

**GENETIC STOCK IDENTIFICATION OF SOCKEYE
AND CHUM SALMON FROM BRISTOL BAY, ALASKA**

by

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Final Report

Outer Continental Shelf Environmental Assessment Program
Research Unit 694

June 1989

ACKNOWLEDGMENTS

This study was funded by the Minerals Management Service, Department of the Interior, through an Interagency Agreement with the National Oceanic and Atmospheric Administration, Department of Commerce, as part of the Alaska Outer Continental Shelf Environmental Assessment Program.

We thank Gary Sonnevil and his staff (Service Fisheries Assistance Office, King Salmon) for collecting the Bristol Bay salmon used in this *study*. Lyman Thorsteinson (National Oceanic Atmospheric Administration) facilitated the entire study. Various employees of the Alaska Department of Fish and Game helped us in different aspects of this project. David Rutz and John Bigelow assisted with electrophoresis. Martha Ronaldson typed the manuscript.

ABSTRACT

To study the Pacific salmon that may be affected by oil and gas development in the North Aleutian Basin, Alaska, we have used electrophoretic methods of protein separation to genetically characterize stocks. In 1987, tissue samples were collected from eleven populations of sockeye salmon and four populations of chum salmon from Bristol Bay drainages. In the laboratory, we analyzed 50 gene loci from each collection to establish genetic baseline data. In comparisons to sockeye salmon sampled from the same drainages in previous years, we found no significant differences in allele frequencies. The genetic identities (Nei) among Bristol Bay sockeye salmon populations are high, all greater than 0.98. Few loci are variable, and only 2% of the total genetic variation is due to differences between populations. Bristol Bay chum salmon sampled have genetic identities of 0.97 or more. Divergence between chum stocks, at 4%, is twice that of sockeye salmon sampled. Computer simulations with maximum likelihood statistics and re-sampling procedures were used to estimate the composition of artificial mixed stocks made up from baseline data. For sockeye salmon, only a few stocks were accurately and precisely identified from mixtures. Chum salmon stocks were more precisely identified, with some bias among the geographically close Inner Bay stocks.

TABLE OF CONTENTS

	Page
Acknowledgments	555
Abstract	557
Introduction	561
Objectives	562
Materials and Methods	563
Sampling and Electrophoresis	563
Genotypic Distributions	564
Allele Frequency Heterogeneity	564
Genetic Variation	564
Genetic Similarity	570
Gene Diversity Analysis	571
Genetic Stock Identification	572
Results	573
Sockeye Salmon	573
Genotypic Distributions	573
Allele Frequency Heterogeneity	574
Genetic Variation	575
Genetic Similarity	577
Gene Diversity Analysis	577
Genetic Stock Identification	577
Chum Salmon	580
Genotypic Distributions	580
Allele Frequency Heterogeneity	583
Genetic Variation	583
Genetic Similarity	585
Gene Diversity	586
Genetic Stock Identification	587
Discussion	590
References	594
Appendix A	597
Appendix B	598
Appendix C	599
Appendix D	599

INTRODUCTION

Offshore **oil** and gas lease sales proposed for the North Aleutian **Basin** have the potential to **impact** several **life** history stages and fisheries of Bristol Bay salmon. These concerns stem from the overlap of proposed leasing areas **with** major salmon migration pathways (Straty 1981; **Thorsteinson** and **Thorsteinson** 1984), the potential **siting** of onshore **facilities** near important rearing habitats for juvenile salmon (**Thorsteinson** 1984; **Isakson** et al. 1986), and a perception of **diminishing** resource availability and harvest incomes resulting from possible oil spills.

An appraisal of the **risk** to **Pacific** salmon from resource development in the North Aleutian **Basin** would be best addressed on a **river by river** (or stock) basis. This form of assessment requires that detectable differences **exist** between stocks of salmon from major drainages **in** the Bristol Bay region. Detectable stock differences can result from **either** genetic and/or environmental factors, and may be identifiable depending on the species of interest and the method of study.

Methods for identifying fish stocks include enumeration and comparison of various morphological and biological characters (e.g., scale and **otolith** growth patterns and parasite infestations). These types of markers can be affected by yearly fluctuations in the environment and must be standardized on an annual basis if population identification is desired (**Ihssen** et al. 1981). Using starch-gel **electrophoresis**, we can

detect differences between individuals as a result of inherited genetic material not subjected to environmental perturbations. These differences have been shown to be stable characteristics within salmon populations (Grant et al. 1980; Utter et al. 1980; **Beacham** et al. 1985).

Genetic stock identification (**GSI**) is based on **electrophoretically** detectable differences in **genotypic** distributions among fish populations. The genotypic distributions result from allele frequency differences at protein-coding gene loci. For anadromous fishes, estimates of stock composition in a mixed fishery are derived by comparing genotypic distributions of a mixed-stock sample against samples taken from discrete freshwater populations (baseline data). The best fit estimates of various stock admixtures are determined by maximum likelihood analysis (**Pella** and **Milner** 1986). Genetic stock identification is being employed in the management of salmon stocks in the **states** of Washington and California, and in British Columbia.

Objectives

The objectives of this segment of the study are:

- 1) to collect electrophoretic gene frequency data from freshwater spawning populations of sockeye (**Oncorhynchus nerka**) and chum salmon (**O. keta**);
- 2) to describe the amount of interannual variation in allele

frequencies based on our work and two previous genetic studies of Bristol Bay salmon populations conducted in different years; and

3) to use this data as a reference baseline to assess whether sufficient detectable genetic divergence exists among populations of Bristol Bay sockeye and chum salmon to permit accurate stock composition estimates in a mixed-stock fishery.

The identification of fish taken from potential development sites can aid in assessing the effects of resource development on specific stocks throughout Bristol Bay. The long range goal of this study includes genetic stock analysis for all five species of Pacific salmon that inhabit the Bristol Bay area.

MATERIALS AND METHODS

Sampling and Electrophoresis

Tissue samples from eleven populations of sockeye salmon and six populations of chum salmon were collected from drainages of Bristol Bay (Tables 1 and 2, Figure 1). **Biologists** from **the** U.S. Fish and Wildlife Service (Service) Fishery Assistance Office in King Salmon used nets to capture adult salmon during their freshwater spawning migration. Samples from these collection sites are thought to be representative of populations that are major contributors to the Bristol Bay sockeye and

Table 1.- Sockeye salmon collection sites, Universal Transverse Mercator (UTM) coordinates, sample size, and dates for the genetic stock identification study, Bristol Bay, Alaska.

Site	UTM coordinates			Number of fish	1987 collection date
	Zone	Latitude	Longitude		
Bear River	4W	6210625W	420625E	100	July 9
Brooks Lake					
Headwaters Cr.	4W	6484375N	671875E	34	August 14
Hidden Creek	5W	6485625N	328750E	33	August 14
Upatree Creek	SW	6488750N	336875E	33	August 15
Egegik River	4W	6437500N	625000E	100	July 7
Igushik River	4W	6545000N	483125E	100	July 10
Kvichak River	5W	6580000N	335000E	100	July 6
Naknek Lake					
Margot Creek	5W	6485000N	350000E	34	August 6
Idavain Creek	5W	6508125N	346250E	33	August 13
Brooks River	5W	6493125N	337500E	33	August 27
Nushagak River	4W	6530625N	574375E	100	July 3
Nelson River	4W	6176250N	368750E	100	July 11
Togiak River	4W	6548750N	423125E	100	July 15
Ugashik River	4W	6281875N	619375E	29	July 16
	4W	6281875N	619375E	71	July 18
Wood River	4W	6468250N	523750E	100	July 8
Total				1100	

chum salmon fisheries.

Individual fish were dissected for samples of muscle, liver, **eye**, and heart tissue, and the samples were placed in polystyrene test tubes.

Table 2.- Chum salmon collection sites, Universal Transverse Mercator (UTM) coordinates, sample sizes, and dates for the genetic stock identification study, Bristol Bay, Alaska.

Site	UTM coordinates			Number of fish	1987 collection date
	Zone	Latitude	Longitude		
Alagnak River	4W	6544375N	671875E	100	August 18
Herendeen Bay					
Portage Creek	4W	6176250N	395000E	34	August 21
Grass Valley	4W	6176250N	399375E	33	August 21
Lawrence Creek	4W	6178750N	399375E	33	August 21
King Salmon River					
unnamed tributary	4W	6450000N	662500E	4	July 13
Gertrude Creek	4W	6450000N	665000E	40	July 18
unnamed tributary	4W	6450000N	662500E	42	July 20
Gertrude Creek	4W	6450000N	665000E	14	July 25
King Salmon River					
Mother Goose Lake	4W	6342500N	598750E	100	July 28-30
Nelson River	4W	6183750N	367500E	50	August 21
Sapsuk Lake	4W	6176250N	368750E	50	August 22
Togiak River	4W	6569375N	431250E	<u>98</u>	July 27
Total				598	

The tissues were immediately placed on ice, then transported to King Salmon where they were frozen. The frozen samples were flown to the Alaska Fish and Wildlife Research Center laboratory in Anchorage where they were stored at -80°C prior to electrophoretic analysis.

