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THE GENETIC POPULATION STRUCTURE OF THE KOKANEE SALMON IN FLATHEAD LAKE, MONTANA

By

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B.S., University of Montana, 1977

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Dean, Graduate School

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Date

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ABSTRACT

Phelps, Stevan R., M.A., 1980 Zoology

The genetic population structure of the kokanee salmon in Flathead Lake, Montana.

Director: Fred W. Allendorf

Collections of kokanee salmon were made during the fall of 1976 and 1977 from spawning concentrations in Flathead Lake, Flathead River and other lakes in northwestern Montana. These fish were examined electrophoretically at 63 loci. A very low amount of genetic variation exists in the kokanee salmon populations in northwestern Montana. Average heterozygosity is 0.006. Only a single locus, PGM-2, is polymorphic. Genetic variation occurs at three other loci, LDH-1, LDH-3 and AGP-1, but at a frequency too low to be useful in population genetics studies.

On the basis of gene frequency data and location, I propose that there are six major subpopulations of kokanee salmon in Flathead Lake. The age at maturity and genotype-phenotype correlations are examined. The implications for the management of this subdivided population is discussed.

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INTRODUCTION

An awareness of the genetic structure of a population is essential if we are to understand the biological diversity within a species, i.e., the amount and type of genetic variation occurring within and among populations. This knowledge can be very important from a fishery management perspective. Such an understanding is necessary if fish management programs are going to take advantage of potentially useful ecological adaptations in future fish management and breeding programs. It is critical to determine whether or not the population in question is separated into distinct reproductively isolated and ecologically specialized units. If the population is subdivided, an additional question becomes how many distinct units are there, and further, in which ways do they differ? In addition, the degree of genetic divergence among populations can be used to estimate the amount of gene flow between them.

Genetic variation in a population can be studied by means of starch gel electrophoresis. This technique provides an effective tool for differentiating demes (i.e., local random mating units) within a species. The major advantages of electrophoresis are the direct relationship between appropriately chosen protein variants and the gene, the relative ease of application, and the efficiency of the technique.

Protein variants that are used for genetic analysis reflect simple genetic differences. Such differences among individuals and populations are expressed in the form of genotypic and allelic frequencies. Frequencies of variants in salmonid populations appear to be stable attributes of these populations and tend to persist at the same levels over many generations (Allendorf and Utter 1979). Morphological variation, on the other hand, is usually affected by an unknown number of genes and unknown environmental components.

Salmonids are well suited for population genetic studies. Extensive gene duplication results in a large number of electrophoretically detectable loci (Allendorf et al. 1975). Inheritance data has confirmed the genetic basis of the isozyme patterns and relatively large sample sizes can be obtained. In addition, salmonids have been extensively studied by classical fisheries research methods. Salmonids show a tendency to evolve genetically discrete, ecologically specialized populations that are differentiated on the basis of such life-history characteristics as time and place of spawning (Foerster 1968). The site-specific homing behavior of most salmonids is an important factor in the maintenance of this diversity (Leggett 1975). Such traits allow for development of reproductively isolated populations. The existence of these genetically distinct sympatric populations

presents an important problem to those responsible for the management of fish populations. Many unique and potentially valuable gene pools have been lost by improper management (Behnke and Zarn 1976).

Within many species, there are considerable phenotypic differences between different natural populations. Usually, the most striking variation is in body size, but differences in other characters such as body shape, color, and spawning age are also common. Differences in such characteristics are seen in the populations of kokanee salmon (Oncorhyuchus nerka), a landlocked form of sockeye salmon, in Flathead Lake, Montana, and surrounding lakes (Hanzel, 1973, 1976, 1977). There are some indications of distinct spawning areas in Flathead Lake and in the incoming river system (Stefanich 1954, Hanzel 1964).

Kokanee salmon in Flathead Lake thus provide an unusual opportunity to study the genetic structure of a natural population of fish. The present population of kokanee salmon in Flathead Lake was started by a planting in 1916 of what was thought to be chinook salmon, O. tshawytscha, from the Quannat Salmon Hatchery in Oregon (Anonoymous 1918). This error was discovered in 1918 when mature kokanee salmon were taken from Lake Mary Ronan (a small lake west of Flathead Lake) which was included in the 1916 stocking. It is impossible to tell whether this original introduction of salmon consisted of sockeye or kokanee salmon or both. Since

that time, this single initial plant has spread throughout the Flathead drainage diverging into lotic and lentic spawning stocks that use discrete spawning areas each fall.

A kokanee salmon hatchery operates at Somers, Montana. In 1934 the Somers hatchery began kokanee salmon egg taking operations from Flathead Lake and started distributing kokanee salmon fry to areas in Flathead Lake and other lakes in northwestern Montana. Eggs are still taken from various areas around Flathead Lake and other lakes where large spawning concentrations occur. Most of the eggs presumably are taken from fish returning to hatchery bay which were released from the hatchery as fry (Hanzel unpublished data). Some lakes and areas in Flathead Lake and the Flathead River receive an annual stocking, while other lakes have not been planted for many years. All the other lakes in this study have populations of kokanee salmon which were derived from the Flathead Lake stock. The recent introduction of kokanee salmon into these lakes in the Flathead River drainage permits the examination and comparison of rates of evolutionary isolation, mechanisms such as genetic drift and differential selection in a group of organisms from an identified origin.

The objectives of this study are the following: (1) Describe the amount of genetic variation within and between kokanee salmon populations in northwestern Montana; (2) Identify any reproductively isolated units of kokanee salmon

in Flathead Lake and the Flathead River; (3) Compare the magnitude of the divergence of these Flathead stocks to populations of kokanee salmon in other lakes to estimate the extent of divergence and identify the genetic factors which affect the rate of this divergence.

MATERIALS AND METHODS

Sample Collection

Mature kokanee salmon were collected from spawning concentrations along the shoreline of Flathead Lake, The Flathead River, and several other lakes in northwestern Montana during the fall in 1976, 1977 and 1978 (Figure 1). The fish were obtained by the use of gill nets (lake samples) and electroshocking (river samples). Sample size ranged from 15-50 individuals each year and varied according to the number of spawning fish present at the time of sampling. The majority of the fish had spawned by the time the samples were collected.

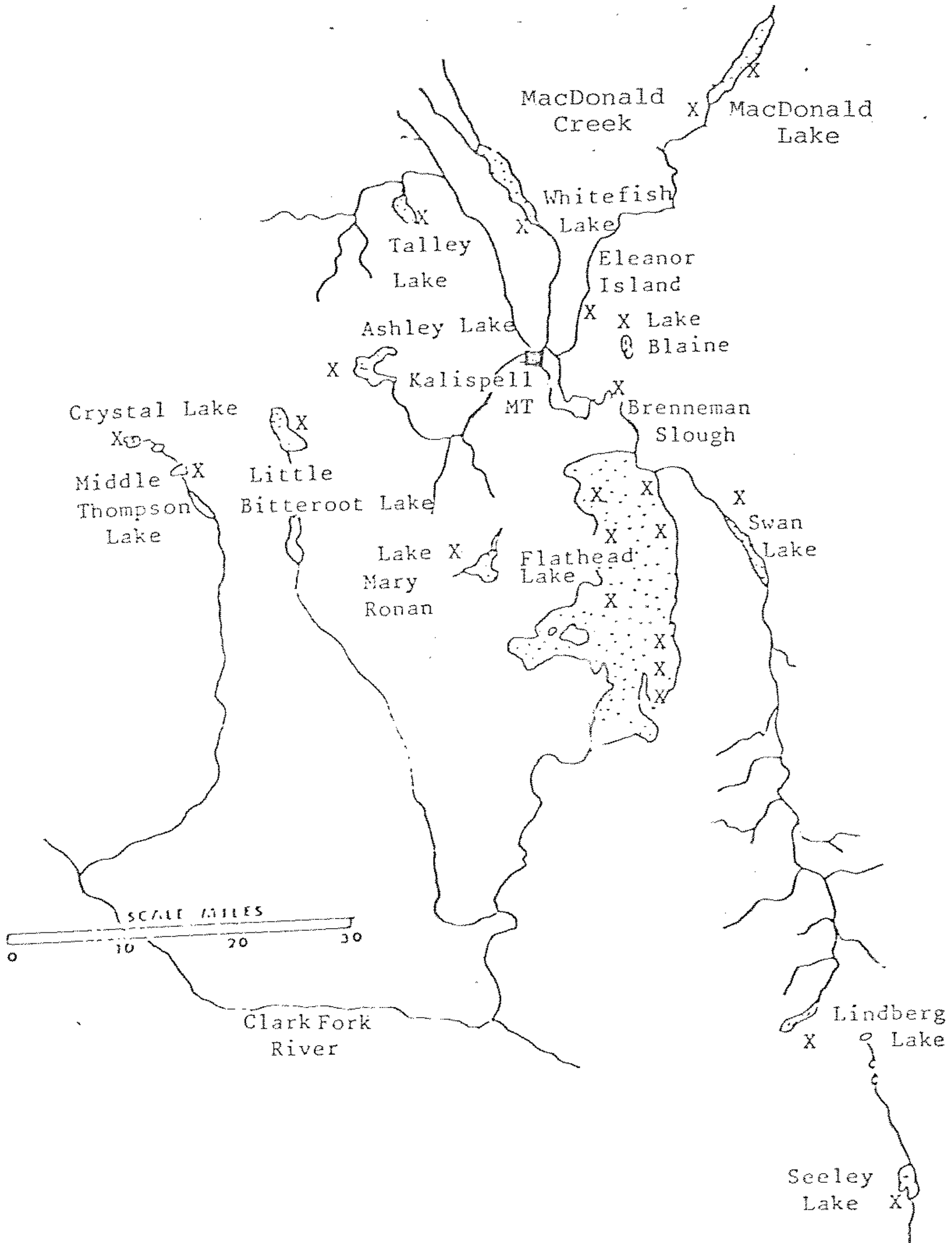
The length, weight, age (otolith), sex, and breeding condition for each fish was recorded by biologists from the Montana Department of Fish, Wildlife, and Parks (Hanzel, unpublished data). Each fish was numbered so that the electrophoretic data could be compared to the morphological data. The head and anterior abdominal cavity were cut from the carcass and frozen until the electrophoretic analysis.

