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VERTEBRATE DEVELOPMENTAL BIOLOGY

Dynamics of Lipid and Fatty Acid Content at Early Ontogenesis Stages in Pink Salmon *Oncorhynchus gorbuscha* (Walbaum, 1792) in a Natural Environment (Indera River, Kola Peninsula)

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Abstract—A comparative study of lipid and fatty acid status at the early stages of pink salmon's ontogenesis has been carried out in prespawn eggs, at the stage of eye pigmentation, and in the hatched prelarvae under ecological conditions of a natural environment (Indera River, Kola Peninsula). It is shown that the high plasticity of pink salmon is due to the activation and combination of complex biochemical mechanisms at the early stages of development, providing both the sensitivity and the sustainability of the species, which contributes to the formation of the species' high potential at high latitudes. Metabolically mature pink salmon eggs are characterized by a high level of total lipids with a high amount of the structural and energetic components that provide optimal development of the embryos. Metabolic processes are activated at the stage of eye pigmentation in autumn, which affects the changes in the spectrum of individual classes of lipids and fatty acids and their ratio indicators. The prelarvae that hatched in winter at temperatures below zero have a low content of phospholipids, triacylglycerols, and 16:1ω-7, 18:3ω-3, and 20:5ω-3 fatty acids as well as high level of cholesterol, cholesterol esters, and a ratio index of 20:4ω-6/18:2ω-6 fatty acids, while the high content of dominant 18:1ω-9 and 22:6ω-3 fatty acids remains the same. Multidirectional changes of lipid and fatty acid levels at the stage of eye pigmentation (the most sensitive stage of embryogenesis) and in pink salmon prelarvae after hatching are caused by specific processes of metabolism intensification of the developing organism.

Keywords: lipids, fatty acids, eggs, stage of eye pigmentation, prelarvae, ontogenesis, pink salmon, White Sea

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INTRODUCTION

Biochemical studies on lipid and fatty acid status of pink salmon *Oncorhynchus gorbusha* (Walbaum, 1792) at early stages of ontogenesis are interesting from the point of view of a search for possible assessment indicators of their further development potential (especially for larvae) and offspring formation. Pink salmon are the most numerous and plastic species of Far East salmon. They are anadromous fishes and have the shortest life cycle among representatives of the family Salmonidae (matures in 22–23 months after spawning) (Zubchenko et al., 2004). In northern latitudes, the spawning migration of pink salmon in the Indera River begins in the middle of June, its spawning occurs in autumn, and embryogenesis occurs in winter at low temperatures and under ice. Embryos of pink salmon develop in spawning nests in rivers with pebbly sediments. Approximately 500 degree-days should pass before larvae hatching. Hatching of juvenile fish occurs from the end of January until the beginning of March depending on climatic conditions. Larvae then stay in the sediments, feed on the content of the yolk sac that is completely absorbed by April (in larvae in rivers of the Kola Peninsula), and withstand a period without food until May. Mass downstream migration of juveniles from the river to feeding areas in the sea begins with ice break-up and the increase in water temperature to 5°C and higher (Zubchenko et al., 2004).

One of the main biochemical criteria of egg maturation and its readiness for fertilization is the content of lipids in the yolk sac and their particular level and the ratio of some lipid classes, including their fatty acids, are indicators of the viability of embryos and larvae (Kryzhanovskii, 1960; Sidorov, 1983; Tocher, 2003). Fatty acids, the components of lipid molecules, participate in energetic processes in cells, in regulation of biochemical reactions and physiological processes, including those associated with age-related aspects. During vitellogenesis, yolk proteins and lipids are accumulated in the oocyte (Wiegand, 1996).

