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Use of the Otolith Complex Method for Stock Identification of Juvenile Pink and Chum Salmon in the Offshore Waters of the Okhotsk Sea During Post-catadromous Migrations

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There is no doubt that identification of the regional stock origin of Pacific salmon in mixed-stock ocean feeding aggregations is a challenge, especially if the stocks have overlapping migration routes. However, the challenge should be answered because the composition of catch, when correctly identified, can enhance the reliability of abundance forecasts for certain species. It is particularly informative when these techniques can be successfully applied to estimating juvenile salmon abundance in the early marine period because this period basically sets the strength of the year-class.

Scientific experience indicates that using morphological differences in otolith structure is promising for stock identification. The otolith, as one of fish's record-keeping structures, has attracted scientific interest for a long time. The formation rate of the otolith's structural elements, influenced in Pacific salmon by environment and genetics, can capture microstructural differences between populations. Normally, individuals from different populations demonstrate different otolith microstructures and relative sizes (Bugaev et al. 2012; Chistyakova et al. 2012). Examination of otolith structural variations in juvenile pink and chum salmon of Kamchatka, Sakhalin, and the northern coast of the Okhotsk Sea has demonstrated reliable differences among stocks. However, if regional variation in otolith microstructure can be used to answer practical questions, this needs to be clarified.

Otolith marking has been used widely in salmon hatcheries of the North Pacific Rim for quite a long time. The North Pacific Anadromous Fish Commission (NPAFC) has a database of all the otolith marks used by hatcheries of the North Pacific that allows for identification of marked hatchery fish in marine catches with almost complete accuracy.

Additional options for salmon stock identification are available by combining analysis of otolith structure collected from wild populations with results of otolith marking of hatchery stocks. We call such approach to analyzing otolith structural diversity as the "otolith complex method". The possibility of identifying juvenile salmon during trawl surveys makes this method particularly timely because otoliths can always be retrieved from fish in the catch, whereas fish scales can be lost in the process of trawling.

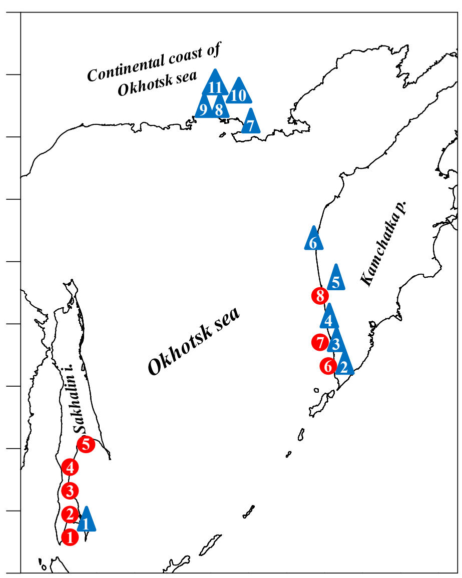

Fig. 1. Locations where baseline otolith samples of pink and chum salmon were collected from rivers of Kamchatka, Sakhalin, and the north coast of the Okhotsk Sea. Pink salmon (red color): Sakhalin: Kura R. (1), Lutoga R. (2), Voznesenka R. (3), Dudinka R. (4), and Poronai R. (5). West Kamchatka: Opala R. (6), Bolshaya R. (7), and Kikhhik R. (8). Chum salmon (blue color): Sakhalin: Ochepukha R. (1). West Kamchatka: Opala R. (2), Bolshaya R. (3), Kikhchik R. (4), Vorovskaya R. (5), and Icha R. (6). The north coast of the Okhotsk Sea: Armansky Hatchery (7), Tauy R. (8), Kava R. (9), Chelomdzha R. (10), and Tauysky Hatchery (11).

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Our purpose was to check how well the otolith complex method works in identifying mixed marine aggregations of juvenile pink and chum salmon during autumn migrations in the Okhotsk Sea.

Materials and Methods

Otolith baseline samples were collected by scientists from the fisheries research institutes of KamchatNIRO, SakhNIRO, MagadanNIRO, and researchers from the Kikhchinsky area, Sevvostrybvod. The baseline pool included mostly otoliths collected from juvenile pink and chum salmon. In some cases, when in-river collection of juvenile fish otoliths was not possible, we used otoliths collected from adult fish. The sampling scheme for otolith samples collected from fish in West Kamchatka, Sakhalin, and the north coast of the Okhotsk Sea is shown in Figure 1. The otolith baseline samples consisted of 735 pink and 819 chum salmon.

