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IDENTIFICATION OF KODIAK ISLAND PINK SALMON POPULATIONS BASED ON BIOCHEMICAL GENETIC VARIATION

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Interim Report

to

Alaska Department of Fish and Game

Contract No. 3813

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Associate Director

TABLE OF CONTENTS

	Page
INTRODUCTION	1
MATERIALS AND METHODS	2
RESULTS	5
Alpha Glycerophosphate Dehydrogenase (AGP)	5
Phosphoglucomutase (PGM)	5
Aspartate Aminotransferase (AAT)	12
Lactate Dehydrogenase (LDH)	12
Malate Dehydrogenase (MDH)	12
Additional Isozymes	13
Breeding Experiments	14
SUMMARY	14
REFERENCES	18

INTRODUCTION

Migratory habits of Kodiak Island pink salmon are highly variable. Bevan (1959) showed that adults tagged on the Northeastern side of Afognak Island were recovered from many locations adjacent to Afognak Island, Kodiak Island and the mainland. This indicates fish captured in one location may not be destined for that same area. Thus, to assure that individual populations are harvested on a basis commensurate with their production, a method is needed to identify individual stocks within a mixed fishery. The accuracy of management decisions by the Alaska Department of Fish and Game (ADF&G) would be greatly enhanced by a rapid, inexpensive method of distinguishing stocks.

Presently, many methods are available to provide data that will potentially distinguish individual stocks, or populations, but only two yield fairly definitive results in a relatively short analytical time. One is scale pattern recognition utilizing a polynomial discriminant function to separate characters. The other is biochemical determination and analysis of population genetic differences. Results from the even-year class (1976) of pink salmon (Donnelly, et al., 1977; Seeb and Wishard, 1977) demonstrated a potential for using genetic markers in stock separation.

The primary objective of this study is the application of biochemical genetic markers to Kodiak Island pink salmon stock separation studies.

Kodiak and Afognak Islands have over 100 spawning populations, of which less than 30 actually contribute the majority of the fish to the total fishery. Previous data (Aspinwall, 1974; Donnelly, et al., 1977) showed considerable amounts of overlap in gene frequencies between populations at each locus. To obtain the needed separation we have looked for additional

polymorphic loci. Also, Aspinwall (1974) reported a significant difference in gene frequencies between year classes of pink salmon. Thus, in order for the biochemical genetic markers to be effectively used as a management tool for the total pink salmon fishery, it was concluded that the experimental approach used in 1976-1977 (Donnelly, et al., 1977) should be repeated on the odd-year class (1977). The Alaska Department of Fish and Game (ADF&G) concurred, and work was continued under Contract No. 3813.

MATERIALS AND METHODS

Horizontal starch gel electrophoresis is a method by which genetic differences among enzymes of individual fish can be analyzed. In this procedure, mixtures of enzymes are placed in a starch gel matrix and made to migrate by applying a low voltage current. Since enzymes have an electrical charge inherent in the components of their structure, each type of enzyme has a characteristic migration distance. Thus, changes in the "typical" migration distance of an enzyme can be recognized by electrophoretic analysis and reflects a change in the gene that codes for that enzyme. Enzymes exhibiting genetically different forms are classified as allozymes or isozymes.

In order to detect these isozymes in the starch gel after migration, it is necessary to stain them. This is accomplished by use of staining techniques which utilize the specific biochemical activity of individual enzymes. Thus, by combining the separation of isozymes and the specific staining characteristics of these molecules, we are able to measure genetic variability among individual fish in a population.

On the basis that a population of fish is an interbreeding unit, the quantity of the variable genes (gene frequency) should be characteristic of a given population. This is a valid premise and the gene frequencies will remain stable over generations provided the following three conditions are met: (1) Large population size; (2) random mating; and, (3) no selection, mutation, or migration. While we cannot be completely assured that all of these are met, work on many other fish species (May, 1975) and our previous work on the pink salmon populations of Kodiak Island indicate no serious discrepancies from expectations.

