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GENETIC STOCK IDENTIFICATION, BASELINE DATA COLLECTION;

WESTWARD REGION, FIELD SUMMARY

By

David R. Sarafin

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ACKNOWLEDGEMENTS

Credit should be given to all ADF&G personnel who worked on or in support of this project. In particular, the efforts of the various people who temporarily filled in as sampling assistants were greatly appreciated. This project could not have proceeded as smoothly without this help. Likewise, the cooperation of the Anchorage Genetics Lab was vital to our efficient operation.

The cooperation of the management staff of the Kodiak and the Izembek National Wildlife Refuges was also greatly appreciated. This project could not have proceeded without their timely approval of sampling and special access requests. Additional credit should be given to the Izembek NWR staff for providing assistance with sampling.

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INTRODUCTION

The Alaska Department of Fish and Game (ADF&G) has initiated a genetic stock identification (GSI) program for chum salmon in various regions of the state. The Kodiak Office is conducting the field sampling to establish baseline data for the Westward Region. For the 1992 season, the North and South Alaska Peninsula and the Kodiak Island Districts were targeted (Figures 1 and 2). This report summarizes the preliminary and field operations of this project.

Tissue samples were to be collected from streams with local stocks thought to be significant contributors to the commercial fisheries of the Alaska Peninsula. The samples would then be sent to the ADF&G Genetics Lab, Anchorage. The Genetics Lab would then conduct analysis and evaluation of the samples.

The actual GSI methodology incorporates protein electrophoresis to examine the composition of genetic variants (alleles) within tissue samples. Data can be collected from the commercial chum salmon catch from specific fisheries and then compared to a baseline of genetic data from various known individual stocks. This can then provide information regarding the various stocks contributing to the commercial harvest.

SAMPLING STRATEGY/RESULTS

In developing this seasons' sampling strategy, the following factors were considered: personnel; stream selection; run timing; bases of operation; sampling site accessibility; sampling methods; and supply logistics. These factors all had to be coordinated to provide an efficient operation.

As project leader, I (David Sarafin, Fisheries Biologist I) was given responsibility for all aspects of this project, under the direction of Pete Probasco, Regional Management Biologist. All field work was conducted by one sampling crew.

Sixteen streams on the Peninsula and six on Kodiak Island were selected to be sampled, in 1992. This seemed a reasonable number for one crew to accomplish, considering the time frame salmon are available for sampling and the funding allotted. These 22 streams were sampled during the period of 30 July to 6 September. Table 1. provides a listing of streams sampled, date, and number of samples collected from each system.

Personnel

One two person crew was initially placed on this project. I was appointed project leader. A Fisheries Technician III, Brian Westgate, was also hired as a crew member, however, after sampling the first two streams he left the project for medical reasons.

Subsequently, being suddenly short-handed, it was fortunate that I received extensive cooperation from various people who assisted with the sampling. In particular, the assistance of our chartered helicopter pilot, Larry Larrivee (Pollux Aviation Limited), enabled the project to function quite efficiently. Larry assisted with sampling on a regular basis. Additional assistance was received from the following people as sampling assistants:

Judy Hamik, Fish Tech. III, Sand Point
Jim McCullough, Area Management Biologist, Sand Point
Dan Miller, Fish Tech. III, Cold Bay
Natalie Schlicten, U.S.F.&W.S., Izembek NWR, Cold Bay
Chris Sundby, Fish Biol. I, Cold Bay
Randy Weber, Aircraft Pilot, Chignik Weir
Mark Weinberger, Fish Biol. I, Sand Point
Scott Weimer, Fish Tech. II, Kodiak

Stream Selection

Streams were selected on the basis of three factors: run size; geographic dispersal; and logistical feasibility of obtaining samples. Our selection goal was to have an even dispersal of streams supporting the largest escapements throughout the districts to be sampled.

Selected streams, estimated escapements, and management districts are presented in Table 2.

