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A BIOCHEMICAL INVESTIGATION OF KODIAK ISLAND PINK SALMON GENE FREQUENCIES

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Interim Report for the Period
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Robert L. Burgner
Director

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INTRODUCTION

Management of the Kodiak pink salmon fishery, when compared with other salmon fisheries, has been quite successful; however, areas of concern do exist. The problem addressed in this report is stock separation. Early in the fishing season, much of the fishing fleet concentrates its efforts at the capes (headlands). Many different races (stream stocks) probably pass these headlands at one time. The Alaska Department of Fish and Game (ADF&G) is then faced with deciding what portions of the catch are destined for specific streams.

A technique for accurately determining racial proportions in mixed fisheries would be a significant aid to management. We proposed (account code number 11-41-1-286) to do biochemical studies on samples collected from approximately 27 spawning streams of Kodiak Island (and adjacent areas) during the summer of 1976, to determine if analysis of gene frequencies defined by biochemical methods could separate stocks in mixed fisheries.

This interim report summarizes the data we have collected and the analysis made to date. No attempt will be made in this report to provide a definite answer to the problem outlined above. We will address this in the final report when the data collection and all analyses are completed. On the basis of the work to date, we do suggest that gene frequency analysis be continued through the following year class.

MATERIALS AND METHODS

Liver, muscle and eye tissues were collected from spawning adult pink salmon by personnel of the Kodiak office of the ADF&G during the summer of 1976. Twenty-nine locations were sampled from Kodiak and surrounding environs. In early 1977 (mainly March) the same group collected emergent fry while on the annual egg dig surveys (used in forecasting). Approximately 5 fry from each dig that had fry were collected and kept frozen.

The adult tissues and the whole body of the fry were subjected to starch gel electrophoresis. Small portions of the tissue, or in the case of the emergent fry the whole animals, were placed in test tubes. The tissue was mashed up and a few drops of distilled water added. The test tubes were centrifuged for several minutes and then some of the supernatant which contains the enzymes was soaked up on a small piece of filter paper. This filter paper was placed in an opening cut lengthwise in the starch gel (May 1975). The starch gel was made from hydrolyzed potato starch and buffer solution (Smithies 1955, Hunter and Markert 1957). The starch gel, with encased filter paper, was subjected to an electric field for several hours, causing the enzymes to migrate according to their electrical charge. After the enzymes traveled a sufficient distance, the starch gel was sliced (May 1975). These slices were placed in trays and stained for specific enzymes. Staining was done according to Shaw and Prasad (1970) and Allendorf (1975).

RESULTS AND DISCUSSION

Table 1 is a list of the different enzyme systems we have stained for on both emergent fry and adults. The expressed patterns of enzymes that showed variation are listed in Figure 1. Tables 2 and 3 show the results of the biochemical analysis to date. It should be noted that the common and variant allele frequencies as well as the estimated variance (times 10^{-3}) are listed by statistical area, location of stock, and enzyme system. The statistical areas (large numbers) are shown in Figure 2. The map (Figure 2) is reproduced from an unpublished stream catalogue of the Kodiak management district. The small numbers are stream numbers. Each enzyme system will be discussed in some detail below. The applicable data are summarized in Tables 1 through 3.

Lactate Dehydrogenase (LDH): Isozymes determined by five loci were stained for, but only the slowest migrating fraction (LDH-1) showed any variation. The variant allele of LDH-1 had a slower migration rate than the common form. Frequencies of the variant allele ranged from 0 to 0.08. Thirteen out of 29 locations showed some variation.

Phosphoglucomutase (PGM): The only variant we found was a faster migrating fraction than that expressed by the common allele. Frequencies for the variant allele ranged from 0 to 0.22; 11 out of 29 locations showed some variation. Adult samples from Pillar Creek and emergent fry samples from the Uganik and Afognak rivers were not readable, probably due to length of time between collection and freezing and/or storage time.

