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ORIGINS OF SALMON SEIZED FROM THE F/V *Arctic Wind*

by

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ABSTRACT

Samples of chum (*Oncorhynchus keta*), sockeye (*O. nerka*), and chinook salmon (*O. tshawytscha*) seized from the F/V *Arctic Wind* were analyzed to determine their region of origin using genetic stock identification (GSI), otolith marks, and parasite analysis. Based on the analysis, the chum salmon samples originated in Russia, 63%; Japan, 14%; western Alaska, 11%; Alaska Peninsula and Kodiak, 6%; PWS/southeast Alaska, 4%, and British Columbia, 1%. The origins of the sockeye salmon sample were Russia, 24%; Alaska/northern British Columbia, 75%; and southern British Columbia/Washington, 2%. The origins of the chinook salmon sample were Russia, 44%; western Alaska, 23%; southcentral Alaska, 6%, and California/Oregon/Washington, 27%. No chinook salmon were detected from southeast Alaska or British Columbia.

INTRODUCTION

The F/V *Arctic Wind* was sighted with approximately 4 miles of nets in the water by a U.S. Coast Guard (USCG) C130 on 1 May, 2000 at 45^{o} 24'N, 171^{o} 58'E (Fig. 1). Section 307(a)(M) of the Magnuson-Stevens Fishery Conservation and Management Act (MSFCMA), 16 U.S.C. 1857(a)(M), implements United Nations General Assembly Resolution 44-225, *Large Scale Pelagic Driftnet Fishing and Its Impact on the Living Marine Resources of the World's Oceans and Seas*, which imposed a global moratorium on large-scale driftnet fishing beyond the Exclusive Economic Zone (EEZ) of any nation. (Large scale driftnet fishing is defined as a method of fishing in which a gillnet composed of a panel or panels of webbing, or a series of such gillnets, with a total length of two and one-half kilometers or more is placed in the water and allowed to drift with the currents and winds for the purpose of entangling fish in the webbing. 16 U.S.C. 1802(23).) On 7 May 2000, the vessel was intercepted and, after a lengthy chase, the vessel was boarded on 9 May 2000 by personnel of the USCG cutter SHERMAN. At the time of interception, approximately one ton of processed salmon was onboard. The boarding team directed the vessel return to the location where it was sighted on the 1ST of May to retrieve the nets it had left behind. A driftnet was found on 10 May, 2000 at 47^{o} 04'N, 179^{o} 17'W. The net was 6.5-8.0 km in length and contained approximately 250-300 salmon, 3 sharks, and 22 seabirds. A second net (5.0-6.5 km long) was located at 47^{o} 06'N, 179^{o} 14'W and contained approximately 425-450 salmon, 5 sharks, and 40 seabirds (Fig. 1). The F/V *Arctic Wind* was registered in Honduras and owned by Sirious Fisheries with a Vessel Agent in Pusan, Korea. The crewmembers were Russian.

Charles Guthrie from the Auke Bay Laboratory (ABL) identified the salmonid catch as sockeye (*O. nerka*), chum (*O. keta*), and chinook salmon (*O. tshawytscha*). Guthrie collected a total of 464 whole and 28 gutted chum salmon (out of a total of about 1500

onboard), all 217 sockeye salmon onboard (61 whole, 146 gutted, and 10 headed and gutted), and all 55 chinook salmon onboard (24 whole and 31 gutted). These samples were shipped to ABL for stock identification using otolith marks, parasites, and genetic characters.

Genetic stock identification relies on differences among stocks in relative frequencies of protein-coding genes detected by allozyme electrophoresis. Genetic baselines have been constructed from potentially contributing stocks around the North Pacific Ocean. A comprehensive genetic baseline for chum salmon has been developed from data provided by various state and federal agencies of Japan, Russia, Canada, and the United States (Kondzela *et al*. 1994; Phelps *et al*. 1994; Wilmot *et al*. 1994; Winans *et al*. 1994; Seeb and Crane 1995a; Urawa *et al.* 1998). A comprehensive genetic baseline for sockeye salmon has been assembled by ABL from various sources. A chinook salmon genetic baseline has been developed by an interagency work group; locus standardization, data sources, and testing of the baseline is described in Teel *et al*. (1999).