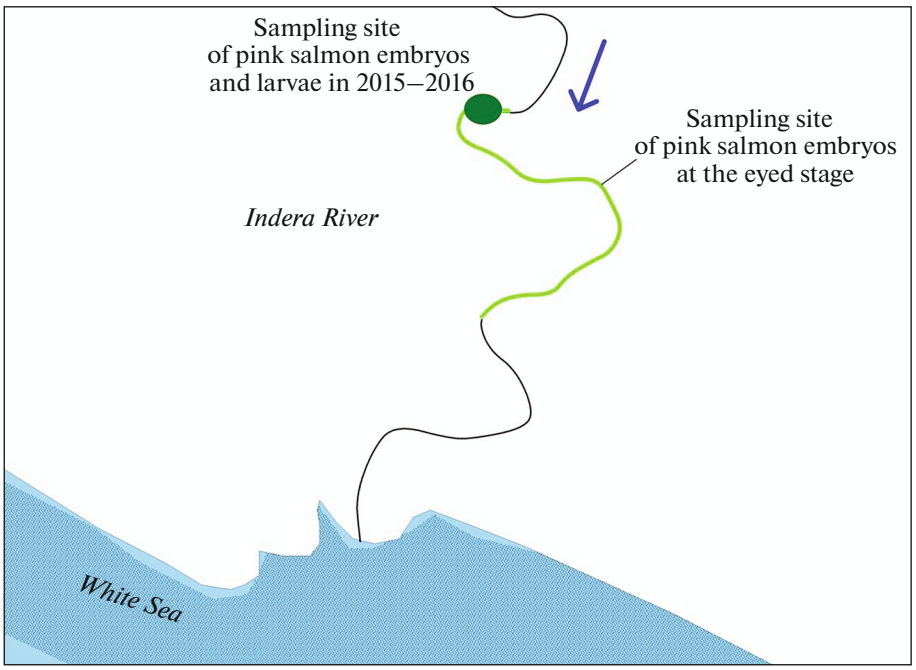

Fig. 1. Sampling sites of embryos and larvae of pink salmon in 2015–2016.

Each stage of ontogenesis is characterized by particular morphological and physiological changes maintained by biochemical reactions and processes of different intensities. By the stage of eye pigmentation (at complete pigmentation of the choroid of eye with melatonin) heat and liver are formed, the liver-yolk system of blood circulation starts to function, and yolk components are utilized more actively (Wiegand, 1996; Ryzhkov and Krupen, 2004). It was earlier established that the decrease in the yolk mass by the stage of pigmentation in embryos of salmon (from the stage of cleavage) is 24.0% (Nefedova, 1989). The stage of eye pigmentation is one of the sensitive (critical) stages of embryogenesis; the metabolic activity with considerable morphophysical and biochemical transformations increases (Pavlov, 1984; Novikov, 2000; Murzina et al., 2012; Nemova et al., 2015). The potential for survival of hatched larvae depends on the initial level of lipids in prespawn eggs and rates of their degradation during embryonic development.

The aims of this work are to study the lipid and fatty acid status at the early stages of pink salmon ontogenesis in prespawn eggs, at the stage of eye pigmentation, and in hatched prelarvae with an account for the ecological conditions of natural environment in the Indera River (Kola Peninsula).

MATERIALS AND METHODS

Individual samples of prespawn eggs were collected a week before spawning in the first decade of August. Mass spawning migration of pink salmon in the Indera River occurs at the beginning of August. The average water temperature varies from 16 to 12°C in this period.

Embryos of pink salmon were collected at the stage of eye pigmentation in the Indera River at a distance of 1.0–1.8 km from the mouth on October 5 (Fig. 1) at water temperature 1.5°C. Embryos developed under ice in natural nests in the river (Fig. 2). More than 200 embryos of pink salmon were samples from one nest in the center of the river channel. The depth in the site was 0.4–0.5 m and the surface current velocity was 0.9–1.0 m/s. The nest was formed by large- and medium-size pebble, gravel, and sand without vegetation. The period of embryonic development lasted from the end of August to the end of January. Hatching of prelarvae began at the end of January and lasted to the beginning of March depending on temperature conditions. Prelarvae were collected in March from two spawning nests located at a distance of 10 m from each other. Water temperature was –0.5°C. The depth of occurrence both of embryos and prelarvae of pink salmon did not exceed 10–15 cm below the surface of the redd. Larvae had a transparent body with an expressed head, body, tail part, and oval elongated yolk sac that was 2/3 of the body length. The yolk sac and area of its attachment to the body was of a brightly orange color.

The lipid status of mature eggs, embryos at the stage of eye pigmentation, and hatched prelarvae was estimated according to the content of total lipids (TL), including phospholipids (PL) fractioned into phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidyl ethanolamine (PEA), phosphatidylcholine

