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Status Report for Genetic Stock Identification of Pacific Rim Chum Salmon

by

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ABSTRACT

Chum salmon populations from the Pacific Rim form mixed-stock aggregations during their ocean residency. In Alaska, migrating chum salmon are harvested incidentally in sockeye salmon fisheries during June in the areas of South Unimak Island and Shumagin Islands (South Alaska Peninsula). Numerous tagging studies have shown that these fish originate from throughout the Pacific Rim. Detection of genetic differences at the molecular level has become possible within the last 30 years, providing a set of genetic information complementary to more traditional approaches stock identification approaches. A comprehensive chum salmon allozyme genetic database has been established through the cooperation of many international institutions to provide information on migration routes and fishery composition. Alaska populations are underrepresented in this database, and the Alaska Department of Fish and Game initiated a genetic stock identification program in 1992 to characterize areas including Norton Sound, the Kuskokwim River system, Bristol Bay, and the North and South Alaska Peninsula. Over 10,000 individuals have been collected to date, and laboratory analyses are ongoing. In addition, mixed-stock fishery samples have been collected from the South Peninsula June fishery during 1993 and 1994. These samples will be analyzed to provide estimates of the stock composition of the South Peninsula fishery through time. A progress report of the results will be available in February, 1995.

INTRODUCTION

Chum salmon (*Oncorhynchus keta)* from North America and Asia migrate into the North Pacific Ocean generally spending four to six 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.

In Alaska, migrating chum salmon are harvested incidentally in sockeye salmon fisheries during June in the area of South Unimak Island and Shumagin Island (South Alaska Peninsula). Numerous tagging studies (e. g. Gilbert and Rich 1925; Aro et al. 1971; Aro 1972; Meyer 1983; Eggers et al. 1991) have shown that significant numbers of chum salmon harvested are not of local origin. Tag recoveries have been reported not only from areas from throughout Alaska, but also from Japan, Russia, British Columbia, and Puget Sound (Brannian 1984; Eggers et al. 1991).

Incidental catch of chum salmon in the sockeye fisheries exacerbates chum salmon allocation issues and may promote conservation problems in certain areas. In Alaska, harvests of chum salmon are fully allocated to commercial, sport and subsistence fisheries. Directed harvests in some areas, such as Norton Sound, Kotzebue Sound, and Yukon Fall run, are below historic levels. Because of the lack of current, sufficiently geographic-

specific data on stock composition of chum salmon caught as bycatch, the potential impacts of the South Alaska Peninsula June fisheries on chum salmon stocks cannot be adequately determined. Methods for identifying more specific geographic origins of chum salmon are needed to address concerns regarding conservation and allocation of chum salmon.

These procedures, which examine direct products of individual genes or the actual genetic material (i.e. DNA) itself, 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 proteins detected by allozyme electrophoresis was first applied to fisheries problems in the early 1970's. (Utter et al. 1974) and has become an important part of many salmonid management programs (e. g. Milner et al. 1981; Wishard 1980; Utter et al. 1987; Winans et al. 1994; Kondzela et al. 1994; Phelps et al. 1994). It was recognized that these underlying genetic differences could be extremely valuable to differentiate stocks in mixtures of Pacific salmon (e. g. Milner et al. 1981; Grant et al. 1980; Seeb et al. 1986; Seeb et al. 1990; Winans et al. 1994; Wilmot et al. 1992), and considerable statistical framework based on maximum likelihood estimates evolved to identify individual stocks within mixtures (Milner et al., 1981; Fournier et al., 1984; Millar, 1987, 1990; Pella and Milner, 1987; Smouse et al., 1990; Gomulkiewicz et al., 1990).

Developing a comprehensive chum salmon GSI database for the North Pacific requires international cooperation. A major step in realizing this goal was taken with the establishment of a Pacific Rim allozyme protein electrophoresis database. This database is currently being maintained by Alaska Department of Fish and Game. It 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, Seattle and Auke Bay, Alaska; Washington Department of Fisheries, Olympia; United States Fish and Wildlife Service, Anchorage; and Alaska Department of Fish and Game (ADF&G), Anchorage; with assistance from the Pacific Research Institute of Fisheries and Oceanography, Kamchatka (KoTINRO) and the Fisheries Agency of Japan.