Otolith marks can aid in determining the origins of salmon. Many hatcheries purposely regulate rearing temperature to mark otoliths of young salmon. Some chum salmon hatcheries in southeastern Alaska and British Columbia release large numbers of thermally-marked fry that can be definitively identified from otoliths sampled later. Japanese and Russian hatcheries have also started releasing thermally-marked chum salmon, but these marked fish are currently small-sized and would not be seen in any numbers in catches until the year 2001. Many hatcheries in the United States and Canada also mark chinook salmon with coded-wire tags with unique numbers that will identify exactly when and where a particular fish was released.

Parasites acquired during freshwater residence of salmon can serve as useful markers in mixed-stock fisheries. Unlike most other management tools for stock separation, parasite markers require no initial tagging because the tag is acquired naturally during their freshwater residence. Parasite fauna reflect differences in the stream of origin, such as habitat, diet, limnology, or presence of intermediate hosts. A few parasites persist for a year or more, are not lost during the transition to saltwater, and have a disjunct distribution. Using the presence or absence of each parasite, certain stocks in the baseline can be eliminated as contributors to a mixed-stock fishery.

METHODS

Samples of tissue from the heart, liver, muscle, and eye were taken from whole fish, and muscle and eye from gutted fish, placed in individual tubes, and frozen at –80^{o}C until electrophoretic analysis. Protein electrophoresis was used to identify genotypes for the 20 loci available in the Pacific Rim chum salmon baseline (Seeb *et al*. 1995; Wilmot *et al*.

1998; Seeb and Crane 1999). Electrophoretic analysis followed procedures described by Aebersold *et al.* (1987) and Harris and Hopkinson (1976), and results are reported using the genetic nomenclature of the American Fisheries Society (Shaklee *et al*. 1990). Specific tissues and buffers used to interpret genetic variation at each locus for chum salmon follow Kondzela *et al*. (1994). Specific tissues and buffers used to interpret genetic variation at 72 sockeye salmon loci follow Guthrie *et al*. (1994), and 45 chinook salmon loci follow Teel *et al*. (2000).

The chum salmon baseline includes 273 populations. This baseline includes representative populations from Japan (10), Russia (18), western Alaska (30), fall Yukon River (10), Alaska Peninsula (42), Prince William Sound/southeastern Alaska (41), British Columbia (44), and Washington (78). The original data can be found in Phelps *et al*. (1994), Kondzela *et al*. (1994), Winans *et al*. (1994), Seeb and Crane (1999a), Wilmot *et al*. (1994), and some recent data from southeast Alaskan wild and hatchery populations (Auke Bay Laboratory, unpublished data). These 18 populations were added to represent the very large returns to these areas in the last few years, and the finding of thermally-marked fish from these areas in the Bering Sea bycatch in the groundfish fisheries (Farley and Munk 1997). The genetic loci used in the analysis are listed in Table 1.