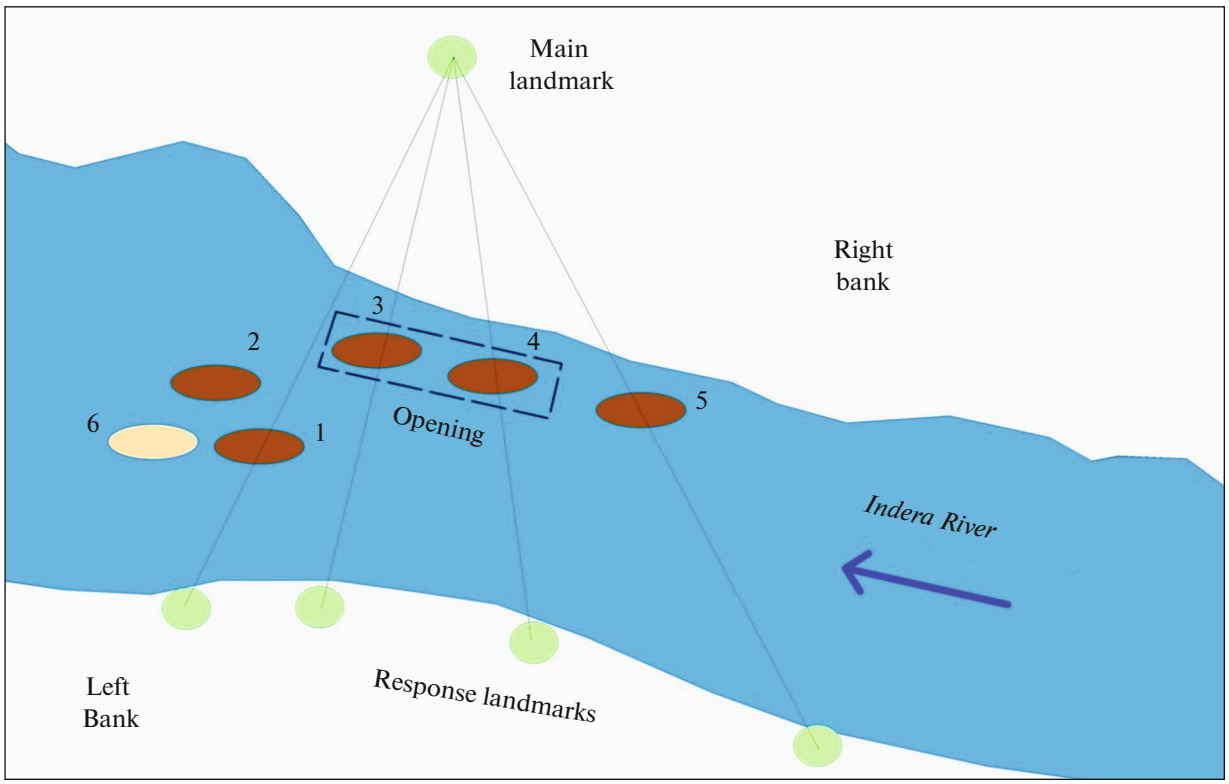

Fig. 2. Scheme natural nest location of pink salmon. (1, 2, 3, 4, 5) Nests of egg sampling in the winter period; (6) a nest in which the sample was taken at the eye stage in October 2015. The dotted line denotes an opening cut through ice; the sample was collected in March 2016.

(PC), lysophosphatidylcholine (LPC) and sphingomyelin (SPM), triacylglycerols (TAG), cholesterol (CHOL), cholesterol esters (CE), and fatty acids (FA) of total lipids: saturated (SFA), monounsaturated MUFA), and polyunsaturated (PUFA) fatty acids. Samples of prespawn eggs, embryos, and prelarvae of pink salmon (300 mg each in 10–30 replicates) were homogenized in a small amount of ethanol (96%), fixed in a chloroform–methanol mixture (2 : 1), and stored at 4°C prior to analysis. The lipids were extracted and purified according to the Folch method (Folch et al., 1957), concentrated by evaporation using a rotary vacuum pump and fractioned on Silufol plates (Kavalier, Czech Republic) in a petroleum ether : diethyl ether : acetic acid solvent system (90 : 10 : 1 by volume). The total amounts of phospholipids, triacylglycerols, and cholesterol esters were determined by the hydroxamate method (Walsh et al., 1965; Sidorov et al., 1972); cholesterol (CHOL) was determined according to the procedure of Engelbrecht (Engelbrecht et al., 1974) and expressed as a percentage of dry matter weight. Phospholipids were fractioned into PI, PS, PEA, PC, LPC, and SPM using high performance liquid chromatography (Arduini, 1996) on a Nucleosil 100-7 steel column (Elsiko, Moscow, Russia). The mobile phase consisted of acetonitrile–hexane–methanol–orthophosphoric acid (918 : 30 : 30 : 17.5). Detection was made by the degree of light absorption at 206 nm. Standard PLs (Sigma Aldrich, United States) were used for lipid identification. The ratio of phospholipid components was determined from areas of chromatographic peaks. Methanolysis of FAs of total lipids was performed (Tsyganov et al., 1976). Methyl esters of fatty acids were separated and identified by the method of gas-liquid chromatography using a Kristall 5000.2 chromatograph (Khromatek, Russia). Behenic acid (22 : 0) was used as an internal standard (Sigma Aldrich, United States), and chromatograms were analyzed using Khromatek Analytik software (Khromatek, Russia).

