elevated mineralization on fish detoxification system. Because whitefish, pike and roach are common in northern European lakes this data may be of importance for ecological monitoring in this region.

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MYOSIN EXPRESSION LEVEL IN WHITE MUSCLE AS A MARKER OF FISH GROWTH

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One of the main questions in biotechnology of aquaculture is the search of convenient markers of fish growth. Methods for growth rate maximizing have been tested for many years in aquaculture. Examples, of such methods are recombinant growth hormone injection, use of transgenic fish, variation in type and regimes of nutrition, etc. That is why it is important to assess accurately the effect of use a new techniques. However, there are numerous ways of assessing growth, from traditional and direct measurements such as length, weight and condition factor, to the newest techniques that measure gene expression. Measuring biological macromolecules has been hypothesized by many investigators to be a more sensitive indicator of overall fish condition than traditional morphometric variables. It is considered that muscle-specific genes (Myogenic regulatory factors, myostatin, myosin) are the most reliable markers for assessment of fish growth. The myosin hard chain (MyHC) gene expression has been tested as possible indicator of muscle growth during the last decade since Overturf and Hardy (2001) demonstrated the positive correlation of myosin heavy chain expression with nutrient intake in rainbow trout. However, gene and protein expression of myosin has not been widely tested in fish. Furthermore, the limited number of studies in the literature present quite contradictory results (Biga et al., 2004, Johansen and Overturf 2006, Imsland et al., 2006, Dhillon. et al., 2008, Koedijk et al., 2010)

Myosin is the most abundant protein in muscle, comprising as much as 25% of the whole organism protein pool (Baldwin and Haddad, 2001) and up to 50% of the muscle protein pool (Watabe and Ikeda, 2006). The chosen in our study myosin heavy chain isoform belongs to class II of myosins. The class II proteins, referred to as conventional myosins, are expressed in the striated muscles and are directly involved in muscle contractions (Regiani, Bottinelli, 2008). The location and abundance of these proteins in white muscle make myosin an ideal candidate for investigating growth in fish. The gene encoding myosin heavy chain is expressed during the entire life of fish (Regiani, Bottinelli, 2008).

To test the myosin mRNA level as a potential biomarker of fish growth we evaluate the relationship of the MyHC mRNA level in white muscle with body mass and fork length of salmonid fish of different ages. We exmined both farmed fish (rainbow trout, *Parasalmo mykiss* W.) and wild fish (Atlantic salmon, *Salmo salar* L., white-fish, *Coregonus lavaretus* L.)**.** Biological characteristics of fish examined are presented in Table 1.

Table 1. Examined biological characteristics of fishes. The values indicate the mean and standard error with minimal and maximal values in brackets (min-max)

Species	Location	Age	Number of fish	Fork length (mm)	Body mass (g)
Rainbow trout	fish farm Lake Onego (Republic of Karelia)	Two-year-old fish (1+)	20	271.9±4.5	317.76±16.72
				(180–360)	(75.9–755.0)
		Three-year-old fish (2+)	15	470.3±4.9	1789,42±44,88
				(354–524)	(789.9–2210)
Atlantic salmon	River Indera (Kola peninsula)	Two-year-old fish (1+)	20	72.6 ± 1.1	3.64 ± 0.17
				(63–82)	(2.30 – 4.83)
		Three-year-old fish (2+)	10	96.9 ± 2	8.25 ± 0.51
				(86–103)	(5.67–10.20)
Whitefish	Lake Kamennoe (Republic of Karelia)	Three-year-old fish (2+)	9	189.6 ± 2.7	56.8 ± 2.38
				(176 – 197)	(50–67)
		Four-year old fish (3+)	9	205 ± 2.8	84.71 ± 4.9
				(196–241)	(73–110)
	Lake Tumasozero (Republic of Karelia)	Six-year old fish (5+)	15	311.7 ± 1.2	340.23 ± 5.4
				(292–325)	(263–390)

MyHC mRNA level were analyzed by quantitative real-time RT-PCR. Total RNA was extracted from the white muscles according to Chomczynski and Sacchi (1987) using a Yellow Solve kit (Clonogene, St. Petersburg, Russia). Total RNA was treated with DNase (10 U/ml; SibEnzyme, Russia). First strand cDNA was synthesized from the total RNA using MMLV reverse transcriptase and random primers (Sileks, Russia). Amplification was performed in an IQ5 Real-Time PCR Detection System (Bio-Rad, USA) using 2.5x reaction mixture for qRT-PCR in the presence of an intercalating dye, SYBR Green I (Syntol, Russia). Primers for the myosin heavy chain, β-actin and EF1 were selected using the Beacon Designer 5.0 software. Primers used for myosin and reference genes were as follows: rainbow trout *MyHC*: forward 5' – GCTGAGAAGGACGAGGAGATG – 3', reverse 5' – GCCTGCCTGTTGGAGTGG – 3' (GenBank, Z48794); rainbow trout *β-actin*: forward 5' – TGGACTTTGAGCAGGAGATGG – 3', reverse 5' – TCGTGGATACCGCAAGACTC – 3' (GenBank, AJ438158); salmon *MyHC*: forward 5' – TTCAGTGGCGTGCTTCTC – 3', reverse 5' – AAGAGGCTGGAGGATGAGG – 3' (GenBank, DN164736); salmon *EF1*: forward 5' – TGGACTGCCTATCAAACATC – 3', reverse 5' – TCTCACTCGCTATGGAACC – 3' (GenBank AJ427629). To analyse *MyHC* mRNA level in white muscle of whitefish the primers for salmon genes were used. Real-time PCR was conducted under the following conditions: 5 min at 95°C; 50 cycles for 30 s at 95°C, 30 s at 56°C, 30 s at 72°C; and then melting of DNA fragments. The concentration of mRNA was assayed using relative standard curve method for quantification (Gahr et al., 2008). The expression levels of *MyHC* gene were normalized for the expression level of a reference gene, *β-actin* and *EF-1*. The data were expressed as the ratio of *MyHC* mRNA concentration of the studied gene to that of β-actin mRNA.