TABLE 1. Enzyme systems tested in adult and emergent fry tissues

Adults		Emergent Fry	
Enzyme	Results	Enzyme	Results
LDH	clear	LDH	clear
PGM	"	PGM	"
CK	"	CK	very weak
AGP	"	AGP	clear
ME	clear (however, genetic crosses will be necessary to interpret the results)	ME	" (however, genetic crosses will be necessary to interpret the results)
MDH A & B	unreadable	MDH A & B	clear
6 PGDH	"	6 PGDH	"
PGI	"	PGI	"
G_6PDH	"	IDH	"
IDH	"	AAT	"
AAT	"	PMI	"
ADH	"	SDH	unreadable
PMI	"		
SDH	monomorphic		
PEP	"		
LAP	nothing showed		

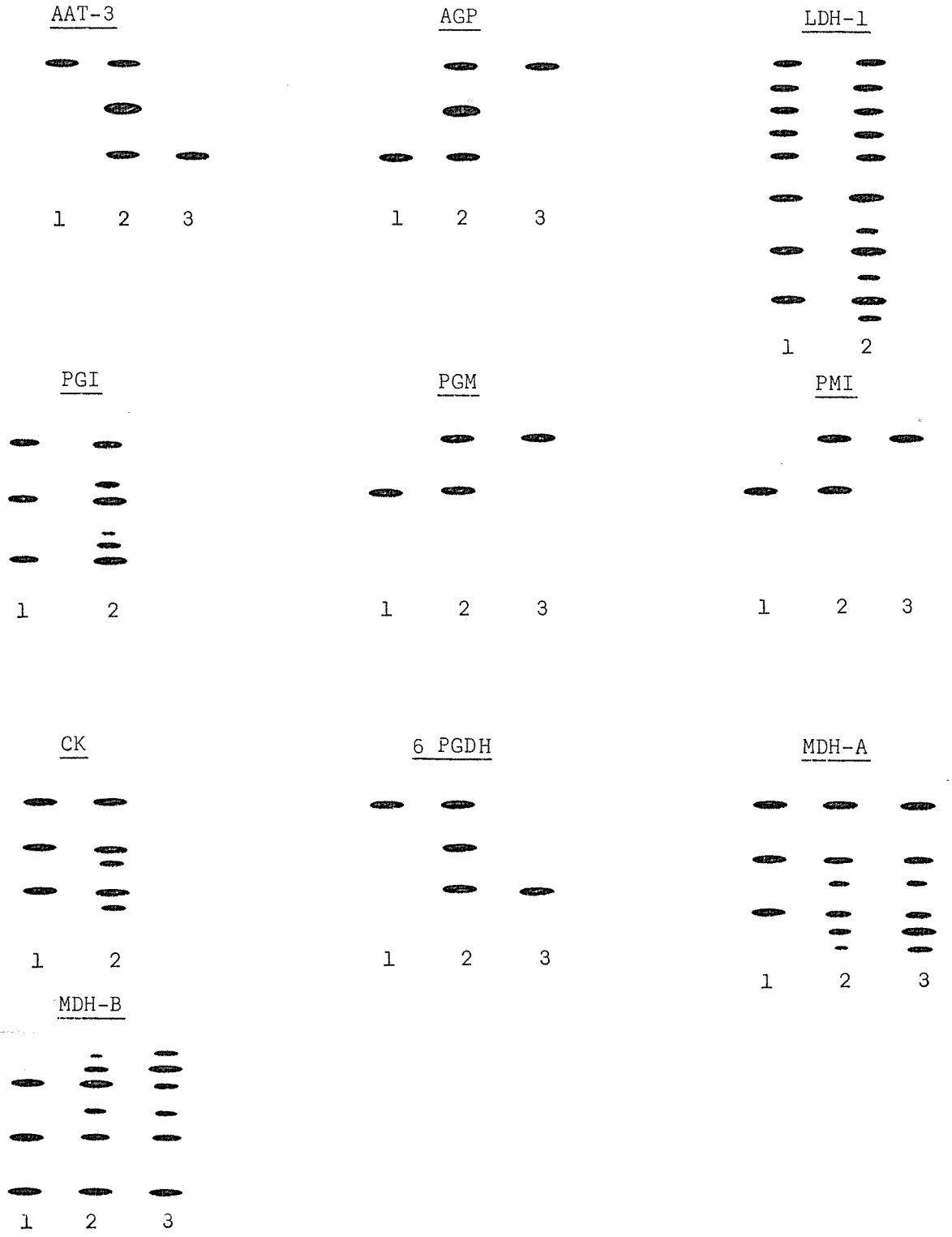

FIGURE 1. Electrophoretic patterns observed in the variant systems of pink salmon adult and emergent fry tissues. The pattern in each system numbered 1 is the common pattern observed, 2 is the heterozygote between the variant and common forms, and 3 is the homozygous variant form, where it was observed.

