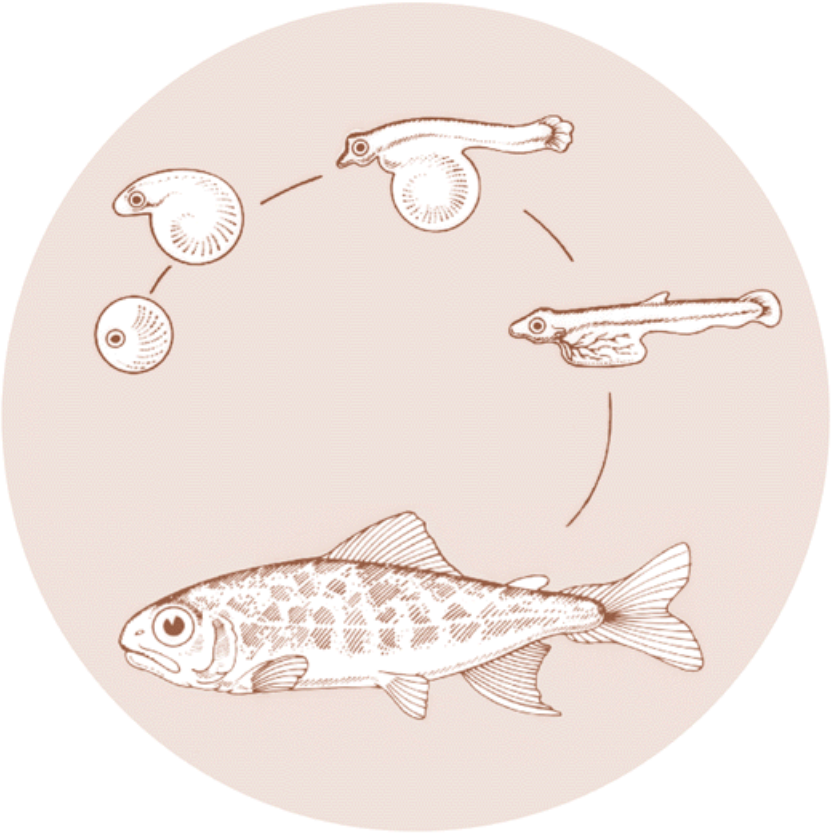
August 1991

A GENETIC MONITORING AND EVALUATION PROGRAM FOR SUPPLEMENTED POPULATIONS OF SALMON AND STEELHEAD IN THE SNAKE RIVER BASIN

Annual Report



DOE/BP-00911-1

This report was funded by the Bonneville Power Administration (BPA), U.S. Department of Energy, as part of BPA's program to protect, mitigate, and enhance fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries. The views of this report are the author's and do not necessarily represent the views of BPA.

This document should be cited as follows:

Waples, Robin S., D.J. Teel, P.B. Aebersold, A Genectic Monitoring and Evaluation Program for Supplemented Populations of Salmon and Steelhead in the Snake River Basin, Annual Report to Bonneville Power Administration, Portland, OR, Contract 89-AI-00911, Project 89-096, 55 electronic pages (BPA Report DOE/BP-00911-1)

This report and other BPA Fish and Wildlife Publications are available on the Internet at:

http://www.efw.bpa.gov/cgi-bin/efw/FW/publications.cgi

For other information on electronic documents or other printed media, contact or write to:

Bonneville Power Administration
Environment, Fish and Wildlife Division
P.O. Box 3621
905 N.E. 11th Avenue
Portland, OR 97208-3621

Please include title, author, and DOE/BP number in the request.

A GENETIC MONITORING AND EVALUATION PROGRAM FOR SUPPLEMENTED POPULATIONS OF SALMON AND STEELHEAD IN THE SNAKE RIVER BASIN

Annual Report

Prepared by:

Robin S. Waples
David J. Teel
Paul B. Aebersold

National Marine Fisheries Services
Coastal Zone and Estuarine Studies Division

Prepared for:

U.S. Department of Energy
Bonneville Power Administration
Environment, Fish and Wildlife
PO Box 3621
Portland, Oregon 97208

Project No. 89-096
Contract No. 89-AI-00911

August 1991

ABSTRACT

This is the first report of research for an ongoing study to evaluate the genetic effects of using hatchery-reared fish to supplement natural populations of chinook salmon and steelhead in the Snake River Basin. The study plan involves yearly monitoring of genetic and meristic characteristics in hatchery, natural (supplemented), and wild (unsupplemented) populations in four different drainages for each species. This report summarizes the first year of electrophoretic data for chinook salmon; electrophoretic data for steelhead and meristic data for both species will be presented in a subsequent report.

Important results include the following: 1) Genetic variation was detected at 35 gene loci, a considerable increase over previous electrophoretic studies of Snake River chinook salmon. A tentative conclusion is that Snake River spring and summer chinook salmon may have somewhat lower levels of genetic variability than are found in lower Columbia River stocks, but the difference may not be as large as suggested by earlier studies. 2) Based on a combined test over all gene loci, statistically significant ($P < 0.001$) differences in allele frequency were found between every pair of samples. Thus, there is genetic evidence for restricted gene flow between streams in the same drainage. However, the differences between populations in this study were relatively small compared to levels of differentiation that have been reported for major groups of chinook salmon throughout the Columbia River Basin. 3) Comparison with data collected in earlier studies for some populations provides insight into genetic changes that have occurred over a 4-8 year period. A much more complete picture of genetic structuring in these chinook salmon populations should emerge as data for the second and third year of samples become available.

CONTENTS

Page

INTRODUCTION 1

METHODS 5

Study Areas 5

Collections 8

Electrophoresis 10

Data Analysis 16

RESULTS AND DISCUSSION 18

Levels of Genetic Variability 18

Population Subdivision 24

Temporal Changes 31

ACKNOWLEDGMENTS 35

LITERATURE CITED 36

APPENDIX 39

INTRODUCTION

In spite of concerted management efforts, the abundance of most Pacific salmon species has been substantially below historical levels in recent years (Fredin 1980; Fraidenburg and Lincoln 1985; Nehlsen et al. 1991). The Columbia River Basin Fish and Wildlife Program (NWPPC 1987) has an interim goal of doubling the abundance of anadromous salmonids in the Columbia River Basin. The program calls for improvements in a variety of areas, including mainstem passage, habitat restoration, and control of disease, but a centerpiece of the program is supplementation--that is, the use of artificial propagation to increase the abundance of naturally-spawning salmon and steelhead. A number of supplementation programs are already underway throughout the basin.

A recent review of supplementation research (Miller et al. 1990) indicates that there are still substantial gaps in our knowledge of how to supplement natural populations effectively. Among the most important, yet least understood, factors to consider are the genetic consequences of releasing hatchery-reared fish into the wild. This is an important consideration because the genetic makeup of native wild stocks was presumably shaped by hundreds or thousands of years of adaptation to local conditions. Transplanted fish may be less well suited to local conditions, and hybridization may cause a reduction in fitness of the native stock through outbreeding depression. Emlen (1990) reviewed some of the evidence for outbreeding depression in other organisms and suggested a model that may be applicable to Pacific salmon. These possibly adverse effects can be reduced by using a stock for outplanting that is genetically similar to the local stock. However, unless the hatchery stock used for outplanting is genetically identical to the natural stock being supplemented, a successful supplementation program will entail some genetic change to the local stock. It is important, therefore, to have a means of assessing the

nature and extent of genetic changes that occur as a result of supplementation.

Unfortunately, traditional monitoring methods are not well suited to determining whether outplanted fish are having any permanent genetic effect on the target stock. Physical tags may indicate whether a fish returns as an adult, but not whether it produces offspring that survive and contribute to subsequent generations. It is possible, for example, to release large numbers of juvenile fish in a stream over a period of many years and, in the end, not know whether a) the natural population has been entirely replaced, b) the current population contains genetic material from both the original population and the outplanted fish, or c) the outplanted fish have had no permanent genetic impact on the natural population (Fig. 1). Hindar et al. 1991) reviewed data from a number of studies of salmonids that show each of these outcomes is possible.

A genetic monitoring program provides the best opportunity for determining which of these scenarios has occurred. Because genetic markers are heritable, they reveal information about the reproductive success of transplanted fish and the degree to which the native and transplanted gene pools have been integrated. Furthermore, the same approach can be used to evaluate the genetic effects of outplants on nearby wild stocks that are not intended to be supplemented.

The current study focuses on the genetic effects of using hatchery-reared fish to supplement natural populations of chinook salmon and steelhead. The experimental design capitalizes on supplementation programs already underway in several areas of the Snake River Basin. The study plan calls for yearly monitoring of genetic and meristic characteristics in hatchery, natural (supplemented), and wild (unsupplemented) populations in four different drainages for each species. Study sites were selected after consultation with personnel from Idaho Department of Fish and Game **(IDFG),** Oregon Department of Fish and Wildlife (ODFW), and

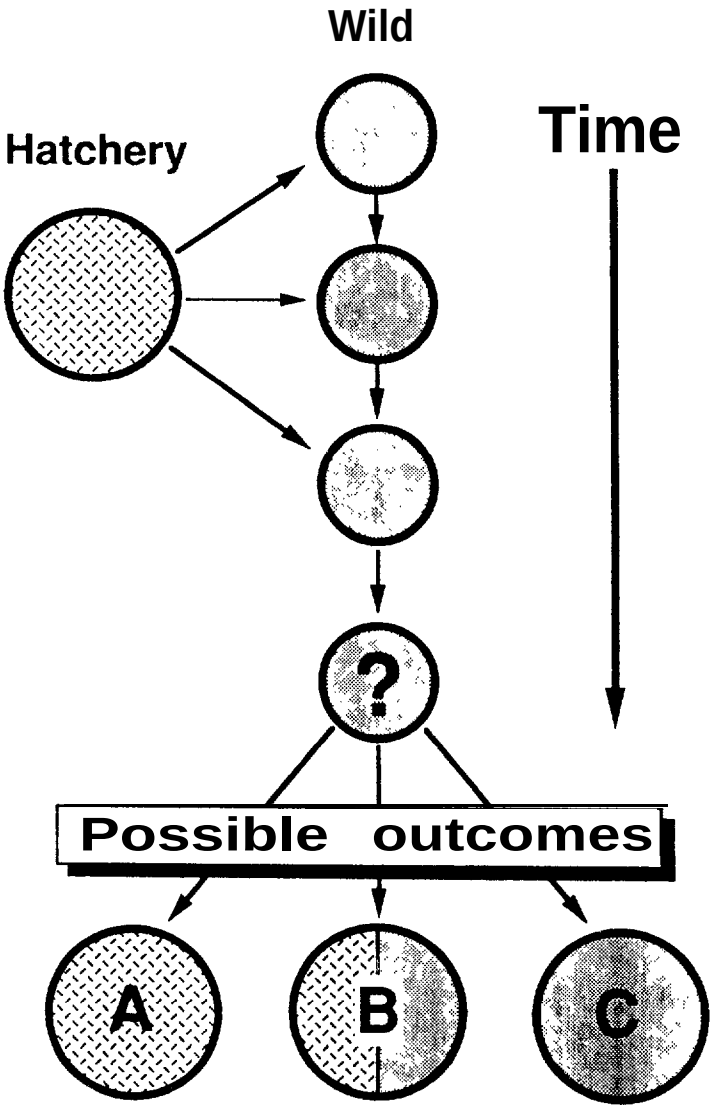


Figure 1.--Schematic diagram of three possible outcomes for a supplementation program in which hatchery fish are outplanted into the wild each year for several years. A: replacement of native gene pool with hatchery stock; B: integration (coexistence or hybridization) of native and hatchery gene pools; C: persistence of native gene pool with little or no permanent genetic effect of hatchery stock. Monitoring genetic markers provides the best means for determining which of these possibilities has occurred.

Washington Department of Wildlife (WDW). Efforts were made to select systems in which supplementation was just beginning or the past effects of supplementation were thought to be minor. After analysis of data for the first 3 years of sampling, an evaluation will be made for each supplementation program of the power to be expected in measuring genetic impacts on the selected natural/wild populations. The ability to measure these genetic effects depends on the existence of sufficient genetic differences between the outplanted hatchery fish and the natural/wild stocks. Results of the evaluation will help to determine the nature and scope of the long-term phase of the monitoring program; in particular, the sampling plan may be modified to concentrate efforts in those programs with the greatest probability of successful resolution.

The species and areas to be studied (chinook salmon and steelhead above Bonneville Dam) were singled out by the Columbia River Basin Fish and Wildlife Program (NWPPC 1987) for highest priority for research. The current research directly addresses a number of concerns in the plan: Section 204(d), monitoring the potential effects of outplanting on natural gene pools; Section 703(e)3, studies to ensure that genetic integrity of spawning stocks is maintained; Section 703(f)(5)(A)(vii), biological monitoring of supplementation programs in the Grande Ronde and Imnaha drainages; and Section 703(h)(l), studies of the best methods for supplementing wild stocks in the upper Snake and Columbia Rivers.

The research will provide information relevant to Major Question II of the Supplementation Technical Work Group Five-Year Work Plan, "What are the effects of supplementation on indigenous populations?" In particular, results from the study will help answer Specific Question 7 from the work plan, "What are the long-term effects of supplementation programs on the genetic characteristics of indigenous stocks?" Specific activities in this area called for by the Five-Year Work

Plan include use of standard genetic techniques to monitor changes in supplemented and non-supplemented populations through a serial sampling program.

Major long-term goals of the study include monitoring the nature and extent of genetic change over time in supplemented and unsupplemented populations and correlating the genetic changes with measures of productivity such as adult-to-adult survival of naturally spawning fish. Because this research focuses on genetic changes that occur over periods of one to a few generations, the primary objectives can only be realized in a multiyear study. This report summarizes the first year of electrophoretic data for chinook salmon. Electrophoretic data for steelhead and meristic data for both species will be presented in a subsequent report.

