Activity of metabolic enzymes and muscle-specific gene expression in parr and smolts Atlantic salmon *Salmo salar* L. of different age groups

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Abstract This study was conducted to characterize the energy metabolism level and the features of muscle growth regulation during the development of Atlantic salmon (*Salmo salar*) inhabiting the Indera River (Kola Peninsula, Russia). The activities of aerobic and anaerobic enzymes (cytochrome *c* oxidase and lactate dehydrogenase) and carbohydrate metabolism enzymes (glucose-6-phosphate dehydrogenase, glycerol-3-phosphate dehydrogenase, and aldolase) were measured in muscle and liver tissue. Gene expression levels of myosin heavy chain (*MyHC*), myostatin (*MSTN-1a*), and myogenic regulatory factors (MRFs—*MyoD1a*, *MyoD1b*, *MyoD1c*, *Myf5*, *myogenin*) were measured in the white muscles of salmon parr of ages 0+, 1+, 2+, and 3+ and smolts of ages 2+ and 3+. Multidirectional changes in the activity of enzymes involved in aerobic and anaerobic energy metabolism with age were shown in the white muscles of the parr. The cytochrome *c* oxidase activity was higher in muscles of underyearlings (0+) and yearlings (1+) and decreased in 2+ and 3+ age groups. The activity of lactate dehydrogenase, in contrast, increased with age. The patterns of changes in expression levels of *MyoD1a*, *MyoD1b*, *myogenin*, *MyHC*, and *MSTN-1a* at different ages of the parr were similar. Particularly, the expression of these genes peaked in the yearling parr (1+) and then decreased in elder groups. The differences were revealed in parameters studied between the parr and smolts. The level of aerobic and anaerobic metabolism enzyme activities was higher in the white muscles of smolts than in parr. The activity of carbohydrate metabolism enzymes was decreased in the smolts' livers. The expression levels of *MyHC*, *MyoD1a*, *MyoD1b*, and *myogenin* were lower in smolts at age 2+ compared to parr. These findings expand our knowledge of age-related and stage-related features of energy metabolism and muscle development regulation in young Atlantic salmon in their natural habitat. The results might be used for monitoring of the salmon population during restoration and rearing.

Keywords Atlantic salmon · Parr · Smolt · Energy and carbohydrate metabolism · Enzymes · Myogenesis · Gene expression

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Introduction

Atlantic salmon (*Salmo salar* L.) is an important animal model to investigate the regulation of growth and development processes in fish during ontogenesis. The life cycle of salmon includes various developmental stages with a complex system of adaptations (Atlantic salmon ecology 2011). Individuals of the same generation can differ significantly based on their size and growth rates, which influences the onset of parr–smolt transformation (smoltification) at different ages (Pavlov et al. 2007; McCormick 2013). The process resulted in the formation of complex age structure of salmon population on duration of river life period. Each age group of young salmon is

characterized by features in metabolism related to changes of stream location, feeding type and regime, activity, and beginning of smoltification.

The most important parameters which have age-related variations and determine the state of individuals and the population as a whole are energy metabolism and growth. A sufficient level of adenosine triphosphate (ATP) synthesis determines active growth and development of fish, especially during early ontogenesis and in the first years of life when high energy costs are needed for the synthesis of structural, functional, and storage compounds (Ozernyuk 2011). The level of energy metabolism can be assessed by measurement of the activities of key enzymes of respiratory chain and glycolysis. Cytochrome c oxidase (COX), the enzyme of the respiratory chain of mitochondria, is used as an indicator of the aerobic capacity (Gauthier et al. 2008). Lactate dehydrogenase (LDH) is the terminal enzyme of anaerobic glycolysis and is used as an indicator of anaerobic capacity (Somero and Childress 1980).

The enzymes involved in the pathways of carbohydrate oxidation could be applied to characterize the level of carbohydrate use in energy metabolism and biosynthesis. Aldolase, an enzyme of glycolysis, converts fructose 1,6-bisphosphate into dihydroxyacetone phosphate (DHAP) and glyceraldehydes 3-phosphate, which are precursors to glycolysis, gluconeogenesis, and lipid formation (Johansen and Overturf 2006). Glycerol-3-phosphate dehydrogenase (GPDH) catalyzes the process of formation of glycerophosphate from DHAP, a precursor of structural and storage lipids (Treberg et al. 2002). Glucose-6-phosphate dehydrogenase (G6PDH) is the key enzyme involved in the pentose phosphate pathway, which leads to the formation of pentoses and reduction of nicotine amide dinucleotide phosphates used in nucleic acids and fatty acid synthesis (Gauthier et al. 2008). Previous researches have shown that the activity of enzymes involved in energy and carbohydrate metabolism are associated with the processes of growth in fish, indicating age-related and seasonal metabolic changes, and correlate with size-weight characteristics of individuals, which have been demonstrated in various fish species (Imsland et al. 2006; Davies and Moyes 2007; Gauthier et al. 2008; Koedijk et al. 2010; Churova et al. 2010, 2015).

According to the study of COX, LDH, GPDH, and G6PDH activities in Atlantic salmon parr, it was found that energy and carbohydrate metabolism differed between groups of underyearling salmon living in the mainstream of the subarctic Varzuga River and its tributary. It was assumed that the noticeable differences in parameters of energy metabolism of juvenile salmon in the first year of life in the river and in its tributary make a basis for subsequent smoltification of parr at different ages 2+, 3+, or 4+ (Pavlov et al. 2007). Changes in respiratory and glycolytic enzyme activities in organs have been demonstrated during smoltification of several salmonid species (Leonard and McCormick 2001; Mizuno et al. 2012). These studies indicated the alteration in energy status during parr–smolt transformation in salmonids.

In most fish, skeletal muscles comprise the larger part of body (about 60% of weight) and therefore play a great role in metabolism of the entire organism and determine total growth rate (Houlihan et al. 1993). The rate and mechanisms of muscle growth in juvenile and adult fish depend on numerous factors, such as genotype, photoperiod, temperature, water flow characteristics, hypoxic, and food availability (Johnston 2006).

