

Genetic Identification of Okhotsk Sea Juvenile Pink Salmon Mixed-Stock Aggregations in the Course of Their Early Marine Period of Life

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Pink salmon is the most abundant Pacific salmon species and one of the most important components of the North Pacific and Bering Sea ecosystem. Post-catadromous juvenile pink salmon leave coastal waters for winter foraging areas after a short stay near the coast. In this paper we identify the region of origin of juvenile pink salmon using RFLP (restriction fragment length polymorphism) analysis of the mtDNA Cytb/D-loop region. Identification was aimed at making quantitative estimates of the contributions of juvenile pink salmon originating from different spawning regions to mixed-stock foraging aggregations in the Okhotsk Sea.

The quantitative evaluation was based of analysis of genetic marker frequencies in samples collected in three autumn trawl surveys in the Okhotsk Sea. Samples were collected by researchers aboard the R/V *Professor Kaganovsky* (TINRO-Center) and the STR *Uzon* (KamchatNIRO) surveys in 2011 and the R/V *TINRO* (TINRO-Center) survey in 2012. Samples were collected at 19 locations in 2011 (Table 1). Regional identification of pink salmon in the mixed-stock aggregation was determined for the total sample, but some data, including September 20–28 and October 18–30 samples, required separate analysis. In 2012 samples were collected at 11 locations (Table 2).

Table 1. Location, period of sample collection, and number (N) of juvenile pink salmon samples collected during cruises in September and October 2011.

Latitude/Longitude	N
20–28 September 2011	
53°01′/152°60′	50
53°00′/154°02′ and 53°02′/155°19′	47
54°04′/153°56′ and 53°60′/152°59′	46
54°00′/152°01′ and 53°60′/151°03′	45
54°00′/150°16′ and 54°00′/148°38′	50
54°60′/148°31′ and 55°00′/149°59′	49
55°00′/150°59′ and 55°00′/152°02′	49
55°00′/152°60′ and 55°02′/153°60′	49
55°01′/155°05′ and 56°01′/154°57′	50
56°33′/153°02′	48
51°04′/153°57′	49
Σ	532
13–30 October 2011	
52°22′/151°08′	35
49°53′/151°06′	50
49°34′/149°13′	50
50°54′/150°39′	50
50°26′/148°55′	50
54°43′/147°39′	47
52°14′/147°47′	50
50°14′/145°39′	50
Σ	382
Total	914

Table 2. Location, period of sample collection, and number (N) of juvenile pink salmon samples collected 7 October – 5 November, 2012.

№	Latitude/Longitude	N
1	53°13'/154°16'	47
2	51°22'/154°51'	50
3	49°41'/151°09'	50
4	55°17'/152°19'	50
5	54°52'/151°05'	49
6	51°08'/149°36'	48
7	48°45'/148°11'	46
8	50°58'/147°44'	50
9	52°37'/148°40'	47
10	52°39'/146°26'	48
11	48°35'/146°04'	48
Total		533

Juvenile pink salmon mixed-stock aggregations collected in 2011 represent fish that would return to native regions for spawning in 2012. Estimation of the error of our method was based on comparing our composition estimates for juveniles collected in 2011 with the magnitude of 2012 adult pink salmon commercial catches obtained in fishing districts located near the natal rivers.

The mtDNA haplotype diversity of the even-year pink salmon brood-line (spawn in even-numbered years) was determined from a baseline comprising 24 local populations of West Kamchatka, Sakhalin, the continental shore of the Okhotsk Sea, and Primorye. Regional identification of fish from the odd-year brood-line (spawn in odd-numbered years) was provided by analysis of a baseline comprising 19 rivers of West Kamchatka, Sakhalin, the continental shore of the Okhotsk Sea, and Kuril Islands. In total, we analyzed the mtDNA haplotype frequencies for approximately 2,500 individuals.

Results revealed 61 haplotype variants (38 composite haplotypes in the even-year brood-line, 32 in the odd-year brood-line, and 9 in both brood-lines) in mature odd- and even-year pink salmon. These data on population diversity of both even- and odd-year pink salmon indicate genetic heterogeneity was expressed clearly and has regional characteristics (Fig. 1). The statistical heterogeneity of the regional groups was visibly higher than the heterogeneity of the populations.