Information regarding escapement numbers was provided by referencing annual management reports for the appropriate area.

In some cases, alternate streams were also selected. This allowed a degree of flexibility in cases of weather, salmon run timing, logistics, or other difficulties.

Timing

Considering the vast area of coverage, coordination of run timing was a crucial factor in developing the sampling strategy. Annual management reports and local finfish management biologists were the sources of information on the actual time of spawning.

From this information, a tentative sequence of sampling locations was determined. Information from recent aerial surveys was also considered. The final decision on the sampling sequence was often made one day prior to the actual sampling of the stream, and occasionally delayed for a weather check, until the morning of sampling.

Bases of Operation/Accommodations

The sampling crew was based out of four different ADF&G offices throughout the sampling area; Kodiak, Chignik, Sand Point, and Cold Bay. From these four locations, all selected streams could be accessed within less than 2 hours of flight time.

The initial sampling trip in Kodiak was operated from a mobile field camp. Two streams were sampled on this trip. All other streams were sampled on day trips being operated from ADF&G offices/bunkhouses as bases/housing facilities.

Beginning in Kodiak, sampling was next conducted from Chignik Weir, Sand Point, Cold Bay, and finally back to Kodiak again.

Access

The majority of the 22 streams were very remote and difficult to access. Of the 22 locations sampled, 18 were accessed by aircraft, 3 by road, and 1 by skiff.

Originally it was hoped to access the streams by fixed-wing aircraft. As a trial, the initial trip to the first two locations on Kodiak was conducted as a mobile camp operation, transported by floatplane (DeHaviland Beaver). Although sampling was successful and accomplished within a reasonable amount of time (5 days), it was also realized that this would not be a feasible method to complete the desired number of streams to be sampled. The use of fixed-wing aircraft is more limiting when considering not only the factor of weather; but, more importantly the factor of run timing. Finding the fish within a reasonable distance of a landing area would require timing of pinpoint accuracy which could not possibly be determined nor relied upon for all the streams involved. Logistical coordination would be just too complex. As it turned out, the availability of the state aircraft on the Peninsula was totally incompatible with this method.

On August 5, the State committed to chartering a small helicopter (Robinson R22) for sampling the streams on the Peninsula. On August 14, the Robinson, piloted by Larry Larrivee, Pollux Aviation Limited, arrived in Sand Point to begin service on this project. The aircraft is a small, two seat helicopter with limited cargo space. Sampling gear and provisions were minimized and compacted accordingly. The Robinson proved to be quite practical and very appropriate for this project. Upon completing the Peninsula streams, the helicopter was shuttled to Kodiak.

While in Chignik, the Meshik R. was sampled as a day trip with access provided by the State's fixed-wing aircraft (Piper Super Cub). Pilot Randy Weber assisted with sampling. Due to the weather being unsuitable for fixed-wing operation, six days were spent in Chignik to sample this one location.

While operating from Sand Point, Zachary Bay was accessed by skiff as a one day sampling trip. From Cold Bay, both Frosty and Russel Creeks were accomplished on the road system. The American River (Kodiak Is.) was also accessed by road.

Special permission was obtained from National Wildlife Refuges, as required.

Sampling

An optimal sample size of 100 fish with a minimum of 50 fish was the desired goal from each stream. In most streams, samples were collected from the spawning grounds. Quite often, these fish were mostly spawned-out. As spawn-outs, they were much easier to catch and to work with; their energy level is reduced and their well-developed teeth tangle in the seine web. This also minimized any impact on the spawning escapement.

Fish were captured primarily by beach seine. Initially a 50x10 ft. nylon web seine was used. This seine weighed approximately 60 lbs. and was a nuisance to work with, and since fish were primarily obtained from shallow spawning grounds, it was much more than we needed. To replace this, a 30x6 ft. seine was constructed by modifying a herring gillnet (monofilament web). This was much more appropriate, very compact and weighing only 15 lbs. However, being constructed of lighter weight material, its use requires more frequent repairs and occasional replacement.

