

FISHERIES RESEARCH INSTITUTE  
College of Fisheries  
University of Washington  
Seattle, Washington 98195

A BIOCHEMICAL INVESTIGATION OF KODIAK ISLAND  
PINK SALMON GENE FREQUENCIES

by

Robert F. Donnelly, Kenneth R. Johnson  
William K. Hershberger, and Donald E. Bevan

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## INTRODUCTION

Presently, the Kodiak pink salmon fishery is considered by many to be one of the best managed salmonid fisheries on the West Coast of the United States. However, there is the question of whether each stock within a river system is harvested commensurate with its population size and reproductive capability. The ability to separate stocks within the mixed fishery would be a significant aid to management personnel in addressing this question.

The Fisheries Research Institute (FRI), under contract to the Alaska Department of Fish and Game (ADF&G), has been investigating possible use of biochemical genetic markers to distinguish between stocks of pink salmon. We have analyzed adult samples from 29 streams and emergent fry from 35 streams. However, due to limited sample size, only 26 (with  $\geq 10$  samples) of the emergent fry streams were included in the analysis. In 16 cases, the emergent fry were the progeny of the stocks sampled for the adults. This permitted a check on our work and gave a better idea of the actual variation within the populations sampled. Exploratory staining was also done with several enzyme systems not previously employed as biochemical markers.

The question of the amount of natural variation in biochemical genetic markers within a stock was addressed for the Kodiak even-year pink salmon cycle. A complete overview of both year classes will be presented in the final report on the odd- and even-year cycle data.

## MATERIALS AND METHODS

Twenty-nine locations from Kodiak and surrounding environs were sampled during the summer of 1976 by personnel of the Kodiak office of the ADF&G. Liver, muscle, and eye tissues were taken from approximately 50 adult salmon at each location. Also, in early spring of 1977, the same group collected emergent fry samples from 35 locations in conjunction with the annual egg and larvae surveys. (Data from surveys are used in the annual pink salmon forecast.) About five fry were collected from each sample site that had fry.

All samples were labeled, frozen, and sent to FRI for laboratory processing. Prior to actual processing, the samples were stored frozen ( $-20^{\circ}\text{C}$ ).

The whole body of the fry and selected adult tissues were used for starch gel electrophoresis. Small portions of the adult tissue or, in the case of the emergent fry, the whole animal were placed in test tubes. A few drops of distilled water were added, the tissue was mashed into a paste with a glass rod, and the samples were centrifuged for several minutes. At this point, some of the supernatant was absorbed into a small piece of filter paper to be placed in a starch gel, or the samples were frozen and stored for later processing.

The electrophoretic support medium (in this case, starch gel) was made from hydrolyzed potato starch and an appropriate buffer solution (Smithies 1955; Hunter and Markert 1957). The starch gel was prepared as outlined by May (1976). A longitudinal cut was made about 2 centimeters from one edge, and the two parts were separated from one another. The small pieces of filter paper containing the supernatant were placed between the two pieces of starch gel. The starch gel with the enclosed filter paper was subjected to an electric current (about 50 milliamperes) for about 15 minutes. At the end of this time, the small pieces of filter paper were removed, ice packs (to reduce heating of the gel) were placed on the starch gel, and electrophoresis was allowed to continue for several hours (usually 2 to 3) until migration of the proteins was sufficient for satisfactory analysis.

After the enzymes had migrated an adequate distance, electrophoresis was terminated. The starch gel was sliced parallel to the supporting glass plate. The slices were placed on trays and stained for specific enzymes. The staining techniques used were from Shaw and Prasad (1970) and Allendorf (1975).

## RESULTS

Tables 1 and 2 list the enzyme systems analyzed in adults and emergent fry, respectively. The observed patterns of the enzymes that showed variation are shown diagrammatically on Figure 1. The results from the data analyses are shown in Tables 3 through 6. The common and variant allele frequencies and 95 percent confidence intervals are listed by statistical area, location of stock, and enzyme system. Figure 2 shows the statistical areas (large numbers) and stream numbers within the statistical areas. Figure 2 is reproduced from an unpublished FRI stream catalog. Summarizations of the enzyme systems are given in some detail below.

## Adults

### Alpha Glycerophosphate Dehydrogenase (AGP)

This enzyme system was one of the most easily analyzed, with all but one population showing readable results. Two populations were monomorphic, and the range of gene frequencies was 0.77 to 1.00 for the common allele. The variant form was faster migrating than that of the common allele.

### Malate Dehydrogenase (MDH)

This system is determined by two groups of duplicated loci (a total of four loci)--one group designated MDH-A and the other MDH-B (Bailey et al.1970). In the Kodiak pink salmon populations, frequencies of the common (fast migrating) form of MDH-A were 0.95-1.00. Three populations contained a very fast MDH-A variant with frequencies of 0.01-0.02 (Fig. 1). The common form of MDH-B was slower migrating than the variant allele, and the frequency of the variant form was 0.05-0.00. In only 8 of 29 populations were good readable results obtained for both MDH-A and MDH-B.

### Lactate Dehydrogenase (LDH)

Isozymes determined by five loci were stained, but only two of the five loci (LDH-1 and LDH-4) showed any variation. The variant allele for LDH-1 was slower migrating than the common form and was present in frequencies ranging from 0.00-0.04. Baumann's Creek (Table 4) was the only population to demonstrate variation at LDH-4. The variant at this locus was slower migrating than the common form and had a gene frequency of 0.02.

### Phosphoglucose Isomerase (PGI)

Only three populations showed readable results and all those were monomorphic. This is a three-locus system, with PGI-1 and 2 being determined by apparently duplicated genes.

### Phosphoglucomutase (PGM)

The common allele was slower than the variant. The gene frequencies of the variant ranged from 0.00-0.21. Two populations (Terror and Afognak Rivers) showed what appeared to be a slow variant at a very low frequency of 0.01 in both cases (Table 5). All population samples provided readable data for this enzyme system.

### Creatine Kinase (CK)

General protein stain proved very satisfactory for staining this system. Twenty-five out of 29 streams stained very clearly with 6 of the 25 populations showing variation. The common form migrated faster than the variant and had a frequency that ranged from 0.96 to 1.00.

### Juveniles

Better results were obtained for more enzyme systems on these fish than the adult tissues. Although the enzymes were more easily analyzed, the average sample size per stream was considerably lower (approximately 20 versus about 50 per stream for the adults). As a result, we are less confident of our data.

### Alpha Glycerophosphate Dehydrogenase (AGP)

All 26 populations showed good readable results. The variant enzyme migrated faster than the common form, with a range in gene frequencies from 0.08-0.28.

### Malate Dehydrogenase (MDH)

As outlined for the adult data, this enzyme system is determined by two duplicated loci (MDH-A and MDH-B). Two variant alleles were found for MDH-A. One variant (faster migrating than the common allele) was found in only three populations: Kaiugnak River, Seven Rivers (lower fork), and Kitoi Creek (Table 3); the frequency of occurrence in these populations was low (0.01-0.02). The other variant allele was slower than the common form and was found in 13 populations (Fig. 1); gene frequencies ranged from 0.01-0.15. Only Kitoi Creek had both variant alleles present in the samples. Two variant alleles were also found for MDH-B (Fig. 1). Sid Olds and Dog Salmon rivers had a rare slow variant allele with frequencies of 0.01 (Table 3). The other variant allele (faster migrating than the common form) was found in all but seven populations. Gene frequencies for the common form were from 0.83-1.00. The MDH enzyme system was successfully analyzed for all populations of juveniles.