Electrophoresis

Horizontal starch gel electrophoresis was conducted according to the methods of Utter, Hodgins and Allendorf (1974). The buffer systems, and staining methods used in this study are described by Allendorf, et al. (1977). The nomenclature used to describe the gene loci and the allele variants

Figure 1

Collection Areas (X)



encoding the enzymes surveyed follows the system proposed by Allendorf and Utter (1979). An abbreviation is chosen to represent each protein. A hyphenated numeral is included to represent the loci coding for this protein with the least anodal mobility designated as 1. The allele variants are designated according to their relative mobility. The migration distance of the most common isozyme is assigned a mobility of 100. Thus, an allele of the most cathodal LDH locus coding for an enzyme migrating one-half as far as the common allele would be designated LDH-1(50).

Muscle, liver and eye tissues were screened for adequte electrophoretic resolution, enzyme activity and genetic variation. A total of 31 enzymes (Table 1) and 69 loci were examined for the above criteria (Table 2). The tissue and buffer system combinations with the best activity and resolution generally agree with Allendorf et al. (1977).

Table 1.

List of enzymes used in the study.

Abbreviation	Number	Common Name
AAT	2.6.1.1	Aspartate aminotransferase
ADA	3.5.4.4	Adenonsine deaminase
ADH	1.1.1.1	Alcohol dehydrogenase
AGP	1.1.1.8	Glycerol-3-phosphate dehydrogenase
AK	2.7.4.3	Adenylate kinase
ALD	4.1.2.13	Aldolase
CPK	2.7.3.2	Creatine phosphokinase
DIA	1.6.2.2	Diaphorase
EST	3.1.1.1	Esterase
FDP	4.1.2.13	Fructose -1,6 diphosphotase
FUM	4.2.1.2	Fumerase
G6PDH	1.1.1.49	Glucose-6-phosphate dehydrogenase
GAPDH	1.2.1.12	Glyceraldehyde-3 phosphate dehydrogenase
GDH	1.1.1.47	Glutamate dehydrogenase
GLYDH	1.1.1.6	Glycerol dehydrogenase
GPT	2.6.1.2	Glutamate pyruvate transaminase
GUS	2.3.1.31	B-glucoronidase
HK	2.7.1.1	Hexokinase
IDH	1.1.1.42	Isocitrate dehydrogenase
LAP	3.4.1.1	Leucine aminopeptidase
LDH	1.1.1.27	Lactate dehydrogenase
MDH	1.1.1.37	Malate dehydrogenase
ME	1.1.1.40	Malic enzyme
MPI	5.3.1.8	Mannose-6-phosphate isomerase
NP	2.4.2.1	Nucleoside phosphorylase
PEP	3.4.1.2	Peptidase
6PGDH	1.1.44	6-Phosphogluconate dehydrogenase
PGI	5.3.1.9	Phosphoglucose isomerase
PGK	2.7.2.3	Phosphoglucomutase kinase
PGM	2.7.5.1	Phosphoglucomutase
PK	2.7.1.40	Pyruvate kinase
SDH	1.1.1.14	Sorbitol dehydrogenase
SOD	1.15.1.1	Superoxide dismutase
SUCDH	1.3.99.1	Succinate dehydrogenase
TPI	5.3.1.1	Triose phosphate isomerase
XDH	1.2.3.2	Xanthine dehydrogenase

Table 2. Designation of loci coding for different enzymes.

Enzyme	Loci	Tissues and Buffer Systems with Adequate Resolution AC	RW	Genetically Variable
AAT	1	L,M	L	NO
	2	L,M	L	NO
	3	L	L	NO
	4	L	L	NO
	5	E,L,M	E,L,M	NO
ADA	?			?
ADH	1		L	NO
AGP	1	M		NO
	2	L,M	L,M	YES
	3	E		NO
AK	1	L		NO
	2	L		NO
	3	E,L,M		NO
ALD	1	M		NO
	2	E		NO
	3	E		NO
CPK	1	L,M	L,M	NO
	2	L,M	L,M	NO
	3	E	E	NO
DIA	1		L	NO
EST	1	L	L	NO
	2	E,L,M	E,L,M	NO
FDP	1		L	NO
	2		L	NO
FUM	1		M	NO
	2		E	NO
GAP	1	M	M	NO
	2	E	E	NO
GDH	1	L		NO
GPT	1	L		NO
	2	L		NO

Table 2. Designation of loci coding for different enzymes, continued.

Enzyme	Loci	Tissues and Buffer Systems with Adequate Resolution		Genetically Variable
		AC	RW	
G6PDH	1	M	L	NO
	2	L	L	NO
GUS	1		L,M	NO
HK	1	L		NO
	2	E,L,M		NO
IDH	1	M		NO
	2	M		NO
	3	L		?
	4	L		?
LAP	1	M		NO
	2	L		NO
LDH	1	M	M	NO
	2	M	M	NO
	3	E,M	E,M	YES
	4	E,L,M	E,L,M	NO
	5	E	E	NO
MDH	1	M	M	NO
	2	M	M	NO
	3	L	L	NO
	4	L	L	NO
ME	1	M	M	NO
	2	L,M	L,M	NO
PEP	1	E,L,M	E,L,M	NO
	2	E,L,M	E,L,M	NO
GPG	1	L,M		NO
	2	L		NO
PGI	1		M	NO
	2		M	NO
	3		E,L,M	NO
PGM	1	M		
	2	L,M	L,M	YES
	3	E	E	(YES)?
PMI	1	E,L,M		NO

Table 2. Designation of loci coding for different enzymes, continued.

Enzyme	Loci	Tissues and Buffer Systems with Adequate Resolution		Genetically Variable
		AC	RW	
SDH	1		L	NO
	2		L	NO
SOD	1		L	NO
TPI	1?	M		NO
	2?	E		NO
XDH	1		L	NO

RESULTS

Electrophoretic Systems

The kokanee salmon in this study have a very low amount of genetic variation. Of the 69 loci examined, only one locus, PGM-2, was genetically variable at a high enough frequency to be considered polymorphic (see Table 1, 2 for abbreviations of enzymes and loci). Three other loci, AGP-2, LDH-1, LDH-3 are genetically variable at a very low frequency (Table 3). Average heterozygosity ($\overline{H}$), the average proportion of heterozygotes per locus, is 0.006. This measure is an estimate of the average proportion of the time an individual receives a different allele from each parent.

