CHUM SALMON GENETIC STOCK IDENTIFICATION:

BASELINE AND FISHERY SAMPLE COLLECTION, 1991 - 1993

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INTRODUCTION

Chum salmon (*Oncorhynchus keta)* from North America and Asia migrate into the North Pacific Ocean, generally spending two to four years in the ocean, before returning to their natal streams to spawn. The salmon form aggregations composed of numerous stocks during their ocean residency and freshwater migrations. Identification of composite stocks in mixtures of chum salmon caught in international waters, in the U.S. Exclusive Economic Zone, and in the major river systems leading to spawning tributaries has been an ongoing challenge for fisheries biologists and management agencies throughout the Pacific Rim.

Detection of genetic differences at the molecular level has become possible within the last 20 years, providing a set of genetic information complementary to more traditional approaches. These procedures, which examine direct products of individual genes or the actual genetic material (i.e. DNA), have been the basis of a new era in understanding genetic differences both within and among populations of all organisms including fishes. Genetic stock identification (commonly abbreviated GSI) using the laboratory technique of protein electrophoresis has become an important part of many salmonid management programs.

Developing a comprehensive chum salmon GSI database for the North Pacific not only requires international cooperation among the nations involved, but also considerable cooperation among state and federal agencies within the U. S. A major step in realizing this goal was taken with the establishment of a coastwide GSI database. This database, originally maintained by the Washington Department of Fisheries, is currently being maintained by the Alaska Department of Fish and Game (ADF&G). It currently includes allozyme data for approximately 20 loci from over 144 collections ranging across the Pacific Rim from Washington State to Japan. These data have been collected by National Marine Fisheries Service (NMFS), Seattle and Auke Bay, AK; Washington Department of Fisheries (WDF), Olympia; United States Fish and Wildlife Service, Anchorage; and ADF&G, Anchorage; and are distributed among cooperating agencies. This database is internally consistent with respect to scoring of alleles (standards of all alleles have been exchanged among those laboratories).

However, many areas are clearly underrepresented in the Alaska collections contributed to the Pacific Rim database. In the Bering Sea region, these include Norton Sound river systems, the Kuskokwim system, and the Nushagak River in Bristol Bay. The North and South Alaska Peninsula stocks have not been well characterized, nor have the stocks of Kodiak Island, Cook Inlet, and Prince William Sound. The objective of this project is to develop a comprehensive chum salmon GSI database with emphasis on Northwest and Southcentral Alaskan stocks. In the future, these data can be used to evaluate the relative contribution of chum salmon stocks to the South Peninsula Area M June fisheries.

This report reviews field collections, from both fishery and spawning populations, conducted between 1991 and 1993. As part of a pilot study, a portion of the South Peninsula Area M commercial catch was sampled in June, 1993. Emphasis is placed on the results from Region IV and the Area M fishery. Collections from the other regions are briefly summarized. More detailed information regarding the laboratory and statistical analyses can be obtained by reference to the Project Operational Plan: Bering Sea Salmon Stock Identification.

BASELINE SAMPLING

Baseline tissue samples have been collected from spawning populations throughout Northwest and Southcentral Alaska (Figure 1). A total of 95 collections have been made, as of December, 1993. Actual sample sizes vary depending on the size of the runs and availability of spawners during the sampling period. A target sample size of 100 per population unit was set. In addition to these collections, complementary studies are being conducted in Southeast Alaska by the National Marine Fisheries Service, Auke Bay, Alaska.

Varying sampling strategies have been employed throughout the different regions of the state, with area and regional personnel handling the collections or cooperating with the genetics laboratory personnel. An extensive effort was conducted in Region IV with personnel assigned specifically to the project. More in-depth details of this region's sampling are provided, since it was conducted as a project in itself.

Tissues samples were collected following the techniques described in the instructions "Collection of Finfish Genetic Samples", (ADF&G Genetics Laboratory, Anchorage) (Appendix A.1). Liquid nitrogen containers were shipped by air to the remote sites to assure excellent sample quality. Individual tissues (muscle, liver, retinal fluid, and heart) were subsampled, placed in 2.0 ml cryotubes, frozen as soon as possible in liquid nitrogen, and remained in liquid nitrogen during storage and shipment to the Anchorage laboratory. Upon arrival in Anchorage, samples were stored in -80° C until subsampled for electrophoretic analysis. All tissues were placed in -80° C archive storage, and these same tissues can then be used for future DNA-level analyses or exchange and standardization among laboratories.