Postnatal muscle growth in fish occurs by both hyperplasia (generation of new myotubes) and hypertrophy (increase in myotube size) which are controlled by sequential expression of certain transcription factors. The main role in myogenesis regulation belongs to specific miogenic regulatory factors (MRFs), the transcription factors of bHLH family: MyoD, Myf5, myogenin, and MRF4 (Watabe 2001). These factors have highly conserved basic helix-loop-helix (bHLH) domain which is linked to the DNA sequence E-box that is found in the promoting region of many skeletal muscle-specific genes. Transcription factors MyoD and Myf5 play a key role in specification and proliferation of myoblasts and myogenin, and MRF4 mediate their differentiation (Watabe 2001). MRF gene expression could be used in description of juvenile and adult myogenesis of fish (Johansen and Overturf 2005). The quantitative expression of MRF in reference to hypretrophic and hyperplastic muscle growth mechanisms were studied in rainbow trout *Oncorhyncus mykiss* (Johansen and Overturf 2005) and pacu *Piaractus mesopotamicus* (Almeida et al. 2008, 2010) at different developmental stages. MRF expression depends on different factors. Studies on myogenesis during salmon embryogenesis have shown that the MRF expression level was affected by incubation temperature, which would define the postnatal muscle growth mechanisms (Johnston et al. 2000; Macqueen et al. 2007). The studies on farmed salmonidae revealed that

MRF messenger RNA (mRNA) levels depend on changes in farmed conditions such as feeding regime and feed composition (Johansen and Overturf 2006; Bower et al. 2009; Overturf et al. 2010).

A key regulator of myogenesis is myostatin (MSTN), a member of transforming growth factor-β (TGF-β). MSTN is a negative regulator of muscle growth and inhibits proliferation and differentiation of muscle cells in mammals (Gabillard et al. 2013). Studies in salmonids have shown that MSTN regulation of muscle growth mechanisms is dependent on the muscle type, developmental stage, and nutritional conditions (Østbye et al. 2001; Johansen and Overturf 2005, 2006).

Myosin, the most abundant protein in muscle, amounts to 25% of total protein in the whole organism and 50% of the muscle proteins (Watabe and Ikeda 2006); thus, it is an advantageous protein for growth studies. It was revealed that myosin heavy chain (*MyHC*) mRNA level correlated with the growth rate of rainbow trout *O. mykiss* (Overturf and Hardy 2001), wolfish *Anarhichas minor* (Imsland et al. 2006), and walleye *Sanders vitreus* (Dhillon et al. 2009). The study in Atlantic salmon indicated that *MyHC* mRNA expression can be a useful marker for evaluating growth and protein accretion (Hevroy et al. 2006). A positive correlation between *MyHC* expression level and weight was revealed in Atlantic salmon parr within different age groups (Churova et al. 2015).

There are numerous studies devoted to metabolism and myogenesis of Atlantic salmon in ontogenesis. However, there is not enough information about age-related differences and alterations during smoltification in the energy status and muscle growth regulation of young salmon in wild. The complex study of energy metabolism and muscle growth processes in wild parr and smolts of different age groups allows for the determination of characteristics of Atlantic salmon development in the freshwater period. Based on this, the study was designed to evaluate the energy and carbohydrate metabolism enzyme activities and expression of genes that control muscle growth in young salmon. In particular, the activities of COX, LDH, G6PDH, GPDH, and aldolase in muscles and the liver and gene expression levels of *MyHC*, myostatin (*MSTN-1a*), and myogenic regulatory factors (*MyoD1a*, *MyoD1b*, *MyoD1c*, *Myf5*, *myogenin*) were studied in white muscles in salmon parr and smolts of different ages.

Materials and methods

Fish samples

Parr of Atlantic salmons (*S. salar* L.) at ages 0+, 1+, 2+, and 3+ and smolts at age 2+ and 3+ inhabited the Indera River (the Kola Peninsula, Russia) were studied. Samples were collected in June 2015 at a water temperature of 11.5 °C. The parr were caught by electric fishing gear (model Fa_2, Norway). To avoid the effect of electrofishing, fingerlings were held for 24 h in cages located in the mainstream portion of the river. Smolts were caught by smolt catches, located in the river 300 m from an estuary. Fish were individually measured and weighed. Tissue pieces were frozen in liquid nitrogen and kept at −80 °C prior to analysis. The mean body weight, total length, and number of fish are presented in Table 1.

This study was carried out at the Scientific Center of Collective Usage Platform of the Institute of Biology of the Karelian Research Centre of the Russian Academy of Sciences.

Enzyme analyses

The activities of enzymes were determined both in white muscles and the liver of parr at age 1+, 2+, and 3+ and smolts of 2+ and 3+ age groups. The enzyme activities in underyearlings (0+) were assayed only in muscles. The activity of liver enzymes in parr at age 0+ was not measured due to the low weight of organ, which was inadequate for sampling.

Tissue samples were weighed and homogenized in 0.05 M Tris-HCl buffer (pH 7.5) containing 40% sucrose

Table 1 Mean (M ± SE) weight and length of parr and smolts of different ages

	Number	Weight (g)	Fork length (cm)
Parr			
0+	7	0.14 ± 0.05	2.75 ± 0.03
1+	8	1.54 ± 0.07	5.68 ± 0.12
2+	8	7.90 ± 0.47	9.67 ± 0.15
3+	5	14.13 ± 0.78	12.20 ± 0.06
Smolts			
2+	9	12.85 ± 1.06	11.66 ± 0.24
3+	9	20.06 ± 1.46	13.71 ± 0.42

and 0.1% Triton X-100. The enzyme activities were assayed spectrophotometrically. Specifically, the activity of cytochrome c oxidase (COX, E.C.1.9.3.1) was measured based on the oxidation of cytochrome c by COX, which was determined from the decrease in absorbance at 550 nm (Smith 1955). Activities of lactate dehydrogenase (LDH, E.C.1.1.1.27, Vassault 1983), glucose-6-phosphate dehydrogenase (G6PDH, E.C.1.1.1.49, Bergmeyer 1983), and glycerol-3-phosphate dehydrogenase (GPDH, E.C.1.1.1.8, Bergmeyer 1983) were assayed at 340 nm. One unit of enzyme activity was defined as the amount of enzyme necessary to elicit a change in absorbance of $1.0 \, U \, min^{-1}$ (1.0-cm light path). The extinction coefficient for NADH + H^+ or NADPH was 6.22 $cm^{-1} \, \mu mol^{-1}$. Aldolase (E.C.4.1.2.13, Bergmeyer 1965) activity was determined based on the colorimetric reaction of products of aldolase and 2,4-dinitrophenylhydrasin in alkaline medium and measured at 535 nm.