A coastwide baseline of 170 populations was constructed for sockeye salmon using data from Russia (ABL, unpublished data; Alaska Department of Fish and Game (Seeb *et al*. In press, Templin *et al*. (1999)), Bristol Bay (Varnavskaya *et al.* 1994; Everett and Wilmot, unpublished data), western and southcentral Alaska Game (Seeb *et al*. In press, Templin *et al*. (1999), southeastern Alaska-British Columbia (Guthrie *et al.* 1994; Wood *et al*. 1994; ABL, unpublished data; Wood-CDFO unpublished data), and Washington (Winans *et al*. 1996) (Table 1). The baseline consisted of 12 populations from Russia, 82 from western and southcentral Alaska, 37 from southeast Alaska, 34 from British Columbia, and 5 from Washington. The locus *PGM-1** in sockeye salmon is treated as a two-state character (common and null allele) in the analysis. The brain parasite *Myxobolus* was incorporated into the analysis and treated as a two-state, non-genetic character (presence or absence) similar to the PGM-1* locus. Data on the rate of infection of *Myxobolus* was available for 88 of the 170 populations used in the analysis (4 from Russia, 18 from western Alaska and the Alaska Peninsula, 16 from southcentral Alaska, 32 from southeast Alaska, 17 from British Columbia, and one from Washington). The Statistical Program for Analyzing Mixtures (SPAM) programs can estimate missing allele frequencies for the other 82 populations incorporating an algorithm based on one developed by Smouse *et al.* (1990). A detailed explanation of the procedure can be found in SPAM Version 3.2: User's Guide, Appendix 14 (ADF&G, 1999; Debevec *et al*. In press).

A chinook salmon genetic baseline made up of 253 populations was constructed and tested by an interagency working group with data from many agencies and is described in Teel *et al*. (1999). Three populations from the Yukon River Drainage were added to the

baseline. The genetic loci used in the analysis are listed in Table 1. sMep-2* is treated as a non-genetic, two-state character. The presence or absence of the brain parasite *Myxobolus* was determined in the chinook salmon, but was not used in the genetic analysis due to the lack of information in most (213 out of 253) populations.

Conditional maximum likelihood estimates (MLE) of stock composition of the seized fish were calculated using the SPAM program (Debevec *et al.*, In press). Standard errors and 90% bootstrap confidence limits of stock composition estimates were determined by 500 resamplings of baseline and mixture samples (Efron and Tibshirani 1986). Simulation studies were conducted on the genetic baselines to evaluate the reliability of stock composition estimates using the simulation procedure contained within SPAM. Simulated baseline samples of sizes equal to actual baseline samples were generated by resampling. Mixture samples, comparable in size to that available and composed of 100% of stocks from a given region (equal proportions by the region's baseline stocks), were simulated from baseline frequencies. Genotypes of individuals in these hypothetical mixtures of known composition were generated from baseline frequencies assuming independence of characters and Hardy-Weinberg equilibrium for genetic loci. The SPAM program calculated the MLE of stock composition for each of 500 simulated sets of baseline and mixture samples, and the average MLEs of regional composition were reported and compared with the true contribution. The 100% simulations were repeated for each region.

The brain was removed from the heads of the sockeye and chinook salmon and examined for the myxosporidian *Myxobolus* as described in Moles *et al*. (1990). Due to their limited time in freshwater, chum salmon do not acquire parasites suitable for identifying their origins. The heads from the chum salmon were removed and sent to the ADF&G Mark, Tag, and Age Laboratory in Juneau, AK to determine the presence of hatchery thermal marks. Examples of thermal marks used in Japan and the Russian Far East were provided to the processing technicians. Each otolith was read once and questionable patterns were read a second and third time (Personal Communication, Ryan Scott, ADF&G). The chinook salmon heads were also examined for the presence of coded-wire tags. All fish were measured for length, whole fish were weighed, and scale samples were taken for aging. The age, length, and weight data will not be analyzed until a later date at which time they will be reported. All three species of salmon were predominately large, maturing fish that would have spawned in fall 2000.

CHUM SALMON

RESULTS

As is characteristic of the estimation methodology, simulations of the chum salmon

baseline showed that the SPAM program underestimated the 100% regional contributions as follows: Japan (95%), Russia (88%), Western Alaska (90%), Fall Yukon (92%), Alaska Peninsula (88%), Prince William Sound (PWS)/southeast Alaska (77%), British Columbia (74%), and Washington (89%). The estimates with the 90% bootstrap confidence intervals are shown in Figure 1A.