### Lactate Dehydrogenase (LDH)

This is also a multi-locus system, with only LDH-1 and LDH-4 demonstrating variation. Five of the 26 populations showed some variation for LDH-1. With

two notable exceptions, the variant form (slower than the common allele) was fairly rare, with gene frequencies ranging from 0.03-0.01. The exceptions were Karluk River and Geographic Creek, with frequencies of occurrence of 0.07 (Table 4). Seven Rivers (upper fork) was the only sample with LDH-4 polymorphism. A fast variant (Table 4) with a low frequency (0.04) was detected.

#### Phosphoglucose Isomerase (PGI)

This enzyme system has three loci designated PGI-1, 2, and 3. Dog Salmon River had a fast migrating variant allele with a frequency of 0.01 at the PGI-1 locus. A PGI-3 variant was noted in very low frequency (0.01) for the Red River sample. All other populations (Tables 4 and 5) demonstrated no variation at any PGI locus.

#### Phosphoglucomutase (PGM)

Even though some variation was noted for the adult samples, none was detected in the juveniles. Therefore PGM was not included for the juvenile samples in Table 5.

#### Creatine Kinase (CK), Isocitrate Dehydrogenase (IDH), and Phosphomannose Isomerase (PMI)

No variation was detectable for any of the juvenile samples. IDH stained well for all populations, while for CK 19 out of 26 sites, and for PMI 16 out of 26 areas gave readable results. Since no variation was found, these enzyme systems were excluded for the juveniles in Tables 5 and 6.

#### 6-Phosphogluconate Dehydrogenase (6-PGDH)

Only one-half (13 locations) gave interpretable results. Of these, four populations showed variation. The gene frequencies of the common form in these populations ranged from 0.95-1.00. The variant form in this case was slower migrating than the common form.

#### Aspartate Aminotransferase (AAT)

Three loci determine the isozymes of this system, but only one (AAT-3) showed variation, and that from only the freshest samples. Three of the 13 populations with readable results were monomorphic. The common allele (faster

migrating than the variant) had gene frequencies of 0.92-1.00. This enzyme was usually most readily detected from eye tissue; however, the juvenile samples gave acceptable results with a mixture of other tissues included.

#### Exploratory Staining

Another aspect of the project was to screen enzyme systems that were not reported previously for pink salmon, with the objective to find additional isozymes that would be useful in stock separation. Juvenile samples were used in this screening since they gave better results for the commonly analyzed enzymes. Table 2 shows all of the enzymes stained. Glucose-6-Phosphate Dehydrogenase ( $G_6PDH$ ), ME, and CK stained well; however, the results could not be interpreted on the basis of simple Mendelian genetics. Specific genetic crosses are needed to clarify this situation. In the case of CK, we tabulated the observed results for the adults without fully understanding the genetic basis; therefore, this enzyme system will be excluded from any use in stock separation until the genetic interpretation is available.

Of the 16 isozymes listed after Sorbitol Dehydrogenase (SDH) in Table 2, 8 gave unreadable results. The others stained well but showed no heterozygosity.

#### Discussion

##### Exploratory Staining

Results of the exploratory staining indicate that additional isozymes could be used for stock separation. Whether these will exhibit polymorphism remains to be seen. We plan to screen several populations of the odd-year cycle to determine this.

##### Sample Size

Samples taken from the 29 adult populations were sufficiently large (approximately 50) for all populations to be included in the statistical analyses. The juvenile sample size, on the other hand, ranged from less than 10 to about 50. Because confidence intervals are a function of sample size, any population with less than ten samples was not analyzed. For small sample sizes, the confidence interval became so big as to make the point estimate of

gene frequency almost meaningless. Therefore, 9 of the 35 juvenile populations were excluded from this analysis.

#### Adult and Juvenile Gene Frequencies

Of 39 different areas analyzed, 16 of these had both adult and juvenile samplings. Significant differences between adult and juvenile gene frequencies were found in five populations. The following list shows the populations and the locus (loci) at which these differences occurred:

<u>Population</u>	<u>Locus</u>
Hurst Cr.	MDH-A and PGM
Terror R.	AGP
Buskin R.	LDH-1
Deadman R.	PGM
Barling R.	PGM

There are a number of possible reasons that could be used to explain these results, e.g., sampling error, selection, sub-populations within the runs, etc.; however, the most logical reason relates to the actual sampling. There are now indications that gene frequencies can vary both spatially and temporally with a given run (Utter, personal communication). The adult samples used in this study were taken from a limited area within most streams and from essentially one point in time. The juvenile samples may have the same shortcoming as the adult samples except the temporal problem is probably reduced when the manner of sampling and emergence conditions are considered. The egg digs are done such that samples were taken with the full spectrum of development, possibly eliminating some of the timing problems; however, sampling was not done in a systematic manner throughout the spawning grounds; thus, we may not have a representative sample of the entire population.

Steps are being taken to alleviate the problem. Larry Malloy of the Kodiak office of ADF&G has agreed to collect adult samples from one river system several times during the spawning run. We also plan to sample the fry more extensively from the same river later in the life cycle.

### Populations within Rivers

The two largest spawning streams on Kodiak Island are Karluk and Red rivers, with recorded spawning escapements of up to 1,000,000 pink salmon. The two rivers appear to support more than one population within each stream. The gene frequencies differed significantly for AGP and PGM between upstream and downstream spawners in both rivers. In addition, the LDH-1 frequencies were significantly different in Karluk River for upstream and downstream populations. Based on this evidence, there seem to be at least two, and possibly more, distinct populations within each of the two river systems.

### Stock Separation Capabilities

The objective of this study was to determine if gene frequency data, as determined by starch gel electrophoresis, would allow separation of separate stocks in a mixed fishery. As often happens, the answer is qualified. Nine of the populations (Table 7) appear separable from the rest of the areas studied and from each other. A mathematical technique for stock separation, maximum likelihood, is available (Grant 1977; Seeb and Wishard 1977) and would undoubtedly enable us to distinguish between the aforementioned populations. However, two problems exist: 1) The data we collected, as discussed in the two previous sections, may be subject to greater intrapopulation variation than is desirable; and 2) the maximum likelihood estimator, as developed, assumes the gene frequencies are known without error. When both of these problems are combined, the maximum likelihood estimator is questionable because of possible compounding errors. Therefore, when stock separation criteria are established, we need to test the classification system with known samples.

### SUMMARY

1. Several additional isozyme systems should be systematically screened in the odd-year cycle of pink salmon.
2. Differences between adult and juvenile gene frequencies may be due to sampling problems.
3. At least two river systems (Karluk and Red rivers) contain two, and possibly more, distinct populations each.

4. Nine populations would appear to be separable from each other and the rest of the populations.

#### CONCLUSIONS

Assuming the data as it now stands are reasonably accurate, the starch gel approach to stock separation of Kodiak pink salmon appears to have significant value. Karluk River is one of the major spawning streams on Kodiak Island and is easily separated on the basis of electrophoretic analysis. Likewise, another major Kodiak producer--a population within Red River (upstream stock)--can be distinguished. Future studies should include the analysis of scale characters and other hard parts. We believe that a combination of factors may at least partially resolve the stock separation problems of pink salmon on Kodiak Island.