The genetic variation at Pgm-2 consists of three phenotypes represented by two alleles (100,120) (Figure 2). These findings agree with those of Utter and Hodgins (1970), who first described the PGM polymorphism in sockeye salmon. Inheritance studies have demonstrated simple Mendelian segregation at this locus (Utter et al. 1973). This locus was the only one genetically variable enough to use for the population genetic analysis.

Several samples possess variation for LDH. A slow allelic variant occurs at Ldh-3, LDH-3(75) (Figure 3). This allelic variant was shown to occur at the LDH-3 locus due to the evidence that the LDH-4 locus, the only LDH locus found in the liver tissue of salmonids, was monomorphic. A null

Table 3. genetic variation at AGP-2, LDH-1, LDH-3

Sample* Area	Sample Year	Sample Size	Number of variant alleles AGP-2 (60)	LDH-1 (50)	LDH-3 (75)	LDH-3 null
MacDonald Creek-FR	1976	50	0	0	0	0
	1977	50	0	1	0	0
Middle Thompson Lake	1976	50	0	0	1	1
	1977	50	0	0	0	0
Crystal Lake	1976	44	0	0	1	0
	1977	22	0	0	0	0
Somers Hatchery-FL	1976	50	0	1	0	0
	1977	50	0	0	1	0
	1978	36	0	0	0	0
Ashley Lake	1976	50	0	0	0	0
	1977	50	0	0	0	0
Talley Lake	1976	50	0	0	0	2
	1977	46	0	0	0	0
Whitefish Lake	1976	49	0	0	1	1
	1977	no sample				
Yellow Bay-FL	1976	50	0	0	0	3
	1977	44	0	0	0	0
Bigfork Bay-FL	1976	49	0	0	0	0
	1977	50	0	0	0	0
Skidoo Bay-FL	1976	19	0	0	0	0
	1977	31	0	0	0	0
Dr. Richards Bay-FL	1976	19	0	0	0	0
	1977	50	0	0	2	2
Woods Bay-FL	1976	46	0	0	1	1
	1977	42	0	0	1	1
West Shore Park-FL	1976	15	0	0	1	0
	1977	18	0	0	0	0
Crescent Bay-FL	1976	26	0	0	0	0
	1977	47	0	0	1	1

Sample* Area	Sample Year	Sample Size	AGP-2 (60)	LDH-1 (50)	LDH-3 (75)	LDH-3 null
Little Bitterroot Lake	1976	50	0	0	0	0
	1977	50	0	0	0	0
	1977	48	0	0	0	0
Brennaman's Slough-FR	1976	50	0	0	0	0
	1977	50	0	0	0	0
Eleanor Island-FR	1976	50	1	0	0	0
	1977	49	0	0	0	0
Swan Lake	1976	50	0	0	0	0
	1977	50	0	0	2	1
Lindberg Lake	1976			no sample		
	1977	50	1	0	0	0
Lake Mary Ronan	1976			no sample		
	1977	17	0	0	0	0
Lake Blaine	1976			no sample		
	1977	23	0	0	0	0
Seeley Lake	1976			no sample		
	1977	18	0	0	0	0
MacDonald Lake	1978	50		no data		

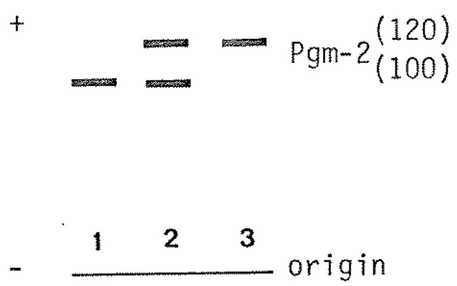
* FR= Flathead River
FL= Flathead Lake

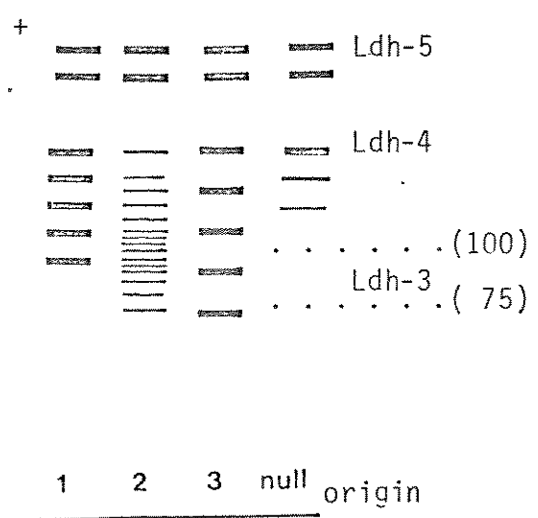
allele at the LDH-3 locus also appears to be present (Figure 3). An electrophoretic pattern similar to what was found by Allendorf et al. (in preparation) at the LDH-1 locus in brown trout, *Salmo trutta*, is observed in the heterozygous state at low frequency. These two variant alleles have not previously been reported. The kokanee salmon in this study also possess a slow allelic variant at the LDH-1 locus, LDH-1(50) (Figure 4). This variant has been reported in another kokanee salmon stock in Lake Washington (Utter et al. 1979). These genetic variants occur at a frequency too low to be useful in the subpopulation discrimination.

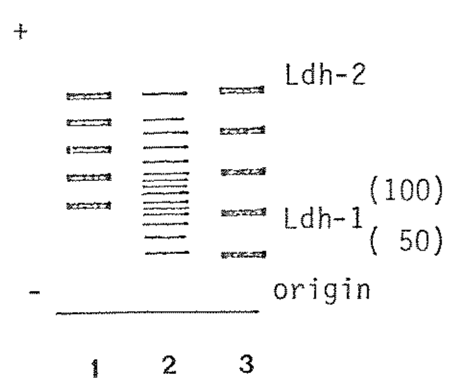
Variation also occurs in two additional enzymes. An AGP-2 variant allele, AGP-2(60), was seen in two fish. The observed variation agrees with what Allendorf et al. (1977) found in brown trout and Engel et al. (1971) reported in rainbow trout *S. gairdneri*. IDH in liver has a variable isozyme pattern but an adequate genetic model could not be devised that would adequately explain the observed variation (Figure 6). Utter (personal communication) has also observed the same variable isozyme patterns in west coast sockeye and kokanee salmon.

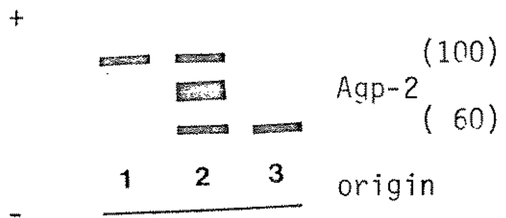
Genotypic Proportions

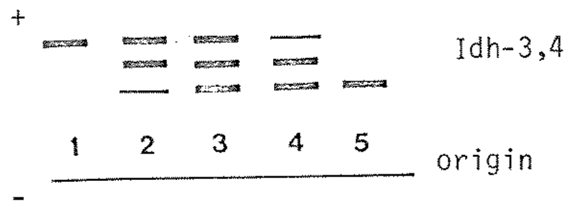
The first step in analyzing gene frequency population data from natural populations, is to see whether the individual samples represent single random mating populations.


+
Pgm-2
(120)
(100)
1 2 3
-
origin


+
Ldh-5
Ldh-4
(100)
Ldh-3
(75)
-
1 2 3 null
origin


+
Ldh-2
(100)
Ldh-1
(50)
-
origin
1 2 3


+
(100)
Agp-2
(60)
1
2
3
origin
-


+
Idh-3,4
1
2
3
4
5
origin
-

To do this, the observed genotypes from each sample are compared to the expected genotypes which would occur under random mating conditions. The Hardy-Weinberg law states that with random mating (i.e., panmixia), the three expected genotypes in a two allele system will be in the proportions ($p^2 + 2\ pq + q^2 = 1$), where p and q are the allelic frequencies. So, the expected genotypes of a population of N = 100 with the gene frequencies of p = .80, q = 120 would be: AA = 64, AA' = 32, A'A' = 4.

Significant deviations from the expected genotypes, such as a deficiency in observed heterozygotes, can be caused by two or more reproductively isolated populations occurring in a single sample (i.e., the Walhund effect). Differential survival associated with a specific genotype can also modify observed genotypic proportions.