Regional stock group estimates (Table 2) indicated that most of the chum salmon sample originated from Russia, 63% (54-73%), mainly from the Kamchatka Peninsula. Japan was the next largest source at 14% (9.0-19%) followed by western Alaska at 11% (4-18%), Alaska Peninsula at 6% (1-12%), and PWS/southeast Alaska at 4% (0-9%). Less than 1% of the sample was estimated to have originated in British Columbia, Washington, or the fall Yukon River group.

Analysis of the chum salmon otoliths by ADF&G showed that no thermal-marked otoliths were observed in the samples taken from the F/V *Arctic Wind*.

DISCUSSION

The accuracy of the original chum salmon genetic baselines of 69 and 77 populations has been tested widely and used in previous mixed-stock analyses including the analysis of chum salmon bycatch in the sockeye salmon fisheries near south Unimak and Shumagin Islands along the southern Alaska Peninsula (Seeb *et al*. 1997a; Seeb *et al*. 1997b; Seeb and Crane 1999; Crane and Seeb 2000), the chum salmon bycatch in the Bering Sea trawl fishery for walleye pollock (Wilmot *et al*. 1998), and a high seas sample of juvenile chum salmon (Urawa *et al*. 1998; Winans *et al*. 1998). The expanded 273 population baseline produced only slightly different results from the previous baselines in the 100% simulations and estimations for Japan, Russian, Western Alaska, and fall Yukon River. However, the amount of misallocation between the Alaska Peninsula, PWS/southeast Alaska, British Columbia, and Washington increased substantially, and suggests further research into the relationship among the populations of these regions. The estimated presence of nearly 77% Asian-origin chum salmon in the catch was plausible given known migration routes (Myers *et al*. 1998) and the location where the F/V *Arctic Wind* was fishing. Also, the small proportion of Alaska-origin fish is consistent with earlier analyses of the Bering Sea trawl fishery (Wilmot *et al*. 1998) and the high seas samples by Urawa *et al*. (1998) and Winans *et al*. (1998). The routine presence of chum salmon from central and southeastern Alaska and British Columbia hatcheries in waters on the Pacific and Bering Sea sides of the Aleutian Islands was previously confirmed by the recovery of fish with thermally-marked otoliths (Farley and Munk 1997).

SOCKEYE SALMON

RESULTS

Data for only14 loci (Table 1) were available across all regions in the 170 population baseline. Data for 10 other variable loci were missing for some baseline populations: Russia (*sAH-1*, PEPC*, PEPLT*, TPI-1,2*, TPI-3*,* and *TPI-4*);* Bristol Bay *(PEPC**); British Columbia (*GPIA*, PEPD-1*, TPI-1,2*, TPI-3*,* and *TPI-4*);* and Washington State (*sMEP-1*, mMEP-1*,* and *PEPD-1**).

Previous work (Wilmot *et al*. 1999) showed that populations from Russia and southern British Columbia/Washington displayed some regional clustering. The populations from southwestern, southcentral, southeastern Alaska, and northern British Columbia formed a large amorphous group. Therefore, only three regional reporting areas were deemed statistically valid: Russia, Alaska and northern British Columbia, and southern British Columbia and Washington.

The estimates of the origins of the sockeye salmon sample by region using only the 14 genetic loci were: Russia, 24% (13-39%); Alaska/northern British Columbia, 75% (57-85%); and southern British Columbia/Washington, 2% (0-8%). When the rate of *Myxobolus* infection was added to the analysis, the estimates were: Russia, 23% (9-34%); Alaska/northern British Columbia, 77% (63-89%); and southern British Columbia/Washington, 1% (0-5%). Examination of the sockeye salmon heads of the confiscated sample for the brain parasite *Myxobolus* showed that 37% (77/207) were infected. Average rate of infection among sampled stocks of the regions were as follows: Russia (80%), Alaska/northern British Columbia (28%), and southern British Columbia/Washington (19%).

