Exxon Valdez Oil Spill
Restoration Project Final Report

Kenai River Sockeye Salmon Restoration

Restoration Project 96255-2
Final Report

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Study History: This study was initiated as Restoration Study Number 59 "Assessment of Genetic Stock Structure of Salmonids." The project effort continued under Restoration Project 93012 "Genetic Stock Identification of Kenai River Sockeye Salmon." In FY94, Restoration Project 93015 was combined with Restoration Project 94255 "Kenai River Sockeye Salmon Restoration." In FY95 and FY96 the project continued under the same title as Restoration Projects 95255 and 96255, respectively. Reports were submitted under the title Assessment of Genetic Stock Structure of Salmonids for Restoration Study Number 59 and under the title Genetic Diversity of Sockeye Salmon (*Oncorhynchus nerka*) of Cook Inlet, Alaska and its Application to Restoration of Injured Populations of the Kenai River for Restoration Projects 93012 and 94255 and under the title Kenai River Sockeye Salmon Restoration for Restoration Project 95255. The final report for the hydroacoustic portion of this project (96255-1) is being submitted independently.

Abstract: Genetic data from sockeye salmon (*Oncorhynchus nerka*) were collected from the Kenai River, a major salmon-producing system impacted by the *Exxon Valdez* Oil Spill, as well as all other significant spawning populations contributing to mixed-stock harvests in Cook Inlet, Alaska. A total of 68 allozyme loci were resolved from 47 putative populations. Allozyme data reveal a substantial amount of genetic diversity among populations. Mixed-stock analyses using maximum likelihood methods with 27 loci were evaluated to estimate the proportion of Kenai River populations in Cook Inlet gillnet fisheries. Simulations indicate that Kenai River populations can be identified in mixtures at a level of precision and accuracy useful for restoration and fishery management. Fishery samples were analyzed both inseason (within 48 h) and postseason. The contribution of Kenai River populations to the Cook Inlet fisheries varied from 16.3% to 90.9%. Samples from fish wheels from the Kenai, Kasilof, Yentna, and Susitna River systems were also analyzed. Microsatellite DNA data were also collected from four populations to assess the ability of this technique to discriminate among populations. Results from this study are currently being used in the management and restoration of Kenai River sockeye salmon injured in the 1989 *Exxon Valdez* oil spill.

Key Words: Alaska, allozymes, Cook Inlet, *Exxon Valdez* oil spill, genetic diversity, *Oncorhynchus nerka*, sockeye salmon.

Project Data: *Description of Data* - The data collected during the course of this project were the relative frequencies of variation within three classes of genetic markers: 1) Allozyme - variant proteins formed by allelic forms of the same locus, 2) Mitochondrial DNA - genetic material found within the mitochondria with strict maternal inheritance and haploid nature, 3) Microsatellites - highly polymorphic variable number of tandem repeat nuclear DNA sequences that are distributed throughout the genome at intervals of approximately 10 kilobase pairs. *Format* - These data are stored in ASCII text format. *Custodian* - Contact Lisa W. Seeb at the Alaska Department of Fish and Game, Division of Commercial Fisheries Management and Development, Genetics Laboratory, 333 Raspberry Rd., Anchorage, Alaska

Seeb at the Alaska Department of Fish and Game, Division of Commercial Fisheries Management and Development, Genetics Laboratory, 333 Raspberry Rd., Anchorage, Alaska 99518. *Availability* - A complete set of the data are reported either in this report (allozyme and microsatellite) or in the final report for restoration projects 93012 and 94255 (mitochondrial DNA). Electronic copies of these data are available upon request.

Citation:

Seeb, L.W., W.D. Templin, K.E. Tarbox, R.Z. Davis, and J.E. Seeb. 1997. Kenai River sockeye salmon restoration, *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Project 96255-2), Alaska Department of Fish and Game, Division of Commercial Fisheries Management and Development, Anchorage, Alaska.

LIST OF APPENDICES

Appendix I.

Seeb, L.W., C. Habicht, W.D. Templin, K.E. Tarbox, R.Z. Davis, L.K. Brannian, and J.E. Seeb. Genetic diversity of sockeye salmon (*Oncorhynchus nerka*) of Cook Inlet, Alaska, and its application to management of populations affected by the *Exxon Valdez* oil spill. Submitted to Transactions of the American Fisheries Society.

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Genetic Diversity of Sockeye Salmon (*Oncorhynchus nerka*) of Cook Inlet, Alaska, and its Application to Management of Populations Affected by the *Exxon Valdez* Oil Spill

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Abstract

Genetic data from sockeye salmon (*Oncorhynchus nerka*) were collected from the Kenai River, a major salmon-producing system affected by the *Exxon Valdez* Oil Spill, as well as all other significant spawning populations that contribute to mixed stock harvests in Cook Inlet, Alaska. The products of 29 enzymes encoded by 67 protein loci were resolved from samples from 47 spawning locations in Upper Cook Inlet. Allozyme data revealed a substantial amount of genetic diversity among populations. Mixed stock analyses using maximum likelihood methods with data from 27 loci were evaluated to estimate the proportion of Kenai River populations in Cook Inlet fisheries. Simulations indicate that Kenai River populations can be identified in mixtures at a level of precision and accuracy useful for fishery restoration and management. Samples from fisheries were analyzed both inseason (within 48 h) and postseason. The contribution of Kenai River populations to the Cook Inlet fisheries varied from 16.4% to 90.9%. Samples from fish wheels on the Kenai, Kasilof, Yentna, and Susitna rivers were analyzed to check the adequacy of the baseline. Results from this study are currently being used in the management of Cook Inlet sockeye salmon populations affected by the *Exxon Valdez* oil spill.

Key Words: *Oncorhynchus nerka*, sockeye salmon, Cook Inlet, Alaska, genetic diversity, allozymes, *Exxon Valdez* Oil Spill

The *T/V Exxon Valdez* hit Bligh Reef in Prince William Sound on March 24, 1989, spilling 11.2 million gallons of oil. In the ensuing days oil spread in a southwesterly direction through the Gulf of Alaska. Oil reached the Cook Inlet region, an area that supports large populations of Pacific salmon and extensive commercial fisheries. Sockeye salmon (*Oncorhynchus nerka*) have been commercially harvested in Cook Inlet since the late 1800s, and harvest levels have ranged from 95,000 to 9.5 million (Rigby et al. 1991; Ruesch and Fox 1994). Over the last 10 years the total value of the fishery has ranged from $12.3 to $111.1 million, and sockeye salmon represented 80.4% to 96.0% of the total of all salmon species harvested (Ruesch and Fox 1994). However, in July 1989, fishing time in the Cook Inlet area was greatly reduced due to the presence of oil from the *Exxon Valdez* spill.

As a direct result of the reduced exploitation, the number of sockeye salmon spawners in the Kenai River system was almost twice the upper bound of the desired escapement goal range. Extremely high escapements can produce enough fry to deplete invertebrate prey populations in rearing lakes, causing high fry mortality and altering the species composition and productivity of prey populations for several years (Schmidt et al. 1995).

In anticipation of a possible decline in the fishery, efforts were begun in 1992 to refine stock identification and management techniques and to increase knowledge of the diversity and abundance of sockeye salmon in Cook Inlet. This information is essential to maintain the productivity of mixtures of stocks in mixed stock harvests (Walters 1975; Kope 1992), assists managers to meet seasonal goals for individual stocks or stock-groups (Fried 1996), and allows managers to assess the impacts of harvest regulations and other restrictions during the season (Mundy 1985; Mundy et al. 1993). By directing the commercial harvest, managers could closely regulate the number of spawning adults in the Kenai River, one of the few ways to manage sockeye salmon fry production and restore the productivity of affected lakes.

Most of the sockeye salmon production in Upper Cook Inlet (UCI) comes from four major river systems. The largest sockeye salmon producer (2.8 million fish annually) is the Kenai River, which drains 5,200 km^2 of the Kenai Peninsula on the east side of UCI (Fig. 1). The Kasilof (1,700 km^2) and Susitna rivers (49,000 km^2) each produce approximately 700,000 sockeye salmon annually. The Kasilof River is on the Kenai Peninsula south of the Kenai River and the Susitna River empties into the north end of the inlet. The Crescent River drainage (200,000 fish) covers 300 km^2 on the western side of the Inlet. The Kenai, Kasilof, and Crescent river systems include large glacial lakes fed by numerous smaller tributaries. The Susitna River system has many smaller lakes, each of which empties into the mainstem through smaller, separate streams. The remainder of the sockeye salmon production in UCI is composed of many minor stocks that contribute between 6% and 31% (15% on average) of the total inlet-wide escapement (Ruesch and Fox 1994).

Cook Inlet sockeye salmon have been the focus of a number of stock identification studies. Extensive efforts were made to delineate populations through scale pattern analyses (Marshall et al. 1987) and parasites (Waltemyer et al. 1993). Neither technique proved adequate. Waltemyer et al. (1996) found that significant temporal and sexual variability within

populations exists with scale pattern analyses and that the technique could not be used on an inseason basis. Genetic markers have proven effective for stock management in recent years: Seeb et al. (1986, 1990) and Shaklee and Phelps (1990) for chum salmon (*O. keta*), White and Shaklee (1991) and White (1996) for pink salmon (*O. gorbuscha*), Wood et al. (1989, 1994) and Beacham et al. (1995) for sockeye salmon. These markers can also be used to discriminate populations in mixed stock aggregations, and a considerable statistical framework (Mixed Stock Analysis: MSA) based on maximum likelihood estimates (MLE) has been developed to identify individual stocks within mixtures (Fournier et al. 1984; Pella and Milner 1987; Wood et al. 1987; Millar 1987, 1990; Pella et al. 1996).

An early genetic study of sockeye salmon focused on Cook Inlet, where Grant et al. (1980) found considerable heterogeneity among populations. In evaluations of their resulting mixed stock model, Grant et al. (1980) demonstrated a high degree of success using three allozyme loci to classify populations from the Kasilof and Susitna river drainages, but incomplete baseline data were thought to confound the Kenai River classifications. Additional data from the Russian River, one of the Kenai River drainages, were presented by Wilmot and Burger (1985). They found significant differences between early and late runs from the Russian River. However, no comprehensive genetic survey of Cook Inlet has been undertaken since the 1970s (Grant et al. 1980). In this study we present genetic data to delineate populations and evaluate the genetic model as a tool for stock identification and restoration of Kenai River sockeye salmon.

Materials and Methods

Baseline samples for allozyme analysis were collected from spawning populations of sockeye salmon by personnel of the Alaska Department of Fish and Game (ADF&G) using gillnets and beach seines. Target sample size for baseline collections was set at 100 to achieve acceptable precision around the allele frequency estimates (Allendorf and Phelps 1981; Waples 1990). Tissue samples from spawning populations were collected from all major sockeye-producing systems of UCI. Approximately 7,000 individual sockeye salmon from spawning populations were sampled from 1992 to 1995 (Table 1; Fig. 1). Most spawning populations were sampled in at least two separate years to check for temporal variation, and some sites were sampled twice within a year to check for differences in run timing.

Mixed stock collections originating from Cook Inlet fisheries (Central District; Fig. 1) were collected in a manner similar to that for spawning samples. Sockeye salmon from the drift gillnet fishery were sampled at processing plants as fishing vessels were offloaded. Collections were made during July in 1992-1996 (Table 1). In 1995, two collections were also taken from set gillnet sites fishing the eastern shore of the Central District. In addition, inriver collections were made at four mainstem fish wheel sites (Yentna River, river mile 4; Susitna River, river mile 80; Kasilof River, river mile 7; and Kenai River, river mile 19; Table 1; Fig. 1). Target mixed stock sample sizes were set at 200 for inriver and 400 for fisheries samples (Wood 1989), although these were not always achieved. Each year two collections from the commercial fishery were processed within 48 h.

Samples of muscle, liver, vitreous humor, and heart were dissected from freshly killed individuals. Individual sample numbers were assigned to uniquely identify all genetic tissues. Tissues were placed into cryovials, and the cryovials were stored in liquid nitrogen until transferred to -80°C storage where they remained until laboratory analysis.

A comprehensive examination for discriminating gene markers was conducted using allozyme electrophoresis. Allozyme techniques followed those of Aebersold et al. (1987); nomenclature rules followed the American Fisheries Society standard (Shaklee et al. 1990). The products of 29 enzymes encoded at 67 allozyme loci were resolved (Table 2). A photographic record of each gel was made, and a collection of mobility standards for all scored alleles was constructed and used to verify alleles.

Of the 67 loci, 23 loci (*ADA-1*; mAH-3*; CK-A1*; CK-C1*; CK-C2*; ESTD*; FBALD-4*; FH*; βGALA*; GAPDH-3*; GAPDH-4*; GAPDH-5*; G3PDH-3*; GR*; mIDHP-2*; LDH-A1*; LDH-B1*; LDH-C*; αMAN*; mMDH-1*; mMDH-2*; mMDH-3*; sMEP-1**) were found to be invariant and were surveyed for only a single year from each site. Statistical analyses for all populations were based on the remaining set of 44 loci. A reduced set of 27 loci (*mAAT-1*; mAAT-2*; mAH-1,2*; mAH-4*; sAH*; ALAT*; GAPDH-2*; G3PDH-4*; GPI-B1,2*; GPI-A*; sIDHP-1*; LDH-B2*; sMDH-A1,2*; sMDH-B1,2*; mMEP-1*; PEPA*; PEPB-1*; PEPC*; PEPLT*; PGM-1*; PGM-2*; TPI-1,2**) were chosen for their information content and ability to be adequately resolved from lesser quality tissues, a common occurrence in fishery samples. This set of loci was used in the majority of the admixture analyses. However, we were unable to resolve some loci (*mAAT-2*; mAH-4*; GPI-B1,2*; G3PDH-4**) from all mixtures. In those cases estimates were based on the remaining loci in the set of 27.

Where possible, multiple collections at the same site were pooled for the analysis following the recommendations of Waples (1990) and White (1996). Genotypes were scored from enzyme phenotypes and then summarized into allele frequency estimates (Appendix A). Because of difficulty scoring the **100/null* heterozygote, only homozygote alternate phenotypes could be scored for null allele variation at *PGM-1**. Hardy-Weinberg expected frequencies were calculated for this locus (Appendix A) and were used for heterogeneity and tree analyses, but phenotypic frequencies were used for the mixture analysis. Frequencies at isoloci (*sAAT-1,2*; mAH-1,2*; G3PDH-1,2*; sMDH-A1,2*; sMDH-B1,2*; GPI-B1,2*; TPI-1,2**) were calculated assuming the variation occurred with equal frequency at both loci. Tests for departure from Hardy-Weinberg equilibrium were made for each population at each single locus to test for random mating within each population ($\alpha = 0.05$; adjusted for the number of tests; Lessios 1992). Isoloci and *PGM-1** were excluded from these tests.

Populations were grouped *a priori* into seven regions for subsequent analyses: Kenai River, Kasilof River, Susitna River, Yentna River, Northeast Cook Inlet, Knik Arm and West Cook Inlet. The first four regions encompass the entire watersheds of three of the four major river systems in UCI. The vast Susitna River watershed, of which the Yentna River is a tributary, was divided into two separate regions to allow finer-scale resolution. Populations within each

river system share common freshwater migration pathways. The last three regions, comprising the remaining UCI river systems, were geographically proximal units. With a few exceptions, the populations within each of these three regions do not share freshwater migration pathways, and one or more nursery or rearing lakes are located in each region. The fourth major river system, Crescent River, is located in the West Cook Inlet region.

Homogeneity of allelic frequencies among the various collections were tested using log-likelihood ratios (modified from Weir 1990) with $\alpha = 0.01$. This 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 populations in the test. The likelihood values can be summed over all loci to obtain a total value at each level of analysis. The total gene frequency dispersion at each locus was subdivided into within- and among-region components in a hierarchical fashion. Hierarchical levels were organized to test for homogeneity (1) among sites within nursery lakes, (2) among nursery lakes within regions, and (3) among river systems/regions. Rejection of the null hypothesis of homogeneity indicates presence of discrete spawning populations. This analysis is a conservative test because the degrees of freedom reflect the entire pattern of diversity around Cook Inlet. In some situations we also performed pair-wise and region-wide analyses, which resulted in fewer degrees of freedom and a finer scale analysis.

To further describe the subdivision of genetic diversity, a hierarchical gene diversity analysis (Nei 1973) was conducted to delineate the distribution of variability among sites within nursery lakes, among nursery lakes within regions, and among regions. Isoloci and *PGM-1** (scored phenotypically) were excluded from the diversity analysis.

Genetic distance measures (Cavalli-Sforza and Edwards 1967), which summarize multi-locus data into a single number, were calculated between all pairs of spawning locations. These values were used to construct a neighbor-joining tree (N-J tree; Saitou and Nei 1987) using PHYLIP (Version 3.5, Felsenstein 1993). This method allows for unequal rates of molecular change among branches. Allele frequency estimates, fit to expected genetic models, and genetic variability and distance measures were calculated using functions written in *S-Plus* (Mathsoft, Inc., Seattle, WA) .

Stock contributions to the mixture samples were estimated via maximum likelihood (MLE; Pella and Milner 1987) using a conjugate gradient searching algorithm with square root transformations (Pella et al. 1996). This algorithm provides good performance with large baselines and small stock differences (Pella et al. 1996). The precision (standard error) of the stock composition was estimated by an infinitesimal jackknife procedure (Millar 1987). Individuals missing data at two or more loci were deleted. Individual population estimates were first calculated, then summed into regional groupings (allocate-sum procedure, Wood et al. 1987).

We conducted simulations in which the mixture was composed entirely of populations from each of the seven reporting regions to evaluate the accuracy of the stock composition

estimates at the regional level. These hypothetical mixtures (N = 400) were generated from the baseline allele frequencies assuming Hardy-Weinberg equilibrium (with the exception of *PGM-1** which was treated as a non-genetic character). The precision (standard error) of the simulated mixtures was estimated by a parametric bootstrap (Efron and Tibshirani 1986), where the observed multilocus genotype frequencies were assumed to be multinomially distributed as were the allele frequencies in the baseline. We performed 100 bootstrap iterations.

To maintain confidence in the estimates, fishery managers wanted at least 90% of the harvest in these simulations to be correctly allocated to the region of origin. Within regions the individual populations were constrained to contribute equally to the sample so that no allowances were made for differential abundances. We also performed simulations varying the contribution of the Kenai River to an mixture sampled from all baseline populations. Contributions varied from 0% to 100% in 10% increments.

Results

Heterogeneity Within Regions

Kenai River

Rearing of sockeye salmon occurs in Upper and Lower Russian lakes, Kenai Lake, Skilak Lake, Hidden Lake, Tern Lake, and Trail Lake (Fig. 1). Spawning occurs in tributaries of these lakes as well as the mainstem Kenai River.

Divergence was detected within the Russian River. Late-spawning populations above and below Russian River Falls were significantly different ($G = 660.5$, $df = 24$, $P < 0.001$). Loci exhibiting distinct discontinuity in allele frequencies between all populations spawning above and below the falls included *sAH*100* (above 0.26 - 0.29; below 0.96), *ALAT*100* (above 0.84 - 0.86; below 0.65), *LDH-B2*100* (above 0.50 - 0.71; below 0.92), and *PGM-1*100* (above 0.00 - 0.01; below 0.38)(Appendix A). The population spawning below the falls more closely resembled populations inhabiting the mainstem Kenai River and populations spawning above the falls formed the most highly divergent group in the analysis (Fig. 2). In addition, temporal differentiation was detected in pairwise comparisons between early- and late-run spawners above the falls, ($G = 93.4$, $df = 12$, $P < 0.001$) with significant heterogeneity found at *LDH-B2**, *mAAT-1**, *mAAT-2**, and *mAH-1,2**.

Overall similarity among populations from the Kenai River drainage is apparent from the N-J tree (Fig. 2). Populations showing high levels of similarity and forming a single cluster included Skilak Lake outlet, populations between Kenai and Skilak lakes (sites 1 - 6), Ptarmigan Creek, Quartz Creek, and Russian River below the falls. Moose Creek joined a larger grouping, which included populations from Susitna River drainages and West Cook Inlet. Other Kenai River populations appeared highly divergent. While the Russian River populations above the falls (both early and late) were the most divergent, Hidden Creek also

was highly distinct, not only from Russian River populations above the falls, but also from the other Kenai River populations. Compared to mainstem Kenai River populations, Hidden Creek was characterized by higher frequencies of *mAAT-2*-73*; *ALAT*100*; and *PGM-2*100* (Appendix A). Moose Creek also was distinct within the drainage having high frequencies of *ALAT*91*.

Kasilof River

Populations returning to the Kasilof River drainage spawn in tributaries and along the shoreline of Tustumena Lake. Five tributaries (Bear, Moose, Glacier Flat, Nikolai, and Seepage creeks; Fig. 1) were sampled. Lake spawners utilizing the beach were also sampled (Tustumena Lake sites 1 and 2). In comparisons among populations, Bear, Moose, and Seepage creeks were statistically indistinguishable ($G = 29.5$, $df = 32$, $P = 0.593$). Relative to other Cook Inlet sockeye salmon populations, the Kasilof River drainage populations were more similar and cluster together on the N-J tree (Fig. 2). Overall heterogeneity within the region when all Cook Inlet populations were considered was not significant (Table 3). As a group, Kasilof River drainage populations exhibited a high frequency of *ALAT*95* (frequencies range from 0.10 to 0.15) and consistent presence of rare alleles (*G3PDH-4*108; GPI-B1,2*132*).

Susitna River Drainages

The Susitna River is composed of the Yentna River and mainstem Susitna River drainages. Within each of these systems are many smaller lakes and tributaries that support sockeye salmon spawning and rearing. Chosen sampling sites were assumed to represent the largest spawning populations within the system, although less is known about populations of the Susitna River than populations from other drainages.

We found extensive divergence within the Susitna River system, both within and between the Yentna and Susitna rivers (Table 3). Within the Yentna River drainage, there was a wide spectrum of loci at which one or more populations have exceptionally divergent allele frequencies (Table 3, Appendix A) . The most dramatic difference occurred at *PGM-2** where frequencies of the **100* allele were 0.25 for Shell Lake and 0.28 for Trinity/Movie lakes; Hewitt/Whiskey lakes had a frequency of 0.63, and the remaining populations had frequencies greater than 0.80. Other loci that displayed a large amount of heterogeneity were *PEPC*105* (generally < 0.02; Hewitt/Whiskey lakes = 0.13; Shell Lake = 0.32), *PGM-1*100* (generally < 0.10; Judd Lake = 0.36), *PEPB-1*130* (generally = 0.00; Trinity/Movie lakes = 0.15), *ALAT*100* (generally < 0.59; Trinity/Movie and Hewitt/Whiskey lakes > 0.70), and *mAAT-1*-100* (generally > 0.84; Judd Lake = 0.62).

Populations in the Susitna River mainstem also showed considerable heterogeneity at several loci (Table 3; Appendix A). At *PGM-1**, most of the populations had frequencies of the **100* allele between 0.15 and 0.40; however, in Red Shirt Lake a frequency of 0.03 was estimated, and the **100* allele was absent in the Stephan Lake collections. Other alleles that

displayed a large amount of heterogeneity were *PEPC*105* (frequencies ranging from 0.003 to 0.17) and *sIDHP-1*94* (generally = 0.00; Stephan Lake = 0.13), and *mAAT-1*-83* (generally > 0.19; Birch Creek = 0.06; Red Shirt Lake = 0.00). The degree of differentiation was most easily seen in the N-J tree (Fig. 2), where Susitna River populations can be found on many different branches clustering with populations from other regions.

Western Cook Inlet

Populations assigned to the Western Cook Inlet region spawn in the river/lake systems that drain the west side of Cook Inlet from the mouth of the Susitna River south to the Crescent River. These are generally cold, high-energy streams fed by the glaciers and snowpack in the mountains along the coast. An exception is the Packers Lake population, which returns to Kalgin Island, a large island located in the middle of the Inlet west of the mouth of the Kasilof River. Unlike the Kenai, Kasilof and Susitna river regions, populations spawning within this region do not generally share a common fresh-water migration pathway to their spawning sites (Fig. 1).

As might be expected from the geography of the region, the Western Cook Inlet populations exhibited considerable regional heterogeneity (Table 3). A large part of the heterogeneity within the region can be attributed to a few loci within a few populations. The *ALAT*95* allele occurred much more frequently in McArthur River (frequency = 0.17) than in the remaining populations (frequency < 0.07). In this region, the *sMDH-B1,2*65* allele occurred only in Coal Creek and Packers Lake, whereas **116* was an allele exclusive to Packers Lake. The frequencies of the null allele for *PGM-1** ranged from 0.54 to 1.00, and the *PGM-2*136* allele frequencies ranged from 0.03 to 0.39 through all the populations in this region.

Northeastern Cook Inlet

Only two sites were sampled in the Northeastern Cook Inlet region: Daniels Lake and Bishop Creek. Both sites are in the Bishop Creek drainage, located north of the mouth of the Kenai River on the Kenai Peninsula (Fig. 1). When sites were compared, heterogeneity was found at *ALAT**, *sAH**, *GPI-A**, and *mAAT-1** between Bishop Creek and Daniels Lake collections (Table 3). Their similarity to each other, though, was greater than their similarity to other populations as shown in the N-J tree (Fig. 2). Northeastern Cook Inlet populations were marked by a high frequency of *PEPLT*88* alleles, a low frequency of *PGM-2*100* alleles, and the lack of *LDH-B2** and *PEPC** variant alleles, which were seen in every other region.