The fixation index, F, can be used to examine the samples with regard to deviations from expected genotypic proportions; ($F = 1 - H_o/H_e$), where H_o is the observed number of heterozygotes and H_e is the expected number (Spiess 1977). F will be positive when there is a deficiency of observed heterozygotes. The significance of the deviation can be calculated by an χ^2 where $F^2N = \chi^2$ (Workman, 1969).

None of the samples had any significant deviations from the expected genotypic frequencies (Table 4). There is also no trend over all populations that would indicate any selection

Table 4

Pgm-2 (100) allele frequencies, observed genotypes, F value and χ^2 test of significance between observed and expected genotypes

Sample* Area	Sample Year	Sample Size	P	AA	AA'	A'A'	F	χ^2
MacDonald Creek-FR	1976	50	0.820	35	12	3	0.195	1.90
	1977	50	0.827	34	13	2	0.084	0.35
Middle Thompson Lake	1976	50	0.900	40	10	0	-0.100	0.50
	1977	50	0.880	38	12	0	-0.125	0.78
Crystal Lake	1976	44	0.682	21	18	5	0.068	0.20
	1977	22	0.705	9	13	0	-0.387	3.30
Somers Hatchery-FL	1976	50	0.700	26	18	6	0.151	1.15
	1977	50	0.820	34	14	2	0.061	0.19
	1978	36	0.640	14	14	5	-0.184	1.22
Ashley Lake	1976	50	0.810	34	13	3	0.164	1.34
	1977	50	0.700	22	26	2	-0.226	2.55
Talley Lake	1976	50	0.800	32	15	2	[illegible]	0.00
	1977	46	0.870	34	12	0	-0.137	0.87
Whitefish Lake	1976	49	0.867	36	13	0	-0.141	0.98
	1977	no sample taken						
Yellow Bay-FL	1976	50	0.750	28	19	3	-0.003	0.00
	1977	44	0.830	30	13	1	-0.033	0.05
Bigfork Bay-FL	1976	49	0.735	25	22	2	-0.140	0.96
	1977	50	0.770	28	21	1	-0.174	1.51
Skidoo Bay-FL	1976	19	0.947	17	2	0	-0.028	0.01
	1977	31	0.823	21	9	1	0.021	0.01
Dr. Richards Bay-FL	1976	19	0.816	12	7	0	-0.194	0.71
	1977	50	0.810	31	19	0	-0.222	2.47
Woods Bay-FL	1976	46	0.783	26	20	0	-0.264	3.20
	1977	42	0.750	24	15	3	0.059	0.15
West Shore Park-FL	1976	15	0.867	11	4	0	-0.115	0.20
	1977	18	0.816	13	5	1	0.147	0.41
Crescent Bay-FL	1976	26	0.808	18	6	2	0.271	1.92
	1977	47	0.862	34	13	0	-0.148	1.03

Table 4

Pgm-2 (100) allele frequencies, observed genotypes,

F value and χ^2 test of significance between

observed and expected genotypes

continued

Sample* Area	Sample Year	Sample Size	P	AA	AA'	A'A'	F	χ^2
Little Bitterroot Lake	1976	50	0.790	32	15	3	0.105	0.55
	1977	50	0.800	31	18	1	-0.114	0.65
	1977	48	0.844	33	15	0	-0.173	1.43
Brennaman's Slough-FR	1976	50	0.850	36	13	1	-0.009	0.00
	1977	50	0.786	31	15	3	0.100	0.49
Eleanor Island-FR	1976	50	0.830	34	15	1	-0.052	0.14
	1977	49	0.837	36	10	3	0.261	3.33
Swan Lake	1976	50	0.750	28	19	3	-0.003	0.00
	1977	50	0.760	31	14	5	0.240	2.88
Lindberg Lake	1976	no sample taken						
	1977	50	0.730	27	19	4	0.046	0.10
Lake Mary Ronan	1976	no sample taken						
	1977	17	0.882	13	4	0	-0.100	0.17
Lake Blaine	1976	no sample taken						
	1977	23	0.826	16	6	1	0.112	0.29
Seeley Lake	1976	no sample taken						
	1977	18	0.750	11	5	2	0.280	1.41
MacDonald Lake	1978	50	0.082	33	16	1	0.075	0.03

* FR= Flathead River
FL= Flathead Lake

against a particular genotype. Therefore, I conclude that each sampling area represents a single, or part of a single, random mating population.

Differences within Sampling Areas

In the absence of differential selection dependent upon sex, both sexes should have the same gene frequency. No significant differences in gene frequency exist between sexes of the same sampling area during 1976 and 1977 (Table 5).

There are no allelic frequency differences between years within all the naturally reproducing spawning areas. However, the gene frequency of the hatchery sample did change significantly between sampling years (Table 5). Thus the hatchery population demonstrates the only significant example of temporal variation. The PGM-2(100) gene frequency changed from .70 in 1976 to .82 in 1977 ($P<0.5$) and dropped to .64 in 1978 ($P<.01$). The cause for this could be the introduction of spawn into the hatchery from other areas in Flathead Lake, River, and other lakes in northwestern Montana. These fish may return to the hatchery at different times during the spawning season and thus the gene frequency changes constantly throughout the spawning season depending upon the proportion of fish from the different areas which are ripe. Young salmon fish released at other areas in Flathead Lake and River may also return to the hatchery because of the imprinting that has occurred at the hatchery at different times due to

X^2 test of significance of gene frequencies between years within samples and between sexes within samples 1df.

Sample Area	#	Year	N of Genes 100, 120	Gene Freq.	Between Years X^2	Sex	N of Genes 100, 120	Gene Freq.	Between Sexes X^{2*}
MacDonald Creek (FR)	1	1976	82 18	.820	0.03	M	43 7	.860	0.61
						F	39 11	.780	
		1977	83 17	.830		M	42 8	.840	0.00
						F	41 9	.820	
Mid. Thompson Lake	2	1976	90 10	.900	0.20	M	48 2	.960	2.78
						F	42 8	.840	
		1977	88 12	.880		M	41 9	.820	2.37
						F	47 3	.940	
Crystal Lake	3	1976	60 28	.682	0.07	M	34 16	.680	0.04
						F	26 12	.684	
		1977	31 13	.704		M	17 7	.708	0.07
						F	14 6	.700	
Hatchery Bay (FL)	4	1976	70 30	.700	3.95	M	34 16	.680	0.05
						F	36 14	.720	
		1977	82 18	.820	7.09	M	41 9	.820	0.07
						F	41 9	.820	
		1978	42 24	.636					
Ashley Lake	5	1976	81 19	.810	3.27	M	40 10	.800	0.00
						F	41 9	.820	
		1977	70 30	.700		M	34 18	.654	0.69
						F	36 12	.750	
Talley Lake	6	1976	80 20	.800	1.67	M	40 10	.800	0.06
						F	40 10	.800	
		1977	80 12	.869		M	42 4	.913	0.86
						F	38 8	.826	

Sample Area	#	Year	N of Genes 100, 120	Gene Freq.	Between Years χ^2	Sex	N of Genes 100, 120	Gene Freq.	Between Sexes χ^{2*}
Whitefish Lake	7	1976	85 13	.867		M	45 5	.900	0.46
						F	40 8	.833	
Yellow Bay (FL)	8	1976	75 25	.750	1.77	M	33 17	.660	3.01
						F	40 8	.833	
		1977	73 15	.829		M	42 8	.840	0.00
						F	31 7	.816	
Bigfork Bay (FL)	9	1976	72 26	.735	0.33	M	31 17	.645	2.97
						F	41 9	.820	
		1977		.770		M	40 10	.800	0.23
						F	37 13	.740	
Skidoo Bay (FL)	10	1976	36 2	.947	3.24	M	16 0	1.000	0.25
						F	20 2	.909	
		1977	51 11	.822		M	12 2	.857	0.00
						F	39 9	.813	
Dr. Richards (FL)	11	1976	31 7	.815	0.01	M	7 1	.875	0.00
						F	24 6	.800	
		1977	81 19	.810		M	41 9	.820	0.00
						F	40 10	.800	
Woods Bay (FL)	12	1976	72 20	.783	0.18	M	36 12	.750	0.29
						F	36 8	.818	
		1977	65 21	.756		M	38 12	.760	0.00
						F	25 9	.735	
Westshore Park	13	1976	26 4	.867	0.44	M	18 4	.818	0.47
						F	8 0	1.000	
		1977	29 7	.806		M	10 4	.714	0.45
						F	19 3	.864	
Crescent Bay (FL)	14	1976	42 10	.808	0.74	M	9 5	.643	2.06
						F	33 5	.868	
		1977	81 13	.862		M	47 7	.870	0.00
						F	34 6	.850	