The importance of this to fish management is that by this analysis we can, with a relatively easy and inexpensive analysis, obtain data on a basic biological characteristic of component populations. Since the genes are a part of the fishes biological make-up, they cannot be lost. Also, because the gene frequencies are characteristic of a population and are stable over time, they provide data that can separate stocks reliably.

One hindrance to the maximum application of this technique is that, compared to the number of genes in an individual, relatively few can be analyzed because techniques have been developed for only a limited number of enzymes (20-30). In addition, not all enzymes we analyze show sufficient genetic variability or genetic divergence to be useful. For instance, in this study 14 enzymes were analyzed, but only five demonstrated useful polymorphism (genetically determined multiple forms). These were, alpha-glycerophosphate dehydrogenase, aspartate amino transferase, phosphoglucomutase, lactate dehydrogenase, and malate dehydrogenase. The effect of this has been to limit the number of populations that can be reliably separated to those that show distinctive variation in one or more of these five enzyme systems.

In this study the five enzyme systems mentioned above were analyzed in adult tissue samples collected from 22 streams on Kodiak and Afognak Islands. These streams were chosen on the basis of the magnitude of their contribution to the odd-year-cycle fishery. Approximately 50 fish were collected from each stream by personnel of the Kodiak office of the ADF&G under the direction of Larry Malloy, fishery biologist. The samples were frozen as soon as possible after collection, and remained frozen (-20°C) until processing for electrophoresis. Small portions (approximately 1 to 2 grams) of liver, muscle tissue and vitreous eye fluid from each fish were placed in three separate test tubes. Since the liver and muscle tissue did not contain sufficient liquid to enable subsequent analysis, a few drops of distilled water were added to the test tubes containing these tissues; these samples were then homogenized and centrifuged to remove cellular debris (eye fluid did not need special handling since it was already a liquid). A small amount of the supernatant from the test tubes was absorbed into a piece of filter paper, termed a wick. The wicks were then placed into previously prepared starch gels (May, 1975). Each gel contained only wicks with one type of tissue sample. All starch gels were subjected to electrophoresis for periods ranging from two to four hours. At the termination of electrophoresis the starch gels were sliced into several layers (usually 5) and each layer was stained for a different enzyme. The staining solutions used were those detailed by Shaw and Prasad (1970). After staining, the phenotype of each fish was recorded for every enzyme system analyzed. Phenotypes were coded onto computer cards and the data was analyzed using existing computer programs.

RESULTS

Table 1 lists the management district by number, stream name, management stream number, enzyme system, gene frequency and 95% confidence interval of the most common allele(s) for each stream. The observed enzyme patterns are illustrated in Figure 1. Figure 2 is an unpublished stream catalog map of the Kodiak management area showing the districts (large numbers) and stream locations by number. Comparison of gene frequencies between year classes is summarized in Table 2.

Sample collections and handling gave better results than the previous year (1976-1977); more enzyme systems gave consistently analyzable results and genetic differences were more apparent. Details of each enzyme system are listed below:

Alpha Glycerophosphate Dehydrogenase (AGP)

Readable results were obtained for all populations. The common allele (gene frequencies ranged from .81 to 1.0) migrated more slowly than the variant form (Table 1). There was no significant difference between year classes (Table 2) using pooled data for each year.

Phosphoglucomutase (PGM)

PGM-1 was also readable for all populations. Gene frequencies (Table 1) of the common allele (slower migrating than the variant form) ranged from .86 to 1.0. The enzyme system did show significant differences between year classes (Table 2).

Table 1. Gene frequencies (C) and 95% confidence intervals (C.I.) for the polymorphic loci; "N" is the sample size and "V" the variant allele frequency.