METHODS

Study Areas

The study involves four supplementation units, or drainages. Chinook salmon in the Grande Ronde and Upper Salmon drainages are generally regarded as spring-run fish, whereas those in the Imnaha and the South Fork drainages are considered to be summer-run fish. In general, each supplementation unit includes a hatchery used in supplementation, a naturally-reproducing population that is supplemented, and a wild population that is not intended to be affected by hatchery releases (Table 1). Exceptions to this pattern are use of the Lostine River as both the wild (pre-1991) and natural (after 1991, when supplementation is scheduled to begin) populations for Grande Ronde spring chinook salmon, and Imnaha River chinook salmon, for which a wild stream has not been identified. Also, Marsh Creek was included to allow comparison with a chinook salmon drainage (Middle Fork of the Salmon River) that is managed entirely for wild fish. A map of the study areas is shown in Figure 2.

Table 1.-- Chinook salmon populations in the genetic monitoring and evaluation program. Sample size is the number of 1988 brood-year juvenile fish collected in 1989-90. A maximum of 100 fish per population were analyzed electrophoretically, with the remainder archived at -80°C for possible future use.

Drainage/population	Run-timing	Classification	Sample size
S. Fork Salmon	Summer		
McCall Hatchery		Hatchery	202
Johnson Creek		Natural	196
Secesh River		Wild	94
Middle Fork Salmon	Spring		
Marsh Creek		Wild	200
Main Fork Salmon	Spring		
Sawtooth Hatchery		Hatchery	211
Upper Salmon River		Natural	200
Valley Creek		Wild	200
Imnaha	Summer		
Imnaha facility		Hatchery	200
Imnaha River		Natural	200
Grande Ronde	Spring		
Lookingglass Hatchery (Rapid River stock)		Hatchery	200
Lostine River		Wild	150

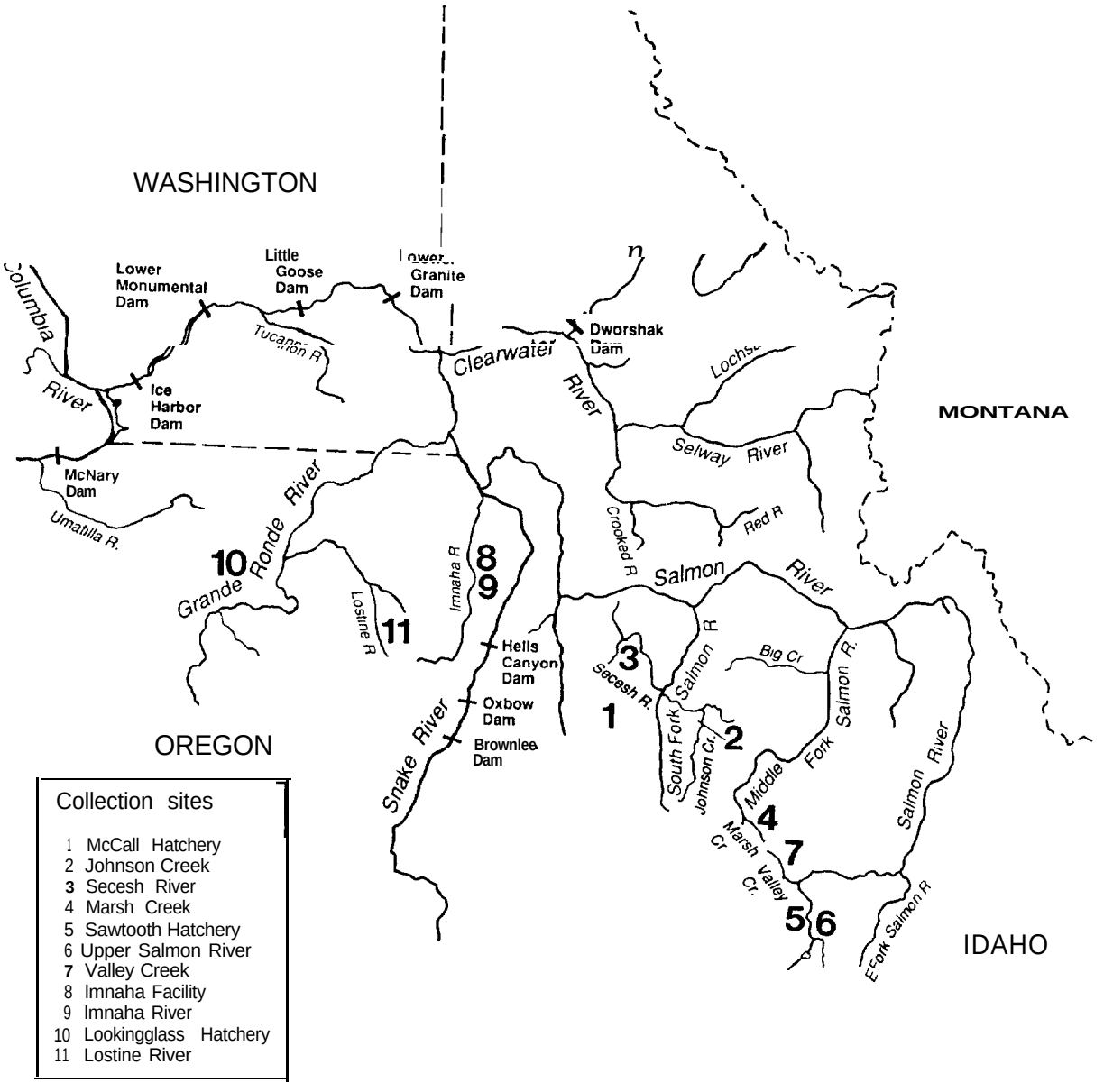


Figure 2.--Map of study areas showing collection sites for chinook salmon samples.

Collections

All samples (wild, natural, and hatchery) were from the 1988 brood year. Collections of wild and natural juveniles were made in August and September 1989. Except as noted below, fish were collected by seine. In general, collections covered stream distances of approximately l/4 to l/2 mile. Seined fish were maintained in live boxes for up to 24 hours before being anesthetized by MS-222 and placed on dry ice. Fish captured by electrofishing were kept alive in a bucket or live box for up to 2 hours before being anesthetized and frozen. As the study is intended to monitor the effects of supplementation on subsequent generations, efforts were made in sampling the supplemented streams to avoid planted fish that were not the result of natural spawning. Hatchery samples were taken between August 1989 and February 1990. Dip nets were used to capture fish from each raceway containing progeny from the targeted stock and brood year. Frozen fish were transported or shipped on dry ice to the National Marine Fisheries Service (NMFS) laboratory in Seattle, where they were transferred to a supercold (-80°C) freezer for storage prior to electrophoretic analysis. Detailed collection information is as follows:

Lostine River

Date: 26 September 1989
Location: Near Strathearn's Pond in spawning ground index area, about 4 miles south of Lostine at River Mile (RM) 11.
Method: Electrofishing
Notes: Sampling was difficult because most juveniles had already moved downstream. Reasonable concentrations of fish were found in a side channel of the river, and the sample was taken there.

Rapid River hatchery stock

Date: 28 February 1990
Location: Lookingglass Hatchery
Notes: Fifty fish were taken from each of four ponds; fish averaged 17-20/lb. Lookingglass fish represent a random sample of entire brood year for Rapid River Hatchery (R. Carmichael[1]).

---

[1]Richard Carmichael, Oregon Department of Fish and Wildlife, Badgley Hall, Eastern Oregon State College, La Grande, OR 97850. Pers. commun., April 1990.

Imnaha River
Date: 29 September 1989
Location: 6 miles south of town of Imnaha at RM 30.5
Method: Trap box at screen 8-57
Notes: Outmigrating juveniles were progeny of naturally-spawning fish that could have originated anywhere upstream from the trap. No juvenile hatchery fish are released above the trap site.

Imnaha hatchery stock
Date: 28 February 1990
Location: Lookingglass Hatchery
Notes: Sample was taken from ponds 16-18; fish averaged 18/lb. Fish were progeny of adults taken at the Imnaha weir.

Johnson Creek
Date: 19 August 1989
Location: About l/4 mile above Ice Hole Campground on lower Johnson Creek; sampled area included a l/4-mile long side channel to the west of the main stream
Method: Electrofishing
Notes: About 6-8 parr were released as possible hatchery fish on the basis of their large size. About 590,000 juveniles from McCall Hatchery were released in Johnson Creek between 8 May and 10 August 1989 (G. McPhearson'). The few large parr found may have been from the August releases. Most of the fish collected were small enough (ca. 50-70 mm FL) that it is unlikely they resulted from the earlier outplantings.

Secesh River
Date: 28 September 1989
Location: About l/2 mile below Warren Road bridge
Notes: Only 94 individuals collected

McCall Hatchery
Date: 1 December 1989
Location: McCall Hatchery
Notes: Sample taken from two raceways containing entire 1988 brood year.

Marsh Creek
Date: 14 August 1989
Location: About l/2 mile above mouth of Capehorn Creek

Valley Creek
Date: 17 August 1989
Location: About l/4 - l/2 mile above confluence with Stanley Creek.

---

[2]Gene McPhearson, Idaho Department of Fish and Game, McCall Hatchery, P. 0. Box 1021, McCall, ID 83638. Pers. commun., August 1990.

Upper Salmon River
Date: 18 August 1989
Location: At Blaine County Bridge on Hwy 93 (border of Custer and Blaine Counties), just above confluence with Alturas Lake Creek.
Notes: At time of sampling, nearest 1989 outplants of Sawtooth Hatchery fish were thought to have been ca. 3 miles downstream, near Fourth of July Creek (R. Kiefer[3]). However, it has since been determined that 51,000 Sawtooth Hatchery fish were released into Alturas Lake Creek in 1989 (Matthews and Waples 1991).

Sawtooth Hatchery
Date: 14 August 1989
Location: Sawtooth Hatchery

Electrophoresis

A maximum of 100 individuals per population were used in the electrophoretic analysis (see Aebersold et al. 1987 for details of procedures); the remainder were archived at -80°C for possible future use. Four tissues (skeletal muscle, liver, heart, and eye fluid including retinal tissue) were sampled from each fish, and extracts were loaded onto starch gels utilizing seven different buffer systems (Table 2). Most of these buffers are described by Aebersold et al. (1987), with the following modifications. The ACE7 and TBCLE buffers include the optional EDTA component. The ACEN7 buffer is the same as the ACE7 buffer with an additional component (β-nicotinamide adenine dinucleotide = NAD^+) added to the gel just prior to degassing at a 0.015% concentration (15 mg per 100 ml), and added to the cathodal electrode buffer at a 0.03% concentration (30 mg per 100 ml).

The TC4 buffer (Dreyfus and Alexandre 1972) consists of 0.223 M tris and 0.083 M citric acid, resulting in a pH of 5.8. The full concentration is used for the electrode buffer, and a 1 to 27.5 dilution of buffer to distilled water is prepared for the gel. After dilution, the pH of the gel buffer is readjusted with 1.0 M HCI.

---

[3]Russ Kiefer, Idaho Department of Fish and Game, 1798 Trout Rd., Eagle, ID 83616. Pers. commun., August 1990.

Table 2.-- List of enzymes surveyed, enzyme numbers, new and old abbreviations for each presumptive gene locus, tissues sampled (M = muscle, L = liver, H = heart, E = eye) and buffers used to resolve these loci, and status for each locus (M = monomorphic, P = polymorphic, NR = not resolved). For polymorphic loci, the earliest published source describing the variation or providing allele frequency data is indicated. Locus names and abbreviations follow the nomenclature guidelines provided by Shaklee et al. (1990a). Descriptions of the buffer systems are found in the text.

Enzyme name	Number	Locus	Previous Abbrev.	Tissue	Buffer	Status	Source'
Aspartate aminotransferase	2.6.1.1	*sAAT-1,2**	GOT-I ,2; AAT-1,2	MH	TEE	P	1
		*sAAT-3**	GOT-3: AAT-	E	TBE	P	1
		*sAAT-4**	AAT-4.	L	TBE	P	2
		*mAAT-I**		HME	ACE7	P	4
		*mAAT-2**		HME	ACE7	NR	
		*mAAT-3**		HME	ACE7	NR	
Acid phosphatase	3.1.3.2	*ACP-1'*		L	TBE	M	
		ACP-2'		L	TBE	M	
Adenosine deaminase	3.5.4.4	*ADA-I'*		E	TBE	P	1
		*ADA-P**		E	TBE	M	
Alcohol dehydrogenase	1.1.1.1	*ADH**		L	ACE7	P	1
Aconitate hydratase	4.2.1.3	*sAH**	ACON-2; AH	L	ACE7	P	1
		*mAH-I**		HME	ACE7	M	
		mAH-2'		HME	ACE7	P	8
		*mAH-3**		HME	ACE7	M	
		*mAH-4**		HME	ACE7	P	4
Adenylate kinase	2.7.4.3	*AK**		ME	ACE7	M	
Alanine amlnotransferase	2.6.1.2	*ALAT**	GPT	M	TBE	NR	
Creatine kinase	2.7.3.2	*CK-AI**	CK-1	M	TBCLE	NR	
		*CK-AZ**	CK-2	M	TBCLE	NR	
		*CK-B**	CK-5	E	TBCLE	NR	
		CK-CI .	CK-3	E	TBCLE	M	
		*CK-C2**	CK-4	E	TBCLE	M	
Esterase	3.1.1.-	*EST-I**		L	TBCLE	NR	
Esterase-D	3.1.-.-	*ESTD**		M	TBCLE	NR	
Fructose-bisphosphate aldolase	4.2.1.13	*FBALD-I**	ALD-1	M	ACEN7	NR	
		*FBA L D-2 **	ALD-2	M	ACEN7	NR	
		*FBALD-3**	ALD-3	E	ACEN7	NR	
		FBALD-4'	ALD-4	E	ACEN7	NR	
Fumarate hydratase	4.2.1.2	*FH**	FUM	M	ACEN7	M	
β-N-Acetylgalactosaminidase	3.2.1.53	*βGALA**		L	ACE7	M	
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*GAPDH-I**	GAP-I	M	ACEN7	NR	
		GAPDH-2'	GAP-3	H	ACEN7	P	8
		*GAPDH-3**	GAP-4	MH	ACEN7	M	
		GAPDH-4"	GAP-5	E	ACEN7	P	8
		*GAPDH-5**	GAP-6	E	ACEN7	M	
Guanine deaminase	3.5.4.3	*GDA-1**		L	TC4	NR	
		*GDA-2**		L	TC4	NR	
u-Glucosidase	3.2.1.20	*aGLU-1"*		L	TC4	NR	
		*αGLU-2**		L	TC4	NR	
N-Acetyl-β-glucosaminidase	3.2.1.30	*βGLUA**	bGA	L	TC4	P	8

