Exxon Valdez Oil Spill Trustee Council

441 W. 5th Ave., Suite 500 • Anchorage, AK 99501-2340 • 907 278 8012 • fax 907 276 7178

November 9, 2007

At this time, we cannot confirm that this report has been peer reviewed or approved for correct formatting by ARLIS.

Federal Trustees
U.S. Department of the Interior
U.S. Department of Agriculture
National Oceanic and Atmospheric Administration

State Trustees
Alaska Department of Fish and Game
Alaska Department of Environmental Conservation
Alaska Department of Law

STATE/FEDERAL RESTORATION SCIENCE STUDIES
ANNUAL STATUS REPORT

Project Title:	ASSESSMENT OF GENETIC STOCK STRUCTURE OF SALMONIDS
Study ID Number:	Restoration Science Study Number 59
Principal Investigators:	Lisa Seeb James Seeb
Assisting Personnel:	Richard Gates Chris Habicht
Lead Agency:	State of Alaska, ADF&G; Commercial Fisheries Management and Development Division
Cooperating Agencies:	None
Inclusive Dates:	March 1, 1992 - February 28, 1993
Date Submitted:	June 15, 1993

Report Note: This is a report of studies that are in progress. All data and analyses provided are incomplete and preliminary. This report does not constitute publication of these data and this report may not be cited or the data used without written authorization of the principal investigators.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDICES

INTRODUCTION

Fishing time in the Upper Cook Inlet area was greatly reduced in 1989 due to the presence of oil from EVOS. As a direct result, sockeye salmon spawning in the Kenai River system exceeded optimal escapement goals by three times. Data collected by NRDA Fish/Shellfish Study 27, *Sockeye Salmon Overescapement*, showed that this overescapement resulted in overproduction and greatly reduced survival of juvenile sockeye salmon during the winter-spring rearing period. This extremely high escapement may have produced enough fry to not only deplete invertebrate prey populations and cause high fry mortality, but also to alter the species composition and productivity of prey populations for several years. Controlling sockeye salmon fry production by closely regulating the number of spawning adults may be the only way to restore the productivity of these rearing areas. However, the number of adult sockeye salmon returning from the 1989 escapement may be so reduced that a severe reduction, or complete elimination, of harvest of this stock may be necessary to ensure even minimally adequate escapements.

Knowledge of stock structure is critical not only to increase the productivity of mixtures of stocks in mixed-stock harvests (Walters 1975), but also to allow managers to assess the impacts of harvest regulations during the season (Mundy 1985; Mundy et al 1992). Marshall et al. (1987) investigated scale patterns analysis as a means to identify river of origin of Cook

Inlet sockeye salmon. However, the accuracy of scale patterns alone has not been reliable, and additional stock identification techniques are warranted.

Genetic stock identification (GSI) analyses have proven extremely effective for stock management in recent years (Seeb et al. 1986; 1990; Shaklee and Phelps 1990; White and Shaklee 1991), and many genetic markers have been found which discriminate stocks of sockeye salmon (e.g., Wilmot and Burger 1985; A.J. Gharrett, University of Alaska Fairbanks, Juneau and Paul Aebersold, National Marine Fisheries Service, Seattle, personal communication). Grant et al. (1980) showed a high degree of success using three markers to classify samples from the Kasilof and Susitna drainages, but incomplete baseline data confounded the Kenai River classifications. Strong supporting evidence (described above and including sockeye salmon data from Bob Davis, Alaska Dept. of Fish and Game, unpublished; and Richard Wilmot, U.S. Fish and Wildlife, Anchorage, unpublished) indicate that GSI analyses including many marker loci and complete baseline data will provide accurate estimates of stock composition for in-season protection of the Kenai River stocks. The objectives of this on-going study are to:

1. Obtain baseline genetic data during 1992-1995 from all significant spawning stocks contributing to mixed-stock harvests of sockeye salmon in Cook Inlet.
2. Obtain genetic data each week from samplings of the various mixed-stock harvests occurring in 1993 and 1994.

