-spawning populations. Multidimensional scaling analyses for both data sets (Figures 4, 5, and 7) indicated Lagoon Creek upstream to be genetically distinct from all other spawning aggregates. The differences observed within Koppen and Lagoon Creeks are particularly interesting and somewhat surprising given the relatively close geographic proximity of the upstream and tidal spawning areas.

When there were discrepancies in results between allozyme and mtDNA data, in all but two cases, mtDNA data were less able to detect differences than were allozyme data. Allozyme data detected various differences among tidal collections (Table 4), many differences between upstream regions (Table 5a), and differences between upstream and tidal collections at Lagoon Creek (Table 6) which were not detected with mtDNA data (Tables 10, 5 and 6). Three hypotheses might explain this discrepancy: higher straying rates in females than in males, bottlenecks or extinctions and recolonizations, or lack of statistical power. Higher straying rates in females could homogenize mtDNA allele frequencies because of strict maternal inheritance, while allozyme heterogeneity might be maintained if males stray little (Allendorf 1994). However, evidence from coded wire tag data indicates that straying rates of pink salmon in PWS are similar for males and females (Habicht, unpublished data). Other studies have observed low mtDNA variation in populations with high allozyme variation and have attributed these results to historical bottlenecks or extinction and subsequent recolonizations (reviewed in Allendorf 1994). However, mtDNA data in this study were variable; we found eight haplotypes of which three had frequencies greater than 5% (Table 9). The last hypothesis for the lack of significant tests in the mtDNA data analysis may have been a lack of statistical power resulting from the lower allele counts observed per population using this single-locus method. We analyzed 40 fish for mtDNA data which translates to 40 haplotypes per population; conversely, we analyzed 100 fish using allozymes which translates to 200 alleles per locus, and we analyzed 40 loci per population.

The two cases where mtDNA data detected differences where allozyme data did not were between Koppen upstream and tidal collections (Table 6) and between AFK Hatchery and the North collections (Table 5a). One hypothesis that would explain this difference is

that mtDNA data were gathered from the first 40 fish collected in each collection while allozyme data were collected from all 100 fish collected. These first 40 fish, especially in the upstream or tidal collections at Koppen Creek, could have been distinct from the second 60 fish which would have resulted in more heterogeneous allozyme data, but more homogeneous mtDNA data within elevations. To test this hypothesis, we analyzed the allozyme data for the first 40 individuals from all these collections. No differences were detected in either comparison, nor did this result appear to be caused by the decrease in statistical power of only analyzing 40 individuals. Alternatively, in these locations males may stray more than females, however, no data exists to test this hypothesis at these locations. Finally, these discrepancies might be the result of sampling error (Type II error). Data from additional year(s) will allow us to determine if these differences hold between years within the same year-classes.

We recognize that the data show the even-year lineage to have a shallow genetic structure (in contrast to the structure of sockeye salmon populations from a similar geographic range in Cook Inlet, Alaska, for example; Seeb et al. 1995a). In both MDS analyses in this study, collections within each of the *a priori* management regions did not cluster into tight, regional groups. Shallow structure is usually an indication of highly dispersive taxa with limited barriers to gene flow (Avise 1994).

Yet population structure and barriers to gene flow do exist for these fish in the face of oil spills, tectonic upheavals, and potential hatchery straying (Habicht et al. in prep.; available in Seeb et al. 1995b). Our goal is to provide the basis for key management decisions by defining the genetic structure of populations from throughout PWS. The commercial harvest of pink salmon fluctuated dramatically between six and 44 million fish during the years since the oil spill because of ecological instability. Maintenance of genetic diversity will play a key role in ameliorating the affects of this instability. Our data confirm that harvest- and hatchery-management decisions made for conservation purposes should best be made on a population-specific rather than species-specific basis. Expansion of this study to include additional even-year collections as well as comparable odd-year collections is continuing; the analysis of data from multiple year classes will allow us to better test the appropriateness of current management regions.

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Table 1. Pink salmon collected from Prince William Sound in 1994. Map numbers refer to Figure 1. All fish were screened for allozyme variation. Forty fish from each collection were screened for mtDNA variation.

Sample #	Map #	Location name	Elevation	Region	Sample Date	N
1	1	Rocky Creek	tidal	Montague	8/29	100
2	2	Armin F. Koernig Hatchery	-	Southwest	9/08	100
3	3	Cathead Creek	tidal	Southwest	8/22	99
4	4	Herring Creek	tidal	Southwest	8/22	100
5	5	Halverson Creek	tidal	Southwest	8/23	100
6	6	Countess Creek	tidal	Southwest	8/23	100
7	7	Chenega Creek	tidal	Southwest	8/22	100
8	8	Totemoff Creek	tidal	Southwest	8/22	100
9	9	Erb Creek	tidal	Southwest	8/24	100
10	10	Mink Creek	tidal	North	8/24	100
11	10	Mink Creek	upstream	North	8/24	100
12	11	Swanson Creek	tidal	North	8/06	100
13	12	Coghill River	tidal	North	8/24	100
14	13	Jonah Creek	tidal	North	8/23	96
15	14	Solomon Gulch Hatchery	-	East	8/12	100
16	15	Duck River	tidal	East	8/16	100
17	16	Millard Creek	tidal	East	8/16	100
18	17	Lagoon Creek	tidal	East	8/14	100
19	17	Lagoon Creek	upstream	East	8/14	99
20	18	Olsen Creek	tidal	East	8/17	100
21	18	Olsen Creek	upstream	East	8/17	100
22	19	Koppen Creek	tidal	East	8/15	100
23	19	Koppen Creek	upstream	East	8/13	100
24	20	Humpback Creek	tidal	East	8/13	100
25	21	Hartney Creek	tidal	East	8/12	100
26	22	Constantine Creek	tidal	Southeast	8/18	92
27	22	Constantine Creek	upstream	Southeast	8/18	100

Table 2. Enzymes, loci, and primary tissue-buffer combinations used to screen for allozyme variation. Enzyme nomenclature follows Shaklee et al. (1990), and locus abbreviations are given.

Enzyme	Enzyme Number	Locus	Tissue	Buffer[1]
Aspartate aminotransferase	2.6.1.1	*sAAT-1,2**	Heart	ACEN 6.8
		*sAAT-3**	Eye	TG
		*sAAT-4**	Liver	TG
		*mAAT-1**	Heart	ACEN 6.8
		*mAAT-2**	Muscle	ACE 6.5
Adenosine deaminase	3.5.4.4	*ADA-1**	Muscle	AC 6.1
		*ADA-2**	Muscle	AC 6.1
Aconitate hydratase	4.2.1.3	*mAH-1**	Heart	ACEN 6.8
		*mAH-2**	Heart	ACEN 6.8
		*mAH-3**	Muscle	ACE 6.8
		*mAH-4**	Muscle	ACE 6.8
		*sAH**	Liver	ACEN 6.8
Adenylate kinase	2.7.4.3	*AK**	Muscle	TG
Alanine aminotransferase	2.6.1.2	*ALAT**	Muscle	TG
Creatine kinase	2.7.3.2	*CK-A1**	Muscle	TG
		*CK-A2**	Muscle	TG
		*CK-B**	Eye	TG
		*CK-C1**	Eye	TG
		*CK-C2**	Eye	TG
Esterase-D	3.1.1.-	*ESTD**	Muscle	ACE 6.5
Formalin dehydrogenase	1.2.1.1	*FDHG**	Heart	ACEN 6.8
Fumarate hydratase	4.2.1.2	*FH**	Muscle	ACE 6.8

Table 2. Continue.

Enzyme	Enzyme Number	Locus	Tissue	Buffer[1]
β-N-Acetylgalactosaminidase	3.2.1.53	*βGALA* *	Muscle	TG
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*GAPDH-1**	Muscle	AC 6.1
		*GAPDH-2**	Heart	ACEN 6.8
		*GAPDH-3**	Heart	ACEN 6.8
		*GAPDH-4**	Eye	TG
		*GAPDH-5**	Eye	TG
Guanine deaminase	3.5.4.3	*GDA-1**	Liver	TG
N-Acetyl-β-glucosaminidase	3.2.1.53	*βGLUA* *	Liver	ACE 6.8
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-1**	Muscle	TG
		*G3PDH-2**	Heart	ACEN 6.8
		*G3PDH-3**	Heart	ACEN 6.8
Glucose-6-phosphate isomerase	5.3.19	*GPI-B1,2**	Muscle	TG
		*GPI-B2**	Heart	TG
		GPI-A *	Muscle	TG
Glutathione reductase	1.6.4.2	*GR**	Heart	TC4
L-Iditol dehydrogenase	1.1.1.14	*IDDH-1**	Liver	TBCL
Isocitrate dehydrogenase (NADP+)	1.1.1.42	*mIDHP-1**	Muscle	ACE 6.5
		*mIDHP-2**	Heart	ACEN 6.8
		*sIDHP-1**	Liver	ACE 6.8
		*sIDHP-2**	Liver	ACE 6.8

Table 2. Continue.

Enzyme	Enzyme Number	Locus	Tissue	Buffer[1]
L-Lactate dehydrogenase	1.1.1.27	*LDH-A 1**	Muscle	TG
		*LDH-A 2**	Muscle	TG
		*LDH-B1**	Heart	TG
		*LDH-B2**	Heart	TG
		*LDH-C**	Eye	TG
αMannosidase	3.2.1.24	*αMAN**	Heart	TG
Malate dehydrogenase	1.1.1.37	*sMDH-A 1,2**	Heart	ACEN 6.5
		*sMDH-B1,2**	Heart	ACEN 6.5
		*mMDH-1**	Heart	ACEN 6.5
		*mMDH-2,3**	Heart	ACEN 6.5
Malic enzyme (NADP+)	1.1.1.40	*mMEP-1**	Muscle	ACE 6.8
		*mMEP-2**	Muscle	ACE 6.8
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	Heart	TG
Nucleoside-triphosphate pyrophosphatase	3.6.1.19	*NTP**	Muscle	ACE 6.5
Dipeptidase	3.4.-.-	*PEPA**	Muscle	TG
Tripeptide aminopeptidase	3.4.-.-	*PEPB-1**	Heart	TG
		*PEPB- 2**	Heart	TG
Proline dipeptidase	3.4.13.9	*PEPD- 1**	Heart	ACEN 6.5
		*PEPD- 2**	Heart	ACEN 6.5
Peptidase-LT	3.4.-.-	*PEPLT**	Muscle	TG
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	Muscle	ACE 6.5

Table 2. Continue.

Enzyme	Enzyme Number	Locus	Tissue	Buffer[1]
Phosphoglycerate kinase	2.7.2.3	*PGK-1**	Muscle	ACE 6.8
		*PGK-2**	Muscle	ACE 6.8
Phosphoglucomutase	5.4.2.2	*PGM-2**	Heart	TG
Superoxide dismutase	1.15.1.1	*sSOD-1**	Heart	ACEN 6.8
		*sSOD-2**	Heart	ACEN 6.8
		*mSOD**	Heart	ACEN 6.8
Triose-phosphate isomerase	5.3.1.1	*TPI-1**	Muscle	TG
		*TPI-2**	Muscle	TG
		*TPI-3**	Muscle	TG
		*TPI-4**	Muscle	TG

Buffers: AC: amine-citric acid buffer, pH 6.8 (Clayton and Tretiak 1972) modified with EDTA (E), NAD (N), or both (Harris and Hopkinson 1976); TBCL: Tris-citric acid gel, pH 8.7 and lithium hydroxide-boric acid electrode buffer, pH 8.0 (Ridgway et al. 1970); TC4: Tris-citric acid buffer pH 5.8 (Schaal and Anderson 1974); TG: Tris-glycine buffer, pH 8.5 (Holmes and Masters 1970).

Table 3. Observed and expected heterozygosities calculated from 38 polymorphic loci.

	Observed Heterozygosity		Expected Heterozygosity	
	H	Std. Dev.	*H*	Std. Dev.
Rocky Ck. T	0.144	0.054	0.150	0.000
A. F. Koernig Hatchery	0.138	0.049	0.144	0.000
Cathead Ck. T	0.132	0.048	0.137	0.000
Herring Ck. T	0.147	0.058	0.148	0.001
Halverson Ck. T	0.147	0.057	0.148	0.000
Countess Ck. T	0.142	0.054	0.144	0.000
Chenega Ck. T	0.145	0.053	0.151	0.001
Totemoff Ck. T	0.159	0.064	0.157	0.000
Erb Ck. T	0.162	0.069	0.152	0.000
Mink Ck. T	0.150	0.061	0.144	0.000
Mink Ck. U	0.140	0.054	0.142	0.000
Swanson Ck. T	0.145	0.054	0.149	0.000
Coghill R. T	0.152	0.059	0.152	0.000
Jonah Ck. T	0.151	0.057	0.158	0.000
Solomon Gulch Hatchery	0.138	0.050	0.143	0.001
Duck R. T	0.158	0.062	0.153	0.001
Millard Ck. T	0.155	0.060	0.154	0.001
Lagoon Ck. T	0.140	0.052	0.144	0.000
Lagoon Ck. U	0.136	0.054	0.139	0.000
Olsen Ck. T	0.138	0.052	0.138	0.000
Olsen Ck. U	0.148	0.056	0.149	0.001
Koppen Ck. T	0.153	0.060	0.152	0.001
Koppen Ck. U	0.146	0.056	0.145	0.001
Humpback Ck. T	0.163	0.070	0.155	0.000
Hartney Ck. T	0.149	0.059	0.151	0.000
Constantine Ck. T	0.153	0.060	0.153	0.001
Constantine Ck. U	0.154	0.060	0.158	0.000

Table 4. Hierarchical analysis of 1994 pink salmon collections in PWS using log-likelihood ratios. Comparisonwise significance levels (α_c) were adjusted for multiple tests done within the same test groups (Test) using sequential Bonferonni adjustments (modified from Miliken and Johnson 1984 and Rice 1989). Experimentwise significance level was set to 0.05. Complete allozyme table with all loci is in Appendix B.

Source of Variaton	DF	Overall	*P*-value	α_c	Test
Between Sources	56	55.13	0.508	0.050	1
Within Sources	1400	1750.02 *	0.000	0.025	1
Wild	1344	1691.30 *	0.000	0.025	2
Between elevations	56	126.50 *	0.000	0.050	3
Within elevations	1288	1564.80 *	0.000	0.025	3
Upstream	224	356.90 *	0.000	0.025	4
Among Regions	112	184.10 *	0.000	0.050	5
Within East Region	112	172.80 *	0.000	0.025	5
Tidal	1064	1207.90 *	0.001	0.050	4
Among Regions	224	289.50 *	0.002	0.025	6
Within Regions	840	918.40 *	0.031	0.050	6
Southwest	336	402.80 *	0.007	0.017	7
North	168	175.10	0.338	0.025	7
East	336	340.50	0.421	0.050	7
Hatchery	56	58.72	0.376	0.050	2

* Significant at experimentwise $\alpha = 0.05$.

Table 5a. Pairwise homogeneity tests, within stream elevation, between regions and hatcheries. Log-likelihood ratios and degrees of freedom (in parentheses) are given below diagonal for allozyme data; χ^2 values from Monte Carlo simulations are given above the diagonal for mtDNA data.

Tidal

	Montague	Southwest	North	East	Southeast	AFK	Solomon Gulch
Montague	-	3.23	5.30	1.84	1.73	1.42	4.39
Southwest	70.6 (54)	-	6.77	9.96	2.43	4.74	5.63
North	67.4 (49)	79.1 (56)	-	14.33	1.62	12.12	10.26
East	65.8 (51)	93.6 (55)*	73.8 (54)	-	4.78	2.60	6.82
Southeast	53.0 (45)	60.4 (54)	66.4 (53)	57.4 (52)	-	4.10	5.10
AFK	43.7 (44)	52.6 (55)	42.4 (48)	45.9 (50)	58.2 (49)	-	4.31
Solomon Gulch	63.3 (42)	73.4 (54)	85.0 (52)	61.8 (51)	65.8 (46)	58.72 (46)	-

Upstream

	North	East	Southeast	AFK	Solomon Gulch
North	-	16.98*	11.15	14.44*	9.86
East	86.0 (47)*	-	7.82	8.39	2.38
Southeast	87.4 (45)*	94.6 (46)*	-	7.11	8.14
AFK	47.4 (44)	67.3 (45)	62.6(45)	-	5.10
Solomon Gulch	68.4 (45)	62.4 (45)	72.8 (44)*	58.72 (46)	-

* Significant at experimentwise $\alpha = 0.05$ (Rice 1989)

Table 5b. *P*-values for pairwise homogeneity tests from Table 5a, within stream elevation, between regions and hatcheries. *P*-values for the log-likelihood ratios are given below diagonal for allozyme data; *P*-values for the χ^2 values from Monte Carlo simulations are given above the diagonal for mtDNA data.

Tidal

	Montague	Southwest	North	East	Southeast	AFK	Solomon Gulch
Montague	-		0.264	0.759	0.582	0.717	0.198
Southwest	0.064	-	0.338	0.104	0.828	0.486	0.400
North	0.042	0.023	-	0.008	0.792	0.031	0.031
East	0.080	0.001*	0.038	-	0.369	0.596	0.245
Southeast	0.193	0.256	0.102	0.282	-	0.316	0.154
AFK	0.484	0.567	0.701	0.639	0.173	-	0.317
Solomon Gulch	0.018	0.041	0.003	0.143	0.029	0.099	-

Upstream

	North	East	Southeast	AFK	Solomon Gulch
North	-	0.004*	0.016	0.005*	0.049
East	0.000*	-	0.152	0.123	0.784
Southeast	0.000*	0.000*	-	0.050	0.029
AFK	0.336	0.017	0.042	-	0.317
Solomon Gulch	0.014	0.044	0.004*	0.099	-

* Significant at experimentwise $\alpha = 0.05$ (Rice 1989)

Table 6. Heterogeneity between paired tidal and upstream collections for allozyme and haplotype frequencies. Log-likelihood tests were performed to test homogeneity of allozyme frequencies. Homogeneity of mtDNA was tested using Monte Carlo simulations; probabilities of exceeding the original χ^2 by chance alone are given.

	Allozyme			mtDNA	
Stream	Log-likelihood	df	*P*	χ^2	*P*
Olsen Ck.	51.80	47	0.2920	2.20	0.9033
Mink Ck.	58.73	47	0.1172	3.94	0.5990
Lagoon Ck.	115.73	43	0.0000*	6.90	0.0223
Koppen Ck.	56.97	46	0.1288	13.56	0.0016*
Constantine Ck.	63.07	51	0.1196	1.17	0.7382

* Significant at experimentwise $\alpha = 0.05$ (Rice 1989)

Table 7. Gene diversity analysis (Nei 1973) by locus between stream elevations, among regions within elevations, among collections within regions, and within collections.