Enzyme name	Number	Locus	Previous Abbrev.	Tissue	Buffer	Status	Source'
Glutamate dehydrogenase	1.4.1,-	GLUDH*		L	ACE7	NR	
Glycerol-J-phosphate dehydrogenase	1.1.1.8	GJPDH-1 .	AGP-1	MH	ACEN7	NR	
		GJPDH-2'	AGP-2	MH	ACEN7	NR	
		G3PDH-3*	AGP-3	H	ACEN7	NR	
		G3PDH-4*	AGP-4	H	ACEN7	NR	
Glucose-&phosphate isomerase	5.3.1.9	GPI-BI*	GPI-I	M	TBCLE	M	
		GPI-92"	GPI-2	M	TBCLE	P	1
		GPI-A'	GPI-3	M	TBCLE	M	
		GPIr*	GPI-H	M	TBCLE	M	
Glutathione reductase	1.6.4.2	GR*		E	TBCLE	P	3
β-Glucuronidase	3.2.1.31	βGUS*		L	TBCLE	NR	
Hydroxyacylglutaihione hydrolase	3.1.2.6	HAGH*	GLO-II	L	TBE	P	2
Hexokinase	2.7.1.1	HK*		M	ACE7	NR	
L-lditol dehydrogenase	1.1.1.14	IDDH-I*	SDH-1	L	TBCL	P	4
		IDDH-2*	SDH-2	L	TBCL	NR	
Isochrate dehydrogenase	1.1.1.42	mIDHP-1.	IDH-1	MH	ACE7	M	
		mIDHP-2.	IDH-2	MH	ACE7	M	
		sIDHP-1*	IDH-3	LE	ACE7	P	5
		sIDHP-2*	IDH-4	LE	ACE7	P	5
L-Lactate dehydrogenase	1.1.1.27	LDH-AI*	LDH-1	M	TBCLE	M	
		LDH-A2*	LDH-2	M	TBCLE	M	
		LDH-B1.	LDH-3	MEH	TBCLE	P	8
		LDH-B2*	LDH-4	LMEH	TBCLE	P	1
		LDH-C*	LDH-5	E	TC4	P	2
Lactoylglutathione lyase	4.4.1.5	LGL *	GLO-I	M	TBCLE	NR	
a-Mannosidase	3.2.1.24	αMAN*		L	TC4	NR	
Malate dehydrogenase	1.1.1.37	sMDH-A1,2*	MDH-I,2	LH	ACE7	M	
		sMDH-B1,2*	MDH-3,4	MH	ACE7	P	1
		mMDH-I*		HM	ACEN7	P	4
		m MDH-2'		HM	ACEN7	P	4
		mMDH-3*		HM	ACEN7	M	
Malic enzyme (NADP')	1.1.1.40	sMEP-1*	MDHP-1 ; ME-1	HL	TC4	P	4
		sMEP-2*	MDHP-2; ME-2	HL	TC4	P	4
		mMEP*		HM	TC4	NR	
Mannose-6-phosphate isomerase	5.3.1.8	MPI*		EHL	TBE	P	1
Nucleoside-lriphosphate pyrophosphatase	3.6.1.19	NTP*	ITP	M	TBCLE	NR	
Dipeptidase	3.4...-	PEPA*	DPEP-1; GL-I	ME	TBE	P	1
Tripepfide aminopeptidase	3.4...-	PEPB-1 .	PEP-3; PEP-LGG: TAPEP-1	ME	TBCLE, TC4	P	1,7
		PEPB-2*	TAPEP-2	ME	TBCLE	M	
Peptidase-C	3.4.-.-	PEPC'	DPEP-2; GL-2	E	TBE	NR	
Proline dipeptidase	3.4.-.-	PEPD-1.	PDPEP-I : PHAP-1	M	TBE	NR	
		PEPD-2*	PDPEP-2; PHAP-2	M	TBE	P	2
Leucyl-tyrosine dipeptidase	3.4.-.-	PEP-LT"		ML	TBE	P	2
Phosphogluconate dehydrogenase	1.1.1.44	PGDH'	6PG	ME	ACE7	M	
Phosphoglycerate kinase	2.7.2.3	PGK-1 .		EM	ACE7	M	
		PGK-2*		EM	ACE7	P	1

Enzyme name	Number	Locus	Previous Abbrev.	Tissue	Buffer	Status	Source'
Phosphoglucomutase	5.4.2.2	*PGM- I**		MEH	ACE7	M	
		PGM-2'		MEH	ACE7	M	
		PGM-3,4'		E	TBCLE	P	3,6
Pyruvate kinase	2.7.1.40	*PK-1'*		H	ACE7	NR	
		PK-2'		HL	ACE7	M	
Purine-nucleoside phosphorylase	2.4.2.1	*PNP- I **	NP-1	E	ACE7	NR	
		PNP-2'	NP-2	E	ACE7	NR	
Superoxfde dismutase	1.15.1 .I	*sSOD-1**	SOD-I	L	TBE	P	1
		*sSOD-2**		LH	TC4	NR	
		*mSOD**	SOD-2	H	TBE	NR	
Tyrosine aminotransferase	2.6.1.5	*TAT-I**		L	ACE7	NR	
		*TAT-P**		L	ACE7	NR	
Triose-phosphate isomerase	5.3.1.1	*TPI-1.1**	TPI-1	EM	TBCLE	M	
		*TPI-1.2**	TPI-2	EM	TBCLE	M	
		*TPI-2.1**	TPI-3	EM	TG	M	
		TPI-2.2'	TPI-4	EM	TG	P	2
Xanthine oxidase	1.2.3.2	*XO'*		L	TBCLE	NR	

"1 = Milner et al. 1983; 2 = Milner et al. 1986; 3 = Utter et al. 1989; 4 = Gall et al. 1989; 5 = Shaklee et al. 1990b; 6 = Waples and Aebersold 1990; 7 = James Shaklee, Washington Department of Fisheries, 115 General Administration Bldg., Olympia, WA 98504. Pers. commun., May 1987; 8 = this report.

When running a TC4 gel, in addition to using the cold-water circulating cooling plate under the gel, a pan of crushed ice is placed on top of the gel. The TG buffer (Holmes and Masters 1970) consists of 0.025 M tris and 0.192 M glycine, resulting in a pH of 8.4. The full concentration is used for both the electrode buffer and the gel buffer.

The seven electrophoretic buffers used in combination with the four tissues resulted in a screening protocol involving 16 gels for each 40 fish analyzed. Forty-six different enzymes, which code for over 100 presumptive gene loci, were screened on these gels. Table 2 lists the enzymes surveyed, the loci that were scored, the tissue(s) and buffer(s) used to resolve each locus, and the status of each locus (monomorphic, polymorphic, or not resolved) in the present data set. For each polymorphic locus, Table 2 also gives the earliest published source describing the type of variation observed in this study. Screening protocols and allele designations follow guidelines developed by the Coastwide Genetic Stock Identification Consortium. This group, which includes personnel from NMFS, Washington Department of Fisheries (WDF), the University of California at Davis, and the U. S. Fish and Wildlife Service, has made a concerted effort over the last several years to standardize methods for collection and reporting of electrophoretic data for chinook salmon.

Locus names and abbreviations follow the American Fisheries Society nomenclature guidelines established by Shaklee et al. (1990a). In general, when multiple gene loci occur for a single enzyme, higher numbers correspond to gene products that migrate farther from the origin on an electrophoretic gel. At each gene locus, one allele (generally the most common) is designated the “100” allele and additional alleles (if any) are designated by numbers that reflect their

electrophoretic mobilities relative to the "100" allele. Positive numbers represent anodal mobility and negative numbers represent cathodal mobility.

Genetic variability was detected at five gene loci not previously shown to be polymorphic in chinook salmon. The new locus with the highest level of variation is mAH-2*, which is detected in eye, heart, or muscle tissue on an ACE7 gel. Four anodally-migrating loci are revealed when staining for this locus; mAH-2* is the second locus from the origin. The variant allele migrates below (slower than) the common allele, measured as 88% mobility of the common allele, and is visualized in the upper portion of the space between *mAH-1** and mAH-2%. Additional alleles reported by Gall et al. (1989) were not observed in this study.

Variation was observed at *GAPDH-2** in one sample (Lostine River). This locus is detected only in heart tissue on an ACEN7 gel. ***GAPDH-3**** also will be resolved on this gel, along with bands resulting from interactions with other GAPDH loci. Enzymatic products from the two loci migrate mid-anodally, appearing as a 5-7 mm wide zone of staining activity, the bottom of the zone being *GAPDH-2** and the top ***GAPDH-3*.*** The variant allele migrates only 22% of the distance covered by the common allele. Variants are identified by the appearance of additional bands below the ***GAPDH-Z*,*** *GAPDH-3** zone. This variation has been observed at much higher frequencies in chinook salmon from other regions.

*GAPDH-4** showed low levels of variation in four stocks. This locus, along with ***GAPDH-5*,*** is expressed only in eye tissue. Gene products from these loci migrate into the upper anodal region of an ACEN7 gel. A five-banded pattern results (typical for tetrameric enzymes such as GAPDH), with ***GAPDH-4**** being represented by the slowest of the five bands. Anodal mobility of the variant allele is 95% of the common allele and is detected as a slight broadening of the *GAPDH-4** band and two of the bands above it.

Low levels of variability were observed in most stocks for *βGLUA**. This is a slow-migrating, anodal locus, best observed in liver tissue on a TC4 gel. The stain for this locus depends on the ability of the enzyme product to fluoresce and is visualized under ultraviolet light. Variants are seen as three-banded patterns typical of dimeric enzymes; the homodimeric band of the variant allele migrates 60% of the distance covered by the common allele.

Low-frequency variation at ***LDH-Bl**** was found in one stock (Johnson Creek). This locus is part of a complex system of five LDH loci that code for tetrameric enzymes. ***LDH-Bl**** is known to interact with *LDH-B2** and ***LDH-C****, resulting in complex banding patterns that vary with the tissue used to detect this locus. On a TBCLE gel, muscle tissue expresses ***LDH-Al**** and ***LDH-A2**** in the slow anodal portion of the gel; the two loci also produce interaction bands. ***LDH-Bl**** and *LDH-B2** are expressed in the mid anodal portion of the gel and also produce interaction products. The variant allele at ***LDH-Bl**** has a 48% mobility relative to the common allele; this places it in the same zone of activity as ***LDH-Al**** and ***LDH-AZ****, making detection difficult in muscle tissue. Eye fluid and retinal tissue also express ***LDH-Bl**** and *LDH-B2** in the mid anodal portion of the gel, as well as ***LDH-C****, a locus unique to the eye. In this tissue, the variants are not obstructed by slow-migrating loci and are easily detected. Furthermore, additional interaction bands between the variant allele and ***LDH-C**** aid in this detection. This allele has recently been observed in chinook salmon from other geographic regions as well.

Data Analysis

Electrophoretic phenotypes visualized on starch gels were interpreted as genotypes according to guidelines discussed by Utter et al. (1987). A chi-square test was used to compare genotypic frequencies at each variable locus in each population

with expected frequencies assuming Hardy-Weinberg equilibrium. This test can be useful in detecting artifactual (nongenetic) variation, and it also may detect population admixture, as population genetics theory indicates that a mixture of different gene pools should result in an apparent heterozygote deficiency.

Allelic frequencies, genetic distance values, and chi-square tests of Hardy-Weinberg genotypic proportions were obtained using the BIOSYS program (Swofford and Selander 1981). The unweighted pair-group method with arithmetic averages (UPGMA) was used with Nei's (1978) unbiased genetic distance values to generate dendrograms depicting genetic affinities among the samples.

In chinook salmon, as in other salmonids, several pairs of duplicated gene loci occur that have alleles with identical electrophoretic mobility. These loci are termed "isoloci." Isoloci present special problems for interpretation and data analysis because genotypes of individual fish cannot be determined unambiguously. Waples (1988) developed a maximum likelihood method to estimate the allele frequencies at the individual loci of an isolocus pair, and the chi-square test he described was used to test for agreement of observed and expected phenotypic proportions at isoloci that were polymorphic. This latter test is the two-locus equivalent of the Hardy-Weinberg test for individual gene loci. However, for reasons discussed by Waples (1988), allele frequency estimates for the individual loci of an isolocus pair may not be suitable for comparison among populations. Therefore, the allele frequencies presented in Appendix Table 1 (and those used in the genetic distance analyses) are mean frequencies computed over both loci of an isolocus pair. In this form the frequencies are also more easily compared with data from previous studies.

RESULTS AND DISCUSSION

Levels of Genetic Variability

Electrophoretic analysis of 46 enzyme systems in chinook salmon produced data for 65 presumptive gene loci that could be scored in all samples (the total is 69 if the 4 isoloci ***[sAAZ'-1,2*; sMDH-A1,2*; sMDH-B1,2*;*** and ***PGM-3,4*1*** are counted as 2 loci each). Thirty-five of the 65 loci that were resolved were variable, or polymorphic (defined as the presence of more than one allele), in at least one sample, with the remaining 30 loci being monomorphic (a single allele expressed) in all samples. In addition, data for a number of additional gene loci were gathered for some, but not all, populations; these data are not reported here. The small size of some of the wild fish made it difficult to resolve some of the enzymes expressed solely or primarily in heart tissue.

