

NPAFC
Doc. 598
Rev. _____

ORIGINS OF SALMON SEIZED FROM THE F/V *PETROPAVLOVSK*

by

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Submitted to the
NORTH PACIFIC ANADROMOUS FISH COMMISSION
by the
UNITED STATES OF AMERICA

May 2002

THIS PAPER MAY BE CITED IN THE FOLLOWING MANNER:

C. M. Kondzela, S. Hawkins, C. M. Guthrie III, and R. L. Wilmot, R. 2002. Origins of salmon seized from the F/V *Petropavlovsk*. (NPAFC Doc. 598) Auke Bay Fisheries Laboratory, Alaska Fisheries Science Center, NMFS, NOAA, 11305 Glacier Highway, Juneau, AK 99801-8626. 10 pp.

ABSTRACT

On 25 July 2001, the Russian factory trawler *Petropavlovsk* was seized in the Bering Sea seven miles into the U. S. EEZ by the U. S. Coast Guard. There was approximately 75 metric tons of pink and chum salmon onboard that the captain claimed were purchased from the Russian coastal fishing fleet off the east coast of Kamchatka. Samples of both pink and chum salmon were analyzed using stock identification methods to determine whether they were of Asian or North American origin. The origins of the chum salmon samples were 92% Asian and 8% North American by the standard maximum likelihood (MLE) method, and 100% Asian by the Bayesian method. The origins of the pink salmon samples were 85% Asian and 15% North American by the MLE method, and 99% Asian and 1% North American by the Bayesian method. Results of the analysis do not refute the F/V *Petropavlovsk* captain's statement that he purchased the salmon in Russian waters.

INTRODUCTION

On 25 July 2001, the 309-foot Russian factory trawler *Petropovlosk*, with 81 crew members on board, was seized in the Bering Sea by the U.S. Coast Guard (USCG) using the cutter *Storis*. The USCG spotted the vessel illegally trawling seven miles into the U. S. Exclusive Economic Zone (EEZ) at 60° 34.5' N, 178° 46.6W (Figure 1). There were approximately 75 metric tons of frozen salmon (*Oncorhynchus* spp.), 10 tons of pollock, and 22 tons of fish meal on board. There were 66 tons of pink salmon (*O. gorbuscha*), 8.6 tons of chum salmon (*O. keta*) and 0.364 tons of char. The salmon were gutted and frozen into blocks. The captain of the vessel claimed that the salmon were purchased from local Russian fishermen while he was anchored up for repairs in Karaginsky Bay on the east coast of Kamchatka.

Chuck Guthrie from the Auke Bay Laboratory (ABL) traveled to the *Petropavlovsk* and confirmed the species identification of the salmon on board and requested that 500 pink and chum salmon be retained for genetic sampling for stock identification. About 2% of the chum were misidentified by the crew and determined to be sockeye salmon (*O. nerka*) during subsequent examination and analysis at ABL. Scales, otoliths, length, and weight were taken from the chum salmon. One hundred ninety eight walleye pollock (*Theragra chalcogramma*) were retained for length determination. Tina Antonson, NMFS enforcement agent, secured and shipped the samples to ABL.

METHODS

Samples of tissue from the muscle and eye were taken from gutted fish, placed in individual tubes, and frozen at -80°C until electrophoretic analysis. Protein electrophoresis was used to identify chum salmon genotypes at 16 loci available in the Pacific Rim chum salmon baseline (Seeb *et al.* 1995; Wilmot *et al.* 1998; Seeb and Crane 1999), and pink salmon genotypes at 19 loci. 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 and pink salmon follow Kondzela *et al.* (1994) and Noll *et al.* (2001).