	Absolute Gene Diversity		Relative Gene Diversity			
	Total	Within Collection	Within Collection	Collections Within Region	Region Within Elevation	Between Elevation
*sAAT-3**	0.3528	0.3504	0.9931	0.0061	0.0008	0.0001
*sAAT-4**	0.5229	0.5184	0.9914	0.0067	0.0018	0.0001
*mAAT-1**	0.0167	0.0166	0.9940	0.0040	0.0020	0.0001
*ADA-1**	0.0028	0.0028	0.9935	0.0035	0.0017	0.0012
*ADA-2**	0.1510	0.1499	0.9929	0.0046	0.0024	0.0001
*sAH**	0.0065	0.0064	0.9943	0.0036	0.0018	0.0004
*mAH-3**	0.0044	0.0044	0.9944	0.0046	0.0008	0.0002
*mAH-4**	0.0734	0.0728	0.9916	0.0058	0.0017	0.0008
*CK-A2**	0.0048	0.0048	0.9949	0.0041	0.0010	0.0000
*FDHG**	0.0151	0.0150	0.9945	0.0041	0.0012	0.0002
*bGALA**	0.2067	0.2051	0.9922	0.0049	0.0027	0.0002
*G3PDH-1**	0.3031	0.3000	0.9898	0.0066	0.0035	0.0000
*G3PDH-2**	0.2494	0.2476	0.9930	0.0038	0.0029	0.0003
*G3PDH-3**	0.0157	0.0156	0.9939	0.0044	0.0012	0.0005
*GDA-1**	0.5150	0.5120	0.9941	0.0043	0.0003	0.0013
*IDDH-1**	0.0060	0.0060	0.9947	0.0036	0.0016	0.0001
*mIDHP-1**	0.0089	0.0088	0.9957	0.0031	0.0011	0.0000
*sIDHP-2**	0.4528	0.4485	0.9906	0.0059	0.0026	0.0008
*LDH-A2**	0.0032	0.0032	0.9878	0.0092	0.0025	0.0004
*LDH-B2**	0.0204	0.0203	0.9945	0.0045	0.0009	0.0001
*mMEP-1**	0.3869	0.3844	0.9935	0.0017	0.0030	0.0018
*NTP**	0.0020	0.0020	0.9920	0.0073	0.0005	0.0003
*PEPB-1**	0.2230	0.2217	0.9939	0.0045	0.0004	0.0012
*PEPD-2**	0.6089	0.6058	0.9951	0.0025	0.0022	0.0003
*PEP-LT**	0.2580	0.2561	0.9928	0.0037	0.0026	0.0008
*PGDH**	0.4386	0.4360	0.9941	0.0033	0.0013	0.0013
*PGM-2**	0.0040	0.0040	0.9950	0.0032	0.0017	0.0001
*mSOD**	0.0204	0.0202	0.9927	0.0059	0.0012	0.0002
*sSOD-1**	0.0160	0.0159	0.9945	0.0047	0.0004	0.0004
*TPI-2**	0.0301	0.0299	0.9934	0.0048	0.0018	0.0000
Overall	0.1640	0.1628	0.9929	0.0045	0.0019	0.0007

Table 8. Restriction enzymes, length of recognition sequence (r), and fragment sizes detected in ND5/ND6 haplotypes.

Restriction Enzyme	r	Haplotype	Fragment sizes (bp)
Apa I	6	A	1300, 1100
		B	1300, 650, 450
BstU I	4	A	1650,750
		B	1200, 750, 450
		C	1150, 750, 500
EcoR V I	6	A	2400
		B	1500, 900
Hinf I	4	A	800, 500, 350, 300, 250[a]
		B	1050, 500, 350, 300, 250
		C	500, 450, 350[a], 300, 250[a]
Rsa I	4	A	1605, 265[b]
Xba I	6	A	2400

[a] There are two fragments of the indicated size in these patterns.
[b] There are three fragments of the indicated size in these patterns.

Table 9. Haplotype counts for 1994 collections from Prince William Sound (T = tidally spawning, U = upstream spawning, H = hatchery). Haplotype designations after Fetzner et al. (in prep.): I = AAAAAA, II = ACAAAA, III = AAABAA, IV = ABAAAA, V = AABAAA, VI = BAAAAA, VII = AAACAA, XV = ACBAAA. Order of restriction enzymes is *Apa I, BstU I, EcoR V, Hinf I, Rsa I, Xba I.* Haplotype diversity (h) and nucleotide diversity (π) are given.

			ND5/ND6 Haplotypes									
	Sampling Site		I	II	III	IV	V	VI	VII	XV	h	π
1	Rocky Creek	T	31	7	2	0	0	0	0	0	0.3709	0.0032
2	Armin F. Koernig	H	28	8	3	0	1	0	0	0	0.4696	0.0042
3	Cathead Creek	T	26	7	2	3	0	2	0	0	0.5430	0.0050
4	Herring Creek	T	34	2	1	2	0	0	0	0	0.3083	0.0026
5	Halverson Creek	T	32	5	3	0	0	0	0	0	0.3430	0.0030
6	Countess Creek	T	26	8	3	2	1	0	0	0	0.5354	0.0049
7	Chenega Creek	T	32	3	3	2	0	0	0	0	0.3506	0.0031
8	Totemoff Creek	T	27	5	5	1	0	1	1	0	0.5177	0.0049
9	Erb Creek	T	31	5	3	0	1	0	0	0	0.3823	0.0034
10	Mink Creek	T	33	2	2	3	0	0	0	0	0.3127	0.0027
11	Mink Creek	U	28	1	3	6	0	1	0	1	0.4861	0.0047
12	Swanson Creek	T	36	4	0	0	0	0	0	0	0.1823	0.0015
13	Coghill River	T	30	4	3	2	0	1	0	0	0.4241	0.0038
14	Jonah Creek	T	35	2	2	1	0	0	0	0	0.2316	0.0020
15	Solomon Gulch	H	27	5	7	1	0	0	0	0	0.5038	0.0047
16	Duck River	T	31	4	4	0	1	0	0	0	0.3835	0.0034
17	Millard Creek	T	29	7	3	0	1	0	0	0	0.4430	0.0039
18	Lagoon Creek	T	33	4	3	0	0	0	0	0	0.3076	0.0027
19	Lagoon Creek	U	26	2	12	0	0	0	0	0	0.4911	0.0045
20	Olsen Creek	T	29	5	4	1	0	1	0	0	0.4532	0.0041
21	Olsen Creek	U	29	6	5	0	0	0	0	0	0.4418	0.0040
22	Koppen Creek	T	29	8	3	0	0	0	0	0	0.4342	0.0038
23	Koppen Creek	U	35	0	1	2	1	1	0	0	0.2329	0.0020
24	Humpback Creek	T	29	6	5	0	0	0	0	0	0.4418	0.0040
25	Hartney Creek	T	37	1	1	0	1	0	0	0	0.1443	0.0012
26	Constantine Creek	T	33	5	1	1	0	0	0	0	0.3063	0.0026
27	Constantine Creek	U	35	4	0	1	0	0	0	0	0.2266	0.0018

Table 10. Analysis of geographic patterns of heterogeneity in mtDNA haplotypes. A total of 10,000 Monte Carlo simulations were performed to compute the probabilities of exceeding the original χ^2 by chance alone.

Region		Test	χ^2	*P*
WILD				
TIDAL				
Within Region				
	Southwest	5	38.10	0.367
	North	5	11.01	0.560
	East	5	27.08	0.680
Among Regions		5	23.88	0.420
All tidal		3	127.23	0.166
UPSTREAM				
Within Region				
	East	4	26.73	<0.001*
Among Region		4	27.79	0.004*
All Upstream		3	57.58	<0.001*
ALL WILD		2	233.70	<0.001*
HATCHERY		2	4.31	0.317
TOTAL PWS		1	251.25	<0.001*

* Significant at experimentwise $\alpha = 0.05$ (Rice 1989)

Table 11. Hierarchical analysis of molecular variation (AMOVA) observed in Prince William Sound pink salmon collections from 1994.

a. Elevation

Variance Component	Observed Partition		P^a	Φ-statistic
	Variance	% Total		
Among elevation	0.001	0.55	0.119	$\Phi_{CT} = 0.006$
Among collections within elevation	0.002	1.07	0.008	$\Phi_{SC} = 0.011$
Within collections	0.209	98.38	0.007	$\Phi_{ST} = 0.016$

b. Region

Variance Component	Observed Partition		P^a	Φ-statistic
	Variance	% Total		
Among regions	0.001	0.55	0.084	$\Phi_{CT} = 0.005$
Among collections within regions	0.002	0.90	0.044	$\Phi_{SC} = 0.009$
Within collections	0.209	98.55	0.003	$\Phi_{ST} = 0.014$

[a] Probability of having a more extreme variance component than the observed value by chance alone.

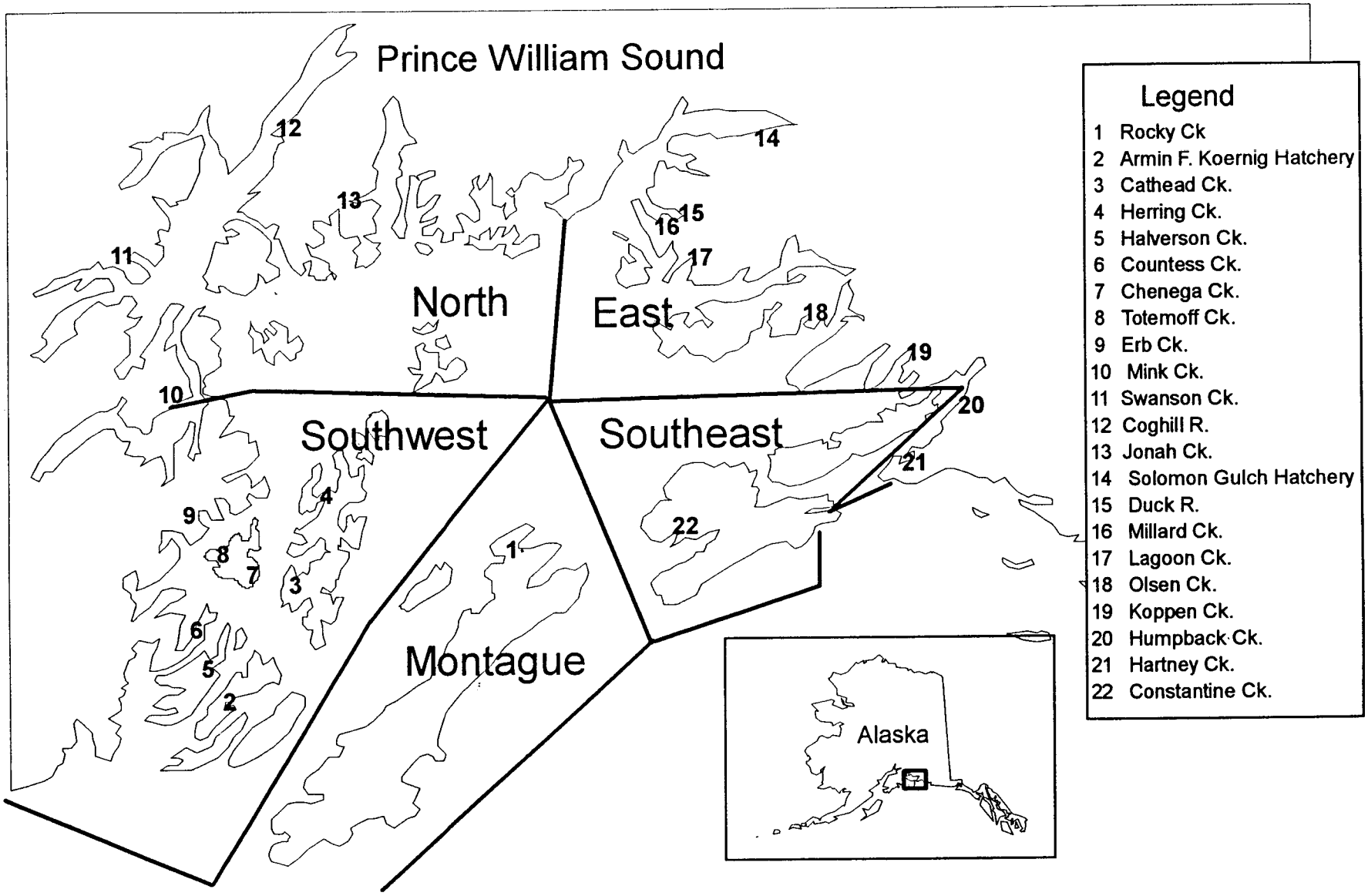

Figure 1. Location of sample collection sites within the major managment regions of Prince William Sound.

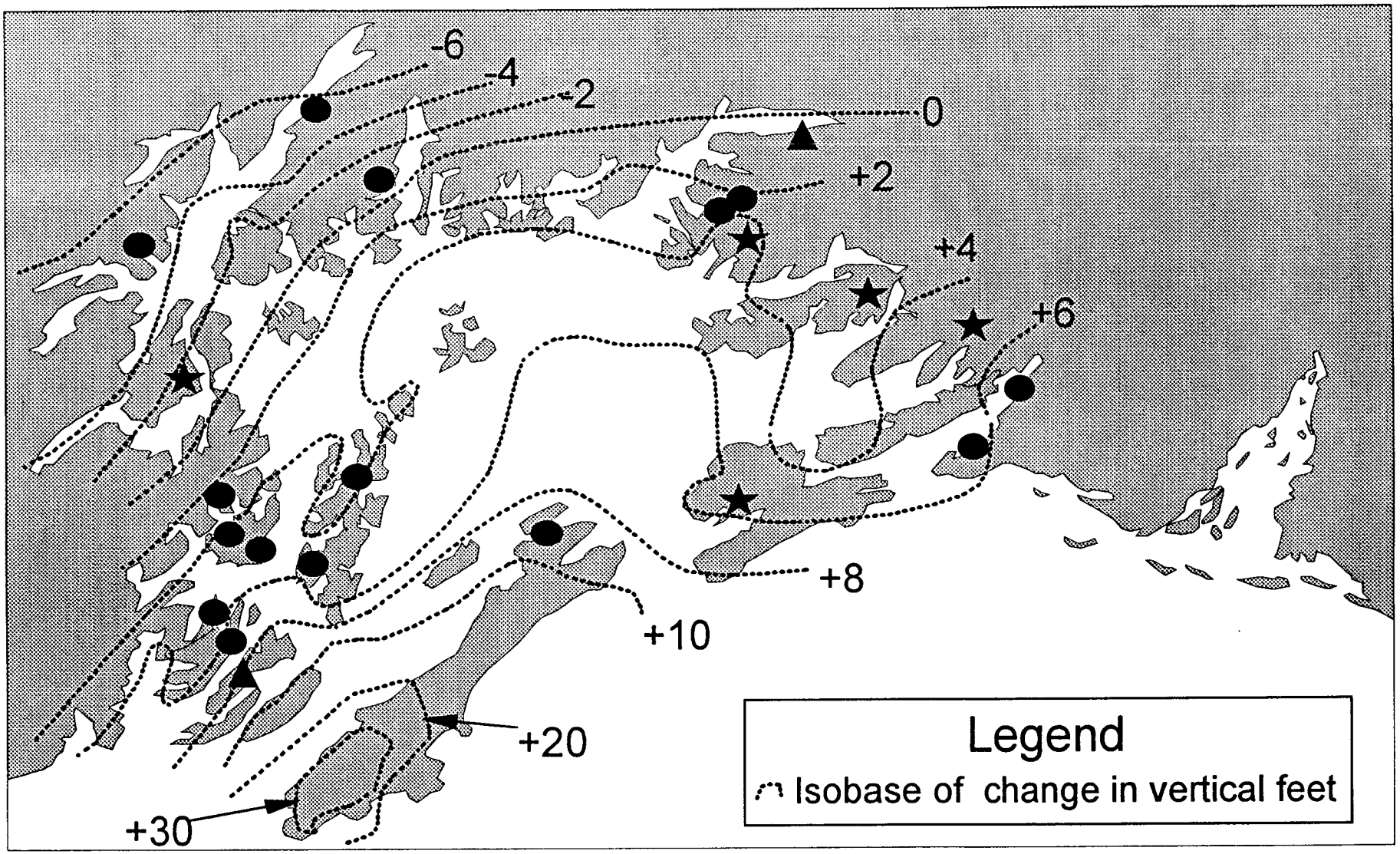

Figure 2. Shift in elevation following the 1964 earthquake in Prince William Sound. Collections with both upstream and tidal samples are indicated with stars, tidal collections are indicated with circles, and hatchery collections are indicated with triangles. Adapted from Plafker and Mayo (1965).

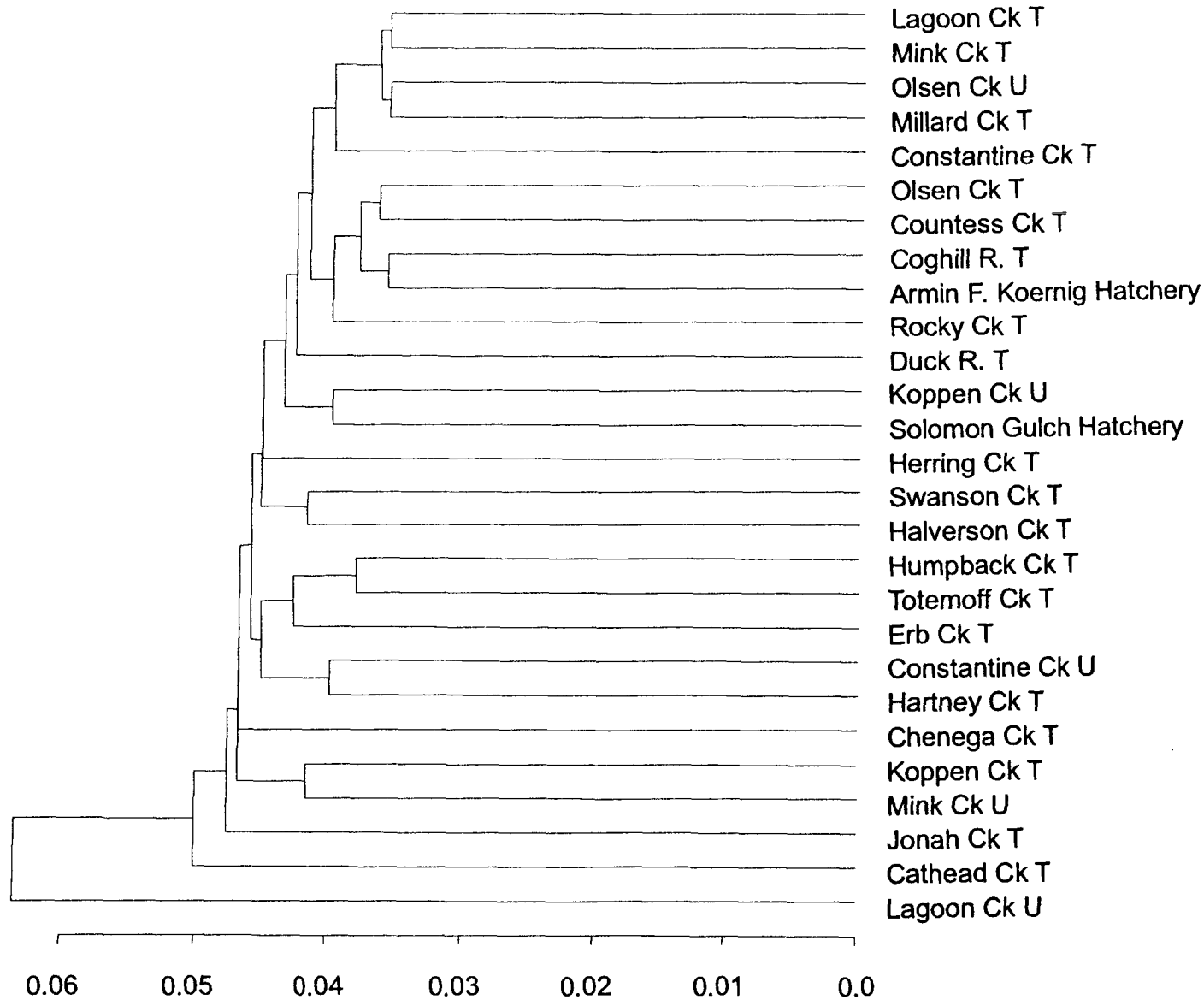

Figure 3. Tree constructed using unweighted pair-group method (UPGMA; Sneath and Sokal 1973) with Cavalli-Sforza and Edwards (1967) chord distances derrived from allozyme variation in pink salmon collections made in Prince William Sound, AK in 1994.

