

Genetic Identification of Juvenile Pink Salmon Improves Accuracy of Forecasts of Spawning Runs in the Okhotsk Sea Basin

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Shpigalskaya, N.Yu., A.I. Kositsina, U.O. Muravskaya, and O.N. Saravansky. 2016. Genetic identification of juvenile pink salmon improves accuracy of forecasts of spawning runs in the Okhotsk Sea basin. N. Pac. Anadr. Fish Comm. Bull. 6: 415–420. doi:10.23849/npafcb6/415.420.

Abstract: Genetic methods were applied to identify the origin of juvenile even-year pink salmon (*Oncorhynchus gorbuscha*), collected from 12 locations in the Okhotsk Sea surveyed by the RV *TINRO* in October and November 2013. According to the results we have obtained, the proportion of the 'northern' group of populations in the Okhotsk Sea basin, including western Kamchatka and the northern continental coast of the Okhotsk Sea, in mixed aggregations of juvenile pink salmon, is relatively small—about 24%. The largest proportion (58%) of the juvenile aggregations was identified as Sakhalin-Kuril stocks. The proportion identified as populations from the Amur River and Primorye was about 17%, with fish of 'unknown' origin making up < 1%. It should be noted that the results we obtained for 2013 did not correspond to the existing regional ratios for pink salmon runs of even-year generations in the Okhotsk Sea basin.

Keywords: pink salmon, Okhotsk Sea, mitochondrial DNA, haplotypes, stock identification

INTRODUCTION

Juvenile pink salmon aggregate in the Okhotsk Sea in their early marine period following emigration from streams in western Kamchatka, the continental coast of the Okhotsk Sea, Sakhalin and the Kuril islands, the Amur River, and Primorye. Estimation of juvenile stock abundance for these regions is based on annual trawl surveys. Materials for genetic analysis have been collected during these surveys since 2009. The first results from the genetic identification of the Okhotsk Sea juvenile pink salmon were obtained in 2010, and now this kind of work is regularly performed (Shpigalskaya et al. 2011a, b; 2012; 2013; Bugaev et al. 2012; Shevlyakov et al. 2014).

The high commercial value of pink salmon requires an accurate forecast of the abundance of spawning populations. This, in turn, requires the identification and assessment of the contribution(s) of regional complexes and principal population systems into mixed marine aggregations during feeding and pre-spawning migrations.

Identification of the regional composition of juvenile Pacific salmon feeding in the Okhotsk Sea in autumn can serve as an additional information source to determine the relative contribution of regional spawning runs and can provide a reliable foundation for an organizational strategy for the fishing fleet.

This work was carried out in order to estimate how the results of regional identification of pink salmon mixed fall aggregations in the Okhotsk Sea correspond to the regional ratios among spawning returns in the basin of the Okhotsk Sea.

MATERIALS AND METHODS

A series of twelve samples was collected during a research cruise of RV *TINRO* in the Okhotsk Sea during October and November 2013 (Table 1, Fig. 1). The baseline survey consisted of the analysis of over 1,900 pink salmon from 29 populations ranging from western Kamchatka, the continental coast of the Okhotsk Sea, Sakhalin and the Kuril islands, the Amur River, and Primorye (Fig. 1). We unfortunately had no samples from the islands of northern Japan in the baseline and thus can only suggest the possible presence of Japanese pink salmon from Hokkaido in the mixed fall aggregations in the Okhotsk Sea, although three individuals were identified on the basis of hatchery otolith markings in a survey in 2012 (Chistyakova and Bugaev 2013b). By contrast, the survey in 2011, revealed no Japanese hatchery otolith marks (Chistyakova and Bugaev 2013a). Thus, it is not possible to estimate the contribution of Japanese juvenile pink salmon to the fall aggregations and how much it might increase the error in the identifications we have made.

Table 1. Location, date of collection, and number (N) of juvenile pink salmon samples collected in the Okhotsk Sea during cruises in October and November 2013.

Samples	Date	Latitude/Longitude	N
1	27 October	51°83'/150°23'	50
2	29 October	51°30'/152°65'	49
3	30 October	50°51'/151°92'	50
4	31 October	49°09'/150°43'	50
5	01 November	48°76'/148°17'	50
6	02 November	48°20'/147°23'	50
7	02 November	47°87'/148°43'	50
8	02 November	47°30'/147°41'	49
9	03 November	47°71'/146°22'	48
10	03 November	46°82'/146°34'	50
11	04 November	48°04'/145°08'	47
12	05 November	49°16'/145°02'	49
Total			592

Tissue samples from the marine and river samples were collected and preserved in 96% ethanol prior to DNA extraction. Standard genetic methods were followed, including the method of proteinase hydrolysis to obtain the total DNA, the polymerase chain reaction (PCR), RFLP-analysis (restriction fragment length polymorphism) of Cyto-B/D-loop region in the mtDNA with the use of six restriction endonucleases (*Cfr*13.I, *Rsa*I, *Msp*I, *Hin*6.I, *Hin*fI, *Dde*I), and electrophoresis in agarose gel (Sambrook et al. 1989; Shpigalskaya et al. 2011a).

