ACTIVITY OF ENZYMES OF ENERGY AND CARBOHYDRATE METABOLISM IN THE ORGANS OF PINK SALMON *ONCORHYNCHUS GORBUSCHA* (SALMONIDAE) DURING SPAWNING MIGRATION

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The article presents the results of the study of the activity of key enzymes of energy and carbohydrate metabolism in pink salmon *Oncorhynchus gorbuscha* spawners during spawning migration from the estuary to the river. A decrease in the activity of carbohydrate metabolism enzymes (lactate dehydrogenase and pyruvate kinase), 1-glycerophosphate dehydrogenase in red muscles and liver, as well as cytochrome c oxidase and aldolase in white muscles in fish at the river stage of the migration route has been shown. Relatively higher values of cytochrome c oxidase activity in gills, glucose-6-phosphate dehydrogenase in red muscles, and aldolase in the liver were found in fish caught in the river. Apparently, as pink salmon spawners move to spawning grounds, metabolic changes occur associated with the redistribution of substrates towards increased use of lipids and proteins of muscle tissue to provide energy for the process of osmoregulation, high physical activity and reproductive function under conditions of complete exogenous starvation.

Keywords: pink salmon, enzyme activity, energy metabolism, carbohydrate metabolism, Indera River.

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INTRODUCTION

Oncorhynchus gorbuscha– one of the most widespread representatives of the genus Pacific salmon, making anadromous migrations from marine habitats to fresh water bodies for spawning (Pacific salmon …, 1991). Sexually mature individuals cover long distances during spawning migration, undergoing significant physiological changes that ensure the maintenance of osmotic and ionic balance of the body (Shrimpton et al., 2005). Salmon are influenced by many abiotic (e.g.

changes in water temperature, salinity, high current velocity) and biotic (e.g. diseases) environmental factors throughout the entire migration route to the spawning grounds (Hinch et al., 2006). Such energy-consuming processes as osmoregulation, movement against the current, reproductive maturation and spawning occur under conditions of limited use of available food resources and transition to endogenous nutrition, since the fish stop feeding even before migrating to fresh water (Pacific salmon …, 1991). Previous studies conducted on salmonid fish species (Salmonidae) – sockeye salmon*O. nerka*, pink salmon, chinook salmon*O. tshawytscha*during their spawning migration, have been focused on the redistribution of endogenous energy reserves of the body (primarily lipid and protein reserves), which ensures the successful completion of the reproductive strategy before the onset of physical exhaustion and death of the fish (Kinnison et al., 2003; Crossin et al., 2003, 2009; Hinch et al., 2006). It has been shown that the mobilization of lipids accumulated during salmon feeding in the sea provides the bulk of the required energy throughout most of the fish migration. Protein and carbohydrate metabolism plays a major role when lipid reserves in the body are almost completely depleted by the end of the migratory route, as well as during periods of "explosive" swimming, when fish make sharp jerks and rapidly gain speed (Gilhousen, 1980; Mommsen et al., 1980). It has been established that the breakdown products of proteins and lipids of muscle tissue (mainly white muscles) are used to synthesize precursors of structural components of developing oocytes (vitellogenin biosynthesis in the liver), while carbohydrates (including those formed from amino acids in the process of gluconeogenesis) are consumed mainly during the spawning process (French et al., 1983;Mommsen, 2004). Thus, regulation of substrate delivery and oxidation in fish organs and tissues is a determining factor that ensures energy availability at different stages of migration. It is known that the functional activity of organs and tissues is determined by their metabolic status, which in turn depends on changes in energy metabolism during salmon migration (Mommsen et al., 1980; Morash et al., 2013). Metabolic changes occurring in the salmon body are essential for their successful physiological adaptation to increased physical exertion and starvation

during spawning migration. Thus, changes in the activity of glycolytic enzymes at the level of gene transcription during white muscle atrophy were detected in migrating sockeye salmon spawners; a metabolic transition of energy supply from anaerobic glycolysis to oxidative phosphorylation was established in them upon arrival of fish at spawning grounds (Miller et al., 2009). Another study (Mommsen et al., 1980) showed a decrease in the activity of metabolic enzymes (hexokinase, 1-glycerophosphate dehydrogenase, pyruvate kinase, lactate dehydrogenase) in white muscles during spawning migration in this species. However, the mechanisms regulating metabolism at the level of changes in the activity of energy and carbohydrate metabolism enzymes in pink salmon spawners migrating to spawn remain poorly understood.

