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RESPONSES OF ANTIOXIDANT STATUS IN THE GILLS OF BROWN TROUT (*SALMO TRUTTA* M. *TRUTTA* L.) WITH ULCERATIVE DERMAL NECROSIS

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Abstract

Antioxidant defence system (activities of superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase), and free radical modification of lipids were determined in the gills from male and female brown trout (*Salmo trutta* m. *trutta* L.) affected by ulcerative dermal necrosis (UDN). In both males and females, lipid oxidation in the gills from UDN-affected trout showed higher values as compared to the respective control. UDN induced an increase of thiobarbituric acid reactive substances (TBARS) levels both in the gills of males and females. UDN caused a decrease in gill antioxidant enzyme activities. This might be due to inactivation of the above-mentioned enzymes by the end products of lipid peroxidation. The importance of the glutathione-mediated antioxidant defence system in protection against UDN-induced oxidative stress was demonstrated.

Key words: ulcerative dermal necrosis, brown trout, *Salmo trutta trutta*, gills, oxidative stress, lipid peroxidation, antioxidant enzymes

INTRODUCTION

Ulcerative dermal necrosis (UDN) is a problem in farming of salmonids (brown and rainbow trout) and various other fish species in the Europe and USA during last years. Skin ulcers on fish are one of the most well-recognized indicators of polluted aquatic environments (Noga 2000). A lot of work was performed regarding UDN in fish (Kane et al. 2000, Law 2001, Bruno et al. 2007, Harikrishnan et al. 2010).
Aetiology of the outbreaks of UDN remains unknown. There has been no conclusive evidence of the involvement of any particular organism as the primary pathogen (Roberts 1993). It is suggested that the fungal infections are triggered by metabolites

of the necrotic epidermal cells (Khoo 2000). Skin samples were tested for standard virus isolation with negative results (Roberts 1993).
Skin ulcers can have many different aetiologies, including infectious agents, toxins, physical causes, immunologic causes, and nutritional and metabolic perturbations (Law 2001). 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).
Law (2001) demonstrated the possible pathways of disease involved in ulcerative lesions of fish. These ulcerative lesions are likely to be initiated by a series of factors that lead ultimately to a breach of the normal barrier function of the skin.
Kane et al. (2000) have observed solitary ulcerative lesions on menhaden sampled from the rivers of North Carolina and Florida. The lesions demonstrated a marked chronic inflammatory infiltrate and granulomas in response to fungal hyphae throughout large areas of exposed necrotic muscle. Gram-negative rod-shaped bacteria were also observed in the lesions, a common finding in ulcers of aquatic organisms. Similar observations in menhaden and other species have been described previously in the literature as ulcerative mycosis, mycotic granulomatosis, red spot disease, and epizootic ulcerative syndrome. Despite the many different known causes of fish lesions, the scientific literature have recently emphasized *Pfiesteria piscicida* and other Pfiesteria-like dinoflagellates (and their bioactive compounds) as the primary causative agent for finfish lesions, particularly mycotic granulomatous ulcers. However, a number of other risk factors besides *Pfiesteria* have been shown to damage epithelium and may also play important roles in skin ulcer pathogenesis (Noga 2000).
Not only skin damage occur via direct contact with toxins, but it may also be induced indirectly from physiological changes that result from exposure not only to toxins but also to other environmental stressors, such as pH and temperature extremes. The multifactorial pathways that operate at both the ecological and the organismal levels as well as the nonspecific response of the skin to insults make it very challenging to link epidemic skin ulcers to any single cause in natural aquatic populations (Noga 2000).
The potential of reactive oxygen species (ROS) to damage tissues and cellular components, called oxidative stress, in biological systems has become a topic of significant interest for environmental toxicology studies (Valavanidis et al. 2006). Oxidative stress results when production of ROS exceeds the capacity of cellular antioxidant defenses to remove these toxic species. Many environmental stressed factors engage signaling pathways that are activated in response to oxidative stress. The same sequences of events are also associated with the a etiology and early pathology of many diseases (Limón-Pacheco and Gonsebatt 2009).
Tissues such as skin and muscle have a limited morphological response to injury. The two most important phenomena that determine the outcome of cell injury appear to be critical cell membrane damage, with associated fluid and ionic imbalances, and

inability of mitochondria, the powerhouse of the cell, to restart ATP synthesis (Law 2001). The balance between prooxidant factors and antioxidant defenses in biological systems can be used to assess toxic effects under UDN.
The depletion of antioxidant defence system and the changes in the activities of various antioxidant enzymes indicative of lipid peroxidation have been implicated in oxidative tissue damage (Limón-Pacheco and Gonsebatt 2009). Our previous studies have shown that UDN seems to be quite capable of causing oxidative stress in liver, muscle, heart, and spawn of brown trout (Kurhalyuk et al. 2009, 2010).
Objective of the present study was to examine the responses of biomarkers of oxidative stress in the gills of male and female brown trout from control (healthy specimens) and UDN-affected trout from the Słupia River (Northern Poland, Central Pomeranian region) during 2007-2009. Activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPX) were measured.

MATERIALS AND METHODS

Fish

Adult brown trout (*Salmo trutta* m. *trutta* L.), 3-5 years of age, were collected from site on the River Słupia, Słupsk, Northern Poland during spawning. Fish-catching took place in exact co-operation from Landscape Park "The valley of Słupia" as well as the Board of Polish Angling Relationship in Słupsk. Sea trout were sampled from November to December, during 2007-2009.