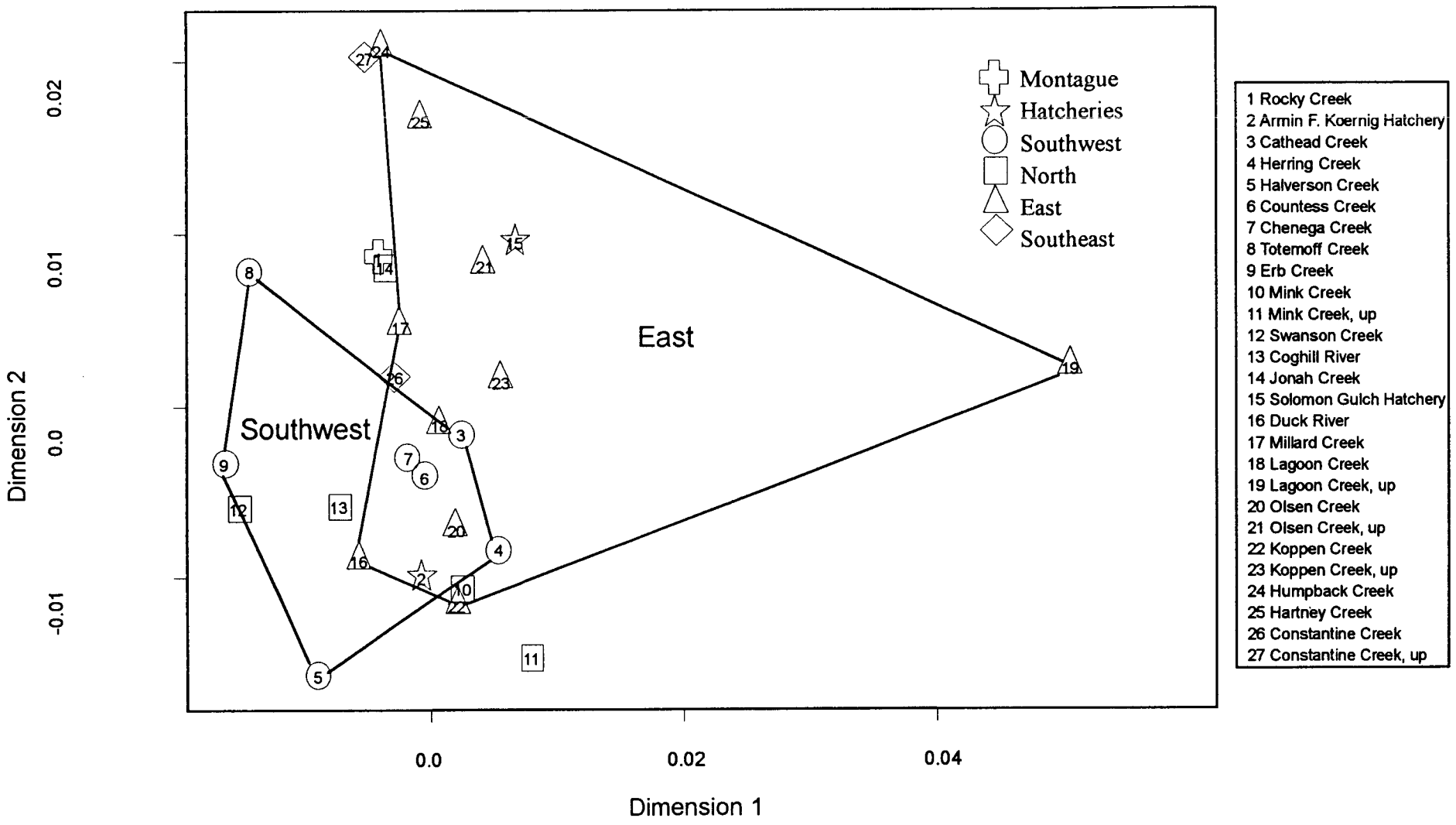

Figure 4. Multidimensional scaling analysis. Cavalli-Sforza and Edwards chord distances, calculated from 38 allozyme loci, were used. Polygons including all Southwest and East collections are superimposed.

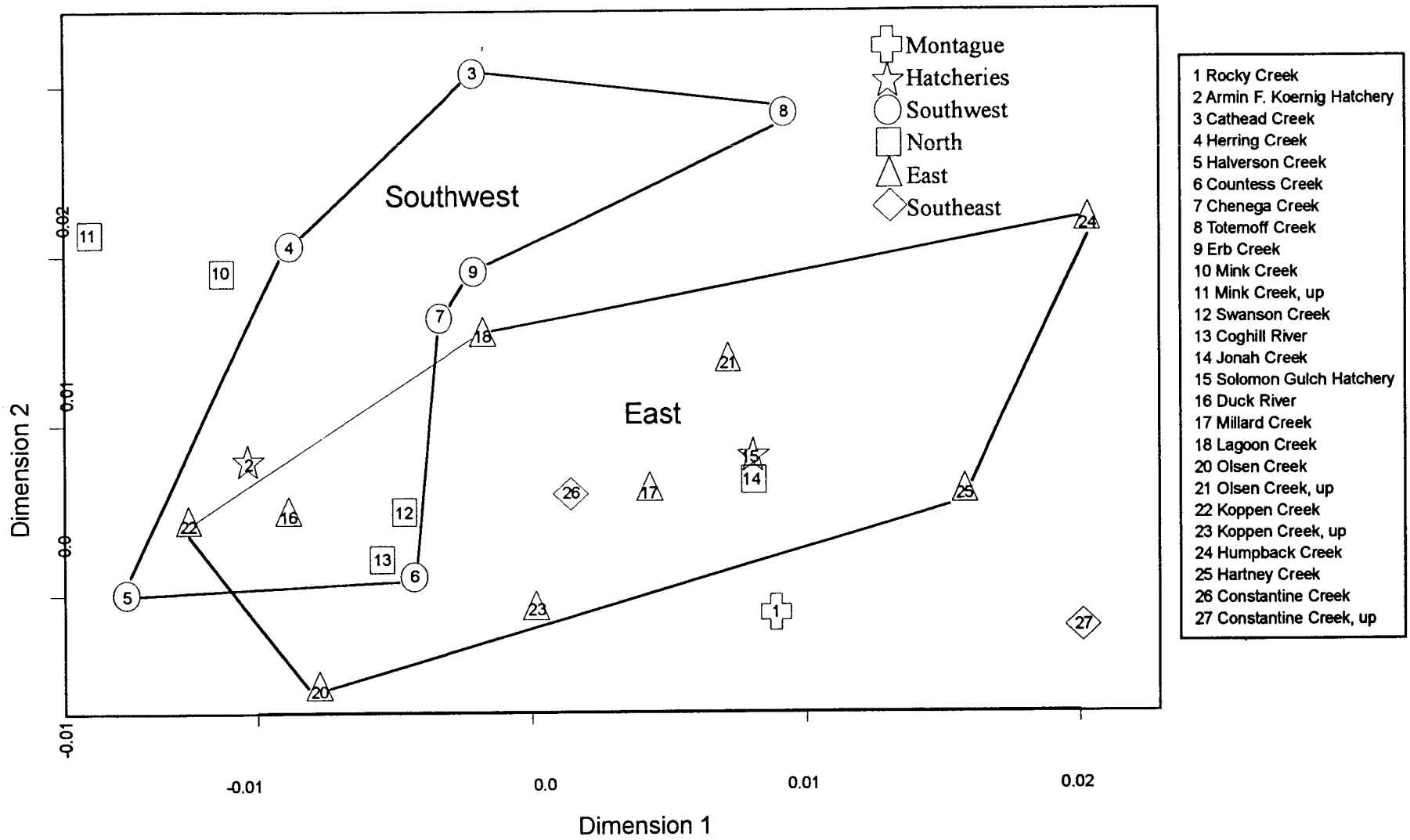

Figure 5. Multidimensional scaling analysis. The upstream collection from Lagoon Creek was excluded to clarify relationships among remaining collections. Cavalli-Sforza and Edwards chord distances, calculated from 38 allozyme loci, were used. Polygons including all Southwest and East collections are superimposed.

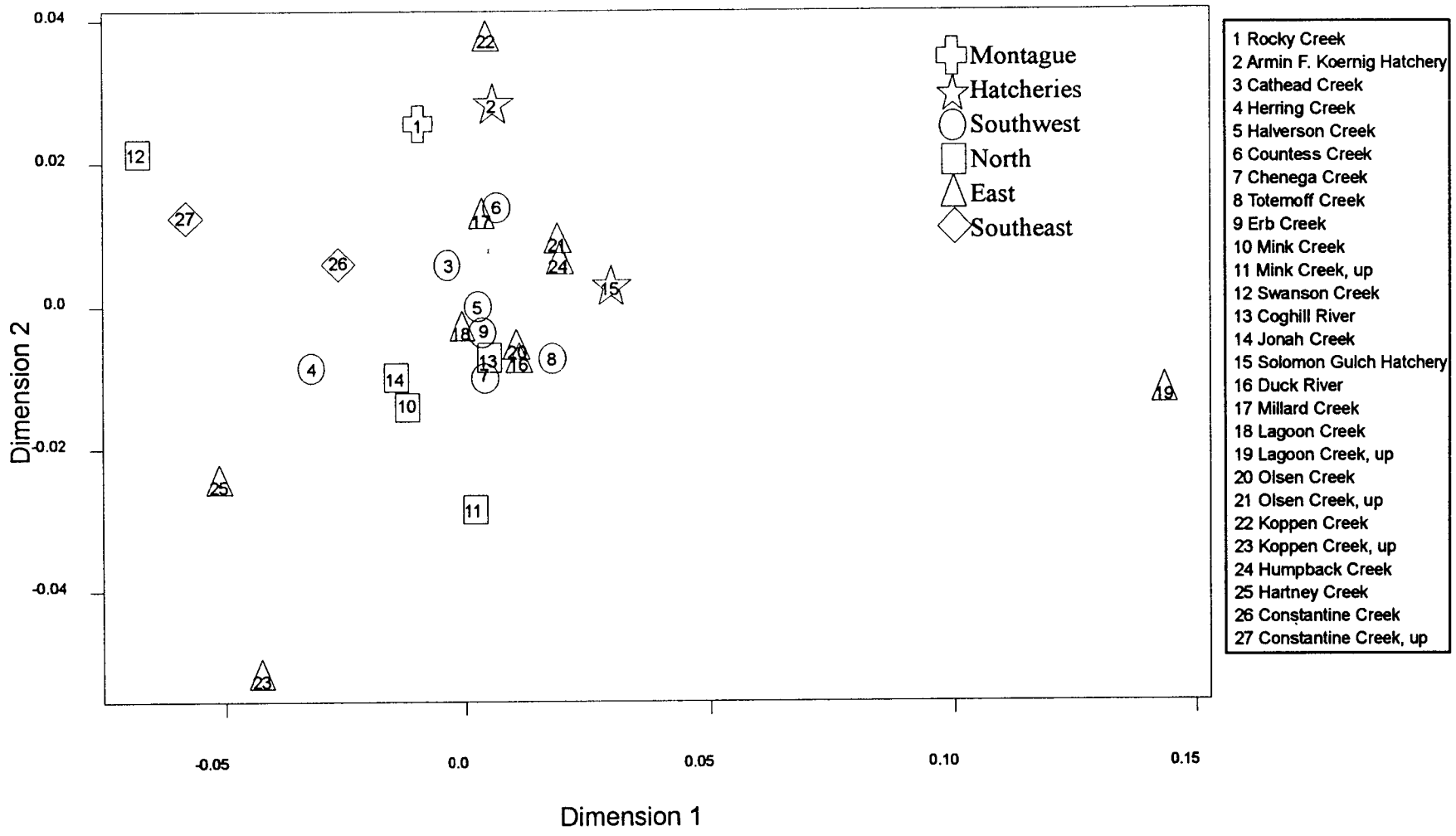

Figure 6. Multidimensional scaling analysis generataed from Φst distances calculated from mtDNA data.

Appendix A. Variation at ND5/ND6 discriminates even- and odd-year pink salmon (*Oncorhynchus gorbuscha*) populations from Alaska.

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Keywords: Mitochondrial DNA, *Oncorhynchus gorbuscha*, PCR-RFLPs, pink salmon, *Exxon Valdez*.

Appendix A. Variation at ND5/ND6 discriminates even- and odd-year pink salmon (*Oncorhynchus gorbuscha*) populations from Alaska.

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Running Title: mtDNA variation in pink salmon

Keywords: Mitochondrial DNA, *Oncorhynchus gorbuscha*, PCR-RFLPs, pink salmon, *Exxon Valdez*.

Abstract--Mitochondrial NADH dehydrogenase subunits 5 and 6 regions were amplified from 160 odd-year and 204 even-year pink salmon (*Oncorhynchus gorbuscha*) from Alaska using the polymerase chain reaction (PCR). Samples included in the analyses were from Norton Sound, Kachemak Bay, Prince William Sound, and Southeast Alaska. Restriction fragment length polymorphism (RFLP) analyses revealed this region of mtDNA to be variable in pink salmon. Haplotype diversity values within populations were relatively high and ranged from 0.097 to 0.636 (mean = 0.466). The most common haplotype (I) was found in all populations examined but was present at a higher frequency in the even-year samples than in the odd-year samples. Overall, haplotype I was detected in 69% of sampled individuals. The frequency of the twelve composite haplotypes were found to be geographically informative across regions, and the frequencies also varied greatly among populations within regions as well as between the even- and odd-year collections.

Introduction

The pink salmon (*Oncorhynchus gorbuscha*) is the most abundant North American species of Pacific salmon (Neave 1967; Heard 1991), thus making it an economic and ecological cornerstone in biological communities of the Pacific Rim. Pink salmon are both anadromous and semelparous: in their natural range, they make long oceanic migrations, home to their natal stream to spawn, and die at age two. Commercial catches of pink salmon exceeded 100 million fish annually in Alaska during this decade. Pink salmon are an important food source for many marine and terrestrial species, and their spawning migration provides a pathway for transferring nutrients from marine ecosystems to nearshore and terrestrial ecosystems.

Pink salmon are unique in the family Salmonidae, having the fixed two-year life span which produces two reproductively isolated lineages in the non-overlapping even- and odd-year classes (Davidson 1934). Their range extends from Puget Sound, Washington, north to the Mackenzie River, which flows into the Beaufort Sea (modified from Heard 1991). Southern populations are primarily limited to odd-years, while even-year populations are common in more northerly rivers where odd-year populations are absent or in low numbers. Rivers in the center of the range are characterized by abundant populations in both even- and odd-years. Previous studies show that differences occur in allozyme frequencies between the even- and odd-year lineages inhabiting the same river (Aspinwall 1974; Utter et al. 1980; Beacham et al. 1988) as well as in morphological and life history characters (Beacham et al. 1988).

Prince William Sound, Alaska, site of the 1989 *Exxon Valdez* oil spill, is approximately the center of the North American range of the pink salmon. These populations were impacted by the *Exxon Valdez* oil spill of 1989, and the effects appear to be rippling through both the even- and odd-year lineages (Bue et al. 1996). In addition to effects of the oil spill, the Prince William Sound populations are subjected to intense pressures from both harvest and adverse interactions with hatchery fish (an average of over 600 million hatchery fry have been released annually from Prince William Sound hatcheries since 1985). For these and other reasons the aggregate census fluctuated an order of magnitude from 1.8 million to

21.0 million annually during recent decades. However, we know little about the genetic structure of these native populations, and a better understanding of the genetic structure of wild stocks inhabiting Prince William Sound is critical to their long-term management and conservation.

Previous studies of the genetic diversity of pink salmon populations rely almost solely on data collected from allozyme electrophoresis (e.g., Aspinwall 1974; Beacham et al. 1988; Gharrett et al. 1988; Shaklee et al. 1991; Varnavskaya and Beacham 1992; Shaklee and Varnavskaya 1994). Results show that pink salmon possess comparatively high allozyme diversity (proportion of polymorphic loci often $\geq$ 33%, heterozygosity $\geq$ 10%), but diversity is not always apparent among geographically adjacent collections (e.g., Gharrett et al. 1988). If heterogeneity is apparent among populations, it may not be partitioned along obvious geographical boundaries (Shaklee and Varnavskaya 1994). Therefore, Zhivotovsky et al. (1994) suggest that the population structure of pink salmon would best be studied by using complementary data sets gathered from more than one technique.

Variation in mitochondrial DNA (mtDNA) haplotypes may offer an additional opportunity for characterizing the structure of salmonid populations. The analysis of restriction fragment length polymorphisms (RFLPs) in mtDNA has sometimes shown resolving power complementary to that of allozyme electrophoresis for salmonids (e.g., Gyllensten and Wilson 1987; Birmingham 1990; Park et al. 1993; Adams et al. 1994; Bickham et al. 1995).

Haplotype diversity has yet to be examined among pink salmon populations. Our goal in this study is to document the levels of variability and diversity in mtDNA in Alaska pink salmon to provide a baseline for future comparative studies both within Alaska and throughout the species range. We hope to utilize markers identified in this study to help resolve the population structure of pink salmon from Prince William Sound affected by the *Exxon Valdez* oil spill.

We chose to examine the mitochondrial NADH dehydrogenase subunits 5 and 6 (ND5/ND6) in pink salmon using the polymerase chain reaction (PCR, Saiki et al. 1988) and utilizing restriction fragment length polymorphisms (RFLP). Previous examinations of many areas of the mitochondrial genome have shown the ND5/ND6 region to be variable in salmonids (Cronin et al. 1993; Park et al. 1993). We examined 14 even- and odd-year populations from across the species range in Alaska, including paired even- and odd- year collections from three streams in Prince William Sound. This allowed for comparisons between years, as well as comparisons within years across Alaska and within Prince William Sound.

Materials and Methods

Samples

We sampled three paired even- and odd-year populations in Prince William Sound and seven additional populations ranging from the northern Bering Sea to the eastern Gulf of Alaska (Figure 1). All tissues were collected on liquid nitrogen or dry ice and stored at -80°C

until analysis. In total, 364 pink salmon specimens were analyzed representing both odd- and even-year populations from: Norton Sound, Kachemak Bay, Prince William Sound, and Southeast Alaska (Table 1).

Total genomic DNA was extracted from 100 mg of liver or muscle tissue. Standard protocols of Proteinase-K and RNase-A digestion, phenol-chloroform extraction, and ethanol precipitation were used (Sambrook et al. 1989). After isolation, the DNA was resuspended in TE buffer (10mM Tris, 0.1 mM EDTA), quantitated by spectrophotometry, and diluted to 50 ng/μl for use in the polymerase chain reaction (PCR, Saiki et al. 1988).

Polymerase Chain Reaction

The entire *ND5/ND6* region of the mitochondrial genome was amplified using PCR and was conducted in 100μl volumes which contained 4mM $MgCl_2$, 1.0μM of each primer, 200μM each dNTP, 2.5U of *Taq* DNA polymerase (Perkin-Elmer Cetus) and 20-50*ng* of sample DNA. The reactions were initially denatured at 94°C for 2.5 minutes followed by amplification using 40 cycles consisting of strand denaturation (94°C, 40 seconds), primer annealing (55°C, 1 minute) and polymerase mediated primer extension (72°C, 3.5 minutes). A final extension of 7 minutes at 72°C was included to minimize partial extension products. Primers used in the reaction were those of Cronin et al. (1993; Table 2). The L and H designations were added and refer to the light and heavy strands of the mtDNA molecule, respectively. These primers are located in the tRNA genes flanking the *ND5/ND6* region. The LND5/ND6 primer is in the $tRNA^{LEU}$ gene and the 3' base is 12896 relative to the rainbow trout (*O. mykiss*) sequence (Zardoya et al. 1994, unpublished). The HND5/ND6 primer is in the $tRNA^{GLU}$ gene and the 3' base is 15337 relative to the rainbow trout sequence.