Of 206 single-locus chi-square tests performed, 12 indicated statistically-significant (2' < 0.05) departures from Hardy-Weinberg expected genotypic frequencies. The incidence of significant tests ranged from none (in Johnson Creek, Valley Creek, Lostine River, and the hatchery sample from the Imnaha River) to 4 of 17 (in the Upper Salmon River sample). The overall proportion of significant departures (5.8%) is about what can be expected to arise from chance. Furthermore, each of the significant tests involved at least one genotypic class with expected frequency less than 1, in which case the test may not be appropriate because the test statistic may not follow the chi-square distribution (e.g., Sokal and Rohlf 1981). Therefore, we did not find evidence for substantial departures from Hardy-Weinberg equilibrium.

The method described by Waples (1988) was used to perform a similar goodness-of-fit, test for the three variable isoloci. In this case, the test is for

agreement between the observed and expected numbers in each phenotypic class. All such tests for ***sAAT-1,2**** and ***sMDH-B1,2**** were non-significant (P > 0.05), whereas for PGM-3,4*, highly significant departures from expectations (P < 0.001) were found in 3 of the 11 samples (Lookingglass Hatchery, Marsh Creek, and Upper Salmon River). This result may reflect the inherent difficulty in scoring isolocus phenotypes for monomeric enzymes such as PGM. For isoloci, some of the phenotypes must be distinguished on the basis of different intensities of the same sets of bands, and, in contrast to dimeric enzymes such as AAT and MDH, monomeric enzymes do not produce intermediate bands that can be helpful in this respect. The results for ***PGM-3,4**** suggest some caution is needed in interpreting allele frequencies reported for this locus.

Allele frequencies for the variable (polymorphic) loci are given in Appendix Table 1; monomorphic loci are identified in Table 2. Of the 35 variable loci, 29 were polymorphic at the 0.99 level (common allele at frequency < 0.99 in at least one sample) and 22 were polymorphic at the 0.95 level. Therefore, with respect to the total number of loci surveyed (65), 54% were variable, 45% were polymorphic at the 0.99 level, and 34% were polymorphic at the 0.95 level.

The 35 variable loci identified in this study represent a considerable increase over previous reports. Schreck et al. (1986) and Utter et al. (1989) reported variation for 13 and 12 loci, respectively, in Snake River spring and summer chinook salmon. Loci with substantial levels of variation not previously reported as polymorphic in Snake River spring and summer chinook salmon include *sAAT-4** (frequency of the "100" allele ranges from 0.716 **in Lostine River to 1.0 in Valley** Creek and Upper Salmon River); *mAH-2** (range 0.807 in Valley Creek to 0.959 in Secesh River); *βGLUA** (range 0.908 in the hatchery sample from the Imnaha River to 1.0 in several samples); *PEP-LT** (range 0.870 in Marsh Creek to 0.985 in Upper

Salmon River); ***sMEP-1**** (range 0.01 in Sawtooth Hatchery to 0.079 in Marsh Creek); ***mMDH-2**** (range 0.49 in Upper Salmon River to 0.80 in Lookingglass Hatchery); and ***PGM-3,4**** (range 0.285 in Marsh Creek to 0.518 in Johnson Creek). In addition, the protocol developed by Shaklee et al. (1990b) was used to identify and report allele frequencies independently for ***sIDHP-I**** and ***sIDHP-2".*** In previous reports involving Snake River (and other) chinook salmon, these loci were considered as part of the isolocus pair ***IDH-3,4.***

Data for two of the polymorphic loci require special consideration. At ***GPI-B2**** and ***sMEP-2*,*** overlapping bands from other gene loci make it difficult to score all phenotypes reliably. For these loci, two phenotypic classes are scored: one that includes only those individuals homozygous for the variant allele (genotype denoted by "22"), and a class that includes individuals homozygous for the common allele (genotype "11") and heterozygotes (genotype "12"). Allele frequency of the variant "2" allele is estimated as the square root of the frequency of the "22" phenotype, with frequency of the common "1" allele estimated as 1.0 minus the estimated frequency of the "2" allele. Under the assumption of random mating, this procedure produces the "best" estimate of allele frequencies, but the variance of this estimate is much higher than the variance for a locus where all genotypes can be identified. In particular, if the "22" genotypes are rare, as was the case for both loci in this study, estimated allele frequencies are very sensitive to small changes in the number of "22" genotypes observed. For example, a sample of 100 fish from a population with frequency 0.1 for the "2" allele is expected to produce $(0.1)^2 x100 = 1$ fish with the "22" genotype, in which case there would be no error in estimating allele frequency from the sample. However, the probability of finding exactly 0 fish with the "22" genotype is about 0.37, in which case the population would be assumed (erroneously) to be monomorphic. In contrast, for a locus at which all

genotypes can be identified, the probability of not observing the “2” allele in a random sample of 100 fish when it is present in the population at frequency 0.1 is less than 1 in a billion.

Although monomorphic loci are not useful for monitoring genetic changes over time, those loci found to be invariant in the samples from the first year will continue to be surveyed for at least l-2 additional years to allow for the possibility that variant alleles may have been missed by sampling error or may occur in different year classes. In addition, some of these monomorphic loci are variable elsewhere in the Columbia River Basin, so lack of variation can provide information of relevance to stock identification and mixed-stock fishery analysis. For example, a variant allele at GPlr* is found at a frequency of at least 0.05 in other Columbia River stocks (Utter et al. 1989), but this locus was monomorphic in the current Snake River samples.

Average heterozygosity (H) values (mean proportion of heterozygous loci per individual) for each population are shown in Table 3. GPI-B2*, sMEP-2*, and the isoloci were not used in computing heterozygosities because individual genotypes for these loci cannot be identified unambiguously; this left 35 - 5 = 30 polymorphic loci and 65 - 5 = 60 total loci in the analysis. Actually, two sets of values are shown in this table: a relative heterozygosity value, based only on the remaining 30 polymorphic loci, and an unbiased heterozygosity value, based on 60 total loci, including those that were monomorphic. Many studies of Pacific salmon have reported relative heterozygosity values, because such studies often focus only on loci known or suspected to be polymorphic. Relative **heterozygosity values are suitable** for comparison within a single dataset, but comparisons with other analyses using different sets of loci can be misleading. For this latter purpose, it is necessary to survey a random **(and preferably large) selection of gene loci. Relative**

Table 3.--Average heterozygosity values for Snake River spring and summer chinook salmon. Unbiased estimates are based on all gene loci resolved, including monomorphic loci; relative estimates are based only on data for polymorphic loci. *GPI-B2**, sMEP-2*, and the three polymorphic isoloci (*sAAT-1,2**, sMDH-B.Z,2*, and PGM-3,4*) were excluded in all computations of heterozygosity.

Sample	Unbiased (60 loci)	Relative (30 loci)	Relative (27 loci)
McCall Hatchery	0.039	0.078	0.081
Johnson Creek	0.037	0.074	0.074
Secesh River	0.040	0.081	0.086
Marsh Creek	0.043	0.087	0.083
Sawtooth Hatchery	0.046	0.092	0.090
Upper Salmon River	0.038	0.077	0.077
Valley Creek	0.049	0.098	0.095
Imnaha River (hatchery)	0.044	0.089	0.091
Imnaha River (natural)	0.040	0.081	0.081
Lookingglass Hatchery	0.034	0.068	0.065
Lostine River	0.050	0.100	0.103
Warm Springs (hatchery)	-	-	0.101
Warm Springs (wild)	-	-	0.096

heterozygosities for 30 loci in the 11 samples ranged from 0.068 in the Lookingglass Hatchery sample to 0.10 in the Lostine River. Unbiased heterozygosities, which in this case were exactly half as large, ranged from 0.034 to 0.05.

Utter et al. (1989) and Winans (1989) noted that Snake River spring and summer chinook salmon stocks as a group are characterized by relatively low levels of genetic variability. Waples (1990) summarized some of Winans' data, which showed a decline in heterozygosity with distance from the mouth of the Columbia River in spring-run but not fall-run stocks. Mean H values for data reported by Winans were about 0.08, 0.06, and 0.04, respectively, for spring-run stocks from the Lower Columbia Willamette, mid-to-upper Columbia, and Snake River (Waples 1990, Table 1). That is, levels of heterozygosity in Snake River stocks were only about half that of lower river stocks.

It is interesting to ask whether this pattern is still found when data for the new samples and additional gene loci are considered. The relatively high levels of variation found at some of the new loci suggest that this may not be the case, but it is difficult to demonstrate conclusively at present. As noted above, comparing relative heterozygosities from different studies is not appropriate, and neither Winans (1989) nor Utter et al. (1989) reported unbiased heterozygosities. Furthermore, many of the loci with newly-reported variation in this study were not surveyed in older studies of lower river stocks. However, two 1987 samples (wild and hatchery) from Warm Springs (an Oregon tributary of the Deschutes River) were analyzed by NMFS for 27 of the polymorphic loci reported here. In Winans' study, these samples had relative heterozygosities of 0.056, about 1.4-2.4 times as large as the values he reported for samples from the Upper Salmon River, Johnson Creek, the South Fork Salmon River, and Rapid River Hatchery. Based on data for 27 polymorphic gene loci, the two Warm Springs samples have relative

heterozygosities of 0.096-0.101, compared to 0.065-0.103 for the 11 new Snake River collections (Table 3). Samples from Valley Creek, Lostine River, Sawtooth Hatchery, and the Imnaha River (hatchery sample) all have relative heterozygosities as high or nearly as high as those found in the Warm Springs samples. A tentative conclusion is that Snake River spring and summer chinook salmon still have somewhat lower levels of genetic variability than found in lower river stocks, but the difference may not be as large as suggested in earlier reports. Additional comparisons with lower river stocks having data for the newly-resolved gene loci are necessary to determine whether this tentative conclusion is correct.

Population Subdivision

Likelihood ratio (G) tests comparing allele frequencies were performed for each pair of samples (11x10/2 = 55 tests total). Every comparison produced highly significant ($P < 0.001$) differences when results were combined for all gene loci. Thus, the hypothesis that spring- and summer-run chinook salmon in the Snake River form a single panmictic unit (or that any pair of populations do) can be convincingly rejected. Interestingly, this also held for the two cases in which a hatchery population was compared with the nearby natural population it was derived from and which it has been outplanted into. The Sawtooth Hatchery - Upper Salmon River comparison yielded an overall G value of 70.1 with 25 df, with significant differences found at 5 loci: *sAAT-3*; sAAT-4*; sIDHP-1*; MPI*;* and ***PEP-LT".*** Allele frequencies in the hatchery and stream samples from the Imnaha River were significantly different at ***sAH*,*** *βGLUA*, HAGH*, sMDH-B1,2*,* and *MPI*,* resulting in an overall G value of 55.3 with 25 df.

More insight into population structuring can be obtained from a dendrogram depicting relationships based on pairwise genetic distance values (Fig. 3). The

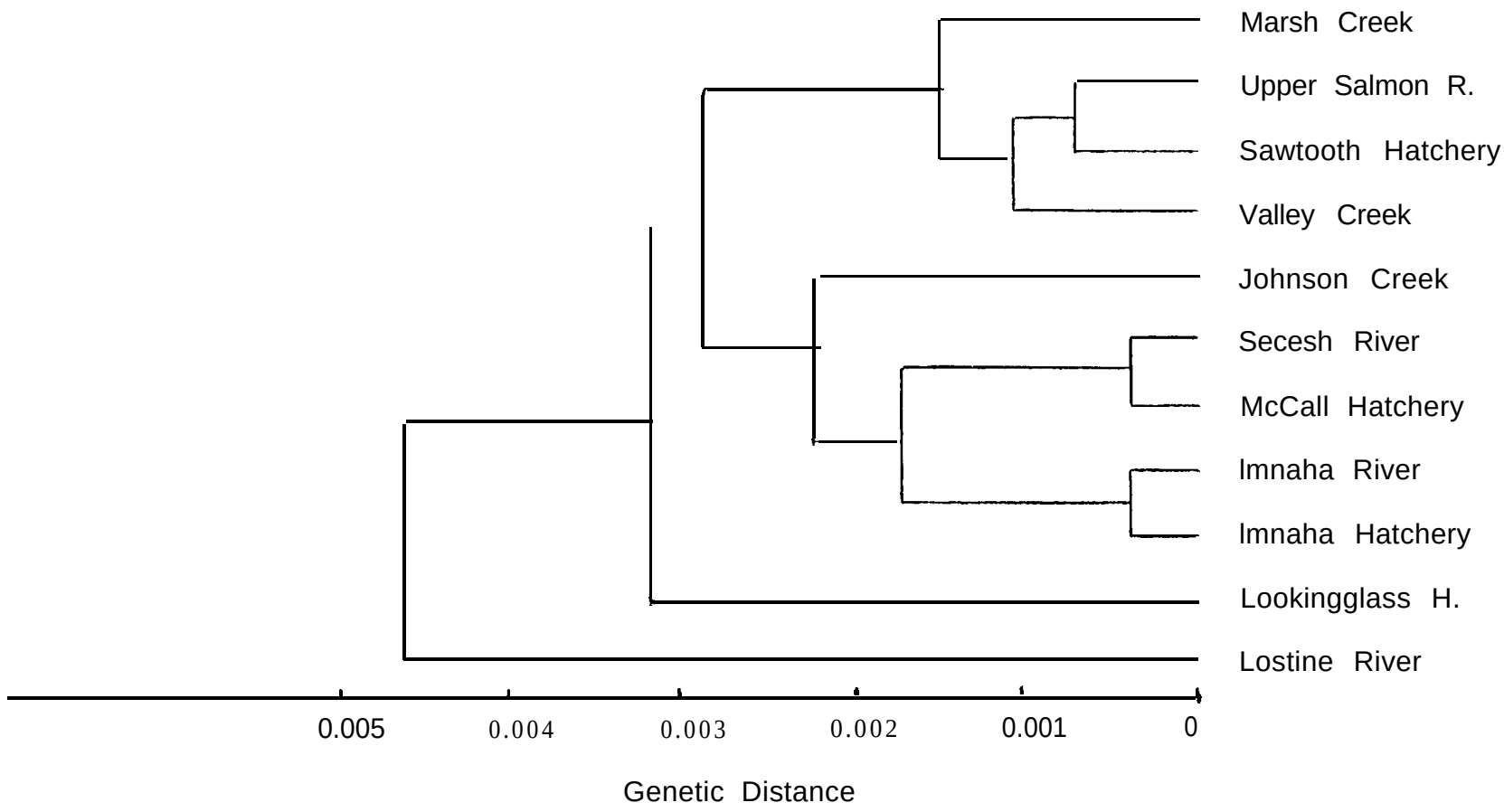


Figure 3.--Dendrogram of pairwise genetic distance values (Nei 1978) for Snake River spring and summer chinook salmon stocks. Dendrogram is based on data for 33 polymorphic loci (excluding ***GPI-B2**** and *sMEP-2**).

clusters that appear in this dendrogram can largely be explained on the basis of geographical proximity. The three spring-run samples from the mainstem Salmon River (Valley Creek, Upper Salmon River, and Sawtooth Hatchery) cluster together, with the spring-run sample from the Middle Fork (Marsh Creek) being only slightly more distinct genetically. In terms of Nei's genetic distance, the two most similar pairs are McCall Hatchery and Secesh River, both on the South Fork of the Salmon River, and the hatchery and natural samples from the Imnaha River. Together with Johnson Creek (the third South Fork sample) these five samples form a cluster that contains all of the populations in this study that are typically classified as summer-run chinook salmon. Lookingglass Hatchery (Rapid River stock) and Lostine River (both classified as spring chinook salmon) are relative outliers in this figure.