Sample Area	#	Year	N of Genes 100, 120	Gene Freq.	Between Years χ^2	Sex	N of Genes 100, 120	Gene Freq.[b]	Between Sexes χ^{2*}
Little Bitterroot Lake	15	1976	79 21	.790		M	39 11	.780	0.00
					0.03	F	40 10	.800	
		1977 (small)	80 20	.800		M	39 11	.780	0.06
					0.94	F	41 9	.820	
		1977 (large)	81 15	.844		M	66 14	.825	0.00
						F	15 1	.937	
Brennaman's Slough (FR)	16	1976	85 15	.850		M	33 7	.825	0.00
					0.04	F	32 8	.800	
		1977	84 16	.840		M	45 5	.900	1.86
						F	39 11	.780	
Eleanor Island (FR)	17	1976	83 17	.830		M	41 9	.820	0.00
					0.63	F	42 8	.840	
		1977	77 21	.786		M	35 15	.700	3.48
						F	42 6	.875	
Swan Lake	18	1976	75 25	.750	0.03				
		1977	76 24	.760		M	34 16	.680	2.69
						F	42 8	.840	
Lindberg Lake	19	1977	73 27	.730		M	55 21	.724	0.00
						F	18 6	.750	
Lake Mary Ronan	20	1977	30 4	.882		M	42 6	.875	0.16
						F	46 4	.920	
Lake Blaine	21	1977	27 7	.794		M	16 4	.800	0.00
						F	22 4	.846	

environmental differences. The samples that I obtained were apparently taken at different times during the spawning run. Hatchery practices also can influence the gene frequency and account for the temporal variation observed. If only fish from a certain lot are released at the hatchery to maintain the brood run, the gene frequency characteristic to that population would be detected the year when they returned to spawn.

Partitioning of Genetic Variation

Since there are no differences between years in the naturally reproducing sample areas, I combined the gene frequency results from the two sampling years to test for the extent of heterogeneity between sample sites by a contingency χ^2 goodness-of-fit test. There is significant heterogeneity between sample areas ($\chi^2 = 47.6_{21df}$). This is not unexpected since twelve of the sampling areas are from lakes that are physically isolated from each other. However, if the sample areas from only the Flathead Lake and River are tested, the χ^2 value (12.2_{9df}) is not significant. This implies that the samples from the Flathead Lake and River do not constitute separate reproductive units. To further examine the samples for possible population subdivision, I used a χ^2 test of all possible pairwise comparisons between sampling areas (2 x 2 contingency tables of allele frequencies) to test the

hypothesis: There is sufficient gene flow between these subpopulations to keep their PGM-2 gene frequency equal. The alternate hypothesis is that the populations are reproductively isolated (i.e., what is the probability that the two populations will have their respective gene frequencies if they are randomly mating.) The null hypothesis is rejected at the .05 level if the χ^2 value exceeds 3.84. (Appendix 2, see also Figure 7.)

Examining the χ^2 values shows that the Bigfork Bay sample is distinct from the Skidoo Bay, Crescent Bay, and the Brennemans Slough populations. Skidoo Bay is distinct from Woods Bay and Bigfork Bay. This indicates that there is a limited amount of population subdivision that occurs in Flathead Lake. The pairwise comparisons also indicate extensive heterogeniety in the twelve lake samples.

Age at Reproduction

The age at maturity in salmon, which has been shown to have a strong genetic component (Calíprice 1969), was examined by a χ^2 test to see if any differences occur between sexes, sampling areas, or years (Table 6). Three sample areas, Brennemans Slough (1976), Yellow Bay (1976) and Talley Lake (1977), had a larger proportion of five year old female spawners than five year old male spawners (χ^2 = 5.25, 5.13, 17.4_{1df}). The Woods Bay sample (1976) had a greater proportion of five year old male spawners than female spawners. In the

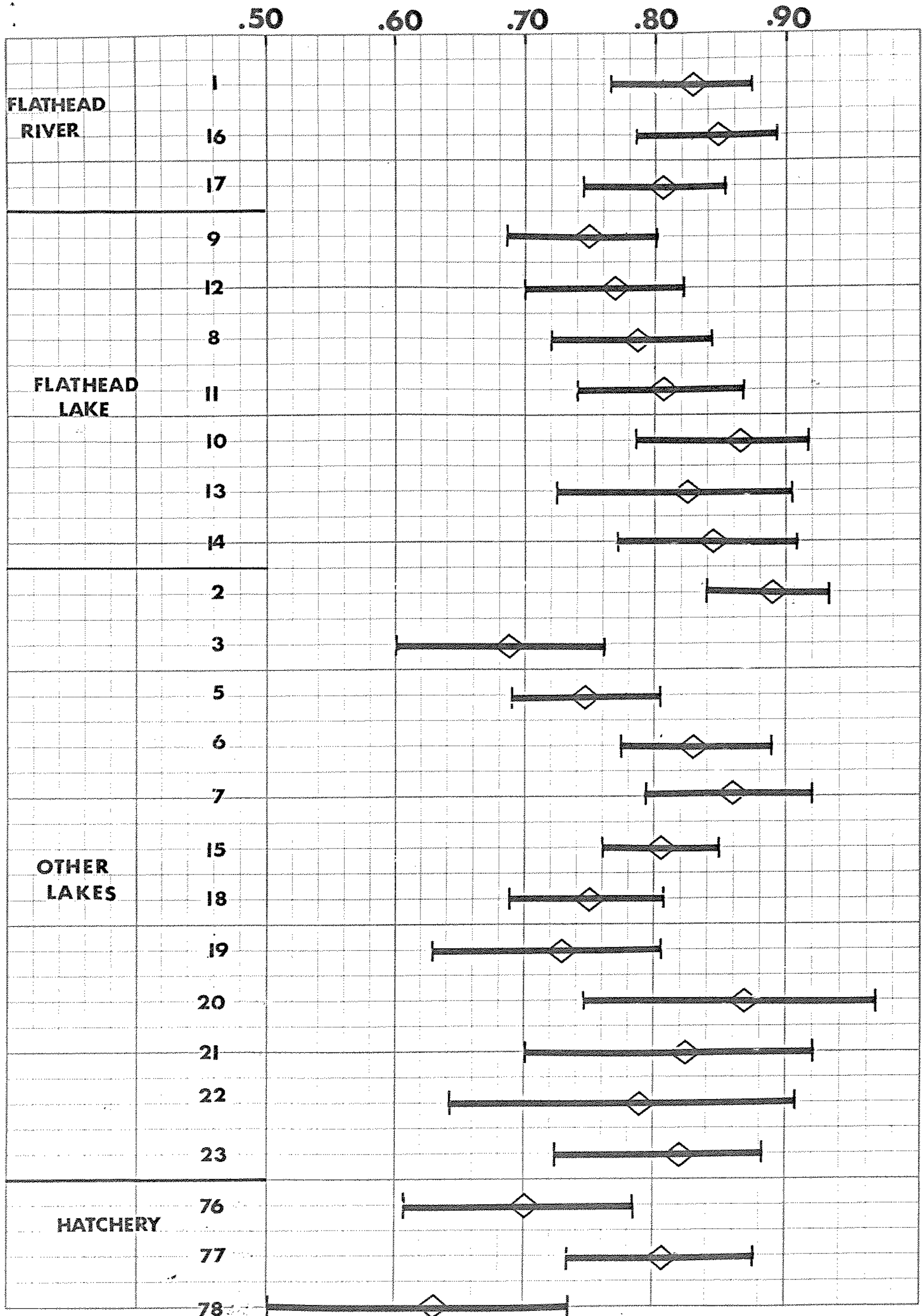
Table 6

AGE at REPRODUCTION

Site	sex	1976 Age 3	1976 Age 4	1976 Age 5	1977 Age 3	1977 Age 4	1977 Age 5
Flathead River							
MacDonald Creek 01	M	1	19	5	0	25	0
	F	3	16	6	0	22	2
Brennemans Slough 16	M	0	20	4	0	21	3
	F	2	12	11	0	20	1
Eleanor Island 17	M	3	24	0	0	23	1
	F	1	18	6	0	22	1
Flathead Lake							
Hatchery Bay 04	M	0	17	8	0	20	3
	F	2	17	5	0	22	0
Yellow Bay 08	M	0	16	9	0	17	6
	F	0	8	17	0	22	3
Bigfork Bay 09	M	0	22	3	0	23	0
	F	1	20	4	0	17	6
Skidoo Bay 10	M	0	5	3	0	5	2
	F	0	8	3	0	19	3
Dr. Richards 11	M	0	3	1	0	20	5
	F	0	12	3	0	23	2
Woods Bay 12	M	0	9	16	0	15	3
	F	0	14	8	0	19	1
W. Shore Park 13	M	0	9	2	0	7	0
	F	0	3	1	0	9	0
Crescent Bay 14	M	0	5	2	0	24	4
	F	0	14	5	0	19	2