Knik Arm

Like the populations in Western Cook Inlet, the Knik Arm populations do not share a common freshwater migration path (Fig. 1). For this reason, sampling sites were chosen based on size of drainage and observed sockeye salmon escapement. The three populations of the region (Nancy Lake, Cottonwood Creek and Fish Creek) were significantly different (Table 3). Cottonwood Creek and Fish Creek clustered together in the N-J tree, but Nancy

Lake was on a separate branch with populations from other regions.

Heterogeneity Among Regions

Observed and expected heterozygosities were calculated for all populations (end of Appendix A). Observed heterozygosities varied from a low of 0.021 in Chilligan River to a high of 0.056 in Stephan Lake. There was no regional trend in heterozygosity level in the populations sampled. All populations conformed to Hardy-Weinberg expectations.

A hierarchical gene diversity analysis was stratified by site, nursery lake, and region. The greatest amount of variation (87.74%) occurred within sites (Table 4). Little variability was detected among sites within nursery lakes (0.38%). However, considerable heterogeneity (7.80%) existed among nursery lakes within regions, the remaining 4.08% of the variability allocated to the among-regions component.

Mixed Stock Analyses

The performance of the MSA model for Cook Inlet sockeye salmon was investigated through simulations. Correct allocation to the Kenai River region, the group of greatest concern, was 91% in the simulation studies, above the 90% goal (Table 5). Northeastern Cook Inlet, Kasilof River, and Knik Arm also were above or close to the goal (99%, 92%, and 88%, respectively). The Yentna River also was near the goal with an allocation of 88%, but the Susitna River misallocated to both the Yentna River and Western Cook Inlet, resulting in a correct allocation of only 77%. When the Susitna and Yentna regions were combined, the allocation rose to 87%. Western Cook Inlet, a heterogenous grouping based on geographic proximity, performed at 86%, below the 90% objective.

A series of simulations was also conducted to test our ability to detect increasing Kenai River presence in the fishery. Simulations were designed so that the Kenai River contribution to the mixture sample varied from 0% to 100% in 10% increments. At low percentages the Kenai River contribution were slightly overestimated, but at higher percentages the contributions were underestimated (Fig. 3).

Maximum likelihood estimates were calculated for all samples collected from the Central District drift gillnet and Eastside set gillnet fisheries. These estimates were then summed by region for use in management (Table 6). In 1992, 1993, and 1994 few samples were taken, and estimated contributions shed little light on the interactions of regions within the fishery (Fig. 4). In 1995 and 1996, five samples were taken from that portion of the season coinciding with the expected presence of Kenai River sockeye salmon. These samples show a marked increase in Kenai River sockeye salmon in the drift gillnet fishery over the periods examined in both years. The harvest of sockeye salmon peaked at 462,625 on July 17 in 1995 and 430,343 on July 19 in 1996 (Table 7). Although the proportion of Kenai River populations in the harvest continued to increase during late July, the total harvest of sockeye salmon in the fishery decreased (Table 7; Fig. 5). Sockeye salmon of Kenai River origin

represented approximately 43% in 1995 and 49% in 1996 of the total Cook Inlet harvest during the sampling periods.

Maximum likelihood estimates were also calculated from samples originating from fish wheel catches (Table 8). Samples were collected from fish wheels in the Kenai, Kasilof, Susitna mainstem, and Yentna rivers (Table 1; Fig. 1). These inriver estimates assumed all contributing populations from a particular drainage were included in the baseline and that there was no straying into the river drainage. Estimates for the Kenai River samples ranged from 63% to 93% across all collections. The lowest value was for July 10, 1994, the earliest sample taken. A similar pattern was observed for the Susitna River mainstem (75% and 92%) and Yentna River (81% to 98%). The lowest value in the Kasilof River was 55%, for the earliest sample in 1994 (July 8-10), however a July 2 sample in 1992 allocated 91% to the Kasilof River. These results may indicate that some early-run populations with unique genetic profiles have not been included in the baseline or that early in the season fish may be entering non-natal systems prior to correctly homing to their natal stream ("nosing in").

Fine-scale estimation was also possible for some populations within some river drainages. A 100% simulation was conducted on the Russian River population above the falls. The simulation result was 99% (S.E. 0.5%) indicating that the Russian River could be identified in mixtures of Cook Inlet populations with a high degree of accuracy and precision. Maximum likelihood estimates for the inriver mixtures from Kenai River were made to estimate the combined early- and late-runs of Russian River sockeye salmon above the falls (Fig. 6). Four estimates were possible in 1994, three in 1995 and one in 1996. The results from 1994 suggest a pulse of early-run fish, a lull, and then a large pulse of late-run fish.

Discussion

The objective of this study was to improve stock-assessment capabilities for sockeye salmon, a prerequisite to protecting and managing populations affected by the oil spill. The allozyme data gave a detailed picture of the genetic diversity of Cook Inlet sockeye salmon, and the data representing 47 putative populations can be used, not only to describe the diversity of the Inlet, but also to assess the contribution of affected populations to mixed stock aggregations.

Genetic Diversity of Cook Inlet Sockeye Salmon

This study represents the first comprehensive analysis of sockeye salmon from Cook Inlet since that of Grant et al. (1980). Grant et al. (1980) identified six informative loci of 26 total loci from 13 populations from Cook Inlet. They documented heterogeneity among both the Kenai and Susitna River drainages, whereas little heterogeneity was detected among Kasilof River populations. Wilmot and Burger (1985) surveyed Russian River populations and documented significant differences between the early- and late-run populations from the Russian River at *LDH-B2** and *sAH**. Our study confirms the previous observations of Grant et al. (1980) and Wilmot and Burger (1985) and greatly expands the database both in terms of loci and number of populations.

Sockeye salmon typically spawn in rivers or smaller creeks associated with nursery lakes, and it has been suggested that the nursery lake is the primary unit of genetic structuring (Utter et al. 1984; Wood et al. 1994). This may reflect the tendency of sockeye salmon to home with great fidelity to their natal streams, presumably to a greater extent than other Pacific salmon (Quinn 1985; Quinn et al. 1987). Juveniles will typically rear from 1 to 2 years in a nursery lake before undergoing smoltification and migrating to the sea.

The Kenai River drainage includes several nursery lakes. Early- and late-run Russian River populations are thought to rear in Upper and Lower Russian Lakes, "mainstem" spawning populations (Skilak Lake outlet, between Kenai and Skilak Lake, Russian River below-the-falls, Quartz Creek, and Ptarmigan Creek) are believed to rear in Kenai and Skilak Lakes, Moose Creek rear in Upper Trail Lake, Tern Lake rear in Tern Lake, and Hidden Creek juveniles rear in Hidden Lake. The genetic diversity among Kenai River populations is clearly far greater than previously documented. Two separate lineages corresponding to an early- and late-run occur above the falls in the Russian River. The falls serve as an effective isolating barrier, populations spawning below the falls join a large aggregation of mainstem populations that rear in Kenai and Skilak Lakes. A third highly divergent lineage is represented by the Hidden Creek population, and additional outliers with distinct genetic profiles occur in Moose Creek and Tern Lake.

In the Kasilof River region, sockeye salmon from four spawning tributaries as well as two beach spawning sites were surveyed from Tustumena Lake. Little heterogeneity among populations rearing in the lake was apparent (Table 3; Fig. 2). Burger et al. (1995) detected a distinct late run of river-spawners that appear near the end of September at the outlet of Tustumena Lake. These outlet-spawners have a distinct genetic profile based on both mitochondrial DNA and allozyme data (Burger et al. In press), but were not included in this study.

The high level of divergence of Susitna River and Western Cook Inlet populations was not unexpected as Grant et al. (1980) also noted significant differences between Susitna River populations. Unlike the Kenai and Kasilof rivers, there are no large nursery lakes that support multiple tributary-spawning populations in these regions. Rather, there are a number of isolated smaller lake systems, and spawning has also been observed in sloughs of the Susitna River that have no obvious access to a nursery lake for early-life rearing. This isolation likely led to the considerable divergence evident in both regions.

The data from the Kenai, Kasilof, and Susitna River drainages support a model of differentiation of populations based on natal spawning areas. In the gene diversity analysis, 7.8% of the variability existed among nursery lakes within regions, but only 4.1% of the variability could be attributed to the among-region component. Wood et al. (1994) reported similar results from a study of variation in 83 distinct spawning sites representing all major sockeye-producing river systems in Canada. They showed extensive differentiation among nursery lakes and attributed it to founder effects and isolation through strict homing behavior. They attributed 7% of the variation to differences among lakes within drainages and lesser

amounts to "among drainages within systems" and "among river system" components.

Divergence within a nursery lake was seen in this study between the early- and late-run Russian River populations. Temporal and geographic divergence within lakes has been noted for other sockeye salmon populations. Wilmot and Burger (1985) reported differences between early- and late-run sockeye salmon returning to Karluk Lake. Varnavskaya et al. (1994) studied the population structure within nine lake systems in North America and Russia and found differentiation among subpopulations exhibiting different run timing (earlier vs. later) or utilizing different spawning habitat (tributary vs. beach). Burger et al. (In press) detected significant differences between the late-run outlet spawners and all other spawners from Tustumena Lake. They attributed the differentiation to precise homing to natal streams, not just to the lake systems.

Mixed Stock Analyses

In addition to describing the genetic diversity present in Cook Inlet, a primary goal of this study was to evaluate and utilize the genetic data for MSA to aid in the management and restoration of Kenai River populations affected by the spill. A total of 27 of the 67 loci were used in the majority of the admixture analyses, which represents a large increase over that available to Grant et al. (1980).

A basic requirement of using genetic data in mixed stock analyses is that all major contributing populations are represented in the baseline. To a large extent, this assumption is met by the extensive genetic information collected by this study. However, unlike other species of Pacific salmon such as chinook salmon (*O. tschawytscha*, Utter et al. 1993), there is little relationship between genetic distance and geographic distance in sockeye salmon populations. Sockeye salmon populations inhabiting the same drainage may be more divergent than populations geographically separated. As a result, exhaustive baseline sampling is needed.

Simulation studies are a useful method to evaluate and refine the MSA model. We primarily used pure or 100% simulations. Bias in the estimated composition is expected to be greatest at the most extreme compositions (0 or 100%) given the constrained maximum likelihood techniques used (no estimates < 0.00 or > 1.00; Pella and Milner 1987). This pattern was evident in the simulations of increasing Kenai River contributions to the fishery (Fig. 3), but the bias was greater at high levels of Kenai River contributions than at low levels. The estimated Kenai River component was within one standard error of the true contribution over the range from 0% to 80%. A series of 100% simulations, thus, provides a rigorous test of the model.

Based on earlier work with sockeye salmon (Wood et al. 1989, 1994), we took a conservative approach by identifying regional reporting units and using the allocate-sum procedure to estimate regional contributions. Previous simulation studies on sockeye salmon have shown that estimates for individual populations may not be reliable (Wood et al. 1989). The

performance of the Kenai River was of particular concern, but it did quite well with a 100% simulation estimate of 91% (S.E. 4.9%). Additional indicators of the accuracy of the method are the misallocations to a particular region. Misallocations to the Kenai River in 100% simulations of other regions were small, ranging from 0% from Northeastern Cook Inlet to 3% from the Kasilof River. The Kasilof River, Northeastern Cook Inlet, and Knik Arm regions also performed well, and pooling the Yentna and Susitna River regions improved performance for the Susitna River populations. The poorest results were obtained for Western Cook Inlet, a very heterogeneous group of populations with genetic affinities to the Yentna and Susitna River populations.

The results for the maximum likelihood estimates of regional contribution to the commercial fishery over the four years varied, not only through time, but also across years with the Kenai River estimate ranging from 16% to 91%. In 1995 the Kasilof River region was the largest contributor early in the season, but by mid July the Kenai River became the predominant contributor. Yearly estimates will vary depending on the relative run strengths, location of sampling, and timing of sampling, but multiyear sampling, particularly with multiple samples within each year, may reveal consistent patterns.

The inriver mixed stock estimates can be used to monitor individual populations within systems. For example, the Russian River and Hidden Creek populations of the Kenai River can be very accurately and precisely estimated and can potentially serve as indicator stocks for management purposes. The inriver samples can also provide an indication of the adequacy of the baseline. However, intrinsic in this application is the assumption that very little straying or "nosing in" occurs. In some cases, the model performs poorly on inriver stock mixtures early in the season (Table 8), but improves dramatically as the season progresses, which suggests that the baseline may be weighted towards populations with middle- or late-run timing. This is probably an acceptable bias because many of the early-timing populations may be very low in abundance (Davis and King 1996). It also could indicate that entrance into a non-natal stream may be more prevalent early in the season.

The allozyme data reveal a substantial amount of genetic diversity among populations of Cook Inlet sockeye salmon. This diversity is distributed both within and among major drainages. In general, the data support a model of population structure based on the nursery lake; however, we did detect significant divergence among both temporal and geographic components within nursery lakes. This diversity probably arises from isolation and genetic drift within nursery lakes and a tendency of sockeye salmon to home with great fidelity.

Application to Fishery Management

The commercial fishery management strategy in Upper Cook Inlet is to regulate the harvest of sockeye salmon by varying fishing time and area to meet a fixed range of escapement objectives. The sockeye season length is mid-June to mid-August and fishing peaks in mid-July. Typically, the fishery operates on Monday and Friday for 12 h. However, this time is adjusted by the ADF&G depending on run strength. Areas open to fishing can also be

adjusted to affect exploitation rates. This management strategy is adjusted as necessary after estimating the number of adults reaching fresh water in the major river systems with sonar (Ruesch and Fox 1994)

Sockeye salmon move into the Central District from the south and tend to delay entering their natal streams. Residence times in the Central District for Kenai River sockeye salmon have a modal value of 11 d early in the season, rapidly declining to 4 d as the season progresses. The average residence time for Kasilof River populations is 9 d at the beginning of the season and declines to 5 d at the end of the season. Susitna River populations, in contrast, hold for 19 d in the early portion of the season; the average time declines to 7 d late in the season (Mundy et. al. 1993).

Approximately 600 drift gillnet vessels fish the offshore waters of the Central District in Upper Cook Inlet. Exploitation rates of the drift gillnet fleet averaged 41% (range 35-45%) for a single 12-h fishing period between 1979 and 1988. Rates have remained relatively stable to the present. In contrast to the drift gillnet fishery, the set gillnet fishery in Upper Cook Inlet concentrates along the east side of Upper Cook Inlet. This fishery targets primarily Kasilof and Kenai River populations and consists of over 1200 35-fathom nets. Exploitation rates in a single 12-h period can be 70% of the fish available to the gear.

Stock abundance, variable residence times which concentrate fish, and high commercial exploitation potential can combine to increase the probability of overharvest in an uninformed mixed stock fishery. Therefore, stock identification in the harvest is essential for long-term management of these fisheries so that each stock can be harvested at its appropriate rate.

The results of the maximum likelihood estimates indicated that Kenai River populations can be identified in mixtures of Cook Inlet sockeye salmon with a level of precision, accuracy, and timeliness useful for fisheries management. The original intent of this study was to determine the Kenai River/non-Kenai River component of the harvest. To evaluate the model, though, populations were initially allocated to seven regions, which were later reduced to six to improve model performance.

The maximum likelihood estimates were first incorporated into inseason fishery management in 1995; results were reported for Kenai River/non-Kenai River components only during the first year. In future years it is likely that four reporting groups corresponding to current management regimes will be used. These groups are Kenai River, Kasilof River, Northern District (Susitna River, Yentna River, Northeastern Cook Inlet, Knik Arm, Coal Creek, Chilligan River, McArthur River), and Western Cook Inlet (those populations spawning south of the Northern District boundary). Evaluation of these groups is being conducted.

Application of genetic data to stock identification in salmon fishery management has several advantages over other methods including stability of allele frequencies over time, ability to process large amounts of samples rapidly, and reasonable costs (Shaklee and Phelps 1990). In comparison to scale patterns or parasites analyses for sockeye salmon in Upper Cook Inlet,

genetic data 1) provides a better understanding of the underlying biological organization, 2) provides more accurate, precise, and less biased stock composition estimates, 3) does not require in-season "known" scale samples, 4) has a similar availability of data to managers, and 5) costs are comparable to scale pattern analysis. The accuracy and precision of the estimates can probably be further improved as additional genetic markers become available. The data collected in this study can be used throughout Cook Inlet as well as within drainages to identify specific population components. These applications are currently underway in Cook Inlet to aid in the management and restoration of sockeye salmon populations affected by the oil spill.

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Table I-1. Sockeye salmon populations sampled for genetic studies. All populations originate from Upper Cook Inlet, 1992-1995.

	Map # and Location	Sample Date	N
	Kenai River Drainage		
1	Russian River (above falls, early)	7/01/92	100
	Russian River (above falls, late)	8/06/92	100
		7/26/93	100
	Russian River (below falls, late)	8/17/93	100
2	Ptarmigan Creek	8/31/92	100
		8/05/93	98
3	Tern Lake	9/01/92	50
		8/24/93	100
4	Quartz Creek	8/13/92	100
		7/27/93	100
5	Between Kenai/Skilak Lake		
	River mile 69.8 (Site 6)	8/18/92	100
		8/13/93	99
		8/27/93	100
	River mile 79.8 (Site 1)	8/11/94	50
		8/22/94	50
	River mile 76.6 (Site 2)	8/12/94	50
		8/23/94	50
	River mile 70.5 (Site 3)	8/12/94	100
		8/23/94	50
	River mile 72.5 (Site 4)	8/23/94	50
	River mile 65.3 (Site 5)	9/09/94	100
6	Hidden Creek	8/03/92	100
		8/04/93	100
7	Skilak Lake outlet		
	River mile 49.6 (north bank)	8/19/92	100
	River mile 47.6 (south bank)	8/13/93	100
		8/27/93	200
		8/20/94	100
		8/30/94	200
		8/29/95	100

Table I-1. Continued.

	Map # and Location	Sample Date	N
8	Moose Creek	7/27/93	100
		7/13/94	100
	Susitna River (Yentna Drainages)		
9	Chelatna Lake	8/20/92	100
		8/02/93	100
10	Yentna River West Fork (Unnamed slough)	9/08/92	100
		9/08/93	100
11	Hewitt/Whiskey Lakes	8/24/92	50
		9/03/93	
12	Shell Lake (Skwentna R.)	8/26/92	100
		9/01/93	100
13	Trinity/Movie Lakes	8/25/92	100
		9/03/93	100
14	Judd Lake (Talachulitna R.)	8/24/92	100
		8/24/93	100
	Susitna River (Mainstem Drainages)		
15	Byers Lake	8/23/93	100
16	Stephan Lake (Talkeetna R.)	9/08/93	100
		8/19/94	25
17	Larson Lake (Talkeetna R.)	8/20/92	100
		8/31/93	100
18	Birch Creek	8/19/93	67
19	Red Shirt Lake	9/15/93	34
20	Slough # 11 (Susitna R.)	9/06/95	50
	Western Cook Inlet Drainages		
21	Coal Creek West Fork (Beluga R.)	9/01/92	100
		8/25/93	100
22	Chilligan River (Chakachatna R.)	9/08/92	100
		9/13/94	50
23	McArthur River (Chakachatna R.)	8/18/93	100

Table I-1. Continued.

	Map # and Location	Sample Date	N
24	Wolverine Creek (Big R.)	7/03/93	100
25	Crescent Lake		
	Site 1 (South Shore)	8/14/94	50
		8/23/95	50
	Site 2 (near outlet)	8/14/94	50
		8/23/95	50
	Site 3	8/23/95	50
26	Packers Lake (Kalgin Island)	7/16/92	100
		7/26/93	100
	Kasilof River Drainage		
27	Bear Creek	8/12/92	100
		8/03/93	100
28	Moose Creek	8/10/92	100
		8/03/93	100
29	Glacier Flat Creek	8/11/92	100
		8/02/93	100
		8/04/94	100
30	Nikolai Creek	7/29/92	100
		7/27/93	100
31	Tustumena Lake (lake spawners)		
	Site 1 (between Glacier Flat and Crystal Ck)	8/31/94	50
	Site 2 (mouth of Crystal Creek)	9/01/94	50
32	Seepage Creek	8/25/94	100
	Northeastern Cook Inlet Drainages		
33	Bishop Creek (Stream 602)	8/23/93	100
34	Daniels Lake (Bishop Ck. Drainage)	9/02/92	100
		8/20/93	100
	Knik Arm Drainages		
35	Nancy Lake (Little Susitna R.)	8/26/93	100
36	Cottonwood Lake (Knik Arm)	8/18/93	100

Table I-1. Continued.

	Map # and Location	Sample Date	N
37	Fish Creek	8/01/92	100
		8/16/93	100
		8/15/94	100
	Inriver Composite Samples		
	Kenai River (fish wheel site, river mile 19)		
	1992	7/13/92	200
	1994-1	7/08-7/14/94	88
	1994-2	7/17-7/18/94	200
	1994-3	7/31-8/01/94	200
	1994-4	8/09-8/11/94	200
	1995-1	7/19-7/21/95	300
	1995-2	7/26/95	300
	1995-3	8/02-8/05/95	300
	1996	8/02/-8/03-96	200
	Kasilof River (fish wheel site, river mile 7)		
	1992-1	7/02-7/03/92	200
	1992-2	7/22-7/23/92	200
	1994-1	7/08-7/10/94	200
	1994-2	7/17/94	200
	1994-3	8/01-8/03/94	98
	Susitna River Mainstem (fish wheel, river mile 80)		
	1992-1	7/26/92	200
	1992-2	8/04/92	114
	Yentna River (fish wheel site, river mile 4)		
	1992-1	7/16/92	200
	1992-2	7/24/92	200
	1994	7/25-26/94	200
	Commercial Fishery Sampling		
	Drift gillnet fishery 1992	7/13/92	200
		7/20/92	200
	Drift gillnet fishery 1993	7/12/93	400
		7/16/93	283
	Drift gillnet fishery 1994	7/08/94	350

Table I-1. Continued.

Map # and Location	Sample Date	N
Drift gillnet fishery 1995	7/04/95	300
	7/10/95	399
	7/17/95	400
	7/24/95	400
	7/31/95	300
Eastside set gillnet fishery 1995	7/07/95	400
	7/20/95	400
Drift gillnet fishery 1996	7/05/96	396
	7/08/96	392
	7/15/96	369
	7/19/96	384
	7/29/96	389

Table I-2. Enzymes or proteins screened in Cook Inlet sockeye salmon. Enzyme nomenclature follows Shaklee et al. (1990), and locus abbreviations are given.

Enzyme or Protein	Enzyme Number	Locus	Tissue	Buffer[1]
Aspartate aminotransferase	2.6.1.1	*sAAT-1,2**	Heart	ACE 7.2
		*sAAT-3**	Eye	TBCL
		*mAAT-1**	Heart	ACE 7.2
		*mAAT-2**	Liver	ACE 7.0
Adenosine deaminase	3.5.4.4	*ADA-1**	Muscle	KG
Aconitate hydratase	4.2.1.3	*mAH-1,2**	Heart	ACE 7.2
		*mAH-3**	Heart	ACE 7.2
		*mAH-4**	Heart	ACE 7.2
		*sAH**	Liver	ACE 7.0
Alanine aminotransferase	2.6.1.2	*ALAT**	Muscle	KG
Creatine kinase	2.7.3.2	*CK-A1**	Muscle	TBCLE
		*CK-A2**	Muscle	TBCLE
		*CK-B**	Eye	ACE 7.0
		*CK-C1**	Eye	ACE 7.0
		*CK-C2**	Eye	ACE 7.0
Esterase-D	3.1.-.-	*ESTD**	Muscle	TBCLE
Fructose-bisphosphate aldolase	4.1.2.13	*FBALD-4**	Eye	ACE 7.0
Formalin dehydrogenase (glutathione)	1.2.1.1	*FDHG**	Liver	TBE
Fumarate hydratase	4.2.1.2	*FH**	Muscle	ACN 7.0
β-N-Acetylgalactosaminidase	3.2.1.53	*βGALA**	Liver	ACE 7.0
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*GAPDH-2**	Heart	ACN 7.0
		*GAPDH-3**	Heart	ACN 7.0
		*GAPDH-4**	Eye	ACE 7.0
		*GAPDH-5**	Eye	ACE 7.0
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-1,2**	Muscle	ACN 7.0
		*G3PDH-3**	Heart	ACN 7.0

Table I-2. Continued.