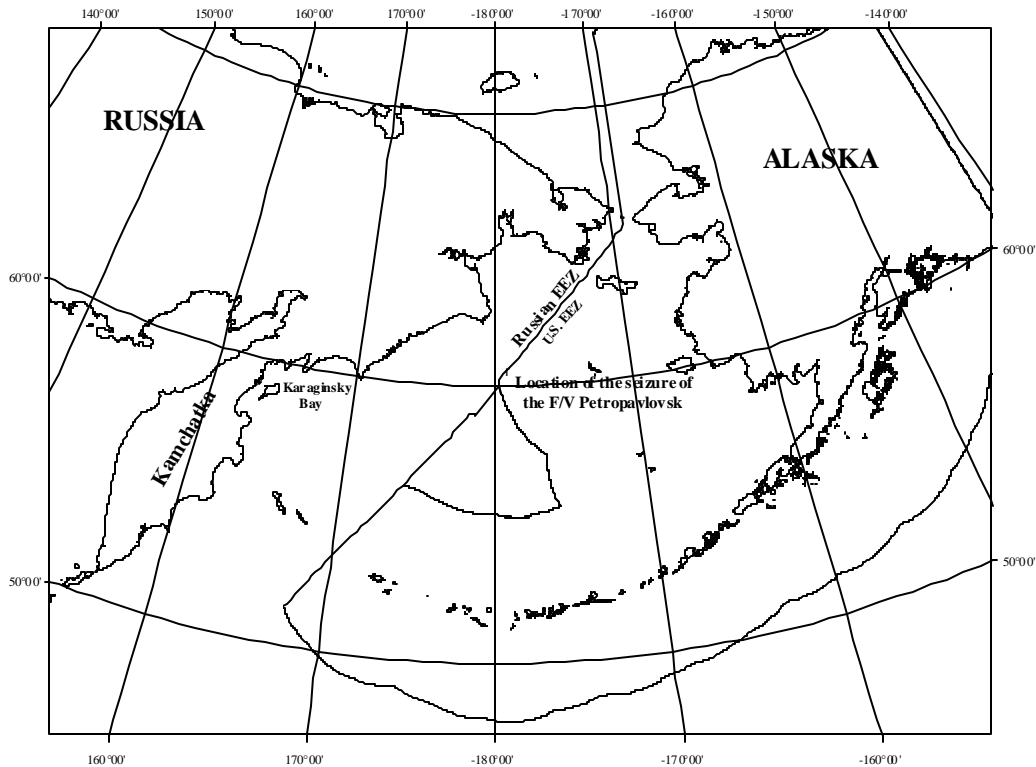


Figure 1. Map of the Bering Sea showing the location of the seizure of the Russian fishing vessel *Petropavlovsk*.

The chum salmon genetic baseline for the Pacific Rim includes 273 populations, with 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 allele frequency 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), including some recent data from southeast Alaskan wild and hatchery populations (ABL, unpublished data). The additional 18 southeast Alaska populations were added due to the very large returns of this region in the last few years, and the finding of thermally-marked fish from this region in the Bering Sea bycatch in the groundfish fisheries (Farley and Munk 1997).

The pink salmon genetic baseline was developed from data analyzed at the ABL, Washington Department of Fish and Wildlife, and the Alaska Department of Fish and Game (ADF&G) Gene Conservation Laboratory. Cooperative standardization of alleles between the various laboratories has not yet been accomplished such as that for the chum salmon genetic baseline. Therefore, many alleles were pooled to insure compatibility of the baseline data from all sources. Regional structuring among the baseline populations was determined using multidimensional scaling (Kruskal, 1964) of Cavalli-Sforza and Edwards (1967) chord distance.

Conditional maximum likelihood estimates (MLE) of stock composition of the seized fish were

calculated using the SPAM program (Debevec *et al.*, 2000). Standard errors and 95% bootstrap confidence limits of stock composition estimates were determined by 1000 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 1000 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 chum and pink salmon mixtures were also analyzed with the Bayesian method developed by Pella and Masuda (2001), and results compared to those obtained from the MLE method. Under the Bayesian method an informative prior for genetic characters of the separate stocks in a mixture is derived from baseline samples. A neutral, low information prior is used for the stock proportions in the mixture. A Gibbs sampler – the data augmentation algorithm – is used to alternatively generate samples from the posterior distributions of genetic parameters of the separate stocks and for the stock proportions in the mixture. The posterior distribution incorporates the information about genetic characters in the baseline samples, including relatedness of stocks, with that in the stock-mixture sample to better estimate genotypic composition of the separate stocks. The mean, mode, and 95% confidence interval for the posterior distributions of stock proportions were reported

RESULTS

Chum Salmon

The current chum salmon genetic baseline for the Pacific Rim contains data for 20 genetic characters across all regions of the North Pacific Ocean. The samples collected from the *F/V Petropavlovsk* had been gutted and we were therefore restricted to obtaining data only from muscle and eye tissue. As a result, data was obtained for only 16 of the 20 loci in the complete baseline (*sAAT-1,2**; *mAAT-1**; *ALAT**; *ESTD**; *GPI-B1,2**; *GPI-A**; *mIDHP-1**; *LDH-A1**; *LDH-B2**; *MPI**; *PEPB-1**; *sMDH-B1,2**; *sMEP-1**; *mMEP-2**; *PEPA**; and *PGDH**).