Sampling

The sampling for analysis from 61 males and 70 females (control group, Fig. 1A) as well as 81 males and 65 females of brown trout affected by UDN (study group, Fig. 1B) was collected directly after catch. The trout were caught from the Słupia River. The animals were quickly captured and killed after being anaesthetized. After catching, microbiological tests were carried out. These tests suggested that *Aeromo-*

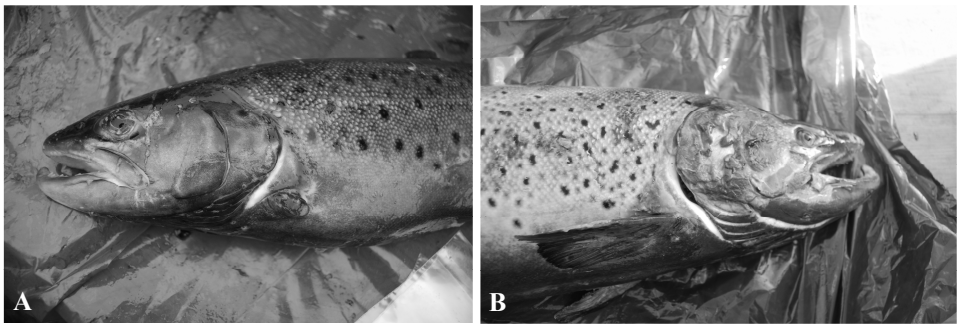

Fig. 1. Specimens of healthy trout (A) and UDN-affected trout (B)

nas hydrophila complex caused ulcerative dermal necrosis (Diagnostyka... 2005). Individuals from both groups were transported to the Department of Animal Physiology**,** Institute of Biology and Environment Protection, Pomeranian University (Słupsk, Poland) in cages with native water and analyzed within a day after the sampling procedure.

Treatment of samples

Specimens in each group were dissected. One fish was used for each preparation. Tissues were homogenized in ice-cold 0.1 M tris-HCl buffer (pH 7.4). The protein content of the samples was determined according to Bradford (1976) using bovine serum albumin as a standard.

Chemicals

Thiobarbituric acid (TBA), oxidized and reduced glutathione (GSSG and GSH), $NADPH_2$, 5.5-dithiobis-2-nitrobensoic acid were purchased from Sigma. Ethylenediaminetetraacetic acid (EDTA), thrichloroacetic acid (TCA), quercetin, hydrogen peroxide, ammonium molibdate, sodium aside, t-butylhydroperoxide, Tween 80 were obtained from Fluka. All other chemicals were of analytical grade.

Analytical methods

All enzymatic assays were carried out at 25±0.5°C using a spectrophotometer Specol 10 (Carl Zeiss Jena, Germany). The enzymatic reactions were started by the addition of the homogenate suspension. Each enzymatic assay was repeated three times for one sample. The analytical methods have high precision and accuracy. Low limit of detection (LOD) and limit of quantitation (LOQ) indicates good sensitivity of proposed methods. The specific assay conditions were as follows.

Lipid peroxidation levels were determined by quantifying the concentration of TBARS, expressed as μmol of malondialdehyde (MDA) per mg of protein, according to Kamyshnikov (2004). The MDA level was calculated by using $1.56 \cdot 10^5$ mM^{-1} cm^{-1} as molar extinction coefficient.

Superoxide dismutase (SOD, E.C. 1.15.1.1). SOD activity was measured using quercetin as the substrate after suitable dilution following the method of Kostiuk et al. (1990). The assay mixture in a total volume of 1 mL consisted of 0.08 mM EDTA and 0.1 M sodium phosphate buffer (pH 7.8) at a 1:1 proportion. Briefly, 0.1 mL of tissue homogenate after dilution was added to 2.3 mL of distilled water, after which 1 mL of assay mixture with EDTA and sodium phosphate buffer was mixed. One activity unit was defined as the amount of enzyme necessary to produce a 50% inhibition of the quercetin (1.4 μM) reduction rates measured at 406 nm in 0 and 20^{th} min. Activity is expressed in units of $SOD \cdot mg^{-1}$ protein.

Catalase (CAT, E.C. 1.11.1.6). CAT activity was determined by measuring the decrease of H_2O_2 in the reaction mixture using a spectrophotometer at the wavelength

of 410 nm by the method of Koroliuk et al. (1988). The reaction was started by addition of 0.1 mL of tissue homogenate to 2 mL of 0.03% H_2O_2 solution and 1 mL of 4% ammonium molibdate. One unit of catalase activity is defined as the amount of enzyme required to clear μmol of $H_2O_2 \cdot min^{-1} \cdot mg^{-1}$ protein.

Glutathione reductase (GR, E.C. 1.6.4.2). GR activity in the tissue homogenate was measured according to the method described by Glatzle et al. (1974). The enzyme assay mixture contained 2.4 mL of 67 mM sodium phosphate buffer (pH 6.6), 0.2 mL of 7.5 mM oxidized glutathione, 0.1 mL of tissue homogenate, and 0.2 mL of 6 mM NADPH. The rate of NADPH oxidation was followed spectrophotometrically at 340 nm. The specific GR activity is expressed as nmol NADPH$\cdot min^{-1} \cdot mg^{-1}$ protein.