3. Use GSI algorithms (e.g., Pella and Milner 1987) to provide estimates of the presence of Kenai River stocks in the different mixed-stock areas so that managers may modify area and time of harvest in order to protect these damaged stocks while targeting surplus Kasilof River and Susitna River stocks.

This status report covers the results from Objective #1 for the period March 1, 1992 through February 28, 1993.

METHODS

Sampling Methods

Baseline and mixed stock samples for allozyme analysis were collected by personnel conducting Restoration Science Study R53 - *Kenai River Sockeye Salmon Restoration*. In addition, mixed stock samples were collected from four mainstem sites (Yentna, Susitna, Kasilof, and Kenai Rivers) and from samplings of drift net fisheries taken at processing plants. Target sample size for baseline collections was set at 100 to maximize the precision around the allele frequency estimates (Allendorf and Phelps 1981; Waples 1990). Mixed stock sample sizes were set at 200 (Pella and Milner 1987) and will be adjusted in 1993 based on the results of simulation studies conducted using the 1992 baseline data.

Personnel were trained in the safe handling of liquid nitrogen prior to any field collections. Additionally, instructions for the use of liquid nitrogen were included as part

of the sampling instructions (Appendix A). Laboratory safety procedures and training followed guidelines outlined in the Genetics Laboratory Hazard Communications Program.

Muscle, liver, eye, and heart were dissected from freshly killed individuals. All tissues were placed in individually labeled cryotubes. Individual sample numbers were assigned to uniquely identify all genetic tissues and the associated parasite information used by Restoration Science Study Number 53. Labels included the following information: 1) species, 2) collection site and/or code, 3) collection date, 4) individual number, and 5) tissue type. Tissues were placed in cryovials and immediately transferred into liquid nitrogen. Tissues were stored on liquid nitrogen until transferred to -80°C storage in Soldotna or Anchorage. Soldotna samples were transferred to the Anchorage laboratory on dry ice or liquid nitrogen and again placed in storage at -80°C where they remained until laboratory analysis. A telephone alarm was connected to the freezers to notify laboratory personnel in the event of a power outage.

Allozyme Methods

A comprehensive examination for discriminating gene markers was conducted using allozyme electrophoresis. All loci resolved in earlier studies were examined, as well as many other loci currently detectable in other *Oncorhynchus* (Seeb and Wishard 1977; Grant et al. 1980; Wilmot and Burger 1985). Allozyme techniques followed those of Harris and Hopkinson (1976), May et

al. (1979), and Aebersold et al. (1987); nomenclature rules followed the American Fisheries Society standard (Shaklee et al. 1990). A photographic record of each polymorphic gel was made. A collection of mobility standards for all scored alleles was constructed and used to verify alleles.

Multiple buffer systems were used. Buffer system abbreviations and descriptions are as follow: 1)**ACE 7.0/ACE7.2;** N-(3-aminopropyl)-morpholine, citrate (pH 7.0/7.2) with EDTA (Clayton and Tretiak 1972); 2) **ACN 7.0;** N-(3-aminopropyl)-morpholine, citrate (pH 7.0) with NAD (Clayton and Tretiak, 1972); 3) **KG** Tris, glycine HCl (pH 8.5) (tray concentration modified to 0.075 M Tris) (Holmes and Masters 1970); 4) **TBCL;** Tris, borate, citrate, LiOH (pH 8.2) (Selander et al. 1971); 5) **TBCLE;** Tris, borate, citrate, LiOH with EDTA (pH 8.2) (Selander et al. 1971); 6) **TBE;** Tris, borate, EDTA (pH 8.7) (Boyer et al. 1963); and 7) **TC4;** Tris citrate, NaOH (pH 5.9). 5.9) (Selander et al. 1971).