Figure 3 is based on data for 33 polymorphic loci (excluding *GPI-B2** and ***sMEP-2*,*** for reasons discussed above). Because isoloci also present some special problems for statistical analysis, and because of the difficulties in scoring one of the isoloci ***(PGM-3,4*),*** we repeated the analysis after omitting the isoloci as well. The topography of the resulting dendrogram (based on 30 gene loci; Fig. 4) is similar to the preceding one with a couple of exceptions: Johnson Creek now clusters with the Upper Salmon River sample, and Lookingglass Hatchery clusters with the South Fork and Imnaha samples before that group is combined with the other Salmon River stocks. These differences found on omitting three loci from the analysis are informative because they help emphasize the caution that should be used in interpreting dendrograms based on genetic or other characteristics. For example, in terms of Nei's genetic distance, the Johnson Creek sample is approximately equidistant from the McCall Hatchery and Upper Salmon River samples. Each of the latter two samples, however, is closer genetically to another sample that it

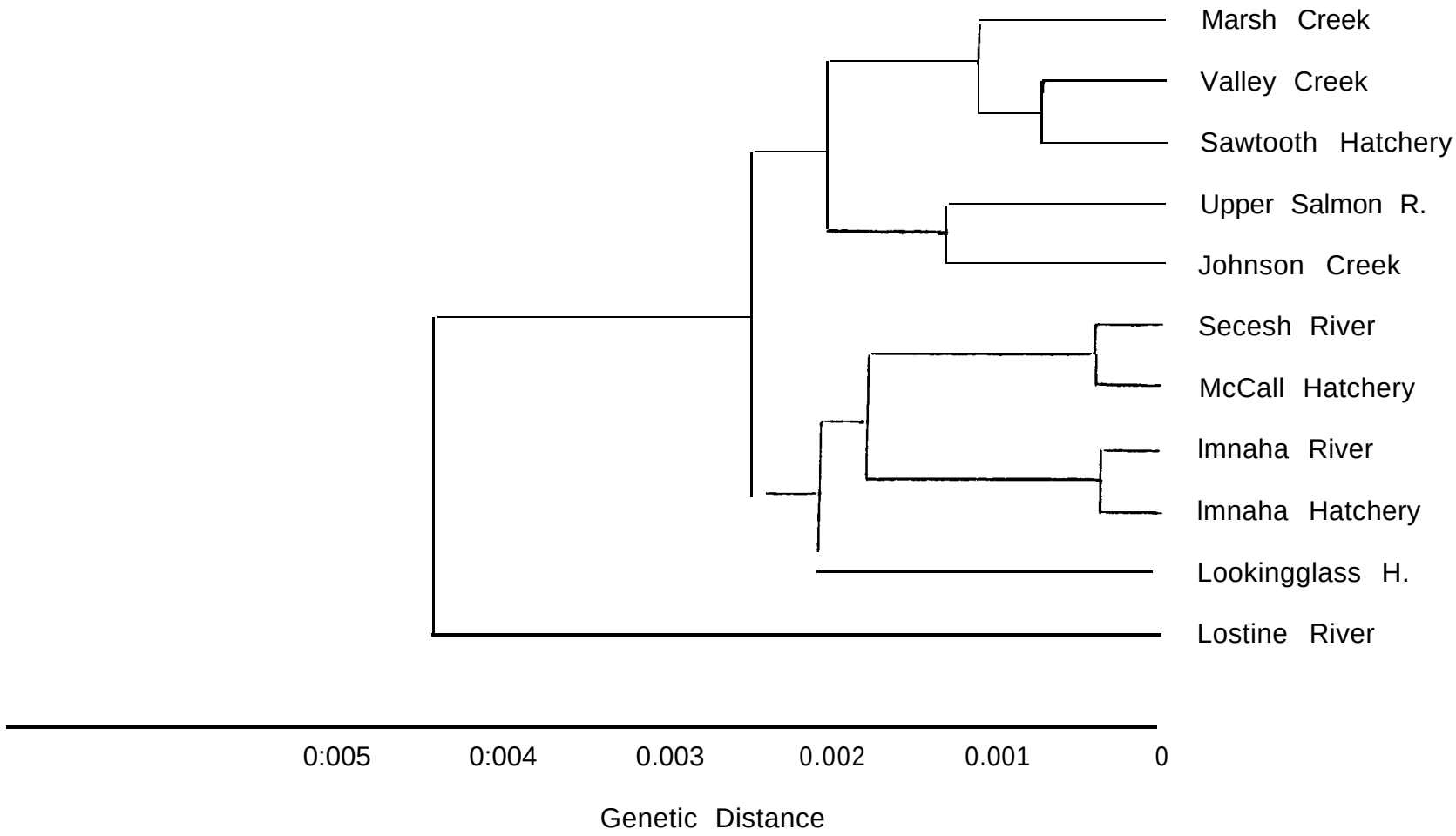


Figure 4.--Dendrogram of pairwise genetic distance values (Nei 1978) for Snake River spring and summer chinook salmon stocks. Dendrogram is based on data for 30 polymorphic loci (excluding *GPI-B2**, ***sMEP-2****, and isoloci).

clusters with initially. Because the initial clusters that are formed can strongly influence the overall topography of the dendrogram, care must be taken to avoid misleading conclusions. Thus, from the first dendrogram it might be concluded that Johnson Creek is very distinct from the Upper Salmon River samples, and from the second dendrogram it might be concluded that Johnson Creek is not at all similar to the other South Fork samples. Both conclusions would be wrong. However, the two dendrograms agree in showing that 1) the Middle Fork sample (Marsh Creek) is not dramatically different from samples from the Upper Salmon River, and 2) the Lostine River is a relative outlier and the most distinct sample genetically. There is also some evidence that summer-run stocks tend to be genetically more similar to each other than they are to spring-run stocks. However, this last point also largely reflects geographical groupings, as the summer stocks included this study are restricted to the Imnaha and South Fork Salmon drainages. A related question--whether spring- and summer-run fish from the same stream are more closely related to each other than they are to stocks of the same run-time in other drainages--cannot be answered with the present dataset.

The distinctness of the Lostine River sample depends largely on a high frequency (0.284) of the “63” allele at sAAT-4*, which was found at much lower frequency (0 - 0.081) in the other samples. More modest differences at ***GAPDH-4*, GR*,*** and ***MPI**** also distinguish the Lostine River sample. As noted in Methods, this sample was taken in a side channel of the river after most of the fish had moved downstream due to early rains. The fish in the side channel may have taken refuge there after migrating from **a variety of locations upstream, but it is also** possible that they represent the progeny of a relatively few adults from nearby **redds. Some support for this hypothesis is provided by gametic disequilibrium** analysis, which examines correlations among alleles at different gene loci. Waples

and Smouse (1990) showed that likely causes of gametic disequilibrium in Pacific salmon populations include 1) a low effective number of breeders responsible for the sample or 2) a mixture of different gene pools. The level of multilocus gametic disequilibrium observed in the Lostine sample was much higher than in any of the other samples (unpublished data). Therefore, we must consider the possibility that non-random sampling of the population as a whole may have contributed to the distinctness of the Lostine River sample. Analysis of samples from subsequent years should help to resolve this issue.

Another perspective on genetic relationships in Snake River chinook salmon can be gained by considering electrophoretic data from throughout the Columbia River Basin. Figure 5 shows a dendrogram of genetic relationships for spring-, summer-, and fall-run stocks from the basin. Note that the scale of this figure differs from that of the previous dendrograms, and that on a coarser scale, the genetic differences among Snake River spring and summer chinook salmon populations appear very modest. These stocks, together with the Carson-stock spring chinook hatcheries, form a group that is genetically most similar to a group of spring chinook salmon samples from the mid and upper Columbia River. This larger group is quite different genetically from two other groups that can be identified on the dendrogram: 1) Willamette River spring chinook salmon and 2) all fall chinook salmon and a few Columbia River spring and summer stocks.

Several interesting patterns in this dendrogram are worth noting. The geographical, morphological, and physiological differences between lower river (Yule") fall chinook salmon and upriver ("bright") stocks are supported by electrophoretic differences between the two groups. Whereas summer chinook salmon in the Snake River are genetically most similar to Snake River spring chinook salmon, the upper Columbia River summer chinook salmon are more similar to upper Columbia River

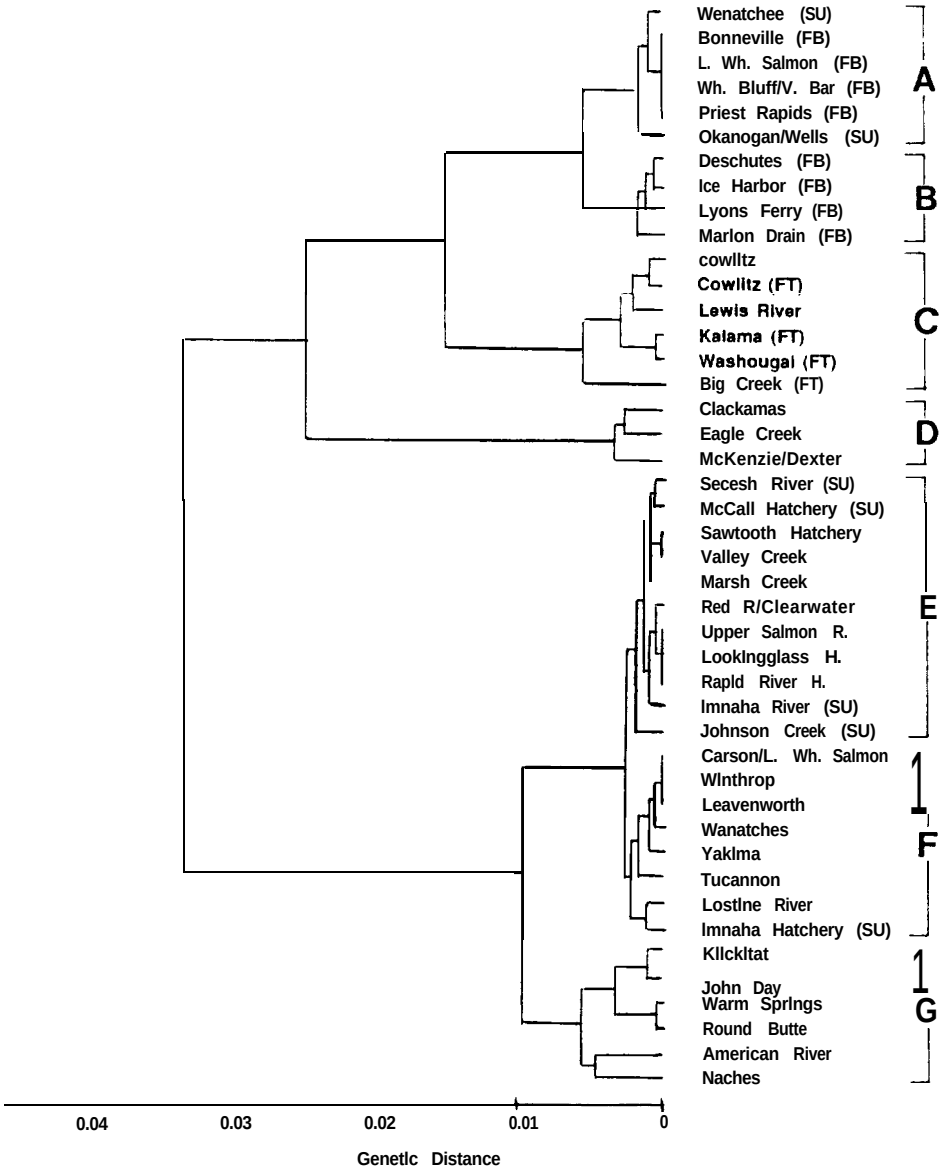


Figure 5.--Dendrogram showing clustering of pairwise genetic distance values (Nei 1978) computed for 21 polymorphic gene loci in chinook salmon from the Columbia River Basin, based on published and unpublished data from NMFS and WDF. Run-time designations in parentheses are SU (summer), FB (fall "upriver bright"), and FT (fall "tule"); others are spring-run stocks. In general, clusters can be characterized by geography and run-timing: A--upper Columbia River summer- and fall-run; B--Snake River fall-run; C--lower Columbia River fall-run; D--Willamette River spring-run; E--Snake River spring- and summer-run; F--upper Columbia River spring-run; G-mid-Columbia River spring-run.

fall chinook salmon. The genetic data are concordant with life history data; in the Upper Columbia River, the summer-run fish mimic the fall-run fish in outmigrating as subyearlings, whereas in the Snake River spring-run and summer-run fish both outmigrate as yearlings (Schreck et al. 1986; Chapman et al. 1991).