Simulations of 100% Asian and North American stocks showed excellent resolution using 16 loci and are comparable to simulations using the 20 loci baseline (Wilmot *et al.* 1998). The MLE simulations for 100% Asian stocks correctly identified region of origin at 90% (84% to 98%) Asian origin, and 10% (2% to 16%) North American origin. Results of the 100% simulations for the North American stocks were 97% (95% to 100%) North American origin and 3% (0% to 5%) Asian origin.

The MLE estimate (Table 1) of the regional composition of the chum salmon from the *F/V Petropavlovsk* was 92% Asian origin (91% to 100%) and 8% North American origin (0% to 9%).

The mode of estimates of the regional composition from the Bayesian program was 100% Asian origin (92% to 100%). Figure 3A displays the posterior distribution of estimates of the Asian proportion.

Table 1. Regional proportions of the chum salmon from the *F/V Petropavlovsk*.

Region	MLE Estimates		Bayesian Estimates		
	Estimate	95% C.I.	Mean Estimate	Mode Estimate	95% C.I.
Asia	0.92	0.913 – 1.000	0.98	0.995	0.916 – 1.000
North America	0.08	0.000 – 0.087	0.02	0.005	0.000 – 0.084

Pink Salmon

Nineteen loci were resolved in the pink salmon samples. They were *sAAT-1,2**; *sAAT-3**; *mAH-3**; *ALAT**; *CK-A1**; *CK-A2**; *GPIA**; *GR**; *G3PDH-1**; *LDH-B1**; *LDH-B2**; *sMDH-B1,2**; *mMEP-1**; *PEPA**; *PEPB-1**; *PEPD-2**; *PEPLT**; *PGDH**; and *PGM-2**.

The multidimensional scaling analysis (Fig. 2) of the odd brood-year pink salmon populations shows three major groupings: a group consisting of all the Japanese and Russian stocks plus the two stocks from northwest Alaska; a second group consisting of stocks from Prince William Sound, southeast Alaska, and northern and central coast British Columbia; and a third group consisting of southern British Columbia and Washington stocks.

Simulations of 100% Asian and North American stocks showed excellent resolution using 19 loci. The MLE simulations for 100% Asian stocks correctly identified region of origin at 97% (94% to 100%) Asian origin, and 3% (0% to 6%) North American origin. Results of the 100% simulations for the North American stocks were 98% (96% to 100%) North American origin and 2% (0% to 4%) Asian origin.

The MLE estimate of the regional composition of the pink salmon from the *F/V Petropavlovsk* was 85% Asian origin (74% to 100%) and 15% North American origin (0% to 26%). The mode of Bayesian estimates was 99% Asian origin (66% to 100%) and 1% North American origin (0% to 34%). Figure 3B displays the distribution of the Bayesian estimates of Asian origin.

Table 2. Estimates of the regional origins of the pink salmon from the *F/V Petropavlovsk*.

Region	MLE Estimates		Bayesian Estimates		
	Estimate	95% C.I.	Mean Estimate	Mode Estimate	95% C.I.
Asia	0.85	0.742 – 1.000	0.92	0.99	0.662 – 0.999
North America	0.15	0.000 – 0.258	0.08	0.01	0.001 – 0.338