RESULTS

Sample Collection

Tissue samples were collected from spawning populations at 25 locations in the Kenai, Kasilof, and Susitna River drainages and the western Cook Inlet. A total of 2,500 individual sockeye salmon were sampled (Table 1). We were able to obtain collections from all populations proposed for sampling with the exception of Redshirt and Stephan Lakes in the Talkeetna

drainage. Collections from Ptarmigan Creek and Tern Lake from the Kenai River drainage were made, although they were not originally included in the sampling plan. Targeted sample size (N = 100) was achieved for all locations with the exception of Tern and Hewitt Lakes for which only 50 individuals were available. A total of 200 individuals were collected from Crescent River. Crescent River was originally considered as an in-river mixed-stock or composite sample, however it is more appropriately treated as a baseline collection representing individuals spawning in Lake Crescent. The great majority of spawning within the system occurs within Lake Crescent.

Composite sampling within river systems were made for the Kasilof, Kenai, Susitna and Yentna River systems (Table 1). The drift fishery was sampled twice from the commercial catch. A sample of 200 was collected on 7/13/92; an additional sample of 160 was collected on 7/20/92 (Table 1).

Allozyme Analysis

An extensive allozyme screening was undertaken to maximize the potential number of available gene markers. A total of 68 allozyme loci were resolved (Table 2). Of the resolvable loci, 30 were polymorphic in at least one individual (*sAAT-1,2; mAAT-1; mAH-1,2; ALAT; CK-B; GAPDH-2; G3PDH-1,2; GPI-B1,2; mIDHP-1; sIDHP-1; LDH-B2; sMDH-A1,2; sMDH-B1,2; mMEP-1; MPI; PEPA; PEPB-1; PEPD-1; PEPLT; PGDH; PGM-1; PGM-2; TPI-1,2*). All gel scoring was conducted directly onto a computer to ensure rapid turnaround,

complete documentation, and immediate availability of summary statistics.

Approximately twelve baseline collections were processed through the initial screenings of all 68 loci as of the end of February, 1993. Allele frequency estimates for these populations will not be reported at this status report pending finalization and standardization across all populations.

The allele frequency estimates for the baseline spawning populations will be available in May, 1993. Simulation studies using these frequencies will begin in April of 1993 prior to the July, 1993, fisheries. These results will be reported in the next status report due at the end of April, 1994, in accordance with the schedule outlined in the detailed study plan.

Considerable progress has been made in developing the analytical and computational techniques to rapidly provide fishery estimates for in-season management. Development of a comprehensive package of genetic analysis programs Windows applications (Microsoft Windows 3.1) has begun and includes the following components: 1) an on-line gel scoring program providing extensive documentation of results and error checking capability, 2) a set of genetic analysis programs providing allele frequency estimates, heterogeneity analyses, and fit to expected genetic models, and 3) revised input into the GIRLS algorithm (Masuda et al. 1991) to allow rapid fishery estimates and a flexible method to conduct multiple simulation studies. The object-oriented genetics applications will work synchronously

within the Windows environment to provide a user friendly interface for data input and complicated analyses so that the geneticists can make a fast turn-around from field samples to fishery estimates. Functional versions of these programs will be completed prior to the July, 1993, in-season fishery.

DISCUSSION

A large number of useful gene markers have been identified in this study. This number far exceeds that documented in earlier studies of sockeye salmon. Grant et al. (1980) observed 26 loci of which six were polymorphic; Wilmot and Burger (1985) collected data from three polymorphic loci of 27 total loci screened; Foote et al. (1989) also only detected three to five polymorphic loci useful for population markers.

Of the 30 polymorphic loci detected in this study, at least 15 will provide power for stock discrimination. Grant et al. (1980) successfully discriminated Kasilof and Susitna stocks of sockeye salmon with only three informative loci. The increased resolution possible from our more complete data set looks promising--not only to allocate catch among major drainages, but possibly to allocate catch to stocks within the EVOS-damaged Kenai River.

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. NOAA Technical Report NMFS 61, U. S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, 19 p.

Allendorf, F. W. and S. R. Phelps. 1981. Use of allelic frequencies to describe population structure. Can. J. Fish. Aquat. Sci. 38:1507-1514.