			LOCUS											
			AGP				PGM				AAT			
District No.	Stream Name	Stream No.	N	C	95% CI	V	N	C	95% CI	V	N	C	95% CI	V
252	Kitoi Cr.	314	72	.81	.73-.86	.19	72	.94	.89-.97	.06	-	-	-	-
"	Marka Cr.	334	49	.85	.76-.91	.15	49	.96	.90-.98	.04	49	.69	.60-.78	.31
"	Afognak R.	342	50	.94	.88-.97	.06	47	.96	.90-.98	.04	38	.74	.63-.82	.26
"	Sharatin R.	371	45	.89	.81-.94	.11	47	.94	.87-.97	.06	44	.74	.64-.82	.26
253	Uganik R.	122	50	.88	.80-.93	.12	50	.97	.92-.99	.03	44	.91	.83-.95	.09
"	Terror R.	331	50	.89	.81-.94	.11	46	.90	.82-.95	.10	46	.77	.68-.85	.23
"	Danger R.	332	50	.91	.84-.95	.09	50	.96	.90-.98	.04	49	.79	.69-.86	.21
255	Karluk R.	101	45	.86	.77-.91	.14	49	.97	.91-.99	.03	13	.77	.58-.89	.23
257	Akalura Lagoon	302	48	.91	.83-.95	.09	48	.98	.93-.99	.02	-	-	-	-
"	Upper Station Cr.	304	47	.84	.75-.90	.16	47	.95	.88-.98	.05	36	.81	.70-.88	.19
"	Narrows Cr.	401	41	.89	.80-.94	.11	40	.90	.81-.95	.10	-	-	-	-
"	Frazer Lake	403	32	.83	.72-.90	.17	30	1.0	.95-.1.0	0.0	-	-	-	-
"	Deadman R.	502	33	.86	.76-.93	.14	33	.88	.78-.94	.12	25	.68	.54-.79	.32
258	Barling Cr.	522	16	.88	.72-.95	.12	16	1.0	.91-1.0	0.0	15	.90	.74-.97	.10
"	Kaiugnak R.	542	29	.91	.81-.96	.09	29	.86	.75-.93	.14	28	.80	.68-.89	.20
"	Seven Rivers	701	44	.91	.83-.95	.09	44	.98	.92-.99	.02	42	.77	.67-.85	.23
259	Pillar Cr.	102	48	.92	.84-.96	.08	49	.94	.87-.97	.06	48	.74	.64-.82	.26
"	Buskin R.	211	47	.87	.79-.93	.13	47	.97	.91-.99	.03	44	.81	.71-.88	.19
"	American R.	231	50	.93	.86-.97	.07	50	.97	.92-.99	.03	50	.85	.77-.91	.15
"	Miam Cr.	412	10	1.0	.86-.1.0	0.0	10	1.0	.86-1.0	0.0	10	.75	.53-.89	.25
"	Hurst Cr.	414	65	.89	.83-.93	.11	65	1.0	.98-1.0	0.0	-	-	-	-
"	Saltery Cr.	415	46	.88	.80-.93	.12	46	.95	.88-.98	.05	43	.74	.64-.82	.26