We used horizontal starch-gel **electrophoresis** of genetically-encoded enzymes to detect differences between populations (Utter et al. 1974). Staining procedures follow the methods described by Aebersold et al. (1987) and Harris and Hopkinson (1976, 1977). The isozyme nomenclature

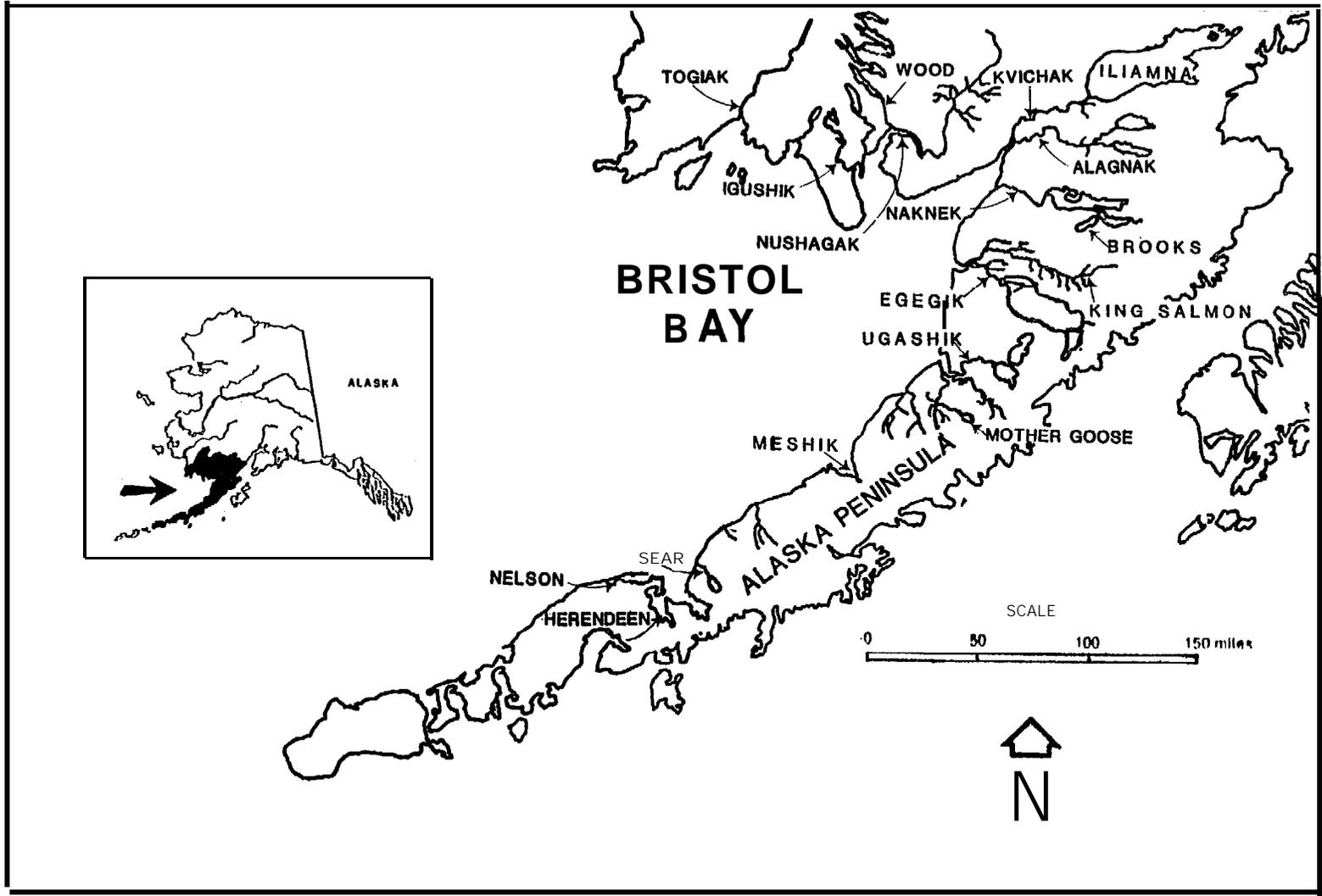


Figure 1. Bristol Bay and the Alaska Peninsula showing the locations sampled for chum and sockeye salmon.

and method of measuring allele nobilities are those of **Allendorf** et al. (1983). We examined 26 enzymes encoded by 50 presumptive gene **loci** using four gel buffers (Table 3).

Genotypic distributions

The **electrophoretic** genotypes for each individual were coded and gene frequencies at each locus were calculated. All polymorphic loci were tested for significant deviations from expected random-mating genotypic proportions (Hardy-Weinberg equilibria) using the **chi-square** analysis.

Allele frequency heterogeneity

A multiple simultaneous log-likelihood ratio statistic (G-test: **Sokal** and **Rohlf** 1981) was used to determine heterogeneity among all collections from each species. G-tests were then used to test for significant differences between allele frequencies of nonspecific populations, pairwise. The significance level was adjusted using the correction of Cooper (1968) to reduce chance statistically significant results due to the number of pairwise tests.

Genetic variation

We used average heterozygosity per locus (H) and percentage of polymorphic loci (P) to measure **electrophoretically-detectable** genetic variation within the study populations. Assuming a population is in

Table 3.- Enzyme **loci** examined **electrophoretically** for 1987 Bristol Bay genetic stock **identification** study of sockeye and chum salmon with Enzyme Commission (**E.C.**) numbers (**IUB** 1984). Tissues are: M (muscle), L (liver), E (eye), and H (heart). Buffers include: AC (Clayton and Tretiak 1972) pH 6.1 to 6.8; EBT (**Boyer** et al. 1963) pH 8.6; TC (**Schall** and Anderson 1974) pH 5.8; RW (**Ridgeway** et al. 1970) pH 8.2. Loci in parentheses are duplicate pairs (**isoloci**).

Enzyme	E.C. number	Loci	Tissue	Buffer
Acid phosphatase	3. 1. 3. 2	Acp1,2	H	TC ,AC
Aconitate hydratase	4. 2. 1. 3	Ah1	L	AC, TC
Adenosine deaminase	3. 5. 4. 4	Adal, 2 ^a	L,H	AC
Adenylate kinase	2. 7. 4. 3	Ak	M	AC
Alanine amino transferase	2. 6. 1. 2	Alat	M	AC
Aspartate aminotransferase	2. 6. 1. 1	Aat(1,2) Aat4	M L	EBT,AC EBT,AC
Creatine kinase	2. 7. 3. 2	Ck1,2 Ck5	M E	RW RW
Esterase-D	3. 1. 1. 1	Es t-D	M,H	EBT
Fructose biphosphate aldolase	4. 1. 2. 13	Ald1,2,3^b	E	AC
Fumarate hydratase	4. 2. 1. 2	Fh	H	TC
Glucose phosphate isomerase	5. 3. 1. 9	Gpi(1,2) Gpi3	M E,M	RW RW
Glutathione reductase	1. 6. 4. 2	Gr	L,H	AC
Glyceraldehyde phosphate dehydrogenase	1. 2. 1. 12	Gap3,4	E	AC
α-glycerophosphate dehydrogenase	1. 1. 1. 8	G3pl G3p1,2,3^c	M H	AC, EBT AC,EBT
Guanine deaminase	3. 5. 4. 3	Gda	L	AC
Isocitrate dehydrogenase	1. 1. 1. 42	Idh1,2 Idh3,4	H,M L	AC AC
Lactate dehydrogenase	1. 1. 1. 27	Ldh1 ,2 Ldh4 Ldh3,4,5	M L E	RW RW RW
Malate dehydrogenase (NAD)	1. 1. 1. 37	Mdh(1,2)^b Mdh(3,4)	L M	AC AC
Malate dehydrogenase (NADP)	1. 1. 1. 40	mMdhp1 ,2 Mdhp1	M H	AC TC ,AC
Mannosephosphate isomerase	5. 3. 1. 8	Mpi	H	EBT

Table 3.- Continued.