Boyer, S. H., D. C. Fainer, and M. A. Naughton. 1963. Myoglobin: Inherited structural variation in man. Science 140: 1228-1231.

Clayton, J. W., and D. N. Tretiak. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Board Can. 29: 1169-1172.

Dempster, A. P., N. M. Laird, and D. B. Rubin. 1977. Maximum likelihood from incomplete data via the EM algorithm. Stat. Soc. B39:1-39

Foote, C. J., C. C. Ward, and R. E. Withler. 1989. Biochemical genetic comparison of sockeye salmon and kokanee, the anadromous and nonanadromous forms of *Oncorhynchus nerka*. Can. J. Fish. Aquat. Sci. 46:149-158.

Fournier, D. A., T. D. Beacham, B. E. Riddell, and C. A. Busack. 1984. Estimating stock composition in mixed-stock fisheries using morphometric, meristic, and electrophoretic characters. Can. J. Fish. Aquat. Sci.

Grant, W. S., G. B. Milner, P. Krasnowski, and F. M. Utter. 1980. Use of biochemical genetic variants for identification of sockeye salmon (*Oncorhynchus nerka*) stocks in Cook Inlet, Alaska. Can. J. Fish. Aquat. Sci. 37: 1236-1247.

Harris, H. and D. A. Hopkinson. 1976. Handbook of enzyme electrophoresis in human genetics. American Elsevier, NY.

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

Masuda, M. S. Nelson, and J. Pella. 1991. The computer programs for computing conditional maximum likelihood estimates of stock composition from discrete characters. USA-DOC-NOAA-NMFS, Auke Bay Laboratory, Auke Bay, Alaska.

May, B., J. E. Wright and M. Stoneking. 1979. Joint segregation of biochemical loci in Salmonidae: Results from experiments with *Salvelinus* and review of the literature on other species. J. Fish. Res. Board Can. 36:1114-1128.

Marshall, S., D. Bernard, R. Conrad, B. Cross, D. McBride, A. McGregor, S. McPherson, G. Oliver, S. Sharr, and B. Van Allen. 1987. Application of scale patterns analysis to the management of Alaska's sockeye salmon (*Oncorhynchus nerka*) fisheries, p. 207- 326, *in* Smith, H. D., L. Margolis, and C. C. Wood [ed.] Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96.

Millar, R. B. 1987. Maximum likelihood estimation of mixed stock fishery composition. Can. J. Fish. Aquat. Sci. 44: 583-590.

Mundy, P. R. 1985. Harvest control systems for commercial marine fisheries management; theory and practice, *in* Mundy, P. R., T. J. Quinn, and R. B. Deriso [ed.] Fisheries Dynamics, Harvest Management and Sampling, Washington Sea Grant Technical Report 85-1. University of Washington, Seattle, Washington.

Mundy, P. R., K. K. English, W. J. Gazey, and K. E. Tarbox. 1992. Evaluation of the harvest management strategies applied to sockeye salmon populations of Upper Cook Inlet, Alaska, using run reconstruction analysis, p. 107-139, *in* Kruse, G., D. M. Eggers, F. J. Marasco, C. Pautzke, and T. J. Quinn, II [ed.] Proceedings of the International Symposium on Management Strategies for Exploited Fish Populations. Alaska Sea Grant College Program, Fairbanks, AK.

Pella, J. J., and G. B. Milner. 1987. Use of genetic marks in stock compositions analysis. p. 247-276 *in* N. Ryman and F. M. Utter, eds. Population genetics and fishery management.

Seeb, J. E., L. W. Seeb and F. M. Utter. 1986. Use of genetic marks to assess stock dynamics and management programs for chum salmon. Transactions of the American Fisheries Society 115:448-454.

Seeb, J. and L. Wishard. 1977. The use of biochemical genetics in the management of Pacific salmon stocks: Genetic marking and mixed fishery analysis. Final report of research to Washington Dept. of Fisheries, Service Contract #792, Olympia, WA, 65 pp.