The 100% simulation studies (Figure 2) showed that if the F/V *Arctic Wind* catch had been composed of equal proportions of the baseline stocks of Russia alone, 82% (90% C.I. of 70-92%) would have been estimated correctly as originating in Russia. Corresponding values for the other regions were: Alaska/northern British Columbia at 91% (81-98%), and southern British Columbia/Washington at 89% (80-97%). Approximately 17% of the Russian fish were misallocated to North American stocks and only 1% to southern British Columbia/Washington. The North American regions misallocated mainly within North America rather than to Russia.

DISCUSSION

In contrast to the chum and chinook salmon genetic baselines, the sockeye genetic baseline has not been thoroughly tested by an interagency working group. The presence of geographical structuring using the 14 loci common to all stocks was only evident for the

Russian and the southern British Columbia/Washington stocks (Wilmot *et al.* 1999). All the Alaska and northern British Columbia stocks formed a large, amorphous group with little indication of further regional structuring. The 100% simulation tests did show success can be expected in estimates for three regions; Russia, Alaska/northern British Columbia, and southern British Columbia/Washington (Figure 2B). Approximately 17% of the Russian fish were misallocated to North America and less than 2% of the North American regions were misallocated to Russia.

The use of the parasite and genetic data together in the analysis had little effect on the results.
Most large stocks of sockeye salmon in Russia have high rates of *Myxobolus* infection (Moles *et al.* 1990), as does Prince William Sound, southeast Alaska, and northern British Columbia (Moles and Jensen 1999; Rutherford *et al.* 1992). *Myxobolus* is nearly absent in western and southcentral Alaska except for Nelson Lagoon on the Alaska Peninsula, Karluk River on Kodiak Island, and Pauls Bay on Afognak Island. The largest change made by adding the parasite data to the analysis was to reallocate approximately 2% of the sample from southern British Columbia/Washington to the Alaska/northern British Columbia group.

The use of genetic and parasite data show the potential for discriminating among stocks of sockeye salmon. However, the database is incomplete for genetic data, and the parasite baseline needs to be expanded. Obtaining data from all regions for the additional 10 variable loci should substantially improve our ability to discriminate between regions within North America. Efforts underway by a number of federal and state agencies in the United States and Canada to develop a microsatellite DNA baseline may hold promise for more reliable stock identification

CHINOOK SALMON

RESULTS

The 100% simulations of the chinook salmon genetic baseline showed that the GSI program could correctly estimate the region of origin as follows: Russia (93%); western Alaska (84%); southcentral Alaska (88%); southeast Alaska 67%); British Columbia (78%); and California/Oregon/Washington (89%). The estimates with the 90% confidence intervals for a simulated mixed-stock sample of N=55 are shown in Figure 2C (black circles). Figure 2C (open circles) shows how the estimates would improve significantly if there were a larger mixture sample size (N = 300).

Regional stock group estimates and the 90% confidence intervals (Table 4) show the largest percentage of the sample were Russian at 44% (29– 58%), followed by California/Oregon/Washington at 27% ((14– 40%), western Alaska at 23% (5–36%), and

southcentral Alaska at 6% (0–25%). None of the sample was estimated to be from southeast Alaska or British Columbia.

Examination of the chinook salmon for the brain parasite *Myxobolus* showed that 40% (21/53) of the sample seized from the F/V *Arctic Wind* were infected. These data were not included in the mixed-stock analysis for chinook salmon due to the availability of information about the rate of infection in only 40 of 268 stocks.

DISCUSSION

An examination and testing of the chinook salmon genetic baseline by Teel et al (1999) showed a greater than 90% accuracy on broad regional groupings. Our simulations using six reporting groups, a mixture sample size of 55, and 22 loci did not achieve the same degree of accuracy. However, as shown in Figure 2C (open circles), increasing the mixture sample size to 300 would have significantly improved our results and this shows how important an adequate mixed-stock sample size is in order to achieve reasonably useful estimates of regional origin.