Glutathione peroxidase (GPX, E.C. 1.11.1.9). The activity of GPX in the tissue homogenate was measured spectrophotometrically as described by Moin (1986). The assay mixture contained 0.8 mL of 0.1 M Tris-HCl with 6 mM EDTA and 12 mM sodium aside (pH 8.9), 0.1 mL of 4.8 mM GSH, 0.2 mL of tissue homogenate, 1 mL of 20 mM t-butylhydroperoxide, and 0.1 mL of 0.01 M 5.5-dithiobis-2-nitrobenzoic acid. The rate of GSH reduction was followed spectrophotometrically at 412 nm. GPX activity is expressed as μmol GSH$\cdot min^{-1} \cdot mg^{-1}$ protein.

Statistical analysis. Results are expressed as mean ± SEM. Significance of differences in enzymes activity in the gills of brown trout (significance level, $p<0.05$) was examined using one-way ANOVA (significance level, $p<0.05$), analysis of variance (test F), Levene's and Tukey's HSD test (test of reasonably important difference for bumpy numerical force of attempt). Correlations between TBARS level and enzymes activities in the gills at the set significance level were determined by the regression method (Zar 1999). Interactions were established by the Pearson test for linear correlation. All statistical calculation was performed on separate data from each individual with STATISTICA version 8.0.

RESULTS

The values of lipid peroxidation for the males and females from control (healthy specimens) and UDN-affected trout are summarized in Figure 2. TBARS levels in

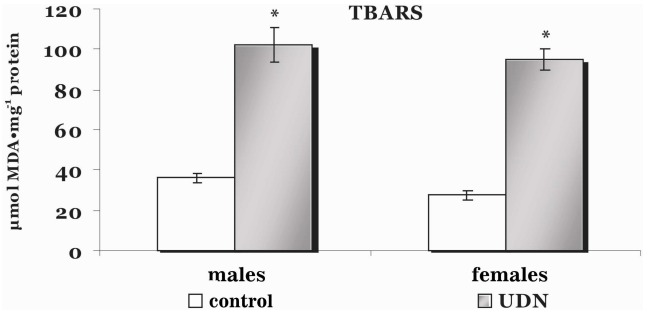

Fig. 2. TBARS level (μmol MDA$\cdot mg^{-1}$ protein) in the gills of males and females from control (healthy specimens) and UDN-affected trout
Each value represents the mean ± SEM
* the significant change was shown as $p<0.05$ when compared with control group values

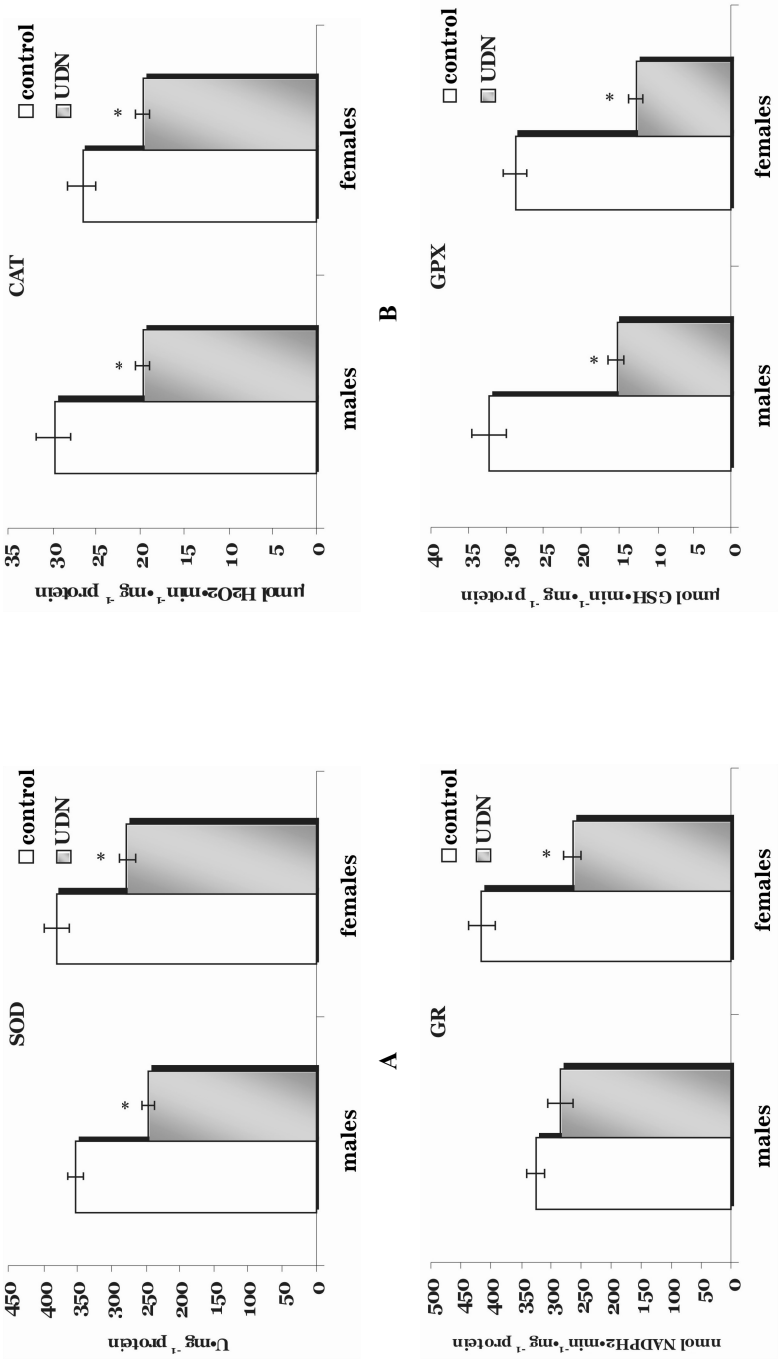

Fig. 3. Superoxide dismutase (A), catalase (B), glutathione reductase (C) and glutathione peroxidase (D) activities in the gills from males and females of brown trout affected by ulcerative dermal necrosis (UDN)
Data are means ± SEM
* The significant change was shown as $p<0.05$ when compared with control group values