Mixed-stock samples of juvenile pink and chum salmon otoliths were collected during the complex trawl survey of the R/V *Professor Kaganovsky* in September-October 2011 in the Okhotsk Sea.

Otoliths were processed in the laboratory where they were set in thermoplastic cement onto glass slides and then polished with fine-grit grinding discs until the central part of the otolith was visible. Otolith images were scanned using the visual-analytic complex LEICA DM 1000 device with 900 pixels/mm resolution.

To determine the character and level of otolith structural variety in native stocks of chum salmon, we used signal wavelet-analysis, which is a version of classical spectral analysis (Astafyeva 1998; Dobeshi 2001; Kuznetsova et al. 2004).

In the course of our analysis, we examined the otolith for the presence of a potential otolith hatchery mark. All potential marks were compared with the NPAFC database of known otolith marks to determine the source populations of the marked fish (NPAFC 2013).

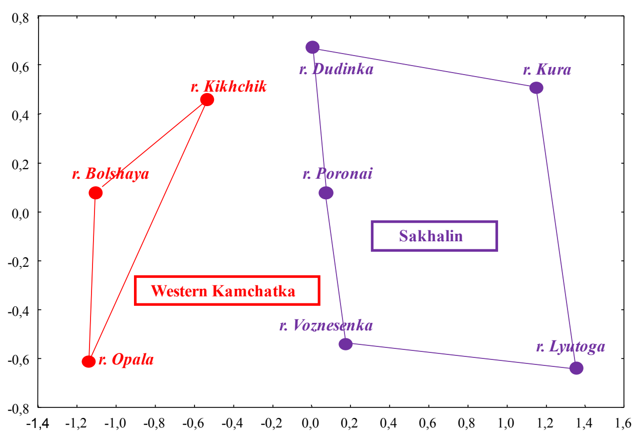

Fig. 2. Centroid plot of populations of pink salmon from West Kamchatka and Sakhalin using multidimensional scaling of otolith characteristics.

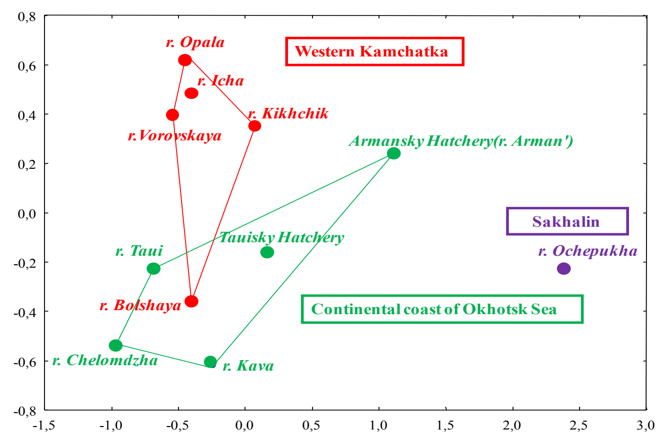

Fig. 3. Centroid plot of populations of chum salmon populations from West Kamchatka, Sakhalin, and the north coast of the Okhotsk Sea using multidimensional scaling of otolith characteristics.

Creation of baselines

Creation of the baselines for analyzing the phenotypic variety of the otoliths was based on principle components analysis. Taking into account the tight geographical connectivity of the Okhotsk Sea sampling area, we assembled baselines only for stocks of three "hot spots" of reproduction for pink and chum salmon, West Kamchatka, Sakhalin, and northern coast of the Okhotsk Sea. The baseline did not include Japan. Later in this paper, we provide results of recaptured otolith-marked fish that is helpful to determine Japanese fish in the mixed samples. This approach works well to provide more accurate estimation of juvenile salmon composition in the catches from the Okhotsk Sea. We determined the regional complexes of pink and chum salmon that were not otolith-marked solely on the basis of otolith structure. Centroid plots of pink and chum salmon otolith baselines based on their geography are shown in Figs. 2 and 3.

Based on Figs 2 and 3, we created two regional complexes of pink salmon stocks: West Kamchatka and Sakhalin and three regional complexes of chum salmon stocks: West Kamchatka, Sakhalin, and the north coast of the Okhotsk Sea.

The next step was to estimate the ability of the baseline models to resolve the components. The method we used was dependent simulation (Tables 1 and 2). The estimated resolution of the baselines was 90.43% for pink and 77.70% for chum salmon.

Table 1. Resolution of the pink salmon otolith baseline estimated using dependent simulation.