#### NEED FOR ADDITIONAL STUDY

A detailed study of the spatial and temporal problems associated with returning spawners needs to be undertaken. To obtain the best genetic data we must first know to what extent the different parts of the runs differ from one another.

Other mathematical procedures should be developed for classification of individual fish. One such possibility is a quadratic discriminate function. With enough polymorphic loci, this function should allow classification of individual fish with a acceptable level of error.

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Table 1. Isozymes tested on adult tissues

Isozyme		Results
Alpha glycerophosphate dehydrogenase	(AGP)	Clear
Lactate dehydrogenase	(LDH)	Clear
Phosphoglucomutase	(PGM)	Clear
Creatine kinase	(CK)	Clear
Malic enzyme	(ME)	Genetic crosses
	Will be necessary to interpret the results	
Malate dehydrogenase	(MDH A & B)	Unreadable
6-Phosphogluconate dehydrogenase	(6-PGDH)	Unreadable
Phosphoglucose isomerase	(PGI)	Unreadable
Glucose-6-phosphate dehydrogenase	(G <sub>6</sub> PDH)	Unreadable
Isocitrate dehydrogenase	(IDH)	Unreadable
Aspartate aminotransferase	(AAT)	Unreadable
Alcohol dehydrogenase	(ADH)	Unreadable
Phosphomannose isomerase	(PMI)	Unreadable
Sorbitol dehydrogenase	(SDH)	Monomorphic
Peptidase	(PEP)	Monomorphic
Leucine aminopeptidase	(LAP)	Nothing showed

Table 2. Isozymes tested on juvenile tissues

Isozyme		Results
Alpha glycerophosphate dehydrogenase	(AGP)	Clear
Lactate dehydrogenase	(LDH)	Clear
Phosphoglucomutase	(PGM)	Clear
Creatine kinase	(CK)	Clear
Malate dehydrogenase	(MDH-A & B)	Clear
6-Phosphogluconate dehydrogenase	(6-PGDH)	Clear
Phosphoglucose isomerase	(PGI)	Clear
Isocitrate dehydrogenase	(IDH)	Clear
Aspartate aminotransferase	(AAT)	Clear
Phosphomannose isomerase	(PMI)	Clear
Malate enzyme	(ME)	Genetic crosses will be necessary to interpret the results
Glucose-6-phosphate dehydrogenase	(G <sub>6</sub> PDH)	
$\beta$ -hydroxybuterate dehydrogenase	(HBDH)	Monomorphic
Sorbitol dyhydrogenase	(SDH)	Monomorphic
Peptidase	(PEP)	Monomorphic
Triose phosphate isomerase	(TPI)	Monomorphic
Glyceraldydyde-3-phosphate dehydrogenase	(G <sub>3</sub> PDH)	Monomorphic
Acid phosphatase		Monomorphic
Aldolase		Monomorphic
Esterase		Monomorphic
Alcohol dehydrogenase	(ADH)	Unreadable
Leucine aminopeptidase	(LAP)	Unreadable
Octanol dehydrogenase	(ODH)	Unreadable
Xanthine dehydrogenase	(XDH)	Unreadable
Adenylate kinase	(AK)	Unreadable
Hexokinase	(HK)	Unreadable
$\beta$ -Glucuronidase		Unreadable
L-Alanine amino transferase		Unreadable

Table 3. Gene frequencies and 95% confidence intervals (C.I.) for AGP, MDH-A, and MDH-B; "c" designates the common form, "v" the variant, and "n" the sample size. Life stage, "A" = adult and "J" = juvenile

Statistical area	Life stage	Location	AGP			MDH-A			MDH-B					
			n	c	95% C.I.	v	n	c	95% C.I.	v	n	c	95% C.I.	v
251	A	Seal Bay Cr.	44	.94	(.87-.98)	.06	-	-	-	-	-	-	-	
	A	Portage Cr.	45	.82	(.73-.89)	.18	-	-	-	-	-	-	-	
	J	Portage Cr.	32	.91	(.81-.96)	.09	31	1.0	(.98-1.0)	.00	31	.98	(.91-1.00)	.02
	J	Malina Cr.	17	.88	(.73-.95)	.12	17	.97	(.85-.99)	.03	17	.94	(.81-.98)	.06
252	A	Afognak R.	50	.86	(.78-.91)	.14	-	-	-	-	-	-	-	
	J	Afognak R.	27	.83	(.71-.91)	.17	27	.97	(.89-.99)	.03	27	.97	(.89-.99)	.03
	A	Sharatin Bay	45	.84	(.76-.91)	.16	-	-	-	-	-	-	-	
	A	Marka R.	44	.89	(.80-.94)	.11	-	-	-	-	-	-	-	
	J	Danger R.	14	.89	(.73-.96)	.11	14	1.0	(.95-1.0)	0.0	14	.96	(.82-.99)	.04
	J	Kitoi Cr.	50	.84	(.76-.90)	.16	50	.97	(.92-.99)	.02	50	.99	(.94-1.0)	.01
253	A	Terror R.	50	.93	(.86-.97)	.07	-	-	-	-	-	-	-	
	J	Terror R.	11	.73	(.52-.87)	.27	11	1.0	(.93-1.0)	0.0	11	.98	(.82-1.0)	.02
	A	Uganik R.	-	-	-	-	50	.97	(.92-.99)	0.3	50	1.0	(.99-1.0)	0.0
	J	Uganik R.	15	.83	(.66-.93)	.17	15	1.0	(.95-1.0)	0.0	15	1.0	(.95-1.0)	0.0
	A	Baumann's Cr.	49	.87	(.79-.92)	.13	-	-	-	-	-	-	-	
	A	Brown's Lagoon	49	.92	(.82-.94)	.08	-	-	-	-	-	-	-	
254	J	Brown's Lagoon	29	.81	(.69-.89)	.19	29	.99	(.92-1.0)	.01	29	.98	(.91-1.0)	.02
	A	Uyak R.	50	.87	(.79-.92)	.13	-	-	-	-	-	-	-	
	J	Uyak R.	12	.83	(.64-.93)	.17	12	.98	(.82-1.0)	.02	12	.98	(.82-1.0)	.02
	A	Zacher R.	39	.87	(.78-.93)	.13	50	.99	(.94-1.0)	.01	50	1.0	(.99-1.0)	0.0
	J	Miam R.	13	.92	(.76-.98)	.08	13	.98	(.84-1.0)	.02	13	1.0	(.94-1.0)	0.0
										fast variant	.01			

Table 3. Gene frequencies and 95% confidence intervals (C.I.) for AGP, MDH-A, and MDH-B; "c" designates the common form, "v" the variant, and "n" the sample size. Life stage, "A" = adult and "J" = juvenile - continued