Region II

Thirteen collections of baseline samples were obtained from Region II, as of December, 1993 (Table 1). Management biologists from the region have been primary coordinators for stream selection and the field sampling. In addition, the Region IV project sampled three Bristol Bay area drainages during the 1993 field season.

Region III

Extensive sampling has taken place throughout Region III; currently, 44 collections have been obtained, as of December, 1993 (Table 2.). As in Region II, the local management biologists assisted with stream selection and field sampling.

The U.S. Fish and Wildlife Service (USFWS) conducted GSI sampling on the Innoko and the Koyokuk Rivers. In addition, since 1987, ADF&G has been involved in a cooperative genetic study with USFWS (Wilmot et al. 1992), and baseline information are available on additional Yukon River systems.

Region IV

Thirty collections from the Alaska Peninsula and eight from Kodiak Island have been sampled, as of December, 1993 (Table 3). Sampling has occurred from late July through early September. Sampling efforts in 1992 were concentrated in the Alaska Peninsula and Kodiak Island areas, while in 1993 the concentration was in the Chignik area and the Alaska Peninsula Mainland District of the Kodiak Management Area

Stream selection was made on the basis of run size and geographic dispersal of location. The selection goal was to have an even dispersal of streams supporting the largest escapements throughout the districts to be sampled.

Considering the vast area of coverage for sampling by one crew, coordination of run timing was a crucial factor in developing the sampling strategy. Area management biologists were the primary sources of information on the actual time of spawning. From this information, a tentative sequence of sampling locations was determined. Reports from recent aerial surveys were also considered.

The sampling crew has generally based out of the ADF&G office closest to the sampling area. These included: Kodiak, Chignik, Sand Point, Cold Bay, and King Salmon. From these locations, selected streams could be accessed within less than two hours of flight time. Other bases of operation have included the State of Alaska *R/V K-Hi-C* (42 foot seiner) of the Kodiak Office, the State of Alaska Fish and Wildlife Protection Service cabin located at Pumice Creek, and various other remote field camps.

The majority of the streams were very remote and difficult to access. Most locations were accessed by chartering a helicopter (Robinson R22). This 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. Other means of access have included fixed-wing aircraft, skiff, and road systems.

An optimal sample size of 100 fish with a minimum of 70 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. A 30 x 8 ft. seine was constructed by modifying a herring gillnet (monofilament web). This seine was quite appropriate for spawning ground sampling, very compact and weighing only 15 lbs. However, being constructed of lighter weight material, its use requires more frequent repairs and occasional replacement. A fish spear was also used in the 1993 season. It was very effective in streams that were too narrow, deep, or 'snaggy' to seine.

The sampling station consisted of a fold out camp stool, a cooler as a second seat, and a collapsible dissection table. Everything was kept compact and lightweight to minimize the difficulties accessing remote sampling sites. A small cooler (18 qt.) containing dry ice was used to temporarily preserve the samples until they could be placed in the liquid nitrogen.

Sampling was most efficient with two people, one person dissecting and the other pipetting the eye fluid and screwing lids on the vials. Vials were labelled and pre-sorted into trays by tissue type and number. Fish were captured, dissected, and tissues were placed on dry ice. After completing the sampling, the tissues were transported to our operational base. The vials were then placed into the liquid nitrogen.

Each stream required at least one full day to obtain a complete sample. While using the helicopter, the regular sampling routine involved day trips to sample each location. The typical sampling day required 10.5 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 1 hour labelling vials for the next sampling site. The sampling assistant normally shared in 7-8 hours of this work schedule.

AREA M COMMERCIAL CATCH SAMPLING

Commercial catch sampling took place at King Cove, June 13-30, 1993. Tender deliveries to the local processing plant were sampled. One delivery to a floating processor was also sampled. Samples were collected from six fishing periods.

Objectives

The primary objectives of this first year's mixed stock sampling were to evaluate the feasibility of sampling within the plant, the feasibility of developing randomized sampling designs, and the quality of samples obtained from fish delivered to the plant. An initial insight into the identification of stock compositions of this area's catch was also desired.