TABLE 2. Gene frequencies and variance estimates (σ^2) of the frequencies from the adult pink salmon tissues collected. "c" designates the common allele and "v" designates the variant allele; also, variance estimates are x 10^{-3}

Statistical area	Location	Sample size	ENZYME SYSTEM							
			LDH-1		PGM		CK		AGP	
			c	v	c	v	c	v	c	v
251	Seal Bay	44	1.00		1.00		1.00		0.94	0.06
	σ^2								0.64	0.64
	Perenosa Cr.	50	1.00		0.99	0.01	0.98	0.02	0.82	0.18
	σ^2				0.099	0.099	0.196	0.196	1.5	1.5
252	Marka Cr.	48	1.00		1.00		1.00		0.89	0.11
	σ^2								1.0	1.0
	Afognak R.	50	0.98	0.02	1.00		1.00		0.87	0.13
	σ^2		0.196	0.196					1.0	1.0
	Sharatin R.	50	0.99	0.01	0.96	0.04	0.99	0.01	0.84	0.16
	σ^2		0.099	0.099	0.38	0.38	0.099	0.099	1.3	1.3
253	Baumann's Cr.	50	0.97	0.03	1.00		1.00		0.87	0.13
	σ^2		0.29	0.29					1.0	1.0
	Terror R.	50	0.97	0.03	1.00		1.00		0.93	0.07
	σ^2		0.29	0.29					0.65	0.65
	Mush Bay	52	0.99	0.01	0.97	0.03	1.00		0.84	0.16
	σ^2		0.095	0.095	0.28	0.28			1.3	1.3
254	Zachar Bay	50	1.00		1.00		1.00		0.87	0.13
	σ^2								1.0	1.0
	Brown's Lagoon	49	0.99	0.01	1.00		1.00		0.90	0.10
	σ^2		0.1	0.1					0.92	0.92
	Uyak Bay	50	0.99	0.01	0.99	0.01	1.00		0.87	0.13
	σ^2		0.099	0.099	0.099	0.099			1.0	1.0

TABLE 2. Adult gene frequencies and variance estimates (σ^2) - continued

Statistical area	Location	Sample size	ENZYME SYSTEM							
			LDH-1		PGM		CK		AGP	
			c	v	c	v	c	v	c	v
255	Karluk Lagoon	50	1.00		0.90	0.10	1.00		1.00	
	σ^2				0.82	0.82				
	Karluk R.	48	0.98	0.02	1.00		1.00		0.93	0.07
	σ^2		0.2	0.2					0.68	0.68
256	Red R.	28	1.00		1.00		*		0.87	0.13
	σ^2								2.0	2.0
	Red Lake	51	1.00		0.78	0.22	0.99	0.01	1.00	
	σ^2				1.7	1.7	0.097	0.097		
257	Upper Station	50	1.00		1.00		1.00		0.89	0.11
	σ^2								0.98	0.98
	Narrow Cr.	29	0.98	0.02	1.00		1.00		0.93	0.07
	σ^2		0.34	0.34					1.0	1.0
	Dog Salmon R.	47	0.99	0.01	1.00		*		0.77	0.23
	σ^2		0.1	0.1					1.9	1.9
	Horse Marine Cr.	52	1.00		1.00		1.00		0.83	0.13
	σ^2								1.4	1.4
	Deadman R.	48	1.00		0.96	0.04	0.98	0.02	0.89	0.11
	σ^2				0.4	0.4	0.2	0.2	1.0	1.0
	Humpy Cr.	39	1.00		1.00		1.00		0.83	0.17
	σ^2								1.8	1.8

*Unreadable.

