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Activities of Energy Metabolism Enzymes in Atlantic Salmon *Salmo salar* L. Smolts and Parr Grown under Different Light Regimens

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Abstract—Activities of enzymes of energy and carbohydrate metabolism in muscles and the liver were studied in Atlantic salmon *Salmo salar* L. smolts and parr grown under continuous or natural lighting and different feeding regimens in autumn followed by a short photoperiod in winter. Enzyme activities were found to differ between test and control salmon groups and between parr and smolts sampled at the end of the winter period. Smolts grown under continuous lighting and round-the-clock feeding differed from other groups by having higher cytochrome *c* oxidase (COX) activity and lower aldolase activity in muscles. Differences in aerobic metabolism in muscles between parr and smolts were found to be the same in all experimental groups, COX and aldolase activities being relatively higher in smolts. The pattern of changes in enzyme activities in the liver from parr to smolts differed between different experimental groups. Based on the results, the photoperiod was assumed to affect the activities of energy metabolism enzymes in salmon juveniles and may eventually affect the completion of smoltification.

Keywords: photoperiod, Atlantic salmon, smolts, parr, activity of enzymes of energy metabolism
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INTRODUCTION

Smoltification (parr–smolt transformation) is the developmental process whereby Atlantic salmon youth prepares to living in seawater. Substantial biochemical, physiological, morphological, and behavioral changes occur in fish during this period [1–5]. The photoperiod is one of the main factors that affects the regulation of smoltification in Atlantic salmon for a long time [6]. A greater photoperiod is used to increase the growth rate and to achieve smoltification earlier in Atlantic salmon farming [7, 8]. When continuous lighting is used, a temporal exposure (for several months) to a short (winter) photoperiod and a subsequent increase in photoperiod have been shown to be necessary for salmon juveniles to develop normally and to complete smoltification successfully [7, 8].

A long-term experiment has been performed to study how continuous lighting affects the growth and development of *Salmo salar* L. youth of the year (0+) in aquaculture in conditions of southern Russia (Republic of North Ossetia–Alania). Salmon youth of the year were exposed to various feeding and lighting regimens from September to November (2022). Data collected in autumn showed [9] that continuous lighting positively affects the weight gain in developing salmon youth and is associated with higher aerobic metabolism in muscles, greater carbohydrate consumption for glycolysis in the liver, and characteristic changes that occur in lipid composition [10] and suggest the start of smoltification. From early December, the salmon youth of all experimental groups were kept under a natural winter photoperiod (without additional lighting) combined with different feeding regimens. Here we report the results of studying the activities of energy and carbohydrate metabolism enzymes in muscles and the liver in Atlantic salmon parr and smolts sampled in early March after the end of exposure to the winter (short) photoperiod.

MATERIALS AND METHODS

The study was carried out at the "Ostrov akvakul'tura" fish farm (Republic of North Ossetia–Alania). Prior to the experiment, fry (hatched March 10–15, 2022) was kept under continuous illumination (24-h light day (24LD)) with LEDs (36 W, 6500 K). Starting from early September, yearlings were kept in experimental conditions. There were tree experimental groups, each including two raising tanks (4 × 1.2 m, 2.5–2.7 m^3): group 1 (24LD + CF) with

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Table 1. Mean weight and mean length of fish collected for the study

Group	Smolts		Parr	
	weight, g	length, mm	weight, g	length, mm
24LD + CF	59.91 ± 2.91	169.28 ± 3.54	53.63 ± 3.46	164.29 ± 2.98
NatLD + DF	59.61 ± 4.11	172.5 ± 3.66	56.61 ± 3.35	168.48 ± 3.57
24LD + DF	63.64 ± 3.74	173.11 ± 3.89	55.31 ± 2.28	161.63 ± 1.73