Statistical area	Life cycle	Location	AGP			MDH-A			MDH-B					
			n	c	95% C.I. v	n	c	95% C.I. v	n	c	95% C.I. v			
255	A	Karluk R.	45	.93	(.86-.97)	.07	49	.98	(.93-1.0)	.02	47	.99	(.94-1.0)	0.1
	J	Karluk R.	20	.85	(.71-.93)	.15	20	1.0	(.96-1.0)	0.0	20	.96	(.85-.99)	.04
	A	Karluk Lagoon	50	1.0	(.97-1.0)	0.0	-	-	-	-	-	-	-	-
256	A	Red R.	28	.88	(.76-.94)	.12	-	-	-	-	-	-	-	-
	J	Red R.	46	.85	(.76-.91)	.15	47	.98	(.93-1.0)	.02	47	1.0	(.98-1.0)	0.0
	A	Red Lake	50	1.0	(.97-1.0)	0.0	-	-	-	-	-	-	-	-
257	A	Narrows Cr.	28	.93	(.83-.97)	.03	-	-	-	-	-	-	-	-
	J	Narrows Cr.	19	.82	(.67-.91)	.18	19	1.0	(.96-1.0)	0.0	19	.97	(.87-1.0)	.03
	A	Deadman R.	45	.89	(.81-.94)	.11	46	.98	(.93-1.0)	.02	46	.99	(.94-1.0)	.01
	J	Deadman R.	36	.88	(.78-.93)	.12	36	.97	(.91-1.0)	.03	36	1.0	(.98-1.0)	0.0
	A	Dog Salmon R.	47	.77	(.67-.84)	.23	-	-	-	-	-	-	-	-
	J	Dog Salmon R.	58	.83	(.75-.89)	.17	61	1.0	(.99-1.0)	0.0	61	.99	(.95-1.0)	.00
Cr.	A	Humpy R.	39	.83	(.74-.90)	.17	-	-	-	-	-	-	-	-
	J	Humpy R.	44	.83	(.74-.89)	.17	44	1.0	(.98-1.0)	0.0	44	1.0	(.98-1.0)	0.0
	A	Horse Marine Cr.	47	.83	(.74-.89)	.17	-	-	-	-	-	-	-	-
	A	Upper Station	37	.89	(.80-.94)	.11	49	.98	(.94-1.0)	.02	49	1.0	(.98-1.0)	0.0

Cr.

Table 3. Gene frequencies and 95% confidence intervals (C.I.) for AGP, MDH-A, and MDH-B; "c" designates the common form, "v" the variant, and "n" the sample size. Life stage, "A" = adult and "J" = juvenile - continued

Statistical area	Life cycle	Location	AGP			MDH-A			MDH-B				
			n	c	95% C.I. v	n	c	95% C.I. v	n	c	95% C.I. v		
258	A	Kiliuda Bay 206	50	.91	(.84-.95)	.09	-	-	-	-	-	-	
	A	Kiliuda Bay 207	46	.89	(.81-.94)	.11	-	-	-	-	-	-	
	J	Kiliuda Bay 207	23	.85	(.72-.93)	.15	.99	(.90-1.0)	.01	.23	.98	(.89-1.0)	.02
	A	Barling R.	50	.92	(.85-.96)	.08	-	-	-	-	-	-	-
	J	Barling R.	10	.75	(.53-.89)	.25	1.0	(.93-1.0)	0.0	11	.93	(.75-.98)	.07
	A	Kaiugnak R.	46	.84	(.75-.90)	.16	.95	(.89-.98)	.04	49	.99	(.94-1.0)	.01
							fast variant .01						
	J	Kaiugnak R.	42	.82	(.73-.89)	.18	.99	(.94-1.0)	.01	42	1.0	(.98-1.0)	0.0
	J	Seven R. (L.fork)	16	.88	(.72-.95)	.12	.98	(.87-1.0)	0.0	16	.95	(.82-.99)	.05
							fast variant .02						
259	J	Seven R. (U.fork)	14	.86	(.69-.94)	.14	.93	(.77-.98)	0.7	14	.98	(.85-1.0)	.02
	A	Hurst Cr.	48	.88	(.79-.93)	.12	.99	(.94-1.0)	.01	50	1.0	(.99-1.0)	0.0
	J	Hurst Cr.	20	.83	(.68-.91)	.17	1.0	(.96-1.0)	0.0	20	.83	(.68-.91)	.17
	A	Sid Olds R.	48	.90	(.82-.94)	.10	-	-	-	-	-	-	-
	J	Sid Olds R.	23	.80	(.67-.89)	.20	.99	(.91-1.0)	.01	25	.96	(.87-.99)	.03
							slow variant .01						
	A	Buskin R.	49	.94	(.87-.97)	.06	.95	(.86-.98)	.05	24	.95	(.85-.98)	.05
	J	Buskin R.	15	.83	(.66-.93)	.17	.93	(.79-.98)	.07	15	.98	(.86-1.0)	.02
	A	Pillar Cr.	36	.78	(.67-.86)	.22	-	-	-	-	-	-	-
262	J	Kinak Cr.	16	.72	(.55-.84)	.28	.98	(.87-1.0)	.02	16	.97	(.84-.99)	.03
	J	Geographic Cr.	15	.87	(.70-.95)	.13	1.0	(.95-1.0)	0.0	15	1.0	(.95-1.0)	0.0

Table 4. Gene frequencies and 95% confidence intervals (C.I.) for LDH-1, LDH-4, and PGI-1; "c" designates the common form, "v" the variant, and "n" the sample size. Life stage, "A" = adult and "J" = juvenile

Statistical area	Life stage	Location	LDH-1			LDH-4			PGI-1					
			n	c	95% C.I. v	n	c	95% C.I. v	n	c	95% C.I. v			
251	A	Seal Bay Cr.	44	1.0	(.97-1.0)	0.0	44	1.0	(.97-1.0)	0.0	-	-	-	
	A	Portage Cr.	50	1.0	(.97-1.0)	0.0	50	1.0	(.97-1.0)	0.0	50	1.0	(.98-1.0)	0.0
	J	Portage Cr.	32	1.0	(.95-1.0)	0.0	32	1.0	(.95-1.0)	0.0	32	1.0	(.98-1.0)	0.0
	J	Malina Cr.	17	1.0	(.92-1.0)	0.0	17	1.0	(.92-1.0)	0.0	17	1.0	(.96-1.0)	0.0
252	A	Afognak R.	50	.98	(.93-.99)	.02	50	1.0	(.96-1.0)	0.0	-	-	-	
	J	Afognak R.	27	1.0	(.95-1.0)	0.0	27	1.0	(.95-1.0)	0.0	-	-	-	
	A	Sharatin Bay	50	.99	(.95-1.0)	.01	50	1.0	(.97-1.0)	0.0	-	-	-	
	A	Marka R.	44	1.0	(.97-1.0)	0.0	44	1.0	(.97-1.0)	0.0	-	-	-	
	J	Danger Cr.	14	1.0	(.90-1.0)	0.0	14	1.0	(.90-1.0)	0.0	14	1.0	(.95-1.0)	0.0
	J	Kitoi Cr.	50	.99	(.95-1.0)	.01	50	1.0	(.97-1.0)	0.0	50	1.0	(.99-1.0)	0.0
253	A	Terror R.	50	.97	(.92-.99)	.03	50	1.0	(.97-1.0)	0.0	-	-	-	
	J	Terror R.	11	1.0	(.87-1.0)	0.0	11	1.0	(.87-1.0)	0.0	11	1.0	(.93-1.0)	0.0
	A	Uganik R.	50	.98	(.93-.99)	.02	50	1.0	(.97-1.0)	0.0	-	-	-	
	J	Uganik R.	15	1.0	(.91-1.0)	0.0	15	1.0	(.91-1.0)	0.0	-	-	-	
	A	Baumann's Cr.	50	.99	(.95-1.0)	.01	50	.98	(.93-.99)	0.0	-	-	-	
slow variant 0.2														
254	A	Brown's Lagoon	49	.99	(.94-1.0)	.01	49	1.0	(.97-1.0)	0.0	-	-	-	
	J	Brown's Lagoon	29	1.0	(.95-1.0)	0.0	29	1.0	(.95-1.0)	0.0	29	1.0	(.97-1.0)	0.0
	A	Uyak R.	50	.99	(.95-1.0)	.01	50	1.0	(.97-1.0)	0.0	-	-	-	
	J	Uyak R.	12	1.0	(.88-1.0)	0.0	12	1.0	(.88-1.0)	0.0	12	1.0	(.94-1.0)	0.0
	A	Zacher R.	50	1.0	(.97-1.0)	0.0	50	1.0	(.97-1.0)	0.0	-	-	-	
J	Miam R.	13	1.0	(.89-1.0)	0.0	13	1.0	(.89-1.0)	0.0	13	1.0	(.94-1.0)	0.0	