Table 1. cont'd

District No.	Stream Name	Stream No.	LOCUS LDH-1 N	C	95% CI	V	LDH-4 N	C	95% CI	V	MDH-A N	C	95% CI	V
252	Kitoi Cr.	314	72	1.0	.98-1.0	0.0	7.2	.98	.94-.99	.02	72	1.0	.98-1.0	0.0
"	Marka Cr.	334	50	.99	.95-1.0	.01	50	1.0	.97-1.0	0.0	49	.97	.91-.99	.03
"	Afognak R.	342	50	1.0	.97-1.0	0.0	50	1.0	.97-1.0	0.0	50	.99	.95-1.0	.01
"	Sharatin R.	371	47	.99	.94-1.0	.01	47	.99	.94-1.0	.01	47	1.0	.97-1.0	0.0
253	Uganik R.	122	50	1.0	.97-1.0	0.0	50	1.0	.97-1.0	0.0	50	.99	.95-1.0	.01
"	Terror R.	331	50	1.0	.97-1.0	0.0	50	1.0	.97-1.0	0.0	50	1.0	.97-1.0	0.0
"	Danger R.	332	50	1.0	.97-1.0	0.0	50	1.0	.97-1.0	0.0	50	1.0	.97-1.0	0.0
255	Karluk R.	101	49	.99	.94-1.0	.01	49	.99	.94-1.0	.01	49	.99	.95-1.0	.01
257	Akalura R.	302	48	1.0	.97-1.0	0.0	48	1.0	.97-1.0	0.0	48	1.0	.97-1.0	0.0
"	Upper Station Cr.	304	-	-	-	-	-	-	-	-	47	1.0	.97-1.0	0.0
"	Narrows Cr.	401	41	1.0	.96-1.0	0.0	41	.99	.93-1.0	.01	41	.99	.87-.98	.01
"	Frazer Lk.	403	50	1.0	.97-1.0	0.0	50	1.0	.97-1.0	0.0	50	1.0	.97-1.0	0.0
"	Deadman	502	33	.97	.90-.99	.03	33	1.0	.96-1.0	0.0	34	1.0	.96-1.0	0.0
258	Barling Cr.	522	16	1.0	.91-1.0	0.0	16	1.0	.91-1.0	0.0	16	1.0	.91-1.0	0.0
"	Kaiugnak R.	542	29	.91	.81-.96	.09	29	1.0	.95-1.0	0.0	29	1.0	.95-1.0	0.0
"	Seven Rivers	701	44	.99	.94-1.0	.01	44	1.0	.97-1.0	0.0	44	1.0	.97-1.0	0.0
259	Pillar Cr.	102	49	1.0		0.0	49	1.0	.97-1.0	0.0	49	1.0	.97-1.0	0.0
"	Buskin R.	211	47	.98	.93-.99	.02	47	1.0	.97-1.0	0.0	47	1.0	.97-1.0	0.0
"	American R.	231	50	.91	.84-.95	0.9	50	1.0	.97-1.0	0.0	50	1.0	.97-1.0	0.0
"	Miam Cr.	412	10	1.0	.86-1.0	0.0	10	1.0	.86-1.0	0.0	10	1.0	.86-1.0	0.0
"	Hurst Cr.	414	65	.99	.97-1.0	.01	65	1.0	.98-1.0	0.0	65	1.0	.98-1.0	0.0
"	Saltery Cr.	415	46	.99	.94-1.0	.01	46	1.0	.97-1.0	0.0	46	1.0	.97-1.0	0.0

Table 1. cont'd

LOCUS (MDH-B)

