

GENETIC ANALYSIS OF SOCKEYE SALMON POPULATIONS
FROM THE WESTWARD REGION
OPERATIONAL PLAN, 2001

By

Mark J. Witteveen
and
William D. Templin

Regional Information Report¹ No. 4K01-46

Alaska Department of Fish and Game
Division of Commercial Fisheries
211 Mission Road
Kodiak, Alaska 99615

September 2001

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INTRODUCTION

An essential aspect of effective management and assessment of Pacific salmon is the determination of stock composition in commercial and other harvests. Reliable estimates of stock composition lead to more accurate run reconstruction estimates resulting in a better understanding of stock-specific productivity. In addition, reliable estimates allow managers to evaluate a specific fishery's effect on individual stocks. Accordingly, considerable effort has been directed towards salmon stock identification, which can be a complicated problem given that a multitude of stocks can contribute to a single fishery.

A comprehensive salmon catch and escapement sampling program was initiated in 1985 for the purpose of estimating stock-specific production of sockeye salmon *Oncorhynchus nerka* within the Westward Region. The subsequent run reconstruction program focuses on sockeye salmon whereby age marker and scale pattern analysis (SPA) of various age classes have been employed to estimate the contributions of sockeye salmon from major systems when possible (Barrett and Nelson 1995; Nelson 1999; Owen and Sarafin 1999; Sagalkin 1999). Stock identification based on SPA assumes that genetically or environmentally influenced growth patterns differ between stocks and that these differences may be detectable in the scales of fish. While this method appears to be reasonably accurate for reconstructing sockeye salmon runs for certain systems, it has been unsuccessful for others.

Stock identification techniques based on genetic variation have been increasingly applied to evaluate stock contributions to fisheries throughout the Pacific Northwest. For example, the National Marine Fisheries Service (NMFS), Auke Bay Laboratory, evaluated the feasibility of using genetic stock identification (GSI) to estimate regional stock contributions of chum salmon *O. keta* in the Bering Sea trawl fishery (Wilmot et al. 1998). Seeb and Crane (1999) used the extensive chum salmon genetic baseline to estimate the stock composition of chum salmon caught in the Alaska Peninsula sockeye salmon fisheries in 1993 and 1994. Recently, Seeb et al. (2000) refined sockeye salmon baseline data for Upper Cook Inlet (UCI) in order to estimate relative contributions of UCI sockeye salmon stocks to selected commercial drift gillnet fisheries in 1997 and 1998.

Genetic stock identification, developed in the 1970s, relies on genetic differences among stocks; therefore, before GSI can be applied to mixed stock fisheries, genetic data must be collected from all potentially contributing stocks. This collection of genetic data from the stock of origin is referred to as a "baseline". The reliability of GSI estimates of stock composition is fundamentally dependent on whether all contributing stocks are represented in the baseline.

The primary objective of this study is to collect baseline data on Westward Region sockeye salmon stocks that contribute substantially to various mixed stock fisheries, serve as a brood stock, or uniquely represent a portion of the region. This study also serves to increase the growing knowledge of sockeye salmon genetic population structure and relationships between stocks and should prove useful in Westward Region stock identification by providing the means to genetically distinguish sockeye salmon populations between geographic regions, management areas, and individual systems.

STUDY AREA

The Westward Region is divided into five salmon management areas (Figure 1): (1) Kodiak Management Area (KMA), (2) Chignik Management Area (CMA), (3) Alaska Peninsula Management Area (Area M), (4) Aleutian Islands Management Area, and (5) Atka-Amlia Management Area. Five species of Pacific salmon are native to the Westward Region: chinook salmon *O. tshawytscha*, sockeye salmon, chum salmon, pink salmon *O. gorbuscha*, and coho salmon *O. kisutch*. The Alaska Department of Fish and Game (ADF&G), Division of Commercial Fisheries, manages commercial salmon fisheries in the Westward Region to achieve biological escapement goals by species into each system while allowing for an orderly harvest of surplus salmon (ADF&G 1996, ADF&G 1998).