The amplification was made with using sets of ready-to-use lyophilized mixture for the PCR (Gene Pak PCR Core, IsoGen Life Sciences Ltd., Moscow, Russia). Ten µl of buffer solution were added into the tubes with the mixture for the PCR, plus 5 µl of genome DNA and 5 µl of the mixture of specific primers:

5' TGAA(G/A)ACCACCGTTGTTATTCAA 3';
 5' TAGGGCCTCTCGTATAACCG 3'.

The PCR of the CytoB/D-loop region has been described earlier (Shpigalskaya et al. 2011a).

For cluster analysis based on genetic chord distances and constructing phylogenetic trees, we used the software

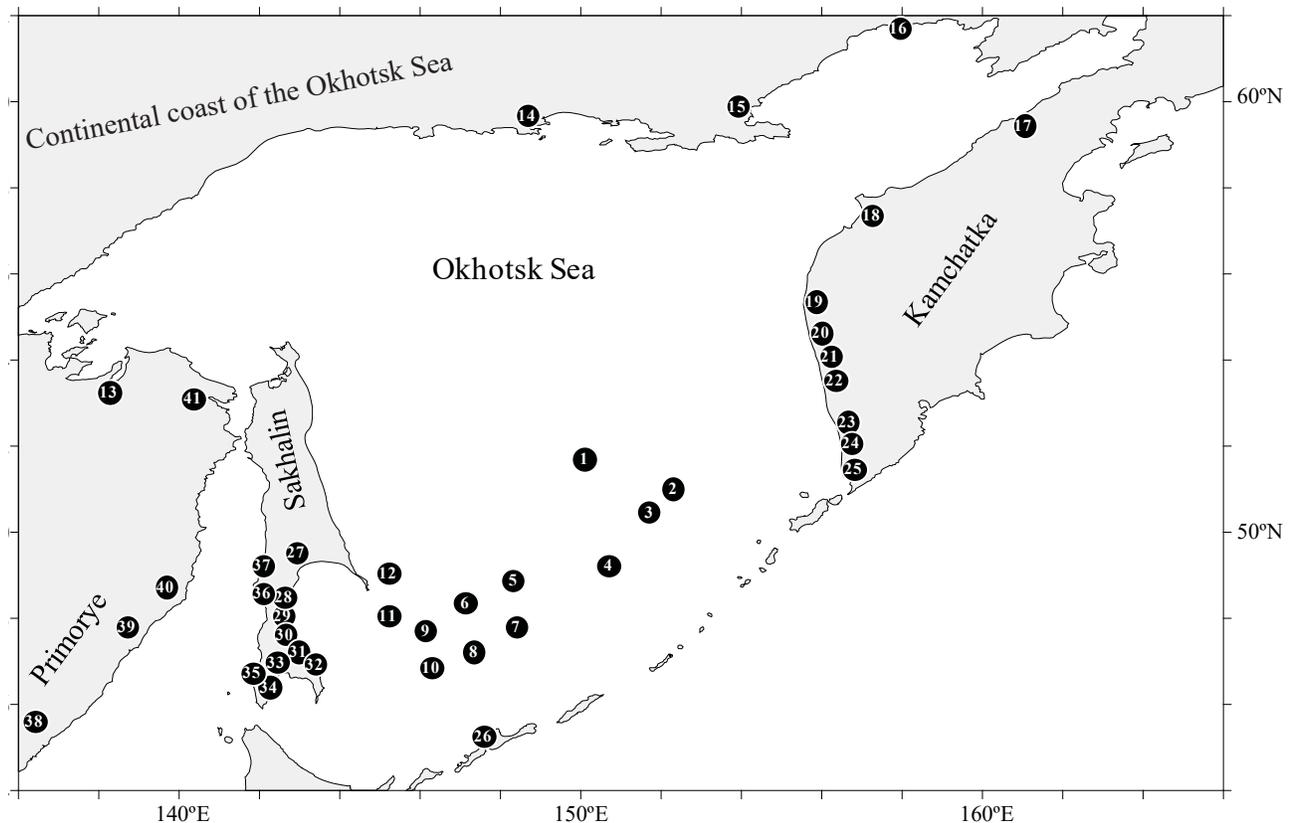


Fig. 1. Location of juvenile pink salmon samples (1–12) collected in the Okhotsk Sea during October and November 2013, and genetic sampling locations for 29 samples of pink salmon collected from locations draining into the Okhotsk Sea basin: continental coast of the Okhotsk Sea—Nikolaya Bay (13), Tauy R. (14), Jama R. (15), Nayahan R. (16); western Kamchatka—Palana R. (17), Khairuzova R. (18), Krutogorova R. (19), Kolpakova R. (20), Vorovskaya R. (21), Kol R. (22), Utka R. (23), Bolshaya R. (24), Opala R. (25); Kuril