continuous lighting (light/dark 24 : 0) and continuous feeding (CF); group 2 (test group, NatLD + DF) with natural illumination (NatLD) and feeding every 2 h during daylight hours (from 6:00 a.m. to 6:00 p.m. in September, from 8:00 a.m. to 6:00 p.m. in October, and from 8:00 a.m. to 5:00 p.m. in November); and group 3 (24LD + DF) with continuous lighting and daytime feeding (DF), as in group 2. In early December, the youths were transferred by group into round 2.1-m^3 tanks (diameter 2 m, height 1 m) at 2800 per tank on average. From December to March, parr and smolts were reared in natural illumination conditions of the region with a short winter day (9–11 h). The light intensity at day was 5500 or 500 lx in cloudy weather. All groups received the same commercial feed in winter; feed amounts were calculated from the amount recommended for the age and the biomass. Water was delivered into the tanks from a well at 2.7–3 L/s, and the water temperature was consequently the same, 10.3–10.8°C, from December to the end of March. The experimental conditions have been described in more detail previously [9, 10].

The smolt fractions on the testing day (March 3) were up to 50% in group 24LD + CF, 40% in group NatLD + DF, and 25% in group 24LD + DF. Parr and smolts were sampled from each experimental group for the study (Table 1).

Activities of energy and carbohydrate metabolism enzymes in muscles (cytochrome *c* oxidase (COX), EC 1.9.3.1; lactate dehydrogenase (LDH), EC 1.1.1.27; and aldolase, EC 4.1.2.13) and the liver (COX; LDH; pyruvate kinase (PK), EC 2.7.1.40; glucose 6-phosphate dehydrogenase (G6PDH), EC 1.1.1.49; glycerol 1-phosphate dehydrogenase (G1PDH), EC 1.1.1.8; and aldolase) were assayed in each individual fish according to standard protocols [11–14]. Enzymatic activities were expressed in terms of μmol of the substrate (product)/min/mg protein. Protein concentrations were measured using the Bradford assay [15]. Statistical analyses were carried out using common variation statistics methods, including the Shapiro–Wilk test, the Kruskal–Wallis test, and subsequent sample comparisons by the Mann–Whitney test. Results were considered significant at $p < 0.05$. Results were presented as M ± SE. The study was carried out using equipment of the Collective Use Center of the Karelian Research Center.

RESULTS AND DISCUSSION

Activities of energy and carbohydrate metabolism enzymes were studied to evaluate the effect of lighting regimens on salmon smolts and parr. The energy status and functional activity of fish organs are possible to infer from the activities of key enzymes involved in anaerobic and aerobic ATP syntheses and glucose oxidation pathways. For example, COX is a crucial enzyme of the mitochondrial respiratory chain, and its activity reflects the level of aerobic metabolism [16]. LDH is a glycolytic enzyme and provides an index of anaerobic metabolism in muscles and gluconeogenesis in the liver [17]. PK is a key glycolytic enzyme that catalyzes conversion of phosphoenolpyruvate to pyruvate; its level characterizes the intensity of the process [18]. Aldolase is a glycolytic enzyme that catalyzes the production of dihydroxyacetone triphosphate and glyceraldehyde 3-phosphate, which are then involved in glycolysis, gluconeogenesis, and lipid syntheses [19]. G1PDH catalyzes the production of glycerol 1-phosphate, which is a precursor of structural and storage lipids [20]. G6PDH is a key enzyme of the pentose phosphate pathway (PPP), which produces pentoses and generates HADPH, which acts as a reducing agent and is utilized in cholesterol and fatty acid biosyntheses [21].

Differences between Groups

Differences in COX and aldolase activities in muscles were observed between fish groups. Smolts of group 24LD + CF showed a higher COX activity in muscles as compared with the other groups (Fig. 1a). A higher COX activity suggests a higher level of aerobic metabolism in muscles for fish grown with continuous lighting. We have previously observed that fish grown with continuous lighting have a higher level of aerobic metabolism in muscles at the stage preceding the introduction of the winter photoperiod, suggesting better energy supply to growth-related processes [9]. Because an increase in growth rate is associated with smoltification-related changes [5], a more successful growth is possible to assume for smolts of group 24LD + CF on evidence of their higher level of aerobic metabolism.