Region	MLE	SD	CI ± 95%	
			Lower	Upper
West Kamchatka	0.9226	0.0577	0.8509	0.9943
Sakhalin	0.8860	0.0706	0.8182	0.9547

Note. The resolution ability is 90.43%.

Table 2. Resolution of the chum salmon otolith baseline estimated using dependent simulation.

Region	MLE	SD	CI ± 95%	
			Lower	Upper
West Kamchatka	0.7201	0.0874	0.5123	0.7721
Sakhalin	0.9300	0.0648	0.6992	0.9403
North coast of the Okhotsk Sea	0.6810	0.0804	0.5265	0.7930

Note. The resolution ability is 77.70%.

We know, of course, that the baselines cannot illustrate completely the phenotypic diversity in otolith structure of wild pink and chum salmon populations originating from the whole Okhotsk Sea basin. However, it is very difficult to collect otolith samples on a scale as large as the Okhotsk Sea basin.

Identification of regional stock complexes based on otolith structure

Identification of principle regional complexes of juvenile pink and chum salmon in the trawl catches of R/V *Professor Kaganovsky* during post-catadromous migrations in the Okhotsk Sea in 2011 are presented in Table 3. Results showed the dominant stock complex of pink salmon originated from West Kamchatka (72.45%). The contribution of Sakhalin stocks was 27.55%. In the case of chum salmon, the principle stock component originated from West Kamchatka (79.83%). Chum salmon stocks from the north coast of the Okhotsk Sea were secondary (20.02%). The percentage of Sakhalin chum salmon was identified at the level of the statistical error. It is clear that at this stage, these results generally reflect the mixing proportions of wild stocks in the basin.

Recovery of otolith-marked fish

Besides wild populations of pink and chum salmon, the Okhotsk Sea also provides a feeding area for juvenile fish released from a number of salmon hatcheries in the Russian Far East and Japan. Most hatcheries are currently otolith-marking salmon that provide for almost absolute likelihood of correctly identifying mixed-stock catches of fish to region of origin (Munk et al. 1993; Akinicheva and Rogatnykh 1996; Akinicheva et al. 1998; Munk and Geiger 1998; Safronenkov et al. 1999; Akinicheva 2001; Akinicheva et. al 2004; Akinicheva 2006;).

Table 3. Identification of the regional stock complexes of juvenile pink and chum salmon collected from trawl catches of the R/V *Professor Kaganovsky* in the Okhotsk Sea, September-October 2011.

Species	No. of Specimens	Parameter	West Kamchatka	Sakhalin	North coast of the Okhotsk Sea
Pink salmon	735	MLE	0.7245	0.2755	–
		SD	0.0348	0.0366	–
Chum salmon	861	MLE	0.7983	0.0015	0.2002
		SD	0.0498	0.0000	0.0520

Juvenile pink and chum salmon with otolith marks from Russian Far East hatcheries (West Kamchatka, Sakhalin, South Kuril Islands, and continental coast of the Okhotsk Sea) and Japan (Hokkaido and Honshu) feed in the Okhotsk Sea during their post-catadromous migration. This is why the next step in our work was to determine the origin of the otolith-marked hatchery juvenile pink and chum salmon in the trawl catches.

Analysis of pink and chum salmon otoliths collected in September-October 2011 trawl catches revealed marks from Russian and Japanese salmon hatcheries. The percentage of otolith-marked individuals was 4.08% of total pink salmon examined and 4.88% of chum salmon examined. The percentage of marked juvenile pink and chum salmon by hatchery origin is shown in Figs. 4 and 5.

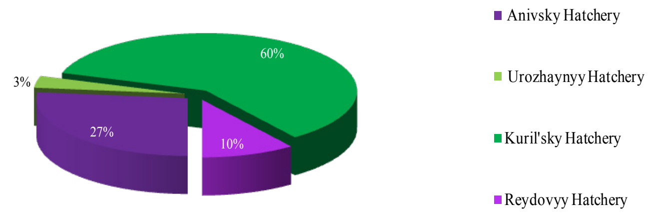

Fig. 4. Percent contribution by hatchery of otolith-marked juvenile pink salmon collected in the trawl catches of the R/V *Professor Kaganovsky* in the Okhotsk Sea, September-October 2011.