Population Genetic Structure of Chum salmon

Identification of stocks in mixtures relies on the existence of underlying genetic differences among stocks. The genetic structure of chum salmon populations has been studied throughout much of the species range in western North America and Asia (Wishard 1980; Okazaki 1981, 1982; Beacham et al. 1985; Winans et al. 1989, 1990, 1994; Wilmot et al. 1992, 1994; Kondzela et al. 1994; Phelps et al. 1994). Results indicate considerable genetic differentiation among major regional groups. Runs of fish returning to the same general area, but showing temporal differences or other life history differences, must also be considered potentially distinct (Phelps et al. 1994; Wilmot et al. 1994). Complementary to the allozyme research, Parks et al. (1993) recently identified unique frequencies of mitochondrial DNA (mtDNA) markers that identify Japanese chum salmon from all other Pacific Rim stocks.

Alaskan populations also generally subdivide along expected geographic lines (Wilmot et al. 1992, 1994; Kondzela et al. 1994). Wilmot et al. (1994) identified at least two distinct genetic units in the Yukon River corresponding to a fall and summer run. Kondzela et al. (1994) was able to characterize three distinct Southeast Alaska island groups as well as

a mainland Southeast Alaska group. These differences have been used to identify the continent of origin of high seas mixed stock fisheries by the National Marine Fisheries Service, Northwest Fisheries Center, Seattle (Winans et al. 1989, 1990, 1994).

Extension of existing database

ADF&G has identified many Alaska areas that are underrepresented in 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. ADF&G initiated a comprehensive chum salmon genetics study in 1993 to supplement the existing database. The objectives of the study are to:

1. Develop a comprehensive chum salmon allozyme database for the North Pacific with emphasis on Northwest and Southcentral Alaskan stocks.
2. Evaluate the performance of genetic stock identification for Pacific Rim chum salmon stocks and evaluate the relative contribution of Pacific Rim chum salmon stocks to the South Peninsula Area M June fisheries.
3. Expand and develop the DNA-level genetic database for the Pacific Rim.

PROGRESS TO DATE

Baseline and Fishery Sample Collection

Extensive baseline collections have recently been collected in Alaska (Table 1). A target sample size of 100 per population unit was set; actual sample sizes varied depending on the size of the runs and availability of spawners during the sampling period. A pilot study to evaluate the feasibility of sampling the South Peninsula June fisheries was undertaken during 1993; a larger fishery sampling program was initiated in 1994 (Table 2, Figure 1). A total of 2,750 and 6,300 individual chum salmon were sampled in the two years, respectively.

Individual tissues were subsampled for both baseline and fisheries analyses, placed in 2.0 ml cryotubes, frozen as soon as possible in liquid nitrogen, and remain in liquid nitrogen during storage and shipment to the Anchorage laboratory. Upon arrival in Anchorage, samples were stored in -80° C until subsampled for allozyme analysis. An archive collection of all tissues is maintained at -80°; these same tissues are available for DNA-level analyses or exchange and standardization among laboratories.

Laboratory Methods

Allozyme laboratory analyses follow the general protocols outlined in Harris and Hopkinson (1974), May et al. (1979), Aebersold et al. (1985). Sixty-three loci, including all those recommended by the Pacific Rim allozyme database, are being analyzed (Table 3). Protocols for RFLP analysis of mtDNA follow those of Parks et al. (1993) with screening currently limited to the region coding for NADH dehydrogenase subunits 5 and 6. Polymorphisms will be detected with the restriction enzyme *AsaI*.

Statistical Analyses:

Statistical analyses objectives are: 1) to further elucidate the population genetic structure of chum salmon with particular attention to the substructure within major southcentral and western Alaska river systems; and 2) to evaluate the adequacy of the Pacific Rim baseline to estimate the composition of South Peninsula June fisheries. We are working both with our own data collected in this study and with the coastwide database.