During the summer of 2001, ADF&G will sample three spawning populations from three river systems in the Alaska Peninsula Management Area.

The Alaska Peninsula Area is referred to as Management Area M and is divided into two subareas (Figure 2); the North Peninsula, consisting of Bering Sea waters extending west from Cape Menshikof to Cape Sarichef on Unimak Island and the South Peninsula, consisting of Pacific Ocean coastal waters extending west of Kupreanof Point to Scotch Cap on Unimak Island. Sockeye salmon are present in about 55 of the 247 salmon spawning systems located within the North and South Peninsula (Murphy 1992). In the North Peninsula, six systems have been selected for genetic baseline sampling because they are considered major sockeye salmon producers: Ilnik River System, Sandy River, Bear Lake, Nelson River, Cinder River, and Meshik River. Orzinski Lake was selected as the primary local sockeye salmon producer in the South Peninsula. During the 2000 season, the Sandy River, Bear Lake, Nelson River, and Orzinski Lake systems were sampled. The Cinder River, Meshik River, and Hoodoo Lake portion of the Nelson River system will be sampled during the 2001 season. If time and funding permit, the Ilnik River System (consisting of four geographically distinct spawning populations) will be sampled in 2001 as well.

OBJECTIVES

Specific objectives of this study are to:

1. Expand and improve sockeye salmon genetic baseline data for Alaska Peninsula stocks with particular emphasis on commercially important stocks,
2. Combine newly collected allozyme baseline data with previously collected statewide sockeye salmon allozyme baseline data,
3. Evaluate the expanded baseline for information on sockeye salmon population genetic structure and its potential use to identify stock groups in mixtures.

METHODS

Baseline Sample Collection

Three spawning locations are intended to be sampled in 2001 to add to the existing sockeye salmon genetic baseline (Table 1). Sockeye salmon tissues will be collected from wild populations on the spawning grounds whenever possible. Actual sample sizes will vary depending on the abundance and availability of spawners during the sampling period, but the target sample size is 100 individuals per spawning location (Allendorf and Phelps 1981). Field operations and costs will be the responsibility of ADF&G Westward Region.

Muscle, liver, heart, and eye tissues will be sampled from individual salmon, placed in 2.0 ml cryotubes and frozen as soon as possible in liquid nitrogen or on dry ice. Collections will remain frozen during storage and shipment to the Anchorage laboratory (see protocols outlined in Appendix A). Upon arrival in Anchorage, samples will be stored at -80° C until allozyme analysis. Remaining tissues after analysis will be stored at -80° C for exchange and standardization among laboratories or for future use in DNA-level analyses. Shipment costs will be equally shared by ADF&G Westward Region and the Gene Conservation Laboratory in Anchorage.

Laboratory Analyses

Laboratory analyses will follow Seeb et al. (2000). Sixty-seven loci will be analyzed by the ADF&G Gene Conservation Laboratory. Enzyme nomenclature will follow recommendations by Shaklee et al. (1990). Genetic data will be entered directly onto personal computer workstations connected to a local network and backed-up onto tape each night. Laboratory operations, data analysis, and related costs will be the responsibility of the Gene Conservation Laboratory in Anchorage.

Statistical Analyses

Statistical analyses will follow Seeb et al. (2000). Allozyme data collected from new baseline populations will be summarized as allele frequencies. Allele designations will be standardized with data available from other studies and a baseline database compiled. A hierarchical analysis using likelihood ratios (modified from Weir 1990) will be performed on the updated baseline to test for genetic population structure. A method adapted from Miliken and Johnson (1984) will be used to test for significance.

Chord distances (Cavalli-Sforza and Edwards 1967) will be calculated between population groups and distances will be used to construct a tree, grouping populations on overall similarity, using numerical taxonomic techniques (UPGMA; Sneath and Sokal 1973). Distances will also be used in a multidimensional scaling analysis, which groups populations so that expected distances between populations in two or three dimensions closely match the observed distances in multidimensional space (MDS; Kraznowski and Marriott 1994).