the gills (F=47.48, p=0.000) from males infected by UDN was significantly higher by 187.6% (p=0.000). UDN induced an increase of TBARS levels in the gills of females by 247.7% (p=0.000), although values of TBARS levels in females were lower than those of healthy fish.

Activities of the antioxidant enzymes are shown in Fig. 3. The significant decrease in the gill SOD activity (F=21.76, p=0.000) were found as a consequence of UDN infection either in males (by 30.4%, p=0.000), or females (by 27.2%, p=0.000), (Fig. 3A). CAT activity (F=14.09, p=0.000) was decreased by 34% (p=0.000) in the gills of UDN-affected males and by 25.9% (p=0.002) from females as compared to controls (Fig. 3B). UDN infection significantly affected GR activity (F=13.16, p=0.000), which was inhibited by 36.4% (p=0.000) in the gills of females, but no differences in GR activity between healthy and UDN-affected males were found (Fig. 3C). Regarding the GPX (F=39.85, p=0.000), its activity was significantly decreased by 52.4% (p=0.000) in the gills of UDN-affected males as compared to the controls, and by 55.7% (p=0.000) as compared to the UDN-affected females (Fig. 3D).

Several correlations between checked parameters were found (Table 1, Fig. 4, 5). Gill TBARS level from healthy males correlated with GR activity (r=0.510, p=0.000), GPX activity (r=0.441, p=0.000), and correlated inversely with CAT activity (r=-0.523, p=0.000). The relationships between gill GR and SOD activities from UDN-affected males was positively (r=0.502, p=0.000), and between gill GR and CAT activities was inverse(r=-0.253, p=0.022), (Fig. 5).

Gill SOD activity from healthy females correlated inversely with TBARS level (r=-0.372, p=0.002), (Fig. 5). Thus, CAT activity was connected with SOD activity

Table 1

Correlative analysis of antioxidative parameters in the gills from males and females of brown trout affected by UDN

Relation	Correlative coefficient, r	Regressive curve	Significant difference level, p
males			
SOD–CAT, control	0.505	y=0.077x+2.79	0.000
SOD–GR, control	-0.290	y=-0.333x+443	0.024
CAT–TBARS, control	-0.523	y=-0.629x+54.22	0.000
CAT–GR, control	-0.278	y=-2.102x+388.19	0.030
CAT–GPX, control	-0.312	y=-0.366x+43.26	0.014
GR–GPX, control	0.308	y=0.048x+16.75	0.016
females			
GR–CAT, control	-0.272	y=-0.22x+35.66	0.023
SOD–CAT, UDN	0.287	y=0.17x+14.96	0.020