Islands—Kurilka R. (26); Sakhalin Island—Poronay R. (27), Dudinka R. (28), Firsovka R. (29), Nayba R. (30), Bakhura R. (31), Ochepukha R. (32), Partizanka R. (33), Urum R. (34), Orlovka R. (35), Ainskaya R. (36), Vodopadnaya R. (37); Primorye—Serebranaya R. (38), Maximovka R. (39), Botchi R. (40), Amur R. (41).

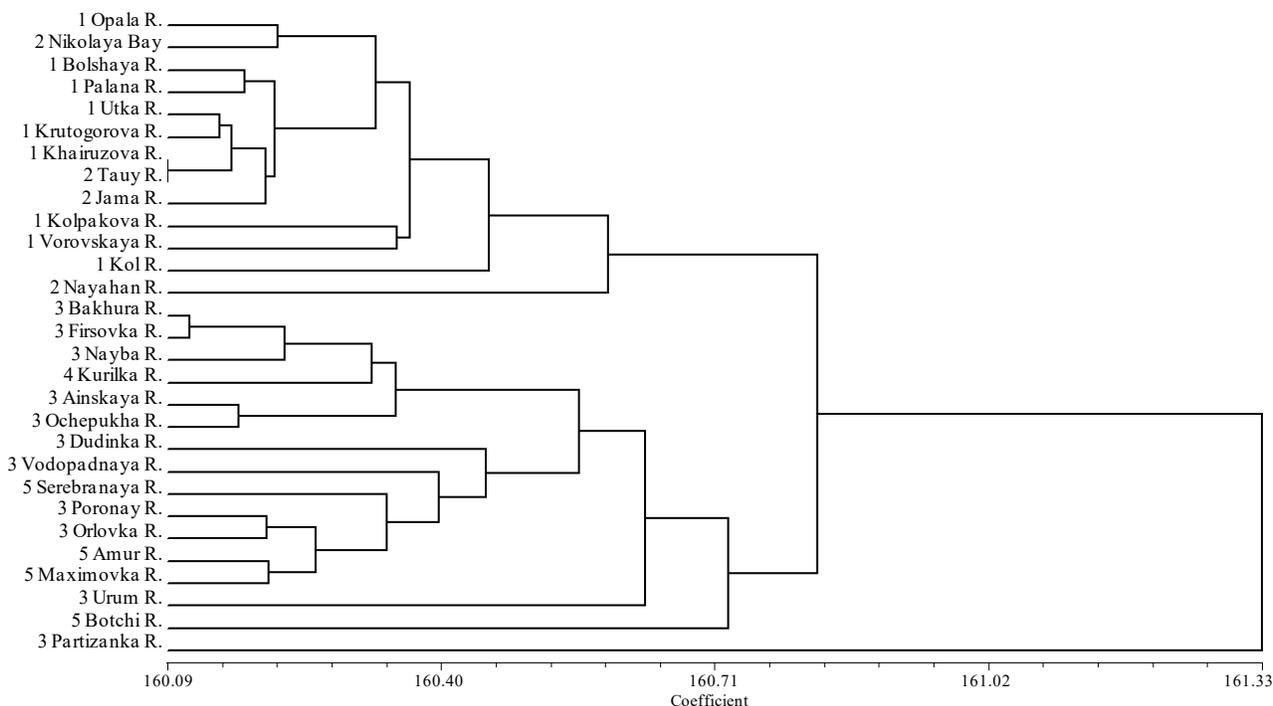


Fig. 2. WPGMA-dendrogram created using genetic chord distances and based on the frequencies of 41 composite haplotypes of 29 even-year pink salmon populations (1—western Kamchatka, 2—continental coast of the Okhotsk Sea, 3—Sakhalin Island, 4—Kuril Islands, 5—Amur River and Primorye); $r = 0.714$, $P < 0.001$ (NTSYS, ver. 2.0; Rohlf 1998).

package NTSYS, ver. 2.0 (Rohlf 1998). Hierarchical analysis (AMOVA) was done using the ARLEQUIN program (Schneider et al. 2000). Baseline simulations for the three regional groupings and estimates of stock proportions present in the juvenile pink salmon from the Okhotsk Sea were made using the program SPAM (Masuda et al 1991; Pella et al. 1996; www.adfg.alaska.gov/index.cfm?adfg=fishing-geneconservationlab.software_spamfaq_citing).