Tissues were collected following the techniques described in the instructions "Collection of Salmonid Genetic Samples", (ADF&G Genetics Laboratory, Anchorage) (Appendix). Liquid nitrogen containers (35VHC) were used for storing samples and shipment to the genetics lab in Anchorage. A small cooler (16 qt.) containing dry ice was used to temporarily preserve the samples until they could be placed in the liquid nitrogen. The time that the samples were on dry ice ranged from 1-10 hours.

Our sampling station consisted of a fold out camp stool, the cooler as a second seat, and a Coleman folding stove rest supporting a small piece of plywood as a dissection table. Everything was kept compact and lightweight to minimize the difficulties accessing remote sampling sites.

Sampling was most efficient with two people; one person dissecting and the other pipetting the eye fluid and screwing lids on the vials. The basic sampling sequence was to:

1. Sort one set of 20 vials by tissue type and number.
2. Capture and kill approximately 20 fish.
3. The dissector then begins with fish #1 by cutting a slit in the eye. The second person then extracts eye fluid, placing into and sealing its labelled vial.
4. The dissector then removes a slice of liver, heart, and muscle, placing each into respective vials, while the other person installs lids.

5. Upon completion of each set of fish, the vials were immediately placed on dry ice, normally within 1-2 hours of the fish's death.

After completing the sampling, the tissues were transported to our operational base. The vials were then sorted and loaded into aluminum canes by tissue type and then placed into the liquid nitrogen container.

Each stream required at least one full day to obtain a complete sample. Once the helicopter was employed, the regular sampling routine involved day trips to sample each location. The average typical sampling day required 12 hours of work: 1 hour morning preparation; 2.5 hours of flight time, 5.5 hours sampling; 0.5 hours transferring samples to nitrogen container; and 2.5 hours labelling vials for the next sampling site. The sampling assistant normally shared in 7-8 hours of this work schedule.

Occasional weather and relocation days interfered with the pursuit of samples. Non-Sampling days usually required a normal 7.5 hour work day to continue the project's efficient operation. Project planning, logistical coordination, gear repairs, and misc. were all accomplished during these periods.

The weekly work schedule was a 7 day work week conducted throughout the project. This schedule was maintained primarily to reduce the costly standby time of the chartered helicopter (3 flight hours/day guaranteed minimum).

Supply Logistics

Supplies required for storage and transport of samples were provided by the Anchorage Genetics Lab. Supplies were requested as needed. Regular communication with the Genetics Lab was vital to the operation of this project. Their cooperation and response to all questions and supply requests was always prompt and complete. Without this efficiency our schedule would have been considerably delayed. Supplies were shipped air freight.

Field gear and supplies were also shipped air freight during relocations between operational bases, except when space was available on existing charters or state aircraft.

Various supplies were purchased for this project. These include: complete camp gear; sampling gear/supplies; aircraft fuel; and food.

RECOMMENDATIONS

Remote sampling of Kodiak Island and the Alaska Peninsula present extremely difficult logistical problems. Access by helicopter is the only feasible method for sampling the majority of

locations. The degree of success of this season's sampling was highly dependant upon the use of the Robinson helicopter. All attempts should be made to acquire its service for future sampling.

Depending upon the extent of next year's sampling and the strategy developed, consideration should be given to working with a two person crew. However, the project leader should be consulted prior to this decision. A steady crew member would have been useful as the project does demand extensive work duties beyond actual sampling, and personnel would not need to be borrowed from other projects to fill in as sampling assistants. The possible assistance from the helicopter pilot could again alleviate the need for a second person.

There remains the need for an improved vial labelling system. Labelling vials currently requires at least 2.5-3 hours of work/location sampled. In addition, the labels are quite prone to peeling off; especially, anytime the sampling was conducted in the rain. It is suggested a search for some type of mechanized heat stamp labelling machine be conducted. Pre-labelled vials would be a great assistance to maintaining a high sampling frequency and in reducing costs of sampling efforts.