Stock contribution to mixed fishery samples will be estimated using a conditional maximum likelihood program (GIRLSEM) developed by National Marine Fisheries Service (NMFS) (Pella and Milner 1987, Masuda et al. 1991). The precision of the stock composition estimates will be determined by bootstrap resampling (Efron and Tibshirani 1986). In bootstrapping, individuals of the stock and mixture samples are randomly resampled with replacement to obtain new samples equal in size to the original samples. Standard errors of stock composition estimates due to sampling errors in the stock and mixture samples can be estimated from the standard errors of composition estimates over resamplings of the bootstrap. Approximately 100 bootstrap resamplings should provide sufficiently accurate estimates of standard error (Masuda et al. 1991). Accuracy graphs will be obtained by constructing simulated samples of mixtures with specific stock proportions and then by bootstrap resampling the baseline to obtain estimates of stock proportions. This same type of simulation will be used to evaluate the effect of mixture sample size on the accuracy and precision of the stock composition estimates and will be used to adjust mixture sample size in succeeding years.

Project status

Baseline allozyme data on over 6,000 chum salmon from Western Alaska and the Alaska Peninsula are nearing completion. Preliminary results indicate significant regional heterogeneity among Alaska populations with the Alaska Peninsula groups being the most divergent. A total of 2,000 fishery samples are currently being analyzed (Table 2). A report of these analyses is being prepared with completion anticipated in February, 1995. In addition to the allozyme work, we plan to utilize mt DNA and the Japanese-specific marker (*N/D 5/6*) identified in Parks et al. (1993) to provide an additional estimate of the Japanese contribution to the South Peninsula June fishery. A subsample of approximately 400 individuals will be screened for this marker, and the results will be compared to those derived from the allozyme genotypes. We also plan to continue research and development of further DNA-level markers, particularly the potentially promising microsatellite markers (Tautz 1989; Valdes et al. 1993).

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Table 1. Collection locations for chum salmon genetic sampled by Alaska Department of Fish and Game, 1991-1994.

Area	Location	N
Noatak River Drainage		
	Sikusuilaq Hatchery	100
	Sikusuilaq Hatchery	100
	Noatak River	100
	Kelly Lake	100
Kobuk River Drainage		
	Salmon River	106
	Other collections	100
Norton Sound		
	Snake River	47
	Snake River	35
	Snake River	24
	Nome River	27
	Nome River	13
	Nome River	53
	Nome River	32
	Solomon River	2
	Unalakleet River	100
	Kwiniuk River	100
	Fish River	100
	Pilgrim River	90
	Tubutulik River	100
Yukon River Drainage		
	Summer Run	
	Andreafsky River, W. Fork	100

Table 1. Continued.

Area	Location	N
	Andreafsky River, E. Fork	100
	Innoko River	88
	Anvik River (mixed)	350
	Anvik River Sonar	6
	Anvik/Beaver Creek	100
	Anvik/Beaver Creek	100
	Anvik/Canyon Creek	50
	Anvik/Otter Creek	100
	Anvik/Swift River	100
	Anvik/Swift River	100
	Anvik/above Swift River	1
	Anvik/Yellow River	100
	Nulato River	100
	Gisasa River	100
	Koyukuk River	100
	Melozitna River	100
	Tanana/Chena River	86
	Tanana/Chena River	100
	Tanana/Salcha River	107
	Tanana/Salcha River	100
	Fall Run	
	Toklat River	60
	Toklat River	155
	Toklat River	200
	Tanana River (mainstem)	97
	Tanana River (mainstem)	100

Table 1. Continued.