Enzyme or Protein	Enzyme Number	Locus	Tissue	Buffer[1]
Aspartate aminotransferase	2.6.1.1	*sAAT-1,2**	Heart	ACE 7.2
		*G3PDH-4**	Heart	ACN 7.0
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-B1,2**	Muscle	TBCLE
		*GPI-A**	Muscle	TBCLE
Glutathione reductase	1.6.4.2	*GR**	Eye	TBCL
Isocitrate dehydrogenase (NADP+)	1.1.1.42	*mIDHP-1**	Heart	ACN 7.0
		*mIDHP-2**	Heart	ACN 7.0
		*sIDHP-1**	Liver	ACE 7.0
		*sIDHP-2**	Liver	ACE 7.0
L-Lactate dehydrogenase	1.1.1.27	*LDH-A1**	Muscle	ACN 7.0
		*LDH-A2**	Muscle	ACN 7.0
		*LDH-B1**	Muscle	TBCLE
		*LDH-B2**	Liver	TBE
		*LDH-C**	Eye	KG
αMannosidase	3.2.1.24	*αMAN**	Liver	TC4
Malate dehydrogenase	1.1.1.37	*sMDH-A1,2**	Heart	ACN 7.0
		*sMDH-B1,2**	Heart	ACN 7.0
		*mMDH-1**	Heart	ACN 7.0
		*mMDH-2**	Muscle	ACN 7.0
		*mMDH-3**	Muscle	ACN 7.0
Malic enzyme (NADP+)	1.1.1.40	*sMEP-1**	Liver	TC4
		*mMEP-1**	Muscle	ACN 7.0
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	Liver	TBE
Dipeptidase	3.4.-.-	*PEPA**	Muscle	TBCLE
Tripeptide aminopeptidase	3.4.-.-	*PEPB-1**	Heart	TBE
Peptidase-C	3.4.-.-	*PEPC**	Eye	KG
Proline dipeptidase	3.4.13.9	*PEPD-1**	Heart	TBE
Peptidase-LT	3.4.-.-	*PEPLT**	Muscle	TBCLE

Table I-2. Continued.

Enzyme or Protein	Enzyme Number	Locus	Tissue	Buffer[1]
Aspartate aminotransferase	2.6.1.1	*sAAT-1,2**	Heart	ACE 7.2
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	Liver	ACE 7.0
Phosphoglucomutase	5.4.2.2	*PGM-1**	Heart	ACE 7.2
		*PGM-2**	Muscle	TBCLE
Superoxide dismutase	1.15.1.1	*sSOD-1**	Liver	TBE
Triose-phosphate isomerase	5.3.1.1	*TPI-1,2**	Eye	KG
		*TPI-3**	Eye	KG
		*TPI-4**	Eye	KG

[1] Buffer system abbreviations and descriptions are : 1) **ACE 7.0** or **ACE 7.2**; N-(3-aminopropyl)-morpholine, citrate (pH 7.0 or 7.2) with EDTA (Clayton and Tretiak 1972); 2) **ACN 7.0**; N-(3-aminopropyl)-morpholine, citrate (pH 7.0) with NAD (Clayton and Tretiak 1972); 3) **KG**; Tris, glycine HCl (pH 8.5; tray concentration modified to 0.075 M Tris; Holmes and Masters 1970); 4) **TBCL**; Tris, borate, citrate, LiOH (pH 8.2; Ridgway et al. 1970); 5) **TBCLE**; Tris, borate, citrate, LiOH with EDTA (pH 8.2; Selander et al. 1971); 6) **TBE**; Tris, borate, EDTA (pH 8.7; Boyer et al. 1963); and 7) **TC4**; Tris citrate, NaOH (pH 5.9; Selander et al. 1971).

Table I-3. Hierarchical log-likelihood analysis of sockeye salmon collections from Upper Cook Inlet, Alaska. Test statistics were derived from simultaneous comparisons of allele frequencies at 44 polymorphic protein loci.

Populations	DF	G	
Among Regions	384	8186.30	**
Within Regions	4928	12067.73	**
Kenai River	1920	6477.84	**
Among nursery lakes	256	5120.00	**
Within nursery lakes	1664	1357.84	
Upper Russian Lake[1]	128	104.94	
Among sites	64	93.41	**
Between years	64	11.53	
Russian River above/late	64	11.53	
Kenai / Skilak lakes	1344	1186.74	
Among sites[2]	576	752.10	**
Between years	768	434.64	
Ptarmigan Creek	64	24.32	
Quartz Creek	64	61.08	
Btwn Kenai / Skilak lakes site 1	64	24.32	
Btwn Kenai / Skilak lakes site 2	64	26.47	
Btwn Kenai / Skilak lakes site 3	64	61.08	
Btwn Kenai / Skilak lakes site 6	128	61.37	
Skilak Lake outlet	320	176.00	
Tern Lake	64	26.47	
Hidden Lake	64	13.10	
Trail Lake (Moose Creek)	64	26.59	
Yentna River	704	2129.20	**
Among nursery lakes	320	2053.00	**
Between nursery lakes	384	76.20	
Chelatna Lake	64	9.13	
Yentna River, west fork	64	9.27	
Hewitt / Whiskey lakes	64	13.56	
Shell Lake	64	10.48	
Trinity / Movie lakes	64	16.95	
Judd Lake	64	16.81	
Susitna River mainstem	448	812.00	**
Among nursery lakes[3]	320	779.10	**

Table I-3. Continued.

Among nursery lakes[3]	320	779.10	**
Between nursery lakes	128	32.90	
Stephan Lake	64	16.36	
Larson Lake	64	16.54	
Western Cook Inlet	768	1786.55	**
Among nursery lakes[4]	320	1605.00	**
Between nursery lakes	448	181.55	
Crescent Lake	320	127.05	
Among sites[5]	192	90.10	
Between years	128	36.95	
Crescent Lake site 1	64	15.43	
Crescent Lake site 2	64	21.52	
Coal Creek	64	30.68	
Chilligan River	64	23.82	
Kasilof River	704	310.36	
Among sites[6]	384	206.70	
Between years	320	103.66	
Bear Creek	64	13.76	
Moose Creek (Tustumena)	64	5.47	
Glacier Flat Creek	64	66.23	
Nikolai Creek	64	18.20	
Northeast Cook Inlet	128	128.54	
Among nursery lakes[7]	64	100.90	**
Between nursery lakes	64	27.64	
Daniel's Lake	64	27.64	
Knik Arm	256	423.24	**
Among nursery lakes[8]	128	345.10	**
Between nursery lakes	128	78.14	
Fish Creek	128	78.14	

* $P < 0.05$; ** $P < 0.01$

[1] Includes Russian River above / early.

[2] Includes Russian River below and Btwn Kenai / Skilake lakes sites 4 & 5.

[3] Includes Byers Lake, Birch Creek and Red Shirt Lake.

[4] Includes McArthur River, Wolverine Lake and Packers Lake.

[5] Includes Crescent Lake site 3.

[6] Includes Tustumena Lake sites 1 & 2 and Seepage Creek.

[7] Includes Bishop Creek.

[8] Includes Nancy Lake and Cottonwood Creek.

Table I-4. Gene diversity analysis of Upper Cook Inlet sockeye salmon collections.

	Absolute gene diversity		Percent relative diversity			
Locus	Total	Within sites	Within sites	Among sites within nurseries	Among nurseries within regions	Among regions
*sAAT-3**	0.0007	0.0007	99.57	0.01	0.35	0.07
*mAAT-1**	0.1706	0.1580	92.82	0.45	4.07	2.66
*mAAT-2**	0.0281	0.0238	84.75	0.75	11.66	2.83
*mAH-4**	0.0010	0.0008	99.23	0.00	0.70	0.07
*sAH**	0.0720	0.0299	41.55	0.14	51.09	7.22
*ALAT**	0.5315	0.4869	91.60	0.35	5.22	2.83
*CK-A2**	0.0008	0.0008	98.78	1.11	0.05	0.06
*CK-B**	0.0004	0.0004	99.40	0.51	0.03	0.06
*FDHG**	0.0002	0.0002	99.79	0.18	0.01	0.02
*GAPDH-2**	0.0049	0.0048	97.55	0.19	1.94	0.32
*G3PDH-4**	0.0023	0.0023	98.95	0.46	0.01	0.57
*GPI-A**	0.0021	0.0021	98.48	0.42	0.69	0.41
*mIDHP-1**	0.0018	0.0018	99.13	0.48	0.09	0.30
*sIDHP-1**	0.0112	0.0105	93.72	0.21	5.25	0.82
*sIDHP-2**	0.0015	0.0014	97.04	0.02	2.79	0.16
*LDH-A2**	0.0007	0.0007	98.97	0.05	0.89	0.10
*LDH-B2**	0.1755	0.1588	90.46	1.00	5.93	2.61
*mMEP-1**	0.0030	0.0029	96.38	0.39	2.58	0.66
*MPI**	0.0019	0.0019	99.18	0.47	0.15	0.20
*PEPA**	0.0061	0.0060	98.73	0.60	0.29	0.38
*PEPB-1**	0.0099	0.0089	89.49	0.16	9.00	1.35
*PEPC**	0.0588	0.0523	88.86	0.11	8.17	2.86
*PEPD-1**	0.0072	0.0070	98.49	0.54	0.47	0.49
*PEPLT**	0.0465	0.0398	85.62	0.09	2.48	11.81
*PGDH**	0.0002	0.0002	99.46	0.00	0.48	0.06
*PGM-2**	0.4033	0.3494	86.63	0.21	6.86	6.30
*sSOD-1**	0.0002	0.0002	99.51	0.00	0.44	0.05
*TPI-3**	0.0042	0.0041	97.07	1.19	1.20	0.54
*TPI-4**	0.0006	0.0006	99.49	0.00	0.47	0.04
Average	1.5469	1.3573	87.74	0.38	7.80	4.08

Table I-5. Results of simulated mixtures of Cook Inlet sockeye salmon from the 1995 baseline with 100 bootstrap resamplings and a simulated sample size of 400. Standard deviations are given in parentheses; row totals equal 1.00. Allocations to correct regions are in bold.

Region	Regional Allocation							
	Kenai	Kasilof	Yentna	Susitna	West Cook Inlet	NE Cook Inlet	Knik Arm	Unknown[1]
Kenai	**0.91**	0.01	0.02	0.02	0.03	0.00	0.01	0.00
	(0.049)	(0.018)	(0.021)	(0.028)	(0.029)	(0.003)	(0.022)	
Kasilof	0.03	**0.92**	0.01	0.01	0.03	0.00	0.00	0.00
	(0.024)	**(0.042)**	(0.017)	(0.020)	(0.032)	(0.000)	(0.008)	
Yentna	0.01	0.00	**0.88**	0.06	0.03	0.00	0.02	0.00
	(0.013)	(0.006)	**(0.065)**	(0.047)	(0.034)	(0.004)	(0.027)	
Susitna	0.01	0.01	0.09	**0.77**	0.08	0.00	0.04	0.00
	(0.011)	(0.024)	(0.063)	**(0.104)**	(0.069)	(0.005)	(0.048)	
Yentna/Susitna	0.01	0.01	**0.87**		0.07	0.00	0.04	0.00
	(0.012)	(0.015)	**(0.072)**		(0.066)	(0.003)	(0.049)	
West Cook Inlet	0.02	0.01	0.03	0.05	**0.86**	0.00	0.03	0.00
	(0.022)	(0.020)	(0.030)	(0.048)	**(0.066)**	(0.001)	(0.042)	
Northeastern Cook Inlet	0.00	0.00	0.01	0.00	0.00	**0.99**	0.00	0.00
	(0.004)	(0.007)	(0.002)	(0.006)	(0.006)	**(0.011)**	(0.003)	
Knik Arm	0.01	0.00	0.02	0.05	0.04	0.00	**0.88**	0.00
	(0.016)	(0.007)	(0.024)	(0.038)	(0.033)	(0.006)	**(0.059)**	

[1] Genotypes in this category have a probability of less than 1.0×10^{-10} of belonging to any population in the baseline.

Table I-6. Results of Cook Inlet Central District drift and set gillnet fishery mixed stock analysis, 1992-1996.

Date	N	Kenai Estimate	Kenai SD	Kasilof Estimate	Kasilof SD	Susitna/Yentna Estimate	Susitna/Yentna SD	W. Cook Inlet Estimate	W. Cook Inlet SD	NE. Cook Inlet Estimate	NE. Cook Inlet SD	Knik Arm Estimate	Knik Arm SD	Unknown[1]
1992[2]														
July 13, 1992	150	0.88	0.077	0.00	0.000	0.10	0.065	0.02	0.046	0.00	0.000	0.00	0.000	0.00
July 20, 1992	200	0.56	0.092	0.10	0.062	0.21	0.080	0.07	0.043	0.01	0.018	0.04	0.064	0.00
1993[2]														
July 12, 1993	337	0.52	0.071	0.03	0.038	0.15	0.062	0.14	0.052	0.00	0.000	0.14	0.040	0.02
July 16, 1993	278	0.82	0.084	0.02	0.055	0.09	0.060	0.02	0.019	0.00	0.000	0.04	0.035	0.01
1994														
July 15, 1994	344	0.53	0.064	0.05	0.059	0.21	0.068	0.08	0.082	0.01	0.015	0.12	0.038	0.00
1995[3]														
Drift gillnet Fishery														
July 3, 1995	298	0.16	0.052	0.43	0.076	0.19	0.079	0.04	0.042	0.02	0.014	0.15	0.042	0.00
July 10, 1995	390	0.32	0.048	0.21	0.062	0.29	0.069	0.07	0.067	0.00	0.000	0.11	0.031	0.00
July 17, 1995	394	0.43	0.054	0.22	0.061	0.07	0.049	0.18	0.063	0.00	0.000	0.10	0.027	0.00
July 24, 1995	390	0.55	0.068	0.05	0.039	0.30	0.059	0.04	0.047	0.00	0.000	0.06	0.021	0.00
July 31, 1995	298	0.86	0.061	0.00	0.000	0.04	0.040	0.07	0.064	0.02	0.012	0.01	0.024	0.00
Set gillnet Fishery														
July 7, 1995	389	0.16	0.056	0.78	0.061	0.05	0.048	0.00	0.018	0.01	0.011	0.00	0.000	0.00
July 20, 1995	297	0.91	0.065	0.02	0.060	0.03	0.053	0.03	0.045	0.00	0.000	0.01	0.012	0.00
1996[4]														
July 5, 1996	396	0.28	0.052	0.37	0.057	0.06	0.049	0.19	0.067	0.00	0.000	0.10	0.027	0.01
July 8, 1996	392	0.30	0.054	0.38	0.056	0.18	0.080	0.04	0.076	0.00	0.000	0.09	0.024	0.02
July 15, 1996	369	0.61	0.073	0.07	0.040	0.21	0.091	0.09	0.075	0.00	0.000	0.00	0.000	0.02
July 19, 1996	384	0.60	0.060	0.23	0.046	0.13	0.051	0.01	0.010	0.00	0.000	0.00	0.007	0.02
July 29, 1996	389	0.63	0.055	0.09	0.044	0.20	0.058	0.04	0.019	0.01	0.012	0.02	0.025	0.01

[1] Genotypes in this category have a probability of less than 1.0×10^{-10} of belonging to any population in the baseline.
[2] *mAAT-2** and *G3PDH-4* were not used in mixed stock analysis.
[3] *GPI-B1,2** was not used in mixed stock analysis.
[4] *mAH-4** was not used in mixed stock analysis.

Table I-7. Catch analysis for drift gillnet fisheries from Cook Inlet Central District that were sampled for sockeye salmon. Harvest, maximum likelihood estimates, catch estimates, and percent of Kenai River harvest are given for 1995-1996.

a. 1995

Date	Drift gillnet harvest	Relative Contribution		Catch		Percent of Kenai River harvest
		Estimate	SD	Estimate	SD	
03-Jul-95	48,490	0.16	0.052	7,758	2,521	1.9
10-Jul-95	225,621	0.32	0.048	72,199	10,830	18.1
17-Jul-95	462,625	0.43	0.054	198,929	24,982	49.7
24-Jul-95	133,462	0.55	0.068	73,404	9,075	18.4
31-Jul-95	56,522	0.86	0.061	48,609	3,448	12.2
Total	926,720			400,899		

b. 1996

Date	Drift gillnet harvest	Relative Contribution		Catch		Percent of Kenai River harvest
		Estimate	SD	Estimate	SD	
05-Jul-96	248,795	0.28	0.052	69,663	12,937	11.0
08-Jul-96	225,565	0.30	0.055	67,670	12,406	10.7
15-Jul-96	353,959	0.61	0.068	215,915	24,069	34.2
19-Jul-96	430,343	0.60	0.060	253,902	25,821	40.2
29-Jul-96	38,845	0.63	0.055	24,472	2,136	3.9
Total	1,297,507			631,622		

Table I-8. Results of inriver mixed stock analyses for Cook Inlet 1992-1996.

		Kenai		Kasilof		Susitna/Yentna		W. Cook Inlet		NE Cook Inlet		Knik Arm		
Population	N	Estimate	SD	Estimate	SD	Estimate	SD	Estimate	SD	Estimate	SD	Estimate	SD	Unknown[1]
Kenai River														
July 13, 1992	199	0.83	0.060	0.00	0.000	0.02	0.036	0.14	0.052	0.00	0.000	0.01	0.012	0.01
July 10, 1994	87	0.63	0.210	0.05	0.139	0.17	0.172	0.15	0.145	0.00	0.000	0.00	0.000	0.00
July 22, 1994	197	0.84	0.087	0.09	0.070	0.06	0.062	0.00	0.000	0.00	0.000	0.00	0.000	0.01
July 31, 1994	155	0.83	0.077	0.00	0.000	0.16	0.075	0.00	0.000	0.01	0.013	0.00	0.030	0.00
August 9, 1994	192	0.93	0.067	0.03	0.054	0.02	0.052	0.01	0.011	0.00	0.000	0.00	0.015	0.00
July 20, 1995	295	0.89	0.067	0.00	0.000	0.05	0.040	0.06	0.054	0.00	0.000	0.00	0.000	0.00
July 26, 1995	298	0.91	0.049	0.03	0.022	0.02	0.040	0.01	0.017	0.00	0.000	0.02	0.017	0.01
August 4, 1995	194	0.86	0.062	0.00	0.000	0.14	0.064	0.00	0.000	0.00	0.000	0.00	0.016	0.00
August 3, 1996	200	0.97	0.054	0.00	0.000	0.01	0.022	0.01	0.048	0.00	0.000	0.00	0.000	0.005
Susitna River Mainstem														
July 26, 1992	199	0.13	0.073	0.00	0.000	0.75	0.117	0.12	0.104	0.00	0.000	0.00	0.000	0.01
August 4, 1992	113	0.04	0.060	0.00	0.000	0.92	0.067	0.01	0.020	0.00	0.000	0.01	0.031	0.03
Yentna River														
July 15, 1992	196	0.08	0.049	0.00	0.000	0.81	0.068	0.00	0.000	0.02	0.025	0.07	0.040	0.02
July 24, 1992	200	0.00	0.000	0.00	0.000	0.96	0.050	0.02	0.054	0.00	0.018	0.01	0.031	0.01
July 25-26, 1994	199	0.00	0.000	0.00	0.000	0.98	0.029	0.00	0.001	0.00	0.000	0.02	0.029	0.00
Kasilof River														
July 2, 1992	196	0.01	0.009	0.91	0.072	0.04	0.043	0.05	0.063	0.00	0.000	0.00	0.000	0.00
July 22, 1992	199	0.00	0.000	0.85	0.065	0.02	0.022	0.13	0.063	0.00	0.000	0.00	0.006	0.00
July 8-10, 1994	197	0.09	0.061	0.55	0.136	0.10	0.068	0.26	0.155	0.00	0.000	0.00	0.000	0.00
July 17, 1994	180	0.03	0.056	0.82	0.082	0.14	0.094	0.01	0.028	0.00	0.000	0.00	0.000	0.00
August 1-3, 1994	96	0.05	0.050	0.80	0.112	0.08	0.088	0.00	0.000	0.00	0.000	0.07	0.053	0.00

[1] Genotypes in this category have a probability of less than 1.0×10^{-10} of belonging to any population in the baseline.

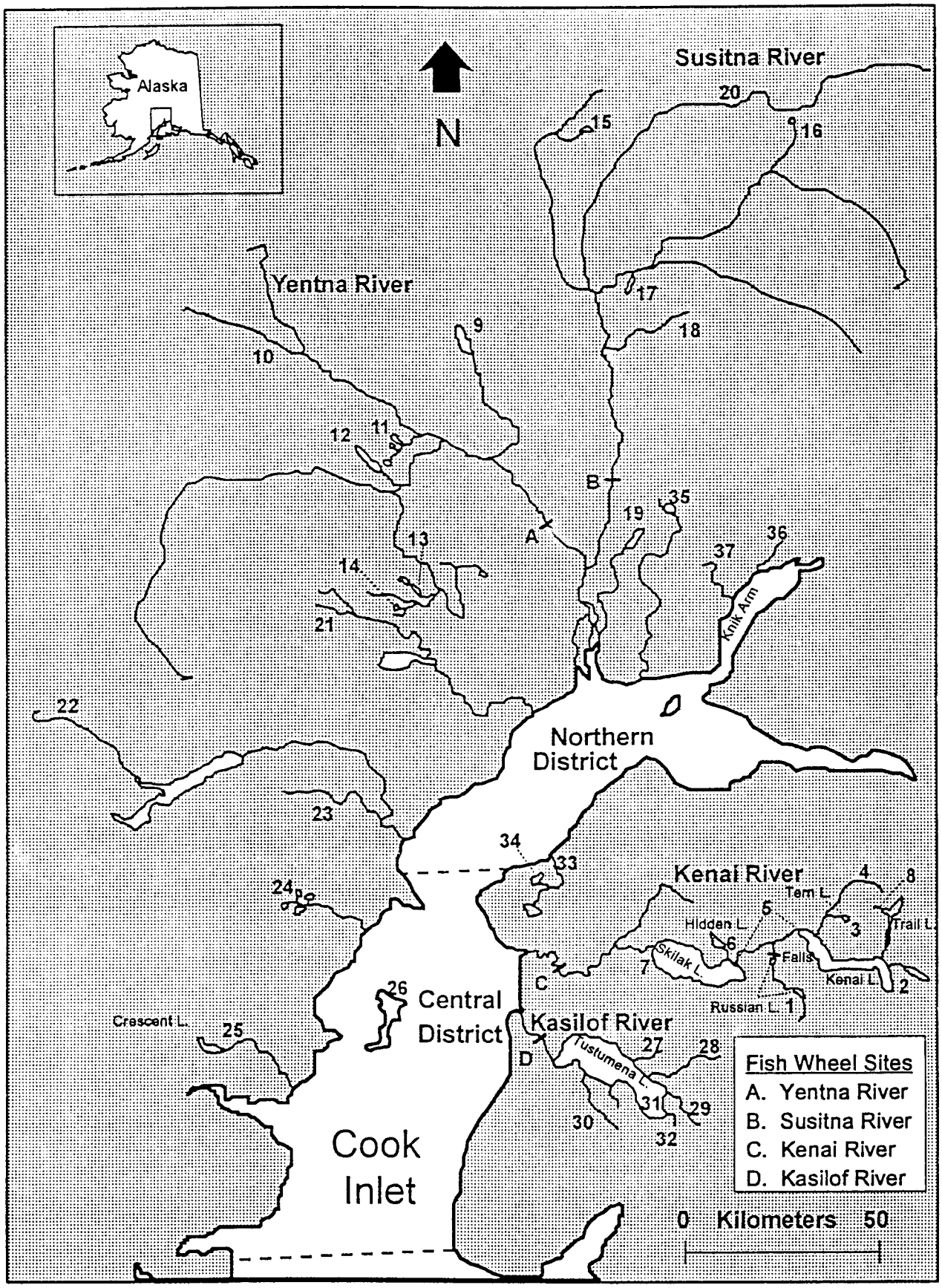

Figure I-1. Sampling location for sockeye salmon originating from Upper Cook Inlet, 1992-1995.

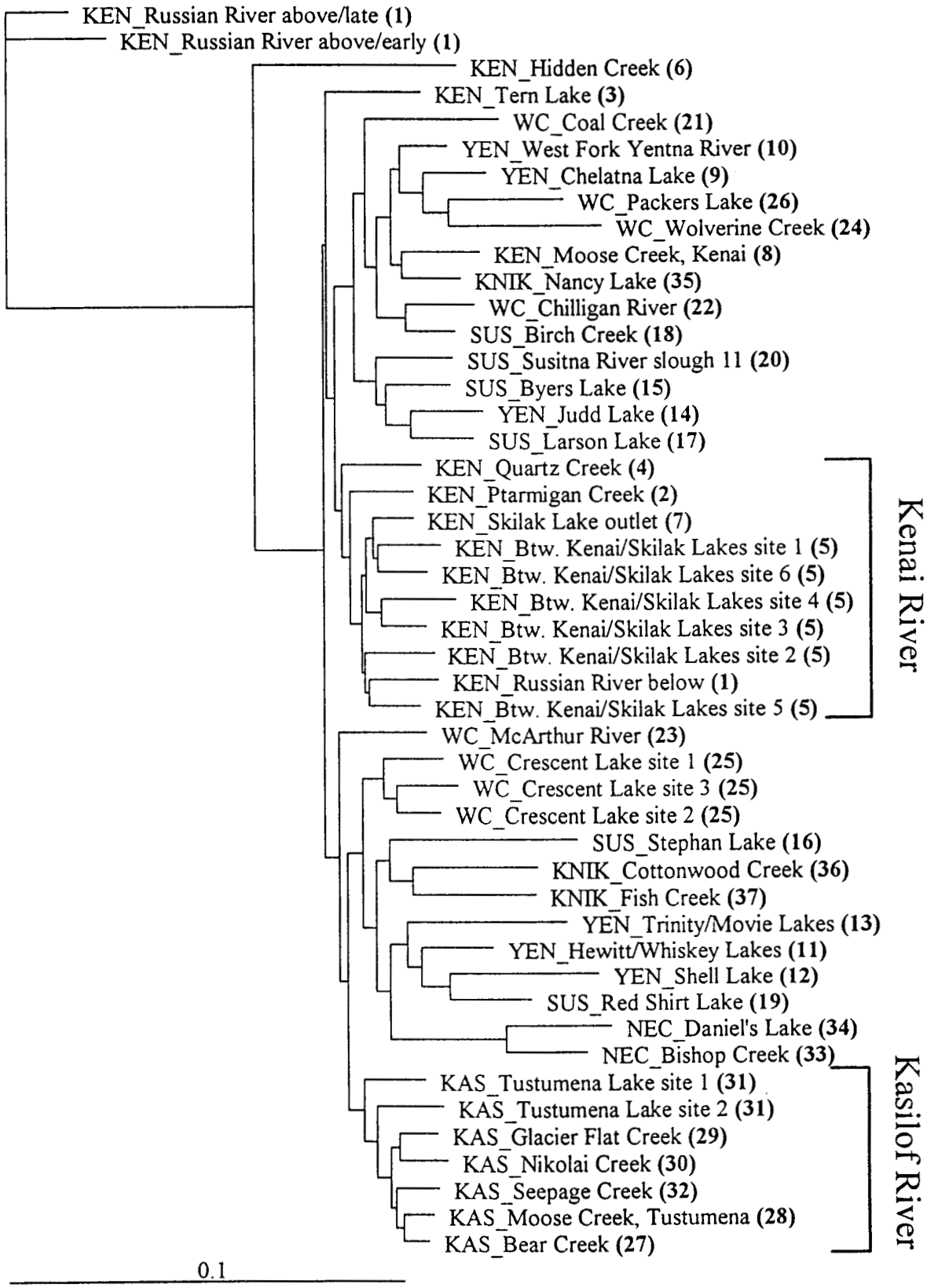

Figure I-2. Neighboring-joining tree for Upper Cook Inlet sockeye salmon using Cavalli-Sforza and Edwards (1967) chord measure of genetic distance.