The aim of our work is to evaluate the activity of enzymes involved in energy and carbohydrate metabolism (cytochrome c *c*- oxidase, lactate dehydrogenase, pyruvate kinase, aldolase, 1-glycerophosphate dehydrogenase, glucose-6-phosphate dehydrogenase) in organs (liver and gills) and tissues (white and red muscles) of pink salmon spawners during their spawning migration from the estuary to the river.

MATERIALS AND METHODS

Mature pink salmon individuals were caught on August 10-15, 2021, during the pre-spawning period along their natural migration route from the Indyora River estuary to its riverine biotopes (table). Fishing in the river mouth pools and riffles (river) was carried out using a casting net, while in the estuary - using a fixed net, installed alternately at two different points: in the saline ("sea") and freshened (estuary) parts. Immediately after capture, fish were placed in flow-through cages with water, the salinity of which corresponded to the capture area, to accumulate the required number of fish.

Characteristics of capture sites, length and weight of pink salmon *Oncorhynchus gorbuscha* spawners caught during spawning migration in the Indyora River on August 10-15, 2021.

Site	Coordinates		Water salinity, ‰	Water temperature, °C	*TL* , cm *	Weight, g *	Number of fish, specimens
	N	E					
"Sea"	66°14′12.0″	37°08′58.8″	32	19.2	45.85 ± 0.64	902.69 ± 0.06	13
Estuary	66°14′28.6″	37°08′55.8″	0↔25**	16.8	47.42 ± 0.87	1122.08 ± 0.09	12
River	66°14′34.6″	Same	0	16.3	48.00 ± 0.77	1093.82 ± 0.04	11

Note. *TL* - total body length, *mean value and its error, **salinity varied depending on tide/ebb (increased during high tide, decreased during low tide) and wind direction.

After accumulation (August 16), several pink salmon individuals were transferred from cages to 127-liter barrels with fresh water (from the Indera River), a mixture of fresh and salt water in a ratio of 1:1 (desalinated water), and salt water (from the White Sea) according to the place of capture and kept for at least 2 hours (but not more than 10–11 hours). The water in the barrels was aerated with a Sera AIR 275 R compressor ("Sera", Germany), its temperature varied within 17–19°C depending on the time of day. Before euthanasia (by piercing the head so that the blade damaged the brain and spinal cord), each fish was anesthetized using clove oil. Then, biological material (white and red muscles, liver, gills) was collected in field conditions, placed in liquid nitrogen, and then stored in the laboratory at −80°C until analysis.

Pink salmon spawners caught in the river and at two estuary sites showed signs of pre-spawning changes and had gonads at stage IV of maturity. Fish were selected according to size and weight characteristics, choosing individuals of average size within a small range of length and weight (table), as well as by sex (males and females constituted approximately equal numbers). Since no sex differences were found in the studied parameters, the samples of females and males were combined.

Pink salmon spawners were caught in accordance with permit No. 51 2021 03 2021 of the Federal Agency for Fishery of the North Sea Territorial Administration dated 06/19/2021.

Determination of enzyme activity and protein concentration

The activity of enzymes in energy and carbohydrate metabolism in pink salmon organs was determined spectrophotometrically (CLARIOstar, "BMG Labtech", Germany) individually for each

specimen. Samples were homogenized in 0.05 M *Tris* -HCl buffer (pH 7.5) using a TissueLyser LT homogenizer ("Qiagen", Germany). Cytochrome *c*- oxidase (CO, EC 1.9.3.1) activity was determined according to Smith's method (Smith, 1955), measuring the increase in the amount of oxidized cytochrome *c* . Total activity of lactate dehydrogenase (LDH, EC 1.1.1.27), glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49), and 1-glycerophosphate dehydrogenase (1-GPDH, EC 1.1.1.8) was determined using standard methods by measuring the amount of reduced nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) (Kochetov, 1980). Pyruvate kinase (PK, EC 2.7.1.40) activity was determined by the amount of NAD formed in a system containing the reduced form of NAD and lactate dehydrogenase (Bücher, 1955). Aldolase activity (EC 4.1.2.13) was determined using Beck's method as modified by Ananyev and Obukhova (Kolb, Kamyshnikov, 1976). Enzyme activity was expressed in μmol substrate (product)/min/mg protein. Protein concentration was determined by Bradford's method (Bradford, 1976).