TABLE 2. Adult gene frequencies and variance estimates (σ^2) - continued

Statistical area	Location	Sample size	ENZYME SYSTEM							
			LDH-1		PGM		CK		AGP	
			c	v	c	v	c	v	c	v
258	Kaiugnak R.	50	1.00		1.00		1.00		0.84	0.16
	σ^2								1.0	1.0
	Barling Cr.	50	1.00		0.95	0.05	0.96	0.04	0.92	0.08
	σ^2				0.5	0.5	0.38	0.38	0.74	0.74
	Kiliuda 207	54	*		0.98	0.02	0.99	0.01	0.89	0.11
	σ^2				0.18	0.18	0.092	0.092	0.91	0.91
	Kiliuda 206	55	1.00		1.00		1.00		0.91	0.09
	σ^2								0.74	0.74
259	Hurst Cr.	50	0.99	0.01	0.89	0.11	1.00		0.88	0.12
	σ^2		0.099	0.099	0.98	0.98			1.1	1.1
	Sid Olds Cr.	52	1.00		0.99	0.01	*		0.90	0.10
	σ^2				0.095	0.095			0.87	0.87
	Buskin R.	50	0.96	0.04	1.00		1.00		0.84	0.06
	σ^2		0.38	0.38					0.56	0.56
	Pillar Cr.	42	0.98	0.02	*		*		0.78	0.22
	σ^2		0.2	0.2					2.0	2.0

* Unreadable.

TABLE 3. Gene frequencies and variance estimates (σ^2) of the frequencies from th
allele and "v" designates the variant allele; also, variance estimates a

Statistical area	Location	Sample size	ENZYME SYSTEM: LDH-1 c*	LDH-1 v	PGI c	PGI v	MDH-A c	MDH-A v	MDH-B c	MDH-B v
251	Seal Bay	14	1.00		1.00		1.00		0.96	0.0
	σ^2								1.4	1.4
252	Afognak R.	27	1.00		*		0.99	0.01	0.98	0.0
	σ^2						0.17	0.17	0.34	0.3
253	Uganik R.	15	1.00		*		1.00		1.00	
	σ^2									
254	Brown's Lagoon	29	1.00		1.00		0.99	0.01	0.98	0.02
	σ^2						0.17	0.17	0.34	0.34
255	Karluk R.	20	0.92	0.08	1.00		1.00		0.96	0.04
	σ^2		1.8	1.8					0.96	0.96
256	Red R.	47	0.99	0.01	0.99	0.01	0.97	0.03	1.00	
	σ^2		0.1	0.1	0.1	0.1	0.3	0.3		
257	Narrow Cr.	19	1.00		1.00		1.00		0.97	0.03
	σ^2								0.77	0.77
	Humpy Cr.	44	1.00		1.00		1.00		1.00	
	σ^2									
258	Kaiugnak R.	42	1.00		1.00		0.98	0.02	1.00	
	σ^2						0.2	0.2		
	Barling Cr.	11	1.00		1.00		1.00		0.86	0.14
	σ^2								5.0	5.0
259	Sid Olds Cr.	25	1.00		0.98	0.02	1.00		0.97	0.03
	σ^2				0.39	0.39			0.58	0.58
	Buskin R.	15	1.00		1.00		1.00		0.96	0.04
	σ^2								1.3	1.3

*Unreadable.