Area	Location	N
	Bluff Cabin Slough	100
	Delta River	100
	Delta River	100
	Porcupine/Sheenjek River	100
	Porcupine/Sheenjek River	64
Kuskokwim Bay Drainages		
	Kwethluk River	100
	Kasigluk River	70
	Kisaralik River	100
	Tuluksak River	100
	Tuluksak River	100
	Aniak River Sonar	100
	Oskawalik River	58
	Kogrukluk River	75
	Kogrukluk River	50
	Stony River	200
	Tatlawiksuk River	100
	Nunsatuk River	100
	Selatna River	10
	Upper Kuskokwim River McGrath	56
	Upper Kuskokwim River McGrath (early)	100
	Upper Kuksokwim River McGrath (late)	100
	Kanektok River	18
	Kanektok River	39

Table 1. Continued.

Area	Location	N
	Kanektok River	100
	Goodnews River Weir	100
Bristol Bay		
	Togiak River	100
	Togiak River	100
	Nushagak River Sonar	89
	Upper Nushagak River	53
	Upper Nushagak River	50
	Upper Nushagak River Mulchatna River	100
	Stuyahok River	45
	Stuyahok River	57
	Alagnak River	84
	Naknek/Big Creek	80
	Egegik Bay/King Salmon River/Whale Mountain Creek	98
	Ugashik Bay/King Salmon River/Pumice Creek	100
North Alaska Peninsula		
	Cinder River/Wiggly Creek	100
	Meshik River/Plenty Bear Creek	93
	Meshik River/Braided Creek	78
	Lawrence River	100
	Nelson Lagoon/Sapsuk River	80
	Joshua Green River (early)	80
	Joshua Green River	100
	Frosty Creek	100

Table 1. Continued.

Area	Location	N
	Trader's Cove Creek	100
	St. Catherines Cove	86
	Peterson Lagoon	86
South Alaska Peninsula		
	Littlejohn Lagoon	87
	Russell Creek	100
	Russell Creek	100
	Belkofski River	87
	Volcano River	64
	Canoe Bay	100
	Zachary Bay	80
	Balboa Bay/Foster Creek	100
	Stepovak Bay/Big River	50
	Stepovak River	100
Chignik		
	Ivanoff River	94
	Kiukta Bay/Portage Creek	100
	Kujulik Bay/Northfork Creek	72
	Aniakchak River/North Fork Creek	100
	Amber Bay/Main Creek	92
	Chiginagak River	75
Alaska Peninsula Mainland District		
	Wide Bay/Kialagvik	100
	Alinchak Bay/E. Bear Bay Creek	100
	Alagogshak River	95

Table 1. Continued.

Area	Location	N
	Hallo Bay/Big River	100
Kodiak Island		
	American River	100
	Gull Cape Creek	100
	Kiliuda Bay/Dog Bay Creek	100
	Sukhoi Lagoon/Big Sukhoi Creek	100
	Sturgeon Lagoon/Sturgeon River	71
	Uganik River	100
	Kizhuyak River	88
	Kitoi Hatchery	100
Cook Inlet		
	McNeil River	60
	Susitna River/Chunilna Creek	100
	Susitna River	100
Prince William Sound		
	WHN Hatchery	92
	Keta Creek	100
	Olsen Creek	100
	Total baseline collections	10,338

Table 2. Genetic sampling for chum salmon from the South Peninsula June fishery, 1993 and 1994 by Alaska Department of Fish and Game. The subsample, which is currently under analysis, is shown.

Year	Geographic Area (ADF&G District)	N	Subsample
1993	Cape Lutke (Unimak)	940	800[1]
	Cape Lazaref to Ikatan Bay (Unimak/Southwestern)	1,810	
	Total 1993	2,750	800
1994	Shumagin Islands (Southeastern)	3,440	0
	Cape Lutke, Cape Lazaref to Ikatan Bay (Unimak/Southwestern)	2,860	1,200
	Total 1994	6,300	1,200
All years	Total	8,900	2,000

[1] Both areas were pooled together, and then a subsample was drawn.

Table 3. Stain protocols used by ADF&G to resolve enzyme coding loci in Alaska chum salmon samples. Enzyme nomenclature follows Shaklee et al. (1990), and locus abbreviations are given.