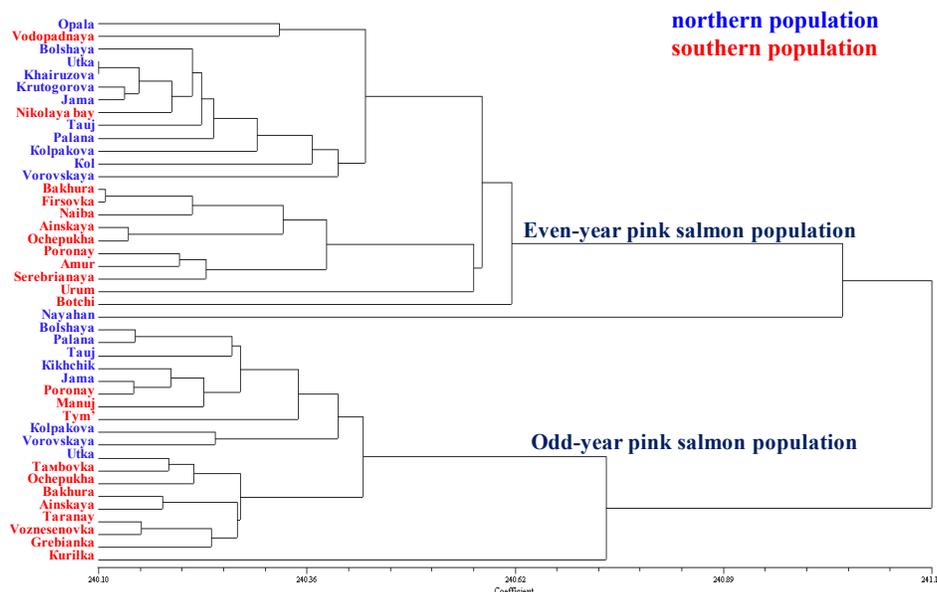


Fig. 1. The unweighted pair-group method with arithmetic mean (UPGMA) dendrogram created using genetic chord distances and based on the frequencies of composite haplotypes of even- and odd-year Asian pink salmon (43 rivers).

Identification accuracy was evaluated using maximum likelihood estimates for individuals based on different frequencies of composite haplotypes from rivers of West Kamchatka and the northern part of the continental shore of the Okhotsk Sea (northern populations) and from Sakhalin, Kuril Islands and Primorye (southern populations; Table 3). Identification accuracy was 92% for northern and 88% for southern populations of even-year pink salmon. For odd-year fish, the accuracy

was lower, 79% for northern and 84% for southern populations, which could indicate lower regional differentiation for this brood-line. Judging on these results, there is no doubt that differentiation of the even-year brood-line is sufficient for genetic analysis at a regional level. As for odd-year pink salmon, differentiation requires further clarification based on analysis of the maximum number of samples available from watersheds representing principal spawning areas.

Table 3. Average percent (standard deviation) 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
Even-year pink salmon			
1	Northern populations	92.0 _(4.45)	11.8
2	Southern populations	7.4	87.8 _(6.72)
	Unknown	0.6	0.4
	Σ	100	100
Odd-year pink salmon			
1	Northern populations	78.6 _(13.06)	15.0
2	Southern populations	20.8	84.2 _(10.86)
	Unknown	0.6	0.8
	Σ	100	100

The RFLP analysis of mtDNA from all the mixed-stock marine samples of juvenile pink salmon revealed 21 composite haplotypes. According to the principle of maximum similarity of composite haplotypes, the frequency distribution in the baseline samples from the two populations was used to estimate the percent regional contribution of the mixed-stock samples.

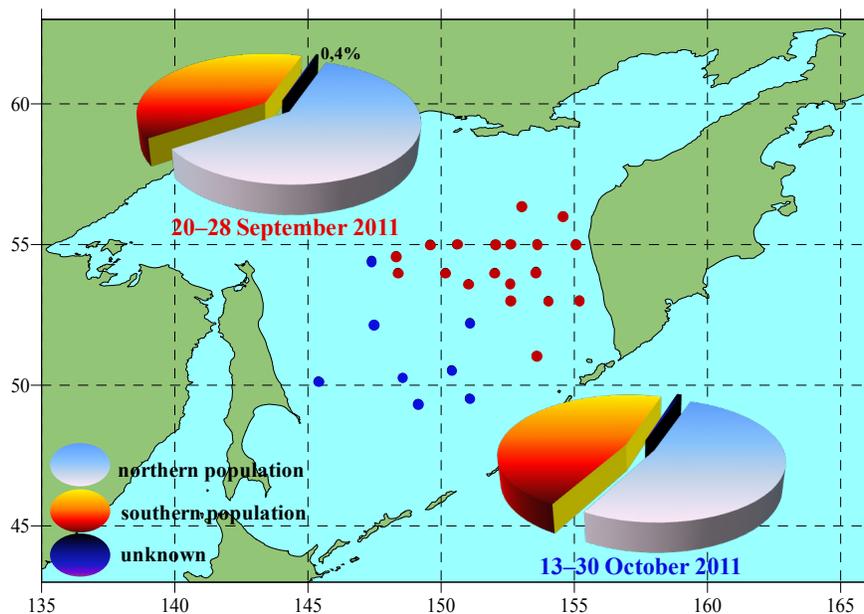


Fig. 2. Regional estimates for pink salmon based on genetic analysis of juveniles in marine feeding aggregations in autumn 2011. Sample location by sampling period is indicated by dot color (September: red, October: blue).