Table 1. Locations Sampled, Kodiak Island and Alaska Peninsula.

DATE	AREA	STREAM	# SAMPLED
7/30-31	W. Kodiak	Sturgeon R.	71
8/1	S.Kodiak	Sukhoi Lgn.	100
8/9	N.Peninsula	Meshik R., Braided Cr.	78
8/13	S.Pen.	Zachary Bay	80
8/15	N.Pen.	Lawrence R.	100
8/16	N.Pen.	Nelson Lgn, Sapsuk	80
8/17	S.Pen.	Canoe Bay R.	100
8/18	S.Pen.	Stepovak Flats, Big River	50
8/20	S.Pen.	Balboa Bay, Foster Cr.	100
8/22	S.Pen.	Belkofski R.	87
8/23	N.Pen.,Unimak I.	St.Catherines Cove	86
8/24	N.Pen.	Joshua Green R.	80
8/25	S.Pen.	Littlejohn Lgn.	87
8/26	N.Pen.,Unimak I.	Peterson Lgn.	86
8/28	S.Pen.	Volcano R.	64
8/29	N.Pen.	Traders Cv.	100
8/30	N.Pen.	Frosty Cr.	100 *
8/31	S.Pen.	Russell Cr.	100 *
9/3	W.Kodiak	Uganik R.	100
9/4	E.Kodiak	Kiliuda Bay, Dog Bay	100 *
9/5	W.Kodiak	Kizhuyak R.	88 *
9/6	E.Kodiak	American R.	100

* 30 extra livers taken; corresponding to samples #1-30.

Table 2. Estimated Average Escapements and Management Districts of Selected Streams.

LOCATION	MANAGEMENT DISTRICTS	STREAM	EST.AVG ESC. *
Kodiak Island	Northeast Kodiak	American R.	8,345
	Eastside Kodiak	Dog Bay,Kiliuda	8,345
	Alitak Bay	Big Sukhoi Cr.	27,817
	Southwest Kodiak	Sturgeon R.	69,542
	Northwest Kodiak	Uganik R.	13,908
	Northwest Kodiak	Kizhuyak R.	11,127
Alaska Peninsula	Northern	Braided Cr.,Meshik	16,400
	Northern	Lawrence R.	15,000
	Northern	Sapsuk R.,Nelson L	5,500
	Northwestern	Joshua Green R.	30,000
	Northwestern	Frosty Cr.	9,700
	Northwestern	St.Catherines Cove	5,500
	Northwestern	Peterson Lgn.	4,500
	Northwestern	Traders Cove	2,800
	Southeastern	Big R.,Stepovak	25,600
	Southeastern	Foster Cr., Balboa	6,100
	Southeastern	Zachary Bay	1,100
	South Central	Canoe Bay R.	46,600
	Southwestern	Volcano R.	16,300
	Southwestern	Belkofski R.	30,700
	Southwestern	Russel Cr.	39,300
	Southwestern	Littlejohn Lgn.	8,400

* Kodiak figures are estimated minimum escapements, and Peninsula figures are average peak escapements (1987-1991).

Figure 1. Kodiak Management District Map depicting Locations of Streams Sampled.