To examine the relationships between *MyHC* mRNA levels and fork length and body mass of fish, the multiple regression analysis was used. The results are presented as the regression equations relating *MyHC* mRNA level in white muscle to fork length and body mass of fish studied (Table 2 and 3).

Table 2. The regression equations relating *MyHC* mRNA level in white muscle of fish studied to fork lenght. *y*, *MyHC* mRNA level; *x*, fork lenght.

Species	Age	n	Equation	r^2	P
Rainbow trout	1+	20	y = –0.876 + 0.006x	0.34	<0.01
	2+	15	y = –1.849 + 0.006x	0.60	<0.001
Atlantic salmon	1+	20	y = –20.133 + 0.334x	0.48	<0.001
	2+	10	y = –3.545 + 0.508x	0.58	0.01
Whitefish (Lake Kamennoe)	2+	9	y = –0.349 + 0.030x	0.34	<0.05
	3+	9	y = –3.876 + 0.216x	0.81	<0.01
Whitefish (Lake Tumasozero)	5+	15	y = –6.818 + 0.240x	0.40	0.01

Table 3. The regression equations relating *MyHC* mRNA level in white muscle of fish studied to body mass. *y*, *MyHC* mRNA level; *x*, body mass. NS, not significant

Species	Age	n	Equation	r^2	P
Rainbow trout	1+	20	y = 0.245 + 0.002x	0.36	<0.01
	2+	15	y = –0.134 + 0.001x	0.54	0.001
Atlantic salmon	1+	20	y = – 2.659 + 1.819x	0.26	<0.05
	2+	10	y = –0.268 + 0.195x	0.62	<0.01
Whitefish (Lake Kamennoe)	2+	9	y = –0.091 + 0.005x	0.68	0.001
	3+	9	y = –0.701 + 0.015x	0.84	<0.001
Whitefish (Lake Tumasozero)	5+	15	y = –0.265 + 0.003x	0.08	NS

Our results demonstrated the positive correlation of *MyHC* mRNA levels with fork length and body mass of rainbow trout, salmon and white-fish from Lake Kamennoe of all age groups studied. There was significant relationship between MyHC gene expression and fork length of whitefish 5+ from Lake Tumasozero. But no relationship was observed between myosin expression and body mass. The last fact is possible to explain by peculiarities of metabolism of fish at this age. The whitefish 5+ were sexually matured (gonad maturity stage V). It is possible to suppose that body mass is determined by gonad mass and store lipid content, and that only fish length reflects the muscle growth of six-year-old fish.

The relationship between *MyHC* mRNA levels and fork length and body mass is stronger in elder fish of all three species studied. MyHC mRNA levels in white muscle increased whit age of rainbow trout and white-fish (fig 1. A and C).

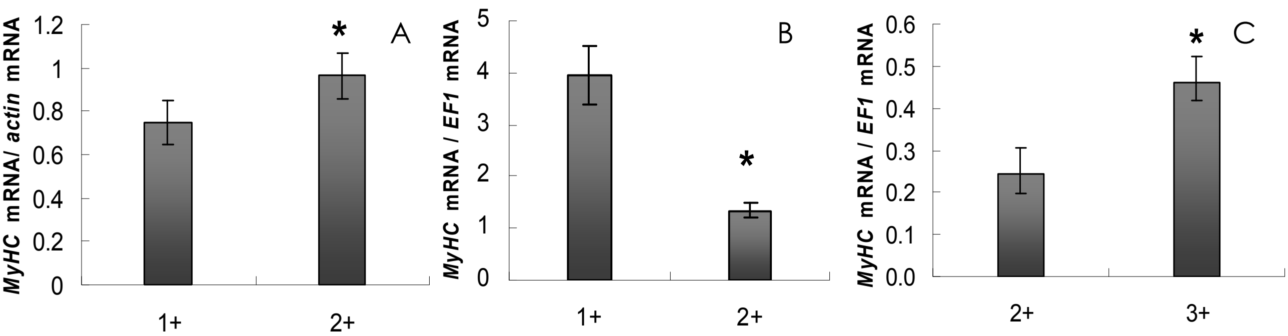

Fig 1. The changes in *MyHC* mRNA levels in white muscle of different age groups of rainbow trout (A), salmon (B), white-fish (C)

That indicates an increase of muscle growth rate with age. However, the level of MyHC gene expression was higher in two-years-old salmon as compared to three-year-old fish (Fig 1. B). It is possible related to switching of myosin isoforms expression. As it is known, in vertebrates, myosin heavy chains are encoded in a family of different genes (Weiss and Leinwand, 1996). For example, in carp, *Cyprinus carpio* L., evidence for at least 28 myosin heavy chain genes have been found (Gerlach et al., 1990). It was shown, that different isoforms are expressed depending on developmental stage (Ennion et al., 1999; Fukushima et al., 2009). Thus, the reduction of the myosin expression level observed with age in our study may reflect the switch to the expression of a different isoform that was undetected due to the specificity of the primers, or could also reflect an actual reduction of expression of myosin heavy chain as salmon grow. This fact should be studied in more detail, especially in aspects of myogenesis regulation.

Therefore, we studied the features of myosin expression in white muscles of fish of different species and ages. According to our results bigger fish within age cohort have a higher level of myosin expression. This indicates that myosin expression levels may be used as biomarker of fish growth to monitor and predict individual growth both in farmed and in wild fish.

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