Restriction Digests and Analyses

An initial screen for variation in the *ND5/ND6* region of mtDNA was conducted on twenty individuals from 11 pink salmon collections using 16 restriction endonucleases. Following amplification, the DNA samples were digested directly with the restriction endonucleases which recognized both four (*BstU I, Dpn II, Hae III, Hinf I*, and *Rsa I*) and six (*Apa I, BamH I, Bcl I, BstE II, EcoR I, EcoR V, Hind III, Kpn I, Pst I, Stu I*, and *Xba I*) base sequences. Each digest was conducted in a total volume of 20μl with 2.5 units of enzyme and then incubated according to the manufacturer's specifications. After incubation, samples were loaded into a 0.8% agarose gel and run for approximately 1.5 hours. A pGEM molecular weight standard (Promega, #G-1741) was run on each gel in order to estimate fragment lengths. Following electrophoresis, gels were stained with ethidium bromide, photographed under ultraviolet light (312*nm*), and scored based on the fragment patterns detected for each individual. Haplotypes were assigned an alphabetic character according to their order of occurrence, with "A" being the most common haplotype detected.

Unbiased estimates of haplotype diversity (h, Nei and Tajima 1981) and nucleotide diversity (π) were estimated to examine the amount of mtDNA variation within populations of pink salmon by using the restriction enzyme analysis package (REAP) of McElroy et al.

(1992). Where the collections allowed, we tested for population subdivision at several hierarchical levels by using Monte Carlo simulations (Rolf and Bentzen 1989). The hierarchical levels we examined were 1) among populations within regions, 2) among regions (Norton Sound, Kachemak Bay, Prince William Sound, Southeast Alaska), and 3) among the even- and odd-year classes. We also performed an analysis of molecular variance (AMOVA) with Φ-statistics (Excoffier et al 1992). Transformed Φ-distances between pairs of populations were used to generated a UPGMA phenogram depicting relationships among populations with the PHYLIP package (Felsenstein 1993). A network connecting the different haplotypes was constructed using site data and the maximum-likelihood RESTML program of PHYLIP.

Sequence Analysis

We sequenced the entire *ND5/ND6* region from a single pink salmon to verify restriction sites detected in the RFLP segment of this study. The amplified *ND5/ND6* PCR products were separated by agarose gel electrophoresis, and the band of interest was excised from the gel. The product was purified from the agarose gel slices using the QIAquick gel extraction kit (Qiagen; Chatsworth, CA) and then further purified and concentrated with Microcon-100 spin columns (Amicon; Beverly, MA). Final concentrations of samples were 30 ng/μl. Sequencing reactions were conducted in 20μl volumes using the Applied Biosystems (ABI) Prism Dye-deoxy terminator kit with AmpliTaq FS. Sequencing reactions contained 3.2 pmol of primer and 225ng of purified template DNA. Primers for sequencing included those listed in Table 1. Samples were run for 14 hours on an ABI 373A automated DNA sequencer using 6% polyacrylamide (8.3M urea) gels. Samples were aligned to the known rainbow trout sequence obtained from Genbank (accession L29771) using the Sequence Navigator program supplied with the ABI sequencer.

Results

Restriction Enzyme Variation

Of the 16 enzymes examined in the initial survey, six (*Apa I, BstU I, EcoR V, Hinf I, Rsa I,* and *Xba I*) were found to be polymorphic (Table 3), three (*BamH I, Kpn I, and Pst I*) failed to recognize any sites within the region, and the remaining seven (*Bcl I, BstE II, Dpn II, EcoR I, Hae III, Hind III, and Stu I*) produced identical fragment patterns in all populations. A total of twelve different composite haplotypes were detected in the *ND5/ND6* region of pink salmon (Table 4). After the initial survey, all remaining populations (Table 2, Figure 1) were examined with the polymorphic enzymes detected in the screening process. The number of sites recognized by any one enzyme ranged from zero to seven. Of the 12 composite haplotypes detected in this study, four were produced by variants at *Hinf I* sites, with all but one of these being detected at low frequencies (≤ 0.05) within populations.

Odd-year Populations

The haplotype diversity (h) values within the eight odd-year populations ranged from 0.385 to 0.636 (mean = 0.524), and nucleotide diversity (π) ranged from 0.015 to 0.022 (mean = 0.019; Table 4).

For the odd-year populations, haplotypes I and II were the most prevalent, with overall frequencies of 0.544 and 0.400, respectively. In half of the odd-year populations examined the frequency of haplotype II surpassed that of haplotype I. Seven additional haplotypes (IV, V, VIII, IX, XI, XII, and XIII) were also detected in the odd-year populations but were at very low frequencies ($\leq$0.025, Table 4). For odd-year populations, haplotypes VIII and IX were found only in Prince William Sound and Kachemak Bay while haplotypes V, XI, XII, and XIII were detected only in populations from Southeast Alaska. However, samples sizes for all these collections were small (N=20), so the possibility that these haplotypes occur throughout Alaska cannot be excluded.

Overall, the odd-year populations had rather heterogeneous composite haplotype frequencies. We were able to detect geographic heterogeneity among the regions examined based on Monte Carlo simulations (χ^2=89.34, P<0.001; Table 5). Within regions, a significant difference was detected between the Norton Sound samples, Nome and Snake Rivers (χ^2=3.75, P=0.023), but no significant differences were detected among populations of Prince William Sound or Southeast Alaska (Table 5).

Even-year Populations

Haplotype diversity values for the even-year populations ranged from 0.097 to 0.442 (mean = 0.312) and nucleotide diversity ranged from 0.003 to 0.013 (mean = 0.010; Table 4). The even-year populations overall contained a higher frequency (0.799) of haplotype I while haplotype II was detected at a much lower frequency (0.088) when compared to odd-years (0.400). Frequency of haplotype II did not exceed 0.150 in any even-year population examined. Five additional haplotypes (III, IV, V, X, and XIV) were detected among the even-year populations but most were found at relatively low frequencies (see Table 4). Three of these (III, X and XIV) were unique to the even-year populations. Among all populations (odd- and even-years) examined, haplotype III was the only haplotype besides I and II which was detected at a relatively high frequency (>0.05) within any one population.

Significant heterogeneity in haplotype frequencies was detected among regions (χ^2= 26.18, P=0.0002; Table 5) for the even-year populations based on Monte Carlo simulations. Tests within Norton Sound and Prince William Sound were possible; no differences in haplotype frequencies were detected among populations within either region. Frequencies of composite haplotypes were also found to be temporally stable among our two even-year Duck River samples (χ^2= 1.69, P=0.6653; Table 5).

Comparison between even- and odd-years

Values for both haplotype and nucleotide diversity were significantly smaller (Wilcoxon rank-sum test [Wilcoxon 1945], P=0.0027 and P=0.0007, respectively) in even-year populations than in odd-year populations, indicating the even-year populations are less diverse in mtDNA variation. Significant heterogeneity in haplotype frequencies was detected in pooled even- versus pooled odd-year comparisons (χ^2=72.52, P=0.0000). We found that 22.4% of the variance (Φ_{ST}=0.224) detected was attributable to between year class differences based on AMOVA analyses. The phenogram (Figure 2) shows that the even-year and odd-year populations form distinct groups. In addition, some geographic clustering is seen among the even-year populations, with branches separating Prince William Sound from the Norton Sound populations. However, this type of pattern was not seen for the odd-year populations which formed two relatively divergent groups, neither of which showed geographic affinities. We constructed a phylogenetic network connecting the different haplotypes based on the restriction site differences detected. All haplotypes detected in this study could be unambiguously assigned to the network, and the differences between haplotypes could be inferred by single restriction site differences (Figure 3a). Differences in haplotype distribution between the even- and odd-year collections are apparent (Figures 3b and 3c). The even-year collections had a very high frequency of haplotype I (0.799), and a low frequency of haplotype II (0.088). It is interesting to note that all the other haplotypes detected among even-year collections are derivatives from haplotype I (Figure 3b). In contrast, we found that haplotypes from the odd-year collections, which had a higher frequency of haplotype II (0.400) and a lower frequency of haplotype I (0.543), were predominantly derived from haplotype II (Figure 3c).

Sequence Analysis

The entire sequence from a single individual exhibiting haplotype VII was obtained (Figure 4) and compared to the known rainbow trout sequence. The sequence divergence between the pink salmon and rainbow trout was 9.8% for *ND5* and 14.8% for *ND6*. The higher value of sequence divergence for the *ND6* gene was due to an 18 bp deletion in pink salmon (starting at position 2105) when compared to the rainbow trout sequence.

Discussion

Intraspecific mtDNA Variability

At the population level (within regions) composite haplotype frequencies varied among the streams examined but was only significantly different in the Norton Sound odd-year populations. Odd-year runs in this region are relatively small, especially when compared to even-year runs, and can fluctuate widely from year to year. In this type of situation, bottlenecks that increase genetic drift can cause rapid population differentiation. This heterogeneity among odd-year Norton Sound streams would seem to suggest that straying is limited among these pink salmon populations or at least below the level necessary to counteract the effects of drift. If this were not the case, one would expect to find

homogeneous haplotype frequencies among streams, especially in intertidal areas, where most straying is thought to occur. It would be interesting to examine if even-year populations from areas where their runs sizes are low (i.e., Washington and southern British Columbia) show this high level of differentiation in mtDNA haplotype frequencies.

Both the odd- and even-year populations showed some level of convergence in composite haplotype frequencies, but significant differences were still detected among regions for both year classes. Haplotype composition also differed among regions suggesting that several haplotypes are unique to a particular region or a particular year class. However, larger sample sizes are necessary to verify with a high level of certainty that the haplotypes are indeed absent.

We were able to detect significant differences between even- and odd-year populations in both haplotype and nucleotide diversity, with values for odd-year populations almost double those estimated for even-year populations. This result is consistent with previous allozyme studies, which found odd-year populations to be more diverse than those in even-years (Gharrett et al. 1990; Shaklee et al. 1991). In addition, we found that the haplotype relationships between even- and odd-year pink salmon to be divergent, with even-year populations having haplotypes derived exclusively from haplotype I while for odd-year populations the majority of haplotypes were derived from haplotype II (Figure 3).

The AMOVA analysis indicated that 22.4% of the variation detected could be attributed to between year-class differences. Values of G_{ST} between year classes, though not directly comparable to Φ_{ST}, have been reported for allozyme data and are quite variable, ranging from estimates of 2.7% - 14.9% (Zhivotovsky et al. 1994; McGregor 1983). The differentiation among year classes is thought to be caused by their temporal separation into independent glacial refugia during the Pleistocene (Aspinwall 1974) and subsequent reproductive isolation. The even-year class is thought to have survived in the Bering Refugia, while the odd-year class may have survived in a southern refuge, possibly in the Columbia River drainage (Withler and Morley 1982). The differences we detected in haplotype affinities would seem to support this hypothesis (Figure 3) and suggest that some lineage sorting has occurred between the even- and odd-year classes.

We were also able to compare sequence obtained from haplotype IX to that of haplotype VII and rainbow trout. Although this second pink salmon haplotype was not observed in any individuals from this study, it did originate from Prince William Sound (Seeb et al. in prep.). We calculated percent sequence divergence between the two haplotypes (*Hinf I* variants); the sequence divergence was estimated to be 1.5%. This comparison suggests that additional variation is present in the ND5/ND6 region of pink salmon and could be helpful in identifying additional restriction enzymes for future RFLP studies conducted on pink salmon. The value obtained for the comparison between pink salmon and rainbow trout sequences was 9.8% for *ND5* and 14.8% for *ND6*, which is akin to results obtained by other researchers that have examined mtDNA diversity in salmonids (Domanico and Phillips 1995; McVeigh and Davidson 1991; Shedlock et al. 1992).

Interspecific mtDNA Variability

Previous studies of mtDNA variation in other salmonid species (Parks et al. 1993; Cronin et al. 1993) have found the *ND5/ND6* region to possess somewhat limited amounts of variation, but more so than other areas of the mtDNA molecule. The current study, however, has detected a considerable amount of sequence divergence (p) in the *ND5/ND6* region in pink salmon (p=0.015 between two distinct haplotypes). Cronin et al. (1993) found sequence divergence to be low among chinook (*O. tshawytscha*) and chum (*O. keta*) salmon populations (p=0.0003-0.0044 and 0.0006-0.0062, respectively), and Parks et al. (1993) suggested a recent bottleneck event as a most likely reason for less variability in mtDNA of chum salmon.

The apparent higher levels of genetic variability observed in pink salmon may be linked to their unique life history. Generational effects (i.e., the length of time between generations) have been suggested as a possible reason for genetic variability among taxa (Sibley et al. 1988; Li et al. 1987). It has been noted that organisms with shorter generation times accumulate mutations at a faster rate and usually have larger population sizes than taxa with longer generation times (Wilson et al. 1987). In comparing Pacific salmon species, pink salmon have the shortest generation time and are the most abundant of the five species (Heard 1991). While there is considerable variability in the ages of returning spawners (anywhere from two to seven years old in chinook salmon), the majority of spawners are three to five years old. This difference may explain the higher levels of diversity detected in pink salmon mtDNA.

mtDNA Variation as a Reflection of Population Structure

One of the objectives of this study was to develop mitochondrial markers to delineate population structure of pink salmon in Prince William Sound and potentially throughout the range of the species. Mitochondrial DNA, as compared to nuclear DNA, has the unique properties of being haploid and transmitted through maternal lines. As a result, the effective population size for mtDNA may be only one fourth that of nuclear genes (Birky et al. 1989), so that the effects of bottlenecks and genetic drift are more pronounced for mtDNA. Further, we may expect additional differentiation in mtDNA if male migration rates are greater than female rates.

Allozyme electrophoresis, reflecting variation in nuclear genes, has been used extensively to distinguish among Pacific salmonid populations (Allendorf et al., 1987). Allozymes sometimes do not discriminate among geographically proximal populations of pink salmon, but they routinely exhibit significant heterogeneity on a broader scale, thus allowing the discrimination of lineages important for management and conservation (Shaklee and Varnavskaya 1994).

Mitochondrial DNA also appears capable of distinguishing pink salmon populations from different regions and in some cases among populations within regions. This study provides only a framework for delineating population structure of pink salmon; additional data are needed before a comprehensive understanding of geographic variability in pink salmon can be obtained from mtDNA. Our data suggest that mtDNA techniques will compliment allozyme and other nuclear markers in discriminating among pink salmon populations, and the smaller effective population sizes in mtDNA as compared to nuclear markers may make it a

more sensitive to technique to reflect historical events of genetic drift.

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Table 1. Primers used in PCR amplifications of the *ND5/ND6* region for RFLP and sequencing analyses (as in text). Numbering system for primers designed in this study are according to the rainbow trout sequence.

Primer Name	Sequence	Reference
LND5/ND6	5'-AATAGTTTATCCRTTGGTCTTAGG-3'	Cronin et al. (1993)
HND5/ND6	5'-TTACAACGATGGTTTTTCATRTCA-3'	Cronin et al. (1993)
L13331	5'-CCTCCTCCTCTTCCTGATTGCCATAA-3'	this study
L13734	5'-GTGGCGGGCATCTTCCTATTAATTCG-3'	this study
L14130	5'-TAGCTGGGTTCTTCTCCAAAGACTC-3'	this study
L14532	5'-TACATAACTTCTCCAACATACTGGG-3'	this study
L14878	5'-AATTAACATTCCCCCTCCATGAGAG-3'	this study

Table 2. Populations, collection year, sample sizes, and regional location of specimens examined in this study.

Map #	Population	Year	N	Region
	Odd-Year			
1	Nome River	1991	20	Norton Sound
2	Snake River	1991	20	Norton Sound
4	Tutka Bay Hatchery	1993	20	Kachemak Bay
5	Swanson Creek	1991	20	Prince William Sound
6	Duck River	1991	20	Prince William Sound
7	Humpback Creek	1991	20	Prince William Sound
8	Gastineau Hatchery	1993	20	Southeast Alaska
9	Little Port Walter Hatchery[a]	1993	20	Southeast Alaska
	Even-Year			
1	Nome River	1994	20	Norton Sound
3	Solomon River	1994	20	Norton Sound
5	Swanson Creek	1994	40	Prince William Sound
6	Duck River	1992	44	Prince William Sound
6	Duck River	1994	40	Prince William Sound
7	Humpback Creek	1994	40	Prince William Sound

[a] Specimens were F1 progeny from 50 wild caught parents used in hatchery experiments.

Table 3. Pink salmon mtDNA restriction fragment sizes and haplotype patterns for polymorphic enzymes examined in this study. Relative frequencies of composite haplotypes within and among populations are given in Table 4.

Restriction Enzyme	Fragment r	size (bp)	Haplotype Pattern
Apa I	6	1240	A B - - -
		1117	A - - - -
		651	- B - - -
		466	- B - - -
BstU I	4	1576	A - - - -
		1200	- B - - -
		1150	- - C - -
		781	A B C - -
		500	- - C - -
		450	- B - - -
EcoR V	6	2375	A - - - -
		1466	- B - - -
		909	- B - - -
Hinf I	4	1057	- B D - -
		850	- - - E -
		803	A - - E -
		550	- - - - F
		500	A B - - F
		362	- - D - -
		351	A B D - F
		258	A B D E F
		239	A[a] B D E[a] F[b]
		90	- - D - -
Rsa I	4	1610	A - - - -
		1100	- B - - -
		405	- B - - -
		298	A B - - -
		274	A B - - -
		187	A B - - -
Xba I	6	2375	A - - - -
		1706	- B - - -
		669	- B - - -

[a]There are two fragments of the indicated size in these patterns.
[b]There are three fragments of the indicated size in these patterns.

Table 4. Distribution of composite haplotypes, haplotype diversity, and nucleotide diversity for the ND5/ND6 region of mtDNA for fourteen populations of Alaskan pink salmon (*Oncorhynchus gorbuscha*) examined in this study.

				Composite Haplotypes[a]											Diversity Values	
Population	Year	N	I	II	III	IV	V	VIII	IX	X	XI	XII	XIII	XIV	Haplotype	Nucleotide
Duck River	1991	20	8	11	-	-	-	1	-	-	-	-	-	-	0.5487	0.0200
Humpback Creek	1991	20	14	4	-	-	-	2	-	-	-	-	-	-	0.4718	0.0173
Swanson Creek	1991	20	9	10	-	1	-	-	-	-	-	-	-	-	0.5590	0.0194
Nome River	1991	20	15	5	-	-	-	-	-	-	-	-	-	-	0.3846	0.0146
Snake River	1991	20	9	11	-	-	-	-	-	-	-	-	-	-	0.5077	0.0193
Gastineau Hatchery	1993	20	7	10	-	-	1	-	-	-	1	-	1	-	0.6359	0.0224
Little Port Walter Hat.	1993	20	13	6	-	-	-	-	-	-	-	1	-	-	0.4974	0.0180
Tutka Bay Hatchery	1993	20	11	7	-	-	-	1	1	-	-	-	-	-	0.5846	0.0209
Odd Year Totals		**160**	**87**	**64**	**0**	**1**	**1**	**4**	**1**	**0**	**1**	**1**	**1**	**0**	**0.5237**	**0.0190**
Duck River	1992	44	33	4	7	-	-	-	-	-	-	-	-	-	0.4086	0.0117
Duck River	1994	40	31	4	4	-	1	-	-	-	-	-	-	-	0.3835	0.0120
Humpback Creek	1994	40	29	6	5	-	-	-	-	-	-	-	-	-	0.4418	0.0134
Swanson Creek	1994	40	36	4	-	-	-	-	-	-	-	-	-	-	0.1823	0.0069
Nome River	1994	20	19	-	-	-	-	-	-	1	-	-	-	-	0.0974	0.0025
Solomon River	1994	20	15	-	-	2	1	-	-	1	-	-	-	1	0.3590	0.0106
Even Year Totals		**204**	**163**	**18**	**16**	**2**	**2**	**0**	**0**	**2**	**0**	**0**	**0**	**1**	**0.3121**	**0.0095**
TOTALS		**364**	**250**	**82**	**16**	**3**	**3**	**4**	**1**	**2**	**1**	**1**	**1**	**1**		

[a]Haplotypes were determined from polymorphic enzymes (*Apa I, BstU I, EcoR V, Hinf I, Rsa I* and Xba I, respectively) and are as follows: I=AAAAAA, II=ACAAAA, III=AAABAA, IV=ABAAAA, V=AABAAA, VIII=BCAAAA, IX=AAADAA, X=AAAFAA, XI=ACAEAA, XII=ACAABA, XIII=ACABAA, and XIV=ABAAAB. Note: VI, and VII were detected in a separate study and thus are not presented here.