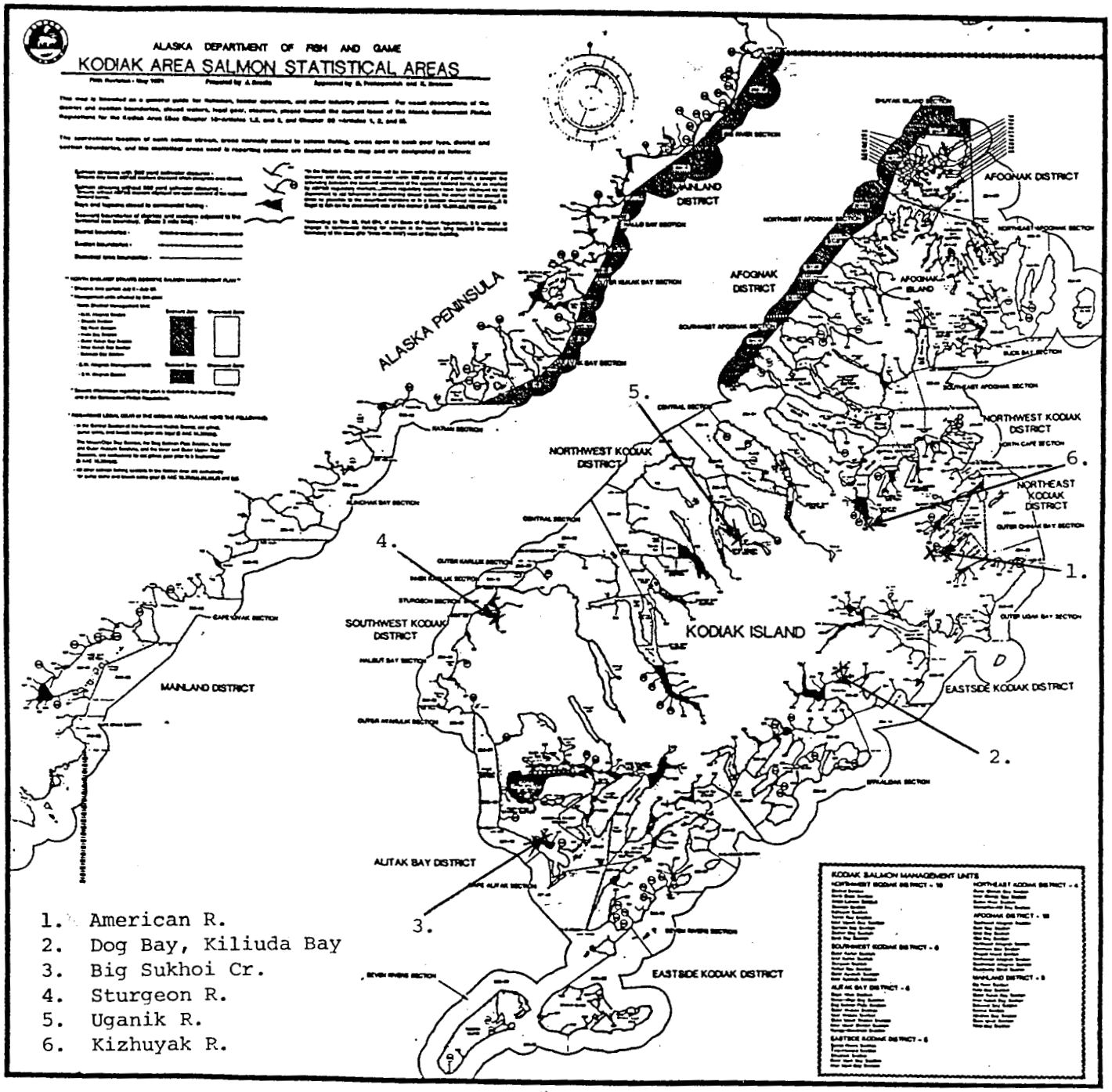