Statistical analysis of results

The analysis of results was carried out using standard methods of variation statistics (Ivanter, Korosov, 2010). Data were tested for normal distribution using the Shapiro-Wilks test. Comparison of samples according to the studied parameters was performed using the Kruskal-Wallis test followed by the Mann-Whitney test. Differences were considered significant at $p < 0.05$. All data are presented as $M \pm SE$ (mean value and its error).

Laboratory analysis was performed using the equipment of the Center for Collective Use of the Federal Research Center "Karelian Research Center of the Russian Academy of Sciences".

RESULTS AND DISCUSSION

Gills (Fig. 1). Significant differences in the activity of CO, a key enzyme in aerobic adenosine triphosphate synthesis, were detected in the gills of pink salmon. The activity of this enzyme in the gills of individuals from the "sea" and estuary was 2.5 times lower than in spawners from the river (

$p < 0.05$) (Fig. 1a), which may be related to the adaptation of aerobic metabolism to changes in salinity and, probably, physical activity. Previously, in a cage experiment, we (Churova et al., 2021) identified a decrease in CO activity in pink salmon smolts kept in the estuary and in the sea compared to individuals that remained in freshwater, as well as when transferring pink salmon larvae from fresh to salt water. Increased CO activity in the gills of fish from the river may also be associated with a possible increase in oxygen consumption during migration. In previous studies conducted on salmonid fish species (Eddy, 1982; Morgan, Iwama, 1999), it was established that metabolism in the gills of fish in freshwater was higher than in saltwater - 1.0-3.9 versus 0.5-2.4% of oxygen consumption at rest.

White muscles (Fig. 2) . In the white muscles of pink salmon individuals at different stages of spawning migration, intergroup differences in the activity values of CO and aldolase were established. CO activity was highest in individuals from the "sea," in fish from the river it was 1.3 times lower ($p < 0.05$) (Fig. 2a). This indicates a decrease in the level of aerobic metabolism in the muscles of pink salmon individuals in the river. During spawning migration in salmonids, white muscles serve as the main source of lipids and proteins to provide energy for biosynthesis processes and generative metabolism (Mommsen et al., 1980; Miller et al., 2009). Since pink salmon do not feed, muscle reserves are depleted, which is also indicated by a decrease in aerobic metabolism.

The activity of aldolase in white muscles was 1.2 times higher ($p < 0.05$) in fish from the estuary compared to that in individuals from the river (Fig. 2c). A similar trend (differences not significant) was observed for LDH activity (Fig. 2b). This may indicate a relatively higher intensity of carbohydrate utilization in the glycolysis process in fish during their migration stage in the estuary, unlike individuals from the river. Previously, it was also established (Maksimovich, 1990) that during spawning migration, a significant enhancement of glycolysis occurs in the white muscles of Pacific salmon. Anaerobic glycolysis is the main mechanism that provides energy for muscle contractions during burst swimming in fish. The lower activity of glycolytic enzymes in spawners from the river

compared to individuals from the estuary may also be associated with the depletion of energy reserves in muscle fibers in fish by the end of their migration route (Miller et al., 2009). It is known that the depletion of white muscle resources, associated with starvation and high physical activity during salmon migration, occurs gradually. The initial stages are characterized by the mobilization of predominantly carbohydrates and lipids, whereas with increasing duration of starvation, the breakdown of muscle proteins is observed, and amino acids become one of the main energy sources for fish along with lipids (Navarro, Gutiérrez, 1995; Nemova, 1996; Morash et al., 2013). Probably, in our study, in pink salmon individuals caught in the river, lipids and proteins were mainly involved in energy metabolism, whereas in fish from the estuary, predominantly carbohydrates and lipids were used.