Table 5. Analysis of geographic patterns of heterogeneity in mtDNA haplotypes. A total of 10,000 Monte Carlo simulations were performed; probabilities of exceeding the original χ^2 by chance alone are given.

Comparisons	χ^2	P
Odd-Year Collections		
Among Regions	89.34	0.0000
Within Regions		
Norton Sound	3.75	0.0234
Prince William Sound	9.44	0.1070
Southeast Alaska	6.80	0.0984
Even-Year Collections		
Among Regions	26.18	0.0002
Within Regions		
Norton Sound	3.26	0.4818
Prince William Sound	10.75	0.2649
Duck River Between Years	1.69	0.6653
Even- vs. Odd-Years	72.52	0.0000
TOTAL	255.25	0.0000

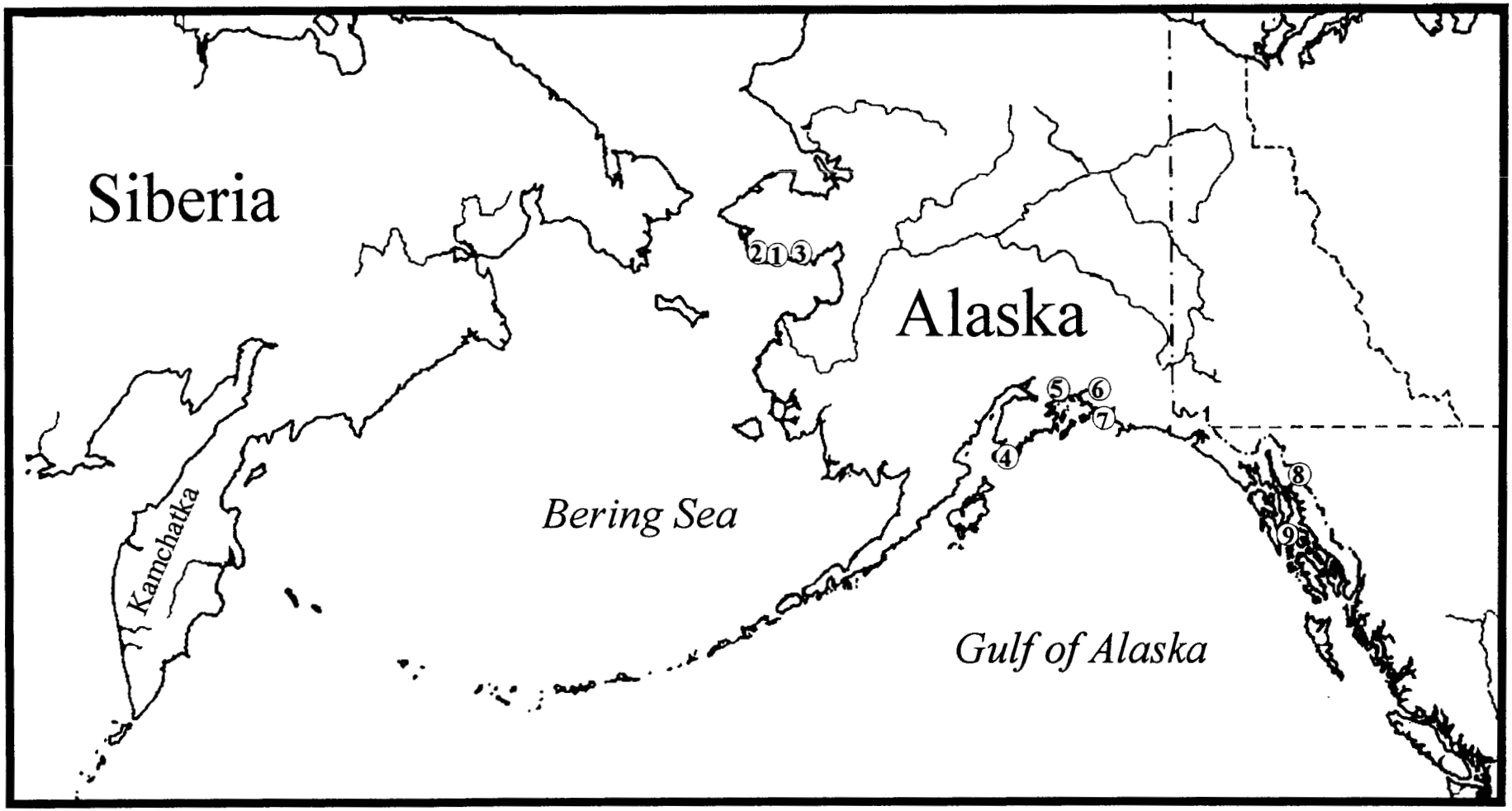
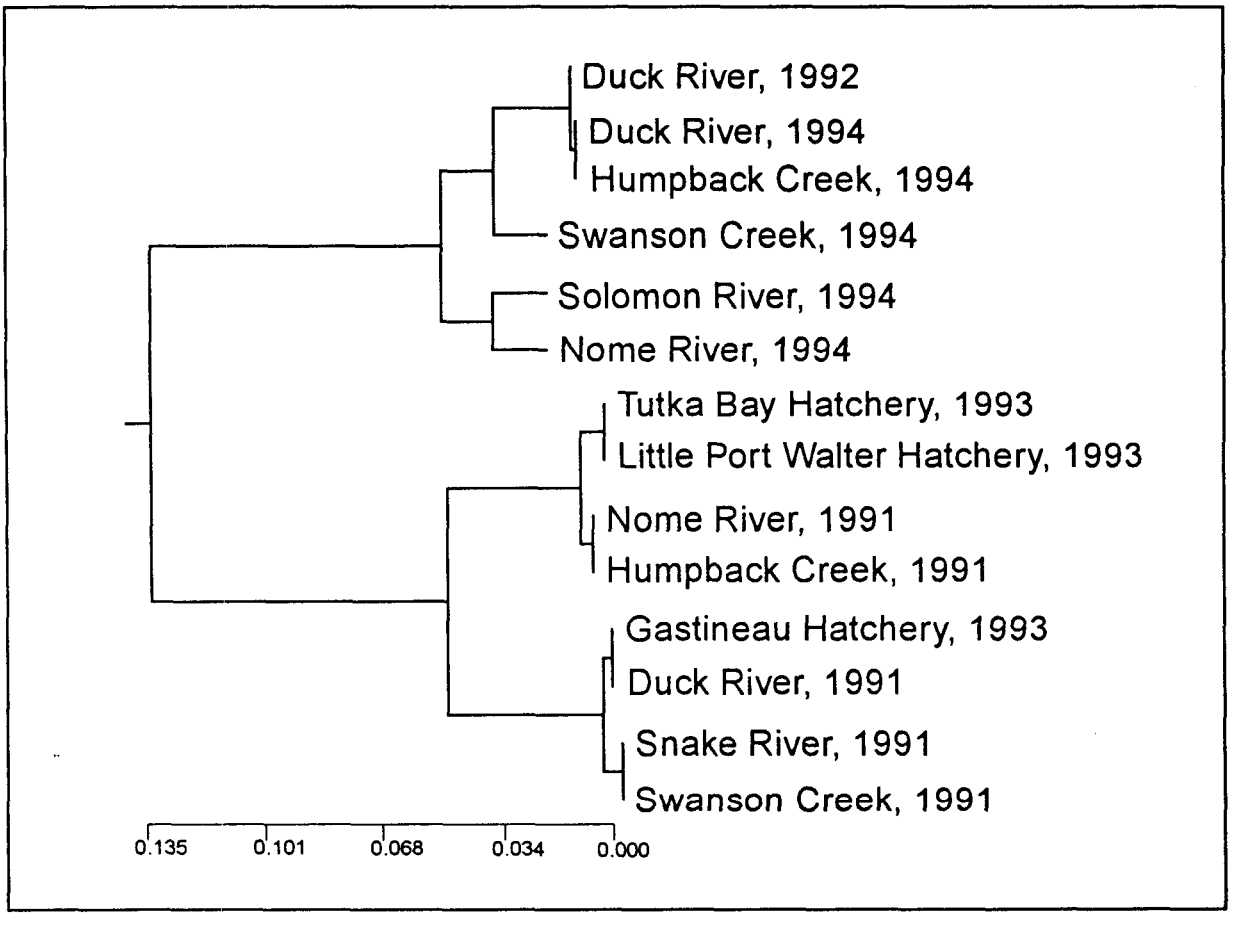


Figure 1. Collection localities of pink salmon populations used in this study. 1) Nome River, 2) Snake River, 3) Solomon River, 4) Tutka Bay Hatchery, 5) Swanson Creek, 6) Duck River, 7) Humpback Creek, 8) Gastineau Hatchery, 9) Little Port Walter Hatchery.

Figure 2. UPGMA phenogram generated from nonlinearly transformed Φ_{ST} distances among populations.



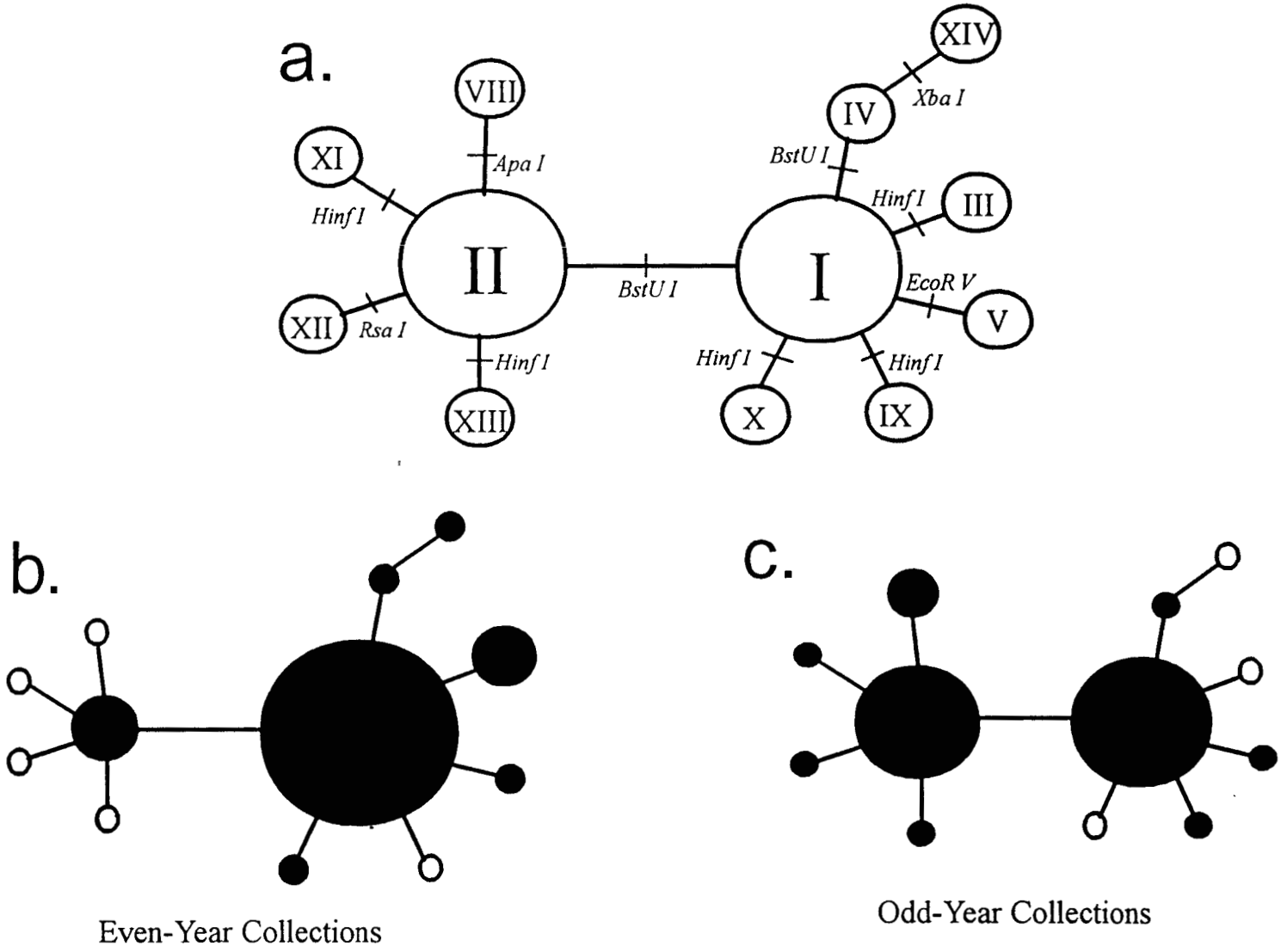


Figure 3. Haplotype network and the distribution of haplotypes within year classes: a. all haplotypes were connected to the network based on single restriction site changes, b. even-year collections, and c. odd-year collections. For the haplotype distributions within the even- and odd-year collections, black circles represent the presence ot that haplotype whereas white circles represent an absence ot the haplotype in that year class. Size of the circles indicates approximate frequencies.

Figure 4. Complete nucleotide sequence of the 2357 base pair (bp) mitochondrial *ND5/ND6* region of pink salmon. The complete *ND5* gene is 1838 bp long (bases 1-1838) and the complete sequence for the *ND6* gene is 522 bp (bases 1834-2357, compliment). There is a four basepair overlap of the two genes at their 3' ends (underlined text). The pink salmon *ND6* sequence contains an 18 bp deletion (starting at position 2105) not found in rainbow trout. This sequence has been submitted to Genbank under accession number U55056.

```
   1 ATGCACCCCA CTACACTCAT CTTAAGCTCA TCCCTTTTAA TAATTTTTAC CCTTCTAATC
  61 TACCCCCTCA TCACCACTCT CACCCCGACC CCTCAACACA AAAACTGATC CCTTACTCAA
 121 GTAAAAACTG CCATCAAAAT GGCCTTCCTC GTAAGCTTAC TCCCCCTTTT TATCTTCCTA
 181 GATCAAGGAA CTGAAACTAT CGTCACTAAC TGGCAATGAA TAAACACCAC AACCTTTGAT
 241 ATTAACCTTA GCTTTAAATT TGACCACTAC TCCATTATTT TTACCCCAAT TGCCCTGTAC
 301 GTAACCTGAT CTATTCTCGA ATTCGCATCA TGGTACATAC ACGCCGATCC AAACATAAAC
 360 CGGTTCTTTA AATATCTCCT CCTCTTCCTG ATTGCCATAA TTATTTTGGT GACCGCCAAC
 421 AATATATTTC AACTATTCAT CGGCTGAGAG GGAGTCGGAA TTATATCGTT CCTCCTCATT
 481 GGGTGATGGC ACGGACGGGC TGATGCTAAC ACAGCTGCCA TACAAGCTGT GATTTATAAC
 541 CGTGTAGGAG ACATTGGACT TATCTTAAGT ATAGCTTGGT TCGCAACAAA CCTTAACTCC
 601 TGAGAAATTC AACAAATATT TGCCTCTTCA AAAGGTCTCG ACCTTACACT CCCTCTTATA
 661 GGCCTCATTC TAGCCGCCAC CGGCAAATCA GCGCAATTTG GACTTCACCC GTGACTTCCC
 721 TCAACGATAG AAGGTCCTAC GCCGGTATCT GCCCTACTAC ACTCCAGCAC CATAATAATC
 781 GCGGGCATCT TCCTGTTAAT TCGACTCCAT CCTCTAATAG AAAACAACCA AACAGCCCTC
 841 ACCACTTGCT TATGCCTAGG AGCCCTAACC ACCCTATTCA CCGCCACCTG TGCCCTAACA
 901 CAAAATGATA TTAAAAAAAT TGTCGCATTC TCTACATCCA GCCAACTAGG ACTTATAATA
 961 GTCACCATCG GACTTAATCA ACCACAGCTA GCCTTTCTCC ACATCTGGCA CTCACGCATT
1021 CTTCAAAGCA ATACTTTTCT TATGTTCCGG TCAATTATTC ACAGTTTAAA CGACGAACAA
1081 GATATTCGAA AAATAGGAGG CATACACAAC CTCACCCCAT TTACTTCCTC CTGCCTTACA
1141 ATCGGGAGTC TTGCACTCAC CGGCACCCCC TTCTTAGCAG GATTTTTCTC CAAAGATGCT
1201 ATTATTGAAG CCTTAAACAC ATCCCACCTC AACGCCTGGG CCCTCACTCT TACCTTACTA
1261 GCCACCTCAT TCACCGCCAT TTATAGCCTC CGAGTTATCT TTTTCGTCTC CATAGGACAC
1321 CCTCGCTTTA CGACAACGGC CCCCATTAAT GAAAATAATC CATCCGTAAT TAACCCTATC
1381 AAACGACTAG CCTGAGGAAG CATCATTGCA GGACTACTAA TTACCTCAAA TTTCCTCCCC
1441 ACCAACACAC CCGTAATAAC TATGCCCACC CACTTGAAAC TGGCCGCTCT CCTAGTTACC
1501 ATCTTAGGCC TTATCATTGC ATTAGAGCTT GCATCACTAA CTAGCAAGCA ATTTAAACTA
1561 CGCCCAACCC TTATACTCTC TAACTTCTCC TACATACTGG GATTCTTCCC CGCTATCATC
1621 CACCGATTAA CCCCCAAACT AAACTTAACT TTAGGACAAG CCATTGCCAG CCAAATGGTT
1681 GATCAAACAT GATTTGAAAA AGTAGGCCCG AAAGGAATTA TTTCAACTCA CCTACCCATA
1741 GTCACAACAA CAAGTAACAT CCAACAAGGC ATAATCAAAA CATACCTCAC TCTATTTTTC
1801 CTTTCGACAA CCCTAGCTGT CCTACTGACA CTAACCTAGA CTGCTCGAAG CGCCCCTCGA
1861 CTCAACCCCC GTGTCAATTC CAGCACCACA AAAAGTGTTA GCAGCAGTAC CCAAGCACAC
1921 GCAATTAACA TTCCCCCTCC ATGAGAGTAC ATCAGCGCCA CCCCGCTCGT ATCCCCACGC
1981 AAGACAGAAA GCTCCTTAAA CTCATCCACC ACTGCTCATG AAGTTTCATA TCATCCACCC
2041 CAAAATAACC CTGCCACTAA TATCACCCCC GCCATGTACA CTACCACATA ACCTAAAACC
2101 GAACGATCCC TTCAAGACTC AGGAAAAGGC TCAGCAGCTA AAGCTGCTGA ATAAGCAAAT
2161 ACCACAAGCA TTCCCCCCAA ATAAATCAAA AATAATACCA AAGATAGAAA AGACCCCCCG
2221 TGACCCACCA AAACACCACA ACCTACACCT GCTGCTACAA CCAATCCCAA AGCAGCAAAG
2281 TAGGGCGCAG GATTGGATGC AACAGTTACA AGCCCTAAAA CCAACCCTAA AAGAAATAAA
2341 GACACAAGAT AAGTCAT
```

Appendix B. Allele frequency estimates of allozymes for pink salmon collected from Prince William Sound, Alaska in 1994. Within the population names, "T" designates collections made in tidal zones and "U" designates collections made in upstream zones.