Seeb, L. W., J. E. Seeb and J. Gharrett. 1990. Genetic marking of fish populations. Pp. 223-239 *in* Whitmore, D. H., eds. Electrophoretic and isoelectric focusing techniques in fisheries management. CRC Press, Boca Raton, FL.

Selander, R. K., M. H. Smith, S. Y. Yang, W. E. Johnson, and J. B. Gentry. 1971. Biochemical polymorphism and systematics in the genus Peromysucs. I. Variation in the old field mouse (*Peromyscus polionotus*). Studies in Genetics VI. University of Texas Publication 7103: 49-90.

Shaklee, J. B., F. W. Allendorf, D. C. Morizot and G. S. Whitt. 1990. Gene Nomenclature for Protein-Coding Loci in Fish. Transactions of the American Fisheries Society 119:2-15.

Shaklee, J. B. and S. R. Phelps. 1990. Operation of a large-scale, multiagency program for genetic stock identification. Amer. Fish. Soc. Symp. 7:817-830.

Walters, C. J. 1975. Optimal harvest strategies for salmon in relation to environmental variability and uncertain production parameters. Can. J. Fish. Aquat Sci. 32: 1777-1784.

Waples, R. S. 1990. Temporal changes of allele frequency in Pacific salmon: implications for mixed-stock fishery analysis. Can. J. Fish. Aquat. Sci. 47:968-976.

White, B. A., and J. B. Shaklee. 1991. Need for replicated electrophoretic analyses in multiagency genetic stock identification (GSI) programs: examples from a pink salmon (*Oncorhynchus gorbuscha*) GSI fisheries study. Can. J. Fish. Aquat. Sci. 48:1396-1407.

Wilmot, R. L. and C. V. Burger. 1985. Genetic differences among populations of Alaskan sockeye salmon. Trans. Amer. Fish. Soc. 114: 236-243.

Table 1. Sockeye salmon populations sampled from Upper Cook Inlet, 1992.

Collection Date(s)	Location	N
Kenai Peninsula		
Kasilof River Drainage		
7/29	Nikolai Creek	100
8/10	Moose Creek	100
8/11	Glacier Flats Creek	100
8/12	Bear Creek	100
Kenai River Drainage		
8/03	Hidden Creek	100
8/13	Quartz Creek	100
8/18	Between Kenai & Skilak Lakes	100
8/19	Outlet of Skilak Lake	100
7/01	Russian River (early run)	100
8/07	Russian River (late run)	100
8/31	Ptarmigan Creek	100
9/01	Tern Lake	50
Bishop Creek Drainage		
9/02	Outlet of Daniels Lake	100
Knik Arm		
Fish Creek Drainage		
7/22	Fish Creek (Big Lake)	100
Susitna River Drainage		
Talkeetna River		
8/20	Larson Creek	100

Table 1. Continued.

Collection Date(s)	Location	N
Yentna River		
8/20	Chelatna Lake	100
8/24	Hewitt Lake	50
9/09	Unnamed Slough (West Fork Yentna)	100
Skwentna River		
8/25	Shell Lake	100
Talachulitna River		
8/24	Judd Lake	100
8/25	Trinity Lake	100
West Side of Cook Inlet		
Beluga River Drainage		
9/01	Coal Creek	100
Chakachatna River Drainage		
9/08	Chilligan River	100
Crescent River Drainage		
7/01-7/27	Crescent River Composite	200
Packers Creek Drainage		
7/16	Packers Lake, Kalgin Island	100

Table 1. Continued.

Collection Date(s)	Location	N
Central District Drift Fishery		
7/13	Commercial Catch	200
7/20	Commercial Catch	160
Composite Sampling		
7/02-7/03	Kasilof River Composite	200
7/22-7/23	Kasilof River Composite	200
7/13-7/14	Kenai River Composite	200
7/26-7/27	Susitna River Composite (sunshine)	200
8/4	Susitna River Composite (sunshine)	114
7/15	Yentna River Composite	200
7/24	Yentna River Composite	200

Total Fish = 4,174

Baseline Fish = 2,500
Composite & Drift Fishery = 1,674

Table 2. Enzymes or proteins screened for allozyme variation in Cook Inlet sockeye salmon. Enzyme nomenclature follows Shaklee et al. (1990), and locus abbreviations are given. Buffer abbreviations are as described in text.