Enzyme	E.C. number	Loci	Tissue	Buffer
Peptidase	3.4.11			
Leucyl-glycyl-glycine		Tapep	H,M	AC
Leucyl-tyrosine		Pep-LT ^c	L	AC
Phosphoglucomutase	2.7.5.1	Pgm2	M	RW
6-Phosphogluconate				
dehydrogenase	1.1.1.44	Pgdh	M,L	AC
Sorbitol dehydrogenase	1.1.1.14	Sdh1 , 2 ^b	L	RW
(Iditol)				
Superoxide dismutase	1.15.1.1	Sod1 ^b	L,H	RW , EBT
Triosephosphate isomerase	5.3.1.1	Tpi1,2 Tpi1,2,3,4	M E	AC AC

^aPolymorphic in chum, but poorly resolved.

^bDeleted from chum baseline screen.

^cAdded to chum baseline screen.

polymorphic loci (P) to measure **electrophoretically-detectable** genetic variation within the study populations. Assuming a population is in random mating proportions, H is defined as the expected frequency of individuals heterozygous (having a variant) at a particular locus:

$$H = 1 - \sum_{i=1}^n p_i^2$$

where n equals the number of alleles and p_i equals the frequency of the **ith** allele. The expected heterozygosity per individual per locus within each population was calculated by summing the single-locus heterozygosities and dividing by the total number of loci studied.

A locus that has its most common allele present in a frequency less than or equal to 0.99 is considered polymorphic. The percent of polymorphic loci (P) is determined by dividing the number of variable loci by the total number examined **electrophoretically** and multiplying by 100.

Genetic similarity

Genetic relatedness between populations was measured using the genetic identity (I) of Nei (1972). When two populations are **electrophoretically** indistinguishable, sharing the same alleles at all loci, their genetic identity is defined as 1.0. Complete genetic divergence (I= 0.0) is indicated by fixed allele substitutions at all loci. Genetic identity values represent the probability of sampling the same allele from two populations and are a normalized measure of genetic relatedness within or between species.

In this study, genetic identity values were calculated using only polymorphic loci, which overestimates the differences between populations. To graphically depict relationships between collections of salmon from Bristol Bay drainages, we used an unweighed pair-group clustering algorithm (**UPGMA: Sneath and Sokal** 1973). The clustering analysis calculates the averaged gene identity values between populations and produces a dendrogram based on the observed **allelic** similarity over all loci studied.

Gene diversity analysis

When measuring genetic divergence, the combined total variation of all stocks of a species in a region can be partitioned to determine how the variation is distributed within and between the stocks. We used two measures for determining the source of genetic variation. First, the hierarchical gene diversity analysis of Chakraborty (1980) partitions the **total** amount of genetic variation within a subdivided population:

$$H_T = H_S + D_{ST}$$

where H_T is the total gene diversity (heterozygosity) if all the samples are considered as a single randomly mating unit, H_S is the average heterozygosity within each subpopulation or stock, and D_{ST} represents that portion of genetic variation due to differences between subpopulations. The relative diversity represents the percent of total variation due to differences between stocks from different drainages.

For this hierarchical statistic, the eleven sockeye salmon collections from different drainages were each treated as different **subpopulations** within a larger geographic region (Bristol Bay). All six chum salmon collections were treated as separate **subpopulations** that were secondarily partitioned into areas of Bristol Bay. Herendeen Bay and Nelson Lagoon chum salmon collections represent the southwest area of Bristol Bay, based on both the genetic identity value and the geographical separation from the other subpopulations. The **Togiak**

River collection represents the northwest area of Bristol Bay. The King Salmon, **Alagnak**, and Mother Goose collections are both genetically and geographically close, and represent the geographic area of inner Bristol Bay. All the separate areas (southwest, northwest, and inner) were then compared at the highest hierarchical level.

Second, the coefficient of genetic divergence (**G_{ST}**) value of Nei (1973) was used as a normalized measure of differentiation among populations from different drainages. A value of 1.0 indicates complete genetic divergence among subpopulations. **G_{ST}** is estimated as $1 - (H_S / H_T)$.

Gene tic stock identification

We tested the effectiveness of the GSI method on populations of Bristol Bay sockeye and chum salmon by constructing artificial mixed-fishery samples of known composition. Artificial mixtures were analyzed relative to baseline data using the maximum-likelihood estimate program provided by Sam Nelson and Jerome **Pella** (National Marine Fisheries Service, Auke Bay, Alaska). Standard deviations were calculated from **re-samplings** via a bootstrapping algorithm (Efron 1982).

First, a mixed-stock fishery was constructed for each species by pooling all individuals (from all drainages) into a single group. The ability of the GSI program to discriminate between stocks, each making an equal contribution, was tested by analyzing this known mixture with 200 **re-sampling** iterations.

Second, incremental mixed-stock simulations were used to determine the accuracy and precision of GSI estimates on a stock-by-stock basis. We constructed artificial mixed stocks by adding percentages of a given population to a mixture at 20% increments (from 0 to 100% as in **Beacham et al. 1985**). The remainder of the artificial mixed stock was constructed of equal contributions of data from the other populations. The GSI program was run 100 times on each mixture using bootstrap **resampling**. Standard deviations for the 100 estimates were used to evaluate the precision of each point estimate.

RESULTS

Sockeye salmon

Of the protein-coding loci studied, we found only four variable loci that could be reliably scored in sockeye salmon: **Pgm2**, **Ldh4**, **Mdh1,2**, and **Mdh3.4**. A polymorphic **Alat** muscle locus used by Grant (1980) was variable in our collections as well, but lacked sufficient resolution to be useful in our analysis. The allele frequencies for all polymorphic loci were calculated for each collection (Appendix A).

Genotypic distributions

The genotypic distribution of the loci studied do not deviate significantly from expected Hardy-Weinberg proportions with the

exception of Pgm2 in Bear River sockeye salmon ($P < 0.025$). The other two variable loci in this populations (Ldh4, Mdh1.2) show no departure from expected proportions so the samples were probably collected from a population mating at random.

MDH phenotypes are derived from duplicated gene pair with indistinguishable nobilities for the alleles at either locus. We treated each duplicate pair as two disomic loci to simplify the analyses; variant alleles were arbitrarily assigned to one locus.

Allele frequency heterogeneity

The multiple-simultaneous G-test analysis for heterogeneity of allele frequencies among all collections of Bristol Bay sockeye salmon indicate that there are sufficient differences that prohibit them from being pooled into a large homogeneous group ($P < 0.001$). The pairwise G-tests support this hypothesis as 31 of 55 comparisons produced significant G-values (Table 4).

Electrophoretic data are available from two previous studies of Bristol Bay sockeye salmon. Based on the two variable loci that could be compared among collections (Ldh4, Pgm2). There are no significant allele frequency differences between Wood River fish collected in 1976 (Grant 1980) and those from this study ($P > 0.05$). The same is true for fish collected from the **Nushagak** River in 1976 (Grant et al. 1980) and those we collected in 1987 ($P < 0.05$). **Wilmot** et al. (1985) have data

Table 4.- PairWise comparisons of allele frequency heterogeneity between sockeye salmon populations. G-values and degrees of freedom were summed over all variable loci that could be compared between populations. The probability values were adjusted to reflect multiple tests (Cooper 1968).

1	Nushagak											
2	Egegik	*										
3	Wood	*	I-is									
4	Bear	**	**	ns	-							
5	Igushik	*	ns	ns	ns	-						
6	Nelson	*	ns	ns	*	ns						
7	Togiak	**	*	**	**	*	*	-				
8	Naknek	*	*	ns	**	**	*	ns	*	-		
9	Ugashik	ns	ns	ns	ns	ns	ns	ns	*	-		
10	Brooks	**	**	**	ns	**	**	**	**	**	**	-
11	Kvichak	*	ns	ns	**	ns	ns	*	ns	ns	**	-
		1	2	3	4	5	6	7	8	9	10	11

* = P<0.05
 ** = P<0.01
 ns = not significant

for 64 fish collected from the Brooks River in 1984. No significant allele frequency differences were detected for Pgm2 and Ldh4 when compared to 1987 spawners taken from the same area (P>0.05).

Genetic variation

Subpopulation heterozygosities (H_S) range from a low of 0.007 in Brooks Lake sockeye salmon to 0.015 in both Togiak and Naknek collections (Table 5). The average subpopulation heterozygosity over all

Table 5.- Heterozygosities for eleven populations of Bristol Bay sockeye salmon. Averaged, single-locus values for H within populations are listed with standard errors. Population heterozygosity (H_S) is based on a total of 50 loci. Average population heterozygosity was calculated by averaging H_S over all populations. P equals the proportion of loci that are polymorphic.