Table 4. Gene frequencies and 95% confidence intervals (C.I.) for LDH-1, LDH-4, and PGI-1; "c" designates the common form, "v" the variant, and "n" the sample size. Life stage, "A" = adult and "J" = juvenile - continued

Statistical area	Life stage	Location	LDH-1			LDH-4			PGI-1					
			n	c	95% C.I. v	n	c	95% C.I. v	n	c	95% C.I. v			
255	A	Karluk R.	47	.98	(.93-.99)	.02	47	1.0	(.97-1.0)	0.0	-	-	-	-
	J	Karluk R.	20	.93	(.80-.97)	.07	20	1.0	(.93-1.0)	0.0	20	1.0	(.96-1.0)	0.0
	A	Karluk Lagoon	50	1.0	(.97-1.0)	0.0	50	1.0	(.97-1.0)	0.0	-	-	-	-
256	A	Red R.	-	-	-	-	-	-	-	-	-	-	-	-
	J	Red R.	39	.99	(.93-1.0)	.01	39	1.0	(.96-1.0)	0.0	39	1.0	(.98-1.0)	0.0
	A	Red Lake	50	1.0	(.97-1.0)	0.0	50	1.0	(.97-1.0)	0.0	-	-	-	-
257	A	Narrows Cr.	29	.98	(.91-1.0)	.02	29	1.0	(.95-1.0)	0.0	-	-	-	-
	J	Narrows Cr.	19	1.0	(.92-1.0)	0.0	19	1.0	(.92-1.0)	0.0	19	1.0	(.96-1.0)	0.0
	A	Deadman R.	48	1.0	(.97-1.0)	0.0	48	1.0	(.97-1.0)	0.0	48	1.0	(.98-1.0)	0.0
	J	Deadman R.	36	1.0	(.96-1.0)	0.0	36	1.0	(.96-1.0)	0.0	36	1.0	(.98-1.0)	0.0
	A	Dog Salmon R.	47	.99	(.94-1.0)	.01	47	1.0	(.97-1.0)	0.0	-	-	-	-
	J	Dog Salmon R.	61	1.0	(.98-1.0)	0.0	61	1.0	(.98-1.0)	0.0	61	.99	(.95-1.0)	.01
	A	Humpy R.	39	1.0	(.96-1.0)	0.0	39	1.0	(.96-1.0)	0.0	39	1.0	(.98-1.0)	0.0
	J	Humpy R.	44	1.0	(.97-1.0)	0.0	44	1.0	(.97-1.0)	0.0	44	1.0	(.98-1.0)	0.0
	A	Horse Marine Cr.	47	1.0	(.97-1.0)	0.0	47	1.0	(.97-1.0)	0.0	-	-	-	-
	A	Upper Station Cr.	49	1.0	(.97-1.0)	0.0	49	1.0	(.97-1.0)	0.0	-	-	-	-
258	A	Kiliuda Bay 206	-	-	-	-	-	-	-	-	-	-	-	-
	A	Kiliuda Bay 207	-	-	-	-	-	-	-	-	-	-	-	-
	J	Kiliuda Bay 207	23	1.0	(.94-1.0)	0.0	23	1.0	(.94-1.0)	0.0	23	1.0	(.97-1.0)	0.0
	A	Barling R.	50	1.0	(.97-1.0)	0.0	50	1.0	(.97-1.0)	0.0	-	-	-	-
	J	Barling R.	11	1.0	(.87-1.0)	0.0	11	1.0	(.87-1.0)	0.0	11	1.0	(.93-1.0)	0.0
	A	Kaiugnak R.	50	.99	(.95-1.0)	.01	50	1.0	(.97-1.0)	0.0	-	-	-	-

Table 4. Gene frequencies and 95% confidence intervals (C.I.) for LDH-1, LDH-4, and PGI-1; "c" designates the common form, "v" the variant, and "n" the sample size. Life stage, "A" = adult and "J" = juvenile - continued

Statistical area	Life stage	Location	LDH-1			LDH-4			PGI-1		
			n	c	95% C.I. v	n	c	95% C.I. v	n	c	95% C.I. v
258 (cont'd)	J	Kaiugnak R.	42	1.0	(.97-1.0) 0.0	42	1.0	(.97-1.0) 0.0	42	1.0	(.98-1.0) 0.0
	J	Seven Rivers (L.fork)	16	1.0	(.91-1.0) 0.0	16	1.0	(.91-1.0) 0.0	16	1.0	(.95-1.0) 0.0
	J	Seven Rivers (U.fork)	14	1.0	(.90-1.0) 0.0	14	.96	(.82-.99) 0.4	14	1.0	(.95-1.0) 0.0
259	A	Hurst Cr.	50	.99	(.95-1.0) .01	50	1.0	(.97-1.0) 0.0	-	-	-
	J	Hurst Cr.	20	1.0	(.93-1.0) 0.0	20	1.0	(.93-1.0) 0.0	20	1.0	(.96-1.0) 0.0
	A	Sid Olds R.	50	1.0	(.97-1.0) 0.0	50	1.0	(.97-1.0) 0.0	-	-	-
	J	Sid Olds R.	25	1.0	(.94-1.0) 0.0	25	1.0	(.94-1.0) 0.0	25	1.0	(.97-1.0) 0.0
	A	Buskin R.	49	.96	(.90-.98) .04	49	1.0	(.97-1.0) 0.0	-	-	-
	J	Buskin R.	15	1.0	(.91-1.0) 0.0	15	1.0	(.91-1.0) 0.0	15	1.0	(.95-1.0) 0.0
262	A	Pillar Cr.	40	.98	(.91-.99) .02	50	1.0	(.97-1.0) 0.0	-	-	-
	J	Kinak Cr.	16	.97	(.84-.99) .03	16	1.0	(.91-1.0) 0.0	16	1.0	(.95-1.0) 0.0
	J	Geographic Cr.	15	.93	(.79-.98) .07	15	1.0	(.91-1.0) 0.0	15	1.0	(.95-1.0) 0.0

Table 5. Gene frequencies and 95% confidence intervals (C.I.) for PGI-3, PGM, and CK; "c" designates the common form, "v" the variant, and "n" the sample size. Life stage, "A" = adult and "J" = juvenile