Parr and smolts displayed similar differences in aldolase activity in muscles; i.e., aldolase activity in fish of group 24LD + CF was lower than in the other

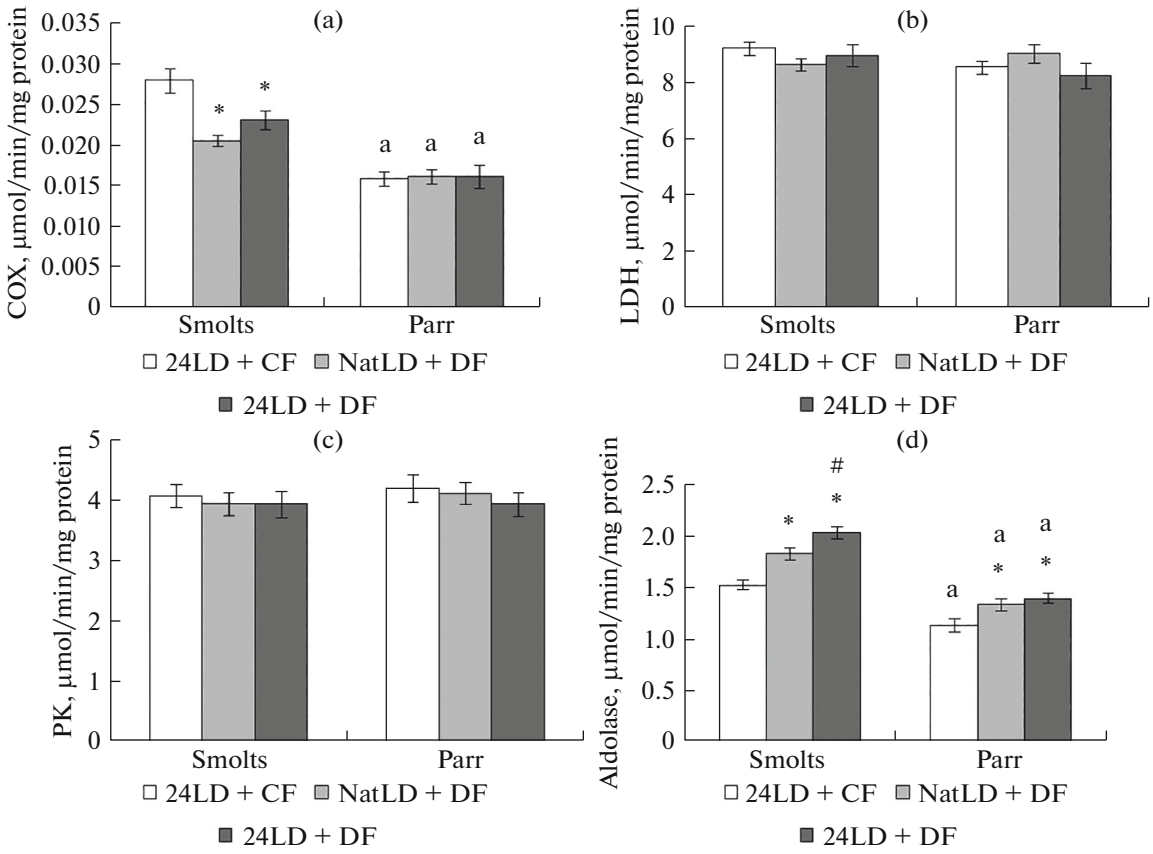

Fig. 1. Activities (μmol/min/mg protein) of (a) COX, (b) LDH, (c) PK, and (d) aldolase in white muscles of Atlantic salmon parr and smolts grown at different lighting and feeding regimens. Groups: 24LD + CF, continuous lighting and continuous feeding; NatLD + DF, natural photoperiod and daylight feeding; 24LD + DF, continuous lighting and daylight feeding. Differences were significant at $p < 0.05$ in comparisons (*) with group 24LD + CF, (#) with group NatLD + DF, and (a) between smolts and parr of the same group.