The results of the experiments were processed by common methods of variation statistics using Excel and Stadia software.

The studies were performed using the equipment of the Center for Collective Use of the Karelian Research Center, Russian Academy of Sciences.

RESULTS

The results of the study of the lipid and fatty acid status of pink salmon prespawn eggs during its embryonic development, at a stage of eye pigmentation, and in hatched prelarvae from two nests are presented in Tables 1 and 2.

Table 1. Content of lipid components (% dry weight) in pink salmon at different stages of development from the Indera River

Parameter	Mature eggs (before spawning)	Eggs at the stage of eye pigmentation	Prelarvae of pink salmon from spawning nests	
date of sampling	August 2015	October 2015	March 2016	
			spawning nests	
			1	2
Number of samples	30	12	7	7
TLs	21.01 ± 0.28	28.2 ± 1.12^	22.31 ± 0.39^^	20.13 ± 0.34*
PLs	10.85 ± 0.30	9.57 ± 0.47^	6.24 ± 0.83^^	7.71 ± 0.44
PI	0.06 ± 0.01	0.06 ± 0.01	0.09 ± 0.02	0.13 ± 0.02
PS	0.01 ± 0	0.04 ± 0.003^	0.13 ± 0.03^^	0.14 ± 0.02
PEA	0.96 ± 0.03	1.06 ± 0.07	1.32 ± 0.19	1.76 ± 0.07
PC	9.58 ± 0.29	8.01 ± 0.40^	4.43 ± 0.60^^	5.33 ± 0.32
LPC	0.16 ± 0.02	0.31 ± 0.07^	0.17 ± 0.02	0.25 ± 0.03*
SPM	0.07 ± 0.01	0.06 ± 0.01	0.08 ± 0.02	0.08 ± 0.02
TAGs	8.57 ± 0.32	17.49 ± 0.80^	13.25 ± 1.27^^	10.61 ± 0.43
EC	0.34 ± 0.05	0.17 ± 0.03^	0.65 ± 0.28^^	0.27 ± 0.15
CHOL	1.25 ± 0.17	0.97 ± 0.20	2.18 ± 0.22^^	1.54 ± 0.15*
CHOL/PL	0.12 ± 0.02	0.11 ± 0.02	0.39 ± 0.06^^	0.21 ± 0.03*
TAG/PL	0.82 ± 0.04	1.85 ± 0.08^	2.69 ± 0.72	1.42 ± 0.11

Values are presented as: M ± m; TL—total lipids; PL—phospholipids; PI—phosphatidylinositol; PS—phosphatidylserine; PEA—phosphatidylethanolamine; PC—phosphatidylcholine; LPC—lysophosphatidylcholine; SPM—sphingomyelin; TAG—triacylglycerols; CE—cholesterol esters; CHOL—cholesterol; ^ differences are significant for eggs before spawning ($p \le 0.05$; ANOVA); ^^ differences are significant for eggs at the stage of eye pigmentation ($p \le 0.05$; ANOVA); * differences are significant for larvae from nests 1 and 2 ($p \le 0.05$; ANOVA).

A high content of total lipids (21% of dry weight) was found in mature eggs before spawning due to the dominance of structural FAs (10.8% of dry weight) and reserve TAGs (8.6% of dry weight). The ratio of reserve and structural lipids (TAG/PL) was 0.82. The proportion of CHOL was 1.25%, and the proportion of its reserve form CE was 3.7 times smaller (0.34% of dry weight). The ratio of structural lipids CHOL/PL was 0.12. PC was dominant (9.58% of dry weight) in the phospholipid spectrum of TL and the proportion of other PL (PEA, LPC, PI, PS, and SPM) did not exceed 1.0% of dry weight.