District No.	Stream Name	Stream No.	Common Allele N	C	95% CI	Fast Allele V	95% CI	Very Slow Allele V	95% CI	Slow variant
252	Kitoi Cr.	314	72	.986	.951-.996	0.00	0.00 -.020	.014	.004-0.49	0.00
"	Marka Cr.	334	49	.969	.914-.990	.026	.008-.079	.005	.001-.047	0.00
"	Afognak R.	342	50	.925	.856-.962	.045	.019-.105	.015	.024-.004	.015
"	Sharatin R.	371	44	.966	.905-.988	.028	.009-.087	.006	.001-.051	0.00
253	Uganik R.	122	50	.950	.888-.979	.015	.004-.062	.025	.008-.079	.010
"	Terror R.	331	50	.970	.916-.990	.010	.002-.055	.020	.006-.070	0.00
"	Danger R.	332	50	.980	.930-.995	.010	.002-.055	.010	.002-.055	0.00
255	Karluk R.	101	49	.949	.886-.978	.015	.004-.064	.036	.013-.093	0.00
257	Akalura R.	302	48	.974	.920-.992	.010	.002-.057	.016	.004 .065	0.00
"	Upper Station Cr.	304	47	.968	.910-.989	0.00	0.00 -.031	.032	.011-.090	0.00
"	Narrows Cr.	401	41	.945	.873-.977	.012	.002-.066	.043	.016-.111	0.00
"	Frazer Lk.	403	50	.975	.923-.992	.015	.004-.062	.010	.002-.055	0.00
"	Deadman R.	502	33	.932	.844-.972	.045	.016-.125	.023	.005-.093	0.00
258	Barling Cr.	522	16	.953	.820-.989	.016	.002-.133	.031	.006-.157	0.00
"	Kaiugnak R.	542	29	.957	.871-.987	.009	.001-.077	.026	.006-.105	.008
"	Seven Rivers	701	44	.955	.889-.982	.017	.004-.071	.023	.006-.079	.005
259	Pillar Cr.	102	49	.954	.893-.981	.015	.004-.064	.031	.011-.086	0.00
"	Buskin R.	211	47	.989	.942-.998	.011	.002-.058	0.00	0.00 -.031	0.00
"	American R.	231	50	.955	.895-.981	.010	.002-.055	.035	.013-.092	0.00
"	Miam Cr.	412	10	.975	.800-.997	0.00	0.00 -.139	.025	.003-.200	0.00
"	Hurst Cr.	414	65	.977	.934-.992	0.00	0.00 -.023	.023	.008-.066	0.00
"	Saltery Cr.	415	46	.973	.916-.992	.005	.001-.050	.022	.006-.076	0.00