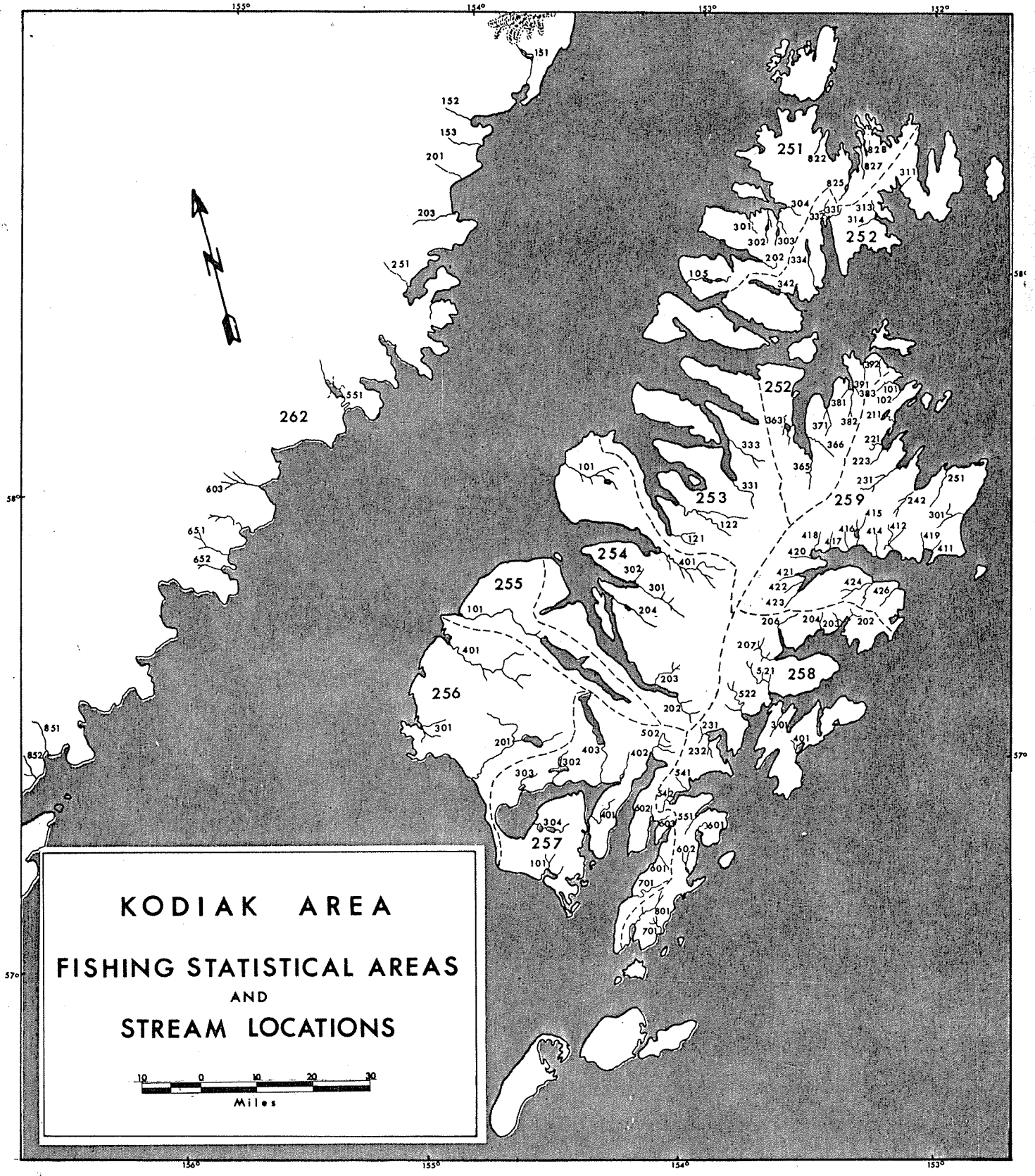

FIGURE 2. Map of Kodiak Island and surrounding area showing statistical areas and stream designations used by Alaska Department of Fish and Game.

Creatine Kinase (CK): The best stain for this enzyme is that used for general protein. The variation noted for CK is unusual since very little has been found in previous work. The protein expressed by the variant allele migrated slower than the common form. Frequencies for the variant allele ranged from 0 to 0.04. Adult samples from four locations were unreadable and six of the remaining locations had variation. This enzyme showed so weakly in the juvelile samples that analysis of the results was impossible.

Alpha Glycerophosphate Dehydrogenase (AGP): This enzyme system is one of the most easily read. All but one location showed variation, and the frequency of the variant allele ranged from 0 to 0.23. Frequencies of the emergent fry samples differed somewhat from the adult samples even though the fry were the offspring of these adults (the 1976 spawners produced the 1977 fry). The variant allele in this case was faster migrating than the common form.

Peptidase (PEP) and Sorbitol Dehydrogenase (SDH): PEP and SDH were both consistently homozygous in the adults, therefore we stopped staining for them. A few juvenile samples were stained for SDH, however, the results proved unreadable.