The UPGMA tree and MDS will be used to examine potential reporting regions for analysis of fishery samples. Simulated mixtures will be used to optimize reporting regions (Seeb et al. 2000). Average mixture estimates will be derived from 1000 simulations, where reporting regions comprise 100% of the mixture ($N = 400$). In each simulation, new baseline and mixture genotypes will be randomly generated from the baseline using Hardy-Weinberg expectations, and each stock in the region will contribute equally to the mixture.

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Table 1. Sockeye salmon systems sampled for the Westward Region genetic baseline pre-2001 and proposed sample locations during 2001.

<i>Management Area Sockeye Salmon System Spawning Location</i>	<i>Sample Size</i>	<i>Dates</i>	<i>Comments</i>	<i>Crew Leader, Crew</i>
Kodiak (Area K)				
Afognak Lake (Litnik)		Pre 1999	Sufficiently sampled	
Malina Creek		Pre 1999	Sufficiently sampled	
Karluk Lake (early run)				
Upper Thumb River	100	Late July 2000	Sufficiently sampled	Foster, Lloyd
Karluk Lake (late run)				
Lower Thumb River	100	Late September 1999	Sufficiently sampled	Nelson, Foster
O'Malley River	100	Late September 1999	Sufficiently sampled	Nelson, Foster
Ayakulik (Red Lake)				
Weir	100	Late July 2000	Sufficiently sampled	Foster, Lloyd
Frazer Lake	100	Pre 1999	Sufficiently sampled	
Upper Station (early run)	100	Mid June 2000	Sufficiently sampled	Sagalkin, weir crew
Upper Station (late run)		Pre 1999	Sufficiently sampled	
Saltery	100	Late August 1999	Sufficiently sampled	Edwards, Foster

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Table 1. (page 2 of 2)

<i>Management Area Sockeye Salmon System Spawning Location</i>	<i>Sample Size</i>	<i>Dates</i>	<i>Comments</i>	<i>Crew Leader, Crew</i>
Chignik (Area L)				
Chignik Lake		Pre 1999	Sufficiently sampled	
Black Lake		Pre 1999	Sufficiently sampled	
North Alaska Peninsula (part of Area M)				
Nelson River System				
Hoodoo Lake	100	Early August 2001	Sample spawning grounds	Tscherich, Dinnocenzo
Sapsuk River	100	Early August 2000	Sufficiently sampled	Tscherich, Dinnocenzo
Bear Lake (early run)	100	Late June 2000	Sufficiently sampled	Tschersich, Boatright
Bear Lake (late run)	100	mid to late August 2000	Sufficiently sampled	Tschersich, Boatright
Sandy Lake	100	Late June 2000	Sufficiently sampled	Tschersich, Brandt
Ilnik Lagoon	400	Late July 2001	Sample spawning grounds at Willie Creek, Wildman Lake, Ilnik River, and Ocean River	Tscherich, Manthey
Cinder River	100	Late July 2001	Sample spawning grounds	Tscherich, Manthey
Meshik River	100	Late July 2001	Sample spawning grounds	Tscherich, Manthey
South Alaska Peninsula (part of Area M)				
Orzinski	100	Early July 2000	Sufficiently sampled	Witteveen, Connolly
Aleutian Islands (part of Area M)				
Summer Bay Lake	100	Mid August 1999	Sufficiently sampled	

Table 2. Westward Region sockeye salmon genetic baseline project timeline and associated personnel to perform the activities.