Popu- lation	Loci				P	H_S
	Ldh4	Pgm2	Mdhl	Mdh3		
Bear	0.095	0.308	0.049	0.000	0.060	0.009
Brooks	0.104	0.226	0.039	0.000	0.060	0.007
Egegik	0.226	0.403	0.010	0.000	0.060	0.013
Igushik	0.164	0.385	0.020	0.000	0.060	0.011
Kvi chak	0.211	0.412	0.010	0.000	0.060	0.012
Naknek	0.314	0.424	0.030	0.000	0.060	0.015
Nelson	0.203	0.370	0.000	0.000	0.060	0.011
Nushagak	0.121	0.476	0.030	0.000	0.060	0.013
Togiak	0.248	0.380	0.030	0.077	0.080	0.015
Ugashik	0.172	0.399	0.020	0.020	0.080	0.012
Wood	0.104	0.385	0.000	0.000	0.060	0.010
Average	0.178	0.379	0.022	0.009	0.064	0.0116
S.E.	(0.070)	(0.065)	(0.016)	(0.023)	(0.080)	(0.002)

collections is 0.012.

The percent polymorphic loci (P) is either 0.060 or 0.080 for each population since only three or four loci out of 50 are variable (Table 5). The Alat muscle locus was not used for these estimates as our data are incomplete.

Genetic similarity

Genetic identity estimates between population pairs exceed 0.980 and are based only on inclusion of polymorphic loci in the data base (Table 6). The mean identity estimate (I) over all comparisons is 0.996 ± 0.003 . No allele substitutions were observed at any locus between any of the populations. The results of the cluster analysis of genetic similarity among the 11 Bristol Bay sockeye salmon populations is presented as a dendrogram (Figure 2).

Gene diversity analysis

Ninety-eight percent of the total gene diversity in Bristol Bay sockeye salmon is due to differences among individuals within populations (Table 7). On average, only 2% of this diversity can be attributed to differences between populations. The estimate of population differentiation, measured as G_{ST} , is **0.03**.

Genetic stock identification

In the mixed fishery simulations with all populations of Bristol Bay sockeye salmon equally represented, the contributions of Egegik, Wood, Igushik, and **Kvichak** stocks are underestimated at zero even though each actually represented 9.1% of the artificial mixture (Figure 3, Appendix c). Nelson and **Nushagak** sockeye salmon are overestimated at 33.3% and **23%**. Brooks, Bear, Naknek, and **Ugashik** contributions are 16.2%, 4.7%,

Table 6.- Matrix of Nei's (1972) gene identity values between 11 populations of Bristol Bay sockeye salmon.

1 Nushagak	-											
2 Egegik	.995	-										
3 Wood	.995	.998	-									
4 Bear	.989	.996	.998	-								
5 Igushik	.995	.999	1.000	.998	-							
6 Nelson	.993	1.000	.999	.998	1.000	-						
7 Togiak	.992	.999	.998	.996	.999	.999	-					
8 Naknek	.993	.999	.994	.991	.997	.997	.998	-				
9 Ugashik	.996	1.000	1.000	.997	1.000	1.000	.999	.997	-			
10 Brooks	.981	.993	.995	.999	.995	.996	.994	.987	.994	-		
11 Kvichak	.996	1.000	.999	.996	1.000	.999	.999	.998	1.000	.992	-	
	1	2	3	4	5	6	7	8	9	10	11	

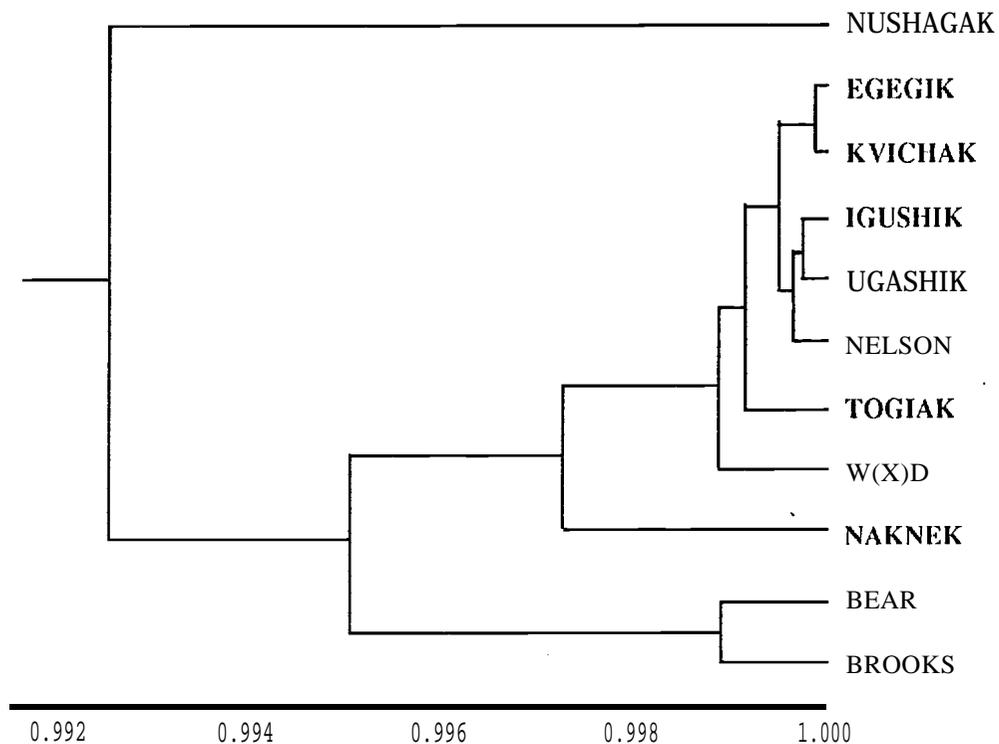


Figure 2.- Dendrogram depicting genetic relationships among 11 populations of Bristol Bay sockeye salmon. Clustering is based on unweighed averages of Nei's (1972) genetic identity values. The values in this table are based on polymorphic loci only.

Table 7.- Distribution of **electrophoretically** detectable gene diversity among 11 populations of Bristol Bay sockeye salmon. The absolute gene diversity averages are based on 50 gene loci (46 that are monomorphic).

Locus	Absolute gene diversity		Relative gene diversity (%)	
	Total (H_T)	Within populations (H_S)	Within populations	Between populations
Ldh4	0.182	0.178	98.0	2.0
Mdhl	0.022	0.021	99.3	0.7
Mdh3	0.009	0.008	97.0	3.0
Pgm2	0.386	0.379	98.0	2.0
Average	0.012	0.012	98.1	1.9
S.E.	(0.060)	(0.058)		

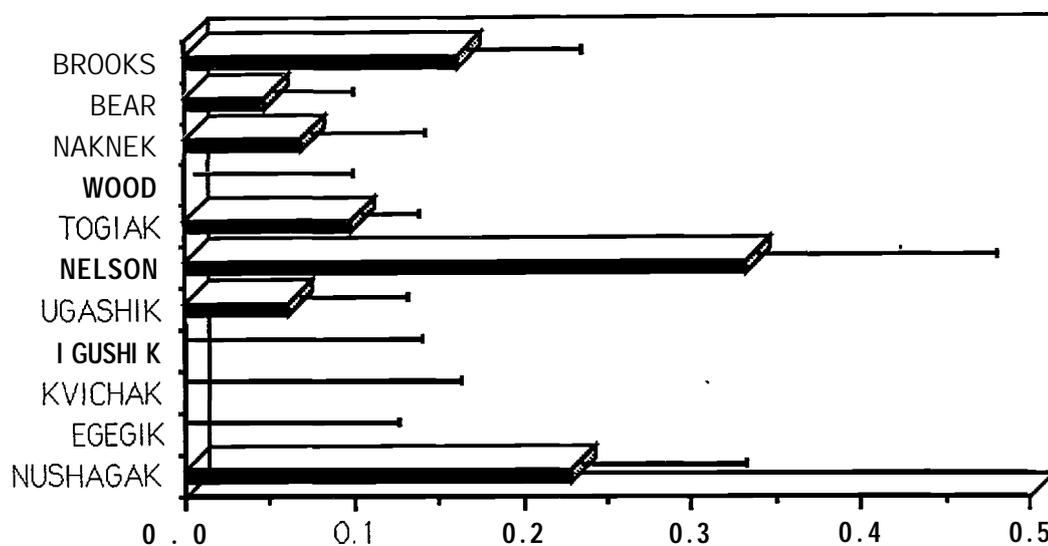


Figure 3.- Percent composition estimates for a mixed fishery (N = 1100) constructed from equal contributions of Bristol Bay sockeye salmon populations. Each population comprised 9.1% of the simulated fishery. Error bars represent one standard deviation calculated from 200 bootstrap resampling iterations.