Enzyme or Protein	Enzyme Number	Locus	Tissue	Buffer
Aspartate aminotransferase	2.6.1.1	*sAAT-1,2*	Heart	ACE 7.2
		sAAT-3	Eye	TBCL
		mAAT-1	Heart	ACE 7.2
		mAAT-2	Liver	ACE 7.0
Adenosine deaminase	3.5.4.4	*ADA-1*	Muscle	KG
Aconitate hydratase	4.2.1.3	*mAH-1,2*	Heart	ACE 7.2
		mAH-3	Heart	ACE 7.2
		mAH-4	Heart	ACE 7.2
		sAH	Liver	ACE 7.0
Alanine aminotransferase	2.6.1.2	*ALAT*	Muscle	KG
Creatine kinase	2.7.3.2	*CK-A1*	Muscle	TBCLE
		CK-A2	Muscle	TBCLE
		CK-B	Eye	ACE 7.0
		CK-C1	Eye	ACE 7.0
		CK-C2	Eye	ACE 7.0
Esterase-D	3.1.1.-	*ESTD*	Muscle	TBCLE
Fructose-biphosphate aldolase	4.1.2.13	*FBALD-4*	Eye	ACE 7.0
Fumarate hydratase	4.2.1.2	*FH*	Muscle	ACN 7.0
β-N-Acetylgalactosaminidase	3.2.53	*βGALA*	Liver	ACE 7.0
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*GAPDH-2*	Heart	ACN 7.0
		GAPDH-3	Heart	ACN 7.0
		GAPDH-4	Eye	ACE 7.0
		GAPDH-5	Eye	ACE 7.0
N-Acetyl-β-glucosaminidase	3.2.1.53	*βGLUA*	Liver	TC4
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-1,2*	Muscle	ACN 7.0
		G3PDH-3	Heart	ACN 7.0
		G3PDH-4	Heart	ACN 7.0

Table 2. Continued.

Enzyme or Protein	Enzyme Number	Locus	Tissue	Buffer
Glucose-6-phosphate isomerase	5.3.19	*GPI-B1,2*	Muscle	TBCLE
		GPI-A	Muscle	TBCLE
Glutathione reductase	1.6.4.2	*GR*	Eye	TBCL
Hydroxyacylglutathione hydrolase (Formaldehyde dehydrogenase[1])	3.1.2.6	*HAGH (FDH)*	Liver	TBE
Isocitrate dehydrogenase (NADP+)	1.1.1.42	*mIDHP-1*	Heart	ACN 7.0
		mIDHP-2	Heart	ACN 7.0
		sIDHP-1	Liver	ACE 7.0
		SIDHP-2	Liver	ACE 7.0
L-Lactate dehydrogenase	1.1.1.27	*LDH-A1*	Muscle	ACN 7.0
		LDH-A2	Muscle	ACN 7.0
		LDH-B1	Muscle	TBCLE
		LDH-B2	Liver	TBE
		LDH-C	Eye	KG
αMannosidase	3.2.1.24	*αMAN*	Liver	TC4
Malate dehydrogenase	1.1.1.37	*sMDH-A1,2*	Heart	ACN 7.0
		sMDH-B1,2	Heart	ACN 7.0
		mMDH-1	Heart	ACN 7.0
		mMDH-2	Muscle	ACN 7.0
		mMDH-3	Muscle	ACN 7.0
Malic enzyme (NADP+)	1.1.1.40	*sMEP-1*	Liver	TC4
		mMEP-1	Muscle	ACN 7.0
Mannose-6-phosphate isomerase	5.3.1.8	*MPI*	Liver	TBE
Dipeptidase	3.4.-.-	*PEPA*	Muscle	TBCLE
Tripeptide aminopeptidase	3.4.-.-	*PEPB-1*	Heart	TBE
Peptidase-C	3.4.-.-	*PEPC*	Eye	KG
Proline dipeptiase	3.4.13.9	*PEPD-1*	Heart	TBE
Peptidase-LT	3.4.-.-	*PEPLT*	Muscle	TBCLE

Table 2. Continued.