Table 6 Continued

Lakes	sex	1976 Age 3	4	5	1977 Age 3	4	5
Middle Thompson 02	M	0	23	0	0	15	5
	F	1	23	0	0	16	1
Crystal 03	M	25	0	0	7	4	0
	F	15	0	4	3	4	2
Ashley 05	M	0	28	0	0	24	0
	F	0	22	0	0	23	1
Talley 06	M	0	26	6	0	25	0
	F	0	12	6	0	11	12
Whitefish 07	M	0	11	14	No sample		
	F	0	17	7			
Little Bitteroot 15	M	4	21	0	13	12	0
	F	1	23	0	10	13	0
	M				20	17	0
	F				7	1	0


.50
.60
.70
.80
.90
FLATHEAD RIVER
1
16
17
FLATHEAD LAKE
9
12
8
11
10
13
14
OTHER LAKES
2
3
5
6
7
15
18
19
20
21
22
23
HATCHERY
76
77
78

other sample areas during both years the proportion of male and female spawners at an age is the same.

Crystal Lake during both sampling years and Little Bitterroot Lake in 1977 have a greater proportion of three year old spawners than the other sampling areas. The fast growth rate and large size of the Crystal Lake kokanee salmon may account for the earlier maturation however, in Little Bitterroot Lake where the population consists of a large and small size fish, there was no difference in the age at spawning.

There was a significantly greater number of five year old spawners in the Flathead Lake and River samples during 1976 than in 1977 ($\chi^2 = 36.6_{1df}$). This was not seen in the samples from the other lakes. The large number of five year old spawners in 1976 probably represents a strong 1971 year class. It is interesting to note that this occurs in both the river and lake spawning areas and indicates that factors controlling survival may be similar for both major spawning areas.

Phenotypic Differences of PGM Genotypes

Are the three different PGM isozymes a factor for size differences in kokanee salmon? (e.g., are the heterozygous fish larger than the homozygous kokanee salmon?) To test this, two morphological parameters, weight and length, were examined to see if these factors are correlated with individual fish genotype.

Weight was not used due to the wide variances within samples. This is probably due to differences in spawning condition of the kokanee salmon when they were collected. Although noticeably fungused or immature fish were not collected, there is still a wide range in the time that the kokanee salmon have been on the spawning grounds and not feeding, and the extent of spawning that has occurred within a population. Approximately 90% of the fish had spawned when the samples were taken.

There is no detectable correlation between the standard length of a kokanee salmon and the PGM genotype of that fish. The data were analyzed in two separate ways, a standard T-test was first used to look for significant differences in mean length between genotypes. First of all, however, the genotypic-length data had to be broken down by each sample area since there were significant differences in mean length due to where the sample was collected. Each sample area had to be broken down into separate age and sex component parts since males were longer than females (mainly due to the large hook jaws which males develop at the time of spawning) and older fish were significantly larger than younger ones. When each collection was broken down this way into separate groups, insufficient sample sizes occurred especially at the PGM 120/120 genotype. Therefore, the power of the T-test was limited.

A Kruskal-Wallis mean ranking non-parametric test was then used since the mean rankings of fish lengths of each genotype in each group are relative and differences in length due to age and sex can be eliminated by comparing the mean rankings in each age and sex group. The mean rankings of kokanee salmon lengths of different sex and ages can be compared to each other by calculating a value X (mean rank - $\frac{N+1}{2}$ = X) (where N is the number of fish of each age-sex group in each collection) X assumes a greater positive value if the mean ranking of a particular genotype is larger than another. For example, if heterozygous kokanee salmon were longer on the average than homozygous fish, the X value of the heterozygous fish would be larger than that of the homozygous fish. A χ^2 test can be used to determine if one genotype occurs above the others a significant amount of times (Table 7). There is no differences between the sexes with respect to the mean ranking of genotypic lengths so the sexes were combined. Differences in mean ranks with respect to age of maturity also proved to be nonexistent. So, the total number of occurrences of X above and below 0 of each genotype were tested (3 x 2 χ^2) and there was no significant difference. Therefore, there is no tendency for kokanee salmon of one genotype to be larger or smaller than those of the other genotypes at the time of reproduction.

TABLE 7

Kruskal-Wallis mean ranking

comparison of X values.

(X=mean rank - (N+1/2))

The numbers represent the number of sampling areas in which the X value is above and below 0 at each age/sex-genotype category.

	Genotype					
Age/Sex	100/100		100/120		120/120	
	$X<0$	$X>0$	$X<0$	$X>0$	$X<0$	$X>0$
3 M	3	1	1	3	0	1
3 F	3	2	2	4	1	0
4 M	14	21	20	14	6	10
4 F	16	17	17	16	2	8
5 M	11	8	10	10	2	2
5 F	13	6	6	12	2	0
X^2_{2df} between sexes	0.58		1.06		0.001	
between ages	4.26		3.12		2.80	

between genotypes 2.05

DISCUSSION

Amount of Genetic Variation

The low amount of genetic variation within these kokanee salmon limited the extent of the genetic data analysis. Since there is only a single polymorphic locus, the ability to detect population subdivision is limited. The power of using population genetics to examine population subdivision depends upon the presence of genetic variation within that population. With only a single variable locus detectable divergence between spawning concentrations can only take place in one dimension. Sockeye salmon contain much lower amount of electrophoretically detectible genetic variation than most other species of salmonids (Utter, Allendorf and Hodgins 1973). Allendorf and Utter (1979) compared the average heterozygosity of nine species of salmonids and found that sockeye salmon were one of the least variable species(Table 8).

The low amount of genetic variation found in sockeye salmon cannot be explained by a low population size. Sockeye salmon historically have had immense spawning migrations up the major North Pacific river systems (Foerster 1968). Steelhead trout on the other hand, is restricted to comparatively small population sizes throughout its range. Numerous small, independent spawning populations in sockeye salmon may make the effective population size much smaller though than previously thought.

Table 8.

Average heterozygosity of the kokanee salmon in northwestern Montana and nine species of salmonids

Kokanee salmon of northwestern Montana $\bar{H}$=0.006

Species		Number of populations	$\bar{H}$	Range of $\bar{H}$
Oncorhynchus				
O. gorbuscha	Pink salmon	6	0.039	.032 - .047
O. keta	Chum salmon	5	0.045	.043 - .048
O. kisutch	Coho salmon	10	0.015	.000 - .025
O. nerka	Sockeye, salmon	10	0.018	.008 - .024
O. tshawytscha	Chinook salmon	10	0.035	.024 - .052
Salmo				
S. apache	Apache trout	1	0.000	
S. clarki	Cutthroat trout			
	Coastal form	6	0.063	.022 - .027
	Interior form	2	0.023	.021 - .025
S. gairdneri	Rainbow trout	41	0.060	.020 - .098
S. salar	Atlantic salmon	2	0.024	.020 - .028

The amount of genetic variation present in the kokanee salmon populations in northwestern Montana is considerably lower than the amount reported for other sockeye and kokanee salmon stocks. Seeb et al. (1978) found 5 variable loci in sockeye and kokanee salmon stocks from Lake Washington. Grant (1977) found 8 polymorphic loci in Alaskian sockeye salmon stocks. Average heterozygosity values ranged from 0.008 to 0.024 in ten populations of sockeye salmon surveyed by Allendorf and Utter (1979). Sockeye and kokanee salmon stocks from the U.S.S.R. also contain a greater amount of genetic variation (Altukhov 1974, Altukhov et al. 1975a, b, Kirpichnikov 1977).