6.9%, and 6.1%, respectively. The estimated contribution of Togiak River sockeye salmon (9.9%) is the most accurate.

From the incremental stock identification simulations, Bear, Brooks, Naknek, and Nushagak sockeye have the most accurate estimates and the smallest standard deviations (Figure 4). Composition estimates for populations within that cluster on the **dendrogram** above 0.999 (**Egegik, Igushik, Kvichak, Nelson, and Ugashik**) are generally poor and have large standard deviations. Togiak and Wood sockeye salmon cluster close to the major group on the dendrogram, but are distinguishable from each other in a mixed fishery due to polymorphism at the **Mdh3.4** locus in the **Togiak** stock.

Chum salmon

Twelve variable loci were scored in chum salmon (Appendix B). More enzyme systems are variable (e.g. Gala, Adal, **Mdh1**, and **Mdh2**), but could not be reliably scored. Duplicated loci [i.e. **Aat(1,2)**; **Mdh(3,4)**] were treated as previously described for sockeye salmon, with variation arbitrarily assigned to one locus of the pair.

Genotypic distributions

Only the genotypic distribution at the Idh3 locus in **Alagnak** chum salmon deviates significantly from random mating proportions ($P < 0.001$). The other variable loci scored in the **Alagnak** collection

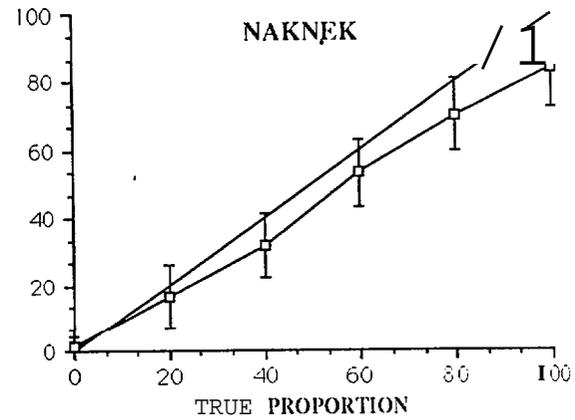
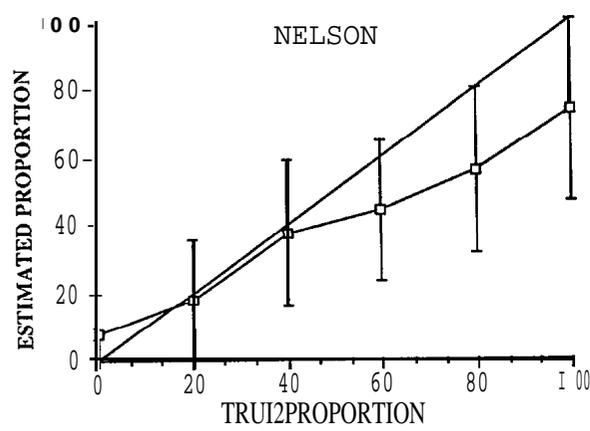
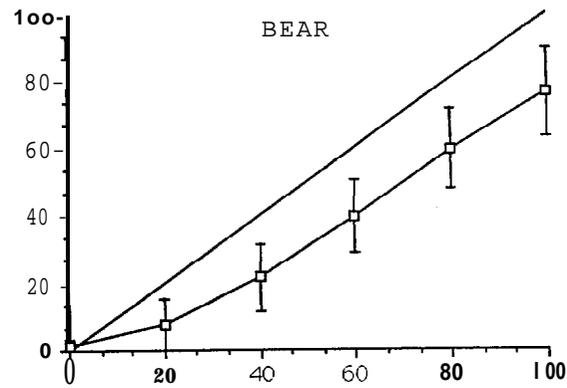
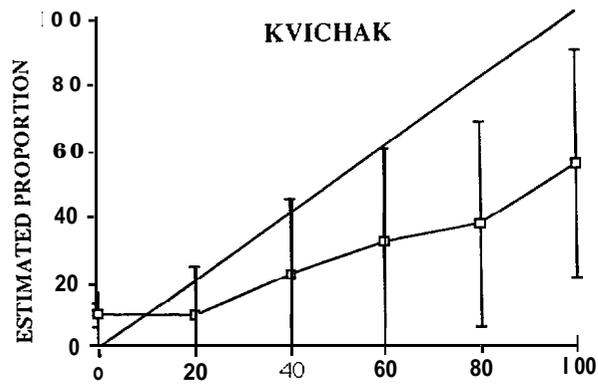
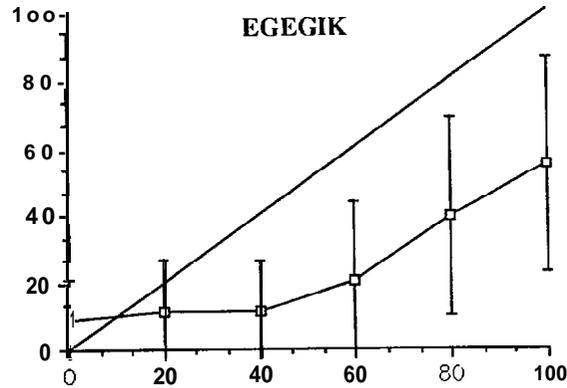
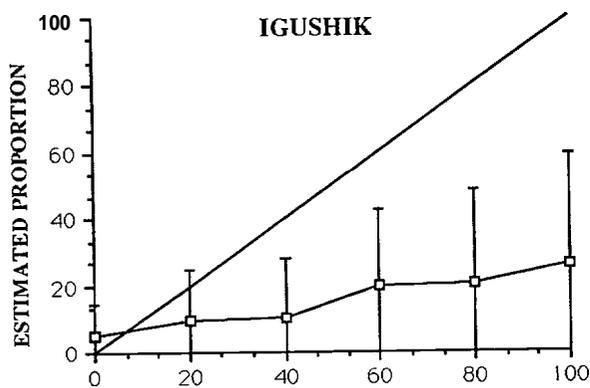


Figure 4.- Graphs of GSI-estimated stock proportions in simulated mixed stocks of Bristol Bay sockeye salmon. Point estimates are the mean of 100 **resamplings** (accompanied with ± 1 standard deviation). All mixtures contained 1100 fish.

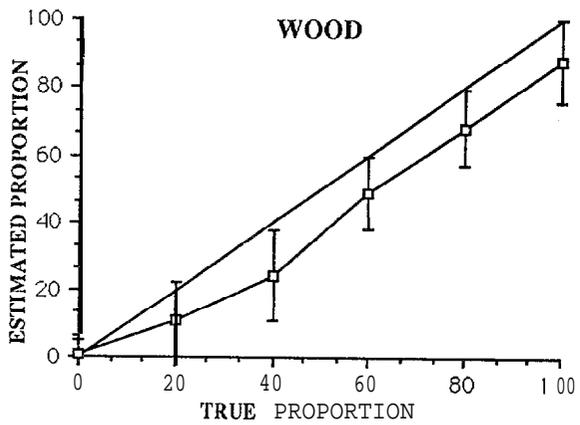
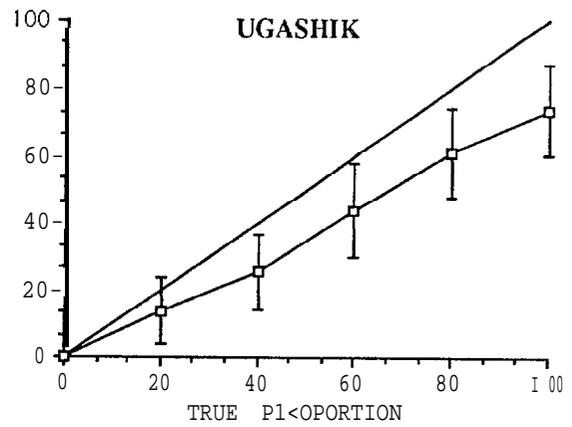
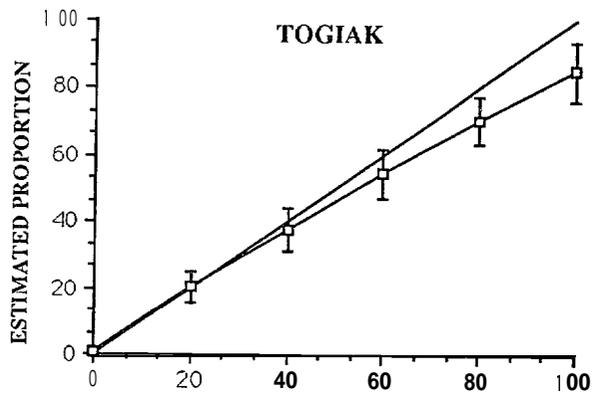
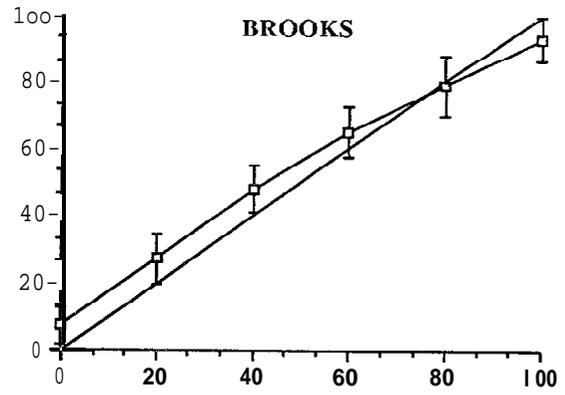
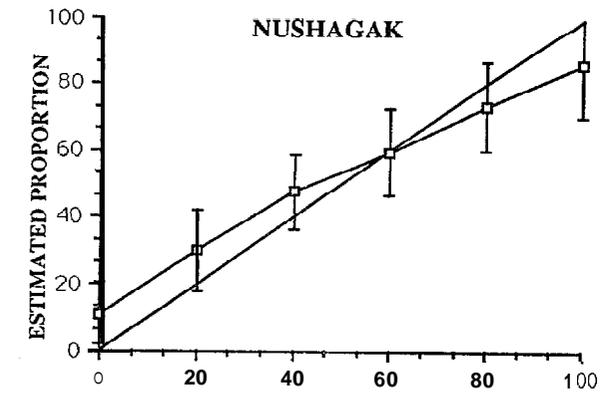


