

## Determining Area of Origin of Pink Salmon Juveniles on Their Catadromous Migration in the Okhotsk Sea in 1995 Using Genetic Stock Identification Techniques.

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The purpose of this study was to reveal distribution patterns and density of even-year pink salmon juveniles in the eastern Okhotsk Sea, to identify the origin of juveniles caught in mixed stock fisheries in the Okhotsk Sea, and to estimate their relative abundance and migration routes. Trawls at 45 stations in the eastern Okhotsk Sea, from September 10th to October 9th, 1995, yielded a total catch of 3,241 pink salmon juveniles. Of those, 775 were measured and weighed, and 200 were analyzed by protein starch gel electrophoresis.

Allelic frequencies in juveniles collected at eight stations showed significant heterogeneity ( $P < 0.01$ ) at four loci - sAAT-3\*, GR\*, PEPB-1\*, and PGDH\*. The genetic baseline used for mixed-stock identification analysis included allelic frequencies at 31 polymorphic loci in 18 stocks from rivers of the Pacific Ocean coast, studied in 1990, 1992, and 1994. The analysis of simulated mixtures which were composed of 100% of fish from one region showed sufficient ability of the baseline to identify stocks from a mixture.

Mean estimates calculated from Maximum Likelihood Estimate (MLE) analysis showed regional contributions from the simulated mixtures of: western Kamchatka - 39.1%, northwestern Kamchatka - 30.3%, Magadan 0.3%, Sakhalin - 4.3%, southern Kuril Islands - 10.9%, Hokkaido 10.7%, and unknown - 4.4%. In two large regional groups, the estimates were: southern Okhotsk coast -  $25.9 \pm 7.5\%$ , northern Okhotsk coast -  $69.7 \pm 7.5\%$ , and unknown - 4.4%. Weighted by the actual abundance of juveniles in the sea, the regional ratio became: western Kamchatka - 57.0%, northwestern Kamchatka - 29.1%, Magadan - 1.6%, Sakhalin - 2.4%, southern Kuril Islands - 3.5%, Hokkaido - 1.9%, and unknown 4.7%; southern Okhotsk coast - 7.8%, northern Okhotsk coast 87.7%, and unknown - 4.7%. Juveniles from Hokkaido and Iturup rivers were found at latitude 55 N, and juveniles from the southwestern Kamchatka rivers were found at latitude 58 N, which indicates that juveniles migrate north after entering the sea. The migration routes of juveniles from the southern Okhotsk coast, Kamchatka and Magadan are discussed.



### INTRODUCTION

Pacific salmon spend significant portion of their life cycle in the Pacific Ocean and its attached seas. Pink salmon, *Oncorhynchus gorbuscha* (Walbaum), juveniles migrate to the sea the same spring they emerge from their redds (Gritzenko et al. 1987), and some still have residual yolk upon entering salt water (Kinas 1988). Because of such an early migration, the mortality of pink salmon may be the highest at this period of their life cycle (Karpenko 1996a,b). Estimation of the survival rate of pink salmon in this period is important in forecasting the abundance of returning adults (Karpenko et al. 1993). The most reliable way for such estimations is to survey the

density of juveniles in the sea, and to calculate approximate abundance after the period of adaptation to sea water when juveniles have moved away off the shore. Such survey studies were performed in the Okhotsk (Shuntov 1989a) and Bering sea (Shuntov 1989b; Erokhin 1990). During the life in the Okhotsk Sea and the Pacific Ocean the survival rates of pink salmon are usually more constant and easier to forecast (Karpenko 1996a). Therefore, the juvenile survey data in the Okhotsk Sea could be very useful in fisheries management.

Pink salmon juveniles stay in the Okhotsk Sea in summer to feed before migrating to the Pacific Ocean in September-November. The Okhotsk Sea is a mixing ground for juveniles from three main natal