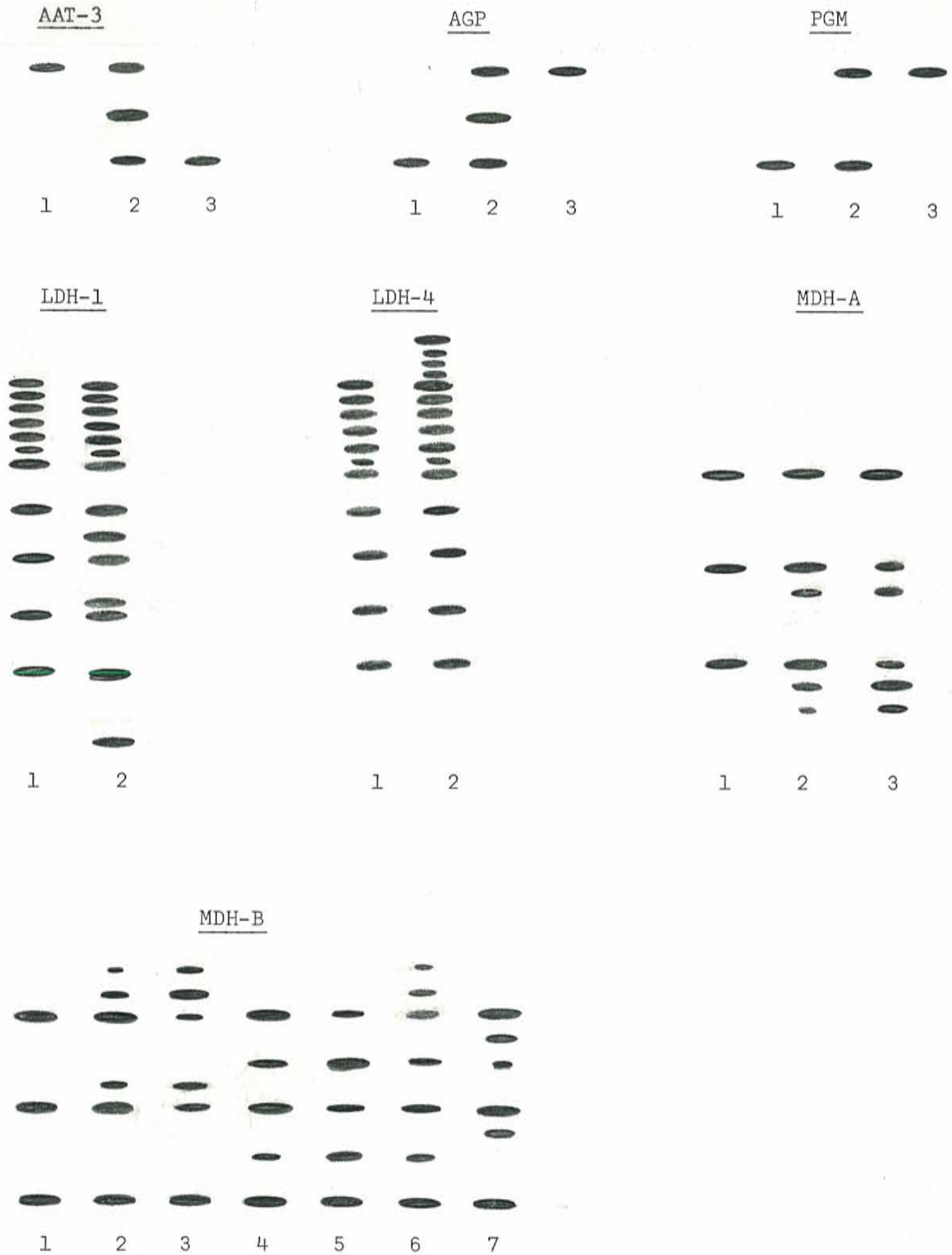

Figure 1. Observed electrophoretic patterns. The common form is No. 1; all other numbers are heterozygous or alternate homozygous forms.

KODIAK AREA

FISHING STATISTICAL AREAS AND STREAM LOCATIONS

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

Table 2. Comparison of 1976 and 1977 adult pink salmon gene frequencies.

Isozyme	Allele	Year	Sample size	Gene frequency	95% Confidence Interval
AGP	common	1976	1254	.888	.875 - .901
		1977	967	.884	.869 - .899
PGM	common	1976	1281	.975	.969 - .982
		1977	964	.953	.944 - .963
MDH-A	common	1976	373	.975	.964 - .986
		1977	993	.996	.994 - .999
MDH-B	common	1976	365	.993	.986 - .999
		1977	989	.964	.955 - .972
MHB-B	fast	1976	365	.007	.001 - .013
		1977	989	.014	.009 - .019
MDH-B	slow	1976	365	.000	.000 - .004
		1977	989	.021	.014 - .027

Aspartate Aminotransferase (AAT)

The only polymorphic locus (AAT-3) was best expressed in the eye vitreous fluid. In contrast to the results obtained for the 1976-1977 adults (Donnelly et al., 1977) most populations (17 out of 22) gave readable results. Gene frequencies for the common allele ranged from .68 to .91 (Table 1). The variant form was slower migrating than the common allele. Comparison between year class was impossible due to lack of 1976-1977 data.

Lactate Dehydrogenase (LDH)

Polymorphism was found in both the LDH-1 and LDH-4 loci. Ten populations exhibited some degree of variation for LDH-1 (Table 1), with the common allele gene frequency ranging from .91 to 1.0. The common form was faster migrating than the variant allele. Variation at the LDH-4 locus was considerably less than LDH-1, with only four populations (Table 1) showing a fast migrating variant allele. The frequency of the slower common form was .98 to 1.0.

Due to the small amount of polymorphism no comparison was made between year classes.

Malate Dehydrogenase (MDH)

The slower migrating loci, MDH-A, demonstrated minor amounts of variation in five populations (Table 1). The gene frequency of the common allele (faster migrating than the variant form) was .97 to 1.0. In contrast we were able to identify three distinct variant alleles for the MDH-B loci; one faster migrating than the common form and two slower. All but four populations had the fast variant with gene frequencies from .005 to .045. The faster migrating of the slow variants was apparent in only four populations

(gene frequencies of .005 to .015) while the very slow variant allele occurred in all but one population (Buskin River), with gene frequencies of .005 to .043 (Table 1).