Figure 4.- Continued

were in expected proportions so the **genotypic** distribution at Idh3 may be a result of chance **oversampling** of the heterozygous genotype.

Allele frequency heterogeneity

Pairwise comparisons show that the **allele frequencies of Alagnak, King Salmon, and Mother Goose chum salmon** populations are not significantly heterogeneous (Table 8). Comparisons between all the other populations are statistically significant (G-test; **P<0.01**).

Genetic variation

The Idh3 locus has the highest measure of variability ($H_T = 0.614$) when averaged over all populations (Table 9). Peptidase loci (**TaPep** and **Pep-LT**) contribute the least to detectable gene variation found in

Table 8.- **Pairwise** comparisons for significant allele frequency heterogeneity between Bristol Bay chum salmon populations. G-values and degrees of freedom were summed over all variable loci that could be compared between populations.

1	King Salmon						
2	Nelson	*					
3	Togiak	*	*				
4	Alagnak	ns	*	*			
5	Mother Goose	ns	*	*	ns		
6	Herendeen	*	*	*	*	*	
		1	2	3	4	5	6

* = $P < 0.01$

ns = not significant

Table 9.- Distribution of **electrophoretically** detectable gene diversity among six populations of Bristol Bay chum salmon. The absolute gene diversity averages are based on 42 loci (30 that are monomorphic).

Locus	Absolute gene diversity		Relative gene diversity (%)		
	Total (H_T)	Within populations (H_S)	Within populations	Between populations in areas	Between populations between areas
Aat1	0.286	0.277	96.8	0.2	3.0
Est-D	0.396	0.325	82.1	0.4	17.6
G3p2	0.225	0.222	98.7	0.2	1.1
Idh1	0.168	0.157	93.3	3.0	3.7
Idh3	0.639	0.612	95.8	2.1	2.1
Ldh1	0.175	0.160	91.6	0.9	7.5
TaPep	0.199	0.188	94.6	3.6	1.8
Pep-LT	0.037	0.036	97*5	0.1	2.4
6Pg	0.041	0.041	99.2	0.3	0.5
Mdh3	0.074	0.073	98.1	0.5	1.4
mMdhp2	0.320	0.309	96.8	0.5	2.7
Mpi	0.197	0.189	96.1	3.1	0.8
Average	0.066	0.062	93.9	1.4	4.7
S.E.	(0.137)	(0.128)			

Bristol Bay chum salmon. The overall population and subpopulation **heterozygosities** (H_T and H_S) are 0.066 and 0.062, respectively (Table 9). The normalized measure subpopulation divergence (G_{ST}) is **0.06**, averaged over all loci.

Genetic similarity

The gene identity values between Bristol Bay chum salmon collections range from 0.966 to 0.997. The greatest amount of divergence (low value of I) is between **the** Herendeen and Togiak River populations (I = 0.966, Table 10). The identity values between Herendeen and Mother Goose; Herendeen and King Salmon; and Togiak and Nelson river populations have nearly the same similarity relationships (I = 0.969 - 0.970). Little divergence is found among the **Alagnak**, King Salmon, and Mother Goose collections.

The dendrogram of genetic relationships shows that Bristol Bay chum populations can be partitioned into two distinct groups (Figure 5): a northern group consisting of King Salmon, Mother Goose, **Alagnak**, and Togiak fish, and a southern group consisting of **Herendeen** Bay and Nelson Lagoon fish. Within the northern group, the **Togiak** fish are

Table 10.- Matrix of **Nei's** (1972) gene identity values between **six** populations of Bristol Bay chum salmon. Values were calculated **using** only polymorphic loci **in** the analysis.

1	King Salmon						
2	Nelson	.974					
3	Togiak	.990	.970				
4	Alagnak	.997	.978	.994			
5	Mother Goose	●997	.969	.992	.997		
6	Herendeen	.970	.986	.966	.976	.969	-
		1	2	3	4	5	6

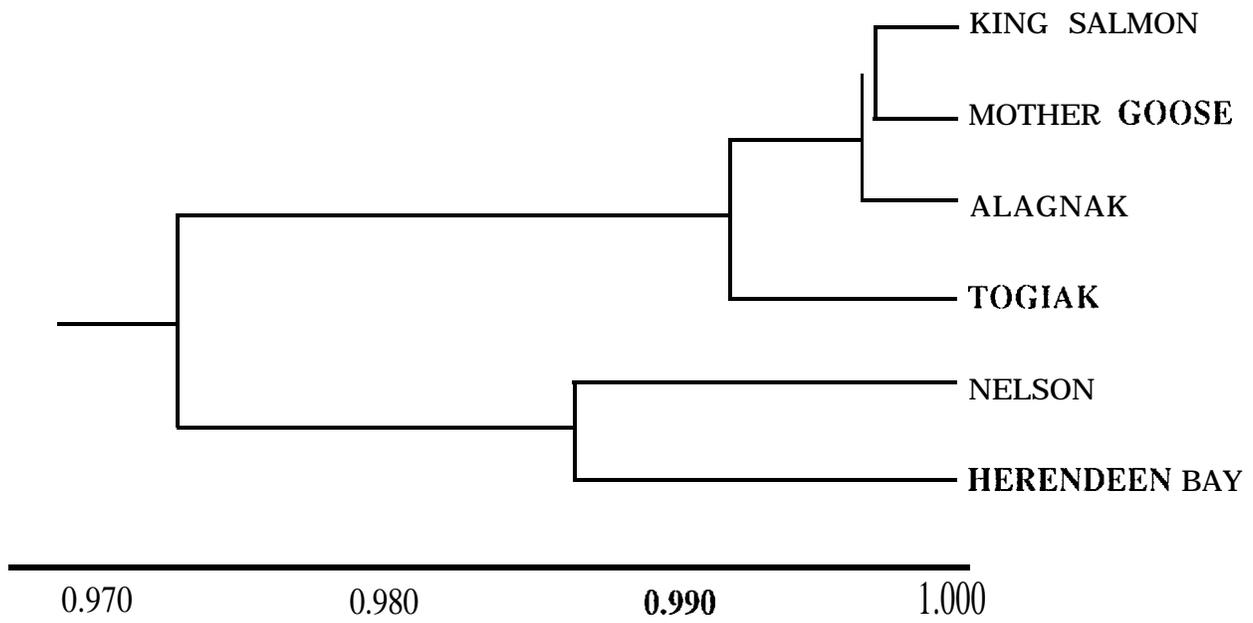


Figure 5.- Dendrogram depicting genetic relationships among six populations of Bristol Bay chum salmon. Clustering is based on unweighed averages of Nei's (1972) genetic identity values using 12 variable protein loci.

distinguishable from the other three populations. The southern group is not only different from the northern group, but also each population within the southern group is distinct.

Gene diversity

Ninety-four percent of the total **gene diversity** exists within

populations of Bristol Bay chum salmon (Table 9). Of the remaining fraction, over 4% is due to differences between areas within Bristol Bay. Relatively little gene diversity (1.5%) is due to differences between populations of the same area of Bristol Bay.