RESULTS AND DISCUSSION

The genetic identification of the even-year juvenile pink salmon generations that returned to spawning regions as parents in 2014 was based on the distribution of composite haplotype frequencies in samples of adult fish from 29 spawning rivers of western Kamchatka, the continental coast of the Okhotsk Sea, Sakhalin and the Kuril islands, the Amur River, and Primorye. The total sample size in the baseline river dataset included 1,910 pink salmon. Analysis of the variety of composite haplotype frequencies at spawning locations was completed earlier (Shpigalskaya et al., in press). We also had demonstrated frequencies of composite haplotypes of pink salmon mtDNA from the basin of the Okhotsk Sea.

There are 41 haplotype variants in the samples examined. Regional characteristics of the variety of mtDNA haplotype frequencies of the even-year pink salmon broodline in the Okhotsk Sea basin have been confirmed. The data

on the population variety of pink salmon even-year generations indicate genetic heterogeneity with a clearly expressed regional character (Fig. 2). Statistical assessments for inter-regional variety are markedly higher than assessments at the interpopulation level (Table 2).

The differences in the composite haplotype frequencies of pink salmon from the rivers of western Kamchatka and the continental coast of the Okhotsk Sea (the regional group of 'northern' populations of the Okhotsk Sea basin), from the rivers of Sakhalin and the Kuril islands (the group of 'southern' populations), and from the Amur River and Primorye were the basis for making a probabilistic assessment of the identification accuracy for the fish from these regions (Table 3). Assessment of the composition of simulated samples has revealed a high (90%) identification accuracy for the northern regional group compared to the southern populations and the Amur River basin and Primorye regional group. The results make possible a highly accurate identification assessment for mixed marine aggregations of pink salmon at the regional level only. It should be noted, however, that this method can overestimate the identification accuracy (Anderson 2010), especially in view of the fact that the fragment CytoB/D-loop we examined is a single locus. To increase the identification accuracy it was necessary to increase the number of informative molecular markers of population variability.

According to our results, the contribution of the 'northern' group of populations of the Okhotsk Sea basin, includ-

Table 2. The relative (%) genetic variation among and within populations and regional groups that was observed in even-year Okhotsk Sea basin pink salmon populations. Variation was estimated by hierarchical analysis of molecular variation (AMOVA).

Analyzed pool	Source of variation	Percentage of variation
All samples	Among populations	5.02
	Within populations	94.98
3 regional groups of populations: northern (west Kamchatka, northern shore Okhotsk Sea), southern (Sakhalin Island, Kuril Islands), Amur R. and Primorye.	Among groups	4.01
	Among populations within groups	1.26
	Within populations	94.73
2 regional groups of populations: northern (west Kamchatka, northern shore Okhotsk Sea, Amur R. and Primorye), southern (Sakhalin Island, Kuril Islands).	Among groups	5.31
	Among populations within groups	1.33
	Within populations	93.36
2 regional groups of populations: northern (west Kamchatka, northern shore Okhotsk Sea, Amur R. and Primorye), southern (Sakhalin Island, Kuril Islands, Amur R. and Primorye).	Among groups	3.21
	Among populations within groups	2.24
	Within populations	94.55

ing western Kamchatka and northern continental coast of the Okhotsk Sea, to mixed aggregations of juvenile pink salmon is relatively small—about 24% (Table 4). The majority (58%) of the juvenile aggregations was identified as Sakhalin-Kuril stock. The contribution from populations of the Amur River and Primorye is about 17%. Fish of unknown origin comprised < 1%. It should be noted that the results we obtained for 2013 were not consistent with the existing regional ratios for pink salmon runs from the even-year generations in the Okhotsk Sea basin. For instance, in recent years the contribution of western Kamchatka even-year pink salmon was similar to (or even exceeded) the contribution of Sakhalin-Kuril pink salmon, whereas the identifications we have provided for juvenile mixed aggregations during the early period of feeding at sea in 2013 show the contribution of the western Kamchatka juvenile fish is visibly lower. The returns of pink salmon in 2014 to the spawning regions within the Okhotsk Sea basin also showed a significant reduction in the western Kamchatka stock. The northern regional group contributed only about 10% of the total catch in the Okhotsk Sea basin. Hence, even if we assume a relatively low (23.7%) contribution of the ‘northern’ group based on the genetic identification of juvenile fish in the early period of feeding at sea, the spawning run forecast error in 2014 is still quite high.