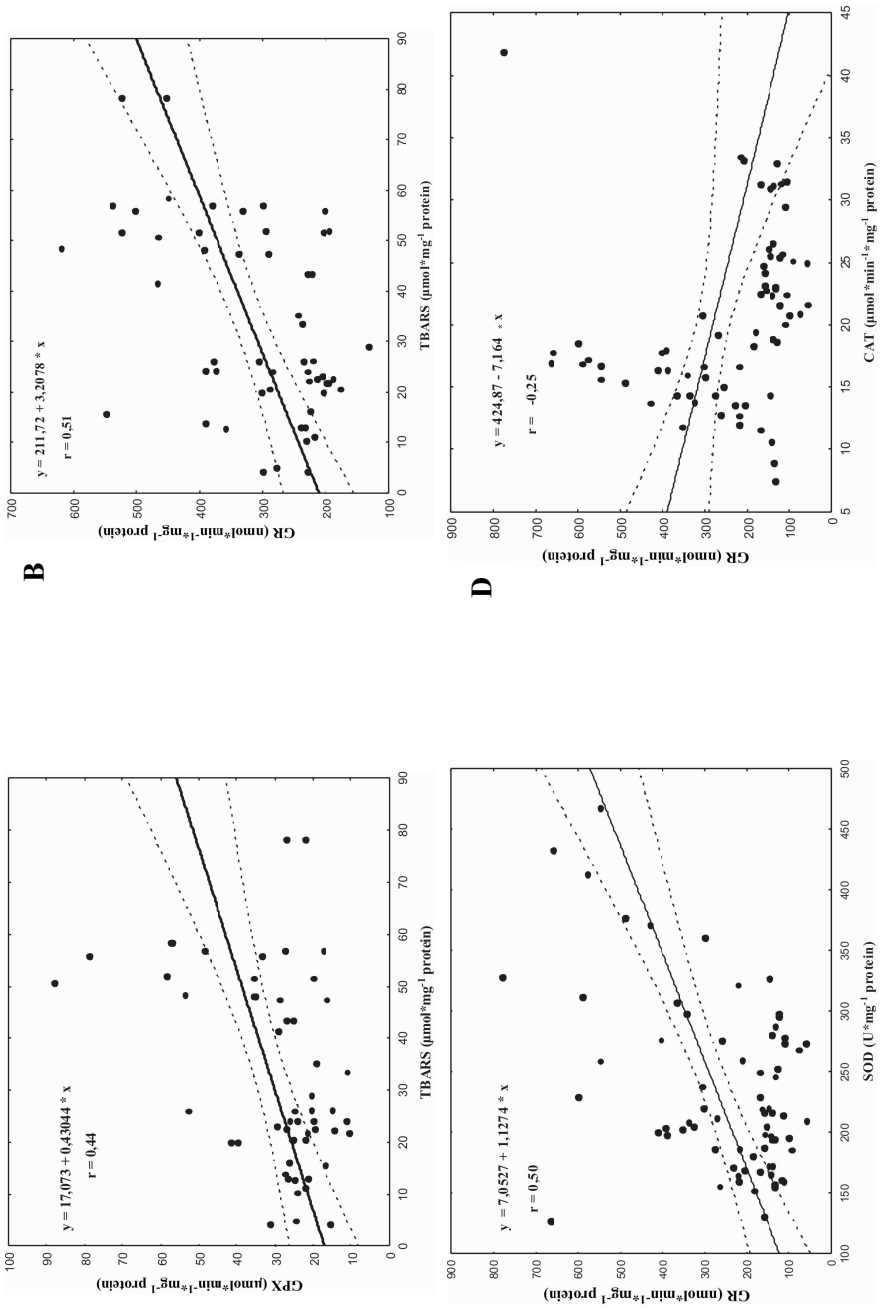

Fig. 4. Correlations between TBARS level and GR activity (A), between TBARS level and GPX activity (B) in the gills from healthy males, and between GR and SOD activity (C) GR and CAT activity (D) in the gills from males affected by UDN

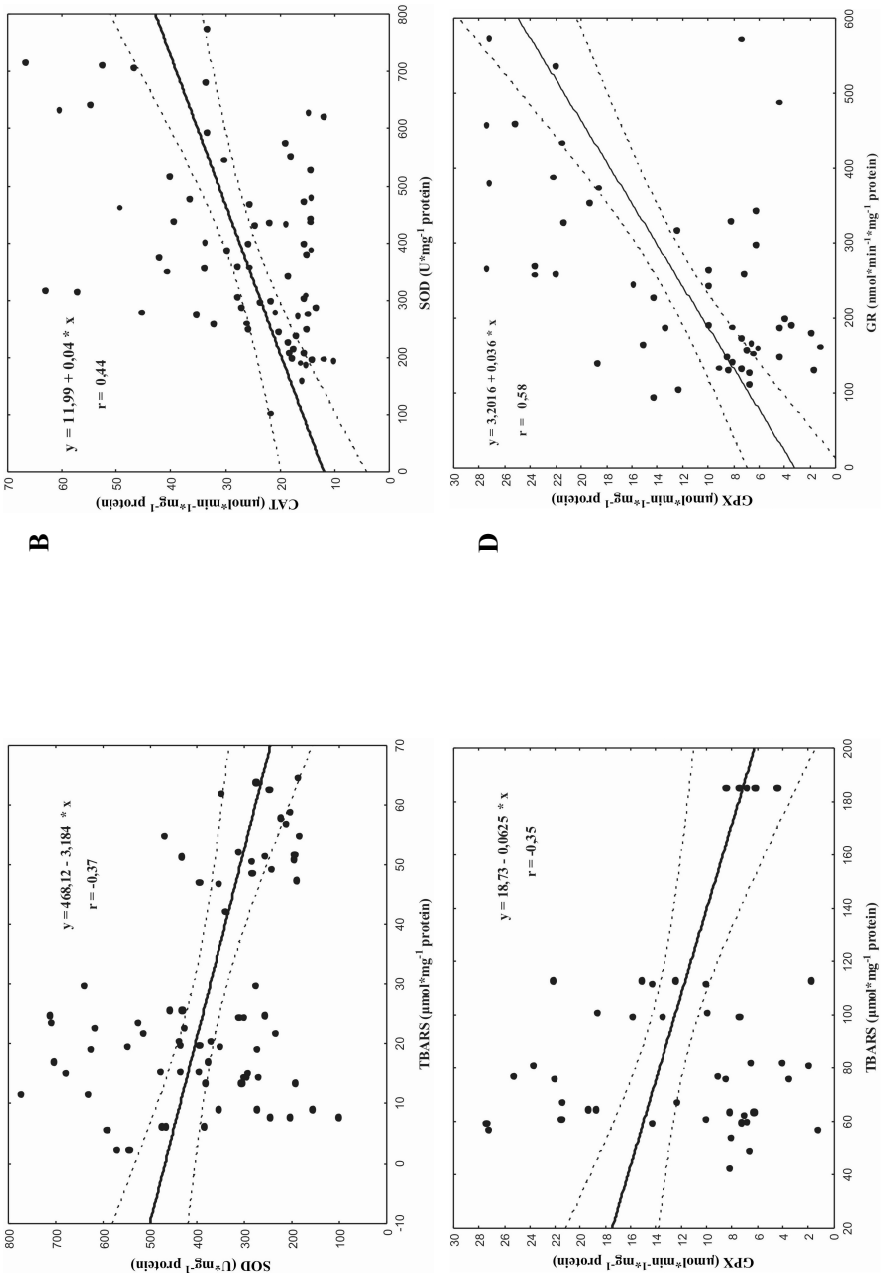

Fig. 5. Correlations between TBARS level and SOD activity (A), between SOD and CAT activity (B) in the gills from healthy females, and between GPX activity and TBARS level (C) between GPX activity and GR activity (D) from females affected by UDN