The activities of enzymes were expressed as international units per gram of tissue ($IU \, g^{-1}$ tissue), where one activity unit equaled the formation of 1 μmol of product per minute.

Myf5, MyoD, myogenin, MSTN-1a, and MyHC mRNA gene expression

Total RNA was isolated from the dorsal white muscle samples using a kit "RNA-extran," analog of TRIzol (Evrogen, Russia) according to the manufacturer's protocol. Then, total RNA was treated with DNase (Sileks, Russia). RNA integrity and quality were assessed by 1% agarose gel electrophoresis and spectrophotometrically at 260/280 nm absorbance ratio (SmartSpec Plus, BioRad, USA). RNA was reverse transcribed using MMLV-reverse transcriptase and random hexamer primers (Evrogen, Russia).

Real-time PCR assay was conducted using the iQ5 real-time PCR detection systems (BioRad, USA). The primers for the *MyHC*, *myogenin*, *MyoD1a*, *MyoD1b*, *MyoD1c*, *Myf5*, *MSTN-1a*, and elongation factor-1 (*Ef-1α*) were selected using the Beacon Designer 5.0 software (Premier Biosoft, USA). The primer sequences are given in Table 2. Amplification of 2 μl complementary DNA (cDNA; 1:5 dilution of RT reaction) occurred using 5 μl qPCRmix-HS SYBR Green 5× (Evrogen, Russia) and 500 nM primers to yield a final volume of 25 μl. The real-time conditions were as follows: DNA denaturation for 5 min at 95 °C; repeat cycles (40): denaturation for 20 s at 95 °C, annealing for 30 s at 60 °C, and

DNA elongation for 30 s at 72 °C. Melt curve analysis verified that the primer sets for each qPCR assay generated one single product and no primer–dimer artifacts.

The relative standard curve method was used for quantification of gene expression (Garikipati et al. 2006). A serial dilution of mixed cDNA from smolts and parr of different ages was used to construct a standard curve for each assay plate and each gene studied. This curve was used to calculate relative abundance of the transcript in each sample. Transcript abundance values were normalized to those of reference gene *Ef-1α*. Each sample was run in triplicate on a single plate. The data are presented as normalized gene expression.

Statistical analysis

Data were analyzed using the Kruskal–Wallis test. Differences between groups were evaluated by Mann–Whitney U test. Spearman's correlation and multiple regression analyses were used to examine relationships between parameters studied and parr's body mass. All results were considered significant at $p < 0.05$. All data are presented as the means ± SE.

Results

Enzyme activity in muscle and liver of parr and smolts of salmon

The results revealed significant age-related changes in enzyme activities in muscles of salmon parr. The COX activity was higher in muscles of underyearlings (0+) and yearlings (1+) and decreased in 2+ and 3+ age groups ($p < 0.05$) (Fig. 1a). The activity of LDH, in contrast, increased with age and was significantly higher in parr of ages 1+ ($p < 0.05$) and 2+ ($p < 0.05$) in comparison to the previous age group (Fig. 1b). The aldolase activity was over two times higher in yearlings than in underyearlings ($p < 0.05$) (Fig. 1c). This enzyme activity was higher at age 2+ compared to 1+ group ($p < 0.05$). The COX activity was negatively related to body mass in parr ($p < 0.05$) (Table 3). The activities of LDH and aldolase were positively correlated with parr's body mass ($p < 0.05$) (Table 3).

The data analysis on the liver enzyme activity of parr did not reveal any significant differences between the studied age groups (Fig. 2). There were no significant

Table 2 Oligonucleotide primers used for RT-qPCR amplification

Gene	Sequence 5′-3′	Size of amplified fragment (bp)	GenBank accession no.
EF-1a	F: TTGCTGGTGGTGTTGGTGAG R: AAACGCTTCTGGCTGTAGGG	154	AF321836.1
Myogenin	F: GTGGAGATCCTGAGGAGTGC R: CTCACTCGACGACGAGACC	147	DQ294029
MyoD1a	F: TGGACTGCCTATCAAACATCC R: TCTCACTCGCTATGGAACC	123	AJ557148
MyoD1b	F: ATTTCGTTCCCTGTCACCTCTG R: ATGTGTTCGTCTTCGTTGTAATGG	152	AJ557150
MyoD1c	F: ACGGCGAAAACTACTACCCTTC R: TAGCTGCTTCGTCTTGCGGA	133	DQ366709.1
Myf5	F: ACGCCATCCAGTACATCGAG R: AGTCAACCATGCTGTCGGAG	132	DQ452070
MyHC	F: TCTCATCCATAGACGCCATC F: AGTTGACTGCCAAGAAGAGG	159	DN164736
MSTN-1a	F: GATTACACGCCATCAAGTCC R: CTCCATCCTTATTGTCATCTCC	159	AJ344158

Forward and reverse primer sequences (5′-3′)

bp base pairs, EF1a elongation factor 1a, Myf5 myogenic factor 5, MyoD1 myoblast determination protein 1, MyHC myosin heavy chain, MSTN-1a myostatin1a

differences in enzyme activity in muscle and liver between age groups in smolts (Figs. 1 and 2).

Comparative analysis of results revealed the differences in enzyme activity between parr and smolts. In muscle, the activity of COX (2+, 3+, $p < 0.05$, Fig. 1a) and LDH (2+, 3+, $p < 0.05$, Fig. 1b) was higher in smolt than in parr of the same age. No differences in aldolase activities were observed between parr and smolts (Fig. 1c).