Genetic stock identification

In the mixed fishery simulation (N = 598), each of the six chum salmon collections represented 16.7% (1/6) of the artificial mixture. Resulting stock contribution estimates for Nelson Lagoon, **Togiak**, Mother Goose, and Herendeen Bay collections are approximately 16% (Figure 6). Estimates for the contributions of the King Salmon and **Alagnak** stocks to the artificial mixture are biased (12.6 and 21.8%) towards each other.

The standard deviations of the stock contribution estimates are small for Nelson, Herendeen, and Togiak chum salmon stocks (2.3%, 2.5%, and 3.4%) while inner Bristol Bay stocks (**Alagnak**, King Salmon, and Mother Goose Rivers) have standard deviations that are twice as large.

For the incremental mixed-stock simulations, Herendeen, Nelson, and **Togiak** chum salmon stocks show consistently smaller standard deviations for each estimate when compared with the other three stocks (Figure 7). There are only three cases where the estimates are not within one standard deviation of the true values (King Salmon at 80%, and Mother Goose at 80% and 100%).

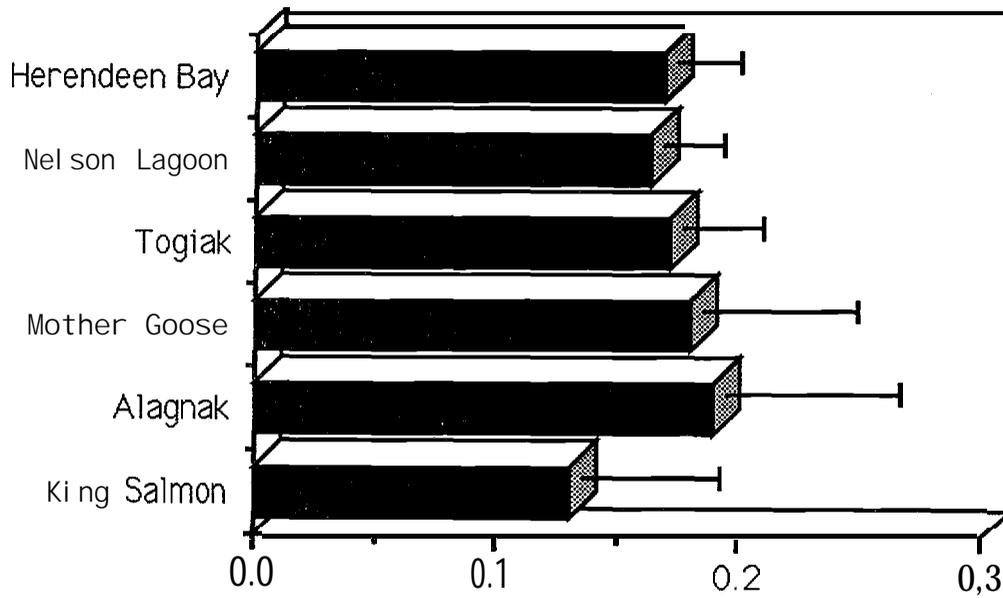


Figure 6.- Percent composition estimates for a single mixed fishery (N = 598) constructed from equal contributions of Bristol Bay chum salmon protein data. Each population comprised 16.7% of the artificial mixture. Standard deviations were calculated from 200 bootstrap resampling iterations.

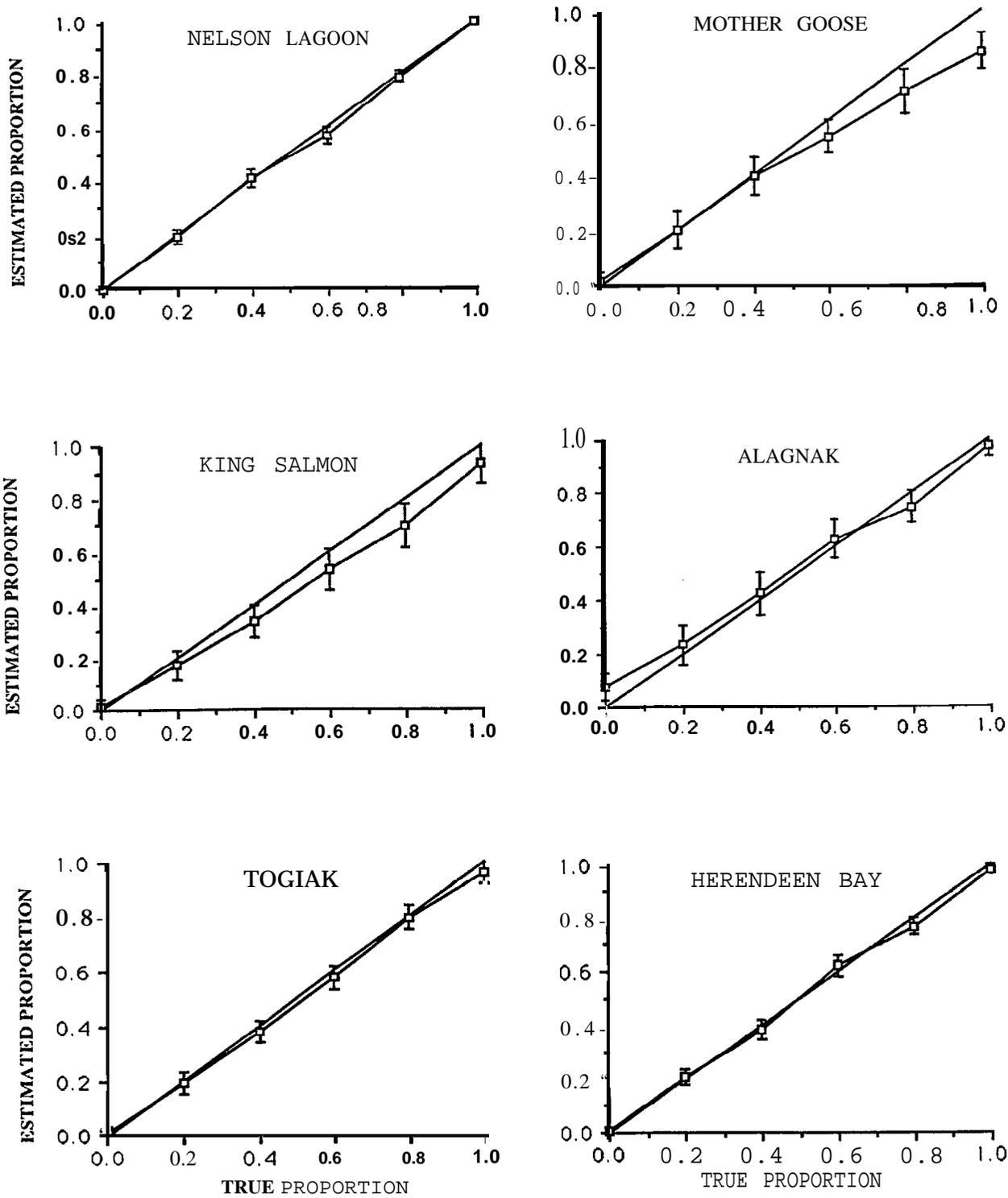


Figure 7.- Graphs of **estimated** stock proportions in **simulated** mixed stocks of Bristol Bay chum salmon. Point estimates are the mean of 100 resamplings (± 1 standard deviation). Each mixture contained 500 fish.

DISCUSSION

The effectiveness of genetic stock identification analyses depends on the amount and distribution of detectable genetic variation within and between stocks of a region. **Little** detectable variation and/or similar patterns of variation between stocks will result in such large confidence limits on the estimates as to make them useless for management decisions. Other considerations include sampling error of both the baseline stocks and of the mixed fishery. The model assumes that all baseline stocks contributing substantially to the mixed fishery have been accurately sampled. If important stocks have not been sampled, or the sample of a stock is not truly representative, then estimates of contribution to the mixed fishery will be biased. Finally, we are assuming that the gene frequencies are stable from year to year and will not have to be validated every year.

Comparisons of our gene frequency results for sockeye salmon with studies **in** previous years (Grant 1980, **Wilmot** et al. 1985) showed that no **significant** differences **exist** between collections from different years. We therefore feel confident that our baseline data for sockeye salmon do not need to be validated yearly. No previous results for chum salmon of the Bristol Bay area are available for comparative purposes, but such comparisons **will** be made **with** samples taken **in** the 1988 **field** season.