Enzyme or Protein	Enzyme Number	Locus	Tissue	Buffer
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH*	Liver	ACE 7.0
Phosphoglucomutase	5.4.2.2	*PGM-1*	Heart	ACE 7.2
		PGM-2	Muscle	TBCLE
Superoxide dismutase	1.15.1.1	*sSOD-1*	Liver	TBE
Triose-phosphate isomerase	5.3.1.1	*TPI-1,2*	Eye	KG
		TPI-3	Eye	KG
		TPI-4	Eye	KG

[1]*HAGH* and *FDH* (Formaldehyde dehydrogenase, E.C. 1.2.1.1) appear to be the same locus.

APPENDIX A

Genetics Laboratory
Alaska Department of Fish and Game, Anchorage

Instructions for Collection of Finfish Genetic Samples

I. General info

We use tissue samples from muscle, liver, heart, and eye from individual fish to determine the genetic characteristics and profile of a particular run or stock of fish. The most important thing to remember in collecting samples is that tissues need to be as fresh and as cold as possible at all times.

II. Sample size

A sample size of 50-100 adult fish is preferred for the baseline electrophoretic study. Samples of juveniles are statistically less desirable and sample sizes will need to be larger than for adults; generally a sample size of 150-200 juveniles is necessary.

III. Tissue sampling

A. General set up

We use four tissues (muscle, liver, eye, and heart) for protein electrophoresis. Working fast is necessary, so it is best to try to get set up in as comfortable a place as possible. You might use a portable table, piece of plywood, or anything to give you a surface at a good height. Before sampling (night before?), label tubes using lab markers or adhesive labels (provided in sampling kit). If using labels, it is best to cover the label with transparent tape to assure the label stays on. Place the prepared tubes in the racks provided. Four separate tubes, corresponding to the four tissues, should be labeled for each individual. The following code should be used:

Species code	see instructions for each project	
Location code	*	see instructions for each project
Individual #	#	i.e. 01, 02, 03....100
Tissue	M,L,E,H	(muscle, liver, eye, heart)

B. Use of liquid nitrogen

We will be using a liquid nitrogen container to immediately freeze the tissues. Inside the liquid nitrogen container are 6 cylindrical canisters. We have shipped special test tubes called "cryotubes" in which to place the samples. These cryotubes have plastic seals and screw on caps to withstand liquid nitrogen storage. Five (white Nalgene) or six (orange Corning) cryotubes are stored in a cane.

The working time of the liquid nitrogen container under normal conditions is 81 days (35VHC) or 50 days (18HC). To prolong the liquid nitrogen, samples can be pre-frozen (if a freezer or dry ice is available) and added in a group to minimize the number of times the container is opened. The liquid nitrogen level can be checked periodically with a flashlight or actually measured with a stick (2.3 liters/inch in 35VHC; 1.25 liters/inch in 18HC).

"Large" 35 VHC container:
35 canes will fit in a canister. Total capacity is 1050 Nalgene tubes or 1260 Corning tubes.

"Small" 18HC container:
19 canes will fit in each of the six canisters. The total capacity is 684 Corning tubes or 570 Nalgene tubes.

Safety with liquid nitrogen:

1. Wear gloves, protective eyewear, and protective footwear when placing samples in container. Liquid nitrogen boils at -196°, and it will spit and boil when samples are added.

2. Do not tip the tank over as it does not seal.

3. Keep lid on liquid nitrogen container at all times when you are not placing samples in it.

4. Use a small cooler with ice, snow, or blue ice to hold canes until an adequate number are collected to be put in liquid nitrogen container. Depending on the conditions and the speed of sampling, place samples in liquid nitrogen within about one hour of sampling.