in the gills of healthy females (r=0.440, p=0.002) and inversely connected with GR activity (r=-0.272, p=0.023), (Fig. 5, Table 1). The relationship between GPX activity and TBARS level in the gills of UDN-affected females was inverse (r=-0.346, p=0.005) and GR activity was positively (r=0.575, p=0.000), (Fig. 5).

DISCUSSION

This work focused on prooxidative changes and antioxidant enzymes activities in healthy and UDN-affected populations of brown trout. To evaluate the effects of UDN infection on the antioxidant defence system and ROS generation in the trout gills, we examined the TBARS level, an end metabolite derived from lipid peroxidation and the activity of some antioxidative enzymes (SOD, CAT, GR, GPX). All these parameters have been described as valuable biomarkers of prooxidant situations in fish (Stephensen et al. 2002, Pandey et al. 2003, Pascual et al. 2003).

Toxic consequences of oxidative stress at the subcellular level include lipid peroxidation and oxidative damage to DNA and proteins. These effects are often used as end points in the study of oxidative stress (Kelly et al. 1998). Based on TBARS levels, our results showed that UDN led to oxidative stress both in males and females, with infected fish showing a 2.9-fold (males) and 3.5-fold (females) increase in the gill TBARS level with respect to control fish. Available information concerning the influence of UDN infection on the antioxidant defence system in fish is scarce.

Species differences in the ability to cope with oxidative stress can provide insight into the mechanisms behind both the mode of toxicity of a specific factor as well as the different ways in which an organism may deal with such stressors (Rau et al. 2004).

During the last four decades evidence has accumulated which strongly supports the concept of a coordinate regulation of cellular pro- and antioxidant mechanisms. The antioxidant defence system is being increasingly studied because of its potential utility it could be used in environmental monitoring systems (Winston 1991, Meyer et al. 2003, Valavanidis et al. 2006, Limón-Pacheco and Gonsebatt 2009). Therefore, study of the protective role of antioxidant compounds on inhibition of the oxidative stress response and correcting the fundamental oxidant/antioxidant imbalance are important vistas for further research.

Impairment in antioxidant enzymes produces an imbalance between pro- and antioxidant systems causing the formation of toxic hydroxyl radicals, with direct consequences on cell integrity and cell function itself (Dorval and Hontela 2003). Our results indicate that UDN leads to enhanced oxidation and oxidative stress in the gills from males and females of brown trout and inhibition of some antioxidant defence mechanisms. Changes in the antioxidant defence system are used as biomarkers of a variety of prooxidant situations in fish, including different diseases, which can negatively affect growth and resistance (Lushchak et al. 2005, Bagnyukova et al. 2006).

Fish respond to disease by altering or adapting their metabolic functions. Alterations found in the activity of antioxidant defence system suggest that changes observed could been adaptive response to ROS. The activity of antioxidant defence system may be increased or inhibited under oxidative stress depending on the intensity and

the duration of the stress applied as well as the susceptibility of the exposed species (Ballesteros et al. 2009). In the present work, the studied enzymes responded in a different way in trout affected by UDN.

Superoxide dismutase is the first enzyme to respond against oxygen radicals. The function of this enzyme is to catalytically convert superoxide radical to oxygen and hydrogen peroxide (Abreu and Cabelli 2010). Excessive hydrogen peroxide is harmful for almost all cell components, so its rapid and efficient removal is of essential importance for organisms. Conversely, hydrogen peroxide acts as a second messenger in signal-transduction pathways. H_2O_2 is degraded by peroxidases and catalases, the latter being able both to reduce H_2O_2 to water and to oxidize it to molecular oxygen (Zamocky et al. 2008). In our case, the activity of SOD and CAT was significantly decreased in the gills from UDN-affected trout. The drop in CAT activity could be explained by the flux of superoxide radicals due to the oxidative stress caused by UDN infection.

The redox system of GSH consists of primary and secondary antioxidants, including glutathione peroxidase, glutathione reductase, glutathione S-transferase, and glucose 6-phosphate dehydrogenase. Alterations in the activities of these enzymes may reflect reduced cellular defence and may serve as markers of many diseases (Rahman et al. 1999). GPX catalyses the reduction of H_2O_2 and lipid hydroperoxides at expense of GSH (Hayes and McLellan 1999).

We observed the inhibition of GPX activity in the gills from UDN-affected trout. The decreased GR and GPX activity indicates it reduced capacity to scavenge H_2O_2 and lipid hydroperoxides produced in this tissue. This test result is in agreement with our previous study in which we reported inhibition of glutathione defence system in liver, muscle, heart, and spawn of UDN-affected trout (Kurhalyuk et al. 2009, 2010). In the present study, the activity of GPX was inhibited in the gills from UDN-affected trout. Gills are the first organ exposed to toxic compounds (Ballesteros et al. 2009) and, therefore, have higher TBARS levels than other organs tested (Kurhalyuk et al. 2009, 2010). Other enzymes showed differential response under UDN infection.