Enzyme or Protein	Enzyme Number	Locus	Tissue	Buffer
Aspartate aminotransferase	2.6.1.1	*sAAT-1,2**	H	ACE7.2 TBE
		*sAAT-3**	E	TBCLE
		*mAAT-1**	H,M	ACE7.2
		*mAAT-2**	L,H	ACN6, ACE7.2
Aconitate hydratase	4.2.1.3	*mAH-1,2**	H	ACE7.2
		*mAH-3**	H	ACE7.2
		*mAH-4**	H	ACE7.2
		*sAH**	L	ACE7.2
Alanine aminotransferase	2.6.1.2	*ALAT**	M	TBE
Creatine kinase	2.7.3.2	*CK-A1**	M	TBCLE
		*CK-A2**	M	TBCLE
		*CK-B**	E	TBCLE
		*CK-C1**	E	TBCLE
		*CK-C2**	E	TBCLE
Esterase-D	3.1.1.-	*ESTD-1**	H	TBE
		*ESTD-2**	H	TBE
Fumarate hydratase	4.2.1.2	*FH**	M	ACE7.2
beta-N-Acetylgalactosaminidase	3.2.53	*bGALA**	H	ACE7.2
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*GAPDH-1**	H	ACE7.2
		*GAPDH-2**	H	ACE7.2

Table 3. Continued.

Enzyme or Protein	Enzyme Number	Locus	Tissue	Buffer
		*GAPDH-3**	H	ACE7.2
N-Acetyl-beta-glucosaminidase	3.2.1.53	*bGLUA**	L	ACN6
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-1**	H	ACE7.2
		*G3PDH-2**	H	ACE7.2
		*G3PDH-3**	H	ACE7.2
Glucose-6-phosphate isomerase	5.3.19	*GPI-B1,B2**	M	TBCLE
		*GPIA**	M	TBCLE
Hydroxyacylglutathione hydrolase[1]	3.1.2.6	*HAGH**	H	TBE
L-Idditol dehydrogenase	1.1.1.14	*IDDH-1**	L	TBCL
		*IDDH-2**	L	TBCL
Isocitrate dehydrogenase (NADP+)	1.1.1.42	*mIDHP-1**	M,H	ACE7.2
		*mIDHP-2**	M,H	ACE7.2
		*sIDHP-1**	L	ACE7.2
		*SIDHP-2**	L	ACE7.2
L-Lactate dehydrogenase	1.1.1.27	*LDHA-1**	M	TBCLE, ACE7.2
		*LDHA-2**	M	TBCL, ACE7.2
		*LDHB-1**	E	TBCLE
		*LDHB-2**	E,L	TBCLE TBCL
L-Lactate dehydrogenase		*LDHC**	E	TBCLE
Malate dehydrogenase	1.1.1.37	*sMDH-A1**	L	ACN6

Table 3. Continued.

Enzyme or Protein	Enzyme Number	Locus	Tissue	Buffer
		*sMDH-A2**	L H	ACN6 AC5.85
		*sMDH-B1,2**	H	ACE7.2 AC5.85
		*mMDH-1**	H	ACE7.2 AC5.85
Malic enzyme (NADP+)	1.1.1.40	*sMEP-1**	M	ACE7.2
		*mMEP-1**	M	ACE7.2
		*mMEP-2**	M	ACE7.2
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	H	TBE
Dipeptidase	3.4.-.-	*PEPA**	M	TBE
Tripeptide aminopeptidase	3.4.-.-	*PEPB-1**	H	TBE, AC5.85
Proline dipeptidase	3.4.13.9	*PEPD**	M	ACE7.2
Peptidase-LT	3.4.-.-	*PEP-LT**	L	ACE7.2
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	H,L	ACE7.2
Phosphoglucomutase	5.4.2.2	*PGM-1**	H,M	ACE7.2
		*PGM-2**	H,M	ACE7.2
Superoxide dismutase	1.15.1.1	*sSOD-1**	L	TBCL
Triose-phosphate isomerase	5.3.1.1	*TPI-1**	E	TBCLE
		*TPI-2**	E	TBCLE
		*TPI-3**	E	TBCLE
		*TPI-4**	E	TBCLE