Catches from two different areas of the South Unimak fishery were to be sampled separately (Figure 2). One sample group was from the catch of the Cape Lutke Section. The other group was to consist of fish caught outside of this section. These fish are predominantly from the Ikatan Bay and Otter Cove Sections of the Southwestern and Unimak Districts (SW/Unimak area). Samples from these areas were separated to examine possible differences in stock composition. For statistical purposes a sample size of 400 individuals per area per opener was established as a goal (800 total per open period).

Methods

ADF&G personnel arrived at the processing plant in King Cove on June 11, 1993, to prepare for and devise a sampling regime. Staff met with the plant superintendent and production manager to discuss their operation, sampling needs, and possible sampling arrangements.

At this plant, fish are pumped directly from the delivering tender to a conveyor belt sorting line. Fish are then sorted into bins by species and grade. From these bins they are immediately sent to the processing lines. Bins are emptied separately, so there is only a short interval that fish are held in some of the bins. This holding period depends upon the extent and duration of deliveries received.

Two different sampling stations were established. The primary station was located at the head of the sorting line. From here fish were sampled directly as they were pumped. Chum were pulled at random from the conveyor and dissected at a cutting table throughout the duration of pumping. Tissue sampling was conducted as in the spawning ground collections. Muscle tissue was taken from the cheek to avoid damaging the product for the plant.

A secondary station was set up in the bins themselves. Bin sampling occurred occasionally when fish were being held or when no deliveries were being pumped.

A two person crew was initially placed on this project. However, after the first two fishing periods it became apparent that the sampling pace would require additional personnel, and so a third person was added to the crew. Sampling with a three person crew was quite

efficient; two people would dissect tissues while the other would continually cap vials and re-supply sampling materials for the dissectors.

Only tenders with pure loads from either of the two designated areas were sampled. Schedules of arriving tenders were posted by the plant's staff. As each tender arrived, the skippers were interviewed to determine the areas that their fish were caught. Loads were sampled which were found to be solely from either the Cape Lutke or the SW/Unimak area.

In trial, sampling was also conducted on-board the floating processor Blue Wave. Fish were pulled as they were off-loaded, placed in a tote, and sampled on-board.

Results and Discussion

A total of 2,622 chum were sampled throughout the six open periods (Table 4). The goal of 400 fish per area/period was not consistently achieved.

The plant's processing is a very rapid pace, efficient, production operation. Fish were available for sampling for only a short period of time. Attempts were made at having fish held for sampling, but this was very difficult to coordinate without significantly impeding the plant's processing operation. Since fish could normally only be sampled during times of actual processing, sampling had to be conducted as a similar production operation.

Available work space was extremely limited. A suitable work station for 3-person sampling was found near the head of the sorting line. This station worked out very well, although it was rather cramped. The secondary station in the bins was not as efficient nor as reliable. Sampling could only be done here when fish were being held, and this cannot be relied upon. However, it is a good location for occasionally sampling additional fish at times when no deliveries are being pumped.

One tender delivery to a floating processor was sampled. This was done during one period in which the only tender with a pure Cape Lutke load was delivering to the floater. Special arrangements were made for the purpose of sampling this load. It also provided an opportunity for exploring the possibilities of sampling directly on board floating processors. Sampling went fairly well; however, work space was even more limited than in the shore-based plant.

Sampling rate and efficiency were much higher when sampling from the line compared to the bin/tote sampling conditions that were present. When sampling from the line, the three person crew became able to sample at a rate ranging from 70 to 85 fish per hour.

With the existing methods, crew size, and goal of sampling 800 fish, 400 from each of the two separate areas each period was not feasible. The crew should be able to consistently sample a full set of 400 fish from the overall catch of each period. An additional problem of separating the two areas was non-pure tender loads. Quite often tenders would pick up catches from both areas and therefore, would not be sampled. This also created a less systematic sampling design since these mixed loads were not sampled, and since some tenders were not sampled after the 400 fish goal was met.

The quality of tissues is also a concern since the fish are being held for variable lengths of time before being delivered to the plant. The tenders do have chilled holds, and the delivered fish were normally at a body temperature of -1 to +2 °C. Once sampled, the tissues were immediately placed on ice and frozen as soon a possible in one of the plant's walk-in freezer areas (-20 °C). The samples were then packed in dry ice and shipped to Anchorage where they are currently stored at -80 °C. The extent of any protein degradation will be evaluated during the lab analysis of these samples in 1994.