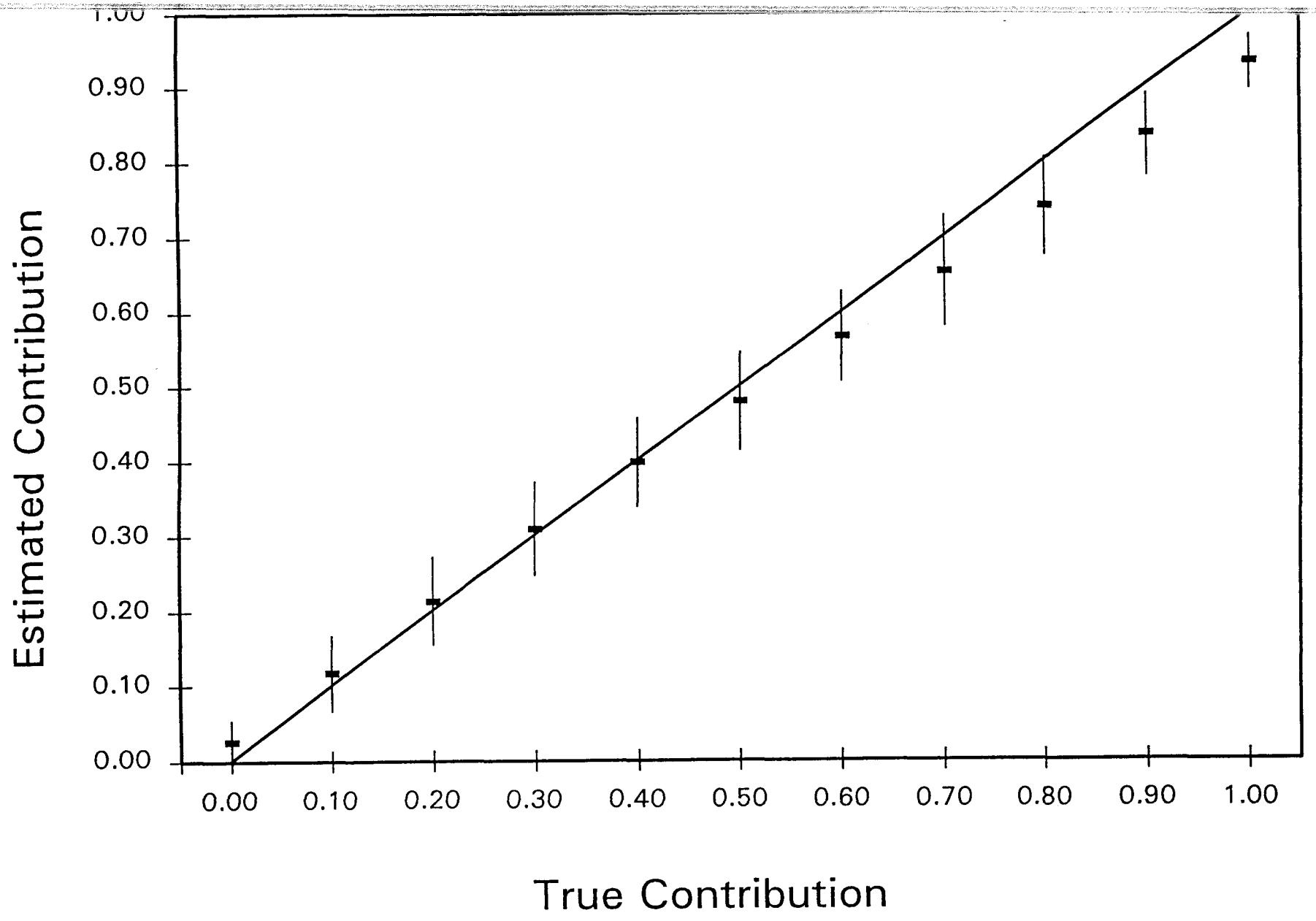

Figure I-3. Estimated contributions to a simulated mixed stock fishery in Cook Inlet with increasing contributions of Kenai River populations. The solid line represents the true contributions, and boxes are the estimated contributions with standard error lines included.

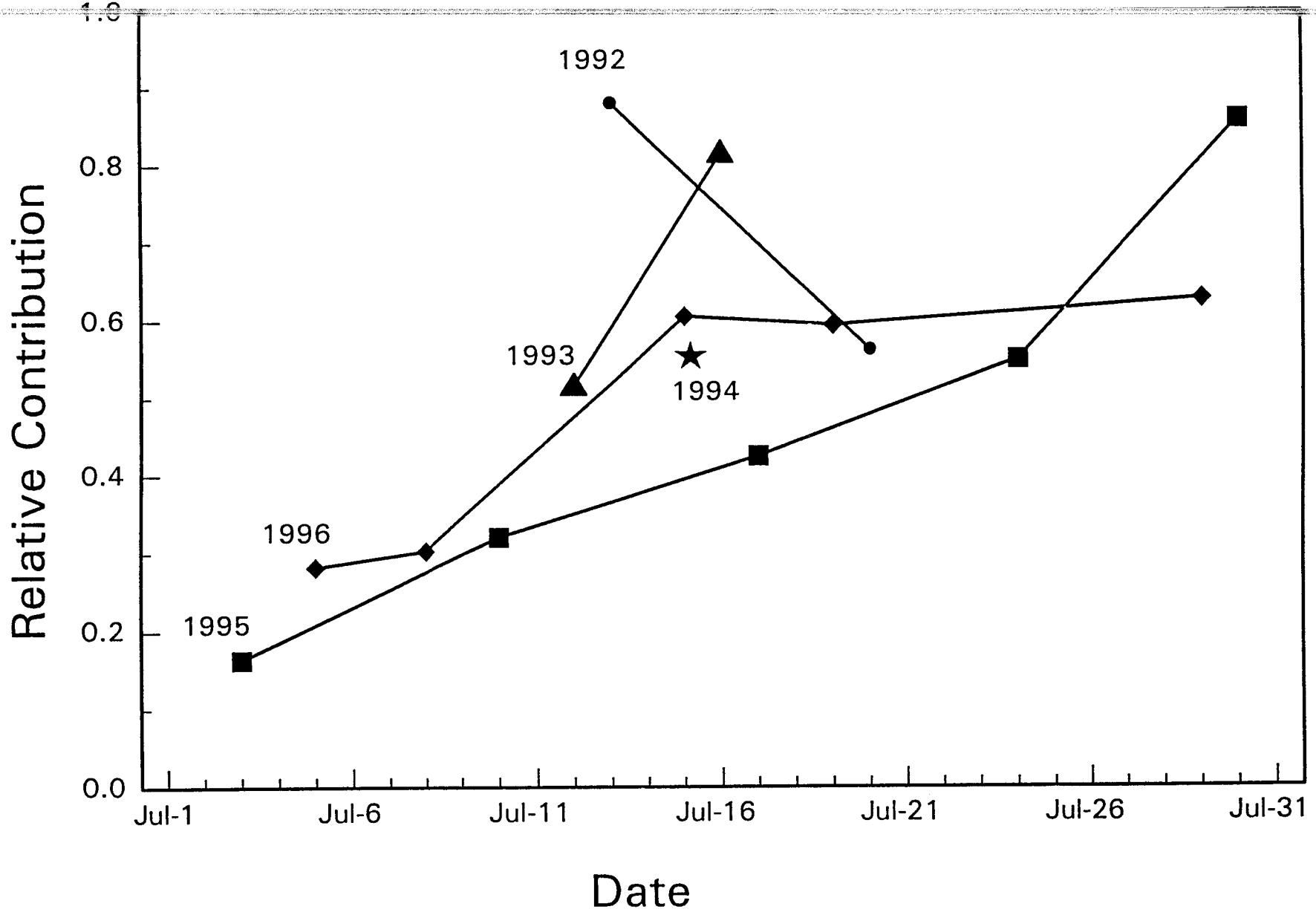

Figure I-4. Relative contribution of Kenai River populations to the Cook Inlet Central District drift gillnet fisheries, 1992-1996.

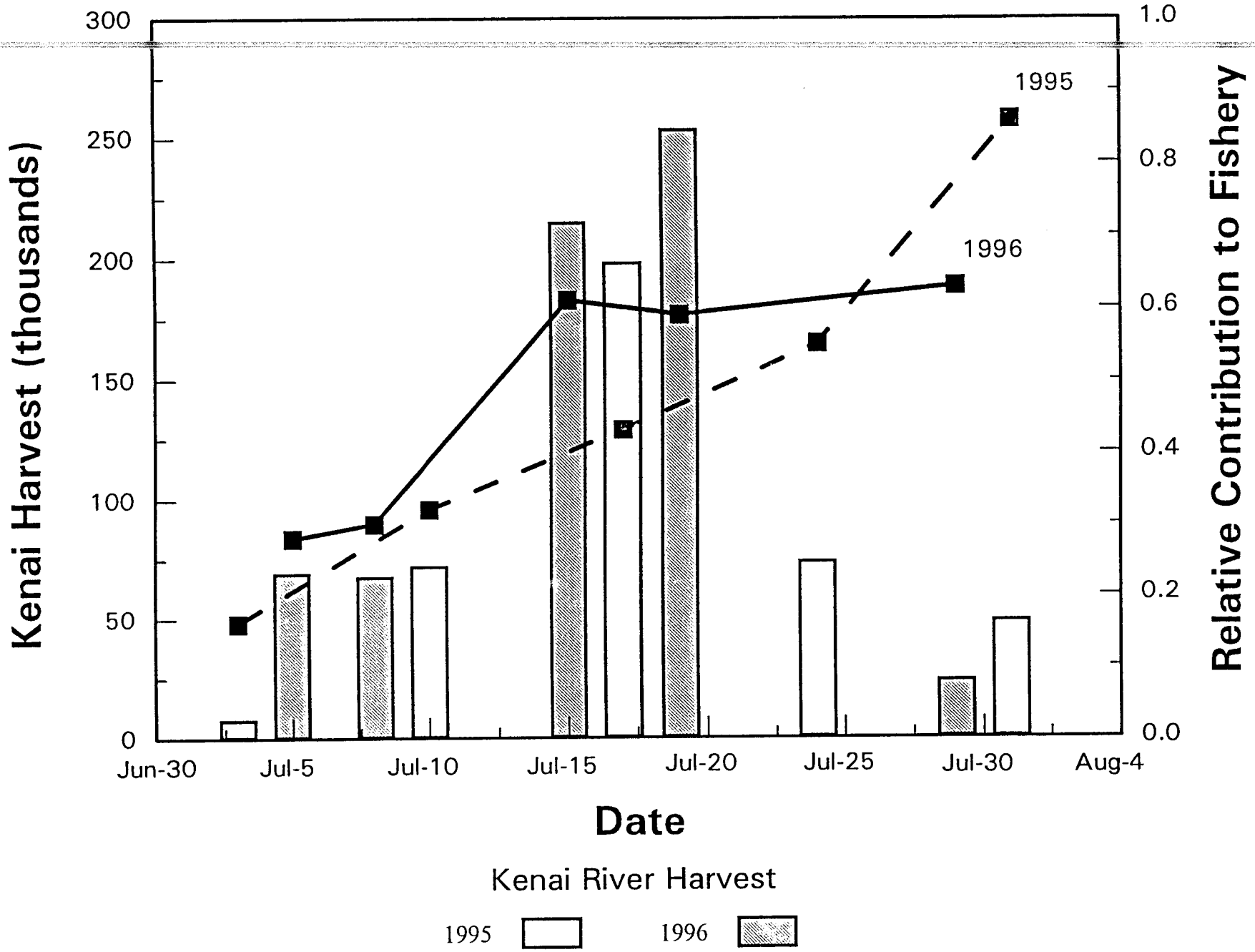

Figure I-5. Estimated harvest (histogram) and relative contribution (line) of Kenai River sockeye salmon in the Cook Inlet Central District drift gillnet fisheries in 1995 and 1996.

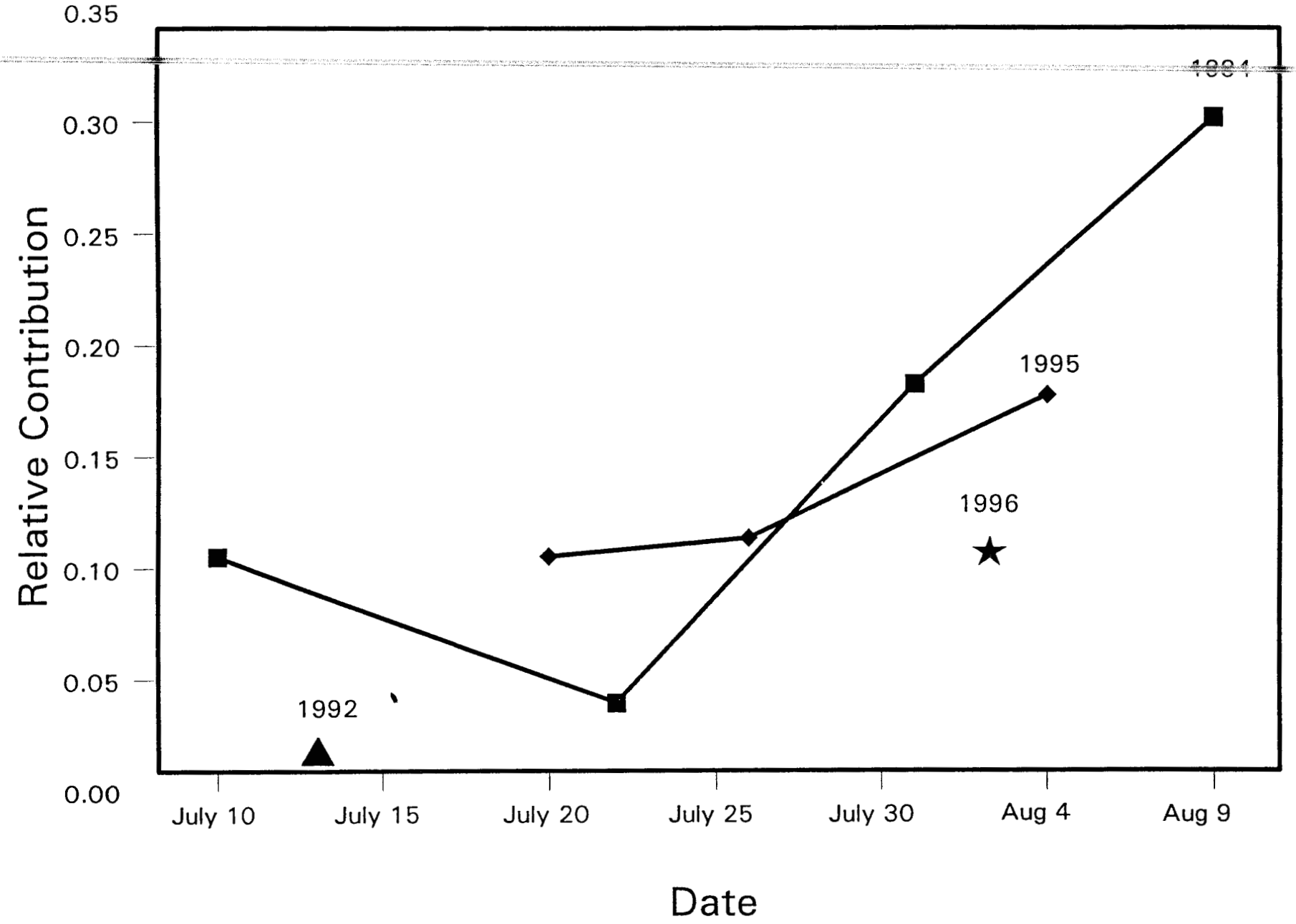

Figure I-6. Relative contributions of Russian River populations to admixtures taken at the Kenai River fish wheel, 1992, 1994-1996.

Appendix I-A. Estimated allele frequencies for Upper Cook Inlet sockeye salmon populations.

Population	*sAAT-1,2*			*sAAT-3*		*mAAT-1*		*mAAT-2*		*mAH-1,2*		*mAH-4*		*sAH*			
	N	77	122	N	117	N	-83	N	-73	N	75	N	114	N	117	83	75
Kenai River																	
Russian River above/early	100	0.000	0.000	100	0.000	100	0.195	98	0.082	100	0.043	100	0.000	100	0.740	0.000	0.000
Russian River above/late	200	0.000	0.000	200	0.000	199	0.083	198	0.169	199	0.000	176	0.000	199	0.706	0.000	0.000
Russian River below	99	0.000	0.000	100	0.000	99	0.076	96	0.005	98	0.038	92	0.000	99	0.046	0.000	0.000
Ptarmigan Creek	198	0.000	0.000	192	0.000	198	0.040	182	0.000	198	0.068	197	0.000	198	0.010	0.000	0.000
Tern Lake	150	0.000	0.000	150	0.000	150	0.030	150	0.013	150	0.022	150	0.010	150	0.003	0.000	0.000
Quartz Creek	199	0.000	0.000	199	0.000	199	0.053	196	0.005	198	0.033	199	0.000	200	0.005	0.000	0.000
Btwn Ken/Ski Lks site 1	100	0.000	0.000	99	0.000	100	0.120	99	0.005	95	0.047	100	0.000	100	0.030	0.010	0.000
Btwn Ken/Ski Lks site 2	100	0.000	0.000	99	0.000	100	0.120	100	0.015	100	0.033	100	0.000	100	0.025	0.000	0.000
Btwn Ken/Ski Lks site 3	150	0.000	0.000	147	0.000	150	0.043	150	0.030	150	0.037	147	0.000	150	0.017	0.000	0.003
Btwn Ken/Ski Lks site 4	50	0.000	0.000	50	0.000	50	0.040	49	0.031	50	0.030	50	0.000	50	0.000	0.000	0.000
Btwn Ken/Ski Lks site 5	100	0.000	0.000	99	0.000	100	0.070	100	0.010	100	0.020	100	0.000	100	0.020	0.000	0.000
Btwn Ken/Ski Lks site 6	290	0.000	0.000	290	0.000	297	0.072	298	0.000	288	0.041	294	0.000	297	0.025	0.002	0.000
Hidden Creek	150	0.000	0.000	197	0.000	200	0.025	199	0.269	200	0.051	200	0.000	200	0.000	0.000	0.000
Skilak Lake outlet	796	0.000	0.000	788	0.000	795	0.094	795	0.004	796	0.032	798	0.000	793	0.018	0.001	0.000
Moose Creek, Kenai	199	0.000	0.000	197	0.000	199	0.030	198	0.013	180	0.065	198	0.000	199	0.020	0.000	0.000
Yentna River																	
Chelatna Lake	200	0.000	0.000	200	0.000	200	0.163	197	0.000	199	0.035	199	0.008	200	0.000	0.000	0.000
West Fork Yentna River	200	0.000	0.000	199	0.000	196	0.140	200	0.000	200	0.024	200	0.000	200	0.005	0.000	0.000
Hewitt/Whiskey Lakes	100	0.000	0.000	99	0.005	100	0.100	100	0.000	100	0.020	100	0.000	100	0.000	0.000	0.000
Shell Lake	198	0.000	0.000	199	0.000	198	0.096	200	0.000	193	0.030	199	0.000	200	0.000	0.000	0.000
Trinity/Movie Lakes	198	0.000	0.000	200	0.000	198	0.104	199	0.000	198	0.005	199	0.000	200	0.000	0.000	0.000
Judd Lake	200	0.000	0.000	198	0.000	199	0.382	200	0.000	199	0.029	199	0.003	200	0.000	0.000	0.000
Susitna River Mainstem																	
Byers Lake	100	0.000	0.000	99	0.000	97	0.258	96	0.000	97	0.054	98	0.000	100	0.000	0.000	0.000
Stephan Lake	125	0.010	0.000	123	0.000	125	0.188	125	0.000	125	0.010	125	0.000	125	0.016	0.000	0.000
Larson Lake	198	0.000	0.000	194	0.000	200	0.310	199	0.000	200	0.009	200	0.000	200	0.000	0.000	0.000
Birch Creek	50	0.000	0.000	66	0.000	67	0.060	67	0.000	67	0.015	67	0.000	67	0.000	0.000	0.000
Red Shirt Lake	34	0.000	0.007	34	0.000	34	0.000	34	0.000	34	0.044	34	0.000	34	0.000	0.000	0.000
Susitna River slough 11	50	0.000	0.000	50	0.000	50	0.210	50	0.000	47	0.032	50	0.000	50	0.000	0.000	0.000
Western Cook Inlet																	
Coal Creek	200	0.000	0.000	200	0.005	199	0.068	191	0.000	200	0.105	200	0.000	198	0.000	0.000	0.000
Chilligan River	150	0.000	0.000	146	0.000	150	0.027	150	0.000	149	0.003	150	0.000	150	0.000	0.000	0.000
MacArthur River	100	0.000	0.000	100	0.005	100	0.030	99	0.010	100	0.073	100	0.000	100	0.000	0.000	0.000
Wolverine Creek	97	0.000	0.000	100	0.000	92	0.114	99	0.005	64	0.133	91	0.000	98	0.010	0.000	0.000
Crescent Lake site 1	99	0.000	0.000	99	0.000	99	0.025	99	0.000	82	0.027	99	0.000	100	0.000	0.000	0.000
Crescent Lake site 2	100	0.000	0.000	100	0.000	100	0.055	100	0.000	92	0.016	98	0.000	100	0.000	0.000	0.000
Crescent Lake site 3	50	0.000	0.000	50	0.000	48	0.063	50	0.000	44	0.000	47	0.000	50	0.000	0.000	0.000
Packers Lake	182	0.000	0.000	180	0.000	182	0.017	180	0.000	98	0.033	181	0.000	181	0.003	0.000	0.000
Kasilof River																	
Bear Creek	119	0.000	0.000	166	0.000	200	0.098	199	0.000	199	0.038	199	0.000	199	0.000	0.000	0.000
Moose Creek, Tustumena	200	0.000	0.000	194	0.000	200	0.075	199	0.000	196	0.040	200	0.000	200	0.000	0.000	0.000
Glacier Flat Creek	220	0.000	0.000	294	0.002	299	0.104	298	0.002	299	0.034	300	0.000	298	0.002	0.000	0.000
Nikolai Creek	200	0.000	0.000	200	0.000	200	0.138	200	0.000	186	0.052	200	0.000	200	0.000	0.000	0.000
Tustumena Lake site 1	50	0.000	0.000	46	0.000	50	0.080	50	0.000	50	0.040	50	0.000	50	0.010	0.000	0.000
Tustumena Lake site 2	50	0.000	0.000	45	0.000	50	0.100	50	0.000	50	0.010	50	0.000	50	0.000	0.000	0.000
Seepage Creek	100	0.000	0.000	100	0.000	100	0.105	100	0.000	100	0.035	100	0.000	100	0.000	0.000	0.000
Northeastern Cook Inlet																	
Bishop Creek	100	0.000	0.000	100	0.000	97	0.160	98	0.000	100	0.000	100	0.000	100	0.000	0.000	0.000
Daniels Lake	199	0.000	0.000	200	0.000	199	0.015	200	0.003	200	0.003	200	0.000	200	0.030	0.000	0.000
Knik Arm																	
Nancy Lake	100	0.000	0.000	99	0.000	100	0.030	99	0.000	99	0.035	100	0.000	100	0.000	0.000	0.000
Cottonwood Creek	95	0.000	0.000	100	0.000	100	0.000	98	0.000	100	0.000	100	0.000	99	0.000	0.000	0.000
Fish Creek	295	0.000	0.000	295	0.000	293	0.014	295	0.000	293	0.004	294	0.000	294	0.000	0.000	0.000

Appendix I-A. Estimated allele frequencies for Upper Cook Inlet sockeye salmon populations.