Statistical area	Life stage	Location	PGI-3			PGM			CK					
			n	c	95% C.I.	v	n	c	95% C.I.	v	n	c	95% C.I.	v
251	A	Seal Bay Cr.	-	-	-	-	44	1.0	(.97-1.0)	0.0	-	1.0	(.97-1.0)	0.0
	A	Portage Cr.	50	1.0	(.97-1.0)	0.0	50	.99	(.95-1.0)	0.1	50	.98	(.93-.99)	.02
	J	Portage Cr.	32	1.0	(.95-1.0)	0.0	-	-	-	-	-	-	-	-
	J	Malina Cr.	17	1.0	(.92-1.0)	0.0	-	-	-	-	-	-	-	-
252	A	Afognak R.	-	-	-	-	50	.99	(.95-1.0)	0.0	50	1.0	(.97-1.0)	0.0
	J	Afognak R.	-	-	-	-	-	-	slow variant	0.1	-	-	-	-
	A	Sharatin Bay	-	-	-	-	50	.96	(.90-.98)	.04	50	.99	(.95-1.0)	.01
	A	Marka R.	-	-	-	-	44	1.0	(.97-1.0)	0.0	44	1.0	(.97-1.0)	.00
	J	Danger R.	14	1.0	(.90-1.0)	0.0	-	-	-	-	-	-	-	-
	J	Kitoi Cr.	50	1.0	(.97-1.0)	0.0	-	-	-	-	-	-	-	-
253	A	Terror R.	-	-	-	-	50	.99	(.95-1.0)	0.0	50	1.0	(.97-1.0)	0.0
	J	Terror R.	11	1.0	(.87-1.0)	0.0	-	-	slow variant	0.1	-	-	-	-
	A	Uganik R.	-	-	-	-	50	.96	(.90-.98)	0.4	50	1.0	(.97-1.0)	0.0
	J	Uganik R.	-	-	-	-	-	-	-	-	-	-	-	-
	A	Baumann's Cr.	-	-	-	-	50	1.0	(.97-1.0)	0.0	50	1.0	(.97-1.0)	0.0
254	A	Brown's Lagoon	-	-	-	-	49	1.0	(.97-1.0)	0.0	49	1.0	(.97-1.0)	0.0
	J	Brown's Lagoon	29	1.0	(.95-1.0)	0.0	-	-	-	-	-	-	-	-
	A	Uyak R.	-	-	-	-	50	.99	(.95-1.0)	0.1	50	1.0	(.97-1.0)	0.0
	J	Uyak R.	12	1.0	(.88-1.0)	0.0	-	-	-	-	-	-	-	-
	A	Zacher R.	-	-	-	-	50	1.0	(.97-1.0)	0.0	50	1.0	(.97-1.0)	0.0
	J	Miam R.	13	1.0	(.89-1.0)	0.0	-	-	-	-	-	-	-	-

Table 5. Gene frequencies and 95% confidence intervals (C.I.) for PGI-3, PGM, and CK; "c" designates the common form, "v" the variant, and "n" the sample size. Life stage, "A" = adult and "J" = juvenile - continued

Statistical area	Life stage	Location	PGI-3			PGM			CK		
			n	c	95% C.I. v	n	c	95% C.I. v	n	c	95% C.I. v
255	A	Karluk R.	-	-	-	47	1.0	(.97-1.0) 0.0	47	1.0	(.97-1.0) 0.0
	J	Karluk R.	20	1.0	(.93-1.0) 0.0	-	-	-	-	-	-
	A	Karluk Lagoon	-	-	-	50	.89	(.81-.94) .11	50	1.0	(.97-1.0) 0.0
256	A	Red R.	-	-	-	-	-	-	-	-	-
	J	Red R.	47	.99	(.94-1.0) .01	-	-	-	-	-	-
	A	Red Lake	-	-	-	45	.79	(.69-.86) .21	50	.99	(.95-1.0) .01
257	A	Narrows Cr.	-	-	-	29	1.0	(.95-1.0) 0.0	29	1.0	(.95-1.0) 0.0
	J	Narrows Cr.	19	1.0	(.92-1.0) 0.0	-	-	-	-	-	-
	A	Deadman R.	48	1.0	(.97-1.0) 0.0	48	.96	(.90-.98) .04	48	.98	(.93-.99) .02
	J	Deadman R.	36	1.0	(.96-1.0) 0.0	-	-	-	-	-	-
	A	Dog Salmon R.	-	-	-	47	1.0	(.97-1.0) 0.0	-	-	-
	J	Dog Salmon R.	61	1.0	(.98-1.0) 0.0	-	-	-	-	-	-
	A	Humpy R.	39	1.0	(.96-1.0) 0.0	39	1.0	(.96-1.0) 0.0	39	1.0	(.96-1.0) 0.0
	J	Humpy R.	44	1.0	(.97-1.0) 0.0	-	-	-	-	-	-
	A	Horse Marine Cr.	-	-	-	47	1.0	(.97-1.0) 0.0	47	1.0	(.97-1.0) 0.0
	A	Upper Station Cr.	-	-	-	49	1.0	(.97-1.0) 0.0	49	1.0	(.97-1.0) 0.0
	258	A	Kiliuda Bay 206	-	-	-	50	1.0	(.97-1.0) 0.0	50	1.0
A		Kiliuda Bay 207	-	-	-	50	.98	(.93-.99) .02	50	.99	(.95-1.0) .01
J		Kiliuda Bay 207 23	-	-	-	-	-	-	-	-	-
A		Barling R.	-	-	-	50	.95	(.89-.98) .05	50	.96	(.90-.98) .04
J		Barling R.	11	1.0	(.87-1.0) 0.0	-	-	-	-	-	-
A	Kaiugnak R.	-	-	-	50	1.0	(.97-1.0) 0.0	49	1.0	(.97-1.0) 0.0	

Table 5. Gene frequencies and 95% confidence intervals (C.I.) for PGI-3, PGM, and CK; "c" designates the common form, "v" the variant, and "n" the sample size. Life stage, "A" = adult and "J" = juvenile - continued

Statistical area	Life stage	Location	PGI-3			PGM			CK				
			n	c	95% C.I. v	n	c	95% C.I. v	n	c	95% C.I. v		
258 cont'd	J	Kaiugnak R.	42	1.0	(.97-1.0) 0.0	-	-	-	-	-	-	-	-
	J	Seven R. (L.fork)	16	1.0	(.91-1.0) 0.0	-	-	-	-	-	-	-	-
	J	Seven R. (U.fork)	14	1.0	(.90-1.0) 0.0	-	-	-	-	-	-	-	-
259	A	Hurst Cr.	-	-	-	44	.89	(.80-.94) .11	50	1.0	(.97-1.0) 0.0	-	-
	J	Hurst Cr.	20	1.0	(.93-1.0) 0.0	-	-	-	-	-	-	-	-
	A	Sid Olds R.	-	-	-	50	.99	(.95-1.0) .01	-	-	-	-	-
	J	Sid Olds R.	25	1.0	(.84-1.0) 0.0	-	-	-	-	-	-	-	-
	A	Buskin R.	-	-	-	49	1.0	(.97-1.0) 0.0	49	1.0	(.97-1.0) 0.0	-	-
	J	Buskin R.	15	1.0	(.91-1.0) 0.0	-	-	-	-	-	-	-	-
A	Pillar Cr.	-	-	-	-	-	-	-	-	-	-	-	
262	J	Kinak Cr.	16	1.0	(.91-1.0) 0.0	-	-	-	-	-	-	-	-
	J	Geographic Cr.	15	1.0	(.91-1.0) 0.0	-	-	-	-	-	-	-	-