<i>Date</i>	<i>Personnel</i>	<i>Activity</i>
July – September 1999	Westward Region Finfish Research and Management Staff and seasonal weir crews	Sample spawning populations
May 2000	Templin	Lab analysis of 1999 samples
July – September 2000	Westward Region Finfish Research and Management Staff and seasonal weir crews	Sample spawning populations
April 2001	Templin	Lab analysis of 2000 samples
May 2001	Templin, Witteveen	Progress report
July – September 2001	Westward Region Finfish Research and Management Staff and seasonal weir crews	Sample spawning populations
December 2001	Templin, Seeb, Crane	Genetic analysis of collections
March 2002	Templin, Witteveen, Seeb	Final report

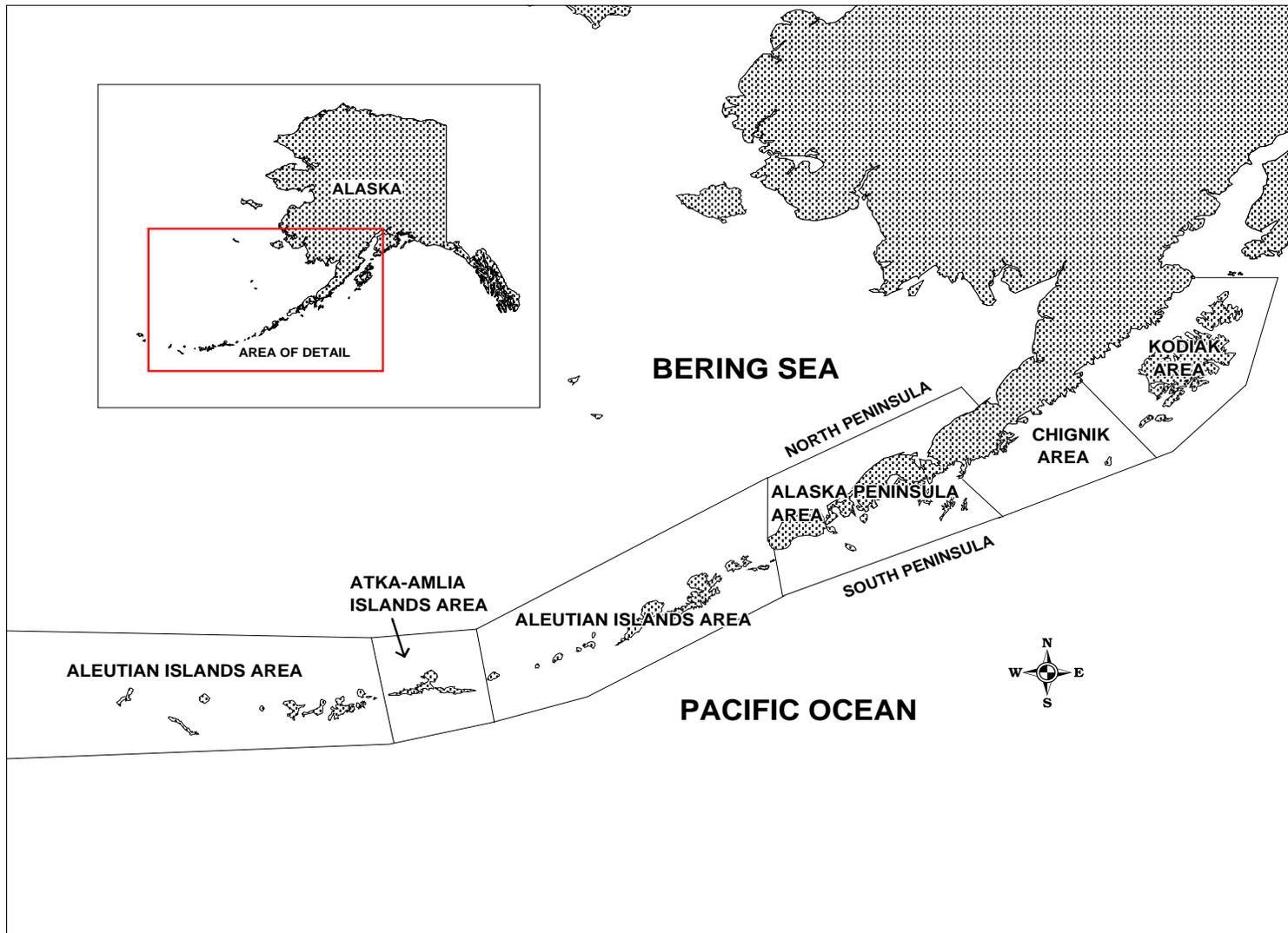


Figure 1. Map of the Westward Region identifying commercial salmon management areas: Kodiak, Chignik, Alaska Peninsula, Aleutian Islands, and Atka/Amlia.