Population		*sAAT-1,2**			*sAAT-3**	
	N	*100*	*83*	N	*100*	*91*
Rocky Ck T	100	0.9975	0.0025	99	0.7929	0.2071
Armin F. Koernig Hatchery	100	0.9900	0.0100	100	0.8000	0.2000
Cathead Ck T	99	1.0000	0.0000	100	0.7900	0.2100
Herring Ck T	97	1.0000	0.0000	100	0.7200	0.2800
Halverson Ck T	100	0.9950	0.0050	100	0.7850	0.2150
Countess Ck T	100	0.9975	0.0025	100	0.7900	0.2100
Chenega Ck T	100	0.9950	0.0050	100	0.6800	0.3200
Totemoff Ck T	100	0.9975	0.0025	100	0.7400	0.2600
Erb Ck T	100	0.9975	0.0025	100	0.7950	0.2050
Mink Ck T	92	0.9918	0.0082	100	0.8300	0.1700
Mink Ck U	100	0.9975	0.0025	99	0.7626	0.2374
Swanson Ck T	100	0.9950	0.0050	100	0.7450	0.2550
Coghill R. T	100	0.9975	0.0025	100	0.7850	0.2150
Jonah Ck T	96	0.9922	0.0078	96	0.7344	0.2656
Solomon Gulch Hatchery	100	1.0000	0.0000	100	0.8300	0.1700
Duck R. T	99	0.9975	0.0025	99	0.8131	0.1869
Millard Ck T	99	0.9975	0.0025	99	0.7525	0.2475
Lagoon Ck T	96	0.9974	0.0026	100	0.8050	0.1950
Lagoon Ck U	95	1.0000	0.0000	98	0.7296	0.2704
Olsen Ck T	100	0.9975	0.0025	100	0.8350	0.1650
Olsen Ck U	99	0.9975	0.0025	100	0.8000	0.2000
Koppen Ck T	99	0.9949	0.0051	100	0.7750	0.2250
Koppen Ck U	100	0.9975	0.0025	99	0.7525	0.2475
Humpback Ck T	100	1.0000	0.0000	99	0.7576	0.2424
Hartney Ck T	99	1.0000	0.0000	100	0.7550	0.2450
Constantine Ck T	93	0.9919	0.0081	91	0.7857	0.2143
Constantine Ck U	100	1.0000	0.0000	98	0.7704	0.2296

Appendix B. Continued.

Population	N	*sAAT-4** 100	210	290	-10	N	*mAAT-1** -100	-83	-108
Rocky Ck T	100	0.4050	0.5900	0.0050	0.0000	100	0.9900	0.0100	0.0000
Armin F. Koernig Hatchery	100	0.4300	0.5650	0.0000	0.0050	100	0.9900	0.0100	0.0000
Cathead Ck T	100	0.5000	0.4950	0.0000	0.0050	100	0.9900	0.0100	0.0000
Herring Ck T	100	0.5150	0.4650	0.0100	0.0100	100	0.9950	0.0050	0.0000
Halverson Ck T	98	0.4133	0.5663	0.0153	0.0051	100	1.0000	0.0000	0.0000
Countess Ck T	100	0.5100	0.4650	0.0100	0.0150	100	0.9950	0.0050	0.0000
Chenega Ck T	100	0.5500	0.4400	0.0050	0.0050	100	0.9850	0.0150	0.0000
Totemoff Ck T	100	0.4450	0.5350	0.0150	0.0050	100	0.9900	0.0100	0.0000
Erb Ck T	99	0.4091	0.5606	0.0202	0.0101	100	0.9850	0.0150	0.0000
Mink Ck T	99	0.5404	0.4444	0.0101	0.0051	100	0.9950	0.0050	0.0000
Mink Ck U	99	0.4747	0.5101	0.0051	0.0101	100	0.9950	0.0000	0.0050
Swanson Ck T	94	0.4468	0.5266	0.0266	0.0000	100	0.9950	0.0050	0.0000
Coghill R. T	98	0.4439	0.5408	0.0102	0.0051	100	0.9950	0.0050	0.0000
Jonah Ck T	96	0.4479	0.5208	0.0208	0.0104	96	0.9792	0.0208	0.0000
Solomon Gulch Hatchery	100	0.4750	0.5100	0.0100	0.0050	100	0.9800	0.0200	0.0000
Duck R. T	100	0.5250	0.4250	0.0350	0.0150	100	0.9950	0.0000	0.0050
Millard Ck T	97	0.4588	0.5052	0.0206	0.0155	100	0.9850	0.0150	0.0000
Lagoon Ck T	100	0.4700	0.5050	0.0200	0.0050	100	0.9950	0.0050	0.0000
Lagoon Ck U	98	0.5204	0.3827	0.0051	0.0918	97	0.9897	0.0103	0.0000
Olsen Ck T	100	0.4300	0.5350	0.0300	0.0050	100	0.9950	0.0050	0.0000
Olsen Ck U	99	0.5101	0.4798	0.0051	0.0051	100	0.9850	0.0150	0.0000
Koppen Ck T	100	0.5050	0.4900	0.0000	0.0050	100	1.0000	0.0000	0.0000
Koppen Ck U	100	0.4250	0.5550	0.0150	0.0050	100	0.9750	0.0250	0.0000
Humpback Ck T	100	0.4500	0.5350	0.0050	0.0100	100	0.9800	0.0200	0.0000
Hartney Ck T	100	0.4650	0.5000	0.0300	0.0050	100	0.9900	0.0100	0.0000
Constantine Ck T	90	0.5167	0.4667	0.0056	0.0111	93	1.0000	0.0000	0.0000
Constantine Ck U	100	0.4350	0.5500	0.0100	0.0050	100	1.0000	0.0000	0.0000

Appendix B. Continued.

Population	N	ADA-1* 100	86	122	N	ADA-2* 100	110	90
Rocky Ck T	100	1.0000	0.0000	0.0000	100	0.8950	0.0400	0.0650
Armin F. Koernig Hatchery	100	1.0000	0.0000	0.0000	100	0.9450	0.0150	0.0400
Cathead Ck T	99	1.0000	0.0000	0.0000	100	0.9600	0.0150	0.0250
Herring Ck T	100	1.0000	0.0000	0.0000	100	0.9300	0.0400	0.0300
Halverson Ck T	100	1.0000	0.0000	0.0000	100	0.9200	0.0450	0.0350
Countess Ck T	100	1.0000	0.0000	0.0000	100	0.9000	0.0300	0.0700
Chenega Ck T	100	1.0000	0.0000	0.0000	100	0.9350	0.0150	0.0500
Totemoff Ck T	100	0.9950	0.0000	0.0050	100	0.9100	0.0450	0.0450
Erb Ck T	100	1.0000	0.0000	0.0000	97	0.9278	0.0309	0.0412
Mink Ck T	100	1.0000	0.0000	0.0000	100	0.9050	0.0400	0.0550
Mink Ck U	100	0.9950	0.0000	0.0050	100	0.9550	0.0150	0.0300
Swanson Ck T	100	0.9950	0.0050	0.0000	100	0.9650	0.0050	0.0300
Coghill R. T	99	1.0000	0.0000	0.0000	98	0.9388	0.0102	0.0510
Jonah Ck T	96	1.0000	0.0000	0.0000	96	0.9323	0.0365	0.0312
Solomon Gulch Hatchery	100	0.9950	0.0000	0.0050	100	0.9200	0.0550	0.0250
Duck R. T	100	1.0000	0.0000	0.0000	100	0.9250	0.0200	0.0550
Millard Ck T	100	1.0000	0.0000	0.0000	100	0.9150	0.0400	0.0450
Lagoon Ck T	100	1.0000	0.0000	0.0000	100	0.9200	0.0200	0.0600
Lagoon Ck U	98	1.0000	0.0000	0.0000	98	0.9286	0.0561	0.0153
Olsen Ck T	99	1.0000	0.0000	0.0000	99	0.9343	0.0404	0.0253
Olsen Ck U	100	1.0000	0.0000	0.0000	98	0.8929	0.0357	0.0714
Koppen Ck T	99	1.0000	0.0000	0.0000	100	0.8650	0.0600	0.0750
Koppen Ck U	100	0.9950	0.0000	0.0050	100	0.8850	0.0650	0.0500
Humpback Ck T	99	1.0000	0.0000	0.0000	99	0.9343	0.0303	0.0354
Hartney Ck T	100	0.9900	0.0000	0.0100	100	0.9050	0.0450	0.0500
Constantine Ck T	92	1.0000	0.0000	0.0000	92	0.8967	0.0217	0.0815
Constantine Ck U	100	0.9900	0.0000	0.0100	99	0.9141	0.0202	0.0657

Appendix B. Continued.

Population	N	sAH* 100	115	88	N	mAH-3* 100	74	125
Rocky Ck T	100	0.9950	0.0000	0.0050	100	0.9950	0.0050	0.0000
Armin F. Koernig Hatchery	100	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Cathead Ck T	99	0.9949	0.0000	0.0051	97	1.0000	0.0000	0.0000
Herring Ck T	100	0.9950	0.0000	0.0050	83	1.0000	0.0000	0.0000
Halverson Ck T	98	0.9847	0.0051	0.0102	98	1.0000	0.0000	0.0000
Countess Ck T	100	0.9900	0.0000	0.0100	100	0.9900	0.0050	0.0050
Chenega Ck T	100	1.0000	0.0000	0.0000	100	0.9950	0.0050	0.0000
Totemoff Ck T	100	0.9950	0.0000	0.0050	100	0.9950	0.0000	0.0050
Erb Ck T	100	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Mink Ck T	98	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Mink Ck U	100	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Swanson Ck T	96	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Coghill R. T	99	1.0000	0.0000	0.0000	100	0.9900	0.0100	0.0000
Jonah Ck T	96	1.0000	0.0000	0.0000	87	1.0000	0.0000	0.0000
Solomon Gulch Hatchery	100	1.0000	0.0000	0.0000	98	0.9898	0.0102	0.0000
Duck R. T	100	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Millard Ck T	100	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Lagoon Ck T	100	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Lagoon Ck U	98	1.0000	0.0000	0.0000	98	0.9949	0.0000	0.0051
Olsen Ck T	100	0.9950	0.0000	0.0050	100	1.0000	0.0000	0.0000
Olsen Ck U	100	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Koppen Ck T	100	0.9950	0.0000	0.0050	100	1.0000	0.0000	0.0000
Koppen Ck U	100	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Humpback Ck T	100	0.9850	0.0000	0.0150	100	0.9900	0.0100	0.0000
Hartney Ck T	100	1.0000	0.0000	0.0000	100	0.9850	0.0100	0.0050
Constantine Ck T	91	0.9890	0.0000	0.0110	93	0.9946	0.0054	0.0000
Constantine Ck U	100	0.9950	0.0000	0.0050	100	0.9900	0.0050	0.0050

Appendix B. Continued.

Population	N	*mAH-4** 100	116	81	N	*CK-A2** 100	108	82
Rocky Ck T	100	0.9600	0.0000	0.0400	100	1.0000	0.0000	0.0000
Armin F. Koernig Hatchery	100	0.9700	0.0000	0.0300	100	1.0000	0.0000	0.0000
Cathead Ck T	98	0.9439	0.0000	0.0561	99	1.0000	0.0000	0.0000
Herring Ck T	80	0.9312	0.0000	0.0688	98	0.9949	0.0051	0.0000
Halverson Ck T	95	0.9842	0.0000	0.0158	98	1.0000	0.0000	0.0000
Countess Ck T	99	0.9747	0.0000	0.0253	100	1.0000	0.0000	0.0000
Chenega Ck T	97	0.9588	0.0000	0.0412	100	0.9850	0.0050	0.0100
Totemoff Ck T	100	0.9600	0.0000	0.0400	100	0.9900	0.0000	0.0100
Erb Ck T	100	0.9750	0.0000	0.0250	98	0.9949	0.0051	0.0000
Mink Ck T	100	0.9850	0.0000	0.0150	100	1.0000	0.0000	0.0000
Mink Ck U	100	0.9800	0.0000	0.0200	100	1.0000	0.0000	0.0000
Swanson Ck T	99	0.9596	0.0000	0.0404	99	1.0000	0.0000	0.0000
Coghill R. T	100	0.9550	0.0000	0.0450	100	1.0000	0.0000	0.0000
Jonah Ck T	85	0.9529	0.0000	0.0471	96	1.0000	0.0000	0.0000
Solomon Gulch Hatchery	98	0.9745	0.0000	0.0255	100	0.9800	0.0000	0.0200
Duck R. T	100	0.9400	0.0000	0.0600	96	0.9948	0.0000	0.0052
Millard Ck T	100	0.9850	0.0000	0.0150	100	0.9900	0.0000	0.0100
Lagoon Ck T	100	0.9750	0.0000	0.0250	100	1.0000	0.0000	0.0000
Lagoon Ck U	98	0.9184	0.0000	0.0816	98	1.0000	0.0000	0.0000
Olsen Ck T	100	0.9800	0.0000	0.0200	98	1.0000	0.0000	0.0000
Olsen Ck U	100	0.9550	0.0000	0.0450	100	0.9950	0.0000	0.0050
Koppen Ck T	100	0.9650	0.0000	0.0350	55	1.0000	0.0000	0.0000
Koppen Ck U	100	0.9650	0.0000	0.0350	100	0.9950	0.0000	0.0050
Humpback Ck T	100	0.9750	0.0000	0.0250	100	0.9950	0.0000	0.0050
Hartney Ck T	100	0.9750	0.0000	0.0250	100	0.9950	0.0000	0.0050
Constantine Ck T	93	0.9516	0.0054	0.0430	93	0.9946	0.0000	0.0054
Constantine Ck U	100	0.9350	0.0000	0.0650	100	1.0000	0.0000	0.0000

Appendix B. Continued.

Population	N	*FDHG** 100	132	57	N	*bGALA** 100	111	91	105
Rocky Ck T	99	1.0000	0.0000	0.0000	96	0.9010	0.0625	0.0260	0.0104
Armin F. Koernig Hatchery	100	0.9950	0.0050	0.0000	98	0.8980	0.0816	0.0051	0.0153
Cathead Ck T	96	0.9844	0.0156	0.0000	87	0.9138	0.0517	0.0230	0.0115
Herring Ck T	99	0.9798	0.0152	0.0051	69	0.9348	0.0362	0.0072	0.0217
Halverson Ck T	100	1.0000	0.0000	0.0000	69	0.9275	0.0290	0.0000	0.0435
Countess Ck T	98	1.0000	0.0000	0.0000	94	0.9043	0.0691	0.0053	0.0213
Chenega Ck T	99	0.9949	0.0051	0.0000	78	0.9167	0.0641	0.0000	0.0192
Totemoff Ck T	98	0.9796	0.0153	0.0051	93	0.8763	0.0645	0.0269	0.0323
Erb Ck T	100	0.9900	0.0050	0.0050	97	0.8763	0.0773	0.0103	0.0361
Mink Ck T	100	0.9900	0.0100	0.0000	98	0.8980	0.0612	0.0153	0.0255
Mink Ck U	94	0.9840	0.0053	0.0106	99	0.8889	0.0909	0.0000	0.0202
Swanson Ck T	99	0.9949	0.0051	0.0000	81	0.9074	0.0432	0.0123	0.0370
Coghill R. T	100	1.0000	0.0000	0.0000	96	0.8958	0.0521	0.0104	0.0417
Jonah Ck T	94	0.9894	0.0106	0.0000	94	0.8457	0.0957	0.0160	0.0426
Solomon Gulch Hatchery	95	1.0000	0.0000	0.0000	68	0.9044	0.0662	0.0000	0.0294
Duck R. T	100	0.9900	0.0050	0.0050	99	0.8485	0.0960	0.0101	0.0455
Millard Ck T	95	0.9947	0.0053	0.0000	97	0.8557	0.0928	0.0206	0.0309
Lagoon Ck T	96	0.9896	0.0052	0.0052	89	0.8764	0.0730	0.0337	0.0169
Lagoon Ck U	85	1.0000	0.0000	0.0000	80	0.9125	0.0562	0.0125	0.0188
Olsen Ck T	99	1.0000	0.0000	0.0000	97	0.9278	0.0412	0.0103	0.0206
Olsen Ck U	100	0.9900	0.0100	0.0000	99	0.8889	0.0606	0.0253	0.0253
Koppen Ck T	96	0.9948	0.0052	0.0000	96	0.8802	0.0990	0.0000	0.0208
Koppen Ck U	94	1.0000	0.0000	0.0000	91	0.8407	0.1044	0.0110	0.0440
Humpback Ck T	97	0.9794	0.0155	0.0052	97	0.8402	0.1082	0.0206	0.0309
Hartney Ck T	99	0.9949	0.0051	0.0000	91	0.8516	0.1044	0.0220	0.0220
Constantine Ck T	93	0.9892	0.0054	0.0054	81	0.9136	0.0247	0.0247	0.0370
Constantine Ck U	93	1.0000	0.0000	0.0000	97	0.8608	0.0928	0.0206	0.0258

Appendix B. Continued.

Population	N	G3PDH-1* 100	-151	-52	N	G3PDH-2* 100	120	90
Rocky Ck T	100	0.8100	0.0000	0.1900	100	0.8450	0.0100	0.1450
Armin F. Koernig Hatchery	100	0.8100	0.0050	0.1850	99	0.8586	0.0354	0.1061
Cathead Ck T	100	0.8600	0.0000	0.1400	100	0.8900	0.0400	0.0700
Herring Ck T	100	0.8600	0.0000	0.1400	96	0.8958	0.0365	0.0677
Halverson Ck T	100	0.8100	0.0000	0.1900	96	0.8802	0.0156	0.1042
Countess Ck T	100	0.8450	0.0050	0.1500	100	0.8650	0.0200	0.1150
Chenega Ck T	100	0.8000	0.0000	0.2000	100	0.8800	0.0350	0.0850
Totemoff Ck T	100	0.8300	0.0000	0.1700	99	0.8687	0.0556	0.0758
Erb Ck T	100	0.7800	0.0000	0.2200	100	0.8350	0.1150	0.0500
Mink Ck T	100	0.8600	0.0000	0.1400	99	0.8788	0.0455	0.0758
Mink Ck U	100	0.8300	0.0100	0.1600	100	0.8600	0.0350	0.1050
Swanson Ck T	100	0.8050	0.0000	0.1950	99	0.8636	0.0253	0.1111
Coghill R. T	100	0.8300	0.0000	0.1700	100	0.8300	0.0500	0.1200
Jonah Ck T	96	0.7135	0.0052	0.2812	96	0.8854	0.0260	0.0885
Solomon Gulch Hatchery	100	0.8050	0.0000	0.1950	99	0.8737	0.0354	0.0909
Duck R. T	100	0.7850	0.0000	0.2150	96	0.8750	0.0365	0.0885
Millard Ck T	100	0.8000	0.0000	0.2000	100	0.8400	0.0350	0.1250
Lagoon Ck T	100	0.8250	0.0000	0.1750	98	0.8163	0.0510	0.1327
Lagoon Ck U	98	0.8929	0.0000	0.1071	97	0.8505	0.0052	0.1443
Olsen Ck T	100	0.8150	0.0050	0.1800	96	0.8646	0.0260	0.1094
Olsen Ck U	100	0.7850	0.0000	0.2150	98	0.8724	0.0306	0.0969
Koppen Ck T	100	0.8200	0.0000	0.1800	100	0.8850	0.0150	0.1000
Koppen Ck U	100	0.8600	0.0000	0.1400	100	0.8600	0.0150	0.1250
Humpback Ck T	100	0.7850	0.0050	0.2100	100	0.8350	0.0450	0.1200
Hartney Ck T	100	0.8100	0.0000	0.1900	100	0.8700	0.0300	0.1000
Constantine Ck T	93	0.8172	0.0000	0.1828	92	0.7989	0.0326	0.1685
Constantine Ck U	100	0.7250	0.0000	0.2750	99	0.8333	0.0354	0.1313

Appendix B. Continued.