Population	*ALAT*				*CK-A2*		*CK-B*		*FDHG*		*GAPDH-2*			*G3PDH-1,2*			
	N	91	108	95	N	125	N	102	N	128	N	50	208	N	-150	-175	0
Kenai River																	
Russian River above/early	100	0.140	0.000	0.005	100	0.000	100	0.000	79	0.000	100	0.000	0.000	100	0.000	0.000	0.000
Russian River above/late	200	0.155	0.000	0.010	197	0.000	200	0.003	176	0.000	198	0.003	0.000	196	0.000	0.000	0.000
Russian River below	100	0.260	0.000	0.090	99	0.000	100	0.000	96	0.000	99	0.000	0.000	99	0.000	0.000	0.000
Ptarmigan Creek	197	0.338	0.000	0.041	197	0.000	198	0.000	196	0.003	197	0.000	0.000	198	0.000	0.000	0.000
Tern Lake	148	0.291	0.000	0.024	148	0.000	150	0.000	148	0.000	150	0.000	0.000	150	0.000	0.002	0.000
Quartz Creek	199	0.475	0.000	0.040	199	0.015	200	0.000	198	0.000	195	0.000	0.000	198	0.000	0.000	0.000
Btwn Ken/Ski Lks site 1	98	0.270	0.000	0.036	100	0.000	100	0.000	100	0.000	100	0.010	0.000	100	0.000	0.000	0.000
Btwn Ken/Ski Lks site 2	98	0.311	0.000	0.036	100	0.000	100	0.000	100	0.000	100	0.000	0.000	100	0.003	0.000	0.000
Btwn Ken/Ski Lks site 3	148	0.304	0.000	0.057	148	0.000	150	0.000	148	0.000	150	0.007	0.000	150	0.002	0.000	0.000
Btwn Ken/Ski Lks site 4	50	0.290	0.010	0.070	50	0.000	48	0.000	50	0.000	50	0.010	0.000	50	0.000	0.000	0.000
Btwn Ken/Ski Lks site 5	100	0.275	0.000	0.035	100	0.000	100	0.000	100	0.000	100	0.000	0.000	100	0.003	0.000	0.000
Btwn Ken/Ski Lks site 6	296	0.284	0.005	0.064	297	0.002	295	0.000	296	0.002	294	0.010	0.000	296	0.003	0.000	0.002
Hidden Creek	200	0.073	0.000	0.118	200	0.000	200	0.000	199	0.000	200	0.000	0.018	200	0.000	0.000	0.000
Skilak Lake outlet	786	0.246	0.001	0.059	795	0.000	800	0.000	797	0.000	796	0.009	0.000	796	0.001	0.000	0.000
Moose Creek, Kenai	197	0.614	0.000	0.003	198	0.000	200	0.000	199	0.000	198	0.000	0.000	200	0.000	0.000	0.000
Yentna River																	
Chelatna Lake	198	0.535	0.000	0.003	200	0.000	200	0.000	200	0.000	196	0.000	0.000	200	0.000	0.000	0.000
West Fork Yentna River	199	0.450	0.000	0.008	197	0.000	199	0.000	200	0.000	200	0.000	0.000	200	0.000	0.000	0.000
Hewitt/Whiskey Lakes	99	0.273	0.000	0.000	99	0.000	99	0.000	100	0.000	100	0.000	0.000	99	0.000	0.000	0.000
Shell Lake	200	0.540	0.000	0.025	200	0.000	199	0.000	193	0.000	199	0.000	0.000	199	0.000	0.000	0.000
Trinity/Movie Lakes	199	0.226	0.000	0.003	180	0.000	200	0.000	200	0.000	198	0.000	0.000	120	0.000	0.000	0.000
Judd Lake	198	0.346	0.000	0.068	180	0.000	199	0.000	200	0.000	200	0.000	0.000	120	0.000	0.000	0.000
Susitna River Mainstem																	
Byers Lake	100	0.225	0.000	0.095	98	0.000	99	0.000	100	0.000	100	0.000	0.000	100	0.000	0.000	0.000
Stephan Lake	125	0.316	0.000	0.132	124	0.000	125	0.000	125	0.000	124	0.048	0.000	125	0.000	0.000	0.000
Larson Lake	199	0.256	0.003	0.015	179	0.000	200	0.000	198	0.000	200	0.000	0.000	96	0.000	0.000	0.000
Birch Creek	63	0.429	0.000	0.000	67	0.000	65	0.000	66	0.000	67	0.000	0.000	67	0.000	0.000	0.000
Red Shirt Lake	34	0.544	0.000	0.015	34	0.000	34	0.000	34	0.000	33	0.000	0.000	34	0.000	0.000	0.000
Susitna River slough 11	50	0.490	0.000	0.070	50	0.000	50	0.000	50	0.000	50	0.000	0.000	50	0.000	0.000	0.000
Western Cook Inlet																	
Coal Creek	200	0.603	0.000	0.023	200	0.000	200	0.000	196	0.000	200	0.000	0.000	200	0.000	0.000	0.000
Chilligan River	150	0.430	0.000	0.000	149	0.000	150	0.000	150	0.000	150	0.000	0.000	150	0.000	0.000	0.000
MacArthur River	98	0.225	0.000	0.168	100	0.000	100	0.000	100	0.000	100	0.000	0.000	100	0.000	0.000	0.000
Wolverine Creek	97	0.887	0.000	0.000	99	0.000	100	0.000	97	0.000	95	0.000	0.000	99	0.000	0.000	0.000
Crescent Lake site 1	100	0.390	0.000	0.070	100	0.000	98	0.000	100	0.000	99	0.000	0.000	100	0.000	0.000	0.000
Crescent Lake site 2	100	0.445	0.000	0.025	100	0.000	100	0.000	100	0.000	96	0.000	0.000	100	0.000	0.000	0.003
Crescent Lake site 3	50	0.460	0.000	0.020	50	0.000	50	0.000	50	0.000	50	0.000	0.000	50	0.000	0.000	0.000
Packers Lake	182	0.659	0.000	0.003	183	0.000	182	0.000	176	0.000	183	0.000	0.000	183	0.000	0.000	0.000
Kasilof River																	
Bear Creek	199	0.342	0.000	0.111	199	0.000	199	0.000	200	0.000	198	0.000	0.000	199	0.000	0.000	0.000
Moose Creek, Tustumena	200	0.323	0.000	0.100	196	0.000	199	0.000	192	0.000	200	0.000	0.000	200	0.000	0.000	0.000
Glacier Flat Creek	298	0.309	0.000	0.143	294	0.002	300	0.000	300	0.000	295	0.002	0.000	299	0.000	0.000	0.000
Nikolai Creek	199	0.307	0.000	0.136	180	0.000	200	0.008	199	0.000	200	0.000	0.000	200	0.000	0.000	0.000
Tustumena Lake site 1	50	0.340	0.000	0.150	50	0.000	44	0.000	50	0.000	50	0.000	0.000	50	0.000	0.000	0.000
Tustumena Lake site 2	50	0.310	0.000	0.150	50	0.000	50	0.000	50	0.000	50	0.000	0.000	50	0.000	0.000	0.000
Seepage Creek	100	0.350	0.000	0.135	100	0.000	97	0.000	98	0.000	100	0.000	0.000	98	0.000	0.000	0.000
Northeastern Cook Inlet																	
Bishop Creek	100	0.620	0.000	0.050	98	0.000	100	0.000	100	0.000	99	0.000	0.000	99	0.000	0.000	0.000
Daniels Lake	200	0.385	0.000	0.145	200	0.000	200	0.000	200	0.000	199	0.000	0.000	198	0.000	0.000	0.000
Knik Arm																	
Nancy Lake	100	0.540	0.000	0.000	100	0.000	100	0.000	100	0.000	99	0.000	0.000	100	0.000	0.000	0.000
Cottonwood Creek	100	0.430	0.000	0.000	99	0.000	100	0.000	96	0.000	100	0.000	0.000	100	0.000	0.000	0.000
Fish Creek	300	0.340	0.000	0.013	298	0.000	300	0.000	236	0.000	294	0.000	0.000	296	0.000	0.000	0.000

Appendix I-A. Estimated allele frequencies for Upper Cook Inlet sockeye salmon populations.

Population	*G3PDH-4*		*GPI-B1,2*			*GPI-A*			*mIDHP-1*			*sIDHP-1*					*sIDHP-2*	
	N	108	N	132	143	N	94	107	N	33	77	N	72	84	61	94	N	92
Kenai River																		
Russian River above/early	100	0.000	100	0.000	0.000	100	0.000	0.000	100	0.000	0.000	100	0.000	0.000	0.000	0.000	100	0.000
Russian River above/late	200	0.000	200	0.000	0.000	200	0.000	0.000	199	0.000	0.000	200	0.000	0.000	0.000	0.000	200	0.000
Russian River below	99	0.000	100	0.000	0.000	99	0.015	0.000	99	0.000	0.000	98	0.005	0.005	0.005	0.000	99	0.000
Ptarmigan Creek	198	0.000	197	0.000	0.000	197	0.000	0.000	198	0.000	0.000	198	0.005	0.010	0.000	0.000	198	0.000
Tern Lake	148	0.000	148	0.000	0.000	148	0.000	0.000	150	0.000	0.000	150	0.000	0.000	0.000	0.000	150	0.000
Quartz Creek	199	0.000	199	0.006	0.000	198	0.000	0.000	199	0.000	0.000	200	0.003	0.000	0.003	0.000	200	0.003
Btwn Ken/Ski Lks site 1	100	0.000	100	0.000	0.000	100	0.000	0.000	100	0.000	0.000	100	0.000	0.000	0.000	0.000	100	0.000
Btwn Ken/Ski Lks site 2	100	0.000	100	0.000	0.000	100	0.000	0.000	100	0.000	0.000	100	0.000	0.020	0.000	0.000	99	0.000
Btwn Ken/Ski Lks site 3	150	0.000	150	0.000	0.000	150	0.000	0.000	149	0.000	0.000	150	0.000	0.007	0.000	0.000	149	0.000
Btwn Ken/Ski Lks site 4	50	0.000	50	0.000	0.000	50	0.000	0.000	50	0.000	0.000	50	0.000	0.000	0.000	0.000	50	0.000
Btwn Ken/Ski Lks site 5	100	0.000	100	0.000	0.000	100	0.000	0.000	100	0.000	0.000	99	0.000	0.000	0.000	0.000	98	0.000
Btwn Ken/Ski Lks site 6	274	0.000	296	0.000	0.000	296	0.000	0.002	294	0.000	0.000	297	0.002	0.003	0.000	0.000	297	0.000
Hidden Creek	200	0.000	200	0.004	0.000	200	0.000	0.013	200	0.000	0.000	200	0.000	0.000	0.000	0.000	200	0.000
Skilak Lake outlet	779	0.000	798	0.000	0.000	798	0.000	0.000	798	0.001	0.000	796	0.003	0.003	0.002	0.001	796	0.000
Moose Creek, Kenai	199	0.000	198	0.001	0.000	198	0.000	0.000	198	0.000	0.000	200	0.003	0.003	0.000	0.000	200	0.033
Yentna River																		
Chelatna Lake	200	0.000	199	0.000	0.000	200	0.000	0.000	200	0.000	0.000	200	0.000	0.000	0.000	0.000	200	0.000
West Fork Yentna River	200	0.000	196	0.000	0.000	196	0.000	0.000	200	0.000	0.000	200	0.000	0.000	0.000	0.000	200	0.000
Hewitt/Whiskey Lakes	100	0.000	100	0.000	0.000	100	0.000	0.000	100	0.000	0.000	100	0.000	0.000	0.000	0.000	100	0.000
Shell Lake	199	0.000	200	0.000	0.000	200	0.000	0.000	200	0.000	0.000	200	0.000	0.000	0.000	0.000	200	0.000
Trinity/Movie Lakes	198	0.000	200	0.000	0.000	200	0.000	0.000	196	0.000	0.000	200	0.000	0.000	0.000	0.000	200	0.000
Judd Lake	200	0.000	200	0.000	0.000	200	0.000	0.000	200	0.000	0.000	199	0.000	0.000	0.000	0.000	200	0.000
Susitna River Mainstem																		
Byers Lake	100	0.000	100	0.000	0.003	98	0.000	0.000	100	0.000	0.000	99	0.000	0.000	0.000	0.000	99	0.000
Stephan Lake	124	0.000	125	0.000	0.000	125	0.000	0.000	124	0.000	0.000	119	0.000	0.000	0.000	0.126	124	0.000
Larson Lake	200	0.000	199	0.001	0.000	199	0.000	0.000	200	0.000	0.000	200	0.000	0.000	0.000	0.000	200	0.000
Birch Creek	67	0.000	67	0.000	0.000	67	0.000	0.000	67	0.000	0.000	67	0.000	0.000	0.000	0.000	67	0.000
Red Shirt Lake	34	0.000	34	0.000	0.000	34	0.000	0.000	34	0.000	0.000	34	0.000	0.000	0.000	0.000	34	0.000
Susitna River slough 11	50	0.000	50	0.000	0.000	50	0.000	0.000	50	0.000	0.000	50	0.000	0.000	0.000	0.000	50	0.000
Western Cook Inlet																		
Coal Creek	200	0.000	200	0.000	0.000	200	0.000	0.000	199	0.005	0.000	197	0.000	0.000	0.000	0.000	197	0.000
Chilligan River	150	0.000	150	0.000	0.000	150	0.000	0.000	150	0.000	0.000	150	0.000	0.000	0.000	0.000	150	0.000
MacArthur River	100	0.000	100	0.000	0.000	100	0.000	0.000	100	0.000	0.000	100	0.000	0.000	0.000	0.015	100	0.000
Wolverine Creek	100	0.000	99	0.003	0.000	99	0.000	0.000	97	0.005	0.000	100	0.000	0.000	0.000	0.000	100	0.000
Crescent Lake site 1	100	0.000	100	0.000	0.000	100	0.000	0.000	99	0.000	0.000	99	0.000	0.000	0.005	0.000	99	0.000
Crescent Lake site 2	100	0.000	100	0.000	0.000	99	0.000	0.000	100	0.000	0.000	100	0.000	0.000	0.000	0.000	100	0.000
Crescent Lake site 3	50	0.000	50	0.000	0.000	50	0.000	0.000	50	0.000	0.000	50	0.000	0.000	0.000	0.000	50	0.000
Packers Lake	179	0.000	183	0.000	0.000	183	0.000	0.000	182	0.000	0.000	182	0.000	0.000	0.000	0.000	180	0.000
Kasilof River																		
Bear Creek	200	0.008	200	0.029	0.000	200	0.000	0.000	198	0.000	0.000	200	0.000	0.000	0.000	0.000	200	0.000
Moose Creek, Tustumena	200	0.005	200	0.038	0.000	200	0.000	0.000	200	0.000	0.005	200	0.000	0.000	0.000	0.000	200	0.000
Glacier Flat Creek	299	0.018	299	0.014	0.000	299	0.000	0.000	300	0.000	0.015	297	0.000	0.000	0.000	0.000	267	0.000
Nikolai Creek	200	0.010	200	0.031	0.000	200	0.000	0.000	199	0.000	0.003	200	0.003	0.000	0.000	0.000	200	0.000
Tustumena Lake site 1	50	0.010	50	0.000	0.000	50	0.000	0.000	50	0.000	0.000	50	0.010	0.000	0.000	0.000	50	0.000
Tustumena Lake site 2	49	0.000	50	0.015	0.000	50	0.000	0.000	46	0.000	0.000	50	0.000	0.000	0.000	0.000	50	0.000
Seepage Creek	100	0.000	100	0.035	0.000	100	0.000	0.000	100	0.000	0.010	100	0.005	0.000	0.000	0.000	100	0.000
Northeastern Cook Inlet																		
Bishop Creek	99	0.000	100	0.000	0.000	99	0.000	0.000	99	0.000	0.000	100	0.000	0.000	0.000	0.000	100	0.000
Daniels Lake	199	0.000	200	0.000	0.000	200	0.000	0.020	199	0.000	0.000	198	0.000	0.000	0.000	0.000	199	0.000
Knik Arm																		
Nancy Lake	100	0.000	98	0.000	0.000	98	0.000	0.000	100	0.000	0.000	100	0.000	0.000	0.000	0.000	100	0.000
Cottonwood Creek	99	0.000	100	0.000	0.000	100	0.000	0.000	97	0.000	0.000	99	0.000	0.000	0.000	0.000	99	0.000
Fish Creek	295	0.003	300	0.001	0.000	300	0.000	0.000	294	0.000	0.000	295	0.020	0.000	0.000	0.000	299	0.000

Appendix I-A. Estimated allele frequencies for Upper Cook Inlet sockeye salmon populations.

	LDH-A2		*LDH-B2*			*sMDH-A1,2*			*sMDH-B1,2*				*mMEP-1*			*MPI*	
Population	N	150	N	110	85	N	64	147	N	65	120	116	N	80	58	N	105
Kenai River																	
Russian River above/early	100	0.000	99	0.505	0.000	100	0.000	0.000	100	0.000	0.000	0.000	92	0.000	0.000	100	0.000
Russian River above/late	196	0.000	197	0.294	0.000	200	0.000	0.000	200	0.003	0.000	0.000	198	0.000	0.000	200	0.000
Russian River below	99	0.000	99	0.076	0.000	100	0.003	0.008	99	0.000	0.000	0.000	99	0.000	0.000	100	0.005
Ptarmigan Creek	198	0.000	198	0.111	0.000	198	0.000	0.008	198	0.000	0.000	0.000	198	0.000	0.000	198	0.003
Tern Lake	148	0.000	150	0.167	0.000	150	0.000	0.000	150	0.000	0.000	0.000	150	0.000	0.000	150	0.000
Quartz Creek	199	0.000	200	0.113	0.000	200	0.000	0.020	199	0.000	0.000	0.000	198	0.000	0.000	199	0.005
Btwn Ken/Ski Lks site 1	100	0.000	100	0.160	0.000	100	0.000	0.003	100	0.000	0.000	0.000	100	0.000	0.000	100	0.010
Btwn Ken/Ski Lks site 2	100	0.000	100	0.120	0.000	100	0.003	0.005	100	0.000	0.000	0.000	100	0.000	0.000	100	0.015
Btwn Ken/Ski Lks site 3	150	0.000	150	0.100	0.000	150	0.000	0.015	150	0.000	0.000	0.000	150	0.000	0.000	150	0.003
Btwn Ken/Ski Lks site 4	50	0.000	50	0.130	0.000	50	0.000	0.020	50	0.000	0.000	0.000	50	0.000	0.000	50	0.000
Btwn Ken/Ski Lks site 5	100	0.000	100	0.055	0.000	100	0.000	0.005	100	0.000	0.000	0.000	100	0.000	0.000	100	0.000
Btwn Ken/Ski Lks site 6	292	0.002	294	0.116	0.000	299	0.001	0.009	297	0.000	0.000	0.000	283	0.000	0.000	296	0.000
Hidden Creek	200	0.000	200	0.028	0.000	200	0.000	0.000	200	0.000	0.000	0.000	200	0.000	0.000	200	0.000
Skilak Lake outlet	791	0.000	799	0.080	0.000	800	0.000	0.008	793	0.002	0.001	0.000	741	0.000	0.001	787	0.003
Moose Creek, Kenai	199	0.000	200	0.090	0.000	200	0.000	0.000	199	0.000	0.000	0.000	199	0.000	0.000	200	0.000
Yentna River																	
Chelatna Lake	200	0.000	200	0.063	0.000	200	0.000	0.000	200	0.000	0.000	0.000	200	0.000	0.000	200	0.000
West Fork Yentna River	197	0.000	200	0.103	0.000	200	0.000	0.000	200	0.000	0.000	0.000	200	0.000	0.000	200	0.000
Hewitt/Whiskey Lakes	99	0.000	99	0.010	0.000	100	0.000	0.000	100	0.000	0.000	0.000	99	0.000	0.000	100	0.000
Shell Lake	200	0.000	200	0.000	0.000	200	0.000	0.000	200	0.000	0.000	0.000	200	0.000	0.000	200	0.000
Trinity/Movie Lakes	199	0.000	200	0.068	0.000	200	0.000	0.000	197	0.000	0.000	0.000	198	0.000	0.000	200	0.000
Judd Lake	197	0.000	200	0.105	0.003	200	0.000	0.000	200	0.000	0.000	0.000	199	0.000	0.000	200	0.000
Susitna River Mainstem																	
Byers Lake	95	0.000	100	0.040	0.000	100	0.000	0.000	100	0.000	0.000	0.000	94	0.000	0.000	100	0.000
Stephan Lake	124	0.000	125	0.084	0.000	125	0.000	0.000	121	0.000	0.000	0.000	123	0.000	0.000	124	0.000
Larson Lake	196	0.000	199	0.050	0.000	200	0.000	0.000	200	0.000	0.000	0.000	200	0.000	0.000	200	0.000
Birch Creek	63	0.000	67	0.008	0.000	67	0.000	0.000	67	0.000	0.000	0.000	67	0.000	0.000	67	0.000
Red Shirt Lake	33	0.000	34	0.000	0.000	34	0.000	0.000	34	0.000	0.000	0.000	33	0.000	0.000	34	0.000
Susitna River slough 11	50	0.000	50	0.070	0.000	50	0.000	0.000	50	0.000	0.000	0.000	50	0.000	0.000	50	0.000
Western Cook Inlet																	
Coal Creek	200	0.000	200	0.023	0.000	200	0.000	0.000	200	0.048	0.000	0.000	200	0.000	0.000	200	0.000
Chilligan River	150	0.013	150	0.030	0.000	150	0.000	0.000	150	0.000	0.000	0.000	148	0.000	0.000	150	0.000
MacArthur River	97	0.000	100	0.140	0.000	100	0.000	0.000	100	0.000	0.000	0.000	97	0.000	0.000	100	0.000
Wolverine Creek	99	0.000	100	0.020	0.000	100	0.000	0.000	100	0.000	0.000	0.000	99	0.000	0.000	100	0.000
Crescent Lake site 1	100	0.000	100	0.110	0.000	100	0.000	0.000	100	0.000	0.000	0.000	100	0.000	0.000	100	0.000
Crescent Lake site 2	100	0.000	100	0.045	0.000	100	0.000	0.000	100	0.000	0.000	0.000	100	0.000	0.000	100	0.000
Crescent Lake site 3	49	0.000	50	0.120	0.000	50	0.000	0.000	50	0.000	0.000	0.000	50	0.020	0.000	50	0.000
Packers Lake	181	0.000	182	0.000	0.000	183	0.000	0.000	183	0.003	0.000	0.018	179	0.048	0.000	182	0.000
Kasilof River																	
Bear Creek	200	0.000	200	0.130	0.000	200	0.000	0.001	199	0.000	0.000	0.000	200	0.003	0.000	200	0.000
Moose Creek, Tustumena	197	0.000	200	0.130	0.000	200	0.000	0.003	200	0.000	0.000	0.000	199	0.000	0.000	199	0.000
Glacier Flat Creek	299	0.000	300	0.127	0.000	300	0.000	0.002	300	0.002	0.000	0.000	300	0.000	0.000	298	0.000
Nikolai Creek	200	0.003	200	0.118	0.000	200	0.000	0.006	200	0.000	0.000	0.000	198	0.000	0.000	200	0.000
Tustumena Lake site 1	50	0.000	50	0.150	0.000	50	0.000	0.000	50	0.000	0.000	0.000	50	0.000	0.000	50	0.000
Tustumena Lake site 2	50	0.000	50	0.040	0.000	50	0.000	0.000	50	0.000	0.000	0.000	50	0.000	0.000	50	0.000
Seepage Creek	100	0.000	100	0.125	0.000	100	0.000	0.003	100	0.000	0.000	0.000	100	0.000	0.000	99	0.000
Northeastern Cook Inlet																	
Bishop Creek	100	0.000	100	0.000	0.000	100	0.000	0.000	100	0.000	0.000	0.000	100	0.000	0.000	100	0.000
Daniels Lake	197	0.000	200	0.000	0.000	200	0.000	0.000	200	0.000	0.000	0.000	200	0.000	0.000	200	0.000
Knik Arm																	
Nancy Lake	100	0.000	100	0.050	0.000	100	0.000	0.000	100	0.000	0.000	0.000	99	0.000	0.000	98	0.000
Cottonwood Creek	99	0.000	100	0.350	0.000	100	0.000	0.000	98	0.000	0.000	0.000	100	0.000	0.000	100	0.000
Fish Creek	297	0.000	300	0.117	0.000	300	0.000	0.006	299	0.000	0.000	0.000	292	0.000	0.000	300	0.000

Appendix I-A. Estimated allele frequencies for Upper Cook Inlet sockeye salmon populations.