In the liver, there were no differences in COX activity (Fig. 2a). Low activity of LDH (Fig. 2b), G6PDH (Fig. 2c), and aldolase (Fig. 2d) was revealed in smolts compared to parr in both 2+ and 3+ age groups ($p < 0.05$). GPDH activity was lower in smolts than in parr at age 2+ ($p < 0.05$) (Fig. 2e).

Muscle-specific gene expression levels in parr and smolts

RT-qPCR results showed age-related and stage-related differences in gene expression levels. The highest MyHC (Fig. 3a), MyoD1a (Fig. 3b), and MyoD1b (Fig. 3c) mRNA levels were revealed for yearling salmon, $p < 0.05$. Their expression level decreased gradually in parr at age 2+ and then in 3+, $p < 0.05$. MyoD1c expression level was highest in 0+ parr then decreased at age 1+ and 3+ (Fig. 3d) ($p < 0.05$). The MyoD1c mRNA level (1.63 ± 0.21) was higher than MyoD1a (1.01 ± 0.04) and MyoD1b mRNA (1.11 ± 0.10) level in the 0+ group ($p < 0.05$).

The level of myogenin gene expression was higher in 1+ parr compared to the 0+ group, $p < 0.05$ (Fig. 3e). The expression level of myogenin was lower in 3+ parr compared to 2+, $p < 0.05$. No significant age-related changes were observed in Myf 5 expression (Fig. 3f).

The MSTN-1a mRNA level was eight times higher in yearlings than in underyearlings, $p < 0.05$ (Fig. 3g). Its expression was lower in groups 2+ and 3+ compared to 1+, $p < 0.05$.

According to comparative analysis, the expression level of MyHC, $p < 0.01$ (Fig. 3a), MyoD1a, $p < 0.01$ (Fig. 3b), MyoD1b, $p < 0.01$ (Fig. 3c), and myogenin, $p < 0.05$ (Fig. 3e), was lower in smolts at age 2+ compared to parr of the same age. There were no differences in Myf5 (Fig. 3f), MyoD1c (Fig. 3d), and MSTN-1a mRNA (Fig. 3g) levels between smolts and parr at age 2+. No differences were observed between parr and smolt in gene expression at age 3+ (Fig. 3).

Discussion

Metabolic enzyme activity in muscles of parr of different age groups

Multidirectional changes in activities of enzymes of aerobic and anaerobic energy metabolism in white muscles in parr with age were shown. Results revealed differences in COX activity in white muscle that are due to changes
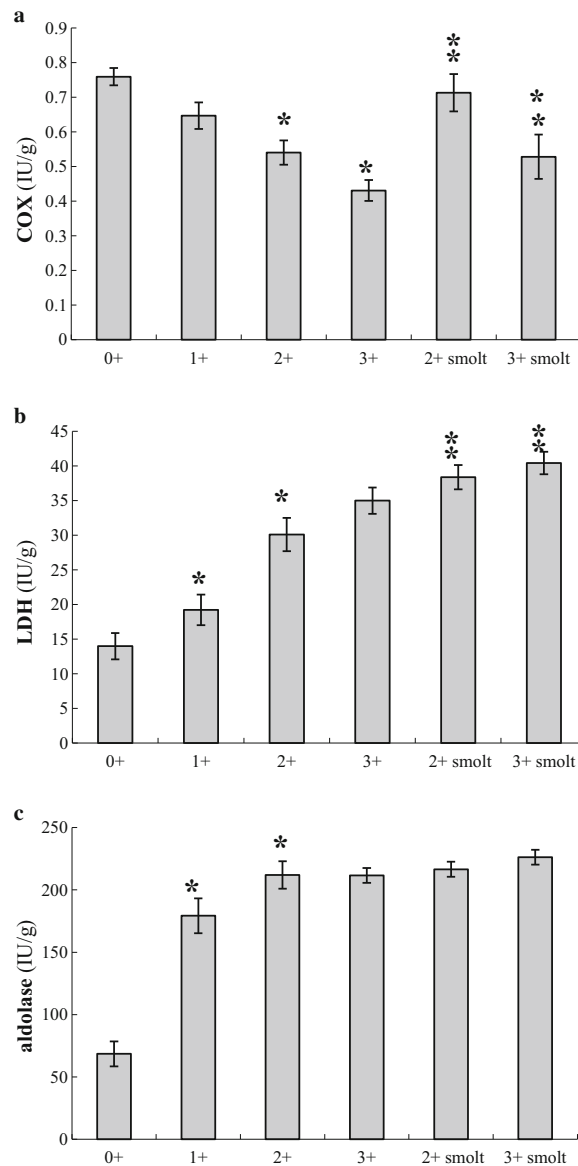
Fig. 1 Enzyme activity (IU g^{-1} tissue). COX (**a**), LDH (**b**), and aldolase (**c**) in muscles of parr (0+, 1+, 2+, 3+) and smolts (2+ smolt, 3+ smolt). *Asterisks* denote significant differences between parr in comparison with previous age group. *Double asterisks* denote significant differences between parr and smolts of the same age

of intensity of aerobic processes in ontogenesis. The level of COX activity was the highest for yearlings and underyearlings and decreased in older groups. Thus, it can be concluded that aerobic capacity is high in parr at age 0+ and 1+. The high level of aerobic synthesis of ATP is of great importance at the early ontogenesis and at first years, when the growth rate is highest and intensive processes of biosynthesis take place (Ozernyuk 2011). It is known that with age and increasing weight, the intensity of oxygen consumption, tissue respiration, and

standard metabolism in whole decreased (Davies and Moyes 2007; Ozernyuk 2011). The growth rate decreased with age, and therefore energy consumption causing a biosynthesis decline.

In contrast to the COX activity, the LDH activity in muscles increased with age and body mass. The LDH activity in the white muscles of fish is related mainly to its involvement in anaerobic glycolysis (Somero and Childress 1980). This process is the main energy supply of the white muscle during intensive contractions.