Red muscles (Fig. 3). In the red muscles of pink salmon specimens, differences in the activity values of G6PDH ($p < 0.05$) and 1-GPDH ($p < 0.05$) were established, which depended on the sampling location. Thus, the activity of 1-GPDH in the red muscles of fish from the "sea" and estuary was 1.3 times higher ($p < 0.05$) than that of specimens from the river (Fig. 3d). The role of 1-GPDH is mainly associated with the formation of glycerophosphate from carbohydrates, which can be used in the synthesis of structural and storage lipids (Treberg et al., 2002). The obtained results may indicate a decrease in the use of carbohydrates in glycerophosphate synthesis in fish from the river. It can be assumed that lipolysis processes occur in fish in the river, associated with the depletion of individuals at the end of the migration route, while in fish in the "sea" and estuary, high 1-GPDH activity may indicate processes of synthesis and storage of lipids in the muscles. In addition, LDH activity in the red muscles of specimens from the estuary was 1.2 times higher than in fish from the river (Fig. 3b). In red muscle fibers, LDH can catalyze both forward and reverse reactions of the interconversion of pyruvate and lactate, thereby participating in both the breakdown (glycolysis) and synthesis (gluconeogenesis) of carbohydrates. It can be assumed that lactate and products of proteolysis of white muscle fibers enter the central metabolism and then into the red muscles, where they act as a substrate

for the formation of glycogen in the process of gluconeogenesis (Maksimovich, 1990). Earlier, when studying the metabolism of sockeye salmon during return migration (Mommsen et al., 1980), it was found that carbohydrates are used during fish spawning, they are restored from the pool of amino acids (mainly alanine) released during proteolysis of white muscles. In pink salmon during prolonged fasting associated with spawning migration and spawning, red muscles (along with the liver) become the most important tissue in which gluconeogenesis occurs, and the content of enzymes of this biosynthesis pathway increases 3-4 times compared to the marine period of migration (Maksimovich, 1990). Based on our data, it can be assumed that the process of carbohydrate resynthesis is most pronounced in fish from the estuary, which is probably due to their lesser depletion compared to individuals from the river. In addition, relatively higher values of LDH activity in red muscles of fish from the estuary may indicate efficient utilization through anaerobic glycolysis of carbohydrates synthesized during gluconeogenesis. As shown earlier (Tseng, Hwang, 2008), this provides a rapid response to changing energy needs of the organism associated with adaptation of water-salt metabolism to constantly changing salinity conditions in the estuary.

Thus, the intensity of some carbohydrate metabolism pathways in red muscles of individuals from the river decreases during migration. However, G6PDH activity in red muscles was lower in pink salmon individuals from the "sea" compared to that in fish from the river and estuary by 1.3 and 1.4 times, respectively ($p < 0.05$) (Fig. 3c). Similar changes (increased G6PDH activity along with decreased 1-GPDH in red muscles) were observed in sockeye salmon individuals as they moved up the river to the spawning site (Mommsen et al., 1980). These results may indicate the importance of maintaining the activity of the pentose phosphate pathway of glucose oxidation when changing environmental salinity conditions during fish migration from the sea to fresh water. This may be related to the need to maintain the level of the reduced form of NADP, which is used in activating the synthesis of steroids that participate in the regulation of osmoregulatory processes in fish under hyperosmotic shock conditions (McCormick, 2001; Ruiz-Jarabo et al., 2019).

The revealed relatively higher activity of G6PDH in fish from the estuary may be associated with the activity of 1-GPDH and thereby reflect an increase in the degree of carbohydrate utilization in the processes of biosynthesis of fatty acids, cholesterol, steroid hormones, sphingolipids (Tian et al., 1998).

Liver (Fig. 4) . In the liver of pink salmon specimens caught in the "sea," higher values of PK activity (1.4 times) and 1-GPDH (1.2 times) were found compared to those in fish from the river ($p < 0.05$) (Fig. 4b, 4d). Since PK is a key enzyme that catalyzes the formation of pyruvate during glycolysis, this result indicates a decrease in the intensity of its synthesis through carbohydrate metabolism in the liver of fish from the river. In a study conducted on pink salmon specimens (Maksimovich, 1990), it was shown that the activity of glycolytic enzymes in the liver of fish decreases during spawning migration. According to Metón et al. (1999), the level of PK activity in the liver reflects feeding conditions, particularly, it decreases during fish starvation. Thus, relatively lower values of PK and 1-GPDH activity may indicate a decrease in the use of carbohydrates in energy supply processes and biosynthesis in fish liver. As mentioned above, for fish that have reached the river stage of the migration route, this may be associated with the depletion of body reserves.