Earlier results of the regional identification of samples collected in mixed aggregations of feeding juveniles of the

even-year generations in 2009 and 2011 were rather similar to the regional abundance ratio of spawners, respectively, in 2010 and 2012 (Shpigalskaya et al. 2011a, 2013). It can be suggested, based on these results and assuming that juvenile pink salmon from different spawning regions in the Okhotsk Sea had already passed the critical stage of adaptation (the highest risk of mortality) in the early marine period in October and November, that the ratios among different regional complexes, assuming a consistent mortality level at sea, remain stable by the time adults return to regions for spawning. In 2014, however, the ratio changed because of a decrease in the northern populations. This result can be explained if we assume that mortality of pink salmon emerging from the rivers of western Kamchatka increased in 2013. It also would be most likely that the mortality of western Kamchatka pink salmon, compared to those from other regions, was higher not only in the early marine period, but in some later phases of the life cycle.

Results of ichthyological research indicate that pink salmon juvenile escapement is well correlated with parental abundance, but poorly correlated with the consequent spawning return (Shevlyakov et al. 2014). This provides evidence that, in general, factors affecting abundance trends for a pink salmon generation take place during the early marine period. It also can be suggested that increasing juvenile mortality during this period is a result of a poor forage base that is insufficient to cover energy requirements

Table 3. Average percent (SD in parentheses) correct and incorrect allocations (read vertically) by region for simulated mixtures based on the number of even-year pink salmon regional groups. Expected value for estimates shown in bold is 100%.

Region		1	2	3
1	Northern populations (west Kamchatka and continental coast of the Okhotsk Sea)	90.2 (6.5)	9.2	22.4
2	Southern populations (Sakhalin Island and Kuril Islands)	4.7	83.0 (8.9)	16.0
3	Amur R. and Primorye	4.5	7.6	61.2 (12.7)
	Unknown	0.6	0.2	0.4

Table 4. Estimated stock compositions (percentage, SD in parentheses) of mixed-stock samples of pink salmon sampled in Okhotsk Sea during October and November 2013.

Samples	Northern populations	Southern populations	Amur River and Primorye	Unknown
1	11.3 (37.09)	31.0 (25.58)	57.7 (37.78)	0.0
2	100.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0
3	75.8 (23.09)	24.2 (25.58)	0.0 (0.00)	0.0
4	17.8 (33.03)	82.1 (33.01)	0.1 (0.07)	0.0
5	0.1 (0.17)	60.8 (31.13)	39.1 (31.13)	0.0
6	30.0 (28.59)	63.7 (20.45)	6.3 (24.41)	0.0
7	00.0 (0.00)	9.5 (9.78)	86.5 (9.78)	4.0
8	69.2 (16.59)	26.7 (16.59)	0.1 (0.12)	4.0
9	20.4 (29.59)	75.4 (23.99)	4.2 (21.43)	0.0
10	1.5 (8.11)	98.4 (8.31)	0.1 (0.26)	0.0
11	30.0 (34.79)	43.7 (18.73)	26.3 (3.,28)	0.0
12	23.2 (17.31)	76.8 (17.31)	0.0 (0.00)	0.0
All samples	23.7 (10.88)	58.3 (8.95)	17.3 (11.57)	0.7

(Shevlyakov et al. 2014). In this way, the smolts that survive under conditions of high coastal mortality appear not to be ready, physiologically, for distant oceanic migrations.

The results we have obtained indicate that the genetic identification of Okhotsk Sea juvenile pink salmon in the fall mixed aggregations can be used to better predict regional forecasts of spawning runs and the assessment of survival and conditions of feeding. Increasing the identification accuracy requires a larger baseline, including the addition of samples from the islands of Japan. Using sequencing results and the molecular markers such as microsatellite loci or SNPs in the analysis also can improve results of the genetic identification of the mixed juvenile pink salmon aggregations in the Okhotsk Sea.

ACKNOWLEDGMENTS

We are grateful to Anastasia Sushkevich and Alexandra Chistyakova for sample collection in the Okhotsk Sea. We thank all our colleagues who provided samples of pink salmon spawning populations situated in remote coastal areas of the Okhotsk Sea.

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