Table 6. Gene frequencies and 95% confidence intervals (C.I.) for 6-PGDH and AAT-3; "c" = the common form, "v" = the variant, and "n" the sample size. Life stage, "A" = adult and "J" = juvenile

Statistical area	Life stage	Location	6-PGDH			AAT-3			
			n	c	95% C.I.	v	n	c	95% C.I.
251	A	Seal Bay Cr.	-	-	-	-	-	-	
	A	Portage Cr.	-	-	-	-	-	-	
	J	Portage Cr.	-	-	-	32	.95	(.87-.98)	.05
	J	Malina Cr.	-	-	-	17	1.0	(.92-1.0)	0.0
252	A	Afognak R.	-	-	-	-	-	-	
	J	Afognak R.	27	.98	(.90-1.0)	.02	.96	(.87-.98)	.04
	A	Sharatin Bay	-	-	-	-	-	-	
	A	Marka R.	-	-	-	-	-	-	
	J	Danger R.	14	1.0	(.90-1.0)	0.0	-	-	
	J	Kitoi Cr.	-	-	-	-	-	-	
253	A	Terror R.	-	-	-	-	-	-	
	J	Terror R.	11	1.0	(.87-1.0)	0.0	-	-	
	A	Uganik R.	-	-	-	-	-	-	
	J	Uganik R.	-	-	-	-	-	-	
	A	Baumann's Cr.	-	-	-	-	-	-	
	-	-	-	-	-	-	-	-	
254	A	Brown's Lagoon	-	-	-	-	.98	(.91-1.0)	.02
	J	Brown's Lagoon	29	1.0	(.95-1.0)	0.0	-	-	
	A	Uyak R.	-	-	-	-	-	-	
	J	Uyak R.	-	-	-	-	1.0	(.88-1.0)	0.0
	A	Zacher R.	-	-	-	-	-	-	
	J	Miam R.	-	-	-	-	1.0	(.89-1.0)	0.0





Table 7. Populations that differ significantly from each other and all other populations

Populations	Locus				
	AGP	MDH-A	MDH-B	LDH-1	PGM
Barling River			x		x
Red River (upstream)	x				x
Karluk River (downstream)				x	x
Hurst Creek			x		
Malina Creek			x		
Kiliuda Bay		x			
Seven Rivers (upper fork)		x			
Buskin River		x			
Kinak Creek	x				

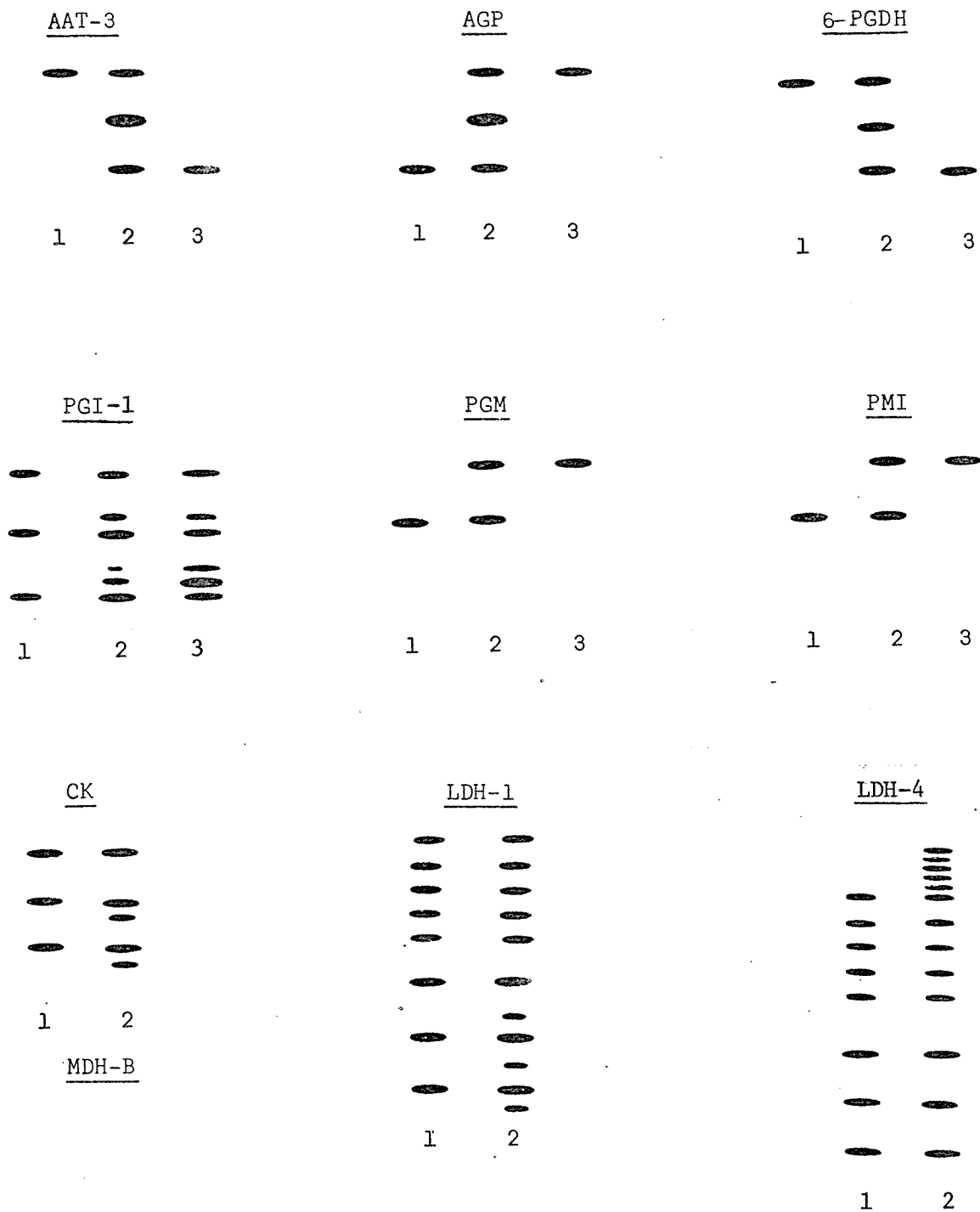


Fig. 1. Observed electrophoretic patterns. The common form is No. 1; all other numbers are heterozygous and alternate homozygous forms.