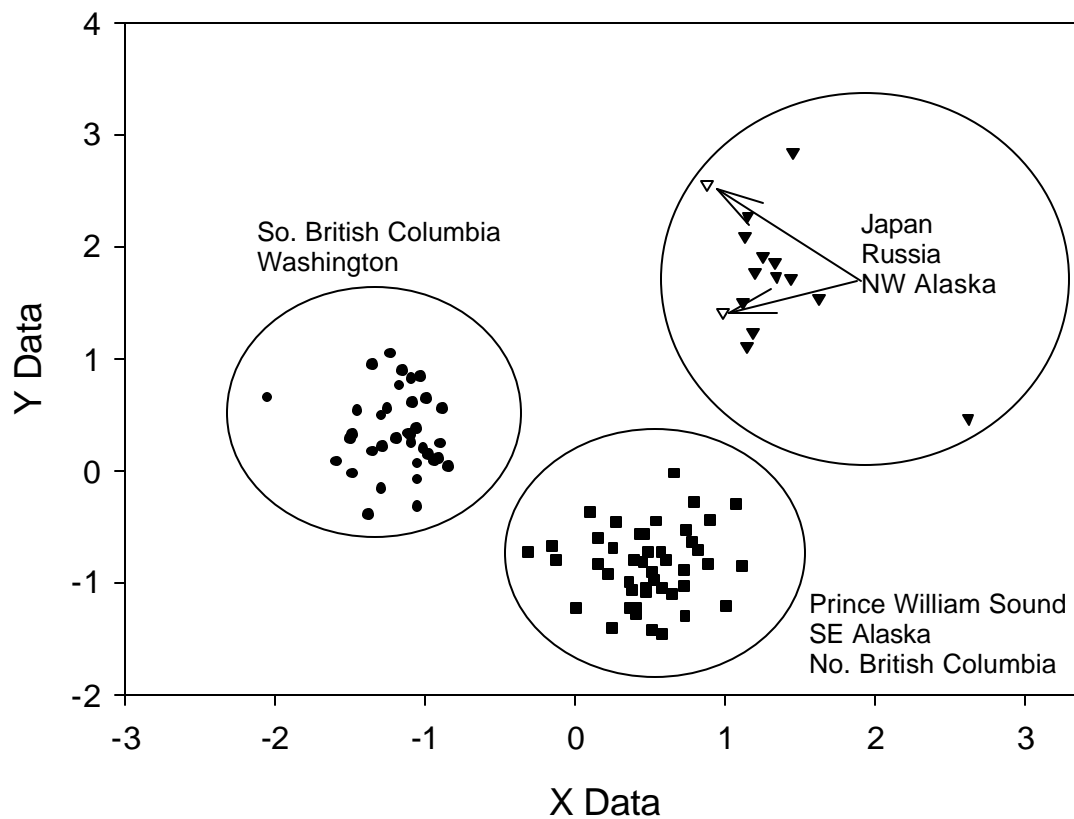


Figure 2. Multidimensional scaling of 95 stocks of Pacific-rim odd brood-year pink salmon using Cavalli-Sforza and Edwards (1967) chord distance calculated from 19 genetic loci.

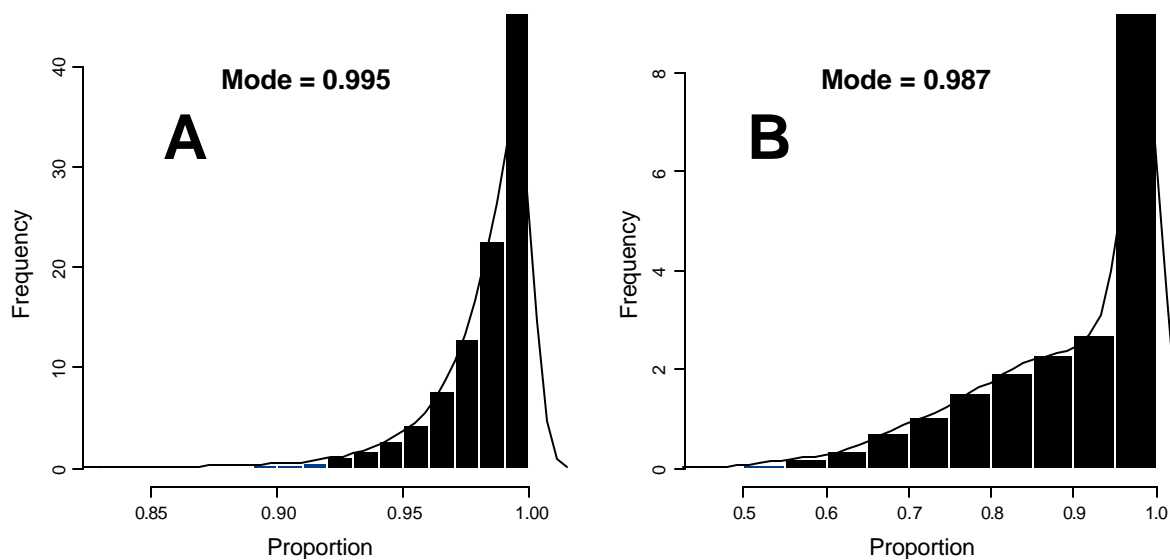


Figure 3. Bayesian posterior distributions of the Asian proportions of chum salmon (A) and pink salmon (B) from the F/V *Petropavlovsk*.

DISCUSSION

Chum Salmon

The Pacific-rim chum salmon genetic baseline has been tested and used extensively (Seeb et al. 1995; Seeb et al. 1997; Wilmot et al. 1998; Urawa et al. 1998; Seeb and Crane 1999; Urawa et al. 1999; Wilmot et al. 1999; Wilmot et al. 2000). This baseline can accurately discriminate sub-areas within both Asia and North America. However, for the purpose of this report, the main objective was to determine whether these fish were of Asian origin in order to verify or disprove the statement by the captain of the F/V *Petropavlovsk* that he purchased these fish from the Russian coastal fisheries. Therefore, we did not attempt to discriminate beyond Asia versus North America origin. A new and more extensive chum salmon genetic baseline is currently being developed and will be used in a later revision to determine origins of the chum samples taken from the F/V *Petropavlovsk* based on smaller geographic regions.