Genetic differentiation of pink salmon allowed for regional identification of the mixed-stock marine samples of juvenile pink salmon (Figs. 2 and 3). Results were obtained from two groups of samples: one group collected in September in the more northerly area and stations close to the Kamchatka Peninsula, and the second group collected in October in the central part of the Okhotsk Sea. In general, the estimated percentage originating from southern populations was less (39.6% in the September samples and 46.9% in the October samples) than the proportion originating from northern populations (Fig. 2). The difference in estimates between the September and October sample groups can result from the different time and area of sampling, but it can also illustrate when juvenile fish emigrate from their native regions and start to leave coastal areas to feed at sea.

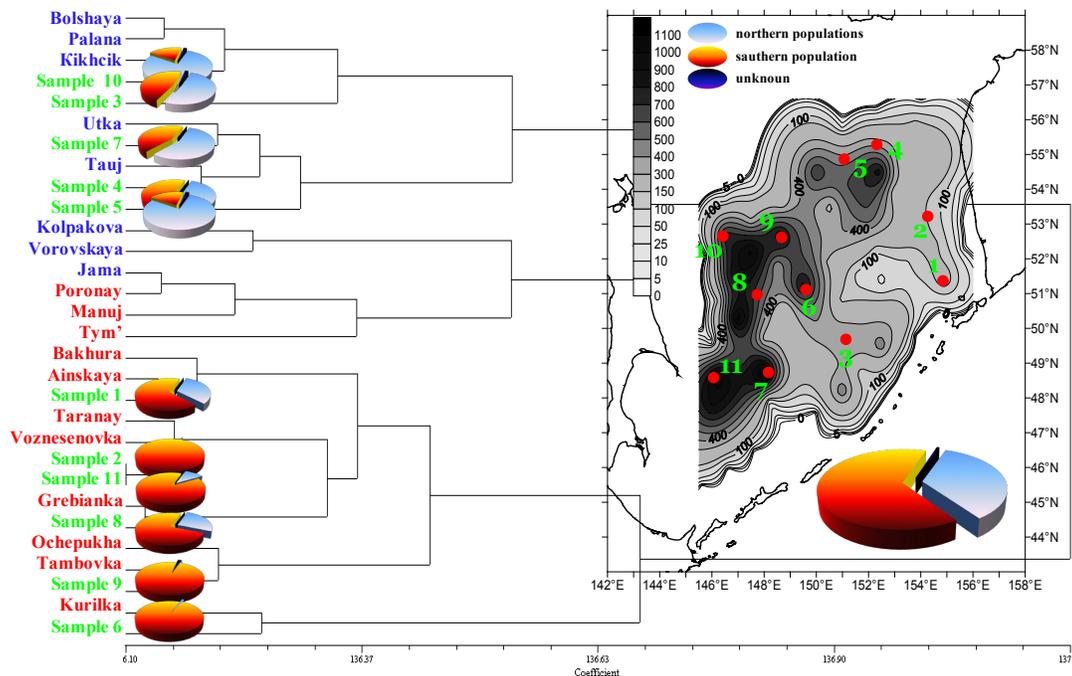


Fig. 3. The unweighted pair-group method with arithmetic mean (UPGMA) dendrogram was created using genetic chord distances and based on the frequencies of composite haplotypes of odd-year Asian pink salmon and samples of juveniles in mixed-stock aggregations.

Estimation of the error of our method was based on comparing our mixing proportion estimates of juvenile pink salmon samples to catches of adults the following year caught in fishing districts in the Okhotsk Sea basin. In a preliminary assessment, the percentage of northern pink salmon populations from West Kamchatka and Magadan Region in the total commercial catch was 57.3%, and the percentage of southern pink salmon populations from Sakhalin, Khabarovsk, and Primorye was 42.7%. These percentages were similar enough to our genetic analysis of juveniles to suggest there is potential for this technique to assess the abundance of juvenile pink salmon stocks in mixed-stock marine aggregations in the early phase of marine foraging.

We analyzed the regional composition of mixed-stock marine aggregations of odd-year juvenile pink salmon collected during the autumn trawl survey in 2012 (Fig. 3). These results showed the portion that was represented by northern populations was rather impressive, > 30% (standard deviation 9%).

In our view, assessment of mixed-stock aggregations of juvenile pink salmon abundance can be done under the following circumstances:

- regional identification accuracy of the odd-year brood-line that is relatively lower than the accuracy of the even-year brood-line;
- sampling time period is strongly influencing composition estimates because the timing of juvenile emergence from rivers and seaward migration varies by region in the Okhotsk Sea basin. The change to an earlier or later sampling period can cause an increase of northern or southern populations in the mixture.