5. Use liquid nitrogen only in well ventilated areas (usually not a problem in the field). Avoid directly breathing the vapor.

6. Hazardous Materials Forms need to be filled out when

shipping a filled liquid nitrogen container by air cargo.

B. Actual sampling

Please take samples from freshly killed fish. We find it easiest to set up four canes simultaneously and organize the samples in canes by tissue. Thus, muscle tissue from fish 1-5 would all be in one cane.

Fill the tubes approximately 3/4 full or to the 1.8 ml mark, leaving air space at the top. Overfilling the tubes can cause them to burst when frozen. Please minimize the amount of blood, dirt, skin, and fat in the sample.

1. Muscle

Muscle samples should be "white" muscle, not muscle from along the lateral line. Use a piece of muscle dorsal to the lateral line. If you have trouble getting the tissue into the tubes, cut it into smaller pieces.

2. Liver

The liver is (generally) located on the fish's left side, just behind the pectoral fin. An L-shaped incision slicing down ventrally behind the pectoral fin then caudally along the belly works well. Please do not include the gall bladder (the small green/yellow sac of fluid attached to the liver).

3. Heart

Once you have taken the liver, it is easy to get the heart by just opening the belly incision towards the head.

4. Eye

There are two ways to take the eyes. If the eyes are small enough (juveniles), they can be placed intact into a cryotube. This is the easiest method. If they are too large, you must pipette out the liquid and black retinal fluid. Using a sharp scalpel, cut a small slit in the surface of the eye, then insert a pipette into the slit and suck out the fluid and black retinal material. Squirt this into the cryotube.
We appreciate your help with the sampling. If you have any questions, please give us a call.

Lisa Seeb	Jim Seeb	Sue Merkouris
267-2249	267-2385	267-2247

APPENDIX B

Maximum likelihood estimation procedure

Consider a mixed-stock sample of N fish that contains G multilocus genotypes. The likelihood function for the observed multilocus genotype frequencies is multinomial

(1)

Millar (1987), where $_i$ is the probability of occurrence of the ith genotype and Y_i is the number of fish in the mixture with the ith genotype. Moreover, the genotype probabilities in the fishery are a linear function of the conditional probabilities of genotype given stock and the S proportionate stock contributions

(2)

Millar (1987), where $_j$ is the proportionate contribution of the jth stock and x_{ij} is the conditional probability of the ith genotype given an individual is from the jth stock. These conditional probabilities are computed using a multinomial probability function for each locus in the analysis, where the multinomial parameters are the allele relative frequencies in the baseline, and the observed allele counts in the mixture sample taken from the fishery. The individual locus genotypes are assumed to be independent so that the multilocus genotype conditional probability is the product of each single locus genotype conditional probability.

Maximum likelihood estimation is carried out by finding the best stock proportions, , that produces the genotype probabilities, , in Equation 2 that best explains the data, Y, in Equation 1.

Several nonlinear optimization algorithms can be used to solve for the optimal stock proportions (Pella and Milner 1987). With the EM algorithm (Dempster et al. 1977), new estimates at iteration $t + 1$ are calculated as:

(3)

where $_i^{(t)}$ is computed by Equation 2 on each iteration. Initially all values are set to $1/S$. Iterations are

continued until the maximum difference between values on successive iterations falls below a specified value.

The program GIRLSEM uses the EM algorithm in the inital search for a solution, then switches to the Gauss-Newton algorithm. Because the likelihood is from the exponential family and due to the linear relation in Equation 2, the Gauss-Newton algorithm reduces to an interatively reweighted least squares algorithm (IRLS) (Masuda et al. 1991).

Moreover, the solution is constrained so that

and .

The algorithm of GIRLSEM is far too complicated to be discussed here, and the reader is referred to the GIRLSEM User Guide (Masuda et al. 1991) for a detailed explanation of its mathematical numerics. Further detailed discussion of the MLE applications can also be found in Pella and Milner (1987), Millar (1987), and Fournier et al. (1984).