Population	N	G3PDH-3* 100	90	N	GDA-1* 100	108	113	82	110
Rocky Ck T	100	0.9900	0.0100	100	0.5350	0.4350	0.0300	0.0000	0.0000
Armin F. Koernig Hatchery	99	0.9949	0.0051	98	0.5306	0.4592	0.0102	0.0000	0.0000
Cathead Ck T	100	1.0000	0.0000	100	0.5600	0.4350	0.0050	0.0000	0.0000
Herring Ck T	99	0.9798	0.0202	100	0.5500	0.4200	0.0300	0.0000	0.0000
Halverson Ck T	100	0.9950	0.0050	97	0.4794	0.5052	0.0155	0.0000	0.0000
Countess Ck T	100	0.9900	0.0100	99	0.5000	0.4747	0.0253	0.0000	0.0000
Chenega Ck T	100	1.0000	0.0000	100	0.5800	0.4100	0.0050	0.0050	0.0000
Totemoff Ck T	99	1.0000	0.0000	99	0.4646	0.5101	0.0253	0.0000	0.0000
Erb Ck T	100	0.9950	0.0050	98	0.5000	0.4745	0.0204	0.0051	0.0000
Mink Ck T	100	0.9900	0.0100	93	0.5430	0.4355	0.0215	0.0000	0.0000
Mink Ck U	100	1.0000	0.0000	98	0.5765	0.4082	0.0153	0.0000	0.0000
Swanson Ck T	98	0.9949	0.0051	96	0.4896	0.5000	0.0104	0.0000	0.0000
Coghill R. T	99	0.9798	0.0202	97	0.5258	0.4536	0.0206	0.0000	0.0000
Jonah Ck T	96	0.9792	0.0208	96	0.5833	0.4115	0.0052	0.0000	0.0000
Solomon Gulch Hatchery	100	0.9950	0.0050	100	0.5950	0.3750	0.0250	0.0050	0.0000
Duck R. T	99	0.9899	0.0101	100	0.5050	0.4800	0.0150	0.0000	0.0000
Millard Ck T	100	0.9900	0.0100	99	0.5152	0.4747	0.0101	0.0000	0.0000
Lagoon Ck T	100	0.9850	0.0150	94	0.5053	0.4840	0.0106	0.0000	0.0000
Lagoon Ck U	97	0.9948	0.0052	98	0.6173	0.3316	0.0510	0.0000	0.0000
Olsen Ck T	99	0.9798	0.0202	98	0.5765	0.4082	0.0153	0.0000	0.0000
Olsen Ck U	100	0.9950	0.0050	96	0.5521	0.4375	0.0104	0.0000	0.0000
Koppen Ck T	100	0.9950	0.0050	100	0.5550	0.4150	0.0300	0.0000	0.0000
Koppen Ck U	100	0.9900	0.0100	100	0.5550	0.4250	0.0200	0.0000	0.0000
Humpback Ck T	100	1.0000	0.0000	97	0.5206	0.4485	0.0309	0.0000	0.0000
Hartney Ck T	100	1.0000	0.0000	99	0.5606	0.3939	0.0404	0.0051	0.0000
Constantine Ck T	93	0.9892	0.0108	90	0.5500	0.4389	0.0111	0.0000	0.0000
Constantine Ck U	100	1.0000	0.0000	98	0.5561	0.4031	0.0357	0.0000	0.0051

Appendix B. Continued.

Population	N	GPI-B1,2* 100	200	25	180	N	IDDH-1* 100	134
Rocky Ck T	100	0.9925	0.0025	0.0000	0.0050	90	1.0000	0.0000
Armin F. Koernig Hatchery	100	0.9925	0.0025	0.0000	0.0050	96	0.9948	0.0052
Cathead Ck T	99	0.9924	0.0000	0.0000	0.0076	89	1.0000	0.0000
Herring Ck T	98	0.9949	0.0000	0.0000	0.0051	91	0.9945	0.0055
Halverson Ck T	100	0.9925	0.0000	0.0000	0.0075	83	0.9940	0.0060
Countess Ck T	100	0.9925	0.0050	0.0000	0.0025	72	1.0000	0.0000
Chenega Ck T	100	0.9900	0.0025	0.0025	0.0050	92	1.0000	0.0000
Totemoff Ck T	100	0.9925	0.0000	0.0000	0.0075	93	1.0000	0.0000
Erb Ck T	100	0.9975	0.0000	0.0000	0.0025	92	0.9891	0.0109
Mink Ck T	100	0.9925	0.0000	0.0000	0.0075	90	1.0000	0.0000
Mink Ck U	100	0.9925	0.0000	0.0000	0.0075	92	0.9891	0.0109
Swanson Ck T	99	0.9949	0.0000	0.0000	0.0051	50	0.9900	0.0100
Coghill R. T	100	0.9925	0.0025	0.0000	0.0050	95	0.9947	0.0053
Jonah Ck T	95	0.9921	0.0026	0.0000	0.0053	49	1.0000	0.0000
Solomon Gulch Hatchery	98	0.9898	0.0000	0.0000	0.0102	93	1.0000	0.0000
Duck R. T	96	0.9896	0.0000	0.0000	0.0104	88	1.0000	0.0000
Millard Ck T	98	0.9974	0.0000	0.0000	0.0026	92	0.9946	0.0054
Lagoon Ck T	100	0.9925	0.0025	0.0000	0.0050	94	1.0000	0.0000
Lagoon Ck U	98	0.9949	0.0000	0.0000	0.0051	92	1.0000	0.0000
Olsen Ck T	100	0.9875	0.0000	0.0000	0.0125	90	0.9944	0.0056
Olsen Ck U	100	0.9900	0.0025	0.0000	0.0075	94	1.0000	0.0000
Koppen Ck T	100	0.9975	0.0000	0.0000	0.0025	50	0.9900	0.0100
Koppen Ck U	100	0.9900	0.0000	0.0000	0.0100	94	1.0000	0.0000
Humpback Ck T	100	1.0000	0.0000	0.0000	0.0000	90	1.0000	0.0000
Hartney Ck T	100	0.9975	0.0000	0.0000	0.0025	87	0.9943	0.0057
Constantine Ck T	93	0.9919	0.0000	0.0000	0.0081	79	1.0000	0.0000
Constantine Ck U	99	0.9975	0.0025	0.0000	0.0000	92	1.0000	0.0000

Appendix B. Continued.

Population	N	*mIDHP-1**			N	*sIDHP-2**			
		100	*53*	*69*		*100*	*125*	*134*	*76*
Rocky Ck T	100	1.0000	0.0000	0.0000	100	0.6400	0.3550	0.0050	0.0000
Armin F. Koernig Hatchery	100	1.0000	0.0000	0.0000	100	0.6750	0.3200	0.0050	0.0000
Cathead Ck T	95	1.0000	0.0000	0.0000	100	0.6400	0.3600	0.0000	0.0000
Herring Ck T	100	0.9850	0.0050	0.0100	99	0.6465	0.3535	0.0000	0.0000
Halverson Ck T	100	0.9900	0.0100	0.0000	99	0.6212	0.3788	0.0000	0.0000
Countess Ck T	100	1.0000	0.0000	0.0000	100	0.6550	0.3450	0.0000	0.0000
Chenega Ck T	100	0.9950	0.0000	0.0050	100	0.6850	0.3150	0.0000	0.0000
Totemoff Ck T	100	0.9950	0.0050	0.0000	99	0.5808	0.4192	0.0000	0.0000
Erb Ck T	100	0.9950	0.0000	0.0050	100	0.6000	0.4000	0.0000	0.0000
Mink Ck T	100	0.9950	0.0050	0.0000	100	0.7150	0.2850	0.0000	0.0000
Mink Ck U	100	0.9950	0.0050	0.0000	100	0.6800	0.3200	0.0000	0.0000
Swanson Ck T	100	0.9950	0.0050	0.0000	100	0.6700	0.3300	0.0000	0.0000
Coghill R. T	100	1.0000	0.0000	0.0000	100	0.6750	0.3200	0.0000	0.0050
Jonah Ck T	96	0.9948	0.0052	0.0000	96	0.6250	0.3750	0.0000	0.0000
Solomon Gulch Hatchery	100	0.9950	0.0000	0.0050	100	0.6350	0.3650	0.0000	0.0000
Duck R. T	100	1.0000	0.0000	0.0000	100	0.6650	0.3350	0.0000	0.0000
Millard Ck T	99	0.9899	0.0051	0.0051	100	0.6650	0.3350	0.0000	0.0000
Lagoon Ck T	100	1.0000	0.0000	0.0000	100	0.6650	0.3350	0.0000	0.0000
Lagoon Ck U	98	0.9949	0.0051	0.0000	97	0.7887	0.2113	0.0000	0.0000
Olsen Ck T	100	1.0000	0.0000	0.0000	99	0.7071	0.2929	0.0000	0.0000
Olsen Ck U	100	0.9900	0.0050	0.0050	100	0.6900	0.3100	0.0000	0.0000
Koppen Ck T	100	0.9900	0.0050	0.0050	100	0.6600	0.3400	0.0000	0.0000
Koppen Ck U	100	1.0000	0.0000	0.0000	100	0.6550	0.3450	0.0000	0.0000
Humpback Ck T	100	0.9950	0.0000	0.0050	100	0.5850	0.4150	0.0000	0.0000
Hartney Ck T	100	1.0000	0.0000	0.0000	99	0.5808	0.4141	0.0000	0.0051
Constantine Ck T	93	0.9892	0.0000	0.0108	93	0.6452	0.3548	0.0000	0.0000
Constantine Ck U	100	1.0000	0.0000	0.0000	99	0.5909	0.4091	0.0000	0.0000

Appendix B. Continued.

Population	N	*LDH-A2**		N	*LDH-B2**				
		100	*148*		*100*	*132*	*74*	*151*	*124*
Rocky Ck T	100	1.0000	0.0000	100	0.9950	0.0000	0.0000	0.0000	0.0050
Armin F. Koernig Hatchery	100	1.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000	0.0000
Cathead Ck T	100	0.9800	0.0200	100	0.9950	0.0000	0.0000	0.0000	0.0050
Herring Ck T	100	1.0000	0.0000	100	0.9900	0.0000	0.0000	0.0000	0.0100
Halverson Ck T	98	1.0000	0.0000	100	0.9750	0.0000	0.0000	0.0000	0.0250
Countess Ck T	100	0.9950	0.0050	100	0.9950	0.0000	0.0000	0.0000	0.0050
Chenega Ck T	100	1.0000	0.0000	100	0.9950	0.0000	0.0000	0.0000	0.0050
Totemoff Ck T	100	0.9900	0.0100	100	0.9900	0.0000	0.0000	0.0000	0.0100
Erb Ck T	97	1.0000	0.0000	100	0.9950	0.0000	0.0000	0.0000	0.0050
Mink Ck T	99	1.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000	0.0000
Mink Ck U	100	1.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000	0.0000
Swanson Ck T	97	1.0000	0.0000	100	0.9850	0.0000	0.0050	0.0000	0.0100
Coghill R. T	100	0.9950	0.0050	97	0.9897	0.0052	0.0000	0.0000	0.0052
Jonah Ck T	96	1.0000	0.0000	96	0.9635	0.0000	0.0000	0.0104	0.0260
Solomon Gulch Hatchery	100	1.0000	0.0000	100	0.9950	0.0000	0.0000	0.0000	0.0050
Duck R. T	98	1.0000	0.0000	100	0.9950	0.0000	0.0000	0.0000	0.0050
Millard Ck T	99	1.0000	0.0000	100	0.9800	0.0000	0.0000	0.0000	0.0200
Lagoon Ck T	100	1.0000	0.0000	100	0.9900	0.0000	0.0000	0.0000	0.0100
Lagoon Ck U	97	1.0000	0.0000	98	0.9949	0.0000	0.0000	0.0000	0.0051
Olsen Ck T	100	1.0000	0.0000	100	0.9850	0.0000	0.0000	0.0000	0.0150
Olsen Ck U	100	1.0000	0.0000	100	0.9850	0.0000	0.0000	0.0000	0.0150
Koppen Ck T	100	1.0000	0.0000	100	0.9900	0.0000	0.0000	0.0000	0.0100
Koppen Ck U	100	1.0000	0.0000	100	0.9950	0.0000	0.0000	0.0000	0.0050
Humpback Ck T	100	1.0000	0.0000	100	0.9850	0.0000	0.0050	0.0000	0.0100
Hartney Ck T	100	1.0000	0.0000	100	0.9900	0.0000	0.0000	0.0000	0.0100
Constantine Ck T	93	1.0000	0.0000	93	0.9892	0.0000	0.0000	0.0000	0.0108
Constantine Ck U	100	1.0000	0.0000	100	0.9800	0.0000	0.0000	0.0050	0.0150

Appendix B. Continued.

Population	N	sMDH-A1,2* 100	148	50	126	158	58
Rocky Ck T	100	0.9675	0.0000	0.0300	0.0025	0.0000	0.0000
Armin F. Koernig Hatchery	100	0.9775	0.0000	0.0225	0.0000	0.0000	0.0000
Cathead Ck T	99	0.9798	0.0051	0.0152	0.0000	0.0000	0.0000
Herring Ck T	100	0.9875	0.0000	0.0125	0.0000	0.0000	0.0000
Halverson Ck T	100	0.9900	0.0000	0.0100	0.0000	0.0000	0.0000
Countess Ck T	100	0.9875	0.0000	0.0125	0.0000	0.0000	0.0000
Chenega Ck T	100	0.9700	0.0025	0.0225	0.0025	0.0025	0.0000
Totemoff Ck T	100	0.9875	0.0000	0.0100	0.0025	0.0000	0.0000
Erb Ck T	100	0.9725	0.0000	0.0200	0.0075	0.0000	0.0000
Mink Ck T	100	0.9775	0.0025	0.0125	0.0075	0.0000	0.0000
Mink Ck U	100	0.9825	0.0000	0.0150	0.0025	0.0000	0.0000
Swanson Ck T	100	0.9900	0.0000	0.0100	0.0000	0.0000	0.0000
Coghill R. T	100	0.9650	0.0000	0.0325	0.0025	0.0000	0.0000
Jonah Ck T	96	0.9844	0.0000	0.0130	0.0000	0.0000	0.0026
Solomon Gulch Hatchery	100	0.9875	0.0000	0.0125	0.0000	0.0000	0.0000
Duck R. T	100	0.9875	0.0000	0.0125	0.0000	0.0000	0.0000
Millard Ck T	100	0.9750	0.0000	0.0225	0.0025	0.0000	0.0000
Lagoon Ck T	100	0.9850	0.0000	0.0150	0.0000	0.0000	0.0000
Lagoon Ck U	98	0.9821	0.0000	0.0153	0.0026	0.0000	0.0000
Olsen Ck T	100	0.9825	0.0000	0.0175	0.0000	0.0000	0.0000
Olsen Ck U	100	0.9900	0.0000	0.0100	0.0000	0.0000	0.0000
Koppen Ck T	100	0.9750	0.0000	0.0200	0.0050	0.0000	0.0000
Koppen Ck U	100	0.9875	0.0000	0.0125	0.0000	0.0000	0.0000
Humpback Ck T	100	0.9875	0.0000	0.0125	0.0000	0.0000	0.0000
Hartney Ck T	100	0.9900	0.0000	0.0075	0.0025	0.0000	0.0000
Constantine Ck T	92	0.9755	0.0027	0.0190	0.0027	0.0000	0.0000
Constantine Ck U	100	0.9625	0.0000	0.0350	0.0025	0.0000	0.0000

Appendix B. Continued.

Population	N	*sMDH-B1,2** 100	124	66	N	*mMEP-1** 100	123
Rocky Ck T	98	0.9949	0.0026	0.0026	100	0.6450	0.3550
Armin F. Koernig Hatchery	100	0.9925	0.0075	0.0000	99	0.7424	0.2576
Cathead Ck T	100	1.0000	0.0000	0.0000	99	0.7172	0.2828
Herring Ck T	100	0.9875	0.0125	0.0000	100	0.7100	0.2900
Halverson Ck T	100	0.9975	0.0025	0.0000	100	0.7400	0.2600
Countess Ck T	100	0.9900	0.0075	0.0025	100	0.7150	0.2850
Chenega Ck T	100	0.9925	0.0025	0.0050	100	0.7350	0.2650
Totemoff Ck T	100	0.9875	0.0100	0.0025	100	0.6650	0.3350
Erb Ck T	99	0.9975	0.0000	0.0025	100	0.7200	0.2800
Mink Ck T	100	0.9975	0.0025	0.0000	100	0.7250	0.2750
Mink Ck U	100	0.9975	0.0000	0.0025	100	0.7800	0.2200
Swanson Ck T	100	0.9925	0.0075	0.0000	100	0.7200	0.2800
Coghill R. T	100	0.9975	0.0025	0.0000	100	0.7400	0.2600
Jonah Ck T	96	0.9948	0.0052	0.0000	96	0.7240	0.2760
Solomon Gulch Hatchery	100	0.9925	0.0050	0.0025	100	0.7850	0.2150
Duck R. T	98	0.9872	0.0128	0.0000	100	0.7350	0.2650
Millard Ck T	100	0.9975	0.0025	0.0000	100	0.7600	0.2400
Lagoon Ck T	100	1.0000	0.0000	0.0000	100	0.7650	0.2350
Lagoon Ck U	98	0.9898	0.0102	0.0000	98	0.7908	0.2092
Olsen Ck T	100	0.9925	0.0075	0.0000	100	0.7650	0.2350
Olsen Ck U	100	0.9850	0.0150	0.0000	100	0.7450	0.2550
Koppen Ck T	100	1.0000	0.0000	0.0000	100	0.7450	0.2550
Koppen Ck U	100	0.9950	0.0050	0.0000	100	0.8200	0.1800
Humpback Ck T	100	0.9925	0.0075	0.0000	100	0.7250	0.2750
Hartney Ck T	100	1.0000	0.0000	0.0000	100	0.7500	0.2500
Constantine Ck T	93	0.9919	0.0054	0.0027	93	0.7688	0.2312
Constantine Ck U	100	0.9850	0.0150	0.0000	100	0.7400	0.2600

Appendix B. Continued.

Population	N	*NTP** 100	130	N	*PEP-B1** 100	138	200
Rocky Ck T	100	1.0000	0.0000	100	0.8700	0.1150	0.0150
Armin F. Koernig Hatchery	100	1.0000	0.0000	100	0.8600	0.1200	0.0200
Cathead Ck T	100	1.0000	0.0000	100	0.9200	0.0700	0.0100
Herring Ck T	89	1.0000	0.0000	100	0.8550	0.1200	0.0250
Halverson Ck T	100	1.0000	0.0000	100	0.8450	0.1300	0.0250
Countess Ck T	100	0.9900	0.0100	100	0.8850	0.0950	0.0200
Chenega Ck T	47	1.0000	0.0000	100	0.8750	0.1100	0.0150
Totemoff Ck T	100	1.0000	0.0000	100	0.8250	0.1350	0.0400
Erb Ck T	100	1.0000	0.0000	100	0.8450	0.1150	0.0400
Mink Ck T	100	1.0000	0.0000	100	0.8650	0.0950	0.0400
Mink Ck U	100	1.0000	0.0000	100	0.8950	0.0900	0.0150
Swanson Ck T	100	0.9900	0.0100	99	0.8485	0.1111	0.0404
Coghill R. T	97	1.0000	0.0000	99	0.8737	0.1061	0.0202
Jonah Ck T	95	1.0000	0.0000	96	0.8802	0.0885	0.0312
Solomon Gulch Hatchery	38	1.0000	0.0000	100	0.8750	0.0800	0.0450
Duck R. T	100	0.9950	0.0050	100	0.8400	0.1450	0.0150
Millard Ck T	100	1.0000	0.0000	100	0.8500	0.1050	0.0450
Lagoon Ck T	90	1.0000	0.0000	100	0.8950	0.0850	0.0200
Lagoon Ck U	61	1.0000	0.0000	98	0.9184	0.0612	0.0204
Olsen Ck T	100	1.0000	0.0000	100	0.9150	0.0700	0.0150
Olsen Ck U	91	1.0000	0.0000	100	0.8800	0.0750	0.0450
Koppen Ck T	91	1.0000	0.0000	100	0.8350	0.1350	0.0300
Koppen Ck U	57	1.0000	0.0000	100	0.9050	0.0850	0.0100
Humpback Ck T	100	1.0000	0.0000	100	0.9000	0.0750	0.0250
Hartney Ck T	66	1.0000	0.0000	100	0.8700	0.0850	0.0450
Constantine Ck T	93	1.0000	0.0000	93	0.8925	0.0914	0.0161
Constantine Ck U	99	1.0000	0.0000	100	0.9050	0.0750	0.0200

Appendix B. Continued.