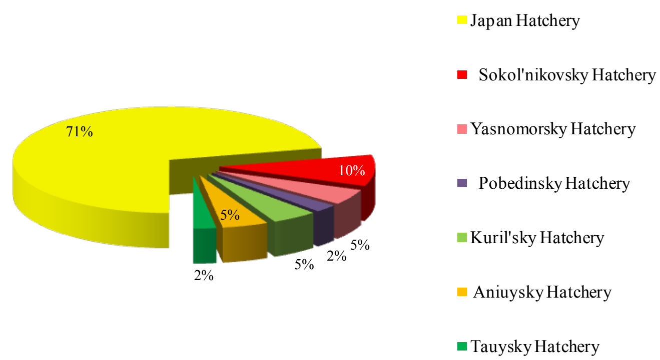

Fig. 5. Percent contribution by hatchery of otolith-marked juvenile chum salmon collected in the trawl catches of the R/V *Professor Kaganovsky* in the Okhotsk Sea, September-October 2011.

Results demonstrate a high percentage (70%) of juvenile pink salmon caught in autumn 2011 originated from Kuril Island hatcheries (Fig. 4). Pink salmon from Sakhalin hatcheries occupy the secondary position (30%). Among chum salmon caught in mixed-stock trawl catches, most otolith-marked fish originated from Japanese hatcheries (71%; Fig. 5). Other otolith-marked chum salmon originated from Russian hatcheries in Sakhalin (17%), Kuril Islands (5%), and the continental coast of the Okhotsk Sea (7%). Because identification of otolith-marked fish is a very accurate method to determine origin of juvenile fish reared in hatcheries, we decided to consolidate results of the theoretical assessment based on regional variation of otolith structure with recovery of otolith-marked juveniles into one "otolith complex method".

Identification of regional stock complexes by the otolith complex method

Identification of juvenile pink salmon stocks in the autumn 2011 trawl catches in the Okhotsk Sea obtained by the otolith complex method is demonstrated in Fig. 6. West Kamchatkan juvenile pink salmon dominated (72.0%) and Sakhalin fish were the secondary component (24.0%) in the catch.

Identification of juvenile chum salmon stocks in the autumn 2011 trawl catches in the Okhotsk Sea obtained by the otolith complex method is demonstrated in Fig. 7. West Kamchatkan juvenile chum salmon dominated (75.0%), as was the case with juvenile pink salmon (Fig. 6). Chum salmon stocks from the continental coast of the Okhotsk Sea (20.8%) was a secondary component, and the percentage of chum salmon originating from Japan was 3.5% (Fig. 7).

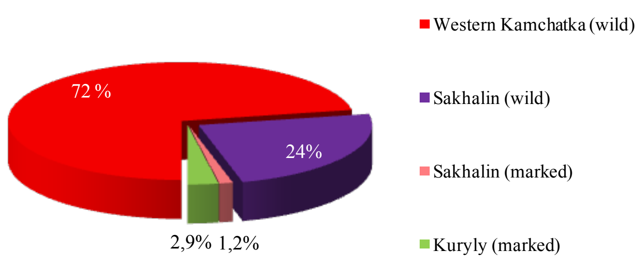

Fig. 6. Stock complex (percentage) of juvenile pink salmon caught in the autumn 2011 trawl catches of R/V *Professor Kaganovsky* in the Okhotsk Sea. Results obtained by the otolith complex method.

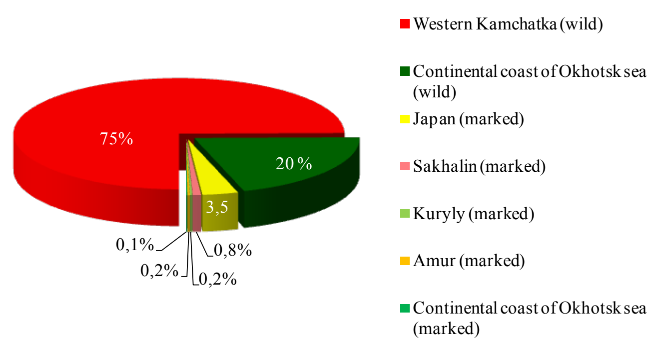

Fig. 7. Stock complex (percentage) of juvenile chum salmon caught in the autumn 2011 trawl catches of R/V *Professor Kaganovsky* in the Okhotsk Sea. Results obtained by the otolith complex method.

In conclusion, we add that we are not proposing to immediately use these results in practice. This investigation is a trial application of a new approach to determine if the stock structure of pink and chum salmon can be determined for mixed marine catches by examining otoliths. In summary, we think this method is promising, especially if used in regions where the abundance of hatchery stocks is significant. Accurate results with the otolith complex method can only be obtained by standardizing and extending baseline sampling sites to encompass all the phenotypic diversity in otolith structure in pink and chum salmon native stocks.

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