Some of the patterns can apparently be explained by hatchery brood-stock practices. For example, the similarity between spring and fall chinook salmon stocks from Cowlitz and Lewis Hatcheries probably results from the arbitrary methods used to decide which returning fish will be spawned with the spring group and which with the fall group (C. Busack”). The very close similarity between the samples from Carson, Leavenworth, Little White Salmon, and Winthrop Hatcheries probably is a consequence of the frequent transfer of eggs or brood stock among these hatcheries.

It should be pointed out that the genetic distance values used to construct the dendrograms in Figures 3-5 are relative in the sense that they are based only on polymorphic gene loci. Inclusion of monomorphic loci would not change the tree topography, but the distance values for all the branch points would be reduced by about one-half.

Temporal Changes

An important goal of this study is to provide a more comprehensive understanding than has been available to date of genetic changes that occur over time in Pacific salmon populations. Changes due to random processes (genetic drift) are inevitable in finite populations, but the nature and extent of genetic change may also reflect the effects of supplementation. Obviously, temporal changes cannot be evaluated from a single year of samples; that is the primary reason that this study

---

[4]Craig Busack, Washington Department of Fisheries, 115 General Administration Bldg., Olympia, WA 98504. Pers. commun., November 1990.

is designed to run for a number of years. Nevertheless, electrophoretic data from samples taken in prior years are available for some of the populations included in this study, and comparison of the new and old data provides some insight into changes that have occurred. Previously analyzed samples that are relevant to the present study include 1981 or 1982 brood year (BY) samples from McCall Hatchery, Johnson Creek, Valley Creek, and the Upper Salmon River, and 1981 and 1984 BY samples from Rapid River Hatchery (Milner et al. 1983; Milner et al. 1986). Allele frequency data for these and comparable samples for BY 1988 are shown in Appendix Table 2. Note that these comparisons involve many fewer gene loci than are shown in Appendix Table 1 because a number of the new polymorphic enzyme systems were not screened in previous years. In addition, for some gene loci included in earlier studies (e.g., PEPB-1*), newly-developed screening protocols have revealed additional alleles not previously detected. In Appendix Table 2, these new alleles have been pooled with other alleles to allow comparisons with older data.

There is little evidence of genetic change in the two samples from McCall Hatchery. Significant allele frequency differences were found at a single gene locus (*PEPB-1**; 0.05 > P > O.Ol), where the frequency of the "130" allele declined from 0.07 to 0.015. However, the overall G value for all loci was non-significant (G = 19.1; df = 14; P > 0.1). A reasonable interpretation of these results is that effective population size was not too small and straying or transfers of genetically distinct stocks into the hatchery were not an important factor during the period 1981-1988. Because broodstock for McCall Hatchery is taken each year from a mixture of wild and hatchery adults on the South Fork of the Salmon River, the hatchery is not a closed population; therefore, these results apply to the effective size and stock history of the combined hatchery/wild population.

Allele frequency changes in the Rapid River Hatchery stock have also been rather small. This is true whether temporal samples from the hatchery are compared (NMFS, unpublished data), or the 1988 brood-year sample from Lookingglass Hatchery is compared to previous data for Rapid River Hatchery (overall G = 25.0; df = 15; P = 0.05). This latter result is not unexpected, as the 1988 brood year Lookingglass Hatchery fish were obtained from Rapid River Hatchery (R. Carmichael").

Genetic changes in samples from the 1981 and 1988 brood years in Johnson Creek were somewhat larger (overall G = 37.1; df = 16; P < O.Ol), and significant changes in allele frequency were found at four gene loci ***(sIDHP-l*, LDH-C*, MDH-Bl,2*,*** and MPI). Possible explanations for the changes include genetic drift in a relatively small population (effective number of breeders less than about 100 per year), non-random sampling from the population, or gene flow from another population. The first hypothesis is not unreasonable given the depressed state of chinook salmon in the Salmon River in the last decade. Non-random sampling must always be considered when temporal (or geographic) changes are observed, but this factor is difficult to evaluate in wild samples. Given that Johnson Creek has been supplemented with McCall Hatchery fish since 1985 (Matthews and Waples 1991), the last hypothesis is also of interest. One way to evaluate this hypothesis is to ask whether the changes between 1981 and 1988 are in the direction expected if they were due to the influence of McCall Hatchery fish. Data for *sIDHP-1** provide some support for this hypothesis: frequency of the "74" allele in the 1988 Johnson Creek sample (0.212) was much closer to that observed in McCall Hatchery (0.206-0.260) than it was in the 1981 Johnson Creek sample (0.098). However,

---

"Richard Carmichael, Oregon Department of Fish and Wildlife, Badgley Hall, Eastern Oregon State College, La Grande, OR 97850. Pers. commun., September 1990.

there is no clear pattern to the change at *MDH-B1,2** and ***MPI,*** and at ***LDH-C**** the difference between the Johnson **Creek and McCall samples was greater for BY 1988** than it was in 1981. Furthermore, there remain substantial differences between McCall Hatchery and Johnson Creek fish at a number of gene loci (see Appendix Table **1).** Therefore, although some genetic influence from McCall Hatchery fish cannot be ruled out, it appears that the outplanted fish had not had a substantial impact on the native Johnson Creek population through BY 1988.

The 1982 Valley Creek sample provides limited information because it included just 22 fish. Most of the allele frequency differences between 1982 and 1988 can probably be explained by sampling error.

The 1981 Upper Salmon River sample is interesting in light of the stock history for this population. In 1979, 914,000 smolts from Rapid River Hatchery stock were released at the present site of the Sawtooth Hatchery weir, and at least 500 returned as adults in 1981 (S. Yundt[6]). Some of these adults were spawned with wild Salmon River fish to form the initial brood stock for Sawtooth Hatchery. The early electrophoretic sample from the Upper Salmon River included 50 juveniles produced by adults spawned at Sawtooth Hatchery in 1981. Not surprisingly, allele frequency profiles in this sample are very similar to those found in 1981 and 1984 brood-year samples from Rapid River Hatchery (Appendix Table 2). The more recent (1988 brood year) sample from the Upper Salmon River is not as similar genetically to the Rapid River samples.

As data for the second and third year of samples become available, a much more complete picture of genetic structuring in Snake River spring and summer chinook salmon populations should emerge. Key questions to address include How

---

[6]Steven Yundt, Idaho Department of Fish and Game, 600 S. Walnut, Box 25, Boise, ID 83707. Pers. commun., May 1990.

stable over time are geographic patterns of genetic differentiation? and How large are temporal differences within populations relative to differences between populations?

ACKNOWLEDGMENTS

Richard Carmichael (ODFW) and Steven Yundt (IDFG) were instrumental in helping to select the study sites and in providing stock history information. Brian Jonasson (ODFW) compiled extensive life-history data for Imnaha and Grande Ronde chinook salmon. We gratefully acknowledge the assistance of Brian Jonasson and Rhine Messmer (ODFW), Russ Kiefer and his staff (IDFG), the NMFS PIT-tagging crew, and personnel at McCall, Sawtooth, and Lookingglass Hatcheries for assistance in collecting juvenile chinook salmon. Cynthia Shiflett, Donald Van Doornik, Frederick (Bud) Welch, Thomas Thornton, Matt Griswold, and Shelley Jay assisted with the electrophoretic analyses. The WDF Genetics Unit (Olympia, WA) kindly allowed us to use unpublished allele frequency data to construct the dendrogram in Figure 5. We thank James Shaklee and Graham Gall for useful comments on an earlier draft.

LITERATURE CITED

Aebersold, P. B., G. A. Winans, D. J. Teel, G. B. Milner, and F. M. Utter.
1987. Manual for starch gel electrophoresis: a method for the detection of genetic variation. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 61, 19 p.

Allendorf, F. W., and G. H. Thorgaard.
1984. Tetraploidy and the evolution of salmonid fishes, p. l-53. In: B. Turner (ed.). Evolutionary Genetics of Fishes. Plenum Press, New York.

Chapman, D., A. Giorgi, M. Hill, A. Maule, S. McCutcheon, D. Park, W. Platts, K. Pratt, J. Seeb, L. Seeb, and F. Utter.
1991. Status of Snake River chinook salmon. Final Report submitted to ESA Administrative Record for Snake River chinook salmon, 531 p. plus Appendix. Available Pacific Northwest Utilities Conference Committee, 101 SW Main Street, Suite 810, Portland, OR 97204.

Dreyfus, J. C., and Y. Alexandre.
1972. Electrophoretic characterization of acidic and neutral amylo-1-4-glucosidase (acid maltase) in human tissues and evidence for 2 electrophoretic variants in acid maltase deficiency. Biochem. Biophys. Res. Commun. 48(4X914-920.

Emlen, J. M.
1990, Inbreeding depression, local adaptation, and outbreeding depression in salmon stocks: some implications for hatchery management, p. 104-107. In: D. L. Park (conv.). Status and Future of Spring Chinook Salmon in the Columbia River Basin--Conservation and Enhancement. U.S. Dep. Commer., NOAA Tech. Mem. NMFS F/NWC-187.

Fraidenburg, M. E., and R. H. Lincoln.
1985. Wild chinook salmon management: an international conservation challenge. N. Am. J. Fish. Manage. 5:311-329.

Fredin, R. A.
1980. Trends in North Pacific salmon fisheries, p. 59-119. ***In:*** W. J. McNeil and D. C. Himsworth (eds.). Salmonid ecosystems of the North Pacific. Oregon State University Press, Corvallis, Oregon. 331 p.

Gall, G., B. Bentley, C. Panattoni, E. Childs, C-f. Qi, S. Fox, M. Mangel, J. Brodziak, and R. Gomulkiewicz.
1989. Chinook mixed fishery project, 1986-1989. Report of Research, University of California, Davis, CA. 192 p. + Appendices.

Hindar, K., N. Ryman, and F. Utter.
1991. Genetic effects of aquaculture on natural fish populations. Can. J. Fish. Aquat. Sci. 48:945-957.

Holmes, R. S., and C. J. Masters.
1970. Epigenetic interconversions of the multiple forms of mouse liver catalase. FEBS Letters 11:45-48.

Matthews, G. M., and R. S. Waples.
1991. Status review for Snake River spring and summer chinook salmon. U.S. Dep. Commer., NOAA Tech. Memo. NMFS F/NWC-200, 75 p.

Miller, W. H., T. C. Coley, H. L. Burge, and T. T. Kisanuki.
1990. Analysis of past and present salmon and steelhead supplementation. Part I. Report to Bonneville Power Administration, Project 88-100, 44 p.

Milner, G. B., D. J. Teel, P. B. Aebersold, and F. M. Utter.
1986. Genetic stock identification. Report to Bonneville Power Administration under contract DE-A179-85BP23520, 90 p. Bonneville Power Administration, P. 0. Box 3621, Portland, OR 97208.

Milner, G. B., D. J. Teel, and F. M. Utter.
1983. Genetic stock identification study. Report to Bonneville Power Administration under contract DE-A179-82BP28044M001, 95 p. Bonneville Power Administration, P.O. Box 3621, Portland, OR 97208.

Nehlsen, W., J. E. Williams, and J. A. Lichatowich.
1991. Pacific salmon at the crossroads: stocks at risk from California, Oregon, Idaho, and Washington. Fisheries 16(2):4-21.

Nei, M.
1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

NWPPC (Northwest Power Planning Council).
1987. Columbia River Basin fish and wildlife program. Portland, Oregon. 246 p.

Schreck, C. B., H. W. Li, R. C. Hjort, and C. S. Sharpe.
1986. Stock identification of Columbia River chinook salmon and steelhead trout. Final Report of Research. Bonneville Power Administration, Portland, Oregon. 184 p.

Shaklee, J. B., F. W. Allendorf, D. C. Morizot, and G. S. Whitt.
1990a. Gene nomenclature for protein-coding loci in fish. Trans. Am. Fish. soc. 119:2-15.

Shaklee, J. B., C. Busack, A. Marshall, M. Miller, and S. R. Phelps.
1990b. The electrophoretic analysis of mixed-stock fisheries of Pacific salmon, p. 235-265. *In:* Isozymes structure, function, and use in biology and medicine. Progress in Clinical and Biological Research. Vol. 344. (Z-1. Ogita and C. L. Markert, eds.) Wiley-Liss, Inc., New York.

Sokal, R. R., and F. J. Rohlf.
1981. Biometry, Second Edition. W. H. Freeman and Company, San Francisco. 859 p.

Swofford, D. L., and R. B. Selander.
1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Heredity 72:281-283.

Utter, F., P. Aebersold, and G. Winans.
1987. Interpreting genetic variation detected by electrophoresis, p. 21-45. ***In: N.*** Ryman and F. Utter [eds.]. Population Genetics and Fishery Management. University of Washington Press, Seattle.

Utter, F. M., G. B. Milner, G. Stahl, and D. J. Teel.
1989. Genetic population structure of chinook salmon, ***Oncorhynchus tshawytscha,*** in the Pacific northwest. Fish. Bull. 87:239-264.

Waples, R. S.
1988. Estimation of allele frequencies at isoloci. Genetics 118:371-384.

Waples, R. S.
1990. Stock structure and gene 'conservation in Columbia River spring chinook salmon, p. 14-21. ***In:*** D. L. Park (conv.). Status and Future of Spring Chinook Salmon in the Columbia River Basin--Conservation and Enhancement. U.S. Dep. Commer., NOM Tech. Mem. F/'NWC-187.