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

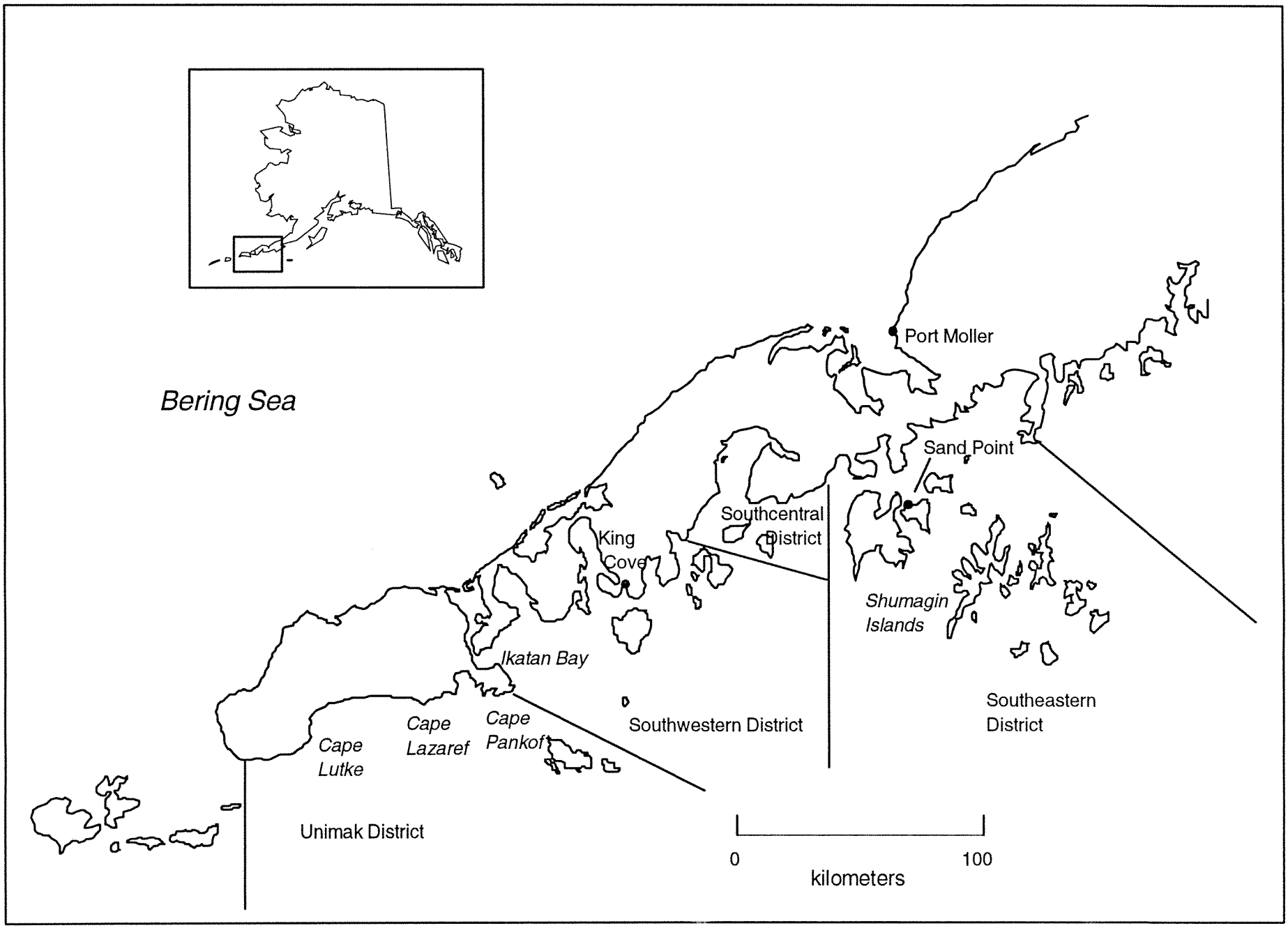

Figure 1. Map of South Alaska Peninsula fishery areas.