This low amount of variation may be the result of a founder effect. Very few survivors of the apparent 1916 planting of kokanee fry into Flathead Lake may have been the ancestors of the present populations. By making a few assumptions, it can be shown that as few as 10 females and N males may have started the original population (the number of males is unimportant as long as we assume that there are enough to fertilize all the females but it can be assumed that N is equal to the number of females). If a four year life cycle is assumed and if each female produces 100 females which live to reproduce the next generation (the life cycle may have been predominately a three year cycle since the kokanee first discovered in 1918 in Lake Mary Ronan were mature) the kokanee population in Flathead Lake would have

reached a level of approximately 1.5 fish/surface acre at the time Elrod (1929) first reported evidence of kokanee salmon in Flathead Lake. This density of kokanee salmon is about the minimum concentration that would have to be present for them to be noticed in Flathead Lake. By the early 1930's, the population would have become large enough, over 150 kokanee salmon/acre to become common. If this reproductive rate persisted, the abundance of kokanee salmon would have neared its peak in 1934 when a commercial fishery was first established for the kokanee salmon (Brown 1971).

Population Structure

In order to detect possible small differences in regional gene frequencies in Flathead Lake, I combined adjacent spawning areas with similar gene frequencies at the Pgm-2 locus. The kokanee salmon in Flathead Lake can then be partitioned into six major groups (Figure 8), (Table 9).

1. Flathead River
2. Northeast--Bigfork Bay and Woods Bay
3. East--Dr. Richards and Yellow Bay
4. Southern--Skidoo Bay
5. West--Crescent Bay and West Shore Park
6. Hatchery

Further subdivision possibly could be detected if additional polymorphic loci were present in the Flathead Lake kokanee salmon population.

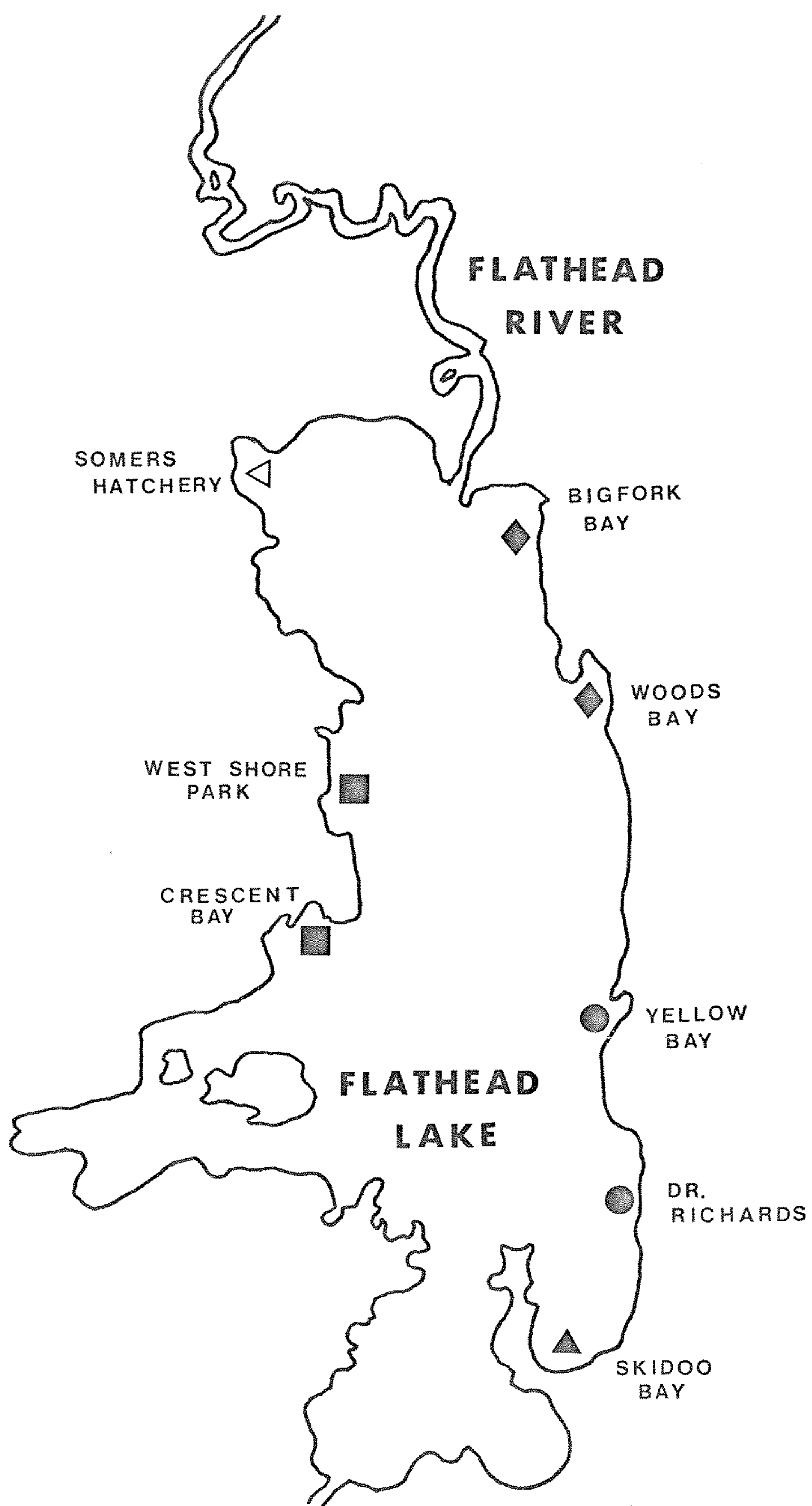

FLATHEAD
RIVER
SOMERS
HATCHERY
BIGFORK
BAY
WOODS
BAY
WEST SHORE
PARK
CRESCENT
BAY
YELLOW
BAY
FLATHEAD
LAKE
DR.
RICHARDS
SKIDOO
BAY

Table 9. χ^2 values between major reproduction areas in Flathead Lake.

		2	3	4	5
1	Flathead River	6.63	1.14	2.09	0.25
2	Bigfork Bay Woods Bay		1.52	7.70	5.58
3	Yellow Bay Dr. Richards			3.94	1.63
4	Skidoo Bay				0.97
5	West Shore Park Crescent Bay				

The Flathead River spawning fish constitute an apparent random mating population. Although Hanzel (1964) reports two pulses of spawners, one spawning in MacDonald creek and the second, a later pulse using sloughs along the lower mainstream of the Flathead River, there is evidently enough gene flow between pulses to maintain similar gene frequencies. Migration also occurs between these river spawning fish and the Lake MacDonald population.

This river deme is statistically different from the northeast spawning population ($p<0.01$) on the basis of the allele frequency at PGM-2. There is an indication of reproductive isolation between the river spawning fish and the lake spawning fish at the Ldh-3 locus. All of the Ldh-3 variants occur in lake spawning fish (Table 3) when each variant, Ldh-3 (75), Ldh-3 null, is tested separately the χ^2's are not significant ($\chi^2 = 2.60_{1df}$, $\chi^2 = 1.66_{1df}$). However, when the total variation at Ldh-3 is tested, the χ^2 is significant ($\chi^2 = 5.53_{1df}$). I also feel that the river spawners constitute a separate reproductive unit distinct from other areas in the lake due to the difference in life history patterns.

Kokanee salmon spawning on the east shore of Flathead Lake can be divided into three separate groups, the northeast, east, and southern population. The northeast spawning areas, Bigfork Bay and Woods Bay, have a significantly lower

frequency of the PGM-2(100) allele than the westshore deme, P<0.025, Skidoo Bay P<0.01, and the Flathead River population as previously discussed. The northeast spawning deme is not statistically different from the Yellow Bay and Dr. Richards samples. These two areas are intermediate in the PGM-2 gene frequencies between the northeast deme and the Skidoo Bay population. The southern or Skidoo Bay population is statistically different from the other east shore populations. This site has the lowest frequency of the PGM(120) allele found in Flathead Lake.