Our work in Bristol Bay has shown that there is adequate genetic

variation between stocks of chum salmon to allow accurate estimates of stock contribution in a mixed fishery. The stock contribution estimates made by the GSI program on the artificial mixed fishery (with every stock equally represented) are close to the true value (Figure 6). The results of the incremental mixed fishery (each stock added at 20% increments) are also very accurate, and with only three exceptions, the estimates are within one standard deviation of the true value (Figure 7). In general, the estimates are most accurate and the standard deviations the smallest when stock contributions are extreme (0 or 100%).

In contrast, composition estimates for sockeye salmon stocks are much less accurate. Only four variable enzymes could be reliably scored in Bristol Bay sockeye salmon. A fifth locus (**Alat**) is variable but could not be resolved on a consistent basis. The amount of genetic variation detected in stocks of Bristol Bay sockeye salmon is low compared to chum salmon stocks, but similar to estimates for sockeye salmon in other studies (Grant 1980, Grant et al. 1980, **Wilmot** et al. 1985, Utter et al. 1984, **Wilmot** and Burger 1985, Wishard 1980).

Estimated contributions of 11 sockeye salmon populations to an artificial mixed fishery (with every stock equally represented at 9%) show how these estimates are biased when attempting to discriminate between genetically similar stocks (Figure 3). Stock contribution estimates for Egegik, Wood, **Igushik**, and Kvichak fish are strongly underestimated. Only the estimated contribution for Togiak River fish

was accurate. **Ugashik**, Naknek, Togiak, Brooks, Bear River sockeye salmon are close, and Nelson and **Nushagak** stocks are overestimated in the artificial mixture.

The results of the incremental mixed-fishery analysis (stocks added at 20% increments) again show the problems encountered with sockeye salmon composition estimates. Estimates for Naknek, **Nushagak**, Brooks, Togiak, and Wood River sockeye salmon are generally within one standard deviation of the true value. The estimated contributions for **Ugashik**, Nelson and Bear River fish are not within the confidence limits, but do increase linearly with their true contribution. The estimates for **Igushik**, Egegik, and **Kvichak** River salmon are poor, with large standard deviations throughout the range of their true contributions (Figure 4).

Our sample size from each system was adequate for accurate gene frequency estimates of the total population in chum salmon. Computer simulations by Wood et al. (1987) found that accuracy in stock composition estimates did not improve substantially by increasing the size of the baseline sample. The situation for sockeye salmon may be different. Part of the problem with the estimates of sockeye salmon stock contributions may be due to more complex breeding structures in certain river systems. The most accurate estimates are for the Naknek River Drainage where we had samples from many tributaries within the system. Only a single collection was taken from the other drainages and each single collection may not accurately reflect the genetic diversity of sockeye populations for these systems. We recommend that

complex systems be sampled more thoroughly in the 1988 field season. This would involve determining the major spawning areas within these large drainages, and sampling from the spawning grounds.

There are two other methods that could greatly improve the contribution estimates. The first would be to intensify our efforts to resolve more enzyme loci so that we get more information from each fish sampled.

Alat is a highly variable enzyme in sockeye salmon and successful resolution should improve our estimates substantially. The second method is to investigate the incidence in sockeye salmon of brain parasites. This method is currently useful in separating sockeye salmon stocks in southeast Alaska (Adam Moles, National Marine Fisheries Service, Juneau, personal communication) when used in conjunction with genetic stock identification techniques. The incidence of this parasite is treated as an additional character and incorporated into the **GSI** program, because it is present in some stocks but not in others.

Successful stock contribution estimates to an offshore mixture of chum salmon now requires only an adequate sample, and an assurance that we have sampled all the major contributors for our baseline. We are continuing our discussions with the fisheries managers in the Bristol Bay region to ensure our baseline is complete. For sockeye salmon, more work on the genetic baseline is necessary to resolve the problems outlined above, so that we can begin to determine stock origins with confidence.

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Appendix A.- Allele frequencies for variable loci in eleven populations of Bristol Bay sockeye salmon. Variation at duplicate loci [Mdh(1,2) and Mdh(3,4)] is attributed to a single locus of the pair for these calculations.

Population	N	Ldh4		Pgm2		Mdh1			Mdh3		
		100	117	100	135	100	147	58	100	122	60
Bear	100	.950	.050	.810	.190	.975	.025	-	1.000	-	-
Brooks	100	.945	.055	.870	.130	.980	-	.020	1.000	-	-
Egegik	100	.870	.130	.720	.280	.995	-	.005	1.000	-	-
Igushik	100	.910	.090	.740	.260	.990	.010	-	1.000	-	-
Kvichak	100	.880	.120	.710	.290	.995	.005	-	1.000	-	-
Naknek	100	.805	.195	.695	.305	.985	.005	.010	1.000	-	-
Nelson	100	.885	.115	.755	.245	1.000	-	-	1.000	-	-
Nushagak	100	.935	.065	.610	.390	.985	.015	-	1.000	-	-
Togiak	100	.855	.145	.745	.255	.985	.015	-	.960	.040	-
Ugashik	100	.905	.095	.725	.275	.990	-	.010	.990	.005	.005
Wood	100	.945	.055	.740	.260	1.000	-	-	1.000	-	-

Appendix B.- Allele frequencies for variable loci in six populations of Bristol Bay chum salmon. Variation at duplicate loci [Mdh(3,4) and Aat(1,2)] is attributed to a single locus of the pair for these calculations.

		Population ^a					
		KS	NN	TK	AK	MG	HN
Aat1	100	.845	.776	.918	.910	.888	.745
	118	.155	.208	.082	.090	.102	.220
	81		.016			.010	.035
	N	100	96	98	100	98	100
Est-D	100	.571	.899	.520	.625	.500	.929
	87	.429	.202	.480	.375	.500	.072
	N	98	99	98	100	100	98
G3p2	100	.935	.832	.852	.949	.910	.869
	90	.065	.168	.148	.051	.090	.131
	N	100	98	98	99	100	99
Idh1	100	.939	.925	.985	.955	.910	.785
	55	.061	.075	.015	.045	.090	.215
	N	99	100	98	100	100	100
Idh3	100	.505	.380	.495	.459	.430	.415
	88	.378	.200	.490	.465	.460	.445
	36	.056	.225	.015	.066	.075	.065
	25	.061	.195		.010	.035	.075
	N	98	100	98	99	100	100
Ldh1	-100	.715	.975	.954	.815	.760	.920
	-50	.285	.025	.046	.185	.240	.080
	N	100	100	98	100	100	100
Mdh3	100	.975	.930	.985	.995	.990	.945
	125	.025	.070	.005		.005	.030
	75			.010	.005	.005	.025
	N	100	100	98	100	100	100
mMdhp2	100	.783	.825	.699	.755	.810	.895
	127	.217	.175	.301	.245	.190	.105
	N	99	100	98	100	100	100
Mpi	100	.935	.960	.857	.939	.934	.805
	90	.065	.040	.143	.061	.066	.195
	N	100	100	98	99	99	100
Tapep	-100	.950	.930	.944	.920	.880	.765
	-185	.050	.065	.051	.070	.115	.230
	-150		.005	.005	.010	.005	.005
	N	100	100	98	100	100	100
Pep-LT	100	.975	1.00	.949	.970	.985	1.00
	82	.025	.000	.051	.030	.015	.000
	N	100	100	98	100	100	100
6Pgdh	100	.965	1.00	.969	.970	.970	.980
	85	.035	.000	.0031	.030	.030	.020
	N	100	100	98	100	100	100

^a KS=King Salmon; NN=Nelson; TK=Togiak; AK=Alagnak; MG=Mother Goose; HN=Herendeen.

Appendix C.- Estimated composition of artificial mixed stock made up of equal contributions of protein data from eleven populations of Bristol Bay sockeye salmon collected in 1987. Standard deviations were calculated from 200 bootstrap resampling iterations.

Population	Estimate	Standard deviation
Nelson River	0.333	0.140
Nushagak River	0.229	0.096
Brooks River	0.162	0.066
Togiak River	0.098	0.033
Naknek River	0.069	0.066
Ugashik River	0.061	0.063
Bear River	0.047	0.047
Egegik River	0.000	0.119
Kvichak River	0.000	0.157
Igushik River	0.000	0.134
Wood River	0.000	0.093

Appendix D.- Estimated composition of artificial mixed stock made up of equal contributions of protein data from six populations of Bristol Bay chum salmon collected in 1987. Standard deviations were calculated from 200 bootstrap **resampling** iterations.

Population	Estimate	Standard deviation
King Salmon	0.131	0.056
Alagnak	0.189	0.071
Mother Goose	0.179	0.063
Togiak	0.171	0.034
Nelson Lagoon	0.162	0.025
Herendeen Bay	0.168	0.027