Waples, R. S., and P. B. Aebersold.
1990. Treatment of data for duplicated gene loci in mixed- stock fishery analysis. Can. J. Fish. Aquat. Sci. 47:2092-2098.

Waples, R. S., and P. E. Smouse.
1990. Evaluation of gametic disequilibrium analysis as a means of identifying mixtures of salmon populations. American Fisheries Society Symposium 7:439-458.

Winans, G. A.
1989. Low levels of genetic variability in spring-run chinook salmon of the Snake River. N. Am. J. Fish. Manage. 9:47-52.

Appendix

Protein Electrophoresis[7]

Protein electrophoresis is a widely used method for studying natural populations. The technique allows one to quantify biochemical differences between individuals and among populations. Because proteins are composed of a series of amino acids, and the amino acid sequence is determined by three-base segments of DNA, differences in proteins can be interpreted in terms of the genes coding for protein structure. Genes coding for a large number of proteins in salmonids and other organisms have been studied in this way.

With a few exceptions, protein electrophoresis focuses on water-soluble enzymes (i.e., proteins that catalyze specific biochemical reactions). Typically, a piece of tissue from an individual is mixed with a small amount of buffer to produce a tissue extract containing the soluble enzymes. For analysis, extracts from a number of individuals can be loaded into a matrix, or **gel** (generally a slab of potato starch somewhat similar in consistency to Jell-o). Application of an electric current ("running the gel") causes the proteins in solution to migrate at a rate determined primarily by their net charge, which, in turn, is determined by the amino acid composition of the enzyme.[8] Most proteins After a period of time (generally several hours>, sections of the gel are treated with a solution containing substrates and

---

[7]This brief summary is intended to help familiarize the reader with some of the terminology used in this report. For a more detailed discussion of protein electrophoresis and its application to salmonids, see Utter et al. (1987).

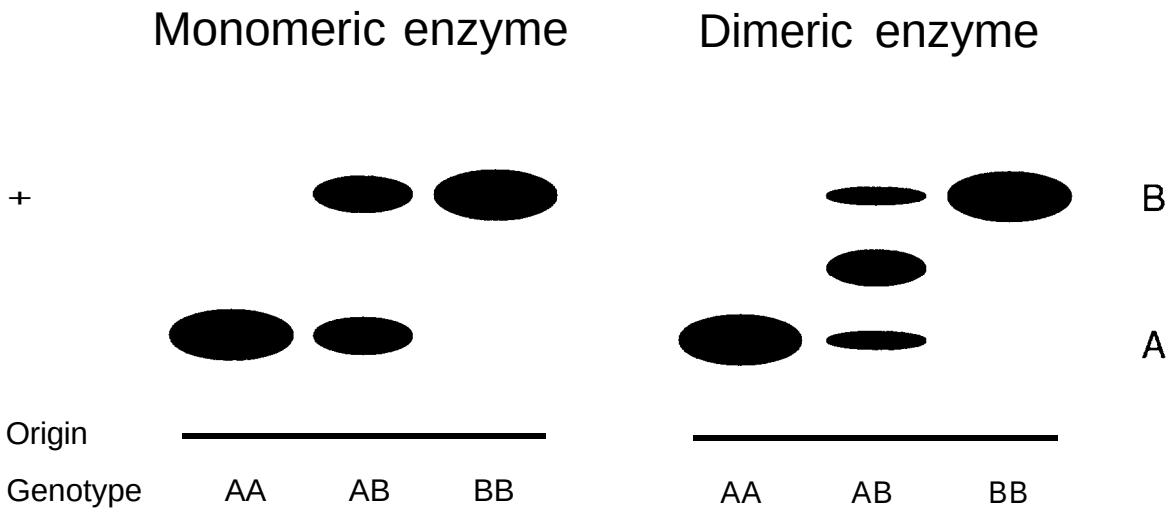
[8]At physiological pH, 5 of the 20 common amino acids carry a net charge (3 with positive charges and 2 negative), the remaining 15 being neutral. Thus, only some amino acid substitutions change the net charge of the enzyme and are detected by routine protein electrophoresis. Some of the "hidden" variation can be detected by adjusting the pH of the gels and buffers or through other methods. Most proteins carry a net negative charge and therefore migrate toward the positive **(anodal)** pole; others, however, migrate **cathodally,** and the direction of migration may vary with the pH of the buffers used.

cofactors necessary for specific enzymatic reactions. Linking dyes that precipitate at the sites of enzymatic activity allow visualization of the distance travelled by enzymes from each individual. Because visualization requires that proteins retain their native configuration and enzymatic ability, care is required throughout the process of sample collection, storage, and analysis. Although some enzymes are relatively stable, others degrade quickly after the organism dies. Analysis of fresh specimens or rapid freezing and storage at -80°C is the best way to ensure adequate sample quality.

Banding patterns visualized on starch gels can be interpreted in terms of genetic variation using guidelines based on principles of protein structure and genetic models of inheritance. The basic data gathered are the **genotypes** for each individual. At each gene locus, a diploid[9] individual has two **alleles,** or alternate copies of the gene. A genotype, then, is simply the enumeration of the two alleles present in the individual. If the two alleles are the same, the individual is termed a **homozygote** for that gene locus; if not, the individual is a **heterozygote. An** individual's multilocus genotype is simply the list of single locus genotypes.

Genotypes are inferred from the banding patterns (i.e., the phenotypes) that appear on electrophoretic gels. For a given gene locus, homozygotes show a single electrophoretic band (representing a single form of the enzyme), whereas heterozygotes show two or more bands representing different forms of the enzyme (Appendix Figure 1).

"Salmonids are ancestrally tetraploid; that is, they are derived from a common ancestor that underwent a doubling of the entire chromosomal complement. However, subsequent loss of duplicated genetic material or divergence of the duplicated segments has restored diploid expression to much of the salmonid genome (Allendorf and Thorgaard 1984). Special analytical problems posed by some of the genes that remain duplicated are discussed in the text.



Appendix Figure 1.-- Schematic diagram of electrophoretic banding patterns characteristic of monomeric and dimeric enzymes. In both cases, two different alleles (A, B) code for subunits of the enzyme.

Interpretation of electrophoretic gels for **monomeric** enzymes that are made up of a single subunit is relatively straightforward. Many enzymes, however, require two or four subunits in their active form; the resulting enzymes are known as **dimers** and **tetramers,** respectively. Although a diploid individual carries only two alleles for each gene locus (producing at most two different kinds of subunits), the subunits can be combined in three (for a dimeric enzyme) or five (for a tetrameric enzyme) different ways. For example, an individual heterozygous for a particular gene locus will produce two types of subunits (call them A and B). If the enzyme is a monomer, the subunits will represent the two only types of the enzyme that are formed; if the enzyme is a dimer, however, the subunits can combine in three different ways (AA, AB, or BB) to form an active enzyme. Therefore, a heterozygote for a dimeric enzyme has a three-banded phenotype, with the band representing the AB **heterodimer** having mobility intermediate to that of the two **homodimers** AA and BB. Thus, the appearance of heterozygotes is distinctive and characteristic for each type of enzyme (Appendix Figure 1).

Additional complications in interpreting banding patterns arise from the occurrence of multiple genes coding for the same enzyme. This is particularly true for salmonids, which still retain expression of many duplicated genes. For example, a gel stained for the enzyme LDH from salmonids may reveal protein products produced by five different gene loci. For dimeric and tetrameric enzymes, a further complication is that subunits from different gene loci may combine to form an active enzyme, leading to additional **interaction** bands that appear on the gel. In many cases, the difficulties in distinguishing products from multiple (and often overlapping) gene loci on a single gel can be reduced by taking advantage of tissue specificity in gene expression. That is, although each cell in an individual contains the same DNA, not all genes are expressed in all cells. For example, of the five

different LDH gene loci, ***LDH-A1**** and LDH-A2* are expressed only in muscle tissue and ***LDH-C**** only in eye, whereas a zone of activity due to the gene locus ***LDH-B2**** will appear on gels using any of the four tissues examined (muscle, liver, heart, and eye; see Table 2).

Generally, different forms of an enzyme coded for by different gene loci are called **isozymes** (for "iso-enzymes"), whereas different forms of an enzyme coded for by the same gene locus are termed **allozymes** (for "allelic enzymes"). The majority of electrophoretic analyses focus on allozyme data for individual gene loci. Genotypes compiled for a sample of individuals provide a means of estimating allele frequencies in the population as a whole, as shown in the following example involving a sample of 50 fish analyzed for a hypothetical gene locus with two alleles ("A" and "B"):

	Genotype		
	AA	AB	BB
Number of fish	32	16	2
Genotype frequency	0.64	0.32	0.04

Once the number of fish with each genotype has been established, computing the allele frequencies in the sample is straightforward. In our example, the 50 fish contain a total of 100 alleles, so the frequency of the "A" allele is (number of "A" alleles)/total = (32x2 + 16x1)/100 = 0.8. A similar exercise shows that the sample allele frequency for the "B" allele is 0.2.

Appendix Table l.--Allele frequency data for 1988 brood-year samples of juvenile Snake River chinook salmon. Locus abbreviations are explained in Table 1. N is the number of fish scored for each locus. Allele mobility designations are explained in text. Alleles screened but not found in any samples are shown in parentheses after locus names.

Locus/ Allele	Marsh Creek	Johnson Creek	Upper Salmon River	valley Creek	Secesh River	Lostine River	Imnaha River	McCall Hatchery	Sawtooth Hatchery	Looking. Hatchery	Imnaha Hatchery
*sAAT-1,2** (105)											
(N)	100	96	99	97	91	100	99	100	99	100	100
100	1.000	0.956	0.977	0.992	0.995	1.000	1.000	0.998	0.975	1.000	1.000
85	0.000	0.044	0.023	0.008	0.005	0.000	0.000	0.003	0.025	0.000	0.000
*sAAT-3** (90)											
(N)	100	97	99	99	92	99	100	100	89	99	98
100	1.000	1.000	0.965	0.990	1.000	1.000	0.995	1.000	1.000	0.980	1.000
113	0.000	0.000	0.035	0.010	0.000	0.000	0.005	0.000	0.000	0.020	0.000
*SAAT-4**(130)											
(N)	98	86	89	97	76		98	68	89	92	95
100	0.985	0.919	1.000	1.000	0.967	0.7%	0.959	0.919	0.966	0.978	0.974
63	0.015	0.081	0.000	0.000	0.033	0.284	0.041	0.081	0.034	0.022	0.026
*mAAT-1** (-77)											
(N)	100	96	99	98	90	96	100	96	100	94	100
-100	1.000	0.990	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.995
-104	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005
*ADA-l**											
(N)	100	97	99	99	92	100	100	100	100	100	100
100	0.910	0.985	0.949	0.894	0.842	0.970	0.995	0.940	0.935	1.000	1.000
83	0.090	0.015	0.051	0.106	0.158	0.030	0.005	0.060	0.065	0.000	0.000
*ADH** (-170)											
(N)	99	97	99	99	92	100	100	100	100	100	100
-100	1.000	1.000	1.000	1.000	1.000	0.985	0.995	1.000	1.000	1.000	1.000
-52	0.000	0.000	0.000	0.000	0.000	0.015	0.005	0.000	0.000	0.000	0.000
*sAH** (69, 108, 116)											
(N)	100	97	99	99	92	100	100	100	100	100	100
100	1.000	1.000	1.000	1.000	1.000	0.995	1.000	0.990	0.995	1.000	0.985
86	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.010	0.005	0.000	0.015
*mAH-2**											
(N)	99	60	98	96	84	100	98	89	98	100	100
100	0.884	0.883	0.918	0.807	0.958	0.900	0.929	0.933	0.918	0.885	0.915
88	0.116	0.117	0.082	0.193	0.042	0.100	0.071	0.067	0.082	0.115	0.085
mAH-4* (112)											
(N)	100	94	99	99	91	100	100	100	100	100	100
100	1.000	1.000	1.000	1.000	1.000	0.990	0.990	0.985	1.000	1.000	0.990
119	0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.015	0.000	0.000	0.010
*GAPDH-2**											
(N)	100	96	99	98	85	100	100	100	100	100	100
100	1.000	1.000	1 000	1.000	1.000	0.995	1.000	1.000	1.000	1.000	1.000
22	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000