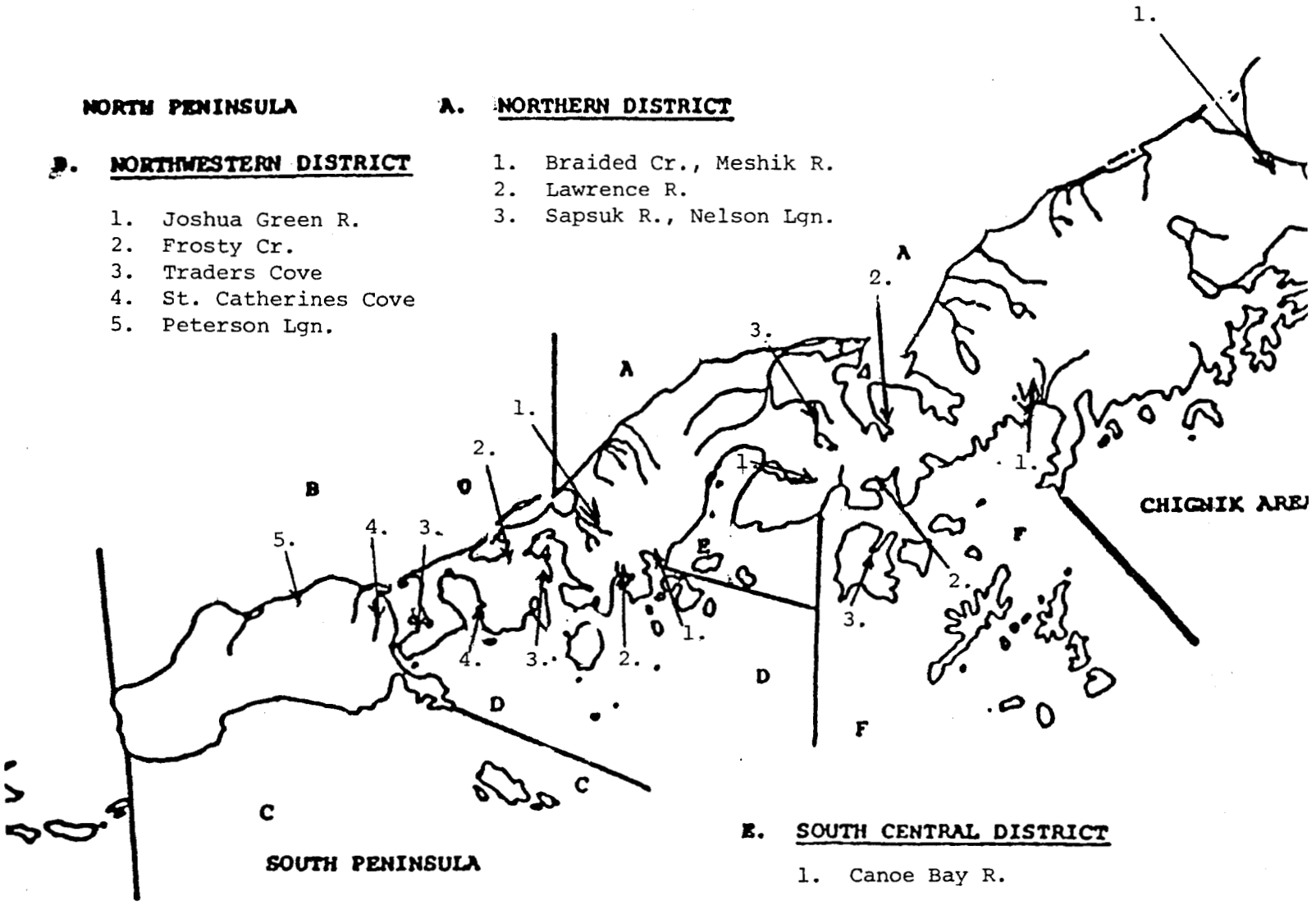


