УДК 57.044:577.3:639.3:612.062

Effect of chloramine-T disinfection on oxidative stress biomarkers in the gill tissue of grayling (*Thymallus thymallus*)

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Chloramine-T is a widely used disinfectant for the treatment of gill diseases of fish in freshwater, and more recently attention has turned to its use in seawater. However, despite the wide use of chloramine-T, few studies have examined its toxicity to fish. The aim of the current study was to examine the effects of disinfection by Chloramine-T on the gill tissue of grayling (*Thymallus thymallus*) using oxidative stress biomarkers (levels of 2-thiobarbituric acid reactive substances and oxidative modified protein derivatives) and antioxidant defense (superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, total antioxidant capacity) to observe the its toxic effects. Our results showed that Chloramine-T non-significantly decrease lipid peroxidation as well as aldehydic and ketonic derivatives of oxidative proteins. No statistically significant alterations in the activities of antioxidant defenses instead catalase and SOD activity in the gill tissue of grayling disinfected by Chloramine-T were noted. Our studies indicated that Chloramine-T in dose 9 mg per L could at least partly attenuate oxidative stress and can be used for prophylactic treatment of grayling. However, more detailed studies on using of these specific biomarkers to monitor the disinfectant treatment in aquaculture are needed.

Key words: chloramine-T, disinfection, grayling *Thymallus thymallus*, gill tissue, lipid peroxidation, oxidatively modified proteins, antioxidant defense, total antioxidant capacity

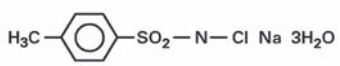
Introduction.

Chloramine-T, as an anti-microbial agent, has had widespread use in a broad range of practices, including medical, dental, veterinary, food processing, and agricultural, as well as a disinfectant for disinfection surfaces and instruments. In agricultural practices, Chloramine-T has been approved as a broadspectrum biocide for foot-and-mouth disease, swine vesicular disease, diseases of poultry, and tuberculosis, while in medicine in the treatment of burns, in whirlpools for the treatment of wounds, and as an oral mouthwash [*Chloramine-T...*].

Chloramine-T is effective for the control of proliferative and bacterial gill disease as well as flexibacteriosis in freshwater, and more recently attention has turned to its use in seawater. Bacterial gill disease is caused by a variety of Gram-negative bacteria (myxobacteria, aeromonads, and pseudomonads). The disease is highly contagious among cultured salmonids and can lead to substantial fish losses. An approved therapeutant to

control bacterial gill disease is needed to enable the production of salmonids for restoration of fish stocks and for sport and commercial fisheries. As a therapeutic agent, it is used as an effective treatment of bacterial gill disease in freshwater or marine aquaria, garden ponds, or other aquatic systems at concentrations ranging from 6.5 to 10.0 mg/L (23.1 to 35.5 μM) and as a preventative, prophylactic, and disinfectant treatment in many fresh water hatcheries [*Chloramine-T*..., Bullock et al., 1991; Powell, Perry, 1996, Thorburn, Moccia, 1993].

Organic chlorine compounds (N-chloro compounds), which contain the =N—Cl group, show microbicidal activity. Examples of such compounds are chloramine-T, dichloramine-T, halazone, halane, dichloroisocyanuric acid, sodium and potassium dichloroisocyanurates and trichloroisocyanuric acid. All appear to hydrolyze in water to produce an imino (=NH) group [Powell, Harris, 2004; Principles and practice of disinfection...].



Chloramine T
(sodium-*p*-toluene-sulphonchloramide)

Their action is claimed to be slower than that of the hypochlorites, although this can be increased under acidic conditions [LeChevallier et al., 1988]. Experiments where equal weights of disinfectants were used suggested that the greater penetrating power of monochloramine compensated for its limited disinfection activity. Studies of LeChevallier and co-workers [1988] showed that monochloramine was as effective as free chlorine for inactivation of biofilm bacteria. In fish studies, chloramine was poorly absorbed from water, and that which was absorbed was rapidly metabolized to the residue marker, p-Toluenesulfonamide. A second, as of yet unidentified, metabolite may also exist [*Chloramine-T*...].

Chloramine-T has a low degree of cytotoxicity and has been used in direct contact with tissues. It is easy to use and effective against many bacteria (both Gram-negative and Gram-positive), viruses (enveloped and naked), fungi, algae, yeast, and parasites. The mode of action of Chloramine-T is thought to be through oxidative processes, quickly destroying cell material or disrupting essential cellular processes. Microorganisms do not develop resistances to Chloramine-T as often happens with antibiotics. In addition, the Chloramine-T ion is highly stable and remains active over an extended period of time. Because Chloramine-T is effective at low concentrations (200 to 300 ppm [710 to 1070 μM]), it is an effective disinfectant without causing tissue cytotoxicity. It may be used as a disinfectant for both skin and for wounds [*Chloramine-T*...].