Locus/ Allele	Marsh Creek	Johnson Creek	Upper Salmon River	Valley Creek	Secesh River	Lostine River	Imnaha River	McCall Hatchery	Sawtooth Hatchery	Looking. Hatchery	Imnaha Hatchery
GAPDH-4"											
(N)	100	77	99	*99*	78	100	100	40	*80*	*99*	*99*
100	1.000	1.000	1.000	1.000	0.974	0.945	0.995	0.988	1.000	1.000	1.000
95	0.000	0.000	0.000	0.000	0.026	0.055	0.005	0.013	0.000	0.000	0.000
βGLUA*											
(N)	*94*	*92*	*99*	*96*	86	*97*	100	100	100	*97*	*98*
100	1.000	1.000	0.980	*0.922*	0.994	*0.969*	0.975	0.995	0.990	*0.995*	*0.908*
60	0.000	0.000	0.020	0.078	0.006	0.031	0.025	0.005	0.010	0.005	*0.092*
GPI-B2 (24, 135)											
(N)	100	97	99	*99*	*92*	100	*99*	100	*97*	100	100
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.859
60	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.141
GR* (110)											
(N)	100	97	99	*99*	92	100	100	100	100	100	100
100	1.000	0.995	1.000	1.000	1.000	0.955	0.995	0.985	1.000	1.000	1.000
85	0.000	0.005	0.000	0.000	0.000	0.045	0.005	0.015	0.000	0.000	0.000
HAGH"											
(N)		66	99	*99*	*89*	100	100	100	99	99	100
100	0.99072	1.000	0.970	*0.949*	*0.955*	0.975	0.990	0.960	0.939	0.944	0.950
143	0.098	0.000	0.030	0.051	0.045	0.025	0.010	0.040	0.061	0.056	0.050
IDDH-1"											
(N)	59	95	57	74	87	100	76	*99*	58	100	*90*
100	0.907	1.000	0.956	0.980	0.994	0.980	0.947	*0.975*	0.897	0.960	*0.978*
0	0.093	0.000	0.044	0.020	0.006	0.020	0.053	0.025	0.103	0.040	0.022
sIDHP-1* (83, 129, 142)											
(N)	100	97	99	*99*	*92*	100	100	100	100	100	100
100	0.870	0.773	0.742	0.848	0.804	0.815	0.835	0.795	0.840	0.920	0.805
74	0.115	0.216	0.217	0.091	0.196	0.185	0.140	0.205	0.115	0.050	0.175
94	0.015	0.010	0.040	0.061	0.000	0.000	0.025	0.000	0.045	0.030	0.020
sIDHP-2* *(50)*											
(N)	100	97	99	*99*	*92*	100	100	100	100	100	100
100	0.965	0.990	0.975	*0.949*	1.000	0.975	1.000	1.000	0.945	1.000	1.000
127	0.035	0.010	0.025	0.051	0.000	0.025	0.000	0.000	0.055	0.000	0.000
LDH-Bl*											
(N)	100	97	99	*99*	*92*	*99*	100	100	100	100	100
100	1.000	0.990	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
48	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LDH-BP" (71, 134)											
		97	99			100	100	100	100	100	100
(N)	100	0.995	0.980	0.9%	0.9;	1.000	1.000	0.990	0.995	*0.990*	1.000
112	0.015	0.005	0.020	0.030	0.027	0.000	0.000	0.010	0.005	0.010	0.000
LDH-C* *(90)*											
(N)	100	97	99	*99*	*92*	*99*	100	100	*97*	*100*	*100*
100	1.000	0.918	1.000	*0.995*	*0.962*	1.000	1.000	0.985	*0.995*	1.000	1.000
84	0.000	0.082	0.000	*0.000*	0.038	0.000	0.000	0.015	0.005	0.000	0.000
106	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Locus/ Allele	Marsh Creek	Johnson Creek	Upper Salmon River	Valley Creek	Secesh River	Lostine River	Imnaha River	McCall Hatchery	Sawtooth Hatchery	Looking. Hatchery	Imnaha Hatchery
sMDH-B1,2* (83)											
(N)	100	97	99	99		100	100	100	100	100	100
100	0.990	0.979	0.985	0.944	0.99927	0.988	0.985	0.993	0.980	0.993	0.943
121	0.010	0.015	0.013	0.056	0.003	0.013	0.015	0.008	0.020	0.008	0.057
70	0.000	0.005	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
mMDH-I*											
(N)	92	49	99	99	13	95	100	92	100	100	100
-100	1.000	1.000	1.000	1.000	1.000	1.000	0.995	1.000	1.000	1.000	1.000
-900	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000
mMDH-2*											
(N)	99	97	97	97	91	100	98	100	97	100	99
100	0.646	0.598	0.485	0.557	0.753	0.735	0.658	0.735	0.526	0.800	0.697
200	0.354	0.402	0.515	0.443	0.247	0.265	0.342	0.265	0.474	0.200	0.303
sMEP-1* (105)											
(N)	95	97	99	97	89	96	98	99	99	100	93
100	0.079	0.077	0.030	0.031	0.017	0.052	0.061	0.035	0.010	0.070	0.043
92	0.921	0.923	0.970	0.969	0.983	0.948	0.939	0.965	0.990	0.930	0.957
sMEP-2*[a]											
(N)	100	97	99	99	91	100	99	100	100	100	95
100	0.900	0.898	1.000	1.000	0.790	1.000	1.000	1.000	1.000	1.000	1.000
78	0.100	0.102	0.000	0.000	0.210	0.000	0.000	0.000	0.000	0.000	0.000
MPI* (113)											
(N)	100	95	99	99	91	100	100	100	99	100	100
100	0.880	0.989	0.939	0.889	0.967	0.770	0.885	0.920	0.884	0.935	0.780
109	0.120	0.011	0.061	0.111	0.033	0.225	0.115	0.080	0.116	0.065	0.220
95	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000
PEPA* (86)											
(N)	100	97	99	99	92	100	100	100	100	100	100
100	0.995	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.995	1.000	1.000
90	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000
PEPB-1*											
(N)	100	94	99	99	92	100	100	100	100	100	99
100	0.945	0.856	0.879	0.904	0.902	0.960	0.915	0.935	0.870	0.805	0.909
130	0.050	0.027	0.091	0.096	0.065	0.015	0.050	0.015	0.090	0.095	0.030
-350	0.005	0.117	0.030	0.000	0.033	0.025	0.035	0.050	0.040	0.100	0.061
PEPD-2”											
(N)	100	97	99	99	92	100	100	100	100	100	100
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.995	1.000	1.000	1000
107	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
PEP-LT*											
(N)	100	97	99	99	92	100	100	100	100	100	100
100	0.870	0.948	0.985	0.919	0.870	0.925	0.965	0.920	0.885	0.945	0.955
110	0.130	0.052	0.015	0.081	0.130	0.075	0.035	0.080	0.115	0.055	0.045
PGK-2*											
(N)	100	97	99	99	92	100	100	100	95	100	100
100	0.065	0.067	0.101	0.187	0.152	0.085	0.100	0.110	0.142	0.085	0.120
90	0.935	0.933	0.899	0.813	0.848	0.915	0.900	0.890	0.858	0.915	0.880

Locus/ Allele	Marsh Creek	Johnson Creek	Upper Salmon River	Valley Creek	Secesh River	Lostine River	Imnaha River	McCall Hatchery	Sawtooth Hatchery	Looking. Hatchery	Imnaha Hatchery
PGM-3,4* (88, 108)											
(N)	100	97	96	98	92	98	96	99	95	96	100
100	0.285	0.518	0.326	0.375	0.462	0.510	0.484	0.429	0.338	0.295	0.508
94	0.715	0.482	0.674	0.625	0.538	0.490	0.516	0.571	0.662	0.705	0.493
sSOD-1* (580, 1260)											
(N)	100	97	97	99	90	99	100	100	100	100	100
-100	0.945	0.974	0.964	0.939	0.956	0.919	0.885	0.980	0.965	0.970	0.890
-260	0.055	0.026	0.036	0.061	0.044	0.081	0.115	0.020	0.035	0.030	0.110
TPI-2.2"											
(N)	100	97	99	99	92	100	100	100	100	100	100
100	0.910	0.954	0.924	0.894	0.897	0.875	0.825	0.875	0.890	0.915	0.850
104	0.090	0.046	0.076	0.106	0.103	0.125	0.175	0.125	0.110	0.085	0.150

"Allele frequencies at GPI-B2* and sMEP-2* are estimated indirectly and thus subject to higher variance than those for other loci; see text for discussion.

Appendix Table 2.--Temporal comparisons of allele frequency in Snake River chinook salmon populations for which old data are available (Milner et al. 1983; Milner et al. 1986). Brood years for samples are indicated below population names; other details are as in Appendix Table 1.

Locus/ allele	McCall Hatchery 1981	McCall Hatchery 1988	Johnson Creek 1981	Johnson Creek 1988	Valley Creek 1982	Valley Creek 1988	Looking. Hatchery 1988	Rapid River H. 1981+84	Upper Salmon River 1981	Upper Salmon River 1988	Sawtooth Hatchery 1988
sAAT-1,2*											
(N)	***50***	100	***56***	98	***22***	97	100	150	***50***	100	99
100	1.000	0.998	***0.964***	0.957	***0.977***	0.992	1.000	1.000	1.000	0.978	0.975
85	0.000	***0.003***	***0.036***	***0.043***	***0.023***	0.008	0.000	0.000	0.000	***0.023***	***0.025***
sAAT-3*											
(N)	***50***	100	***56***	100	***22***	99	99	150	***50***	100	***89***
100	1.000	1.000	1.000	1.000	1.000	0.990	0.980	0.990	***1.000***	0.965	1.000
113	0.000	0.000	0.000	0.000	0.000	0.010	***0.020***	0.010	***0.000***	***0.035***	0.000
ADA-1*											
(N)	***50***	100	***56***	100	***22***	99	100	150	***48***	***99***	100
100	***0.900***	0.940	1.000	0.985	***0.932***	0.894	1.000	0.990	***0.969***	***0.949***	0.935
83	0.100	***0.060***	0.000	0.015	***0.068***	0.106	0.000	0.010	0.031	0.051	***0.065***
sAH*											
(N)	***50***	100	***53***	99	***22***	99	100	***148***	***50***	100	100
100	1.000	0.990	1.000	1.000	1.000	1.000	1.000	***0.993***	1.000	1.000	0.995
86	0.000	0.010	0.000	0.000	0.000	0.000	0.000	***0.007***	0.000	0.000	***0.005***
GR*											
(N)	***50***	100	***56***	100	***22***	99	100	150	***50***	100	100
100	1.000	0.985	1.000	0.995	1.000	1.000	1.000	0.997	1.000	1.000	1.000
85	0.000	0.015	0.000	***0.005***	0.000	0.000	0.000	***0.003***	0.000	0.000	0.000
HAGH*											
(N)	***50***	100	***56***	***68***	***22***	99	99	144	***48***	100	99
100	***0.970***	0.960	0.991	1.000	***0.955***	0.949	0.944	0.913	0.917	0.970	0.939
143	***0.030***	***0.040***	0.009	0.000	***0.045***	0.051	***0.056***	***0.087***	***0.083***	***0.030***	0.061

Locus/ allele	McCall Hatchery 1981	McCall Hatchery 1988	Johnson Creek 1981	Johnson Creek 1988	Valley Creek 1982	Valley Creek 1988	Looking. Hatchery 1988	Rapid River H. 1981+84	Upper Salmon River 1981	Upper Salmon River 1988	Sawtooth Hatchery 1988
sIDHP 1*											
(N)	50	100	56	*99*	22	99	100	150	50	*99*	100
100″	0.740	0.795	0.902	0.786	0.910	0.909	0.950	0.920	0.840	0.783	0.885
74	0.260	0.205	0.098	0.214	0.090	0.091	0.050	0.080	0.160	0.217	0.115
sIDHP-2*											
(N)	50	100	56	99	22	99	100	150	50	99	100
100	1.000	1.000	1.000	0.990	0.910	0.949	1.000	1.000	1.000	0.975	0.945
127	0.000	0.000	0.000	0.000	0.090	0.051	0.000	0.000	0.000	0.025	0.055
LDH-B2*											
(N)	50	100	56	98		99	100	150	50	99	100
100	1.000	0.990	1.000	0.995	0.9%	0.970	0.990	0.990	0.980	0.980	0.995
112	0.000	0.010	0.000	0.005	0.023	0.030	0.010	0.010	0.020	0.020	0.005
LDH-C”											
(N)	50	100	56	100	22	99	100	150	48	100	97
100	0.970	0.985	0.982	0.920	1.000	0.995	1.000	1.000	1.000	1.000	0.995
84[b]	0.030	0.015	0.018	0.080	0.000	0.000	0.000	0.000	0.000	0.000	0.005
106	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000
MDH-Bl,2*											
(N)	50	100	56	98	22	99	100	149	49	99	100
100	0.995	0.993	1.000	0.980	0.989	0.944	0.993	1.000	1.000	0.985	0.980
121	0.005	0.008	0.000	0.015	0.011	0.056	0.008	0.000	0.000	0.013	0.020
70	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.003	0.000
MPI*											
(N)	50	100	56	96	22	99	100	150	50	99	99
100	0.960	0.920	0.946	0.990	0.795	0.889	0.935	0.940	0.890	0.939	0.884
109	0.040	0.080	0.054	0.010	0.205	0.111	0.065	0.060	0.110	0.061	0.116
PEPA*											
(N)	50	100	56	100	22	99	100	150	50	99	100
100	1.000	1.000	1.000	1.000	0.977	1.000	1.000	1.000	0.990	1.000	0.995
90	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.010	0.000	0.005

Locus/ allele	McCall Hatchery 1981	McCall Hatchery 1988	Johnson Creek 1981	Johnson Creek 1988	Valley Creek 1982	Valley Creek 1988	Looking. Hatchery 1988	Rapid River H. 1981+84	Upper Salmon River 1981	Upper Salmon River 1988	Sawtooth Hatchery 1988
PEPB-1*											
(N)	50	100	56	***94***	22	***99***	***100***	148	50	***100***	100
100	0.930	0.985	0.991	0.973	0.818	***0.904***	***0.905***	0.892	0.860	0.910	0.910
130	0.070	0.015	0.009	0.027	0.182	***0.096***	***0.095***	0.108	0.140	0.090	0.090
PEP-LT*											
(N)	50	100	56	100	22	***99***	100	150	50	100	100
100	***0.890***	0.920	***0.929***	0.950	0.841	0.919	0.945	0.963	0.970	0.985	0.885
110	0.110	0.080	0.071	0.050	0.159	0.081	0.055	0.037	0.030	0.015	0.115
PGK-2*											
(N)	50	100	56	100	22	***99***	100	150	50	99	95
100	0.080	0.110	0.045	0.065	0.205	0.187	0.085	0.103	0.090	0.101	0.142
90	0.920	0.890	***0.955***	0.935	0.795	0.813	0.915	0.897	0.910	0.899	0.858
sSOD-I*											
(N)	50	100	56	***97***	22	***99***	100	150	48	98	100
-100	***0.980***	0.980	0.973	***0.974***	0.886	***0.939***	0.970	0.913	0.948	0.964	0.965
-260	0.020	0.020	0.027	0.026	0.114	0.061	0.030	0.087	0.052	0.036	0.035

"Includes "94" allele
[b]Includes "90" allele
"Includes "-350" allele