Since the F/V *Arctic Wind* was intercepted only 526 km south of Adak, Alaska, large percentages of the catch from western Alaska and California/Oregon/Washington are plausible. Coded-wire tag recoveries in the Bering Sea and Gulf of Alaska groundfish fisheries have shown significant numbers from both areas (Myers *et al*. 1998). The presence of Russian chinook salmon in the sample is also credible. Myers *et al*. (1998) reports that the combined results of tag, scale, and parasite data indicate extensive overlap in the oceanic ranges of Russian and North American chinook salmon in the North Pacific Ocean from 160^{o}E to 145^{o}W.

The use of the brain parasite *Myxobolus* could add to the accuracy of the analysis for chinook salmon. Urawa *et al*. (1998) reported that *Myxobolus* is commonly found in Asian stocks (57 – 94%) and rarely in North American stocks except for those from Vancouver Island, B.C. The presence of nearly 40% infection in the samples seized from the F/V Arctic Wind supports a large Russian contribution, as indicated by the genetic analysis.

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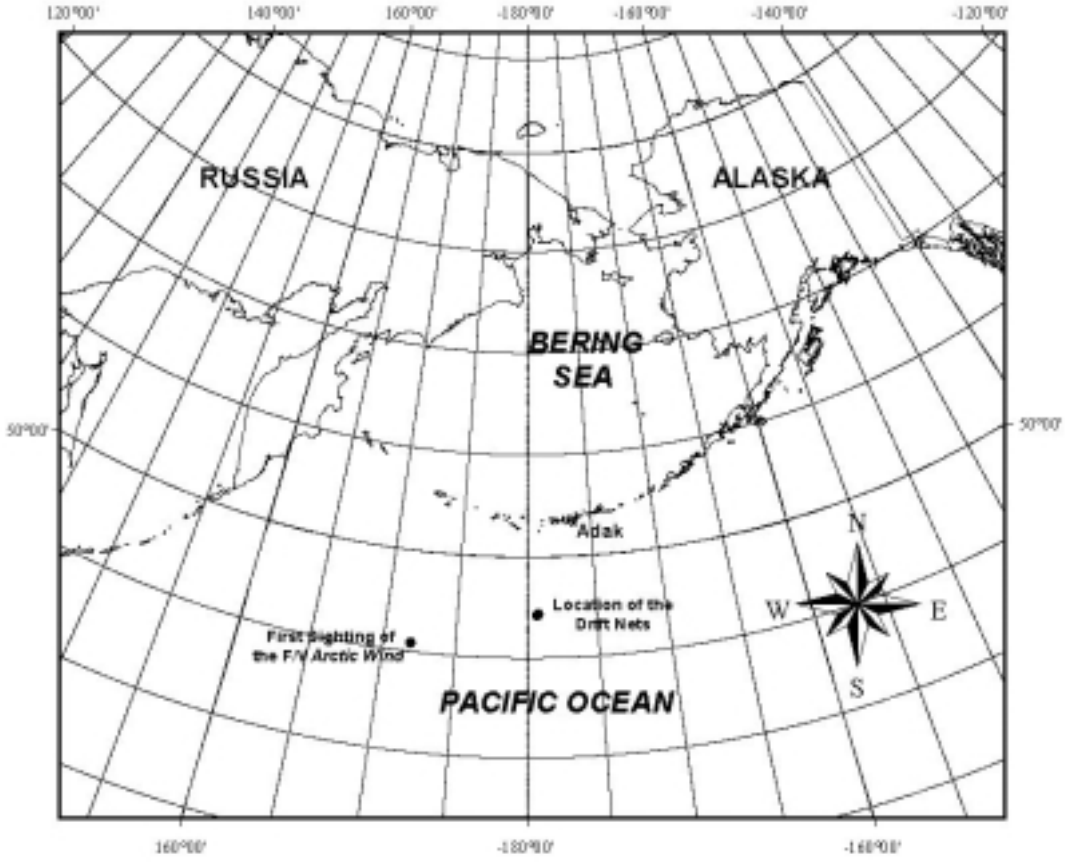

Figure 1. Location of the first sighting of the F/V *Arctic Wind* on May 1, 2000 and the location of the drift nets.