Table 3 Linear regression and coefficient of correlation between activities of COX, LDH, and aldolase in white muscles and mass of salmon parr

Variable (y)	Mass (x)	Coefficient of correlation (mass)
COX activity	$y = 0.722 - 0.026 \times x$ $R^2 = 0.66*$	$-0.81*$
LDH activity	$y = 16.138 + 1.732 \times x$ $R^2 = 0.72*$	$0.85*$
Aldolase activity	$y = 143.365 + 6.515 \times x$ $R^2 = 0.36*$	$0.61*$

$*p < 0.05$, significant relationship

Therefore, with age and body mass gain, the rate of parr anaerobic metabolism increases. It was shown that the level of LDH activity reflects the degree of physical activity in fish and their swimming characteristics. Many fish species were found to have an age-related and size-related increase in the intensity of anaerobic energy metabolism, as well as in the activity of LDH and other glycolytic enzymes (Somero and Childress 1980; Davies and Moyes 2007). Atlantic salmon parr at age 1+, 2+, and 3+ are characterized by high physical activity as they inhabit areas with high current velocity and have to actively resist the current. Therefore, an increase in the LDH activity of the muscles with age and body mass may be aimed to increase the energy supply for swimming activity and retention of parr in the current. The aldolase activity in the muscles increased in age groups along with LDH activity. It indicates that changes in the level of carbohydrate utilization correspond to the tendency of the level of anaerobic metabolism changes in the muscles in the age groups.

Similar studies on enzyme activity in parr were conducted in the summer period of the previous year. The pattern of COX, LDH, and aldolase activity changed in the muscle of parr at ages 1+ and 2+ corresponding to the present results (Churova et al. 2015). The present and previous results indicate the age-dependent variation of energy metabolism in muscles, which are characteristic for the parr inhabiting the Indera River. These results might be used in monitoring and health assessment of the salmon population.

Muscle-specific gene expression in parr of different age groups

MRF play the key role in postembryonic myogenesis as regulate specification, proliferation, and differentiation of myogenic precursor cells (MPCs). Transcription factors MyoD and Myf5 are required for myoblast determination, and myogenin is involved in the differentiation and fusion of myoblasts to form myofibers (Watabe 2001). According to studies on rainbow trout and pacu, they are differently expressed in postembryonic development (Johansen and Overturf 2005; Almeida et al. 2010). The results of the present study have shown specific patterns of age-dependent changes in muscle-specific gene expression involved in myogenesis regulation in salmon parr.

It was noticeable that paralogs of MyoD1 were differently expressed in salmon age groups. In the result of tetraploidization, salmonids expressed three MyoD1 paralogs—MyoD1a, MyoD1b, and MyoD1c—that have subfunctionalized and exhibited distinct expression patterns during development and in different fiber types (Macqueen and Johnston 2006; Macqueen and Johnston 2008). In salmon primary cell culture, MyoD1 paralogs are differentially expressed during myotube maturation, and that suggests that *Myod1b* and *Myod1c* are primarily expressed in proliferating cells and *MyoD1a* in differentiating cells. Based on this, it is possible that *Myod1b* and *Myod1c* regulate cell cycle and *MyoD1a* is involved in terminal differentiation (Bower and Johnston 2010). According to our results, the *MyoD1a* and *MyoD1b* gene expression peaked in 1+ parr, but the *MyoD1*c expression was highest in underyearlings and then decreased. Moreover, the *MyoD1c* mRNA level was higher than *MyoD1a* and *MyoD1b* mRNA level in the 0+ group. In our study on brown trout *Salmo trutta* L. (Churova et al. not published), the similar result on the highest *MyoD1c* expression level in underyearlings was revealed. It is possible that paralogs differently expressed in the ontogenesis and *MyoD1c* is upregulated at the earlier stages (in parr with mass 0.14 g). It was shown that MyoD paralogs differently responded to meal distribution after fasting; most especially, Myod1c expression level was elevated after refeeding in rainbow trout (Valente et al. 2012). The MyoD paralogs differently respond to amino acids after starvation that was shown on myogenic cell culture. In response to amino acids, *MyoD1c* and *MyoD1b* increased significantly and *MyoD1a* did not respond markedly (Bower and Johnston 2010). On juvenile and adult salmon (with average mean mass 304.8 and 4297 g), it was shown that *MyoD1a* was upregulated in fast muscles in comparison to *MyoD1b/c*, but *MyoD1c* was upregulated in slow muscles (Macqueen and Johnston 2006).
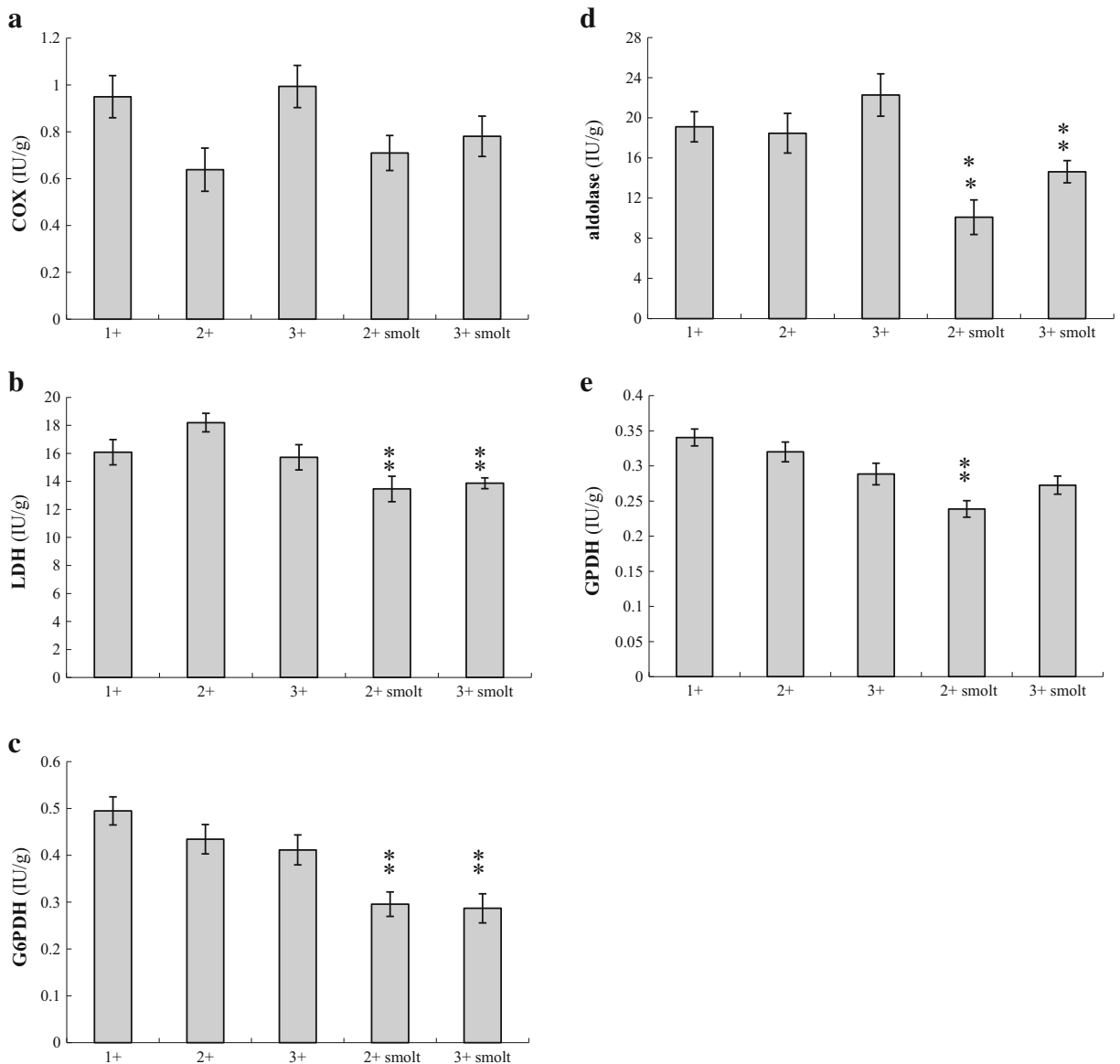
Fig. 2 Enzyme activity (EA, IU g^{-1} tissue). COX (**a**), LDH (**b**), G6PDH (**c**), aldolase (**d**), and GPDH (**e**) in the liver of parr (1+, 2+, 3+) and smolts (2+ smolt, 3+ smolt). *Double asterisks* denote significant differences between parr and smolts of the same age