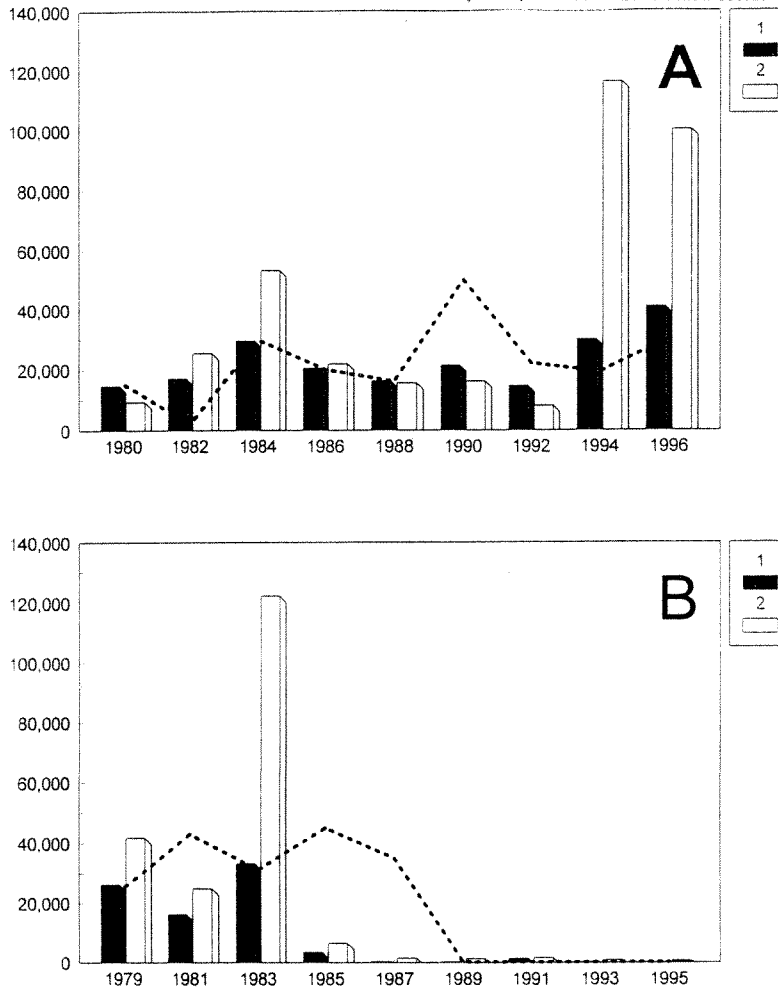
regions: West Kamchatka, East Sakhalin Island, and the northern Okhotsk Sea coast (Magadan Coast).

Pink are the most abundant of Pacific salmon, and they are of significant commercial importance for many Pacific rim countries. They spend approximately a year in freshwater, and a year in the ocean. Their life cycle consists of exactly two years between spawning of consequent generations (Shuntov et al. 1993a,b). Generations spawning in odd years never interbreed with generations spawning in even years. Thus, the species is divided into two genetically different broodlines (Salmenkova et al. 1981; Beacham et al. 1985, 1988; Gagalchy 1986; Jivotovsky et al. 1989; Efremov 1991; Makoyedov et al. 1993), which must be treated separately for research and management purposes. Both even-year and odd-year broodlines experience great fluctuations in abundance (Varnavskaya et al. 1995), making forecasting difficult. Previous miscalculations in forecasting were made in 1985 and 1987 when a great decline in odd-year pink salmon abundance occurred in southwestern Kamchatka rivers (Fig. 1).

In 1983 approximately 100 million of pink salmon spawned in this area; consequently managers expected

a similar return in 1985. Instead, an extremely low return of pink salmon occurred in 1985. Overcrowding on the spawning grounds in 1983, resulting in low survival of eggs and fry, is considered the main cause for the decline. A low return of pink salmon in 1985 was expected to produce a low return in 1987. However, an autumn 1986 survey of juveniles in the Okhotsk Sea revealed a high abundance of pink juveniles on the Kamchatka shelf, and the inference was made that fry survival was high and resulted in a high forecast for the 1987 return (Fig. 1). The expected return did not occur, and led us to believe that Kamchatkan juvenile pink salmon share the Kamchatka shelf with juveniles from other spawning areas, such as those from Magadan or Sakhalin. The main problem was to identify the juveniles from different areas and to estimate their relative abundance on Kamchatka shelf. Such information could be useful for estimations of survival rates of juveniles from different areas, and to reveal the migration routes of juveniles in Okhotsk Sea. The only way to collect such information was to identify genetic markers suitable for mixed-stock identification of juveniles from different spawning areas.

Fig. 1 Catches (1), forecast (---), and spawners (2) of pink salmon of even (A) and odd (B) year broodlines from the rivers of Okhotsk Sea coast of Kamchatka in 1979-1996. X-axis – years. Y-axis – thousand tons.



An Asian pink salmon genetic baseline was developed in a cooperative study by American, Canadian, Japanese, and Russian scientists, from 1988 to 1994. (Varnavskaya and Beacham 1992; Varnavskaya 1992; Shaklee and Varnavskaya 1994; Noll et al. 1995; Noll et al. in press).

The purpose of the present study is to determine the distribution and density of even-year pink salmon juveniles in the eastern Okhotsk Sea, to identify the juveniles originating from different spawning areas of the Okhotsk Sea coast in mixed-stock fisheries, and to estimate their relative abundance and migration routes.