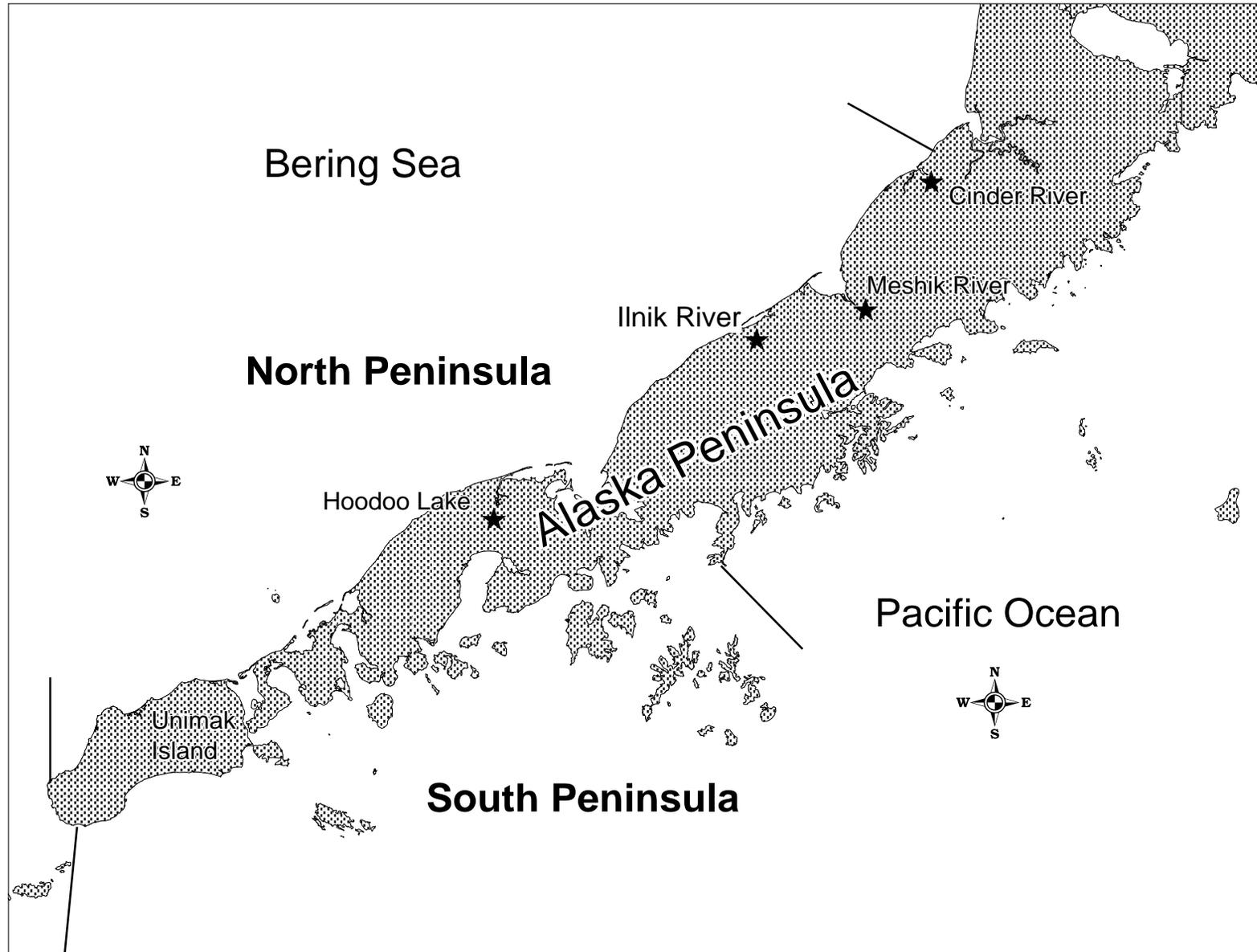


Figure 2. Map of the Alaska Peninsula Management Area (Area M) showing proposed sockeye salmon systems to be included in the Westward Region genetic baseline.

