BIOMARKERS OF LIPID PEROXIDATION IN VARIOUS TISSUES OF FURUNCULOSIS-AFFECTED SEA TROUT (*SALMO TRUTTA* M. *TRUTTA* L.) FROM BALTIC SEA BASIN

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Furunculosis induced by motile aeromonads is a problem in the farming of salmonids (brown and rainbow trout) and various other fish species in the Europe during last few years. Motile aeromonads cause diverse pathological conditions that include acute, chronic, and covert infections (Cipriano and Austin, 2011). Motile aeromonad septicaemias generally are mediated by stress. Elevated water temperature (Esch and Hazen, 1980), a decrease in dissolved oxygen concentration, or increases in ammonia and carbon dioxide concentrations have been shown to promote stress in fish and trigger motile aeromonad infections (Walters and Plumb, 1980).

Central Pomeranian rivers are a unique place where salmonids spawn (Bartel, 2000, 2001; Pelczarski, 2000; Radtke et al., 2007; Dębowski et al., 2008). In recent years, it has been noticed that in the Pomeranian rivers, the number of valuable fish species (sea trout, brown trout, grayling, etc.) with skin lesions of various intensities has increased, i.e. ulcerations and skin defects, turning into nodular swellings covered with mold (Pietrzak, 2009; Bartel et al., 2009). Literature data indicate that a weakened fish organism has a weak immune system, the amount of mucus decreases with the increase of bacteria growth, and the fish is quickly infected with fungi and bacteria, which in extreme cases can cover the entire body surface (Khoo, 2000; Řehulka, 2002; Kozińska, 2001, 2007; Kozińska and Pękala, 2007, 2012; Harikrishnan et al., 2009). Our previous studies have shown that furunculosis seems to be quite capable of causing oxidative stress in the liver, muscle, heart, and spawn of brown trout (Kurhalyuk et al. 2009-2015; Tkachenko et al., 2011, 2014-2020).

The current study aimed to examine the lipid peroxidation in the various tissue (muscles, gills, liver, heart, milt/spawn) from healthy specimens of sea trout (*Salmo trutta* m. *trutta* L.) and naturally furunculosis-affected trout sampled from the Słupia river belonging to Baltic sea basin where adult specimens are spawning (northern Poland, Central Pomeranian region). Biomarkers of lipid peroxidation [2-thiobarbituric acid reactive substances (TBARS)] in the various tissue of healthy and furunculosis-affected trout were assayed.

Adult sea trout (*Salmo trutta* m. *trutta* L.), 3-5 years of age, were collected from sites on the Słupia river (Słupsk, northern Poland). Fish-catching took place in exact co-operation with Landscape Park "The valley of Słupia" as well as the Board of Polish Angling Relationship in Słupsk. The sampling for analysis from healthy males and females, as well as females of sea trout affected by furunculosis, was collected directly after the catch. After catching, microbiological tests were carried out. These tests suggested that the *Aeromonas hydrophila* complex caused furunculosis. The pathogen was isolated from the infected sea trout. Specimens in each group were dissected. One fish was used for each preparation. Each sample was homogenized in cold Tris-HCl buffer (100 mM, pH 7.2) to obtain a 10% (w/v) tissue homogenate. The protein content of each sample was determined using the Bradford method (1976) and bovine serum albumin as the standard. Lipid peroxidation level was determined by quantifying the concentration of thiobarbituric acid reactive substances (TBARS) according to Kamyshnikov (2004). The mean ± S.E.M. values were calculated for each group to determine the significance of the inter-group differences. To assess the differences between the studied groups, the Kruskal-Wallis one-way analysis of variance by ranks test was used (significance level, $p < 0.05$) (Zar, 1999). All statistical calculation was performed on separate data from each individual with STATISTICA package, version 13.3 (StatSoft, Cracow, Poland).

Disturbance of the balance between the production of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hypochlorous acid, hydroxyl, alkoxyl, and peroxyl radicals and

antioxidant defenses against them produces oxidative stress, which amplifies tissue damage by releasing prooxidative forms of reactive iron that are able to drive Fenton chemistry and lipid peroxidation and by eroding away protective sacrificial antioxidants (Gutteridge, 1995). Results of our study revealed that TBARS levels were significantly higher in the muscle tissues (by 8.9%, $p = 0.001$), gills (by 37.7%, $p = 0.010$), and liver (by 139.2%, $p = 0.000$) of males affected by furunculosis compared to the healthy specimens. Decreased TBARS level in milt to (282.2 ± 41.37) nmol/mg protein in males affected by furunculosis against (756.31 ± 85.67) nmol/mg protein in healthy trout was found. Decreased TBARS levels in the gills (by 45.5%, $p = 0.005$) and increased TBARS levels in spawn (by 179%, $p = 0.031$) of infected females compared to healthy females were noted.

Lesions in fish are associated with a variety of organisms including parasites and bacterial, viral, and fungal infectious agents. In addition, trauma, suboptimal water quality, and other abiotic stress factors may result in the loss of homeostasis (Kane et al., 2000). In recent years, skin ulcer epidemics have been either experimentally or epidemiologically linked to exposure to a number of xenobiotic chemicals as well as to biotoxins. Some of these agents have led to serious concerns about the health of aquatic ecosystems (Noga, 2000).

Systemic infections are characterized by diffuse necrosis in several internal organs and the presence of melanin-containing macrophages in the blood (Ventura and Grizzle, 1988). Internally, the liver and kidneys are target organs. The liver may become pale, or have a greenish colouration, while the kidney may become swollen and friable. These organs are apparently attacked by bacterial toxins and lose structural integrity (Huizinga et al., 1979). Many fish infections result in the activation of lipid peroxidation. For example, The effect of *Clinostomum detruncatum* metacercaria infection on the activities of the antioxidant enzymes (superoxide dismutase and catalase) in the muscle of the freshwater fish *Rhamdia quelen* was analyzed by Belló and co-workers (2000). Enzyme activities were similar in infected and uninfected fishes. However, the chemiluminescence was almost 2-fold higher in the muscle of infected fishes than in the muscle of uninfected ones. These results indicate that parasite infection induces oxidative stress and a higher level of membrane damage in the fish muscle due to an imbalance between pro-oxidants and non-enzymatic antioxidants (Belló et al., 2000). Also, oxidative stress biomarkers in the African sharptooth catfish (*Clarias gariepinus*) associated with infections by adult digeneans and water quality was evaluated by Dumbo and co-workers (2020). Overall the trends observed in the data of these researchers showed that the parasites have a negligible effect on oxidative stress in host fish and the trends observed for all variables (water quality, stress biomarkers, body condition, and parasite infections) showed a strong seasonal pattern. The status of oxidative stress in the gill tissues of goldfishes (*Carassius auratus*) parasitized by *Dactylogyrus* spp. was evaluated by Mozhdeganloo and Heidarpour (2014). The results of the present study revealed that parasitized goldfishes showed more severe oxidative stress and lipid peroxidation than non-parasitized fishes and enhanced lipid peroxidation may be linked to gill damage in goldfishes parasitized by *Dactylogyrus* spp. (Mozhdeganloo and Heidarpour, 2014).

In conclusion, TBARS levels were significantly higher in the muscle tissues, gills, and liver of males affected by furunculosis. Decreased TBARS level in milt in males with furunculosis compared to the healthy trout was found. Decreased lipid peroxidation in the gills and increased lipid peroxidation in the spawn of infected females compared to healthy females were noted. Thus, biomarkers of lipid peroxidation cannot be specific biomarkers in the various tissues of furunculosis-affected trouts.

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