**MATERIAL AND METHODS**

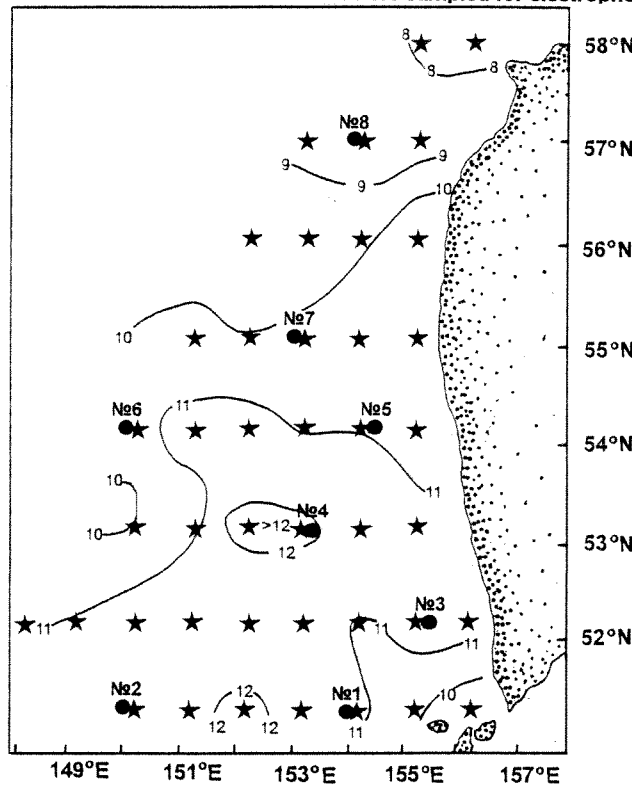
Juvenile pink salmon were caught by trawling at 45 stations in the eastern Okhotsk Sea (Fig. 2, Table 1) from September 10th until October 9th, 1995. Trawling was conducted from south to north

between latitudes 51°N - 58°N, and from east to west and back between longitudes 148°E - 156°E (Fig. 2). Surface water temperatures were measured at each station. A total of 3,241 juveniles were caught, 775 were measured and weighed, 200 of these were analyzed by protein starch gel electrophoresis. (Table 1, Fig. 2). Four tissues (skeletal muscle, heart muscle, liver, and eye retina) were sampled from each freshly caught fish and preserved at -20°C. Long-term storage was in liquid nitrogen. The analysis was conducted at Auke Bay Laboratory by horizontal starch gel electrophoresis (Aebersold et al. 1987). The tissue samples were homogenized in a mixture containing reagents to break down mitochondria, so as to incorporate in the analysis enzymes fixed on the mitochondrial membrane (Aebersold et al. 1987). The proteins were fractionated by starch gel electrophoresis using six buffer systems (Table 2). The active enzyme zones on the gel were revealed by

**Table 1. Average body lengths and weights of pink salmon juveniles sampled from mixed-stock catches in Okhotsk Sea in 1995.**

Area of Okhotsk Sea	Body length (cm)		Body mass (g)		Number of fish
	Average	Min-Max	Average	Min-Max	
South-West	18.6	15.5-25.5	94.7	34-170	208
South-East	18.9	12.6-25.0	68.7	15-160	197
West, central part	22.2	18.0-25.0	118.3	55-175	134
East, central part	20.4	14.3-25.3	88.8	27-165	94
North-West	18.1	17.2-20.2	67.3	53-95	19
North-East	19.1	13.9-28.0	697	22-28	113

**Fig. 2 Trawl stations and average water surface temperatures in eastern Okhotsk Sea in September-October, 1995. Stars designated with numbers - the trawl stations where fish were sampled for electrophoretic analysis.**



**Table 2. Enzyme and loci, screened in even-year broodline pink salmon juveniles from mixed-stock catches in Okhotsk Sea in 1995.**

Enzyme	Subunit structure	IUBNC number	Loci	BS	Tissue
Aspartate aminotransferase	dimer	2.6.1.1	<i>sAAT1,2</i>	2,3,4	MH
			<i>mAAT1</i>	2,3,4	MH
			<i>mAAT2</i>	2,3,4	L
Adenosine deaminase	monomer	3.5.4.4	<i>ADA-2</i>	3	MH
Creatine kinase	dimer	2.7.3.2	<i>CKA1</i>	3	M
			<i>CKA2</i>	3	M
Formaldehyde dehydrogenase	dimer	1.2.1.1	<i>FGDH</i>	4	MH
Glycerol-3-phosphate dehydrogenase	dimer	1.1.1.8	<i>G3PDH1</i>	6	M
Glucose-6-phosphate isomerase	dimer	5.3.1.9	<i>GPIA</i>	3	MH
Isocitrate dehydrogenase	dimer		<i>mIDHP1</i>	4	M
Lactate dehydrogenase	tetramer	1.1.1.14	<i>LDHA1</i>	2,3	M
			<i>LDHA2</i>	2,3	M
			<i>LDHB1</i>	2,3	MHE
			<i>LDHB2</i>	2,3	MLE
			<i>LDHC</i>	2,3	E
Malate dehydrogenase NAD	dimer	1.1.1.37	<i>sMDHA1,2</i>	4,6	LHE
			<i>sMDHB1,2</i>	4,6	M
Malate dehydrogenase NADP	tetramer	1.1.1.40	<i>mMEP1</i>	1	MH
Mannosephosphate isomerase	monomer	5.3.1.8	<i>MPI</i>	2	H
Peptidases		3.4.*.*			
Glycyl-leucine(GL, DPEP)	dimer		<i>PEPA</i>	2	M
Leucyl-glycyl-glycine(LGG, TAPEP)	dimer		<i>PEPB1</i>	2	MLE
Phenylalanine-proline(PP, PDPEP)	dimer		<i>PEPD1</i>	2,4	MH
			<i>PEPD2</i>		MH
6-Phosphogluconate dehydrogenase	dimer	1.1.1.44	<i>PGDH</i>	4	MHLE
Phosphoglucomutase	monomer	5.4.2.2	<i>PGM2</i>	4	MHLE
Pyruvate kinase		2.7.1.40	<i>PK2</i>	4	MHE
Triosephosphate isomerase	dimer	5.3.1.1	<i>TPI1</i>	2,3	MHE
			<i>TPI2</i>	2,3	MHE
			<i>TPI3</i>	2,3	MHE
			<i>TPI4</i>	2,3	mHE

