LACTATE AND PYRUVATE AS MARKERS OF ANAEROBIC METABOLISM IN THE SKELETAL MUSCLE GROWTH IN SEA TROUT (*SALMO TRUTTA* M. *TRUTTA* L.)

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Changing living conditions, which is most characteristic of salmonids due to their changes in the habitat from fresh to saline water and vice versa during spawning, involves the alterations in the physiological and biochemical state of fish and their adaptive potential. Therefore, the use of biochemical indicators is adequate for the evaluation of developmental- and habitat-induced fish metabolism. These include lactate and pyruvate concentrations. Lactate as a marker of anaerobic metabolism accumulates as the end product of glycolysis when oxidative phosphorylation and the Krebs cycle are inhibited. Since pyruvate is reduced to lactate by lactate dehydrogenase in anaerobic conditions, the simultaneous determination of pyruvate and lactate levels is valuable for the assessment of the predominance of aerobic or anaerobic metabolic pathways. Assessment of the lactate and pyruvate ratio (L/P ratio) reveals the prevalence of aerobic/anaerobic metabolism reflecting the cytosolic ratio of the reduced and oxidized NAD (NAD^+ and $NADH^+$). Therefore, the L/P ratio is a more reliable parameter for estimating the cellular energy state, called the adenylate energy charge concerning ATP, ADP, and AMP concentrations (Zoremba et al., 2014). Thus, our study aimed to evaluate developmental-induced changes in the lactate and pyruvate levels, as well as the L/P ratio in the skeletal muscle growth in sea trout.

The study material was sampled in the years 2008 - 2014 from 372 specimens of the sea trout in various developmental stages: parr (n = 113), smolts (n = 122), adults (n = 25, i.e. 13 males, 12 females), spawners (n = 113, i.e. 33 males, 53 females), and kelts (n = 24, i.e. 12 males, 12 females). The trout specimens in the parr and smolt developmental stages were caught in Pomeranian rivers: Glazna, Skotawa, Kamienna, and Kwacza near Słupsk city. Among the adults, spawners, and kelt forms, the sexual dimorphism is well-expressed; the analysis was carried out according to the sex since the phenotypic males and females possessed testicular and ovarian structures. We have analyzed various stages of fish development those the changes in the neurohormonal levels determining sex were expressed (testicular and ovarian structures, eggs). We have investigated the complex of morphological parameters of fish (mean body mass, mean body length) and aging according to Jensen and Johnsen (2011).

Adult specimens of the sea trout were caught in the estuary of the Słupia river (Ustka city, 54°35′N 16°51′E), while the sexually mature spawners and kelts (males and females) were caught in the Słupia river (Słupsk city, Pomeranian Voivodeship, northern Poland; 54°27′57″N 17°1′45″E). The fish was collected using the electric fishing method, with the help of a power generator with a DC adapter, in close cooperation with the Landscape Park "Dolina Słupia" and the District Board of the Polish Angling Association in Słupsk (environmental license DROS.AR.MW.6052-16/10).

Percussion stunning followed by the destruction of the brain was used for fish euthanasia. The tissues were sampled for analysis. Tissue samples were taken, frozen, and homogenized in the laboratory of the Department of Zoology and Animal Physiology, Institute of Biology and Earth Sciences, Pomeranian University in Słupsk. The authors are grateful to the authority of Landscape Park "*Dolina Słupia*" and the District Board of the Polish Angling Association in Słupsk for providing logistic support for collecting the samples.

Słupia river is one of the best-known anglers rivers of Middle Pomerania. The Słupia river belongs to the coastal rivers. The whole of its basin lies in the area of the Pomeranian Voivodship (northern Poland). Słupia has the character of a mountain river, its sources are located near Sierakowska Huta, 178 m above sea level. The middle course of the Słupia river is located in the area of the Polanowska Upland, and the lower one is on the Damnica Upland, the Słupsk Plain, and the Słowiński Coast. The river ends its course in Ustka city, entering the Baltic Sea. The analysis

allowed us to present the quality of water in Słupia in the area of Słupsk city by the European standard EN ISO 8689-2 and the Water Framework Directive as the first class of purity (Obolewski, 2010).

The trout muscle tissue samples were homogenized in an ice-cold buffer (100 mM Tris-HCl, pH 7.2). The level of lactate was determined by reaction with hydroquinone, while pyruvate was assessed with dimethylamino benzaldehyde (Herasimov and Plaksina, 2000). The absorbance was measured at 420 nm and 430 nm, respectively. The calibration curve for lactate (0.1-5 mM) and pyruvate level (0.1-5 mM) was used, and results were expressed in nmol per mg of tissue protein. The results were expressed as mean ± S.D. All variables were tested for normal distribution using the Kolmogorov-Smirnov test ($p>0.05$). The homogeneity of variance was assessed using Levene's test. The significance of differences in the parameters' value, and between all studied groups was determined using one-way analysis of variance (ANOVA) and multifactorial analysis of variance (MANOVA). For unequal observations, Tukey's post-test was used (Zar, 1999). Statistical analysis was carried out in a double way: biomarkers of oxidative stress were compared with those in each developmental stage of the sea trout, while metabolism biomarkers were analyzed separately.

In our study, the increased lactate levels and a higher L/P ratio in the muscle tissue of parr and smolts compared to other developmental stages were observed. This increase in the lactate levels and L/P ratio is similar to an increase in the production of lactic acid via aerobic to anaerobic metabolic pathways. These changes may be related to the almost complete dissociation of lactic acid in aqueous solutions to lactate and protons accompanied by severe acidosis. The increasing stress-induced hypoxia caused by the changing from freshwater to saltwater during the parr and smolt stages can decrease ATP production and stimulation glycolysis. These mechanisms are triggered by phosphofructokinase mechanisms and NAD initiating the conversion of pyruvate to lactate. In our study, the lowest L/P ratio in the muscle tissue of adults (both males and females) was observed. This stage is characterized by enhancing the adaptive mechanisms of the oxygen-redistribution process in the sea habitat. In our study, an increase in the L/P ratio was observed in spawners. This increase reflects the switch of the muscle cytosolic redox potential from the aerobic pathway to anaerobic glycolysis. These results indicate that energy-related cellular components and metabolic enzymes (e.g., succinic dehydrogenase) may compensate for adaptive mechanisms without any serious damage in muscle tissue (Phypes and Pierce, 2006).

The current study revealed the impact of the sex and different development stages of the sea trout (parr, smolts, spawners, adults, and the kelt stage) on the effective formation of adaptive oxygen-dependent mechanisms. The increased lactate levels and a higher L/P ratio in the muscle tissue of parr and smolts compared to other developmental stages were observed. This increase reflects the switch of the muscle cytosolic redox potential from the aerobic pathway to anaerobic glycolysis. These results indicate that energy-related cellular components and metabolic enzymes (e.g., succinic dehydrogenase) may compensate for adaptive mechanisms without any serious damage of muscle tissue. The differences in the lactate and pyruvate levels are associated with the reorganization of the energy-related metabolic pathways (anaerobic and aerobic pathways) during spawning. This process is one of the ways to the adaptation of fish organisms.

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