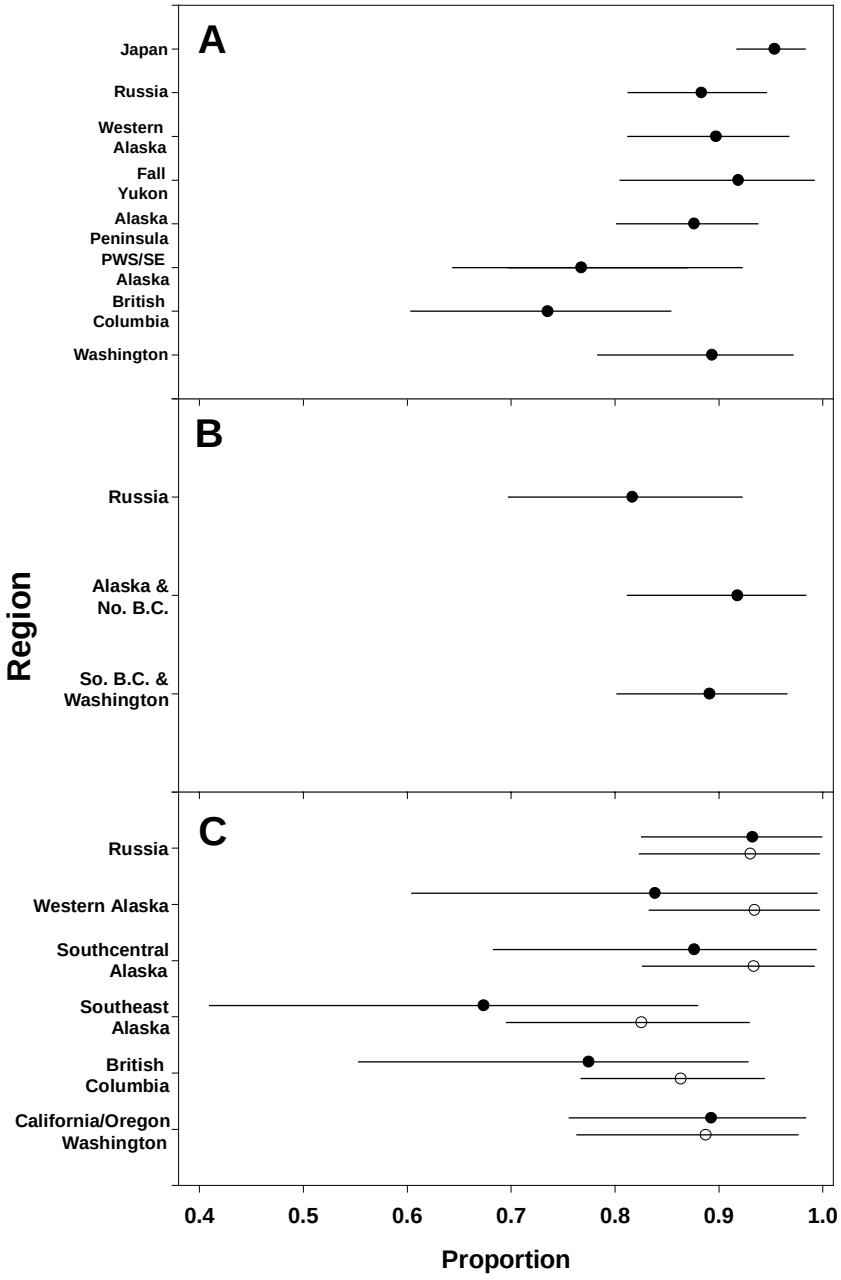

Figure 2. 100% simulation results for reporting groups for (A) chum salmon, (B) sockeye salmon, and (C) chinook salmon. Point estimates and 90% confidence intervals are given for each reporting group. The simulated mixture samples were set to equal the actual samples taken from the F/V *Arctic Wind*, chum salmon (N=300), and sockeye salmon (N=207). For chinook salmon the black circles represent the actual sample size (N=55), and the open circles show how the estimates and 90% confidence intervals would improve with an adequate mixture sample size (N=300).

Table 1. Genetic loci used for each species in the stock identification program.

Chum	Sockeye	Chinook
*sAAT-1,2**	*sAAT-1,2**	*mAAT-1**
*mAH-3**	*mAAT-1**	*sAAT-1,2**
*mAAT-1**	*ALAT**	*ADA-1**
*ALAT**	*GPIB-1,2**	*sAH**
*ESTD**	*sIDHP-1**	*mIDHP-2**
*GPIB-1,2**	*sIDHP-2**	*sIDHP-1**
*GPIA**	*LDHB-1**	*sIDHP-2**
*mIDHP-1**	*LDHB-2**	*LDHB-2**
*sIDHP-2**	*MPI**	*mMDH-2**
*LDHA-1**	*sMDHA-1,2**	*sMDHA-1,2**
*LDHB-2**	*sMDHB-1,2**	*sMDHB-1,2*
*MPI**	*PGM-1**	*sMEP-2**
*PEPB-1**	*PGM-2**	*MPI**
*sMDHB-1,2**	*sSOD-1**	*PEPA**
*sMEP-1**	*Myxobolus*[1]	*PEPD-2**
*mMEP-2**		*PGDH**
*PEPA**		*PGK-2**
*G3PDH-2**		*PGM-1**
*sMDHA-1**		*PGM-2**
*PGDH**		*sSOD-1**
		*TPI-3**
		*TPI-4**

[1] *Myxobolus* in sockeye is a brain parasite and is a non-genetic character.