	PEPA			*PEPB-1*			*PEPC*		*PEPD-1*			*PEPLT*			*PGDH*		*PGM-1*		
Population	N	106	92	N	130	163	N	105	N	113	94	N	88	114	N	90	N	null	-180
Kenai River																			
Russian River above/early	100	0.000	0.000	100	0.000	0.000	100	0.000	100	0.000	0.000	100	0.000	0.000	100	0.000	100	1.000	0.000
Russian River above/late	200	0.000	0.000	200	0.000	0.000	200	0.000	200	0.000	0.000	200	0.000	0.000	200	0.000	200	0.995	0.000
Russian River below	100	0.000	0.000	100	0.005	0.000	100	0.000	99	0.025	0.000	100	0.005	0.010	99	0.000	99	0.611	0.010
Ptarmigan Creek	198	0.005	0.000	198	0.015	0.000	198	0.003	198	0.015	0.000	198	0.003	0.013	198	0.000	198	0.677	0.000
Tern Lake	150	0.003	0.000	149	0.044	0.000	149	0.003	147	0.000	0.000	149	0.024	0.000	150	0.000	150	0.778	0.000
Quartz Creek	200	0.005	0.013	195	0.015	0.000	200	0.003	199	0.000	0.000	196	0.015	0.013	200	0.000	199	0.724	0.000
Btwn Ken/Ski Lks site 1	100	0.000	0.010	100	0.000	0.000	100	0.010	100	0.015	0.000	100	0.015	0.025	100	0.000	100	0.748	0.000
Btwn Ken/Ski Lks site 2	100	0.005	0.000	99	0.000	0.000	100	0.005	100	0.020	0.000	100	0.020	0.000	100	0.000	100	0.656	0.000
Btwn Ken/Ski Lks site 3	150	0.010	0.023	150	0.000	0.000	150	0.010	150	0.010	0.000	150	0.003	0.010	150	0.000	150	0.663	0.007
Btwn Ken/Ski Lks site 4	50	0.000	0.020	49	0.000	0.000	50	0.000	50	0.030	0.000	50	0.010	0.000	50	0.000	50	0.693	0.010
Btwn Ken/Ski Lks site 5	100	0.000	0.005	100	0.000	0.000	100	0.000	100	0.020	0.000	100	0.005	0.015	100	0.000	100	0.633	0.000
Btwn Ken/Ski Lks site 6	297	0.002	0.012	295	0.000	0.000	295	0.002	296	0.002	0.000	297	0.010	0.022	297	0.000	297	0.693	0.000
Hidden Creek	200	0.000	0.000	199	0.000	0.000	200	0.000	199	0.000	0.000	200	0.098	0.000	200	0.000	198	0.943	0.000
Skilak Lake outlet	800	0.002	0.012	796	0.000	0.000	794	0.007	793	0.006	0.000	795	0.003	0.004	800	0.000	800	0.672	0.000
Moose Creek, Kenai	200	0.000	0.000	194	0.000	0.003	198	0.008	199	0.000	0.000	194	0.003	0.000	200	0.000	198	0.887	0.000
Yentna River																			
Chelatna Lake	197	0.000	0.000	200	0.000	0.000	199	0.000	200	0.000	0.000	195	0.005	0.000	200	0.000	200	0.962	0.000
West Fork Yentna River	200	0.000	0.000	197	0.000	0.000	199	0.005	200	0.005	0.000	199	0.028	0.000	200	0.000	200	0.929	0.000
Hewitt/Whiskey Lakes	100	0.000	0.000	100	0.000	0.000	99	0.126	100	0.000	0.000	99	0.000	0.000	100	0.000	99	0.932	0.000
Shell Lake	200	0.000	0.000	200	0.000	0.000	186	0.315	200	0.000	0.000	199	0.000	0.000	200	0.000	200	0.902	0.000
Trinity/Movie Lakes	199	0.000	0.000	200	0.153	0.000	197	0.018	200	0.000	0.000	198	0.000	0.000	200	0.000	200	0.951	0.000
Judd Lake	200	0.000	0.000	200	0.000	0.000	199	0.000	200	0.000	0.000	200	0.000	0.000	200	0.000	200	0.640	0.000
Susitna River Mainstem																			
Byers Lake	100	0.000	0.000	99	0.000	0.000	98	0.005	99	0.000	0.000	100	0.020	0.000	99	0.000	100	0.812	0.000
Stephan Lake	125	0.000	0.000	123	0.000	0.000	124	0.169	125	0.000	0.000	120	0.000	0.000	125	0.000	125	1.000	0.000
Larson Lake	200	0.000	0.000	196	0.000	0.000	200	0.003	200	0.000	0.000	200	0.000	0.000	200	0.000	200	0.606	0.000
Birch Creek	67	0.000	0.000	67	0.000	0.000	67	0.030	67	0.000	0.000	67	0.008	0.000	67	0.000	66	0.718	0.000
Red Shirt Lake	34	0.000	0.000	34	0.000	0.000	34	0.103	33	0.000	0.000	34	0.000	0.000	34	0.000	34	0.970	0.000
Susitna River slough 11	50	0.000	0.000	49	0.000	0.000	50	0.020	50	0.000	0.010	50	0.030	0.000	47	0.000	50	0.849	0.000
Western Cook Inlet																			
Coal Creek	200	0.000	0.000	199	0.000	0.000	200	0.000	199	0.000	0.003	200	0.003	0.000	200	0.000	200	0.537	0.000
Chilligan River	150	0.000	0.000	149	0.000	0.000	149	0.010	150	0.000	0.000	145	0.000	0.000	150	0.000	150	0.799	0.000
MacArthur River	100	0.000	0.000	100	0.000	0.000	98	0.046	100	0.000	0.000	100	0.000	0.000	100	0.000	100	0.787	0.000
Wolverine Creek	100	0.005	0.000	99	0.000	0.000	99	0.000	100	0.000	0.000	98	0.000	0.000	100	0.000	100	0.938	0.000
Crescent Lake site 1	100	0.000	0.000	100	0.000	0.000	99	0.056	100	0.000	0.000	92	0.000	0.000	100	0.000	99	0.870	0.000
Crescent Lake site 2	100	0.000	0.000	97	0.000	0.000	100	0.080	99	0.000	0.000	97	0.000	0.000	100	0.000	100	0.849	0.000
Crescent Lake site 3	50	0.000	0.000	50	0.000	0.000	50	0.060	50	0.000	0.000	50	0.000	0.000	50	0.000	50	0.837	0.000
Packers Lake	182	0.000	0.000	182	0.000	0.000	181	0.000	183	0.000	0.000	182	0.000	0.000	182	0.006	183	0.997	0.000
Kasilof River																			
Bear Creek	200	0.000	0.000	199	0.000	0.000	200	0.005	200	0.000	0.000	200	0.000	0.000	200	0.000	200	0.880	0.000
Moose Creek, Tustumena	197	0.000	0.000	194	0.000	0.000	198	0.000	200	0.000	0.000	198	0.000	0.000	198	0.000	200	0.883	0.000
Glacier Flat Creek	298	0.000	0.000	299	0.000	0.000	300	0.030	300	0.002	0.000	299	0.000	0.000	300	0.000	300	0.883	0.000
Nikolai Creek	200	0.000	0.003	200	0.000	0.000	200	0.033	200	0.005	0.000	200	0.000	0.000	200	0.000	200	0.911	0.000
Tustumena Lake site 1	50	0.000	0.000	50	0.000	0.000	50	0.000	50	0.000	0.000	50	0.000	0.000	50	0.000	50	0.849	0.000
Tustumena Lake site 2	50	0.010	0.000	50	0.000	0.000	49	0.010	50	0.000	0.000	50	0.000	0.000	50	0.000	50	0.883	0.000
Seepage Creek	100	0.000	0.000	100	0.000	0.000	91	0.011	99	0.000	0.000	100	0.000	0.000	100	0.000	100	0.894	0.000
Northeastern Cook Inlet																			
Bishop Creek	100	0.000	0.000	100	0.000	0.000	100	0.000	99	0.000	0.000	99	0.232	0.000	100	0.000	100	0.800	0.000
Daniels Lake	200	0.000	0.000	196	0.000	0.000	199	0.000	199	0.000	0.000	198	0.290	0.000	200	0.000	200	0.787	0.000
Knik Arm																			
Nancy Lake	100	0.000	0.000	100	0.000	0.000	99	0.030	100	0.000	0.000	100	0.015	0.000	100	0.000	100	0.878	0.000
Cottonwood Creek	100	0.000	0.000	99	0.000	0.000	97	0.113	99	0.000	0.000	100	0.000	0.000	100	0.000	100	0.943	0.000
Fish Creek	300	0.000	0.000	297	0.000	0.000	299	0.099	298	0.000	0.002	300	0.163	0.000	300	0.000	293	0.988	0.000

Appendix I-A. Estimated allele frequencies for Upper Cook Inlet sockeye salmon populations.

	PGM-2			*sSOD-1*		*TPI-1,2*			*TPI-3*		*TPI-4*			Heterozygosity	
Population	N	136	57	N	48	N	-173	-82	N	98	N	106	97	Observed	Expected
Kenai River															
Russian River above/early	100	0.105	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.000	0.000	0.0505	0.0528
Russian River above/late	200	0.125	0.000	200	0.000	200	0.000	0.000	200	0.000	200	0.000	0.000	0.0460	0.0482
Russian River below	100	0.200	0.000	93	0.000	100	0.000	0.000	100	0.000	100	0.000	0.000	0.0429	0.0419
Ptarmigan Creek	198	0.136	0.000	198	0.000	198	0.000	0.000	197	0.000	198	0.000	0.000	0.0407	0.0393
Tern Lake	150	0.337	0.000	150	0.000	150	0.000	0.000	150	0.000	149	0.000	0.007	0.0408	0.0410
Quartz Creek	199	0.186	0.000	200	0.000	200	0.000	0.000	200	0.003	200	0.000	0.000	0.0423	0.0425
Btwn Ken/Ski Lks site 1	100	0.220	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.000	0.000	0.0481	0.0469
Btwn Ken/Ski Lks site 2	100	0.225	0.000	100	0.000	99	0.000	0.000	99	0.005	99	0.000	0.000	0.0434	0.0452
Btwn Ken/Ski Lks site 3	150	0.210	0.000	150	0.000	150	0.000	0.000	150	0.000	150	0.000	0.000	0.0409	0.0423
Btwn Ken/Ski Lks site 4	50	0.230	0.000	50	0.000	50	0.000	0.000	50	0.000	50	0.000	0.000	0.0411	0.0426
Btwn Ken/Ski Lks site 5	100	0.205	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.000	0.000	0.0327	0.0343
Btwn Ken/Ski Lks site 6	297	0.199	0.000	297	0.000	296	0.000	0.000	296	0.000	296	0.000	0.000	0.0441	0.0421
Hidden Creek	200	0.008	0.000	199	0.000	200	0.000	0.000	200	0.000	200	0.000	0.000	0.0345	0.0346
Skilak Lake outlet	800	0.246	0.000	800	0.000	800	0.000	0.000	793	0.001	798	0.000	0.000	0.0401	0.0398
Moose Creek, Kenai	200	0.260	0.000	199	0.000	200	0.000	0.000	200	0.000	200	0.000	0.005	0.0412	0.0406
Yentna River															
Chelatna Lake	200	0.130	0.003	200	0.000	200	0.000	0.000	197	0.000	193	0.000	0.000	0.0324	0.0347
West Fork Yentna River	197	0.195	0.000	200	0.000	199	0.000	0.000	199	0.000	198	0.000	0.000	0.0382	0.0385
Hewitt/Whiskey Lakes	100	0.375	0.000	100	0.000	99	0.000	0.000	99	0.000	99	0.000	0.000	0.0372	0.0374
Shell Lake	200	0.750	0.000	200	0.000	199	0.000	0.000	199	0.000	199	0.000	0.000	0.0417	0.0436
Trinity/Movie Lakes	200	0.718	0.000	200	0.000	200	0.000	0.000	200	0.000	198	0.000	0.000	0.0382	0.0375
Judd Lake	200	0.150	0.000	200	0.000	157	0.000	0.000	197	0.000	197	0.000	0.000	0.0435	0.0424
Susitna River Mainstem															
Byers Lake	100	0.185	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.000	0.000	0.0408	0.0407
Stephan Lake	125	0.264	0.000	124	0.000	124	0.000	0.000	125	0.000	125	0.000	0.000	0.0555	0.0568
Larson Lake	200	0.320	0.000	198	0.000	199	0.000	0.000	199	0.000	199	0.000	0.000	0.0435	0.0382
Birch Creek	67	0.187	0.000	67	0.000	67	0.000	0.000	67	0.000	67	0.000	0.000	0.0260	0.0286
Red Shirt Lake	34	0.412	0.000	34	0.000	34	0.000	0.000	34	0.000	34	0.000	0.000	0.0382	0.0376
Susitna River slough 11	50	0.200	0.000	50	0.000	50	0.000	0.000	50	0.000	50	0.000	0.000	0.0451	0.0432
Western Cook Inlet															
Coal Creek	200	0.143	0.000	198	0.000	200	0.000	0.009	200	0.000	193	0.003	0.000	0.0427	0.0410
Chilligan River	150	0.073	0.000	148	0.000	150	0.000	0.000	150	0.000	150	0.000	0.000	0.0211	0.0219
MacArthur River	100	0.220	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.000	0.000	0.0452	0.0435
Wolverine Creek	100	0.030	0.000	100	0.005	100	0.000	0.000	99	0.000	99	0.000	0.000	0.0294	0.0273
Crescent Lake site 1	100	0.275	0.000	100	0.000	100	0.000	0.000	100	0.050	100	0.000	0.000	0.0401	0.0408
Crescent Lake site 2	100	0.380	0.000	100	0.000	100	0.000	0.000	100	0.005	100	0.000	0.000	0.0391	0.0384
Crescent Lake site 3	50	0.390	0.000	50	0.000	50	0.000	0.000	50	0.020	50	0.000	0.000	0.0391	0.0413
Packers Lake	182	0.245	0.000	180	0.000	182	0.000	0.000	182	0.000	182	0.000	0.000	0.0304	0.0299
Kasilof River															
Bear Creek	200	0.333	0.000	199	0.000	200	0.001	0.000	200	0.000	200	0.000	0.000	0.0452	0.0463
Moose Creek, Tustumena	200	0.305	0.000	200	0.000	200	0.000	0.000	200	0.000	200	0.000	0.000	0.0433	0.0451
Glacier Flat Creek	300	0.322	0.000	250	0.000	295	0.000	0.000	300	0.000	300	0.000	0.000	0.0466	0.0478
Nikolai Creek	200	0.313	0.000	200	0.000	200	0.000	0.000	200	0.000	200	0.000	0.000	0.0530	0.0518
Tustumena Lake site 1	50	0.330	0.000	50	0.000	49	0.000	0.000	49	0.000	49	0.000	0.000	0.0422	0.0452
Tustumena Lake site 2	50	0.380	0.000	50	0.000	50	0.000	0.000	50	0.010	50	0.000	0.000	0.0378	0.0403
Seepage Creek	100	0.340	0.000	100	0.000	98	0.000	0.000	97	0.005	98	0.000	0.000	0.0460	0.0485
Northeastern Cook Inlet															
Bishop Creek	100	0.680	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.000	0.000	0.0395	0.0425
Daniels Lake	200	0.733	0.000	200	0.000	199	0.000	0.000	199	0.000	199	0.000	0.000	0.0427	0.0421
Knik Arm															
Nancy Lake	100	0.230	0.000	100	0.000	100	0.000	0.000	100	0.000	100	0.000	0.000	0.0355	0.0333
Cottonwood Creek	100	0.250	0.000	100	0.000	98	0.000	0.000	100	0.000	100	0.000	0.000	0.0439	0.0413
Fish Creek	300	0.417	0.000	294	0.000	298	0.000	0.000	297	0.000	298	0.000	0.000	0.0464	0.0464

Appendix II.

Allendorf, F.W., K.L. Knudsen, J.E. Seeb, and L.W. Seeb. Concordance of genetic divergence among sockeye salmon populations for allozyme, nuclear DNA, and mtDNA markers. Submitted to Molecular Ecology.

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Concordance of genetic divergence among Cook Inlet sockeye salmon populations for allozyme, nuclear DNA, and mtDNA markers

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Running Title: Sockeye salmon genetic population structure

Abstract

We examined genetic variation at 21 allozyme loci, 11 nuclear DNA loci, and mtDNA in four spawning populations of sockeye salmon *Oncorhynchus nerka* from Cook Inlet in Alaska to test for differences in the patterns of divergence among different types of markers. We were specifically interested in testing the suggestion of others that natural selection at allozyme loci compromises the effectiveness of these markers for describing the amount and patterns of gene flow among populations. We found concordance among markers in the amount of genetic variation within population and the amount of genetic differentiation among populations, with the striking exception of one allozyme locus (*sAH*), which exhibited more than three times the amount of among-population differentiation. We conclude that it is important to examine many loci when estimating genetic differentiation to infer historical amounts of gene flow and patterns of genetic exchange among populations. It is less important whether those loci are allozymes or nuclear DNA markers.

Introduction

Knowledge of the genetic structure of subdivided populations is fundamental for understanding the genetics of natural populations. The patterns of genetic structure are determined by the effects of gene flow, natural selection, genetic drift, and mutation. A population is said to be subdivided when it consists of multiple subpopulations among which gene flow is somewhat restricted. Under complete isolation, the rate of genetic differentiation among subpopulations is governed by the combined effects of mutation and effective population size. Gene flow among subpopulations retards the process of differentiation until a steady-state is reached between the opposing effects of gene flow and genetic drift. Gene flow and genetic drift will effect all loci uniformly if the allelic variation is selectively neutral and the mutation rate is much lower than the migration rate. Natural selection will affect loci differently depending upon the intensity or pattern of selective differentials.

Protein electrophoresis has been the primary empirical method for describing the genetic population structure of natural populations over the last 25 years (Lewontin 1991). The patterns of gene flow among subpopulations have been inferred by assuming that the observed patterns of genetic divergence are determined by the interaction of genetic drift and gene flow. This approach assumes that mutations rates are too low to affect the observed patterns and effective selective neutrality of the observed allozymes.

Allozymes have been used to estimate gene flow in studies directed both toward management and conservation of fish populations. An extensive series of studies of marine fishes using allozymes have suggested that gene flow is sufficient to maintain homogeneity of allele frequencies across large geographic distances (Grant & Utter 1980; Winans 1980; Grant & Utter 1984; Grant *et al.* 1984; Mork *et al.* 1985; Grant *et al.* 1987; Seeb & Gunderson 1988). However, apparent barriers to gene flow have also been observed. Several of the studies from the North Pacific concluded that gene flow was restricted between the North Pacific and the Bering Sea across the Alaska Peninsula.

Allozymes have been used extensively for studies of gene flow among salmonid populations (Allendorf & Waples 1996). In sockeye salmon *Oncorhynchus nerka* for example, Wood (1995) estimated gene flow between inlet and outlet spawning subpopulations in lakes throughout the Pacific Rim; his estimates suggested that very little genetic exchange occurred among the subpopulations. Allozyme data have played a major role in the determination of species status and reproductive isolation under the Endangered Species Act (Waples 1995). These studies all assume that the observed patterns revealed by allozymes largely reflect historical patterns of gene flow and drift.

Several recent papers have provided an important challenge to the utility of allozyme markers for describing historical patterns and amounts of gene flow between populations. Karl & Avise (1992) reported similar patterns of genetic differences for mitochondrial DNA (mtDNA) and four single copy nuclear DNA (nDNA) loci examined with restriction fragment length polymorphism (RFLP) analysis in the American oyster (*Crassostrea virginica*) along the east

coast of North America. In contrast, allozyme studies had not detected these genetic differences among these populations. Karl & Avise (1992) concluded that the pattern observed for the mtDNA and nDNA markers reflected the historical patterns of isolation and gene flow among these populations, while this pattern is obscured in the allozymes because of "balancing selection" at the allozyme loci.

Pogson *et al.* (1995) found very similar results in a marine fish, the Atlantic cod (*Gadus morhua*). Very little genetic divergence was detected at 10 allozyme loci. In contrast, highly significant divergence was found at 17 loci examined by restriction fragment length polymorphisms (RFLP) detected with anonymous DNA clones. These authors concluded that this difference between these type of markers is the prevalence of genetic drift acting on the DNA polymorphisms and natural selection acting at the protein level. They also generalized their results and suggested that the low level of genetic divergence observed among population of other marine fishes may have a similar basis.

In this paper, we examine genetic variation at 31 polymorphic nuclear loci and at mtDNA in four subpopulations of sockeye salmon from Cook Inlet in Alaska to test for differences in the patterns of divergence among different types of markers. Understanding the patterns of genetic diversity for Cook Inlet sockeye salmon populations has been the focus of a number of studies since the mid 1970's because of the economic importance of these fish (Grant *et al.* 1980; Wilmot & Burger 1985; Burger *et al.* 1997; Seeb *et al.* in press). Both allozyme and mtDNA data reveal a substantial amount of genetic diversity among populations, and the data support the hypothesis that the nursery lake is the primary unit of reproduction (Seeb *et al.* in press), a hypothesis used to explain diversity patterns in other portions of the species' range (Wood *et al.* 1994). Sockeye salmon generally spawn in rivers or smaller creeks associated with nursery lakes, and typically exhibit an obligate one- to two-year lacustrine freshwater rearing phase prior to undergoing smoltification and migration to the sea. This life history may contribute to the tendency of sockeye salmon to home with great fidelity to their natal streams (Quinn 1985).

The primary purpose of the present paper is to test for concordance in patterns of genetic differentiation at three types of genetic markers (allozymes, nDNA, and mtDNA) in sockeye salmon from Cook Inlet. Notable differences in the patterns of divergence among these markers would suggest that natural selection is affecting these markers in different ways. McDonald (1994) has discussed this approach for detecting natural selection in protein and DNA polymorphisms. He concluded that it is important to examine as many loci as possible even in as few as two populations; we examined 32 polymorphic nuclear loci and mtDNA in four populations. We are specifically interested in testing the suggestion by Karl & Avise (1992) and Pogson *et al.* (1995) that natural selection at allozyme loci compromises the effectiveness of these markers for describing the amount and patterns of gene flow among populations.

Materials and methods

Sample collection

Adult sockeye salmon were collected in 1992 from four spawning areas surrounding Cook Inlet, Alaska (Fig. II-1). These samples are a subset of those included in Seeb *et al.* (in press) that were chosen to represent the major subpopulations contributing to the Cook Inlet fishery (Grant *et al.* 1980). Two populations, Skilak Lake and Russian River, originated from the Kenai River Drainage. The Russian River population was sampled above the Russian River falls and was from the later returning segment of the population (late run, sampled 6 August 1992). The third sample, Moose Creek, originated from a tributary to Tustumena Lake which in turn drains into Cook Inlet through the Kasilof River. The fourth sample was collected from an unnamed slough along the West Fork of the Yentna River.

Tissue samples (muscle, liver, eye, and heart) from each individual were collected on liquid nitrogen or dry ice and kept frozen until analysis (-80 °C). Fifty individuals from each site were analyzed.

Total genomic DNA was extracted from liver or heart tissue using a high salt precipitation method (Gentra System, Minneapolis, MN) following the manufacturers instructions. The resulting DNA was quantitated and diluted for use in PCR reactions.

Allozymes

Allozyme analyses followed the general techniques of May *et al.* (1979) and Aebersold *et al.* (1987); the tissue and gel protocols were those of Seeb *et al.* (in press). Allele and locus nomenclature followed the American Fisheries Society standard (Shaklee *et al.* 1990). A total of 67 allozyme loci were surveyed. Enzymes assayed, enzyme number, and locus abbreviations are as follow: aspartate aminotransferase (2.6.1.1) (*sAAT-1,2; sAAT-3; mAAT-1; mAAT-2*); adenosine deaminase (3.5.4.4) (*ADA-1)*; aconitate hydratase (4.2.1.3) (*mAH-1,2; mAH-3; mAH-4; sAH)*; alanine aminotransferase (2.6.1.2) (*ALAT*); creatine kinase (2.7.3.2) (*CK-A1; CK-A2; CK-B; CK-C1; CK-C2*); esterase-D (3.1.1.-) (*ESTD)*; fructose-biphosphate aldolase (4.1.2.13) (*FBALD-4*); formaldehyde dehydrogenase (1.2.1.1) (*FDH*); fumarate hydratase (4.2.1.2) (*FH*); β-N-acetylgalactosaminidase (3.2.1.53) *(βGALA*); glyceraldehyde-3-phosphate dehydrogenase (1.2.1.12) (*GAPDH-2; GAPDH-3; GAPDH-4; GAPDH-5)*; glycerol-3-phosphate dehydrogenase (1.1.1.8) (*G3PDH-1,2; G3PDH-3; G3PDH-4);* glucose-6-phosphate isomerase (5.3.1.9) (*GPI-B1,2; GPI-A*); glutathione reductase (1.6.4.2) (*GR*); isocitrate dehydrogenase (NADP+) (1.1.1.42) (*mIDHP-1; mIDHP-2; sIDHP-1; sIDHP-2*); lactate dehydrogenase (1.1.1.27) (*LDH-A1; LDH-A2; LDH-B1; LDH-B2; LDH-C*); α-mannosidase (1.1.1.37) (*αMAN*); malate dehydrogenase (*sMDH-A1,2; sMDH-B1,2; mMDH-1; mMDH-2; mMDH-3*); malic enzyme (NADP+) (1.1.1.40) (*sMEP-1; mMEP-1*); mannose-6-phosphate isomerase (5.3.1.8) (*MPI*); dipeptidase (3.4.-.-) (*PEPA*); tripeptide aminopeptidase (3.4.-.-) (*PEPB-1*); peptidase-C (3.4.-.-) (*PEPC*); proline dipeptidase (3.4.13.9) (*PEPD-1*); peptidase-LT (3.4.-.-) (*PEPLT*); phosphogluconate dehydrogenase (1.1.1.44) (*PGDH*);

phosphoglucomutase (5.4.2.2) (*PGM-1; PGM-2*); superoxide dismutase (1.15.1.1) (*sSOD-1*); triose-phosphate isomerase (5.3.1.1) (*TPI-1,2; TPI-3; TPI-4*).