Figure 2. Alaska Peninsula District Map depicting Locations of Streams Sampled.



A. NORTHERN DISTRICT

1. Braided Cr., Meshik R.
2. Lawrence R.
3. Sapsuk R., Nelson Lgn.

B. NORTHWESTERN DISTRICT

1. Joshua Green R.
2. Frosty Cr.
3. Traders Cove
4. St. Catherines Cove
5. Peterson Lgn.

C. UNIMAK DISTRICT

D. SOUTHWESTERN DISTRICT

1. Volcano R.
2. Belkofski R.
3. Russel Cr.
4. Littlejohn Lgn.

E. SOUTH CENTRAL DISTRICT

1. Canoe Bay R.

F. SOUTHEASTERN DISTRICT

1. Big R., Stepovak Flats
2. Foster Cr., Balboa Bay
3. Zachary Bay

Appendix A.1. Selected stream numbers and GPS coordinates of sampling sites (as available).

LOCATION	STREAM NAME	STREAM NUMBER	COORDINATES N.LAT / W.LONG
Kodiak Island	-Kizhuyak R.	259-365	Not Recorded
	-American R.	259-231	' '
	-Dog Bay, Kiliuda	258-204	' '
	-Big Sukhoi Lgn.	257-102	' '
	-Uganik R.	253-122	' '
	-Sturgeon R.	256-401	' '
Alaska Peninsula	-Meshik R.	317-7-B	' '
	-Lawrence R.	314-20-07	' '
	-Sapsuk R.,Nelson	313-30-03	' '
	-Joshua Green R.	312-40-01-B	' '
	-Frosty Cr.	312-20-05	' '
	-St.Catherines Cov	311-60-01	54°53.88'/163°29.15'
	-Peterson Lgn.	311-32-10	54°55.55'/164°09.92'
	-Big R., Stepovak	281-25-04	55°54.11'/159°45.57'
	-Foster Cr.,Balboa	281-80-09	N. R.
	-Zachary Bay	282-35-05	' '
	-Canoe Bay R.	283-24-06	' '
	-Volcano R.	284-36-08	55°14.39'/161°59.98'
	-Belkofski R.	284-42-07	55°11.63'/163°08.97'
	-Russel Cr.	284-67-02	N. R.
	-Littlejohn Lgn.	284-20-13	55°00.26'/162°53.93'
	-Traders Cove	311-60-08	54°54.24'/163°15.64'

Appendix B.1. Collection of Salmonid Genetic Samples, ADF&G Genetics Laboratory, Anchorage, March, 1992.

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 is necessary.

III. Protein electrophoresis 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 the provided lab markers or adhesive labels. 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		K	King (chinook)
		C	Chum
		S	Sockeye
		P	Pink
		H	Coho
Location code	*	see instructions for each project	
Individual #	#		i.e. 01, 02, 03....100
Tissue	M,L,E,H		(muscle, liver, eye, heart)

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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" to place the samples in. These cryotubes have plastic seals and screw on caps to withstand liquid nitrogen storage. Five (white Nalgene) or six cryotubes (orange Corning) 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 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 (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 with Nalgene tubes and 1260 with Corning tubes.

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

Safety with liquid nitrogen:

1. Wear gloves, protective eyewear, and protective footwear when placing samples in container. Liquid nitrogen boils at -196^{o}, 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 after about one hour of sampling.

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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 the liquid nitrogen container back to Anchorage.

C. 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.

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. Fill the tube 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. Please minimize the amount of blood, dirt, skin, and fat in the sample. If you are having trouble getting the tissue into the tubes, cut into smaller pieces.

2. Liver

The liver is (generally) located near 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). Again, fill the tubes only about 3/4 full.

3. Heart

Once you have taken the liver, it is easy to get the heart by just opening the belly incision towards the head. Leave air space in the tubes as before.

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

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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, again filling only 3/4 full or to the 1.8 ml line. Pipettes bulbs are included in the sampling kit.

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

Lisa Seeb
267-2249

Jim Seeb
267-2385

Penny Crane
267-2140

Laboratory
267-2247

Appendix C.1.

Address:

Pollux Aviation Limited (Helicopter Charter)
Larry Larrivee, Pilot
325 South Bartlett Circle
Wasilla, Alaska 99687-9228

Ph: 907-277-0511, 439-5419
Fax:907-272-3486