Glutathione defence system activity can decrease by negative feed back either from excess of substrate or damage induced by oxidative modification under UDN infection. A reduced activity of glutathione defence system in tissue of UDN-positive fish could indicate that its antioxidant capacity was exceeded by the amount of hydroperoxide products and might reflect a possible failure of the antioxidant system in the gills of UDN-affected fish.

Given that TBARS level is considered a valuable indicator of oxidative damage of cellular components, our results suggest that UDN enhanced ROS generation in the gills of males and females and that antioxidant defence system were not totally able to effectively scavenge them, thus leading to lipid peroxidation. In this sense, inhibition of antioxidant defence system could be somehow indicative of a failure in antioxidant defences. Under a situation that enhances UDN-generated oxidative stress, it may be expected that an increase in both ROS generation and ROS-scavenging mechanisms occurs.

In conclusion, this study suggests that UDN causes changes in oxidative stress intensity in the gills of affected trout. Moreover, the increase of lipid peroxidation

modifies antioxidant defence system and causes inhibition of SOD, CAT, GPX, GR activities under UDN-induced oxidative stress.

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REFERENCES

Abreu I.A., Cabelli D.E., 2010. Superoxide dismutases – a review of the metal-associated mechanistic variations. *Biochim. Biophys. Acta*, 1804 (2), 263-274.

Bagnyukova T.V., Chahrak O.I., Lushchak V.I., 2006. Coordinated response of goldfish antioxidant defences to environmental stress. *Aquatic Toxic.*, 78 (4), 325-331.

Ballesteros M.L., Wunderlin D.A., Bistoni M.A., 2009. Oxidative stress responses in different organs of *Jenynsia multidentata* exposed to endosulfan. *Ecotoxicol. Environ. Saf.*, 72 (1), 199-205.

Bradford M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72, 248-254.

Bruno D., Crumlish M., LaPatra S., Noguera P., Verner-Jeffreys D., 2007. Workshop on salmonid skin diseases. European Association of Fish Pathologists. 13th International Conference on Fish and Shellfish Diseases, Grado, Italy 18th September, 2007, pp. 7-10.

Diagnostyka bakteriologiczna. (Bacteriological diagnostics), 2005. (Ed.) E. Szewczyk, PWN, Warszawa, (in Polish).

Dorval J., Hontela A., 2003. Role of glutathione redox cycle and catalase in defence against oxidative stress induced by endosulfan in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*). *Toxicol. Appl. Pharmacol.*, 192 (2), 191-200.

Glatzle D., Vuilleumier J.P., Weber F., Decker K., 1974. Glutathione reductase test with whole blood, a convenient procedure for the assessment of the riboflavin status in human. *Experientia*, 30 (6), 665-667.

Harikrishnan R., Balasundaram Ch., Moon Y.-G., Kim M.-Ch., Kim J.-S., Dharaneedharan S., Heo M-S., 2010. Phytotherapy of ulcerative dermatitis induced by *Aeromonas hydrophila* infection in goldfish (*Carassius auratus*). *Acta Vet. Hung.*, 58 (1), 29-37.

Hayes J.D., McLellan L.I., 1999. Glutathione and glutathione-dependent enzymes represent a coordinately regulated defence against oxidative stress. *Free Rad. Res.*, 31 (4), 273-300.

Kamyshnikov V.S., 2004. Spravochnik po kliniko-biokhimicheskim issledovaniyam i laboratornoy diagnostike. (Reference book on clinic and biochemical researches and laboratory diagnostics). MEDpress-uniform, Moscow, (in Russian).

Kane A.S., Dykstra M.J., Noga E.J., Reimschuessel R., Baya A., Driscoll C., Paerl H.W., Landsberg J., 2000. Etiologies, observations and reporting of estuarine finfish lesions. *Mar. Environ. Res.*, 50 (1-5), 473-477.

Kelly K.A., Havrilla C.M., Brady T.C., Abramo K.H., Levin E.D., 1998. Oxidative stress in toxicology: established mammalian and emerging piscine model systems. *Environ. Health Perspect.*, 106 (7), 375-384.

Khoo L., 2000. Fungal diseases in fish. *Sem. Avian Exotic Pet. Med.*, 9, 102-111.

Koroliuk M.A., Ivanova L.I., Maiorova I.G., Tokarev V.E., 1988. Metod opredeleniya aktivnosti katalazy. (A method of determining catalase activity). *Lab. Delo*, (1), 16-19, (in Russian).

Kostiuk V.A., Potapovich A.I., Kovaleva Zh.V., 1990. Prostoy i chuvstvitelny metod opredeleniya aktivnosti superoksiddismutazy, osnovannyy na reakcii okisleniya kvercetina. (A simple and sensitive method of determination of superoxide dismutase activity based on the reaction of quercetin oxidation). *Vopr. Med. Khim.*, 36 (2), 88-91, (in Russian).

Kurhalyuk N., Tkachenko H., Pałczyńska K., 2009. Antioxidant enzymes profile in the brown trout *(Salmo trutta trutta*) with ulcerative dermal necrosis. *Bull. Vet. Inst. Pulawy*, 53, 813-818.