The pattern of *myogenin* expression levels in different age groups was similar to *MyoD1a* and *MyoD1b* expression levels and peaked in 1+ parr and then decreased with ages. As in our study, the concurrent increase in *TMyoD2* (that is referred as *Myod1b* by Macqueen and Johnston 2008) and *myogenin* gene expression at the same development stage was shown in rainbow trout *O. mykiss* (Johansen and Overturf 2005) and *MyoD* and *myogenin* in pacu *P. mesopotamicus* (Almeida et al. 2010). Coupled changes of *myogenin*

and *MyoD* expression levels are described by the mechanisms of regulation of muscle-specific genes. It is known that MyoD activation leads to robust expression of target genes, such as myogenin, M-cadherin, MyHC, and muscle creatin kinase. MyoD activates myogenin expression by directly binding to its regulatory elements (Berkes and Tapscott 2005). In a study on rainbow trout, it was shown that *myogenin* expression was restricted to small-diameter muscle fibers and was therefore indicative of muscle hyperplasia (Rescan et al. 1995; Rescan
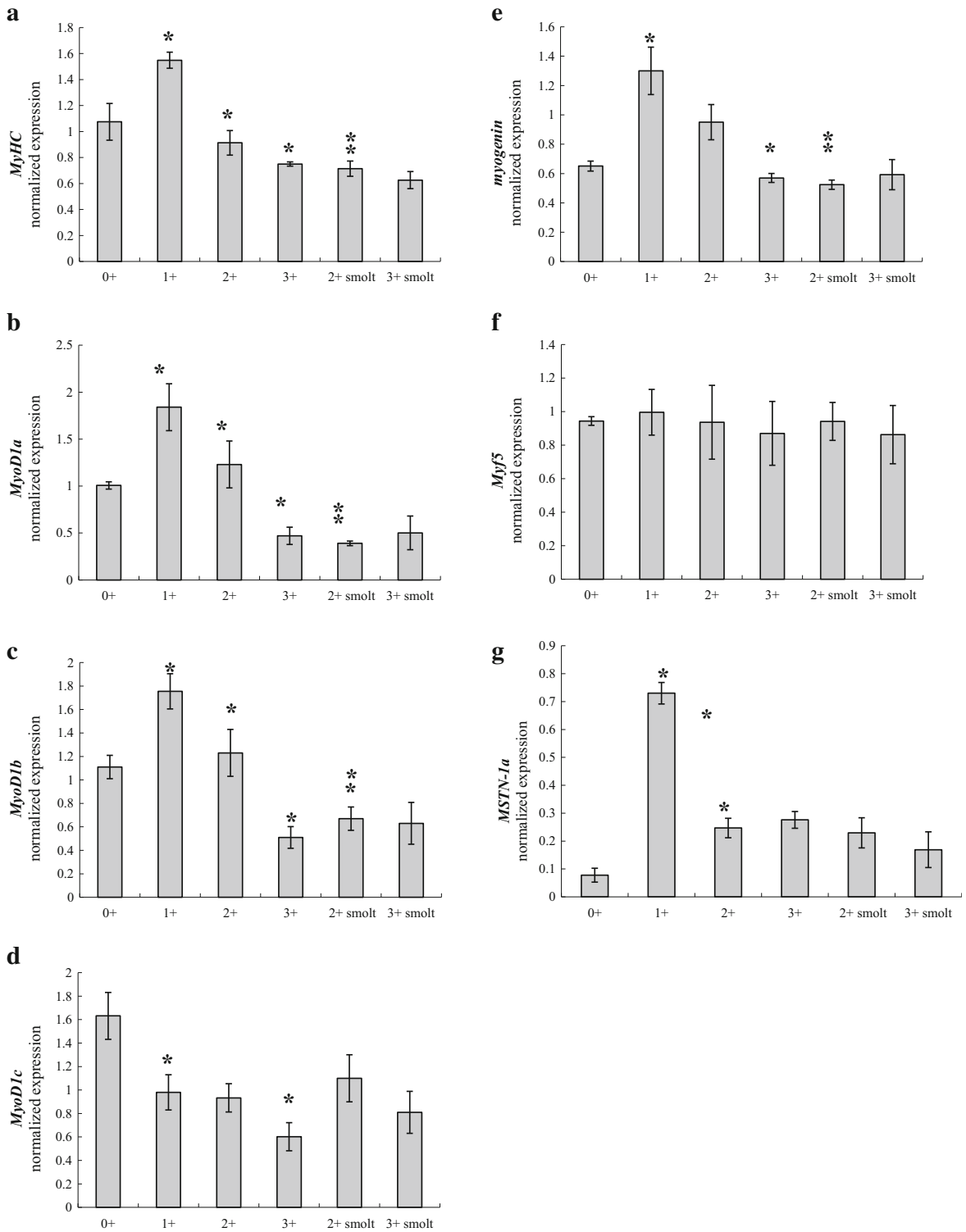
Fig. 3 Expression of growth-related genes. *MyHC* (**a**), *MyoD1a* (**b**), MyoD1b (**c**), MyoD1c (**d**), *myogenin* (**e**), *Myf5* (**f**), and *MSTN-1a* (**g**) in parr (0+, 1+, 2+, 3+) and smolts (2+ smolt, 3+ smolt).