Significant differences in gene frequencies were noted between year classes for both MDH-A and MDH-B (Table 2).

Additional Isozymes

In addition to the above five enzyme systems nine more were analyzed. The following is a list of seven of the nine that were all monomorphic:

Isozyme	Abbreviation (if any)
Acid phosphatase	
Creative kinase	CK
Esterase	Est
Peptidase	Pep
Phosphogluco isomerase	PGI
Phosphomannose isomerase	PMI
6-phosphogluconate dehydrogenase	6-PGDH

This contrasts with the data collected for the previous year class (1976-1977) where four of the above isozymes (CK, PGI, PMI and 6-PGDH) all showed some variation. The remaining three enzyme systems were monomorphic for both years. Two additional enzymes (Malate Enzyme and Glucose-6-phosphate dehydrogenase) showed variation; however, the genetic interpretation is difficult. We anticipate that the breeding experiments conducted this year will help clarify this situation by determining if these additional polymorphic enzymes exhibit genetically determined variation. If so, they can be used in conjunction with the enzyme systems already being used in this study (AGP, PGM, AAT, LDH, and MDH) to further distinguish pink salmon stocks.

Breeding Experiments

Several enzyme systems (notably malate enzyme and glucose-6-phosphate dehydrogenase) possess what appears to be genetically determined variation; however, the results cannot be interpreted with any known Mendelian model (personal communication, F. Utter). Therefore, genetic breeding experiments were conducted at Kitoi Bay hatchery (Table 3), with the purpose of determining whether inheritance of these allozymes follows Mendelian patterns. The progeny from the crosses are being reared at the Kitoi Bay hatchery until at least the button-up stage. Information concerning this phase of the study is not available at the time of this report, but it will be included in the final report.

SUMMARY

Specific staining was done on many enzymes; however, only five demonstrated useful variation. AGP and AAT-3 were highly polymorphic and easily read. Three other loci (PGM, LDH-1 and LDH-4) had low levels of variation. Also, MDH had several variant alleles; however, actual variant allele frequencies were relatively low. All other enzymes observed fell into three categories: (1) monomorphic; (2) unreadable; or (3) in need of additional genetic work to clarify the interpretation.

Significant differences between some of the streams were observed (Table 1); however, further statistical analysis is needed to determine whether these differences will be useful in stock separation.

Aspinwall (1974) shows significant differences between year classes. Our results generally confirm Aspinwall's (1974) findings. However, we recorded a very slow MDH-B variant that Aspinwall (1974) appears to have

Table 3. Summary of genetic crosses performed on pink salmon at Kitoi Bay hatchery.

Isozyme	Cross number	Female Homozygote	Female Heterozygote	Female Alternate homozygote	Male Homozygote	Male Heterozygote	Male Alternate homozygote
AGP	1	x				x	
	2		x				x
	3		x		x		
	4			x		x	
	5	x			x		
	6		x			x	
MOH-B	1	x				x	
	2	x			x		
IDH	1		x			x	
	2	x					x
	3	x			x		
PGM	1	x			x		
	2		x		x		
	3	x				x	
LDH-4	1	x			x		
	2		x		x		
ME	1	x			x		
	2	x				x	
	3		x			x	
	4		x		x		x
	5	x					
	6			x	x		

Table 3. cont'd

Isozyme	Cross number	Female			Male		
		Homozygote	Heterozygote	Alternate homozygote	Homozygote	Heterozygote	Alternate homozygote
G_6PDH	1		x		x		
	2	x				x	
	3	x			x		
	4		x			x	
Est	1	x			x		
	2	x				x	
AAT-3	1	x			x		
	2	x				x	
CK	1	x			x		
	2	x				x	

classified as a fast MDH-A variant. When these differences are taken into account, our results and those of Aspinwall (1974) show reasonable agreement.

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