**BS** – buffer system: 1 - TRIS - citrate buffer, pH 5.8 (TC-4) (Dreyfus and Alexander 1972), 2 - TRIS-EDTA-borate buffer, pH 8.5 (TBE) (Boyer et al. 1963), 3 - TRIS-citrate-lithium-borate buffer, pH 8.0 (TBCL) (Ridgway et al. 1970), 4 - N-(3-aminopropyle)-morpholine-citrate-EDTA buffer, pH 6.1 (ACE) (Clayton and Tretiak 1972), 5 - TRIS-Glycine buffer, pH 8.9 (0.04M TRIS, 0.12M glycine for gel and electrode buffer), 6 - N-(3-aminopropyle)-morpholine-citrate buffer (AC) (Dreyfus and Alexander 1972). Tissue designation: M - muscle, H - heart, E - eye, L - liver.

standard stain procedure with agar cover (Aebersold et al., 1987). The enzyme coding loci and alleles were designated using current nomenclature (Shaklee et al. 1990).

The list of analyzed enzymes and laboratory conditions for each are presented in Table 2. Genetic interpretation of screened allozyme variation was made according to previous studies (Beacham et al. 1985, 1988; Gharrett et al. 1988; Shaklee et al. 1991).

Statistical analysis of the resulting data was performed using BIOSYS (Swafford and Selander 1981) and NTSYS (Rohlf 1992) computer programs. The significance of departures from Hardy-Weinberg-Castle equilibrium was estimated using a chi-square goodness-fit test. We used likelihood ratio analysis (G-test; Sokal and Rohlf 1981) to test for heterogeneity in allelic frequencies among samples of juveniles collected at different times and locations in

the Okhotsk Sea.

Conditional Maximum Likelihood estimates (MLE) were calculated from the mixed fisheries using GIRLS program (Pella and Milner 1987; Masuda et al. 1991). This approach had already been used to determine the origin of mixed-stock fisheries of adult pink salmon in the Okhotsk Sea in August, 1994 (Varnavskaya and Davydenko 1995).

The baseline with which the juvenile mixture was compared for stock identification was selected from the even-year pink salmon baseline collected in 1988-1994 (Noll et al. 1995, in press, plus our unpublished data). It included allelic frequencies at 31 variable loci from 18 pink salmon populations from the Okhotsk Sea coast (Tables 2,3). The genetic frequencies from geographically close and genetically similar populations were pooled to increase the number of loci for better accuracy and precision. For the MLE-analysis the populations were combined in regional groups: Hokkaido (Japan), eastern Sakhalin, Iturup (southern Kuril Islands), north-western Kamchatka, south-western Kamchatka, and Magadan (northern coast of the Okhotsk Sea).

A series of mixtures were simulated from the baseline using the program SIMULATR (Masuda et al. 1991). SIMULATR generates random samples from the baseline data, and creates mixtures of specified size and composition. Each simulated mixture was then analyzed using the Conditional Maximum Likelihood Estimate (MLE). Simulated mixtures were created in two ways: an equal

percentage of abundance from each of the 18 populations and a percentage of 100% of each regional group of populations (total of 8 mixtures). In simulation 2 the actual contribution approaches the limit of the MLE, 0 or 100%. Results are presented as average estimates.

## RESULTS

Water surface temperatures at examined stations in the Okhotsk sea (Fig. 2) averaged 10°C in September and October 1995. Regionally, northern Okhotsk Sea stations averaged 8.5°C, and southern stations averaged approximately 11°C. Central stations were the warmest, with surface temperatures averaging 12°C. This central, warmer area exhibited the greatest abundance (Fig. 3) and largest average body size (Table 1) of pink salmon juveniles. Other large schools of pink salmon juveniles were found in the northern part of the Okhotsk Sea, but no juveniles were found near shore to Western Kamchatka in September and October.