APPENDIX

ADF&G Genetics Laboratory, Anchorage

I. GENERAL INFORMATION

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 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. List of sampling supplies

Cryotube- small (2 ml) plastic vile or test tube. The cryotubes have plastic seals and screw on caps to withstand liquid nitrogen storage

Cryotube rack- white plastic block with holes in it for holding cryotubes

Cryocane- long aluminum cane. Five or six cryotubes are snapped onto the cryocane and placed in the liquid nitrogen canister

Pipettes-plastic droppers for sampling eye fluid (vitreous humor).

Forceps-tweezers

Liquid nitrogen dewer- large bottle in wooden box. It holds liquid nitrogen that can keep the samples very cold for an extended period of time

Liquid nitrogen canister- cylindrical canister that fits inside the dewer. The dewer will arrive filled with six canisters

B. General set up

To insure that the tissues are kept fresh and cold, working fast is necessary. It is important to have your sampling area and supplies set up **before** the fish are caught.

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1. Label the empty plastic cryotubes (night before?) with the adhesive labels provided in sampling kit. Four separate tubes, corresponding to the four tissues (muscle, heart, eye, and liver), should be labeled for each individual. The color on the side of each label should help keep the tissues separate. Place the cryotubes in the cryotube racks in an order that will allow you to work quickly. We find it easiest to set up by individual. For example, all the tissues for fish number one is in row number one of the cryotube rack.

2. 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.

3. Have the caps for the tubes set out along with the sampling tools provided.

C. Actual sampling

Please take samples from freshly killed fish. From each fish gather a small amount of muscle, heart, liver, and eye and place in separate cryotubes label with the same number (Fish #1 has it's tissues loaded in cryotubes labeled muscle #1, liver #1, etc.).

Fill the tubes with tissue approximately 3/4 full or to the 1.8 ml mark, leaving air space at the top and cap securely. **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" skeletal muscle, not muscle from along the lateral line. Use a piece of muscle dorsal (above) to the lateral line. If you have trouble getting the tissue into the tubes, cut it into smaller pieces.

2. Liver: An incision down the belly from the anus to between the gills helps to retrieve the liver and heart quickly. The liver is (generally) located on the fish's left side, just behind the pectoral fin. 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 or knife, 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. Scraping the tip of the pipette around the eye helps to mix up the fluid making it easier to suck the fluid out. Squirt this into the cryotube.

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It is important to wipe your knife, other sampling tools, and sampling area off before sampling the next fish to avoid cross contamination between fish.

Once tubes have been filled, place them in liquid nitrogen within 20 minutes of sampling. Put the cryotubes in the cryocanes. We find it easiest to set up four cryocanes simultaneously and organize the samples in canes by tissue. Thus, muscle tissue from fish 1-5 would all be in one cane. Place the cryocanes in a liquid nitrogen canister and into the liquid nitrogen dewer.

D. Use of liquid nitrogen

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). Send canister back if less than 4 inches of liquid nitrogen remains in the can.

"Large" 35 VHC container:

30 cryocanes will fit in each of the six canisters. Five cryotubes will fit on a cryocane comfortably or six in a pinch. Total capacity is 900 - 1080 cryotubes.

"Small" 18HC container:

Seventeen cryocanes will fit in each of the six canisters. The total capacity is 510 - 612 cryotubes.

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.

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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.

E. Data to Record.

We would like sex of the fish recorded. Data forms will be included in the sampling kit for this purpose. However, if your project includes taking scales, and recording age and length and you are using data sheets of your own, if you would prefer to photocopy your own data sheets and send us a copy once back from the field, this will be fine.

We appreciate your help with the sampling. If you have any questions, please give us a call.

Bill Templin
267-2234

Penny Crane
267-2140

Judy Berger
267-2247

Laboratory 267-2247