Of the 67 loci, 21 polymorphic loci were detected. The common allele in all cases was **100*. Thirteen were non-duplicated loci (loci and observed alleles: *mAAT-1*-100, *-83; mAAT-2*-100, *-73; sAH*100, *117; ALAT*100, *91, *95; G3PDH-4*100, *108; GAPDH-2*100, *50; LDH-B2*100, *110; MPI*100, *105; PEPA*100, *92; PEPD-1*100, *113; PEPLT*100, *88; PGM-1*100, *null; PGM-2*100, *136*). Eight were duplicated (loci and observed alleles: *mAH-1,2*100, *75; GPI-B1,2*100, *132; sMDH-A1,2*100, *147; sMDH-B1,2*100, *65, *120)*.

mtDNA

MtDNA was analyzed by restriction fragment length polymorphism (RFLP) performed on PCR amplified products. The primers of Cronin *et al.* (1993) and Park *et al.* (1993) were used to amplify the NADH 5/6 regions. PCR reactions were conducted in a total volume of 100 ul and contained the following: 3 mM $MgCl_2$, 200 uM each dNTP, 1 uM each primer, 2.5 U *Taq* DNA polymerase and 0.7 - 1.0 ug of DNA template. Cycling conditions included an initial denaturation at 97 °C for 20 sec., 57 °C for 30 sec., and 72 °C for 2 min. A final extension was performed at 72 °C for 5 min.

Thirteen restriction enzymes were surveyed: *ApaI, KpnI, StuI, TaqI, Hha I, HinfI, AseI, Ava II, BstEII, BstUI, EcoRI, EcoRV*, and *Sau96I* following the manufacturers recommendations (New England Biolabs). Restriction fragments were separated on a 0.8% agarose gel containing ethidium bromide, and the resulting banding patterns were visualized under UV light. Distinct single endonuclease patterns were designated by a letter code and then used in combination to describe composite RFLP genotypes.

Polymorphisms for mtDNA were observed with five restriction enzymes. The polymorphic restriction sites and their respective haplotypes and fragment sizes are as follows: *ApaI* "A" 1500 900, "B" 900 800 700; *HinfI* "A" 750 675 500, "B" 800 750 500; *KpnI* "A" 2400,"B" 1200; *StuI* "A" 1500 900,"B" 900 800 700; and *TaqI* "A" 1000 575 250, "B" 575 500 250.

Microsatellites

Microsatellite loci were analyzed by PCR amplification in which one primer was end labeled with ^{32}P. The resulting products were electrophoresed in a 7% denaturing polyacrylamide gel and visualized by autoradiography. Each 10ul reaction contained the following components: MgCl2, dNTPs, unlabeled primer forward and reverse), and one primer 5'end-labeled with gamma^{32}P in proportions to optimize the reaction, 0.4 units Taq DNA polymerase with supplied polymerase buffer (Perkin Elmer), and 30-40 ng DNA template. Reaction conditions for each locus can be supplied on request. Annealing temperature varied with the primer and ranged from 42-57 °C.

Primers for microsatellite loci were those of Estoup *et al.* (1993; μSat60), Olsen *et al.* (1996; Ots2 and Ots3), Morris *et al.* (1996; Omy77), and Sakamoto *et al.* (1994; Fgt1). Primers are typically named after the species from which they are derived: Omy (rainbow trout, *Oncorhynchus mykiss*) and Ots (chinook salmon, *Oncorhynchus tshawytscha*. The μSat60 primer is derived from brown trout (*Salmo trutta*), and the Fgt1 primer is derived from rainbow trout but named for fish GT-repeat marker. Locus names are the primer pair name in upper-case and italics (e.g., *OMY77*) to make them analogous to the nomenclature for allozyme loci (Shaklee *et al.* 1990).

RAPDs

Variable RAPD loci were detected by PCR amplification with 10-base oligonucleotide primers from Operon Technologies, Inc. Products were electrophoresed in agarose gels containing ethidium bromide and photographed over UV light. Reaction conditions and component mixes for each primer were uniform for all populations with controls to assure accurate scoring and consistent amplification of the same bands. Reaction components were 200uM of each dNTP, 2.5 mM MgCl2, 6uM primer, 0.35 units Taq DNA polymerase with supplied buffer (Perkin Elmer), and 40 ng DNA template in a total volume of 20uL. Amplification profiles were initial denaturation at 96 °C (2 min) followed by 45 cycles of 93 °C (1 min), 36-40 °C (depending on the primer) (1 min), 72 °C (1 min) with a final 5 min extension at 72 °C.

We followed nomenclature for zebrafish (*Brachydanio rerio*) in designating locus names for RAPD markers (Johnson *et al.* 1996). The formal name consists of the name of the 10 nucleotide long primer followed by the approximate size of the amplification product. Thus, the locus *20A.760* is amplified by primer A20 and results in a 760-bp amplification product. A slash and an *s* are added at the name of the name to indicate allelic PCR products that are of different length.

Results

Duplicated loci

Extensive gene duplication in salmonids as a result of their polyploid ancestry (Allendorf & Thorgaard 1984) makes genetic interpretation of molecular variation more difficult than in diploid species. Isoloci (two loci resulting from a duplication event that share alleles with identical electrophoretic mobility) are especially problematic. All individuals have four gene copies at an isolocus, and it is difficult to determine how many copies (doses) of a particular allele are present in an individual. In addition, genotypes cannot be determined unambiguously, and there is no way to assign observed variation to a particular locus of the pair without extensive experimental matings (Waples 1988).

We detected isoloci in both allozymes and microsatellites. Isozymes encoded by isoloci included *mAH-1,2*, *GPI-B1,2*, *sMDH-A1,2*, and *sMDH-B1,2*. One of the five microsatellites primer sets (Fgt1) that we used revealed phenotypes that indicated isoloci (*FGT1-1,2*). Most

individuals had three or four alleles of different sizes at *FGT1-1,2*. For example, the following numbers of each genotype were found in the Moose Creek sample: **186/190/192/198* (2); **190/190/190/190* (2); **190/190/190/198* (4); **190/190/192/198* (2); **190/190/194/194* (2); **190/190/194/198* (7); **190/190/198/198* (4); **190/190/198/200* (1); **190/192/192/194* (1); **190/192/194/198* (3); **190/194/194/200* (1); **190/194/198/198* (6); **190/194/198/202* (1); **190/198/198/198* (1); **190/198/200/200* (1); **192/198/198/198* (1); **194/194/198/198* (2); **194/198/198/198* (4); **194/198/198/202* (1); **196/198/198/198* (1); **198/198/198/198* (2); **198/198/198/200* (1).

The most conservative approach to estimate allele frequencies at isoloci is to not make any assumptions about inheritance, and simply estimate allele frequencies by a direct count of the number of alleles expressed by each individual in a population sample (Allendorf & Danzmann 1997). This procedure is equivalent to assuming equal allele frequencies at two disomic loci or treating the isoloci as a single tetrasomic locus (Leary *et al.* 1987). Waples (1988) has developed statistical methods for estimating allele frequencies individually at each of the two isoloci with a maximum likelihood procedure to "identify the set of allele frequencies at the individual gene loci with the highest probability of producing the observed phenotypic distribution" (Waples 1988).

Allozymes

Individual genotypic data for codominant allozyme loci (all loci except *PGM-1*) are summarized into allelic frequencies (Table II-1). Log-likelihood tests for fit to Hardy-Weinberg proportions were performed for all loci. No sample departed significantly from expected proportions.

Allele frequencies at isoloci were estimated by a direct count of the number of alleles present in each individual and assuming the variation occurred at equal frequencies at both loci (Table II-1). Allele frequencies were not estimated using the method of Waples (1988) because little variation was present at isoloci; the common allele at all isoloci was never less than 0.95 (Table II-1).

A polymorphism was found for the presence or absence of the *PGM-1* enzyme product. This was assumed to be caused by a null allele that either did not produce a polypeptide or the product is enzymatically inactive. Such null alleles have been found to be relatively common in salmonids because of their polyploid ancestry (Leary *et al.* 1993). Allele frequencies at *PGM-1* were estimated by treating the absence of product as being homozygous for the null allele and assuming Hardy-Weinberg proportions (Allendorf *et al.* 1983; Lynch & Milligan 1994).

Microsatellites

Allele frequencies at four non-duplicated polymorphic microsatellite loci are presented in Table II-2. Conformance to expected Hardy-Weinberg proportions was tested using a Monte

Carlo pseudo-probability procedure (Zaykin & Pudovkin 1992). Only one locus in one sample showed a significant departure from Hardy-Weinberg proportions. There is a significant excess of homozygotes at *OTS2* in the Russian River sample (F_{IS}=0.177). This excess of homozygotes is caused almost entirely by three homozygotes for a single allele (*OTS2*91*).

Frequencies of the nine alleles at *FGT1-1,2* were estimated initially by counting the numbers of alleles present in each individual and assuming that both loci had equivalent frequencies (Table II-3). We also used the method of Waples (1988) to estimate allele frequencies at the two loci separately (Table II-3). We binned allele into three size classes: **1* (186-190 bp); **2* (192-196 bp); **3* (198-202 bp) since this method uses a maximum of three alleles. None of the four samples differed significantly from Hardy-Weinberg proportions using the procedure of Waples (1988).

All four population samples show similar patterns of allele frequency at *FGT1-1* and *FGT1-2* (Table II-3). Alleles **1* and **3* are at similar frequencies at both loci, while allele **2* tends to be at a much higher frequency at locus *-1* than *-2*. However, the designation of locus *-1* or *-2* is arbitrary. That is, there is no way to determine from population samples if locus *-1* in one population is the same as locus *-1* or locus *-2* in another population.

RAPDs

Four PCR products with RAPD primers were polymorphic for presence or absence. Allele frequencies were estimated as at *PGM-1* by assuming that these polymorphisms resulted from a single locus in Hardy-Weinberg proportions (Table II-4). A fifth RAPD polymorphism (*12B.1300/s*) was caused by an apparent size polymorphism resulting in three genotypes. The common allele had a PCR product of approximately 1300-bp and the alternative allele had a product of approximately 1450-bp. This polymorphism was treated as a single codominant locus. All samples were in agreement with Hardy-Weinberg proportions at *12B.1300/s*.

mtDNA

Six mtDNA haplotypes were detected in the four samples (Table II-5). Three haplotypes (*I*, *II*, *V*) were most common. All populations had one of the three haplotypes at a frequency of at least 0.46. Russian River showed little variability with a frequency of 0.98 for haplotype *I*. The other three haplotypes (*VI*, *VII*, *VIII*) were rarer with no population frequency exceeding a frequency of 0.08. Haplotypes *II* and *V* differed by a single site change from haplotype *I*. Haplotype *VI* differed by a single site from haplotype *V*, while both haplotype *VIII* and *VII* differed by a single site from haplotype *II*.

Overall

A summary of the amount of genetic variation found within and between population samples at individual nuclear loci is presented in Table II-6. Wright's (1951) fixation index (F_{ST})

was estimated using FSTAT (Goudet 1995). Isoloci were excluded from this analysis because of difficulties in estimating H_T, F_{ST}, and H_S. As expected, much greater allelic variation was detected at the microsatellite loci in comparison to the allozymes.

All of the populations, with one exception, have similar amounts of genetic variation (Table II-7). There is evidence of reduced genetic variation in the Russian River sample at mtDNA and microsatellites. This difference is most dramatic for mtDNA. The *I* haplotype is nearly fixed (0.98) in the Russian River sample; in comparison, no single haplotype occurs at a frequency greater than 0.60 in the other samples. The Russian River sample also has the lowest heterozygosity and average number of alleles at the microsatellite loci. This reduction in genetic variation is not apparent at allozymes or RAPDs.

A relatively high proportion of the genetic diversity is attributable to genetic differences among population samples when one considers that these samples come from a small geographical area. The overall F_{ST} is 0.125 for nuclear loci and 0.295 for mtDNA (Table II-7). Much of this divergence among samples is due to the distinctiveness of the Russian River sample (Fig. II-2). The Russian River sample is the most divergent at all nuclear markers (Fig. II-2) and at mtDNA (Table II-5).

Discussion

Isoloci

Microsatellites encoded by isoloci are extremely difficult to use for population genetic analysis. Accurate estimation of allele frequencies at isoloci requires determining the numbers of copies of each allele in individuals (Waples 1988). Isoloci at allozymes are routinely used for population genetic analysis because there is a correspondence between band intensity and doses of an allele present (Shaklee & Phelps 1992; Allendorf & Danzmann 1997). In addition, the presence of heteromeric isozymes also aids in estimating doses for enzymes (Allendorf *et al.* 1975; Waples 1988). However, at microsatellite loci it is difficult to determine how many doses of each allele are present because the amount of PCR product may not accurately reflect the number of allelic doses present (Wagner *et al.* 1994). The many alleles present at most microsatellite loci will also make analysis and allele frequency estimation much more difficult. For a tetrasomic locus with *n* alleles, there are (*n*-3)!/(*n*-1)!4! different genotypes (p. 610, Hartl & Clark 1989). Thus, there are 495 possible genotypes at *FGT1-1,2* with nine alleles.

The best general way to deal with duplicated microsatellite loci is to not use them for population genetic analysis. There are enough microsatellite markers available so that a sufficient number of markers can be obtained without using duplicated microsatellites. Approximately 25% of isozyme markers in rainbow trout are encoded by isoloci (Allendorf & Thorgaard 1984). We would expect the proportion of microsatellites encoded by isoloci to be somewhat less than this because of their higher mutation rate. Nevertheless, we would still expect a substantial proportion (perhaps 10%) of microsatellites to be encoded by isoloci in

salmonids because recombination between homeologs will transfer alleles between loci (Allendorf & Danzmann 1997). Duplicated microsatellite loci in salmonids can be used in other applications (e.g., paternity and kinship analysis). However, it is critical that the inheritance of such loci be tested in the population being investigated because of the possibility of residual tetrasomy in some populations and not others (Allendorf & Danzmann 1997).

Isoloci are also a potential problem for RAPDs or other types of dominant/recessive markers that depend upon the presence of absence of fragments. Allele frequencies at such markers are commonly estimated by assuming Hardy-Weinberg proportions at a disomic locus.

Variation within populations

Allelic diversity is much more sensitive to population bottlenecks than heterozygosity (Allendorf 1986; Leberg 1992). The disagreement between markers in detecting reduced genetic variation in the Russian River sample reflects this distinction. Both the microsatellite and mtDNA show great reduction in allelic diversity in the Russian River sample.

The reduced genetic variation in the Russian River population is compatible with several different explanations. Large glaciers invaded Southcentral Alaska, and what is now Cook Inlet, approximately 25,000 years ago and lasted until approximately 9,000 years ago in the late-Wisconsin glaciation (Reger & Pinney 1996). These events likely played an important role in the colonization of sockeye salmon in the major Cook Inlet drainages.
It is probable that the Kenai and Kasilof Rivers were open for colonization before many of the other drainages in Cook Inlet were free of glacial ice (Reger & Pinney 1996). While the upper Kenai and Kasilof Rivers were still blocked with glacial ice, suitable habitat for spawning sockeye salmon (in the form of impounded lakes and their resulting outwash) existed near the outlets of both rivers. The Russian River valley was probably one of the last to become free of glacial ice. In addition, the presence of an imposing water fall two miles from its confluence with the mainstem of the Kenai River may also have limited the number of founders and may continue to restrict gene flow.

Spawning escapements to the Kenai River and its Russian River tributary have been monitored routinely since 1968 by the Alaska Department of Fish and Game. Escapements into the mainstem Kenai River (including Skilak Lake) have varied from a low of 51,000 adults in 1969 to a high of 1,407,000 adults in 1987 (Fried 1996). Russian River escapements have varied over the same time period from 24,640 adults to 136,970 adults. During periods of high flows, a velocity barrier can severely limit the migration of sockeye salmon over the falls and lead to high mortality rates among returning adults. For example, Engel (1972) documented a minimum mortality below the falls of 10,000 to 12,000 adults in 1971 as a result of high water from a late spring breakup coupled with exceptionally heavy rains. Examination of the carcasses suggested that females suffered greater mortality than males.

Thus, the barrier falls may historically have caused drastic periodic reductions in effective population size in the Russian River population. In addition, the increased mortality on females might lead to even further reductions in effective population size for mtDNA.

Variation among populations

The variation in F_{ST} among loci and types of markers is one of the most powerful methods for determining if natural selection is playing a major role in determining the amount of genetic divergence among populations (McDonald 1994; Bowcock *et al.* 1991; Beaumont & Nichols 1996). Under selective neutrality, all loci will be similarly affected by the demographic properties of the populations (effective population size, migration, etc.). The amount of variability among polymorphisms in this case will only be due to chance. However, if natural selection is having a major effect, then some loci may have a higher or smaller F_{ST}, depending upon the mode of natural selection. If, has been suggested, some types of markers are more strongly affected by selection than others, then we would expect to find differences in the mean or variability in F_{ST} for different markers.

Differences in the number of alleles at a locus is a source of bias in estimating F_{ST} (McDonald 1994). This concern can be especially problematic when comparing allozyme markers with microsatellites because of the much greater allelic diversity found at microsatellites. We have used two methods to avoid this problem. First, we treated all loci as two allele polymorphisms by using the frequency of the overall most common allele and pooling all other alleles to estimate $F2_{ST}$, as recommended by McDonald (1994). This solution is statistically appropriate, but a great deal of information is lost. Therefore, we also treated each allele individually as a separate "marker" (Bowcock *et al.* 1991).

Both types of nDNA markers tended to have lower F_{ST} and $F2_{ST}$ than the allozymes (Tables II-6 and II-7). However, almost all of this effect is due to a single locus: *sAH* (Fig. II-3). There is no difference in the distribution of $F2_{ST}$ for the three types of markers, with the exception of *sAH* (Fig. II-3). We have excluded all loci with a total heterozygosity (H_T) of less than 0.10 in this comparison because loci with little genetic variation cannot have high F_{ST} values (Beaumont & Nichols 1996).

Treatment of each allele individually supports the conclusion of overall similarity in F_{ST}, with the exception of *sAH* (Figure II-4). None of the alleles have an F_{ST} value of greater than 0.25, except for *sAH* with an F_{ST} of 0.713. Figure II-4 also shows the maximum value that F_{ST} can take as a function of total heterozygosity for a two allele polymorphism using the algorithm of Goudet (1994). This effect makes intuitive sense because F_{ST} can be thought of as the reduction in heterozygosity (H_S) at a locus because of allele frequency differences among populations. If only a few copies of an allele occur, there is a little effect on H_S whether or not the copies occur in the same subpopulation because homozygotes for rare alleles are so infrequent.

Greater F_{ST} values are expected for mtDNA than nDNA at drift-migration equilibrium because of the smaller effective population size of a mitochondrial marker. Our estimation of migrants per generation (mN) based upon an F_{ST} of 0.125 at nuclear loci is 1.75, assuming the island model of migration where $F_{ST}=1/(4mN+1)$; m is the proportion of migrants, and N is the effective population size of each subpopulation (Slatkin 1995). This estimate is very close to the comparable value based upon F_{ST} at mtDNA (0.295) of 2.39 (Birky *et al.* 1983).

Mutation

The different mutation rates of allozymes and tandem repeat loci, such as microsatellites, may also effect the amount of genetic differentiation between populations (e.g. Jin & Chakraborty 1995). The expected effect itself will depend upon the model of mutation used and whether differentiation is a result of divergence following complete isolation or drift-migration equilibrium. In the case of complete isolation and the infinite allele model of mutation (IAM), one would expect loci with higher mutation rates to show greater divergence (Bowcock *et al.* 1991). However, constraints on allele size at VNTR loci under the stepwise mutation model (SMM) may reverse the direction of this effect under some conditions (Nauta & Weissing 1996).

In the case of drift-migration equilibrium, the effect of mutation will depend also upon the relative magnitude of migration and mutation rates. Greater differentiation would be expected at loci with higher mutation rates if novel mutations drifted to high frequencies in some populations to produce so-called "private alleles" (Slatkin 1985). There is no suggestion in our data that novel mutations at microsatellite loci have led to high frequency private alleles (Tables II-2 and II-3).
Thus, the differentiation among populations is best interpreted under the drift-migration equilibrium model and is apparently not affected by differences in mutations rates between classes of markers.

Conclusions

Our results indicate concordance among markers in the amount of genetic variation within population and the amount of genetic differentiation among populations, with the striking exception of *sAH*. Three of the four populations have similar amounts of genetic variation within them. The Russian River has reduced allelic diversity at both microsatellites and mtDNA. This effect was not detected at allozymes or with RAPDs; however, this is most likely due to the greatly reduced power to detect this effect because of the reduced number of alleles at these markers.

There is no tendency for differences between markers in the amount of differentiation as measured by F_{ST} in a comparison of either loci (Figure II-3) or individual alleles (Figure II-4). In addition, the direction and magnitude of differentiation at mtDNA relative to the nDNA markers is very close to that predicted assuming selective neutrality. The pattern of differentiation among populations is also concordant for all four types of markers (Figure II-2

and Table II-5). The Russian River population is the most divergent for all markers. The relationships among the remaining three populations does differ, but this is not significant because of the relatively small differentiation among these three populations for all markers.

The simplest interpretation of these data is that these loci, excluding *sAH*, are acting as if they are selectively neutral. The amount of differentiation among populations at *sAH* is obviously exceptional. One interpretation of this discrepancy is that it is caused by natural selection at *sAH* or a tightly linked region. This possibility has been suggested previously by Wilmot & Burger (1985) in their study of sockeye salmon populations from the Russian River and Karluk River on Kodiak Island.

An alternative explanation must be considered before we conclude that natural selection is acting at *sAH*. Fluctuations in effective population size over time are expected to increase the variance of F_{ST} among loci (Bowcock *et al.* 1991; Beaumont & Nichols 1996). The greater differentiation at *sAH* is caused exclusively by an exceptionally high frequency of the **117* allele in the Russian River sample (Table II-1). This allele is at a frequency of less than 0.05 throughout Cook Inlet (Seeb *et al.* in press). The reduced allelic diversity of this sample suggests that this population has gone through a "recent" bottleneck during which rare alleles were lost. This same bottleneck may have resulted in a dramatic increase in the frequency of the *sAH*117* allele.

The current data do not allow us to distinguish between the two possible explanations (natural selection or genetic drift) for the greater divergence seen at *sAH*. Regardless of the mechanism, this locus is the exception. That is, there is no indication of a general difference between allozymes and DNA markers that would suggest that allozymes are not appropriate for estimating patterns and amounts of gene flow among population samples, as suggested by some authors (Karl & Avise 1992; Pogson *et al.* 1995).

The differences between allozymes and nDNA reported previously also are driven by exceptional loci. For example, the greater divergence at nDNA loci reported by Pogson *et al.* (1995) is caused largely by two of 17 nuclear RFLP loci (*GM738* and *GM798*) that have exceptionally high F_{ST} values (Beaumont & Nichols 1996). In addition, seven of the 10 allozyme loci used by Pogson *et al.* (1995) have average heterozygosities less than 0.04 and, therefore, cannot have F_{ST} values greater than 0.07 (Figure II-4). All of the nDNA loci used by Pogson *et al.* (1995) have heterozygosities of 0.10 or greater. Scribner *et al.* (1994) found greater overall F_{ST} values at six allozyme loci than at four nDNA loci, but this difference was driven by one allozyme locus with exceptionally high F_{ST} (*Pgd*).

The results of Karl & Avise (1992) provided one of the most dramatic examples of discordant patterns in their study of divergence at 14 allozyme and four nDNA loci in oysters. In contrast, McDonald *et al.* (1996) recently examined an additional six nDNA loci in these same oyster populations and found patterns of differentiation similar to that found at the 14 allozyme loci.

There is compelling evidence of differences between some loci in the amount of differentiation among populations, such as we have found for *sAH* in sockeye salmon. Those exceptional loci are good candidates for loci at which natural selection may be acting to affect the amount of differentiation among populations. However, there is not compelling evidence for consistent difference between allozyme and nDNA loci that require an explanation of different regimes of natural selection at these two classes of loci. In using estimates of differentiation among loci to infer historical amounts of gene flow and patterns of genetic exchange it is important to examine many loci. It is less important whether those loci are allozymes or nDNA markers.

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Author Information Box

This paper is the result of a collaboration between the Wild Trout and Salmon Genetics Laboratory of the University of Montana and the Genetics Laboratory of the Alaska Department of Fish and Game. Fred Allendorf and Kathy Knudsen study the transmission, population, and evolutionary genetics of salmonid fishes. The genetic population structure of Cook Inlet sockeye salmon are being studied by Lisa and Jim Seeb to provide information to help manage the fishery in order to minimize the effects of the *Exxon Valdez* oil spill.