Allelic frequencies in juveniles collected at eight stations (Fig. 4) were significantly heterogeneous at four loci — sAAT-3\*, GR\*, PEP-B1\*, and PGDH\*. The total for all loci was  $P < 0.01$ . This suggests that juveniles caught in different locations of the Okhotsk Sea originated from genetically different populations.

Before analyzing mixed-stock juvenile fisheries, simulations, each composed of 100% of fish from a single region, were run to estimate the accuracy and

Table 3. Populations of even-year broodline pink salmon included in database (31 variable loci) for identification in mixed-stock catches of juveniles in Okhotsk Sea in 1995.

Region	N	Population	Number of collections	Number of fish	Years
Japan	1	Kushiro River	1	100	1988
	2	Tokadzu River	1	100	1988
	3	Yurrappu	1	100	1988
Sakhalin	4	Monetka	2	200	1990
	5	Znamenka	2	200	1990
	6	Lutoga	3	300	1990,92
	7	Pugachevka	2	200	1990,92
	8	Dolinka	2	100	1990,92
	9	South-Eastern rivers	9	900	1992
Kuril Islands	10	Rivers of Iturup Island	3	240	1994
West Kamchatka	11	Kik-Chik	1	80	1990
	12	Utka	2	160	1990,92
	13	Kol	3	240	1990,92
	14	Homutina	2	160	1990,92
	15	Pymta	2	160	1990,92
North-West Kamchatka	16	Hairusova, Kovran	4	200	1990,94
Magadan Coast of Okhotsk Sea	17	Uglekank	1	80	1990
	18	Tau	1	80	1990
Total			42	3600	1988,90,92,94

Table 4. Log-likelihood analysis (G-test) of genetic heterogeneity among collections of juveniles from Okhotsk Sea in 1995.

Loci	Number of fish	q	G-test	IDF	P
<i>sAAT1,2</i>	199	0.992	5.9	7	non
<i>sAAT-3</i>	180	0.969	21.9	7	<0.01
<i>mAAT-4</i>	139	0.525	1.4	6	non
<i>ADA-2</i>	188	0.952	15.5	14	non
<i>FGDH</i>	172	0.994	8.0	14	non
<i>FH</i>	170	0.977	18.5	14	non
<i>G3PDH1</i>	199	0.915	6.1	7	non
<i>GDA</i>	194	0.474	13.8	14	non
<i>GR</i>	197	0.926	18.3	7	<0.01
<i>mMEP1</i>	196	0.806	7.1	7	non
<i>PEPB1</i>	186	0.852	33.3	7	<0.001
<i>PEPD2</i>	195	0.618	5.1	14	non
<i>PGDH</i>	196	0.834	17.1	7	<0.05
Total	-	-	180.9	132	<0.01

precision of the baseline for stock identification analysis. The results of the simulations showed that with our baseline data we were able to reveal in the mixture the portion mainly not less than 5-10%.

To determine the distribution of juveniles from different natal rivers in the eastern Okhotsk Sea in September and October, MLE analysis was used to calculate percent composition for each mixed-stock collection (Fig. 4). Off the southwestern end of Kamchatka (stations 1, 2 and 3, Fig. 4), juveniles from Sakhalin comprised about 16, 18 and 6%, from Hokkaido - 9, 23 and 6%, and from Iturup - 4, 2 and 26%. Juveniles from the southern Okhotsk Sea Coast (Sakhalin, southern Kuril Islands, and Japan) comprised nearly 50% over these three collections (Fig. 4). A collection from the station 7 was of a similar composition. At all four above mentioned stations (1, 2, 3, and 7) the juveniles were of the lowest abundance (25 fish per trawl) (Fig. 3). At all other stations fish from Kamchatka predominated (up to 95%). At stations 4 (300 fish per trawl), 5 (100 fish per trawl), and 8 (100 fish per trawl) juveniles were very abundant (Fig. 3), and fish from the southwestern Kamchatka rivers predominated in collections (Fig. 4).

Estimates of percent origin of juvenile pink salmon in the Sea of Okhotsk were made based on average of individual station percent origins weighted by the number of fish per trawl. The results were: south-western Kamchatka - 57.0%, northwestern Kamchatka - 29.1%, the Magadan coast - 1.6%, Sakhalin - 2.4%, southern Kuril Islands - 3.5%, Hokkaido - 1.9% and unknown 4.7%. For the total, the southern Okhotsk Coast aggregate comprised 7.8%, the northern Okhotsk Coast aggregate comprised 87.7%, and unknown - 4.7%