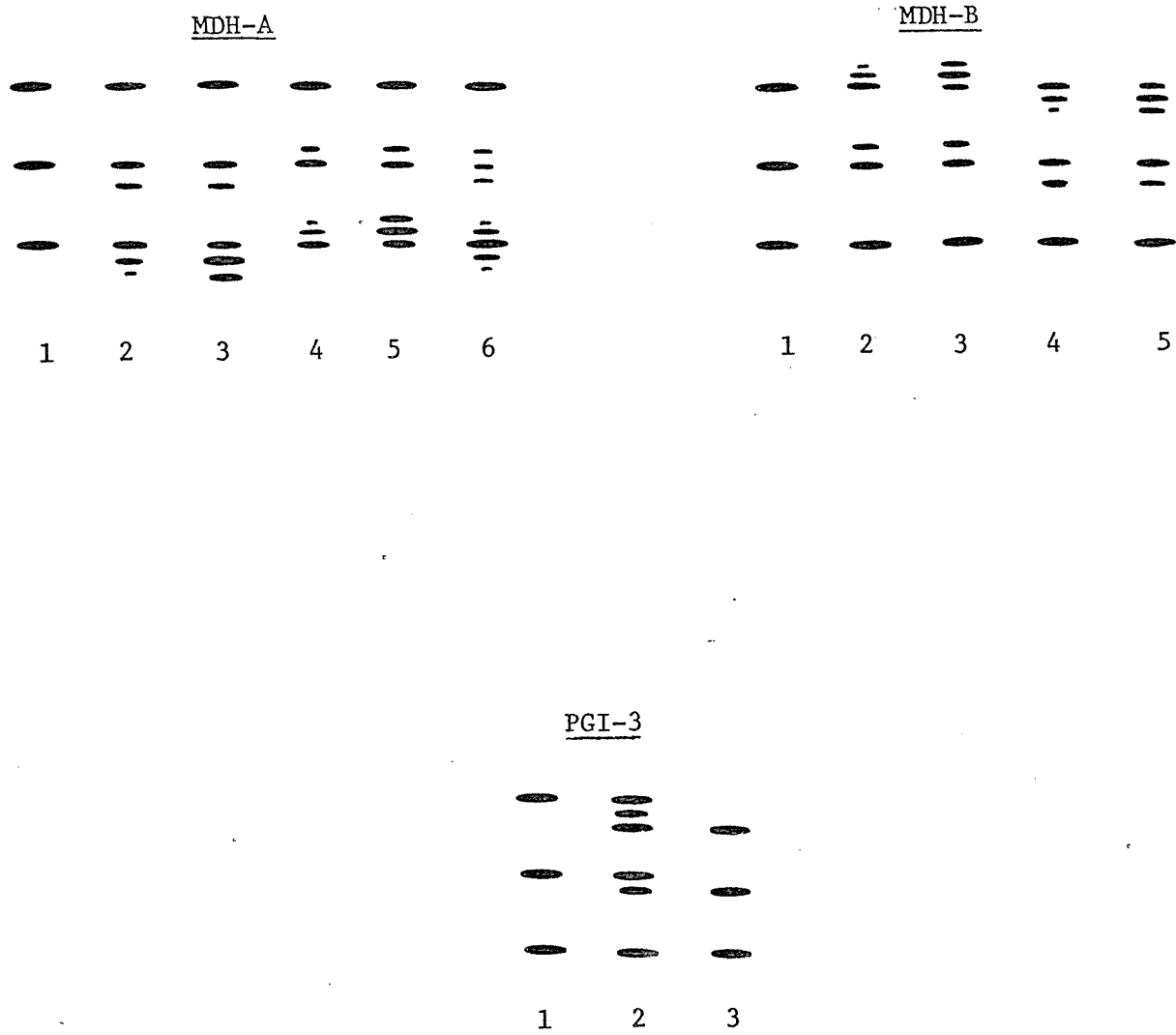


Fig. 1. Observed electrophoretic patterns. The common form is No. 1; all other numbers are heterozygous and alternate homozygous forms. - Continued.

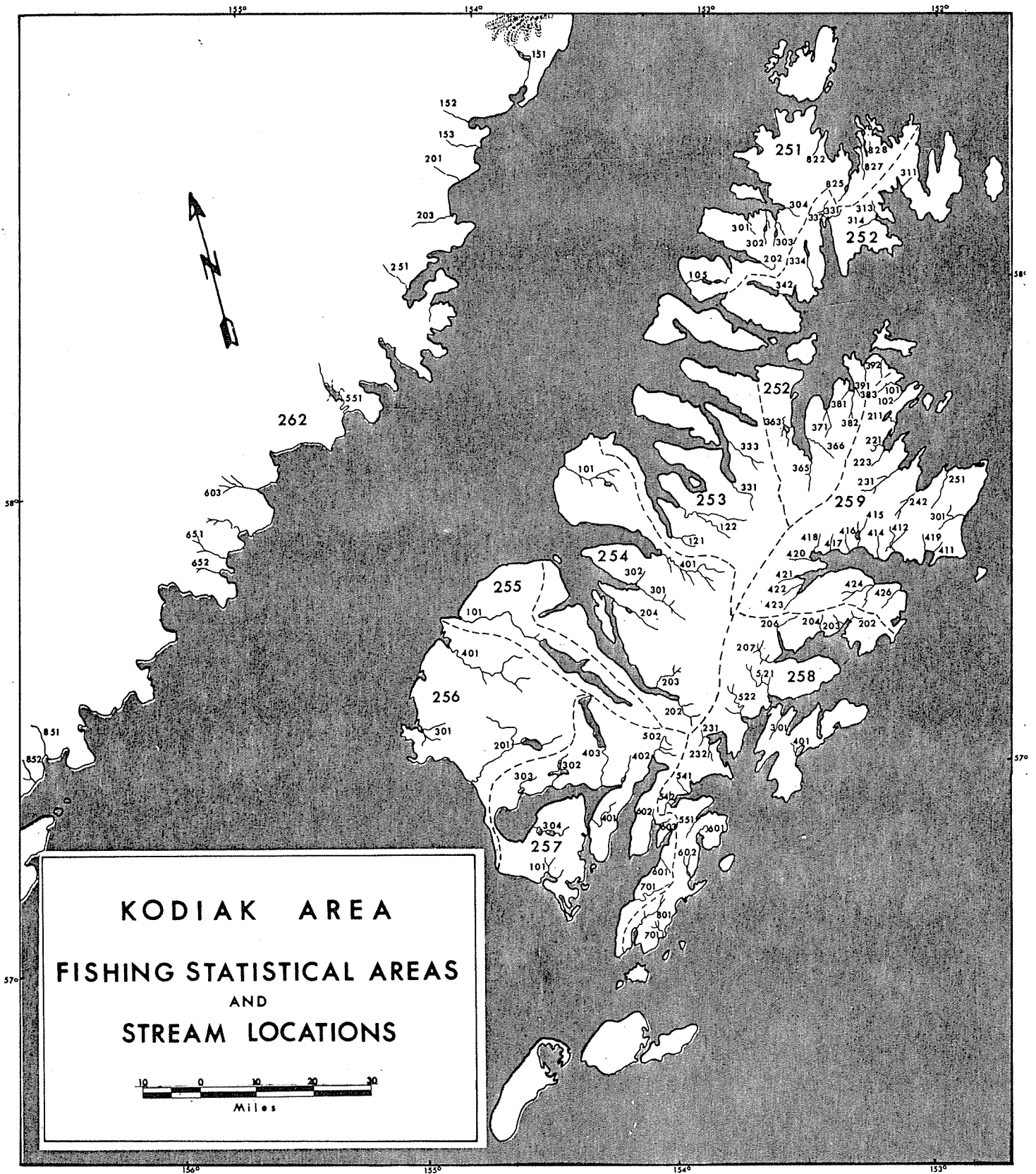


Fig. 2. Map of Kodiak Island and surrounding area showing statistical areas and stream designations used by Alaska Department of Fish and Game.