Asterisks denote significant differences between parr in comparison with previous age group. *Double asterisks* denote significant differences between parr and smolts of the same age

et al. 2015; Montfort et al. 2016). Gene expression profiling of trout regenerating muscle shows that *MyoD1b*, *MyoD1c*, and *myogenin* were upregulated during muscle regeneration and in hyperplastic growth zones (Montfort et al. 2016). That is why, it is possible to suggest that high expression of *Myod1* and *myogenin* expression in yearlings indicated the hyperplastic muscle growth.

In contrast to *MyoD1a* and MyoD1b mRNA levels, the *Myf5* gene expression did not differ between age groups. It is likely that the changes in expression level of *MyoD1* are more significant for myogenesis regulation in parr at different ages. According to the study of Johansen and Overturf (2005), expression of *MyoD* and *Myf5* peaked independently from each other at different stages of rainbow trout development. It was suggested by the authors that MyoD and Myf5 might complementarily control muscle growth processes.

The high *MyoD1a*, *Myod1b*, and *myogenin* mRNA levels in parr at age 1+ and decrease in the older age groups corresponded with the high expression levels of *MyHC*. It was shown that mRNA *MyHC* levels, which is the most abundant muscle protein, correlate with the growth rate of several fish species (Overturf and Hardy 2001; Imsland et al. 2006; Dhillon et al. 2009) as well as protein accretion (Hevroy et al. 2006). But there are controversial studies that did not show the link between *MyHC* expression level and rapid growth in atlantic cod (Koedijk et al. 2010) and rainbow trout following growth hormone treatment (Biga et al. 2004) and fed different diet (Alami-Durante et al. 2010). The correlation of *MyHC* mRNA level with growth rate would depend on different factors, such as size of fish, type of diet (feed content), protein synthesis, and degradation rate (Koedijk et al. 2010; Biga et al. 2004; Alami-Durante et al. 2010). The study on protein degradation system in salmon parr from River Indera (the same research as present) revealed the decrease in proteasome activity in parr 1+ and 2+ in comparison to the previous age group (Kantserova et al. 2017). In the study of Overturf and Gaylord (2009), proteasome enzymatic activity decreased significantly in the muscle of rainbow trout as fish size increased. It was suggested by authors that muscle proteasome activity decrease with trout size would benefit muscle accretion and weight gain and that the fish is becoming more efficient in building muscle as it grows. It allows us to conclude that high level of *MyHC* expression along with MRF high expression in yearling salmon reflects the intensive growth processes

and muscle weight gain. In the previous study on Atlantic salmon from River Idera (Churova et al. 2015), the decrease of *MyHC* mRNA levels at age 2+ compared to 1+ was observed. Moreover, in that study, it was revealed that the RNA/DNA ratio and protein concentration were highest at age 1+ and decreased at age 2+. Both study results indicate that there is a high protein synthesis level and growth rate in salmon yearlings (1+). The low *MyHC* mRNA levels along with decreased COX activity at age 2+ and 3+ indicate the decline in muscle growth rate.

The high levels of *MyHC*, *MyoD1a*, *MyoD1b*, and *myogenin* expression in yearlings were accompanied by high *MSTN-1a* mRNA level, the negative regulator of muscle growth. Simultaneous increase of expression of *MyoD* and *myogenin* with *MSTN* on the same development stages was shown in rainbow trout (Johansen and Overturf 2005). One potential explanation is that MSTN is expressed in response to high MRF expression levels, which are the necessary regulation mechanism to attenuate hyperplasia and hypertrophy and control muscle growth (Johansen and Overturf 2005, 2006). In mammals, MSTN inhibits myoblast proliferation and differentiation through a tumor growth factor-B pathway involving the activin receptor, Act RIIB, and the phosphorylation of Smads 2 and 3. Myogenin is probably one from the major physiological targets of MSTN (Joulia et al. 2003; Johnston et al. 2008). The MyoD binding sites were found in MSTN promoter (Johnston et al. 2008). However, according to recent studies, the mechanism of MSTN action on growth in fish probably differs from mammals. MSTN is conserved as two genes in most teleost and four genes in salmonids and is found to be expressed in muscles and other tissues. Consequently, it may possess different functions (Østbye et al. 2001; Gabillard et al. 2013). Studies using rainbow trout myosatellite cells have shown that MSTN inhibits proliferation and has no effect on differentiation (Seiliez et al. 2012) or, on the contrary, consequently stimulates it (Garikipati and Rodgers 2012). The study on comparison of wild, domesticated, and growth hormone transgenic coho salmon showed high expression levels of both *MSTN* and *MyoD* with *myogenin* in fast-growing fish (Overturf et al. 2010). It was concluded by the authors that MSTN expression increases in faster growing fish when there is sufficient energy but is not negatively acting to regulate muscle growth. The feeding experiments in fish have shown that the *MSTN* gene is differently expressed in periods of fasting and