Only the above six enzyme systems (with one exception to be discussed later) showed consistently readable results in the adult tissues. We believe that many of the less stable enzyme systems deteriorated while in storage (in some cases up to eight months). The emergent fry were much fresher and showed a greater number of active enzymes. Some systems,

notably CK and SDH, did not stain well in the fry, either due to masking from materials in other tissues (fry are too small for use of separate tissues) or incomplete activation in these young fish. The other enzyme systems we stained were very clear. The rest of the enzymes to be discussed were tested using emergent fry.

Phosphomannose Isomerase (PMI) and Isocitrate Dehydrogenase (IDH): These two enzymes exhibited no variation. PMI in some locations was unreadable (4 of 12 locations) while IDH stained well in all cases. Since these enzymes showed no variation, they were not included in Table 3.

Phosphoglucomutase (PGM): Variation was noted for the adults; however, so far we have not found variation in the emergent fry. This enzyme, therefore, was not included in Table 3.

Aspartate Aminotransferase (AAT): This is a three locus system with only AAT-3, expressing variation, and that from the freshest samples. Probably for this reason three out of 12 locations gave unreadable results. Only two areas demonstrated variation. The variant is expressed as a slower migrating fraction than the common form, and was found in a range of frequencies from 0 to 0.08.

6-Phosphogluconate Dehydrogenase (6PGDH): The number of locations that showed variation was small, three out of twelve. The variant allele migrated slower than the common and frequencies of the variant allele ranged from 0 to 0.05. The readability of this enzyme was not as good as desired, but interpretable results were obtained.

Phosphoglucose Isomerase (PGI): This enzyme has three loci of which two were invariant. The PGI-1 locus did show a variant allele (fast migrating) for two locations out of 12. The frequencies for the variant allele ranged from 0.0 to 0.02.

Malate Dehydrogenase (MDH): This system can be divided into two groupings MDH-A and MDH-B, and each group is defined by two loci (duplicated locus); therefore, four loci can be analyzed. MDH-A frequencies for the variant allele were from 0 to 0.03 with four of the 12 locations showing some variation. MDH-B demonstrated more variation (8 of 12 locations) and a wider range of gene frequencies for the variant allele (0 to 0.14). The variant for MDH-A was slower migrating while that for the MDH-B was faster than the common form.

Malic Enzyme (ME): This enzyme was easily stained for in both adults and emergent fry; however, we were uncertain of the interpretation. It was fairly resistant to long storage time and showed what appeared to be considerable variation. Genetic crosses are needed to fully explain the results we obtained.

SUMMARY

Staining was done for seventeen enzyme systems on the adult tissues and 12 for the emergent fry. Only six of the 17 showed consistent results for the adults and two of these had no variation. The emergent fry proved better since 10 of 12 produced readable results, with 7 out of the 10 showing some variation.

Storage time and/or length of time between collection and freezing of the samples appears to have an adverse effect upon the more unstable enzymes. The adult samples were stored up to eight months and the emergent fry less than two months.

Malic enzyme shows promise of being a useful marker in stock separation, but genetic crosses are needed for better understanding of the electrophoretic patterns.

Several enzyme systems, AGP, PGM, MDH-B, and AAT, have shown enough variation in gene frequencies to be of continued interest in stock separation for the even year class. However, preliminary work we did last year indicates more enzyme systems than those listed above should be used in the analysis of fish from the odd year cycle.

The final report will contain more extensive analysis. The adult gene frequencies will be compared with those from their emergent fry to test consistency between generations. Also, evaluation of starch-gel electrophoresis as a useful tool for stock separation will be made.

NEED FOR ADDITIONAL STUDY

We feel strongly that the odd year class should be analyzed in the same manner as the even. Aspinwall (1974) has demonstrated quite a disparity between year classes. Therefore the gene frequencies of the even year are probably not useful as baseline data for the odd year cycle.

As we stated earlier the malic enzyme system may be useful in stock separation; however, the results cannot be interpreted on the basis of present information. Genetic crosses should be made at Kodiak this summer in an attempt to explain the observed electrophoretic patterns.

Finally, various hard parts (scales, otoliths, opercular bones, etc.) should be analyzed to determine if data collected from them could be used to supplement the genetic data.

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