groups (Fig. 1d). The result agrees with our previous data that a lower aldolase activity in muscles (and in the liver) in November was characteristic of fish of group 24LD + CF [9]. It is possible to assume that carbohydrates are predominantly utilized in energy metabolism in fish of the group with natural illumination, while other substrates may additionally be used in fish of group 24LD + CF. Because various stages were characterized by a lower aldolase activity in muscles in fish of group 24LD + CF, additional lighting and round-the-clock feeding probably caused metabolic changes and a redistribution of storage substances. In contrast, smolts of group 24LD + DF displayed a higher aldolase activity as compared with the other groups, suggesting a high level of carbohydrate utilization in energy metabolism. LDH and PK activities in parr and smolts did not differ between groups.

As for the liver enzymes in smolts, the glucose oxidation enzymes G1PDH and G6PDH were found to differ in activities between groups. The liver G6PDH activity in the liver in smolts of group 24LD + CF was lower than in the other groups, while fish of group 24LD + DF had a higher activity as compared with the other groups (Table 2). A higher G1PDH activity in the liver was observed in fish of group 24LD + CF as compared with smolts of group 24LD + DF (Table 2). It is possible to assume that the intensity of glucose utilization in different biosynthesis pathways (PPP and glycerol 1-phosphate production) differs between the two fish groups. Fish of group 24LD + CF were probably completing the period of energy-demanding metabolic changes associated with smoltification to start accumulating energy resources. The idea agrees with data on the proportions of lipid fractions [22]. A higher LDH activity in the liver points to more intense restoration of energy resources in smolts of group 24LD + CF as compared with fish of the other groups (Table 2), being associated with the intensity of gluconeogenesis.

Comparisons between Parr and Smolts

The parr–smolt transformation in the Atlantic salmon *Salmo salar* L. is a set of metabolic and behavioral changes, which affect lipid and carbohydrate metabolism, osmoregulation, oxygen transport,

Table 2. Relative enzymatic activities (M ± m, μmol/min/g protein) in the salmon liver as dependent on the lighting and feeding conditions

	Lighting and feeding conditions		
	24LD + CF	NatLD + DF	24LD + DF
	COX		
Smolts	0.084 ± 0.004	0.087 ± 0.009	0.098 ± 0.009
Parr	0.108 ± 0.009[a]	0.104 ± 0.005	0.098 ± 0.005
	LDH		
Smolts	1.76 ± 0.10	1.38 ± 0.09*	1.44 ± 0.07*
Parr	1.74 ± 0.17	1.77 ± 0.06[a]	1.74 ± 0.10[a]
	PK		
Smolts	0.0167 ± 0.0010	0.0155 ± 0L.0010	0.0177 ± 0.0016
Parr	0.0104 ± 0.0005[a]	0.0107 ± 0.0004[a]	0.0121 ± 0.0007[a]
	G6PDH		
Smolts	0.0249 ± 0.0010	0.0295 ± 0.0010*	0.0318 ± 0.0005*[#]
Parr	0.0292 ± 0.0008[a]	0.0308 ± 0.0014	0.0295 ± 0.0011
	G1PDH		
Smolts	0.0215 ± 0.0004	0.0201 ± 0.0010	0.0165 ± 0.0013*
Parr	0.0207 ± 0.0013	0.0190 ± 0.0012	0.0204 ± 0.0012[a]
	Aldolase		
Smolts	0.0311 ± 0.0013	0.0295 ± 0.0016	0.0286 ± 0.0017
Parr	0.0328 ± 0.0018	0.0297 ± 0.0018	0.0294 ± 0.0017

Differences were significant at $p < 0.05$ in comparisons (*) with group 24LD + CF, (#) with group NatLD + DF, and (a) between smolts and parr of the same group.

growth, and rheotaxis [4, 5]. The period when smoltification is completed is characterized by a decrease in the coefficient of fatness, an increase in resistance to water salinity, a faster growth, a higher Na^+/K^+-ATPase activity in the gills, and a higher growth hormone concentration in the plasma [5].