Both the MLE and Bayesian estimates (Table 1) of the origins of the chum salmon samples taken from the F/V *Petropavlovsk* show these fish were predominately of Asian origin (MLE = 91.7% and Bayesian = 100%). Estimates of North American origin were 8.3% and 0% respectively. At their closest point, Russia and Alaska are only slightly more than 100 kilometers apart. Possibly some North American fish could occur on the Asia side of the Bering Sea. However, all the chum salmon samples were beginning to change color indicating they were close to entering spawning streams. Two likely and related explanations for positive estimates of some North American fish are: 1) the stock identification programs have a bias in that they tend to over-estimate stocks in very low abundance, and under-estimate stocks in very high abundance, and 2) some chum salmon stocks in Russian and western Alaska have been shown to be genetically similar (Seeb and Crane 1999). Therefore, some Russian origin chum salmon are probably misclassified to Alaskan stocks.

Pink Salmon

The multidimensional scaling analysis (Figure 2) shows three very distinct groups of odd-year pink salmon: a group consisting of stocks from southern British Columbia; a second group consisting of stocks from northern British Columbia, southeast Alaska, and Prince William Sound; and a third group consisting of stocks from Japan, Russia, and two stocks from northwest Alaska (the Nome and Snake rivers). This situation mirrors genetic similarity between some Russian and western Alaska chum salmon stocks. The Nome and Snake rivers are only about 350 km from the Russian coast.

As with chum salmon, we restricted the determination of the origin of the pink salmon samples to Asia and North America. In addition to this being the question of main concern, the genetic baseline for odd brood-year pink salmon has not been thoroughly analyzed, tested, and agreed upon by the various scientists that have collected the baseline data. Alleles at many loci had to be pooled to insure compatibility among data sets. Pooling probably resulted in a loss of discriminating ability between major regions and masked even finer discrimination within these major regions. Therefore, we felt it was not appropriate to push the analysis beyond this level of discrimination.

Both the MLE and Bayesian estimates (Table 2) of the origin of the pink salmon samples taken from the F/V *Petropavlovsk* show these fish were predominately of Asian origin (MLE = 85.4% and Bayesian mode = 99%). Estimates of North American origin were 14.6% and 8.0% respectively. As with the chum salmon samples, the pink salmon samples showed signs of maturation. Given the geographic proximity of Russia and Alaska, some North American fish could be present in the western Bering Sea. However, given the close genetic affinity between the pink salmon stocks of northwestern Alaska and Russia, some misclassification of Russian origin fish to Alaskan stocks may have occurred in estimation. As was stated above, the bias of the stock identification programs to over estimate stocks of low abundance and under estimate stocks of high abundance could explain the apparent presence of North American origin fish even if absent.

Maximum Likelihood Estimates Versus Bayesian Estimates

Pella and Masuda (2001) indicate that among the advantages of the Bayes method, the mode of the posterior distribution could be used as an estimator of stock composition. The mode appeared to be less biased than the mean in their simulations. Here the mode is the regional composition with the highest posterior probability. If the mode is used in this analysis, the contributions of Asian stocks increase to near 100% for both species. Given that the inherent bias of the methodology would underestimate the predominant Asian component, the Bayesian mode is a sensible estimator that has no logical counterpart in the MLE method other than the MLE point estimate.

CONCLUSIONS

The analyses of the chum and pink salmon taken from the F/V *Petropavlovsk* indicate they were predominately of Asian origin.

ACKNOWLEDGMENTS

James Shaklee of Washington Department of Fish and Wildlife and Jim Seeb and Bill Templin of Alaska Department of Fish and Game provided baseline data for odd brood-year pink salmon .

We would like to thank the following for logistics support in obtaining the samples: NMFS Enforcement: Tina Antonson, Mike Mahaffey, Jeff Passer and Gary Gailbreth, USCG, including the *Storis* boarding party; Michael Ingrassia who helped drag cases of fish out of the hold; and a special thanks to Capt. Vince O'Shea for helping the biologist depart Cold Bay.

We also thank at ABL: John Pohl, Hanhvan Nguyen and Ellen Martinson for sample processing, and/or laboratory assistance; and Tom Dress, Russ Senkovich, and Mike Anderson for moving totes of fish. Michele Masuda and Jerry Pella provided invaluable assistance for properly applying the Bayesian methodology to the analysis of the data, and for reviewing this manuscript.

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