Kurhalyuk N., Tkachenko H., Pałczyńska K., 2010. Lipid peroxidation and antioxidant defence system in spawn of brown trout (*Salmo trutta* m. *trutta* L.) affected by ulcerative dermal necrosis. *Arch. Pol. Fish.*, 18, 115-122.

Law M., 2001. Differential diagnosis of ulcerative lesions in fish. *Environ. Health Perspect*., 109, Suppl. 5, 681-686.

Limón-Pacheco J., Gonsebatt M.E., 2009. The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress. *Mutat. Res.*, 674 (1-2), 137-147.

Lushchak V.I., Bagnyukova T.V., Lushchak O.V., Storey J.M., Storey K.B., 2005. Hypoxia and recovery perturb free radical processes and antioxidant potential in common carp (*Cyprinus carpio*) tissues. *Int. J. Biochem. Cell Biol.*, 37 (6), 1319-1330.

Meyer J.N., Smith J.D., Winston G.W., Di Giulio R.T., 2003. Antioxidant defences in killifish (*Fundulus heteroclitus*) exposed to contaminated sediments and model prooxidants: short-term and heritable responses. *Aquat. Toxicol.*, 65 (4), 377-395.

Moin V.M., 1986. Prostoy i spetsyficheskiy metod issledovaniya aktivnosti glutationperoksidazy v eritrotsytah. (A simple and specific method for determining glutathione peroxidase activity in erythrocytes). *Lab. Delo*, (12), 724-727, (in Russian).

Noga E.J., 2000. Skin ulcers in fish: Pfiesteria and other etiologies. *Toxicol Pathol.*, 28 (6), 807-823.

Pandey S., Parvez S., Sayeed I., Haque R., Bin-Hafeez B., Raisuddin S., 2003. Biomarkers of oxidative stress: a comparative study of river Yamuna fish *Wallago attu* (Bl. & Schn.). *Sci. Total Environ.*, 309 (1-3), 105-115.

Pascual P., Pedrajas J.R., Toribio F., López-Barea J., Peinado J., 2003. Effect of food deprivation on oxidative stress biomarkers in fish (*Sparus aurata*). *Chem. Biol. Interact.*, 145 (2), 191-199.

Rahman Q., Abidi P., Afaq F., Schiffmann D., Mossman B.T., Kamp D.W., Athar M., 1999. Glutathione redox system in oxidative lung injury. *Crit. Rev. Toxicol.*, 29 (6), 543-568.

Rau M.A., Whitaker J., Freedman J.H., Di Giulio R.T., 2004. Differential susceptibility of fish and rat liver cells to oxidative stress and cytotoxicity upon exposure to prooxidants. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, 137 (4), 335-342.

Roberts R.J., 1993. Ulcerative dermal necrosis (UDN) in wild salmonids. *Fish Res.*, 17, 3-14.

Stephensen E., Sturve J., Förlin L., 2002. Effects of redox cycling compounds on glutathione content and activity of glutathione-related enzymes in rainbow trout liver. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, 133 (3), 435-442.

Valavanidis A., Vlahogianni T., Dassenakis M., Scoullos M., 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicol. Environ. Saf.*, 64 (2), 178-189.

Winston G.W., 1991. Oxidant and antioxidants in aquatic animals. *Comp. Biochem. Physiol. C,* 100 (1-2), 173-176.

Zamocky M., Furtmüller P.G., Obinger C., 2008. Evolution of catalases from bacteria to humans. *Antioxid. Redox Signal.*, 10 (9), 1527-1548.

Zar J.H., 1999. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

STAN OBRONY ANTYOKSYDACYJNEJ W SKRZELACH TROCI WĘDROWNEJ (*SALMO TRUTTA* M. *TRUTTA* L.) Z WRZODZIEJĄCĄ MARTWICĄ SKÓRY

Streszczenie

W pracy przedstawiono wyniki badań dotyczących występowania stresu oksydacyjnego w tkance skrzeli samców i samic troci wędrownej (*Salmo trutta* m. *trutta* L.) z wrzodziejącą martwicą skóry. Pod względem badawczym skrzela stanowią wartościową tkankę, która w największym stopniu narażona jest na oddziaływanie niekorzystnych czynników środowiska. Uzyskane wyniki badań wykazały zróżnicowanie poziomu intensywności procesów lipoperoksydacji (ocenianych przez poziom TBARS produktów) oraz aktywności enzymów antyoksydacyjnych (dysmutazy ponadtlenkowej, katalazy, reduktazy i peroksydazy glutationowej) w okresie tarła u osobników troci wędrownej zdrowych i chorych z wrzodziejącą martwicą skóry. Pobór materiału badawczego z rzeki Słupi (Północna Polska) odbył się we ścisłej współpracy z Dyrekcją Parku Krajobrazowego „Dolina Słupi" oraz Zarządem Okręgu Polskiego Związku Wędkarskiego w Słupsku w latach 2007-2009.

Wyniki naszych badań sugerują istotne zwiększenie poziomu procesów peroksydacji lipidów w tkance skrzeli troci – dla samców trzykrotne, dla samic – o 3,5 raza. Wspomniane schorzenie wywołuje stres oksydacyjny w skrzelach ryb z inhibicją mechanizmów antyoksydacyjnej obrony. Obniżenie aktywności podstawowych enzymów antyoksydacyjnych z jednoczesną intensyfikacją procesów peroksydacji lipidów obniża wydajność procesów tarłowych tego gatunku ryb.