## DISCUSSION

Pink salmon juveniles from Hokkaido and Iturup rivers were found at latitude 55°N and those from South-Western Kamchatka rivers were found at latitude 58°N. This indicates that pink salmon juveniles are migrating far north of their natal rivers using ocean currents which flow north along the Kuril and Kamchatka shelf (Davydov 1975). Kuril and Hokkaido pink salmon juveniles migrate north along the Kuril and Kamchatka shelf, turn to the south somewhere at 53-55°N latitude, and then head to the south (Fig 5). Having already covered a significant distance through rich feeding grounds, they grow to 21-24 cm while traveling north, and gain larger size on their way south. Migrating simultaneously in the same currents are small southwestern Kamchatkan juveniles (Fig. 5). These juveniles continue the northerly migration to Shelekhov Bay and Gijiga Inlet after the southern juveniles turn south around 55°N. In Shelekhov Bay, two currents meet and mix, providing ample nutrients and propitious feeding and growth for juveniles (Davydov 1975). Kamchatkan juveniles probably remain in Shelekhov Bay for a substantial period of time (Erokhin 1990). This is confirmed by the fact that the stock composition of juveniles in this zone (Fig. 4) resembled the actual ratio of abundance of northern and southern Kamchatkan even-year pink salmon stocks (Varnavskaya et al. 1995). Juveniles from Magadan Coast may also migrate in the northeastern direction to Shelekhov Bay and mix with juveniles from Kamchatka. Determining an accurate estimate of Magadan region juveniles in a mixture is difficult because the rivers of that region yield a low

Fig. 3 The density of schools of juveniles in eastern Okhotsk Sea in September-October, 1995. Numbers represent the quantity of fish caught in one trawl.

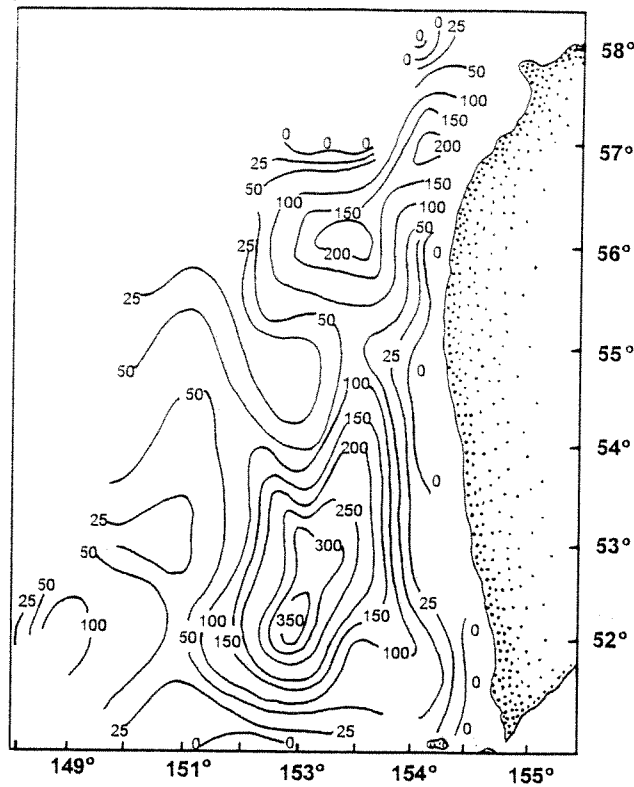


Fig. 4 The percentage of juveniles from six spawning regions in mixed samples calculated by MLE-analysis based on allelic variation at 31 loci in eastern Okhotsk Sea in September-October, 1995.