When comparing the lipid spectrum in embryos at the stage of eye pigmentation with that in prespawn eggs of pink salmon, we found that the content of TLs increased 1.3 times (during the period from August to the beginning of October) mainly due to energetic TAGs, and the content of PLs slightly but significantly decreased due to PCs. The proportion of a minor LPC, a metabolic derivative of PC, increased twice. As a result of such changes, the coefficient TAG/PL increased 2.25 times. The level of structural CHOL in embryos at the stage of eye pigmentation compared to that in prespawn eggs did not significantly change, but the proportion of its reserve form, CE, decreased 2.0 times.

In the FA spectrum of total lipids in prespawn eggs and embryos at the stage of eye pigmentation, a high content of total PUFA (41.9 and 48% of the total amount of FAs, respectively) with the prevalence of PUFA of the ω-3 family (37.8 and 43.92% of the total FAs, respectively) due to physiologically active eicosapentaenoic, 20:5ω-3, and docosahexaenoic, 22:6ω-3, FAs in high and equal proportions. The proportion of PUFA of the ω-3 family increased (1.16 times) by the stage of eye pigmentation. Low and equal proportions of PUFA of the ω-6 family (3.37 and 3.34% of the total FAs, respectively), including metabolically important linoleic 18:2ω-6 and its derivative arachidonic 20:4ω-6 FAs, were found in prespawn eggs and embryos at the stage of eye pigmentation. It is established that the coefficients of the 18:3ω-3/18:2ω-6 and ω-3PUFA/ω-6PUFA ratios characterizing the direction of metabolism of PUFA of the ω-3 and ω-6 families in prespawn eggs and embryos of pink salmon were 0.61 and 11.4, 0.85 and 13.15, respectively.

Table 2. Content of lipid components (% of the sum of FAs) in mature eggs of pink salmon and during early ontogenesis in the Indera River

Parameter	Mature eggs (before spawning)	Eggs at the stage of eye pigmentation	Prelarvae of pink salmon from spawning nests	
date of sampling	August 2015	October 2015	March 2016	
			spawning nests	
			1	2
Number of samples	30	15	7	7
14:0	2.29 ± 0.06	1.95 ± 0.02^	2.19 ± 0.07^^	1.87 ± 0.03*
16:0	10.73 ± 0.13	9.64 ± 0.06^	10.84 ± 0.22^^	12.01 ± 0.18*
18:0	4.47 ± 0.10	4.16 ± 0.03^	4.01 ± 0.08^^	4.41 ± 0.10*
Sum of SFAs	18.82 ± 0.23	16.93 ± 0.09^	18.49 ± 0.21^^	19.59 ± 0.33*
16:1ω-9	0.73 ± 0.02	0.64 ± 0.004^	0.78 ± 0.01^^	0.81 ± 0.01
16:1ω-7	7.33 ± 0.18	6.52 ± 0.02^	6.11 ± 0.03^^	6.26 ± 0.07
18:1ω-9	23.19 ± 0.53	21.08 ± 0.08^	21.60 ± 0.10^^	21.35 ± 0.16
18:1ω-7	4.67 ± 0.10	4.10 ± 0.01^	4.15 ± 0.07	3.81 ± 0.04*
20:1ω-9	1.86 ± 0.11	1.34 ± 0.04^	1.99 ± 0.08^^	1.52 ± 0.02*
Sum of MUFAs	39.24 ± 0.72	35.04 ± 0.09^	36.15 ± 0.19^^	35.10 ± 0.26*
18:2ω-6	1.63 ± 0.03	1.57 ± 0.01	1.68 ± 0.04^^	1.47 ± 0.01*
20:4ω-6	0.87 ± 0.02	0.88 ± 0.004	0.99 ± 0.01^^	1.01 ± 0.02
Sum of ω-6 PUFAs	3.37 ± 0.05	3.34 ± 0.02	3.55 ± 0.05^^	3.32 ± 0.03*
18:3ω-3	0.99 ± 0.03	1.34 ± 0.02^	1.00 ± 0.01^^	1.12 ± 0.01*
18:4ω-3	0.89 ± 0.05	1.15 ± 0.01^	0.88 ± 0.03^^	1.11 ± 0.01*
20:4ω-3	2.14 ± 0.06	3.03 ± 0.03^	2.07 ± 0.09^^	2.73 ± 0.04*
20:5ω-3	14.93 ± 0.46	16.63 ± 0.07^	14.98 ± 0.24^^	13.82 ± 0.15*
22:5ω-3	4.60 ± 0.09	5.07 ± 0.03^	5.27 ± 0.14	4.48 ± 0.04*
22:6ω-3	13.68 ± 0.38	16.12 ± 0.05^	16.25 ± 0.28	17.46 ± 0.37
Sum of ω-3 PUFAs	37.80 ± 0.89	43.92 ± 0.16^	41.01 ± 0.12^^	41.30 ± 0.52
Sum of PUFAs	41.91 ± 0.90	47.99 ± 0.17^	45.30 ± 0.13^^	45.27 ± 0.55
ω-3/ω-6	11.28 ± 0.29	13.16 ± 0.08^	11.55 ± 0.18^^	12.44 ± 0.08*
ω-6/ω-3	0.09 ± 0	0.08 ± 0.001^	0.09 ± 0.001	0.08 ± 0.001*
16:0/18:1ω-9	0.47 ± 0.01	0.46 ± 0.002	0.50 ± 0.01^^	0.56 ± 0.01*
18:3ω-3/18:2ω-6	0.61 ± 0.02	0.85 ± 0.02^	0.60 ± 0.02^^	0.76 ± 0.005*
SFA/PUFA	0.46 ± 0.02	0.35 ± 0^	0.41 ± 0.005^^	0.43 ± 0.01
20:4ω-6/18:2ω-6	0.54 ± 0.01	0.56 ± 0	1.24 ± 0.1^^	1.86 ± 0.02*
22:6ω-3/18:3ω-3	13.98 ± 0.45	12.11 ± 0.22^	16.30 ± 0.13^^	15.63 ± 0.36