Table 2. Estimates of regional origins and 90% bootstrap confidence intervals for the chum salmon sample seized from the F/V *Arctic Wind*.

Region	Estimate	90% C.I.
Japan	0.137	0.085 - 0.193
Russia	0.630	0.537 - 0.733
Western Alaska	0.110	0.044 – 0.183
Fall Yukon	0.003	0.000 – 0.016
Alaska Peninsula	0.060	0.009 – 0.122
PWS/southeast Alaska	0.041	0.003 – 0.087
British Columbia	0.010	0.000 – 0.033
Washington	0.001	0.000 – 0.019

Table 3. Estimates of regional origins and 90% confidence intervals for the sockeye salmon sample seized from the F/V *Arctic Wind* without and with the use of the brain parasite *Myxobolus*.

Region	Estimate	90% C.I.
Russia		
w/o *Myxobolus*	0.239	0.134 – 0.394
with *Myxbolus*	0.226	0.094 – 0.339
Alaska/No. British Columbia		
w/o *Myxobolus*	0.746	0.568 – 0.849
with *Myxbolus*	0.769	0.632 – 0.892
So. British Columbia/Washington		
w/o *Myxobolus*	0.015	0.000 – 0.079
with *Myxbolus*	0.005	0.000 – 0.052

Table 4. Estimates of regional origins and 90% confidence intervals for the chinook salmon sample seized from the F/V *Arctic Wind*.

Region	Estimate	90% C.I.
Russia	0.444	0.286 – 0.583
Western Alaska	0.233	0.047 – 0.360
Southcentral Alaska	0.055	0.000 – 0.247
Southeast Alaska	0.000	0.000 – 0.023
British Columbia	0.000	0.000 – 0.004
California/Oregon/Washington	0.269	0.144 – 0.402