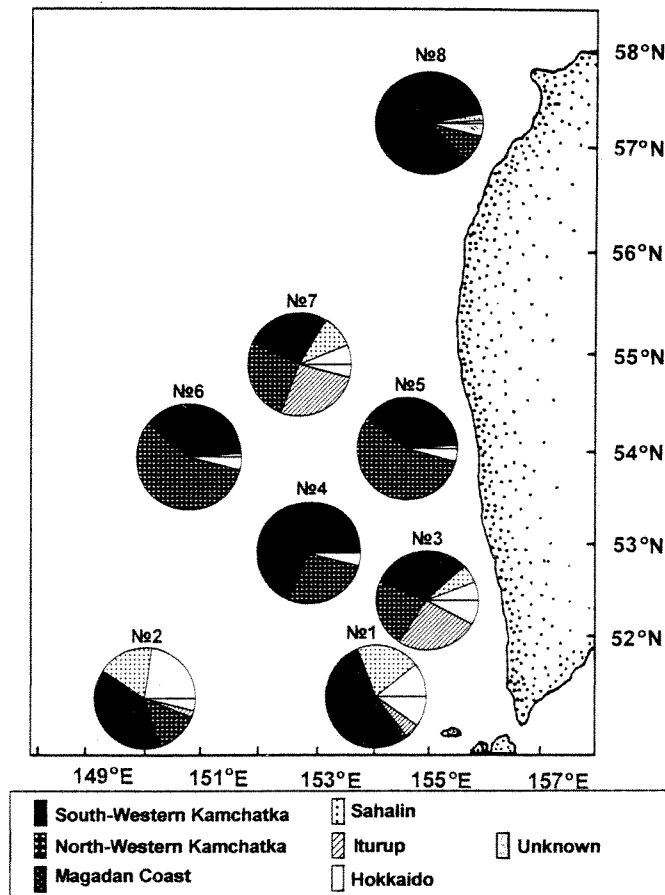
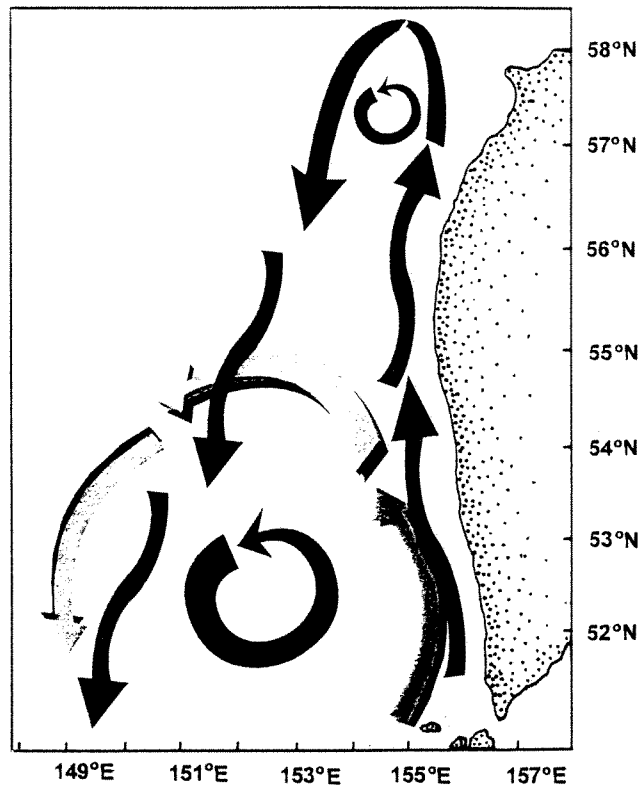


Fig. 5 The scheme of migration routes of pink salmon juveniles in eastern Okhotsk Sea in September-October, 1995. Light arrows - southern Okhotsk Sea group (Hokkaido, Sakhalin, Iturup), dark arrows - northern Okhotsk Sea group (Magadan and Kamchatka coast).



abundance of pink salmon in the even year. The largest portion of Magadan juveniles (4%) was found at station 8, located near the feeding area in Shelekhov Bay (Fig. 4). After a prolonged stay in Shelekhov Bay, the juveniles migrate south, using a route further offshore than the Kamchatka coastline route.

In the central part of the Okhotsk Sea there is a zone of higher temperatures (Figs. 3, 5). Both southern and northern migrations of juveniles are likely to use this area for an extended time. The stock composition in this area is similar to the average estimates for the entire Okhotsk Sea in 1995. Though the sample size was small which resulted in big standard error for each collection, the data still allowed us to reveal the migration routes of pink salmon in the Okhotsk Sea (Figs. 4, 5).