Values are given as: M ± m; SFAs—saturated fatty acids; MUFAs—monounsaturated fatty acids; PUFAs—polyunsaturated fatty acids. The samples also contained <1% FAs: 15:0, 17:0, 24:0, 14:1ω-9, 14:1ω-7, 14:1ω-5, 16:1ω-5, 20:1ω-7, 14:2ω-9, 16:2ω-9, 18:2ω-9, 14:2ω-7, 16:2ω-7, 18:2ω-7, 18:3ω-6, 20:2ω-6, 20:3ω-6, 22:3ω-6, 22:4ω-6, 22:5ω-6, 14:2ω-5, 18:2ω-4, 18:3ω-4, 18:4ω-4, 16:2ω-3, 16:3ω-3, 16:4ω-3, 18:2ω-3, 20:3ω-3, 22:4ω-3; ^ differences are significant for eggs before spawning; ^^ differences are significant for the stage of eye pigmentation; * differences are significant in larvae from nests 1 and 2 ($p \le 0.05$; ANOVA).

MUFAs are the second after PUFAs in respect to their content (39.2 and 35.0% of the total FAs, respectively); among them, oleic acid, 18:1ω-9, constitutes the highest proportion, while 16:1ω-7 and 18:1ω-7 FAs are found in smaller amounts. The proportion of all PUFAs decreased by the stage of eye pigmentation in the studied embryos. It should be noted that the content of 18:1ω-9 FA is the highest (23.19 and 21.1% of the total FAs, respectively) among all found fatty acids both in prespawn eggs and embryos of pink salmon at the stage of eye pigmentation.

The lipid and fatty acid spectra of developing embryos at the stage of eye pigmentation were compared with those in prelarvae of pink salmon from two spawning nests in the Indera River. A decrease in the TL content due to structural PLs (including PC and LPC) and reserve TAGs, an increase in the level of minor PLs—such as PS, PI, and PEA as well as structural CHOL and its reserve form, CE—an increase in the proportion of SFA, MUFA (due to 18:1ω-9 FA), and PUFA of the ω-6 family, and a decrease in the proportion of PUFA of the ω-3 family (due to 20:5ω-3 and 22:5ω-3 FAs) was observed in prelarvae compared to embryos at the stage of eye pigmentation. It should be noted that the content of physiologically important docosahexaenoic 22:6ω-3 FA did not change in prelarvae of pink salmon. All differences were significant.

The study of prelarvae from two nests (located at a distance of 10 m from each other) demonstrated a small but significant difference between them in respect to the TL level, including CHOL and LPC, and CHOL/PL ratio. Variations of reserve TAGs, CE, some PL classes (except LPC), and TAG/PL ratio were insignificant. It should also be noted that prelarvae from different nests slightly but significantly differed in the proportion of almost all bound fatty acids and significant values of their ratios (PUFAs ω-3/ω-6, 16:0/18:1ω-9 and 20:4ω-6/18:2ω-6).