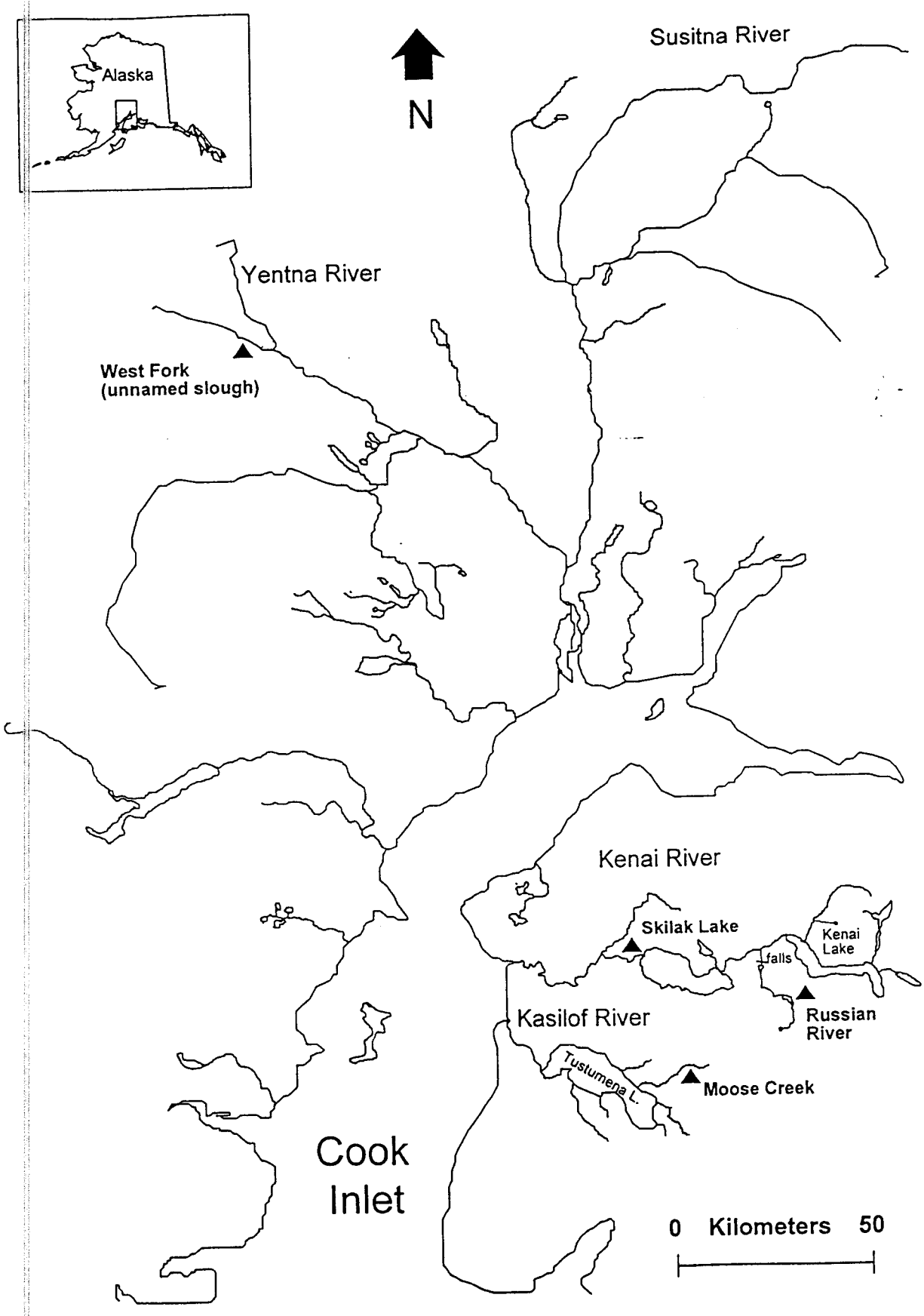

Figure II-1. Sample locations of sockeye salmon from Cook Inlet, Alaska.

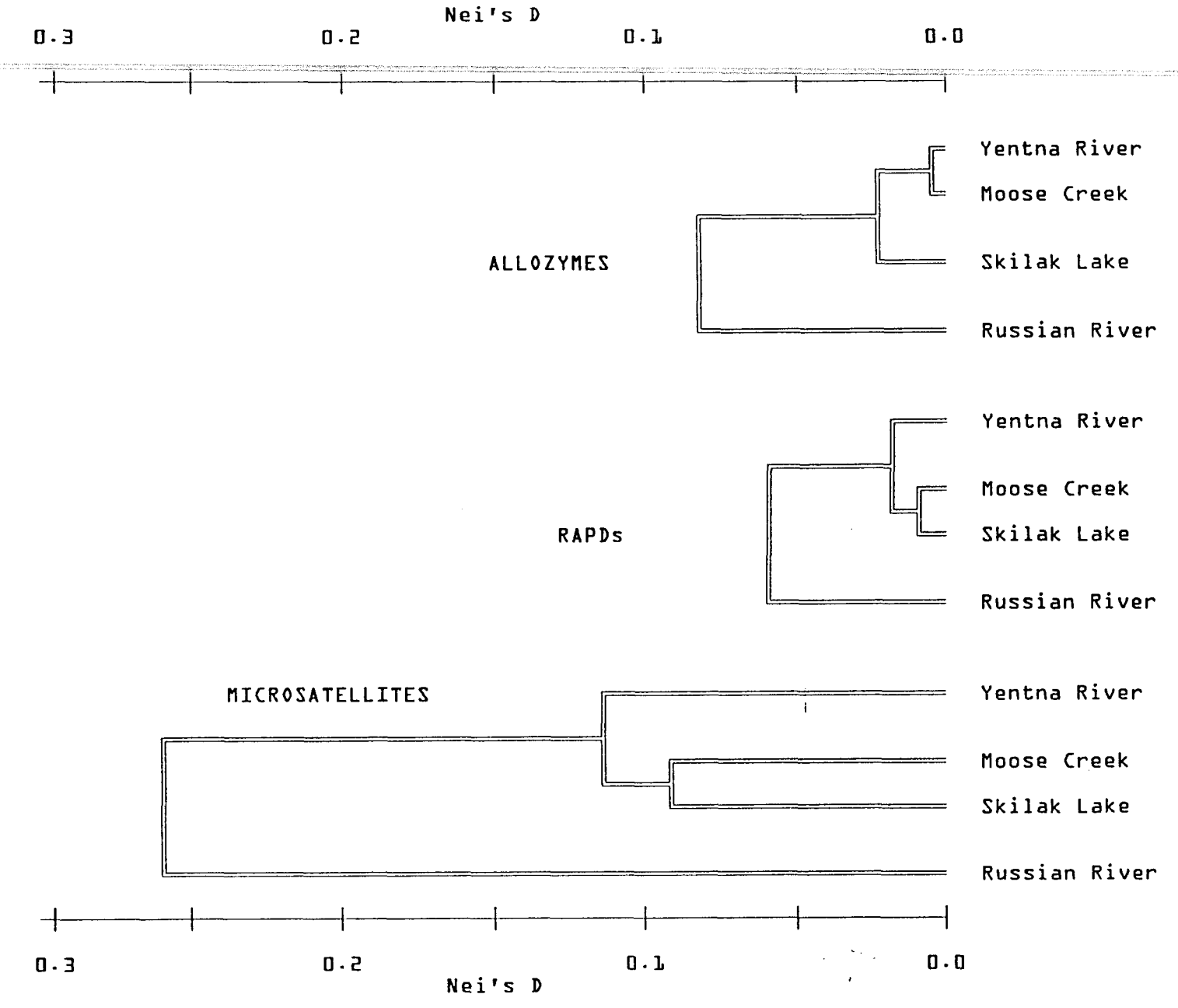

Figure II-2. Dendrogram (UPGMA) sockeye salmon based upon Nei's (1978) unbiased D based upon allele frequencies at three different sets of nuclear markers.

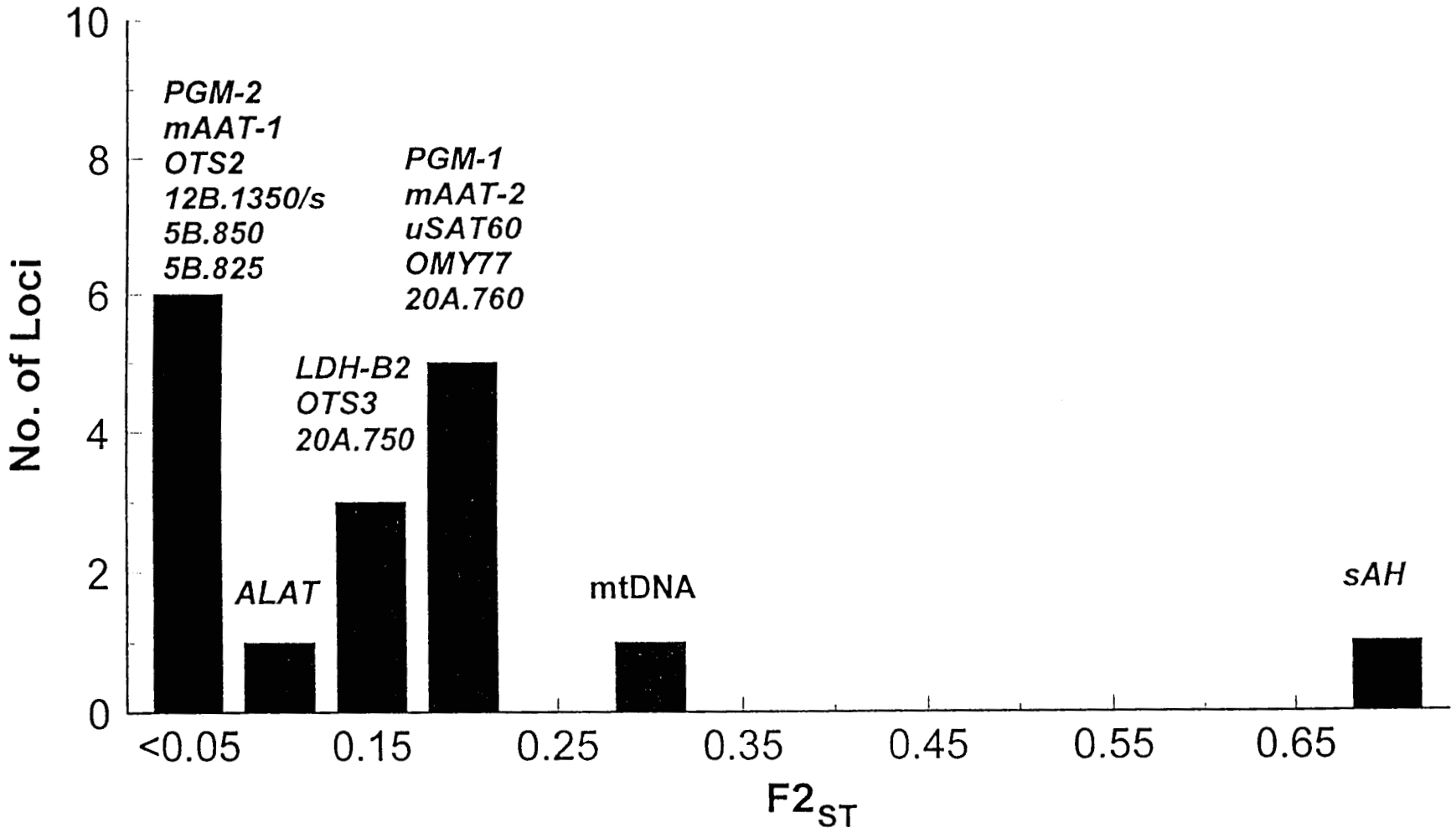

Figure II-3. Distribution of $F2_{ST}$ values for all loci with an H_T greater than 0.10.

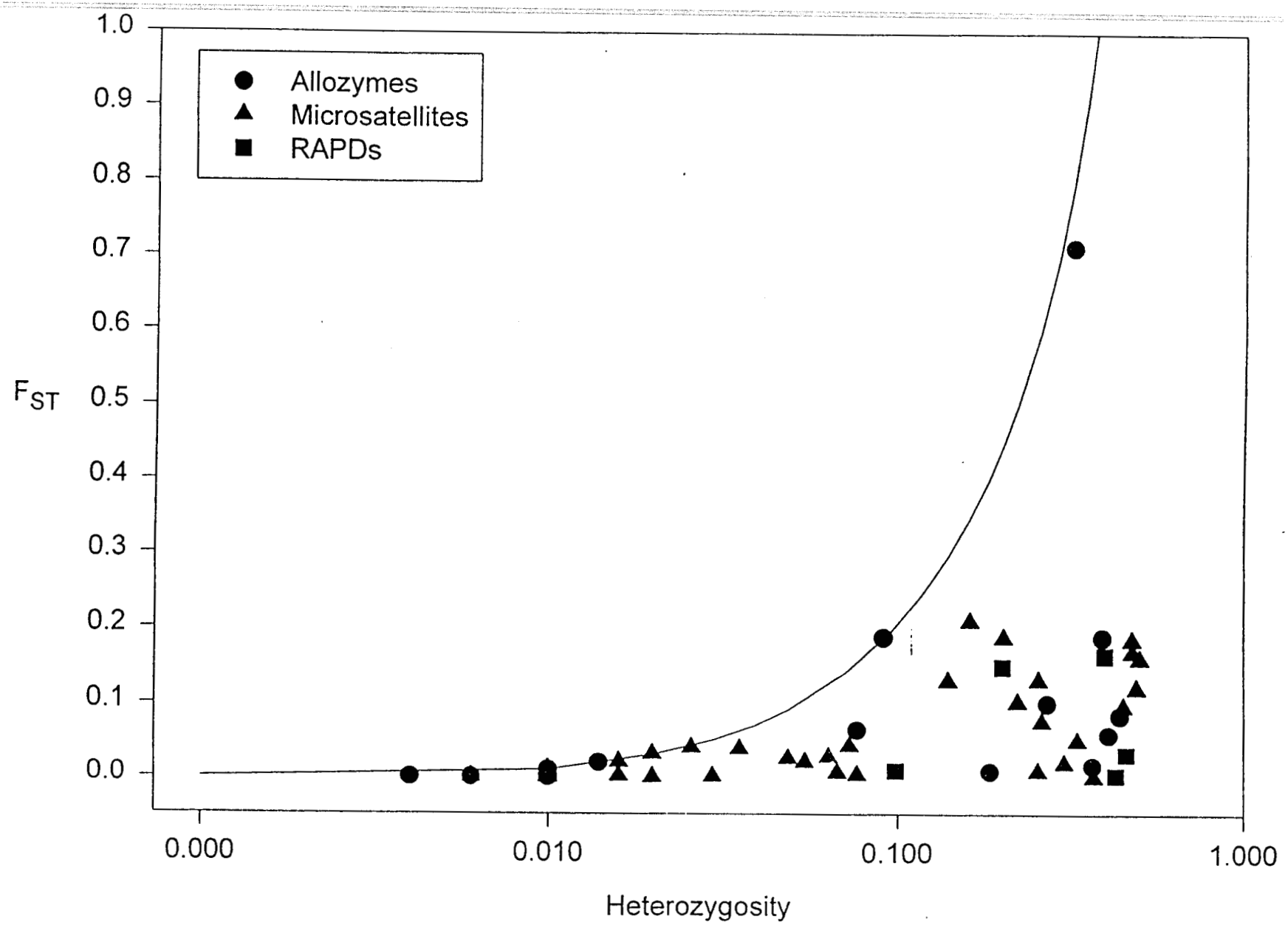

Figure II-4. Relationship between total heterozygosity and F_{ST} for each allele individually by pooling all other alleles at the same locus. The solid line shows the maximum F_{ST} for a locus with two alleles.

Table II-1. Allelic frequencies at 21 allozyme loci in sockeye salmon. Frequencies are for the **100* allele unless otherwise noted.

Sample	*mAAT-1*	*mAAT-2*	*mAH-1,2*	*sAH*	*ALAT* **100*	*ALAT* **91*	*ALAT* **95*
Moose	0.930	1.000	0.959	1.000	0.520	0.360	0.120
Russian	0.890	0.806	1.000	0.240	0.860	0.120	0.020
Skilak	0.929	1.000	0.969	0.960	0.708	0.271	0.021
Yentna	0.844	1.000	0.965	1.000	0.620	0.380	0.000

	G3PDH-4	*GAPDH-2*	*GPI-B1,2*	*LDH-B2*	*PEP-A*	*PEP-D1*	*PEP-LT*
Moose	0.990	1.000	0.950	0.880	1.000	1.000	1.000
Russian	1.000	1.000	1.000	0.660	1.000	1.000	1.000
Skilak	1.000	0.980	1.000	0.940	0.970	0.990	1.000
Yentna	1.000	1.000	1.000	0.880	1.000	0.990	0.980

	sMDH-A1,2	*sMDH-B1,2* **100*	*sMDH-B1,2* **65*	*sMDH-B1,2* **120*	*MPI*	*PGM-1*	*PGM-2*
Moose	0.995	1.000	0.000	0.000	1.000	0.140	0.660
Russian	1.000	0.995	0.005	0.000	1.000	0.020	0.800
Skilak	0.995	0.990	0.005	0.005	0.990	0.640	0.770
Yentna	1.000	1.000	0.000	0.000	1.000	0.073	0.810

Table II-2. Allelic frequencies at four microsatellite loci in sockeye salmon.

	OMY77									
	**95*	**97*	**99*	**101*	**103*	**105*	**107*	**109*	**111*	**113*
Moose	0.010	0.031	0.061	0.010	0.306	0.010	0.398	0.051	0.000	0.122
Russian	0.000	0.000	0.000	0.000	0.200	0.000	0.680	0.000	0.010	0.110
Skilak	0.000	0.000	0.041	0.010	0.337	0.061	0.235	0.041	0.000	0.276
Yentna	0.000	0.000	0.000	0.000	0.700	0.000	0.240	0.050	0.010	0.000

	OTS2												
	**79*	**89*	**91*	**95*	**97*	**99*	**101*	**103*	**105*	**107*	**109*	**111*	**113*
Moose	0.000	0.000	0.170	0.000	0.000	0.000	0.240	0.230	0.060	0.200	0.070	0.020	0.010
Russian	0.000	0.000	0.090	0.290	0.220	0.010	0.000	0.270	0.010	0.090	0.010	0.010	0.000
Skilak	0.000	0.050	0.130	0.030	0.060	0.020	0.200	0.210	0.050	0.210	0.010	0.030	0.000
Yentna	0.010	0.000	0.200	0.030	0.020	0.000	0.170	0.260	0.040	0.240	0.020	0.000	0.010

Table II-2. Continued.

	OTS3										
	**76*	**90*	**92*	**94*	**96*	**98*	**100*	**102*	**104*	**106*	**108*
Moose	0.198	0.000	0.417	0.000	0.031	0.333	0.000	0.000	0.000	0.021	0.000
Russian	0.220	0.000	0.770	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000
Skilak	0.327	0.010	0.429	0.000	0.082	0.112	0.010	0.000	0.010	0.000	0.020
Yentna	0.083	0.000	0.688	0.042	0.021	0.146	0.000	0.021	0.000	0.000	0.000

	µSAT60								
	**115*	**119*	**121*	**123*	**125*	**127*	**129*	**131*	**137*
Moose	0.010	0.430	0.040	0.100	0.000	0.010	0.000	0.410	0.000
Russian	0.010	0.130	0.000	0.020	0.000	0.000	0.000	0.790	0.050
Skilak	0.000	0.310	0.320	0.030	0.010	0.000	0.000	0.330	0.000
Yentna	0.020	0.480	0.090	0.000	0.000	0.000	0.010	0.400	0.000

Table II-3. Allelic frequencies at the *FGT1-1,2* isoloci. Frequencies in the top table were estimated by assuming equal allele frequencies at both loci. Frequencies in the bottom table were estimated using the method of Waples (1988) by binning alleles into three size classes: **1* (186-190 bp); **2* (192-196 bp); **3* (198-202 bp).

	FGT1-1,2								
	**186*	**188*	**190*	**192*	**194*	**196*	**198*	**200*	**202*
Moose	0.010	0.000	0.340	0.050	0.165	0.005	0.395	0.025	0.010
Russian	0.019	0.000	0.463	0.000	0.188	0.000	0.331	0.000	0.000
Skilak	0.000	0.000	0.335	0.037	0.287	0.005	0.314	0.016	0.005
Yentna	0.000	0.018	0.292	0.060	0.262	0.006	0.363	0.000	0.000

	FGT1-1			*FGT1-2*		
	**1*	**2*	**3*	**1*	**2*	**3*
Moose	0.296	0.350	0.354	0.427	0.076	0.497
Russian	0.345	0.246	0.409	0.617	0.129	0.254
Skilak	0.263	0.577	0.159	0.393	0.090	0.518
Yentna	0.298	0.485	0.217	0.280	0.182	0.539

Table II-4. Allele frequencies at five RAPD loci in sockeye salmon.

	20A.760	*20A.750*	*5B.850*	*5B.825*	*12B.1300/s*
Moose	0.908	1.000	0.990	0.602	0.650
Russian	0.470	0.800	0.940	0.810	0.760
Skilak	0.690	1.000	0.959	0.592	0.646
Yentna	0.850	0.750	0.896	0.594	0.720

Table II-5. Composite haplotype frequencies at mtDNA in sockeye salmon. Composite haplotypes were generated from polymorphic restriction enzymes and include *Apa I, Hinf I, Kpn I, Stu I,* and *Taq I,* respectively. Haplotypes are: *I*=AAAAA, *II* = BAAAA, *V*=AAAAB, *VI* = AABAB, *VII* = BAABA, *VIII* = BBAAA.

	I	*II*	*V*	*VI*	*VII*	*VIII*
Moose	0.280	0.140	0.460	0.080	0.040	0.000
Russian	0.980	0.000	0.020	0.000	0.000	0.000
Skilak	0.520	0.260	0.140	0.080	0.000	0.000
Yentna	0.340	0.600	0.040	0.000	0.000	0.020

Table II-6. Summary of genetic variation at non-duplicated nuclear loci in sockeye salmon. *A* is the number of alleles; H_T is the total heterozygosity; F_{ST} is the fixation index; $F2_{ST}$ is the fixation index calculated by pooling all the alleles but the most common together; H_S is the expected heterozygosity assuming Hardy-Weinberg proportions.

					Moose		Russian		Skilak		Yentna	
Locus	*A*	H_T	F_{ST}	$F2_{ST}$	*A*	H_S	*A*	H_S	*A*	H_S	*A*	H_S
Allozymes												
mAAT-1	2	0.183	0.008	----	2	0.132	2	0.198	2	0.134	2	0.266
mAAT-2	2	0.091	0.188	----	1	0.000	2	0.316	1	0.000	1	0.000
sAH	2	0.320	0.713	----	1	0.000	2	0.368	2	0.078	1	0.000
ALAT	3	0.460	0.072	0.085	3	0.592	3	0.248	3	0.429	2	0.476
G3PDH-4	2	0.005	0.000	----	2	0.020	1	0.000	1	0.000	1	0.000
GAPDH-2	2	0.010	0.010	----	1	0.000	1	0.000	2	0.040	1	0.000
IDH-B2	2	0.269	0.101	----	2	0.213	2	0.453	2	0.114	2	0.213
LEP-A	2	0.014	0.020	----	1	0.000	1	0.000	2	0.059	1	0.000
LEP-D1	2	0.010	0.000	----	1	0.000	1	0.000	2	0.020	2	0.020
LEP-LT	2	0.010	0.010	----	1	0.000	1	0.000	1	0.000	2	0.040
MPI	2	0.005	0.000	----	1	0.000	1	0.000	2	0.020	1	0.000
PGM-1	2	0.341	0.188	----	2	0.389	2	0.040	2	0.465	2	0.243
PGM-2	2	0.365	0.016	----	2	0.453	2	0.323	2	0.358	2	0.311
Microsatellites												
OMY77	10	0.682	0.142	0.168	9	0.733	4	0.490	7	0.756	4	0.454
OTS2	13	0.846	0.048	0.000	8	0.820	9	0.786	11	0.853	10	0.811
OTS3	11	0.602	0.093	0.120	5	0.682	3	0.362	8	0.697	6	0.502
SSAT60	9	0.639	0.129	0.159	6	0.642	5	0.360	5	0.699	5	0.607
RAPDs												
A0A.760	2	0.394	0.164	----	2	0.167	2	0.498	2	0.428	2	0.255
A0A.750	2	0.199	0.149	----	1	0.000	2	0.320	1	0.000	2	0.375
B.850	2	0.102	0.009	----	2	0.020	2	0.113	2	0.079	2	0.186
B.825	2	0.455	0.032	----	2	0.479	2	0.308	2	0.483	2	0.482
2B.1350/s	2	0.425	0.001	----	2	0.455	2	0.365	2	0.457	2	0.403

Table II-7. Summary of genetic variation and genetic population structure in sockeye salmon as estimated with different techniques.

					Moose		Russian		Skilak		Yentna	
	Loci	A	F_{ST}	$F2_{ST}$	A	H_S	A	H_S	A	H_S	A	H_S
Allozymes	13	2.1	0.198	0.202	1.6	0.121	1.5	0.150	1.9	0.133	1.5	0.121
Microsat.	4	10.8	0.100	0.119	7.2	0.719	5.0	0.500	7.6	0.751	6.2	0.594
RAPDs	5	2.0	0.072	0.072	1.8	0.244	2.0	0.321	1.8	0.289	2.0	0.340
Nuclear	22	3.6	0.125	0.137	2.7	0.258	2.2	0.253	2.9	0.281	2.5	0.257
mtDNA	1	6	0.295	0.353	5	0.682	2	0.039	4	0.636	4	0.522