refeeding. In rainbow trout, the high *MSTN* mRNA levels during the refeeding period was associated with high *myogenin* expression that possibly is a mechanism to attenuate the muscle growth (Johansen and Overturf 2006). In a study on pacu during a fasting period, high *MSTN* gene expression indicated lower satellite cell proliferation activity, which was confirmed by low *MyoD* mRNA levels (Nebo et al. 2013). Thus, the MSTN expression is affected by several factors, but precise mechanisms of action require further investigation.

Comparative analysis of enzyme activity
and muscle-specific genes expression in parr and smolts

The parr–smolt transformation, or smoltification, is a developmental process in salmon to prepare for life in the sea. Significant biochemical, physiological, morphological, and behavioral changes occur in the fish throughout this period. The alterations affect lipid and carbohydrate metabolism, osmoregulation, oxygen transport, growth, and rheotaxis (Stefansson et al. 2008; McCormick 2013). In particular, the intensification of oxidative processes is revealed in smolts. In Atlantic salmon, standard and active metabolic rates are 50% higher in smolts than in parr (Maxime et al. 1989), suggesting that this elevation might be related with the increase in respiratory enzyme activity and mitochondrial proliferation caused by an increase in thyroid hormone levels (Leonard and McCormick 2001). Decrease in hepatic glycogen content was observed in masu salmon *Oncorhyncus masou* (Mizuno et al. 2012), in Atlantic salmon (Wendt and Saunders 1973), and coho salmon *Oncorhynchus kisutch* (Sheridan et al. 1985), that could be related with decreased glycogen synthesis and increased glycogenolysis in the liver (Sheridan et al. 1985). It is known that in salmon smolts, total body and muscle lipid content and fatty acid synthesis decreased and lipolysis increased (Sheridan et al. 1985; Stefansson et al. 2008; Pavlov et al. 2009). That is why smolts at the period of downstream migration and ocean entry are "energy deficient" (Sheridan et al. 1985; Stefansson et al. 2008).

According to data analysis from the present study, there were differences in aerobic and anaerobic metabolism in muscles between parr and smolts at age 2+. The higher activity of COX in smolts than in parr indicates that the increase in energy costs during smoltification is necessary for supporting adaptive reactions of metabolism (Stefansson et al. 2008).

The increase in LDH activity in the muscle of smolts in comparison to parr is possibly related to their migration activity. The LDH activity indicates the activation of glycolysis which is necessary for energy supply, allowing for physical activity during the migration downstream the river (Stefanson et al. 2008). Higher activity of the glycolysis enzyme, phosphofructokinase, in muscle was observed during smoltification in smolts than in parr (Leonard and McCormick 2001).

In the liver, LDH activities were decreased in smolts compared to parr at the same ages. This may indicate a decline in intensification of lactate–pyruvate conversion and consequently reduction in lactate-dependent gluconeogenesis (Leonard and McCormick 2001). Such a decrease in liver LDH was revealed in Atlantic salmon (Leonard and McCormick 2001) and masu salmon *Onchorhinkus masou* (Mizuno et al. 2012). The activity of G6PDH, GPDH, and aldolase in the liver decreased, that indicated the decrease of carbohydrate use in biosynthesis (pentose phosphate pathway and synthesis of lipids from glycerophosphate). These changes can be related with a decrease in glycogen synthesis and intensification of glycogenolysis (Sheridan et al. 1985).

As for muscle growth regulation, smolts at age 2+ differ from parr by low *MyHC*, *MyoD1a*, *MyoD1b*, and *myogenin* expression levels. It is an evidence of decrease in muscle growth. The MRF expression levels depend on a variety of factors, such as food quantity and quality and characteristics of total metabolism (Johansen and Overturf 2006; Bower et al. 2009; Alami-Durante et al. 2010; Nebo et al. 2013). In the present research, the smolts in the period of downstream migration were studied, and differences were observed which were caused by changes in metabolism during migration. Firstly, differences in MRF expression could be related to changes in food composition and quantity. This is supported by a study of Atlantic salmon from the Varzuga River (Kola Peninsula, Russia); this study revealed that during downstream migration, smolts do feed not intensively compared to parr (Shustov and Belyakova 2012). Taking in consideration the low level of energy reserves in smolts during migration (Stefansson et al. 2008), it is obvious that the delivered nutrients are used first of all for energy requirements then on growth processes. Moreover, the changes in protein turnover take place in smolts. It was revealed that in contrast to lipids and carbohydrates, there were no changes in protein content in salmon smolts and postsmolts during migration (Stefansson et al. 2008).

Our colleagues observed the decrease in protein degradation rate in migrating smolts compared to parr of salmon in the Indera River (Kantserova et al. 2017). These changes could also affect on the muscle-specific gene expression.

Conclusion

This study demonstrated age-related and stage-related features of aerobic and anaerobic capacity and muscle growth regulation in parr and smolt of Atlantic salmon in the wild. Each age group differs from one another by energy enzyme activities and MRFs, *MSTN* and *MyHC* expression levels. Alteration in energy metabolism in smolts during downstream migration was associated with elevation of aerobic and anaerobic capacities in the muscles and the decline of carbohydrate oxidation in the liver. Smolts differed from parr by having a decline in myogenesis regulation and muscle growth processes in relation to metabolic alterations during smoltification and migration. These findings expand our knowledge on the mechanisms of growth and development in wild salmon. The results might be used for monitoring the salmon population during restoration.

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