Differences in aerobic metabolism in muscles were detected between parr and smolts in our data analysis and were the same in all experimental groups. Muscle COX activity in smolts was higher than in parr, indicating that energy expenditures increased during smoltification as necessary for supporting the adaptive metabolic reactions and growth processes [4]. In particular, oxidation processes are intensified in smolts; i.e., standard and active metabolic parameters in smolts are 50% higher than in parr in Atlantic salmon [23]. An increase in intensity is presumably associated with higher activities of respiratory enzymes and mitochondrial proliferation, which result from an increase in thyroid hormones [24].

Muscle LDH activity was not observed to change from parr to smolts (Fig. 1b). The result differs from data obtained in natural salmon populations, where muscle LDH activity in migrating smolts is higher than in parr [25]. The difference is explained by the fact that additional energy expenditures support physical activity in fish migrating downstream a river in nature, but are unnecessary in salmon youth grown in aquaculture [4, 5].

Muscle aldolase activity increased in smolts compared with parr in all groups (Fig. 1d). The finding indicates that carbohydrate utilization in aerobic and anaerobic metabolism increases in smolts.

The pattern of changes in liver enzyme activities from parr to smolts differed between different experimental groups. For example, a decrease in COX and G6PDH activities in smolts compared with parr was observed in group 24LD + CF, while LDH and G1PDH activities decreased in group 24LD + DF (Table 2). The difference suggests a difference in the intensities of biosyntheses and gluconeogenesis and makes it possible to assume that fish from different groups were at different temporal stages of completing smoltification. Smoltification-related changes proceed slower in fish of group 24LD + DF, as has been shown by analyzing the lipid and fatty acid compositions with regard to the proportion of smolts and parr [22, 26]. A comparison of the growth conditions

for the two groups indicates that the feeding regimen (24-h feeding or feeding in daylight hours) determined a certain rearrangement of metabolism in conditions of continuous lighting.

Group NatLD + DF also showed a lower LDH activity (Table 2), suggesting a decrease in lactate-dependent gluconeogenesis [23]. A decrease in liver LDH during smoltification has similarly been observed in another study of Atlantic salmon [23] and in the masu salmon *Oncorhynchus masou* [27].

An increase in liver PK activity in smolts compared with parr was common for all fish groups (Table 2). The change suggests an increase in the intensity of glucose utilization in energy metabolism and biosynthetic processes [18].

To summarize, 24-h feeding and 24-h lighting regimens used to grow salmon youth cause certain metabolic rearrangements that facilitate a more intense fish growth and subsequent smoltification. A period with a short winter light day promoted smoltification after the use of the 24-h lighting regimen. Activities of enzymes involved in aerobic and aerobic metabolism and glucose oxidation pathways were assayed in fish youths grown in the experimental conditions. The results make it possible to assume that the differences observed in the intensities of biosynthetic processes and gluconeogenesis in the liver and aerobic metabolism in muscles point to different stages of completing smoltification in fish that grew and developed in conditions differing in photoperiod and feeding regimen. Regardless of the experimental group, smolts were found to differ from parr in having more intense aerobic metabolism and higher carbohydrate utilization in muscles and higher glycolysis in the liver.

Our findings complement the data on the role that environmental factors play in biochemical adaptations that occur during growth and smoltification in salmons in aquaculture.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

CONFLICT OF INTEREST

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

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