RECOMMENDATIONS

Baseline Sampling

The sampling of remote spawning grounds can present extremely difficult logistical problems. In many cases, access by helicopter can be the most practical and feasible method for obtaining baseline samples, especially in smaller drainages or those with shallow spawning grounds. The degree of success of the extensive sampling conducted in the Westward Region was highly dependant upon the use of the Robinson helicopter. Consideration should be given to more extensive use of this or similar helicopters for sampling in other areas of the state.

Area M Commercial Catch Sampling

As data become available from the 1993 pilot study, sampling designs will likely be modified. Of particular concern is the possible distinct differences of stock composition between chum salmon caught in the Cape Lutke Section and those caught elsewhere. If a consistent pattern of differentiation is found to exist, then it may be worthwhile to continue sampling the two areas separately. However, if variation is found to be inconsistent or of a seemingly random nature, then the necessity of this separation should be re-evaluated to possibly alleviate its related complications in sampling. Existing methods could normally complete a sample size of 400 per open period from the overall catch being processed at the King Cove plant.

Sample quality is another important consideration. If this is found to be unsatisfactory, adjustments to sampling methods may be in order, perhaps necessitating sampling directly on the "grounds" (on-board floating processors).

Sampling the Shumagin Islands catch is tentatively planned for 1994. This could possibly be conducted at the processing plant located in Sand Point. Methods similar to those implemented in King Cove may be appropriate.

REFERENCES

Wilmot, R. L., R. Everett, W. J. Spearman, and R. Baccus. 1992. Genetic stock identification of Yukon River chum and chinook salmon 1987 to 1990. Progress Report, U.S. Fish and Wildlife Service, Anchorage, AK. 132 pp.

Table 1. Region II collection locations for chum salmon genetic samples. Locations are indicated by the symbol (△) on Figure 1.

Area	Location	N	Date Sampled	Map Reference △ # (Fig.1)
Bristol Bay	Togiak R.	100	8/93	1
	Nushagak R. (sonar)	89	8/92	2
	Nushagak R. (upper)	53	8/92	3
	Nushagak R.	50	7/93	3
	Stuyahok R.	45	8/92	4
	Stuyahok R.	57	7/93	4
	Alagnak R.	84	8/92	5
	Naknek R., Big Cr.	80	7/31/93	6
	Egegik Bay, King Salmon R., Whale Mtn.Cr.	98	7/30/93	7
	Ugashik Bay,King Salmon R., Pumice Cr.	100	8/4/93	8
Cook Inlet	Susitna R.,Chunilna Cr.	100	9/93	9
Prince William Sound	WHN Hatchery	100	7/92	10
	Olsen Cr.	100	7/92	11

Table 2. Region III collection locations for chum salmon genetic samples. Locations are indicated by the symbol (□) on Figure 1.

Area	Location	N	Date Sampled	Map Reference □ # (Fig.1)
Noatak River Drainage	Sikusuilaq Hatchery	100	9/91	1
	Sikusuilaq Hatchery	100	9/93	1
	Noatak R.	100	9/91	2
	Kelly Lake	100	9/91	3
Kobuk R. Drainage	Salmon R.	106	9/91	4
Norton Sound	Snake R.	35	8/93	5
	Nome R.	40	4/91	6
	Nome R.	53	8/93	6
	Solomon R.	2	8/93	7
	Unalakleet R.	100	6-8/92	8
Yukon River Drainage	Summer Run			
	Andreafsky R.,W.Fork	100	7/93	9
	Andreafsky R.,E.Fork	100	7/93	10
	Innoko R.	88	7/93	11
	Anvik R.(mixed)	350	7/91	12
	Anvik R.(sonar)	6	7/92	12
	Anvik/Beaver Cr.	100	7/92	12
	Anvik/Beaver Cr.	100	7/93	12
	Anvik/Canyon Cr.	50	7/93	12
	Anvik/Otter Cr.	100	7/93	12
	Anvik/Swift R.	100	7/92	12
	Anvik/Swift R.	100	7/93	12
	Anvik/above Swift R.	1	7/92	12
	Anvik/Yellow R.	100	7/92	12
	Koyukuk R.	100	7/93	13
	Tanana/Chena R.	86	7/92	16
	Tanana/Salcha R.	107	7/92	17

Table 2. Continued.