Population	N	PEP-D2* 100	120	80	N	PEPLT* 100	108	90
Rocky Ck T	100	0.5250	0.2350	0.2400	100	0.8800	0.0600	0.0600
Armin F. Koernig Hatchery	100	0.5250	0.2550	0.2200	100	0.8750	0.0850	0.0400
Cathead Ck T	100	0.5500	0.2150	0.2350	99	0.8737	0.0707	0.0556
Herring Ck T	100	0.5600	0.2550	0.1850	100	0.8250	0.1050	0.0700
Halverson Ck T	100	0.5400	0.2250	0.2350	100	0.8100	0.1250	0.0650
Countess Ck T	100	0.5350	0.2400	0.2250	100	0.8650	0.0800	0.0550
Chenega Ck T	100	0.5200	0.2200	0.2600	100	0.8450	0.1100	0.0450
Totemoff Ck T	100	0.5200	0.2200	0.2600	100	0.8150	0.1450	0.0400
Erb Ck T	100	0.5650	0.1850	0.2500	100	0.8600	0.1100	0.0300
Mink Ck T	100	0.5250	0.2250	0.2500	100	0.8300	0.1300	0.0400
Mink Ck U	100	0.6250	0.1900	0.1850	100	0.8300	0.1000	0.0700
Swanson Ck T	100	0.5100	0.2150	0.2750	100	0.8150	0.1000	0.0850
Coghill R. T	100	0.4650	0.2400	0.2950	100	0.8200	0.1250	0.0550
Jonah Ck T	96	0.4740	0.2396	0.2865	96	0.8385	0.0677	0.0938
Solomon Gulch Hatchery	100	0.5000	0.2700	0.2300	100	0.8850	0.0600	0.0550
Duck R. T	100	0.5400	0.2350	0.2250	100	0.8500	0.1100	0.0400
Millard Ck T	100	0.5500	0.2100	0.2400	100	0.8800	0.0500	0.0700
Lagoon Ck T	100	0.5700	0.2550	0.1750	100	0.8600	0.0850	0.0550
Lagoon Ck U	98	0.5102	0.3061	0.1837	98	0.9337	0.0357	0.0306
Olsen Ck T	100	0.5050	0.2100	0.2850	99	0.8788	0.0707	0.0505
Olsen Ck U	100	0.5350	0.2450	0.2200	100	0.8850	0.0600	0.0550
Koppen Ck T	100	0.5600	0.2250	0.2150	100	0.8600	0.0900	0.0500
Koppen Ck U	100	0.5700	0.2200	0.2100	100	0.8750	0.0950	0.0300
Humpback Ck T	100	0.5150	0.2450	0.2400	100	0.8350	0.0950	0.0700
Hartney Ck T	100	0.4700	0.2800	0.2500	100	0.8950	0.0500	0.0550
Constantine Ck T	93	0.5108	0.2258	0.2634	93	0.8495	0.0753	0.0753
Constantine Ck U	100	0.4900	0.2200	0.2900	100	0.8650	0.0650	0.0700

Appendix B. Continued.

Population	N	PGDH* 100	108	96	86	93
Rocky Ck T	100	0.7400	0.0000	0.2200	0.0400	0.0000
Armin F. Koernig Hatchery	100	0.7350	0.0000	0.2300	0.0350	0.0000
Cathead Ck T	96	0.7135	0.0000	0.2604	0.0260	0.0000
Herring Ck T	100	0.7200	0.0000	0.2500	0.0300	0.0000
Halverson Ck T	100	0.6800	0.0000	0.2900	0.0300	0.0000
Countess Ck T	100	0.7700	0.0000	0.2200	0.0100	0.0000
Chenega Ck T	100	0.7300	0.0000	0.2300	0.0350	0.0050
Totemoff Ck T	100	0.7350	0.0000	0.2350	0.0300	0.0000
Erb Ck T	100	0.7050	0.0000	0.2700	0.0250	0.0000
Mink Ck T	100	0.6650	0.0000	0.3000	0.0350	0.0000
Mink Ck U	100	0.6200	0.0000	0.3250	0.0500	0.0050
Swanson Ck T	100	0.7450	0.0000	0.2400	0.0150	0.0000
Coghill R. T	100	0.7100	0.0000	0.2650	0.0250	0.0000
Jonah Ck T	96	0.6823	0.0000	0.2865	0.0312	0.0000
Solomon Gulch Hatchery	100	0.7050	0.0000	0.2600	0.0350	0.0000
Duck R. T	100	0.7200	0.0000	0.2650	0.0100	0.0050
Millard Ck T	100	0.6950	0.0000	0.2800	0.0250	0.0000
Lagoon Ck T	100	0.6850	0.0000	0.2650	0.0500	0.0000
Lagoon Ck U	97	0.5773	0.0000	0.3196	0.1031	0.0000
Olsen Ck T	100	0.7250	0.0000	0.2450	0.0250	0.0050
Olsen Ck U	100	0.6750	0.0050	0.2700	0.0450	0.0050
Koppen Ck T	100	0.6850	0.0000	0.2900	0.0250	0.0000
Koppen Ck U	100	0.7100	0.0000	0.2750	0.0150	0.0000
Humpback Ck T	100	0.6650	0.0000	0.2850	0.0500	0.0000
Hartney Ck T	100	0.6850	0.0050	0.2850	0.0200	0.0050
Constantine Ck T	93	0.6935	0.0000	0.2796	0.0269	0.0000
Constantine Ck U	97	0.7062	0.0000	0.2577	0.0361	0.0000

Appendix B. Continued.

Population	N	PGM-2* 100	155	25	178
Rocky Ck T	100	1.0000	0.0000	0.0000	0.0000
Armin F. Koernig Hatchery	100	0.9950	0.0050	0.0000	0.0000
Cathead Ck T	100	1.0000	0.0000	0.0000	0.0000
Herring Ck T	100	1.0000	0.0000	0.0000	0.0000
Halverson Ck T	100	1.0000	0.0000	0.0000	0.0000
Countess Ck T	100	1.0000	0.0000	0.0000	0.0000
Chenega Ck T	100	1.0000	0.0000	0.0000	0.0000
Totemoff Ck T	100	1.0000	0.0000	0.0000	0.0000
Erb Ck T	100	1.0000	0.0000	0.0000	0.0000
Mink Ck T	100	1.0000	0.0000	0.0000	0.0000
Mink Ck U	100	0.9900	0.0050	0.0000	0.0050
Swanson Ck T	100	0.9950	0.0050	0.0000	0.0000
Coghill R. T	100	1.0000	0.0000	0.0000	0.0000
Jonah Ck T	96	0.9948	0.0000	0.0052	0.0000
Solomon Gulch Hatchery	100	1.0000	0.0000	0.0000	0.0000
Duck R. T	100	0.9950	0.0050	0.0000	0.0000
Millard Ck T	100	1.0000	0.0000	0.0000	0.0000
Lagoon Ck T	100	0.9950	0.0000	0.0050	0.0000
Lagoon Ck U	98	0.9949	0.0051	0.0000	0.0000
Olsen Ck T	100	0.9950	0.0050	0.0000	0.0000
Olsen Ck U	100	1.0000	0.0000	0.0000	0.0000
Koppen Ck T	100	0.9850	0.0100	0.0000	0.0050
Koppen Ck U	100	1.0000	0.0000	0.0000	0.0000
Humpback Ck T	94	1.0000	0.0000	0.0000	0.0000
Hartney Ck T	100	0.9850	0.0100	0.0050	0.0000
Constantine Ck T	93	1.0000	0.0000	0.0000	0.0000
Constantine Ck U	100	0.9950	0.0050	0.0000	0.0000

Appendix B. Continued.

Population	N	*mSOD** *100*	*185*	*118*	*16*	*54*
Rocky Ck T	100	1.0000	0.0000	0.0000	0.0000	0.0000
Armin F. Koernig Hatchery	100	0.9900	0.0100	0.0000	0.0000	0.0000
Cathead Ck T	100	0.9900	0.0000	0.0000	0.0100	0.0000
Herring Ck T	100	0.9950	0.0050	0.0000	0.0000	0.0000
Halverson Ck T	99	0.9949	0.0000	0.0000	0.0051	0.0000
Countess Ck T	100	0.9900	0.0100	0.0000	0.0000	0.0000
Chenega Ck T	100	0.9650	0.0200	0.0000	0.0150	0.0000
Totemoff Ck T	99	1.0000	0.0000	0.0000	0.0000	0.0000
Erb Ck T	100	0.9900	0.0100	0.0000	0.0000	0.0000
Mink Ck T	100	0.9900	0.0100	0.0000	0.0000	0.0000
Mink Ck U	100	0.9950	0.0050	0.0000	0.0000	0.0000
Swanson Ck T	99	1.0000	0.0000	0.0000	0.0000	0.0000
Coghill R. T	100	0.9850	0.0150	0.0000	0.0000	0.0000
Jonah Ck T	96	0.9948	0.0052	0.0000	0.0000	0.0000
Solomon Gulch Hatchery	100	0.9950	0.0000	0.0050	0.0000	0.0000
Duck R. T	100	0.9750	0.0200	0.0000	0.0000	0.0050
Millard Ck T	100	0.9600	0.0300	0.0050	0.0050	0.0000
Lagoon Ck T	100	0.9850	0.0100	0.0000	0.0050	0.0000
Lagoon Ck U	98	0.9949	0.0051	0.0000	0.0000	0.0000
Olsen Ck T	100	0.9950	0.0050	0.0000	0.0000	0.0000
Olsen Ck U	100	0.9900	0.0100	0.0000	0.0000	0.0000
Koppen Ck T	100	0.9850	0.0150	0.0000	0.0000	0.0000
Koppen Ck U	100	0.9900	0.0100	0.0000	0.0000	0.0000
Humpback Ck T	100	1.0000	0.0000	0.0000	0.0000	0.0000
Hartney Ck T	100	0.9900	0.0100	0.0000	0.0000	0.0000
Constantine Ck T	93	0.9839	0.0108	0.0000	0.0054	0.0000
Constantine Ck U	100	0.9950	0.0050	0.0000	0.0000	0.0000

Appendix B. Continued.

Population	N	sSOD-1* 100	176	15	120	140	N	TPI-2* -100	110
Rocky Ck T	100	0.9900	0.0000	0.0000	0.0100	0.0000	100	0.9800	0.0200
Armin F. Koernig Hatchery	100	0.9850	0.0000	0.0050	0.0100	0.0000	100	0.9850	0.0150
Cathead Ck T	100	0.9950	0.0000	0.0000	0.0050	0.0000	100	1.0000	0.0000
Herring Ck T	100	0.9900	0.0000	0.0100	0.0000	0.0000	100	0.9950	0.0050
Halverson Ck T	100	0.9700	0.0000	0.0100	0.0150	0.0050	100	0.9900	0.0100
Countess Ck T	100	0.9850	0.0050	0.0050	0.0050	0.0000	100	0.9900	0.0100
Chenega Ck T	100	0.9850	0.0050	0.0000	0.0100	0.0000	100	0.9800	0.0200
Totemoff Ck T	99	1.0000	0.0000	0.0000	0.0000	0.0000	100	0.9850	0.0150
Erb Ck T	100	0.9850	0.0050	0.0000	0.0100	0.0000	100	0.9850	0.0150
Mink Ck T	100	0.9950	0.0050	0.0000	0.0000	0.0000	100	0.9850	0.0150
Mink Ck U	100	1.0000	0.0000	0.0000	0.0000	0.0000	100	0.9950	0.0050
Swanson Ck T	100	0.9950	0.0000	0.0000	0.0050	0.0000	100	0.9600	0.0400
Coghill R. T	100	0.9850	0.0000	0.0000	0.0050	0.0100	100	0.9850	0.0150
Jonah Ck T	96	0.9896	0.0000	0.0000	0.0104	0.0000	96	1.0000	0.0000
Solomon Gulch Hatchery	100	0.9900	0.0000	0.0100	0.0000	0.0000	100	0.9850	0.0150
Duck R. T	100	0.9850	0.0000	0.0050	0.0100	0.0000	100	0.9900	0.0100
Millard Ck T	100	0.9950	0.0000	0.0000	0.0050	0.0000	100	0.9700	0.0300
Lagoon Ck T	100	1.0000	0.0000	0.0000	0.0000	0.0000	100	0.9950	0.0050
Lagoon Ck U	98	1.0000	0.0000	0.0000	0.0000	0.0000	98	0.9949	0.0051
Olsen Ck T	100	0.9850	0.0000	0.0050	0.0100	0.0000	100	0.9900	0.0100
Olsen Ck U	100	1.0000	0.0000	0.0000	0.0000	0.0000	100	0.9750	0.0250
Koppen Ck T	100	0.9850	0.0000	0.0000	0.0150	0.0000	100	0.9850	0.0150
Koppen Ck U	100	0.9850	0.0000	0.0050	0.0100	0.0000	100	0.9800	0.0200
Humpback Ck T	100	0.9950	0.0050	0.0000	0.0000	0.0000	100	0.9750	0.0250
Hartney Ck T	100	0.9900	0.0000	0.0000	0.0100	0.0000	100	0.9900	0.0100
Constantine Ck T	93	0.9946	0.0000	0.0000	0.0054	0.0000	93	0.9677	0.0323
Constantine Ck U	100	0.9950	0.0000	0.0000	0.0050	0.0000	100	0.9750	0.0250

Appendix B continued

Source of Variation	DF	*GPI-B1,2*	DF	*IDDH-1*	DF	*mIDHP-1*	DF	*sIDHP-2*	DF	*LDH-A2*	DF	*LDH-B2*	DF	*sMDH-A1,2*	DF	*sMDHB-1,2*
Between Sources	2	0.57	1	0.00	2	1.90	1	0.02	1	1.25	2	3.36	3	0.96	2	0.24
Within Sources	50	43.28	25	25.36	50	45.83	25	48.11	25	32.01	50	40.09	75	54.43	50	71.86
Wild	48	41.20	24	24.01	48	44.45	24	47.28	24	32.01	48	38.71	72	53.26	48	70.28
Between elevations	2	0.12	1	0.20	2	0.91	1	3.97	1	3.59	2	0.65	3	2.82	2	5.55
Within elevations	46	41.08	23	23.81	46	43.54	23	43.31	23	28.42	46	38.06	69	50.44	46	64.73
Upstream	8	9.64	4	6.49	8	6.29	4	18.92	4	0.00	8	8.90	12	8.01	8	14.03
Among Regions	4	6.81	2	6.49	4	2.47	2	9.50	2	0.00	4	7.43	6	7.56	4	11.92
Within Regions	4	2.83	2	0.00	4	3.82	2	9.42	2	0.00	4	1.47	6	0.45	4	2.11
East	4	2.83	2	0.00	4	3.82	2	9.42	2	0.00	4	1.47	6	0.45	4	2.11
Tidal	38	31.44	19	17.32	38	37.25	19	24.39	19	28.42	38	29.16	57	42.43	38	50.70
Among Regions	8	3.43	4	2.16	8	10.93	4	3.54	4	11.86	8	7.47	12	12.85	8	12.07
Within Regions	30	28.01	15	15.16	30	26.32	15	20.85	15	16.56	30	21.69	45	29.58	30	38.63
Southwest	12	10.31	6	7.09	12	14.42	6	6.32	6	13.83	12	5.61	18	15.67	12	20.54
North	6	3.10	3	2.90	6	1.75	3	3.64	3	2.73	6	13.74	9	9.60	6	1.55
East	12	14.60	6	5.17	12	10.15	6	10.89	6	0.00	12	2.34	18	4.31	12	16.54
Hatchery	2	2.08	1	1.35	2	1.38	1	0.83	1	0.00	2	1.38	3	1.17	2	1.58

Source of Variation	DF	*mMEP-1*	DF	*NTP*	DF	*PEPB-1*	DF	*PEPD-2*	DF	*PEPLT*	DF	*PGDH*	DF	*PGM-2*	DF	*mSOD*
Between Sources	1	1.33	1	0.59	2	0.63	2	2.02	2	1.99	2	1.10	1	0.04	2	2.02
Within Sources	25	33.22	25	20.70	50	58.38	50	49.02	50	73.40	50	66.84	25	25.20	50	66.38
Wild	24	32.22	24	20.70	48	54.77	48	48.77	48	72.05	48	66.35	24	23.82	48	63.61
Between elevations	1	9.21	1	1.97	2	7.74	2	3.14	2	5.79	2	14.86	1	0.55	2	4.35
Within elevations	23	23.01	23	18.73	46	47.03	46	45.63	46	66.26	46	51.49	23	23.27	46	59.26
Upstream	4	5.11	4	0.00	8	7.34	8	16.79	8	15.99	8	22.59	4	3.08	8	0.82
Among Regions	2	1.74	2	0.00	4	1.31	4	12.48	4	7.99	4	3.67	2	0.86	4	0.41
Within Regions	2	3.37	2	0.00	4	6.03	4	4.31	4	8.00	4	18.92	2	2.22	4	0.41
East	2	3.37	2	0.00	4	6.03	4	4.31	4	8.00	4	18.92	2	2.22	4	0.41
Tidal	19	17.90	19	18.73	38	39.69	38	28.84	38	50.27	38	28.90	19	20.19	38	58.44
Among Regions	4	12.72	4	2.12	8	5.05	8	7.71	8	15.31	8	5.66	4	10.11	8	14.62
Within Regions	15	5.18	15	16.61	30	34.64	30	21.13	30	34.96	30	23.24	15	10.08	30	43.82
Southwest	6	3.45	6	7.41	12	13.64	12	6.89	12	13.03	12	7.63	6	0.00	12	23.42
North	3	0.23	3	5.47	6	2.49	6	2.20	6	10.56	6	4.41	3	2.75	6	4.45
East	6	1.50	6	3.73	12	18.51	12	12.04	12	11.37	12	11.20	6	7.33	12	15.95
Hatchery	1	1.00	1	0.00	2	3.61	2	0.25	2	1.35	2	0.49	1	1.38	2	2.77

Appendix B continued

Source of Variaton	DF	*sSOD-1*	DF	*TPI-2*	DF	Overall	*P*-value
Between Sources	3	4.45	1	0.00	56	55.13	0.50778
Within Sources	75	69.22	25	36.25	1400	1750.02	0.00000
Wild	72	66.11	24	36.25	1344	1691.30	0.00000
Between elevations	3	3.67	1	0.05	56	126.50	0.00000
Within elevations	69	62.44	23	36.20	1288	1564.80	0.00000
Upstream	12	9.05	4	6.05	224	356.90	0.00000
Among Regions	6	2.47	2	3.00	112	184.10	0.00002
Within Regions	6	6.58	2	3.05	112	172.80	0.00020
East	6	6.58	2	3.05	112	172.80	0.00020
Tidal	57	53.39	19	30.15	1064	1207.90	0.00134
Among Regions	12	11.72	4	5.42	224	289.50	0.00207
Within Regions	45	41.67	15	24.73	840	918.40	0.03057
Southwest	18	20.47	6	7.01	336	402.80	0.00717
North	9	8.37	3	11.26	168	175.10	0.33788
East	18	12.83	6	6.46	336	340.50	0.42132
Hatchery	3	3.11	1	0.00	56	58.72	0.37609