DISCUSSION

At early stages of ontogenesis, different directions of changes in the level of lipids and FAs may be due to specific processes of metabolism intensification in a developing organism, especially at the stage of eye pigmentation, the most sensitive stage of the embryonic development. An increase in the TL content in embryos of pink salmon, mainly due to storage TAG (two times) and the TAG/PL ratio compared to those in prespawn eggs, may be caused by the decrease in the concentration of proteins that may be used in endogenous feeding of fish embryos. This is consistent with an increase in the activity of proteolytic enzymes (cathepsin D) during embryogenesis in salmon as was reported earlier (Nemova et al., 2017). The studies on loach demonstrated that glycolysis was used as an energy source at low oxygen absorption at early stages of embryogenesis (Milman et al., 1977; Ozernyuk, 1985).

The decrease in the environmental temperature by the period of eye pigmentation in embryos (from 10°C in August before spawning and to 0.5°C by the beginning of October at the stage of eye pigmentation) contributed to the increase in the degree of the unsaturation of lipids by increasing the level of PUFAs of the ω-3 family (including 20:5ω-3, 22:5ω-3, and 22:6ω-3 FAs) and reducing the CHOL/PL ratio, which affects the level of metabolic activity of membrane enzymes and thus activates the metabolism of lipids and FAs (Hochachka and Somero, 2002; Tocher, 2003). Such changes were reported earlier when studying the development of Atlantic salmon (Murzina et al., 2012; Nemova et al., 2015). The level of storage TAGs is determined by the need of the organism to preserve (or store) energy reserves that are necessary in the most critical periods of the embryonic development, such as eye pigmentation. Accumulation and storage of a considerable amount of lipids (especially energetic) in eggs and embryos of pink salmon, as well as in other species of salmon, depend not only on the duration of the incubation period of embryos (to 7 months as in Atlantic salmon *Salmo salar* and to 5 months in pink salmon) but on the ecological conditions: larvae hatch in early spring at low temperature and limited food resources. Accumulation of a certain level of reserve energetic lipids in prespawn eggs and their increase in embryos at the stage of eye pigmentation may be considered as an adaptive strategy that provides further optimal development of an embryo and increase in larvae survival during the endogenous feeding period. The level of structural CHOL in embryos compared to that in prespawn eggs did not significantly change, but the proportion of its reserve form, CE, which is a reserve form in the organism not only for cholesterol but also for fatty acids (which enter the structure of the CE molecule) decreases by two times.

The detected decrease in PC and increase in the level of LPC metabolically bound with it at the stage of eye pigmentation in pink salmon is, probably, caused by the activation of the hormone-sensitive cytosolic phospholipase A2 under the effect of growth factors (Prokazova et al., 1998). The optimal LPC accumulation in embryos, which increases the biomembrane permeability for ions and molecules (Osadchaya et al., 2004) and the decrease in the CHOL/PL ratio, promotes the decrease in the biomembrane microviscosity and increase in the functional activity of receptors and membrane-bound enzymes (Netyukhailo and Tarasenko, 2001). Such changes in the values of the LPC/PC and CHOL/PL were found for Atlantic salmon at the stage of eye pigmentation (Nemova et al., 2015).

A high content of oleic 18:1ω-9, eicosapentaenoic 20:5ω-3, and docosahexaenoic 22:6ω-3 FAs in lipids of eggs before fertilization and at the stage of eye pigmentation confirms their important functional role in the organism. The literature shows that there is a correlation between the content of 22:6ω-3 fatty acid and survival of developing eggs and fish larvae, including

pink salmon (Yuneva et al., 1990; Tocher, 2003). Furthermore, 22:6ω-3 FA is vitally important for the function of the brain and eyes (Puskas et al., 2004; Arts and Kohler, 2009).

During embryonic development, the content of some FAs is subject to stage variations associated with changes (growth) in morphological structures of eggs (Nefedova, 1989; Dantagnan et al., 2007; Murzina et al., 2012). The decrease in the MUFA level (mainly, due 18:1ω-9 and 16:1ω-7 FAs) indicates their use by an embryo as an energy source when the environmental temperature decreases (Tocher, 2003; Conceicao and Tandler, 2018). In eggs before spawning and at the stage of eye pigmentation, an equally low level of arachidonic 20:4ω-6 FA compared to its metabolic precursor linoleic 18:2ω-6 FA indicates a low activity of linoleoyl-CoA-desaturase, which plays a key role in its transformation into 20:4ω-6 FA, a source of intracellular endohormones (Bell et al., 2002). The found changes, such as the use of MUFA and preservation of PUFA of the ω-3 family and the decrease in the CHOL/PL ratio and increase in LPC, may be one of the factors of regulation of the optimal level of microviscosity of a lipid component depending on physiological needs of an embryo.