Area	Location	N	Date Sampled	Map Reference □ # (Fig.1)
Yukon River Drainage	Fall Run			
	Tanana River			
	Toklat R.	60	11/91	14
	Toklat R.	155	10/92	14
	Toklat R.	200	10/93	14
	Tanana R.(main)	97	11/92	15
	Tanana R.(main)	100	11/93	15
	Bluff Cabin Slough	100	11/92	18
	Delta R.	100	11/91	19
	Delta R.	100	11/92	19
	Porcupine/Sheenjek R.	100	9/92	20
	Porcupine/Sheenjek R.	64	9/93	20
Kuskokwim Bay/River Drainage	Tuluksak R.	100	7/93	21
	Aniak Sonar	100	7/92	22
	Kogrukluk R.	75	7/92	23
	Kogrukluk R.	50	7/93	23
	Upper Kuskokwim R.	53	7-8/92	24
	Kanektok R.	18	8/92	25
	Kanektok R.	39	6-7/93	25
	Goodnews Weir	100	8/91	26

Table 3. Region IV collection locations for chum salmon genetic samples. Locations are indicated by the symbol (○) on Figure 1.

Area	Location	N	Date Sampled	Map Reference ○ # (Fig.1)
North Alaska Peninsula	Cinder R., Wiggly Cr.	100	8/4/93	1
	Meshik R., Plenty Bear Cr.	93	8/2/93	2
	Meshik R., Braided Cr.	78	8/30/92	3
	Lawrence R.	100	8/15/92	4
	Nelson Lgn., Sapsuk R.	80	8/16/92	5
	Joshua Green R. (early)	80	8/24/92	6
	Frosty Cr.	100	8/30/92	7
	Trader's Cove Cr.	100	8/29/92	8
	St.Catherines Cove	86	8/23/92	9
	Peterson Lagoon	86	8/26/92	10
South Alaska Peninsula	Littlejohn Lagoon	87	8/25/92	11
	Russell Cr.	100	8/31/92	12
	Russell Cr.	100	8/30,31/93	12
	Belkofski R.	87	8/22/92	13
	Volcano R.	64	8/28/92	14
	Canoe Bay R.	100	8/17/92	15
	Zachary Bay	80	8/13/92	16
	Balboa Bay, Foster Cr.	100	8/20/92	17
	Stepovak Bay, Big R.	50	8/18/92	18
	Stepovak R.	100	8/25/93	19
Chignik	Ivanoff R.	94	8/23/93	20
	Kiukta Bay, Portage Cr.	100	8/21/93	21
	Kujulik Bay, Northfork Cr.	72	8/22/93	22
	Aniakchak R.,North Fork Cr.	100	8/3/93	23
	Amber Bay, Main Cr.	92	8/26/93	24
	Chiginagak R.	75	8/20/93	25
Alaska Peninsula Mainland District (Kodiak Mgt.Area)	Wide Bay, Kialagvik Cr.	100	8/11/93	26
	Alinchak Bay,E.Bear Bay Cr.	100	8/11-13/93	27
	Alagogshak R.	95	8/12,13/93	28
	Big River (Hallo Bay)	100	8/14/93	29
Kodiak Island	American R.	100	9/6/92	30
	Gull Cape Cr.	100	9/14/93	31
	Kiliuda Bay, Dog Bay Cr.	100	9/4/92	32
	Sukhoi Lgn., Big Sukhoi Cr.	100	8/1/92	33
	Sturgeon Lagoon/River	71	7/30,31/92	34
	Uganik R.	100	9/3/92	35
	Kizhuyak R.	88	9/5/92	36
	Kitoi Hatchery	100	7/23/93	37

Table 4. Commercial catch samples taken for genetic analysis at King Cove, 1993.

Section/District	Open Fishing Date(s)	N
Cape Lutke	6/13	200
	6/15-17	84
	6/19-20	404
	6/22	252
	6/26-27	0
	Total	940
SW/Unimak	6/13	119
	6/15-17	411
	6/19-20	370
	6/22	257
	6/26-27	440
	6/29	85
	Total	1,682
Fishery Total		2,622