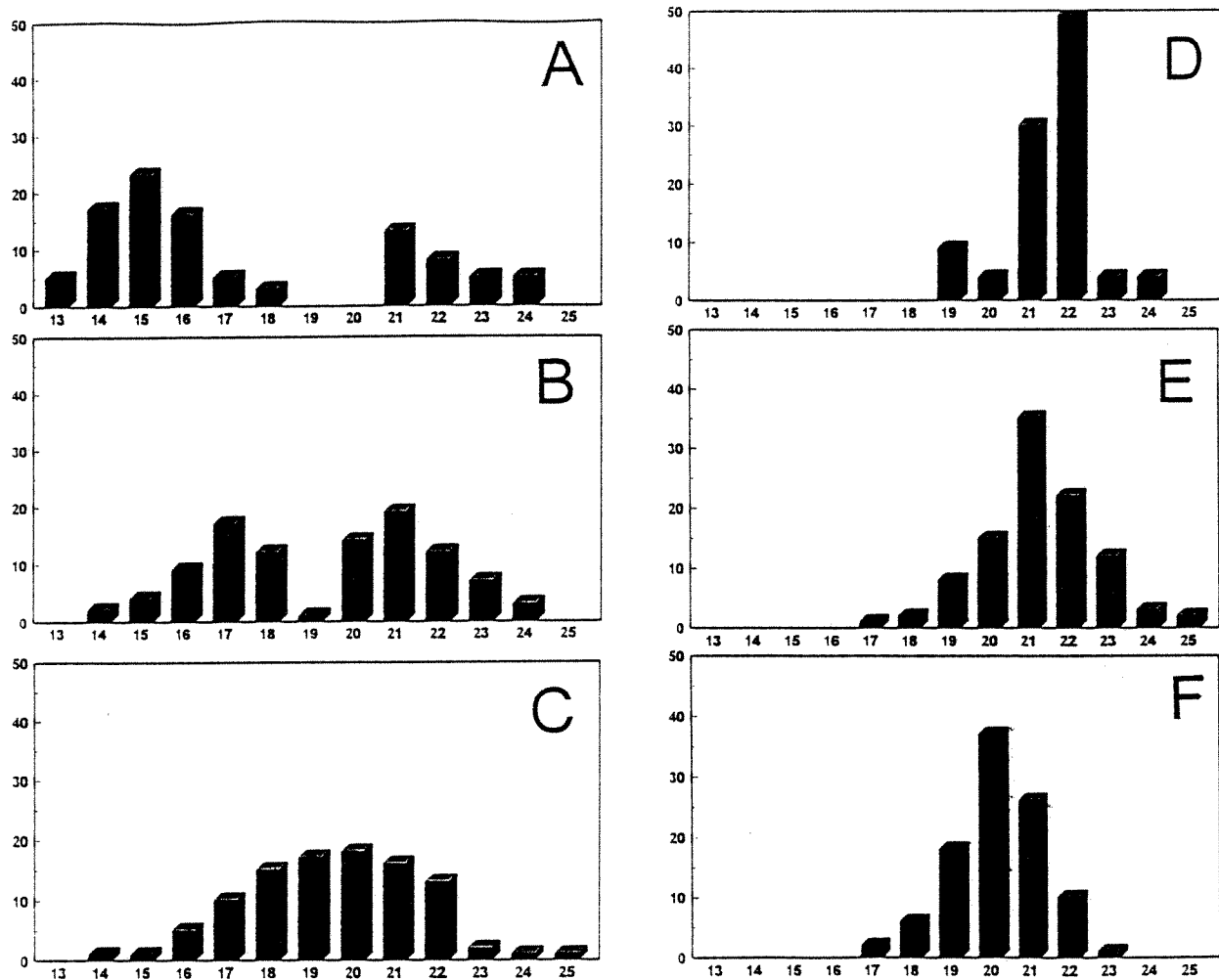
The scheme of migration routes of juveniles in the eastern Okhotsk Sea (Fig. 5), constructed from the stock identification results (Fig. 4), is supported confirmed by the analysis of body length and weight distribution of juveniles caught along latitude 52°N. Six collections of juveniles were analyzed for body length and weight (A, B, C, D, E, and F), caught near 155°E, 153°E, 152°E, 151°E, 150°E, and 149°E, respectively (Fig. 6). Collection A, at 155°E near the south-western coast of Kamchatka, presented two

distributions for body length: 13-18 cm., and 21-24 cm. This confirmed the hypothesis that two populations of juveniles existed in this area; small Kamchatka juveniles, and larger Hokkaido-Kuril-Sakhalin juveniles. The Hokkaido-Kuril-Sakhalin juveniles are believed to be almost twice the size of the Kamchatka juveniles because they have been at sea for a longer time as inferred from the migration time necessary to arrive in the southeastern Sea of Okhotsk. The Kamchatkan juveniles have just recently entered the sea from West Kamchatka rivers. The group of large bodied fish represented approximately 35% of the collection. The stock composition estimate of juveniles from this area (station 3), showed an approximate 38% representation of southern (Hokkaido, Kuril, and Sakhalin) juveniles (Fig. 4).

At 153E and 152E (Fig. 6, B, C) in the central warmer zone the body length distributions were flat and had a negative excess, which indicated, that there was a mixture of all juveniles of all body sizes, as it was supposed to be according to our conclusion of juveniles mixing over there and staying for feeding and growth. At 151°E, 150°E, and 149°E (Fig. 6, D, E, F) the body length distributions looked normal, or had a positive excess, and indicated the presence of larger fish, which were, according to our conclusion,



Fig. 6 Body length distributions in collections of pink salmon juveniles caught along 52°N (A - 155°E, B - 153°E, C - 152°E, D - 151°E, E - 150°E, F - 149°E). X-axis – body length (cm), Y-axis – percentage of fish.



on their southward migration out of the Okhotsk Sea.

Though juvenile pink salmon from Sakhalin were detected in mixtures in the eastern Okhotsk Sea, the percentage does not account for the total abundance of Sakhalin juveniles. We conclude that perhaps only the juveniles from Aniva Bay, and, maybe, partly Terpenie Bay (southern Okhotsk coast of Sakhalin), migrate north together with juveniles from Hokkaido and Iturup along Kuril shelf to Kamchatka shelf, and the remaining portion of the Sakhalin fish migrate north along the Siberian coastline, joining schools of juveniles from the Amur River and rivers of the western Okhotsk coast. To reveal their migration routes we need sampling in the western Okhotsk Sea.

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