Additional studies of lipid and FA spectra in two groups of pink salmon prelarvae developing in different closely located nests (not more than 10 m from each other) under similar environmental conditions made it possible to find (small but significant) differences in the TL level, including some classes of lipids and FAs and their ratios. Thus, a low level of CHOL, low CHOL/PL ratio, and increased LPC in prelarvae in one of the nests (no. 2) assumes a decrease in their biomembrane viscosity and increase in the level of metabolic activity that is indicated by the increase in values of 16:0/18:1ω-9, 20:4ω-6/18:2ω-6, and 18:3ω-3/18:2ω-6 ratios. The first of the parameters characterizes the intensity of the lipid exchange, the second parameter indicates the increase in bioconversion of linoleic 18:2ω-6 FA to arachidonic 20:4ω-6 FA, which is a source of intracellular hormones, and the third parameter is the ratio of essential FAs, which affects the optimization of metabolic processes in a developing organisms according to its needs (Arkhipov, 1980; Sargent et al., 1995; Sergeeva and Varfolomeeva, 2006). The differences between pink salmon prelarvae from different but closely spaced nests indicate the formation of different quality lipid status that may affect their further growth, development, and differentiation of juveniles.

A comparative study of the lipid status of prespawn eggs of pink salmon at the stage of eye pigmentation and hatched prelarvae demonstrated the decrease in the PL level, including PC and increase in minor phospholipids (PI, PS, and PEA) and CHOL and the value of the CHOL/PL ratio, especially in prelarvae. The accumulation of PI and PS as minor PLs induces the activity of membrane enzymes, for example the complex of Na^+,K^+-ATPase associated with osmoregulation that is important when the habitat changes (Boldyrev et al., 2006; Bystriansky and Ballantyne, 2007) and may indicate the beginning of their preparation for migration to the marine environment. Hatched prelarvae stay in the bottom sediments (until May) and feed on the content of the yolk sac. In prelarvae, the content of not only PLs but also reserve TAGs and 16:1ω-7, 18:3ω-3, and 20:5ω-3 fatty acids decreased, but the content of CHOL, CE, and CHOL/PL ratio (more in nest 1 than in nest 2) increased, which may be one of the factors regulating the optimal level of microviscosity of a lipid component depending on their physiological needs in a certain period of development.

The index of 20:4ω-6/18:2ω-6 bioconversion and the level of arachidonic 20:4ω-6 FA as a source of physiologically active endohormones was higher in hatched prelarvae. The detected changes in lipid and FA characteristics in prelarvae of pink salmon were caused by the increase in the intensity of the lipid exchange (16:0/18:1ω-9 FAs ratio), which was higher in nest 2. In hatched prelarvae, a high content of dominating oleic 18:1ω-9 and docosahexaenoic 22:6ω-3 FAs, which are necessary for further growth and development because of their importance and high need of them, is maintained.

Thus, eggs of pink salmon accumulate a large reserve of structural and energetic lipids by the beginning of spawning, which provide optimal development of an embryo. Metabolic processes' activation occurs at the stage of eye pigmentation, which causes changes in the spectrum of some classes of lipids, FAs, and values of their ratios: the content of TAG, LPC, and PS, PUFA of the ω-3 family, and value of TAG/PL increase; PC, CHOL/PL ratio, and all MUFAs and SFAs decrease; PUFAs of the ω-6 family did not change. The intensity of lipid exchange increases in prelarvae as a result PL, reserve TAGs and 16:1ω-7, 18:3ω-3, and 20:5ω-3 FAs decrease, but the level of CHOL, CE, and CHOL/PL ratio and 20:4ω-6/18:2ω-6 fatty acids increases. A high content of dominant oleic 18:1ω-9 and docosahexaenoic 22:6ω-3 FAs functionally important as energy sources and plastic reserve for further development of pink salmon larvae remains.

Multidirectional changes in the level of lipids and FAs are caused by specific processes of metabolism intensification in a developing organism at the stage of eye pigmentation. Quantitative and qualitative differences in some lipid and FA parameters and their ratios were found in prelarvae of pink salmon from two closely located nests that makes it possible to consider them as an example of ecologo-biochemical adaptations associated with features of development and formation of the organism's response to the effect of environmental factors, which determines the juveniles'

further strategy of development and formation of its different phenotypic quality.

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