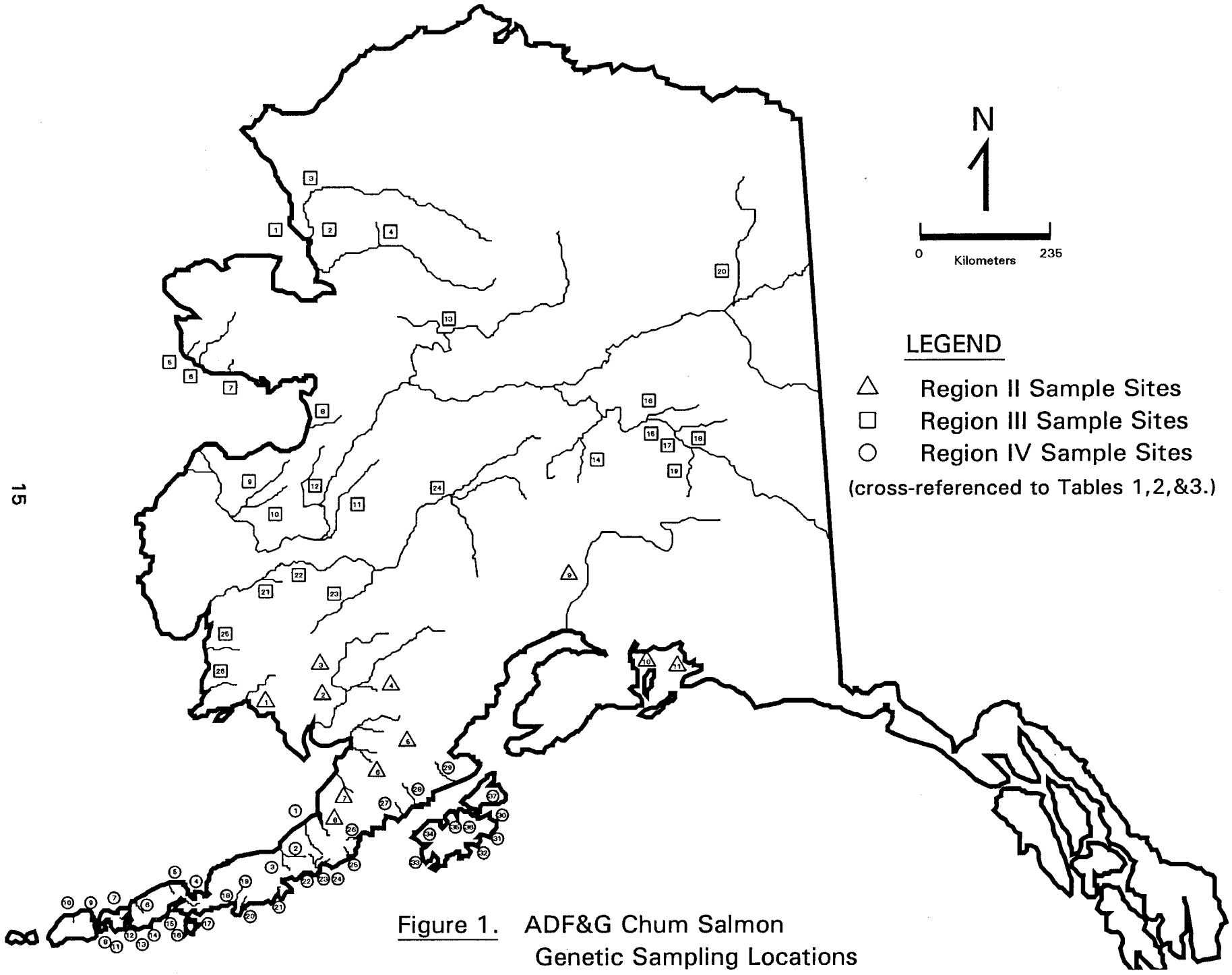

Figure 1. ADF&G Chum Salmon Genetic Sampling Locations

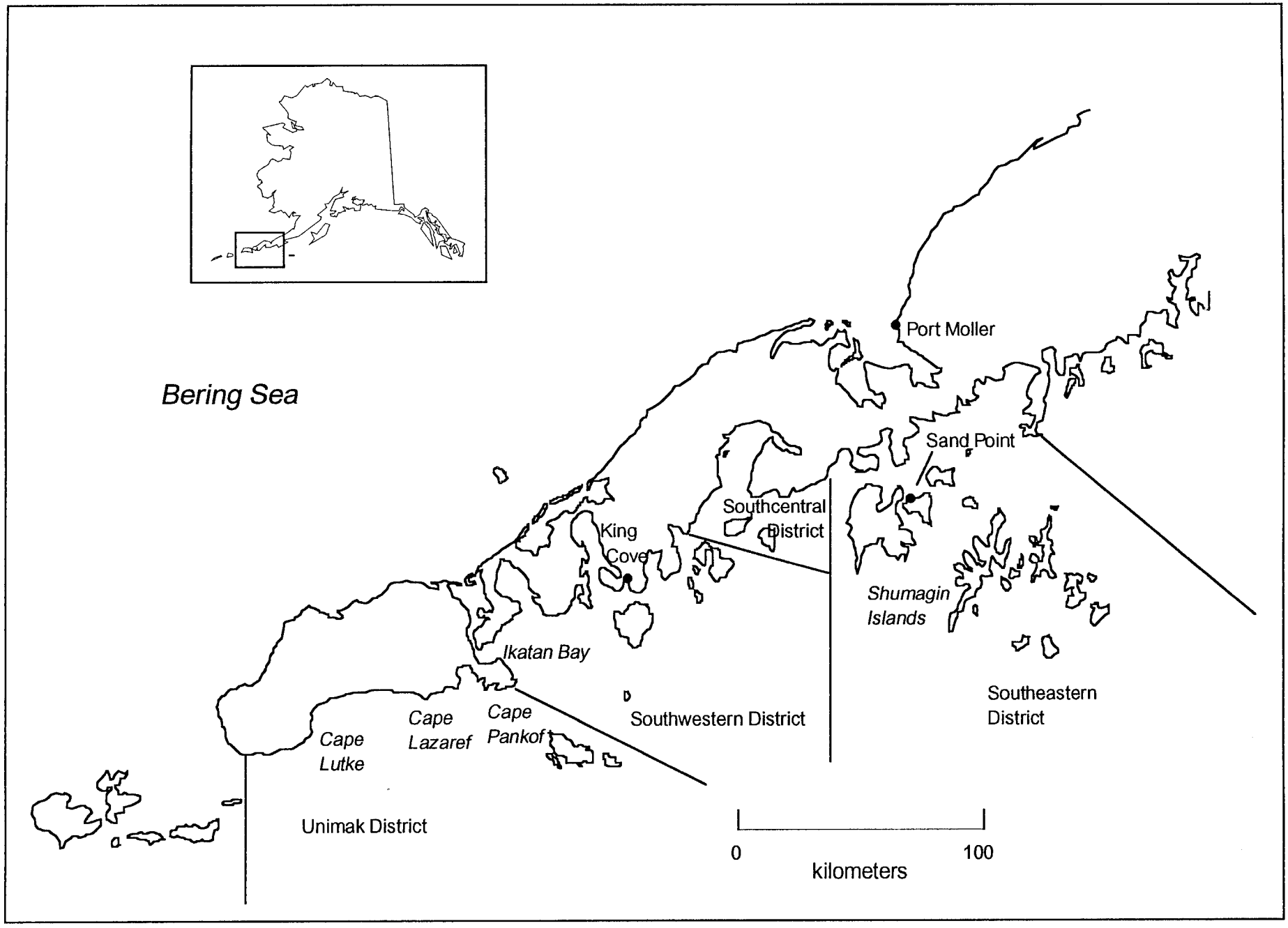

Figure 1. South Alaska Peninsula Fishery Management Districts.

Appendix A.1. 25 May 1993

Collection of Finfish Genetic Samples

ADF&G Genetics Laboratory, Anchorage

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 with the adhesive labels provided in sampling kit. Place the prepared tubes in the racks provided. Four separate tubes, corresponding to the four tissues, should be labeled for each individual.

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 to six tubes are stored in a cane.

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The working time of the liquid nitrogen container under normal conditions is 81 days (35VHC) or 50 days (18VHC). 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 18VHC).

"Large" 35VHC container:
30 canes will fit in each of the six canisters. 5 cryovials will fit on a cane comfortably or 6 in a pinch. Total capacity is 900 - 1080 tubes.

"Small" 18VHC container:
17 canes will fit in each of the six canisters. 5 to 6 cryovials will fit on a cane. The total capacity is 510 - 612 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.

-Continued-

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.

Once tubes have been filled, place them in liquid nitrogen within 20 minutes of sampling.

Be sure to wipe your knife off with a paper towel before sampling the next fish.

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

-Continued-

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. 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.

C. 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.

Lisa Seeb	Jim Seeb	Penny Crane
267-2249	267-2385	267-2140

Laboratory 267-2247