There is no indication that any of these eastern sampling sites are actually conglomerates of several populations, since an excess of heterozygotes occurs at most sites. Woods Bay and Bigfork Bay in 1976 and Dr. Richards and Bigfork Bay in 1977 each had a marked excess of heterozygotes which was not observed in the other Flathead Lake and River populations.

The westshore sites, Crescent Bay and West Shore Park, have gene frequencies similar to each other. It is impossible to conclude that these sites are reproductively isolated from each other, however, if they were just one population using a large spawning area I would expect the gene frequencies to vary in the same direction in 1977 which did not occur. The small samples obtained and corresponding small population size may have caused the opposite oscillation of gene frequencies to occur.

Genetic Factors Affecting Population Structure

There are several factors which influence the rate of gene frequency change in natural populations. Migration tends to make gene frequencies between populations similar. On the other hand, genetic drift, population bottlenecks and selection can all play major rolls in the divergence of populations and maintenance of population heterogeniety.

The amount of migration between the major reproductive areas in Flathead Lake can be roughly estimated by comparing the gene frequencies in these areas with those of other lakes in northwestern Montana. One would suspect that lakes that are continually stocked and also that the sample areas in Flathead Lake would be close in gene frequency to each other while those lakes that have not been planted for many years would show the most divergence. This however, is not the case (Table 10). The sample areas in Flathead show as much gene frequency variation as isolated lakes. Even those lakes which are stocked continually have large differences in gene frequencies.

This continual stocking of hatchery fish into the various spawning areas along the shoreline of Flathead Lake and into backwater areas in the Flathead River may be a major force in the disruption of the genetic isolation that comes about by the return of mature salmon to their natal area to spawn. The hatchery plantings, however, may not be as strong of a disruptive force as generally assumed since imprinting

TABLE 10

Kokanee salmon stocking

practices and Pgm-2 gene frequency

Sample Area	Gene frequency Pgm-2(100)	Gene frequency range
<u>Annual stocking</u> (one or more plantings per generation)		
Crystal Lake	.68	
Lindberg Lake	.73	
Lake Mary Ronan	.88	.68 - .88
Seeley Lake	.79	
Whitefish Lake	.86	
<u>Recent stocking</u> (within three generations of sampling)		
Lake Blaine	.82	
Little Bitterroot Lake	.81	.81 - .83
Talley Lake	.83	
<u>Old stocking</u> (no plantings since the early 1950's)		
Ashley Lake	.75	
Swan Lake	.75	
Middle Thompson Lake	.89	.75 - .89
MacDonald Lake	.82	
<u>Flathead Lake and River</u>*		
Brennaman's Slough	.84	
Bigfork Bay	.75	
Crescent Bay	.84	
Eleanor Island	.81	
MacDonald Creek	.82	.75 - .87
Dr. Richards Bay	.81	
Skidoo Bay	.87	
Yellow Bay	.78	
West Shore Park	.83	
Woods Bay	.77	

* Spawning areas in Flathead Lake were stocked until 1969. Stocking resumed in 1976 at Brennaman's Slough and Bigfork Bay with plantings of fish marked with a florescent die.

probably occurs at the hatchery before the fry are planted. The homing instinct towards the planted area therefore may not occur, and there may be considerable straying of the hatchery kokanee salmon when they mature. Random straying will also tend to keep gene frequencies similar throughout Flathead Lake and River. A flowrescent marking project (Hanzel 1976) is now underway to study if the hatchery fry return to the stocked areas to spawn.

There appears to be a very low amount of migration occurring between the sample areas within Flathead Lake and thus the gene frequency differences are maintained through isolation. Also, the hatchery plantings may vary considerably in gene frequencies from year to year and therefore could create significant differences within samples from recently stocked lakes. The hatchery plantings on the other hand may not survive and this would not affect the gene frequency at all.

Factors creating population divergence may not presently be important in affecting gene frequencies. Kokanee population sizes in Flathead Lake have remained large since the early 1950's and no large fluctuations that would create bottlenecks have been reported. Individual spawning areas do vary in the number of spawners from a few hundred to over ten thousand in MacDonald Creek, however, no large fluctuations in individual demes were noticed. The variation in spawning age may considerably dampen yearly fluctuations. A strong

year class therefore will contribute a large proportion of the spawners to several years and may be an important factor in the consistancy in gene frequency from one year to the next.

Fitness Differences of PGM Genotypes

Genotypic selection does not appear to be an important factor influencing the Flathead drainage kokanee salmon populations. Altukhov (1975) however, reported finding an excess of heterozygotes at the PGM locus in sockeye salmon. He attributed this to an adaptive advantage of the heterozygotes which forms a typical pattern of a balanced polymorphism in the population. Kirpichnikov (1977) also reported that subpopulations of adult sockeye salmon have an appreciably greater number of heterozygotes than theoretically expected, but a deficit was detected in 1 yr.-olds entering the ocean. He concludes that selection changes over the life of the sockeye salmon, the heterozygous fish having a higher fitness at the time of reproduction.

An excess of heterozygotes was not found in the kokanee salmon populations in northwestern Montana. None of the deviations from expected Hardy-Weinberg proportions are significant. The percentage of samples exhibiting an excess and deficiency of heterozygotes was 55% and 45% respectively. No trends between years or groups of sample areas were evident.

A cline, a gradual directional change in a specific trait, is present among the east shore populations, with the frequency of the PGM(120) allele increasing the futher north the sample is located. Utter et al. (1973) also found the same cline from Puget Sound up to Bristol Bay. Kirpichnikov (1977) did not observe this cline but attributed this to the stabilizing selection that was detected. Both authors, however, reported a similar cline for the LDH-4 locus. It was reported by Kirpichnikov (1977) that isozyme most frequently found in more northerly areas was less resistant to heat and more active at low temperatures than the predominantly southern isozyme.

The cline present in Flathead Lake, however, probably does not reflect real biological differences. The probability of a cline occurring at random is .125*. If the PGM(120) allele was being selected in the more northerly areas, the Flathead River populations and other lakes should also have a high frequency of the 120 allele. There also appears not to be the differences in temperature or other limnological parameters between areas in Flathead Lake (Gaufin et al. 1976) that would be present in the large geographical areas where the allelic clines in sockeye salmon populations were first described.

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*The probability of a cline occurring at random with one parameter, i.e., the Pgm-2 gene frequency, is equal to ½ to the power of the number of samples in the cline -1 times 2 (The cline goes in both directions). $\frac{1}{2}^4$ x 2 = .125.

MANAGEMENT IMPLICATIONS

The recognition of the subdivision that exists in the kokanee salmon population in Flathead Lake is important with regard to the management of this species. This subdivision indicates the presence of reproductively isolated units within Flathead Lake. Therefore, the kokanee salmon in Flathead Lake cannot be managed as one large population, but as separate distinct units within one large lake.

The development of small ecologically specialized reproductive units allows the use of multiple niches especially at the time of spawning when intraspecific competition is probably the highest. As a result, factors affecting survival and response to environmental stress may differ and thus these subpopulations evolve differently. This is an important consideration with regard to which fish stocks are chosen to provide gametes for hatchery plantings.

The lack of variation in these kokanee salmon may be helpful in the future management of this species. Several management problems could be addressed by studies incorporating the genetic marking of certain stocks. By using fish with unique genetic marks, questions such as, what is survival of hatchery plantings, can be addressed. This genetic marking technique would lend itself well to the current fluorscent marking project to determine whether kokanee salmon return to the areas in which they were stocked to spawn.

Genetic markers in various kokanee salmon subpopulations in Flathead Lake also can be used to estimate the relative contribution of each subpopulation to the total kokanee salmon fishery. This allows identification of the spawning areas which produce the most fish in the lake. These areas can be protected an enhanced if an increase in fish production is desired. Areas ~~in~~ which under-produce their potential can also be identified and corrective measures taken to increase production. The movement patterns of juvenile salmon the extent of interpopulation schooling may also be addressed. This would compliment the current hydro-accustical sounding research on the identification and location of juvenile kokanee salmon schools.

Ecological studies combined with population genetics and other techniques will allow for an adequate picture of factors affecting the kokanee salmon population in Flathead Lake. These fish are a valuable resource and further studies are needed to protect and enhance their survival.

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