Aldolase activity was 1.2 times lower ($p < 0.05$) in the liver of fish from the estuary compared to that in specimens caught in the river (Fig. 4e). It has been shown (Llewellyn et al., 1998) that an increase in aldolase activity in the liver may indicate an increase in the intensity of gluconeogenesis. According to previous studies, during starvation and spawning migration, gluconeogenesis enzymes are activated in the fish liver, and the glycogen content in hepatocytes increases sevenfold compared to the marine period of migration (Maksimovich, 1990). This may be related to the prolonged muscle load in pink salmon specimens during the river period of migration, as physical activity in fish is the main factor determining the intensity of gluconeogenesis. In addition, it has been suggested that during migration, carbohydrates are stored (in the form of glycogen), which are then used during spawning (Mommsen et al., 1980; Barciela et al., 1993; Miller et al., 2009;). Under conditions of complete

exogenous starvation, the activation of gluconeogenesis allows salmon to provide themselves with the necessary energy substrates and plastic substances.

Thus, the obtained data indicate that pink salmon spawners have adaptive rearrangements in aerobic and anaerobic metabolism and the functioning of the pentose phosphate pathway depending on the environmental salinity and the stage of anadromous migration. A decrease in the activity of CO and aldolase in white muscles, LDH and 1-GPDH in red muscles, as well as PK and 1-GPDH in the liver was shown in pink salmon individuals that reached the river. At the same time, fish caught in the river showed increased activity of CO in the gills, G6PDH in red muscles, and aldolase in the liver. The identified changes in the activity of the studied enzymes suggest that during the river stage of the spawning migration, pink salmon spawners under starvation conditions generally experience a decrease in the intensity of glucose oxidation pathways, most likely, there is a redistribution of energy substrates toward the use of lipids and proteins from muscle tissue, coordinated with the process of gluconeogenesis in the liver, which provides the necessary energy potential for the successful completion of the reproductive strategy. Probably, as the fish move toward the spawning grounds, the identified changes in the activity pattern of these enzymes will intensify.

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COMPLIANCE WITH ETHICAL STANDARDS

Fish capture and all manipulations were carried out in accordance with Russian and international principles and norms of humane treatment of animals, environmental protection, wildlife conservation, rational use of biological resources and safe conduct of biological research, and generally accepted methodological recommendations for working with experimental animals. Protocols using animals were approved by the Bioethics Commission of the Institute of Biology of

the Karelian Research Centre of the Russian Academy of Sciences (meeting protocol No. 4 dated 04.04.2024).

CONFLICT OF INTEREST

The authors of this work declare that they have no conflict of interest.

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FIGURE CAPTIONS

Fig. 1. Relative activity of cytochrome c- *c*- oxidase (a), lactate dehydrogenase (b) and aldolase (c) in the gills of pink salmon *Oncorhynchus gorbuscha* spawners caught in the Indera River on 10-15.08.2021 at sites with different salinity (see table for site characteristics). Here and in Fig.2-4: *1* - "sea", *2* - estuary, *3* - river; (⊥) - standard error. Differences are significant ($p < 0.05$) from the sample from site: * *1* , # *2* .

Fig. 2. Relative activity of cytochrome c- *c*- oxidase (a), lactate dehydrogenase (b) and aldolase (c) in white muscles of pink salmon *Oncorhynchus gorbuscha* spawners caught in the Indera River on 10-15.08.2021 at sites with different salinity.

Fig. 3. Relative activity of cytochrome c- *c*- oxidase (a), lactate dehydrogenase (b), glucose-6-phosphate dehydrogenase (c), 1-glycerophosphate dehydrogenase (d) and aldolase (e) in red muscles of pink salmon *Oncorhynchus gorbuscha* spawners caught in the Indera River on 10-15.08.2021 at sites with different salinity.

Fig. 4. Relative activity of cytochrome c- *c*- oxidase (a), pyruvate kinase (b), lactate dehydrogenase (c), glucose-6-phosphate dehydrogenase (d), 1-glycerophosphate dehydrogenase (e) and aldolase (f) in the liver of pink salmon *Oncorhynchus gorbuscha* spawners caught in the Indera River on 10-15.08.2021 at sites with different salinity.