However, despite the wide use of Chloramine-T, only few studies have examined its toxicity to fish [Powell, Harris, 2004]. Therefore, the aim of the current study was to examine the effects of disinfection by Chloramine-T on the gill tissue of grayling (*Thymallus thymallus* (Linnaeus, 1758)) using oxidative stress biomarkers (levels of 2-thiobarbituric acid reactive substances and oxidative modified protein derivatives) and antioxidant defense (superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, total antioxidant capacity) to observe the its toxic effects. The endpoints obtained from this study will be useful to monitor the effects of disinfectant bathing with Chloramine-T for this species of fish.

Materials and methods.

Fish. Twenty clinically healthy grayling were used in the experiments. The study was carried out in a Department of Salmonid Research, Inland Fisheries Institute near the village of Żukowo, Poland. Experiments were performed at a water temperature of 16±2 °C and the pH was 7.5. The dissolved oxygen level was about 12 ppm with additional oxygen supply. All biochemical assays were carried out at Department of Zoology and Animal Physiology, Institute of Biology and Environmental Protection, Pomeranian University in Slupsk (Poland).

The fish were divided into two groups and held in 250-L square tanks (70 fish per tank) supplied with the same water as during the acclimation period (2 days). On alternate days, the water supply to each tank was stopped. In the disinfectant exposure, grayling (n=10) were exposed to Chloramine-T in final concentration 9 mg per L. Control group of grayling (n=10) were handled in the same way as Chloramine-T exposed groups. Fish were bathed for 20 min and repeated three times every 3 days. Two days after the last bathing fish

were sampled. Fish were not anesthetized before tissue sampling.

Gill tissue isolation. Gill tissue was removed from grayling after decapitation. One grayling was used for each homogenate preparation. Briefly, gill tissue were excised, weighted and washed in ice-cold buffer. The minced tissue was rinsed clear of blood with cold isolation buffer and homogenized in a glass Potter-Elvehjem homogenising vessel with a motor-driven Teflon pestle on ice. The isolation buffer contained 100 mM tris-HCl; pH of 7.2 was adjusted with HCl.

Analytical methods. All enzymatic assays were carried out at 25±0.5 °C using a Specol 11 spectrophotometer (Carl Zeiss Jena, Germany). The enzymatic reactions were started by adding the homogenate suspension. The specific assay conditions are presented subsequently. Each sample was analyzed in triplicate. The protein concentration in each sample was determined according to Bradford [1976] using bovine serum albumin as a standard.

TBARS assay for lipid peroxidation. The level of lipid peroxidation was determined by quantifying the concentration of TBARS with the Kamyshnikov method (2004) for determining the malondialdehyde (MDA) concentration. The nmol of MDA per 1 mg of tissue protein was calculated by using $1.56 \cdot 10^5$ mM^{-1} cm^{-1} as extinction coefficient.

The carbonyl derivatives content of protein oxidative modification (OMP) assay. The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of amino acid reaction with 2,4-Dinitrophenylhydrazine (DNFH) as described by Levine and co-workers (1990) and as modified by Dubinina and co-workers [1995]. DNPH was used for determining carbonyl content in soluble and insoluble proteins. Carbonyl groups were determined spectrofotometrically from the difference in absorbance at 370 nm (aldehydic derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}) and expressed in nmol per mg of tissue protein.

Superoxide dismutase activity assay. Superoxide dismutase (SOD, E.C. 1.15.1.1) activity was assessed by its ability to dismutate superoxide produced during quercetin auto-oxidation in an alkaline medium (pH 10.0) by Kostiuk and co-workers [1990] method. Activity is expressed in units of SOD per mg of tissue protein.

Catalase activity assay. Catalase (CAT, E.C. 1.11.1.6) 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 and co-workers [1988]. One unit of catalase activity is defined as the amount of enzyme required for decomposition of 1 μmol H_2O_2 per min per mg of tissue protein.

Glutathione reductase activity assay. Glutathione reductase (GR, EC1.6.4.2) activity in the tissue was measured according to the method described by Glatzle and co-workers [1974]. The GR activity was expressed as nmol NADPH per min per mg of tissue protein.

Glutathione peroxidase activity assay. Glutathione peroxidase (GPx, EC1.11.1.9) activity was determined by detecting the nonenzymatic utilization of GSH (the reacting substrate) at an absorbance of 412 nm after incubation with 5,5-dithiobis-2-nitrobenzoic acid (DTNB) according by the method of Moin [1986]. GPx activity is expressed as μmol GSH per min per mg of tissue protein.

Total antioxidant capacity assay. The TAC level in the sample was estimated by measuring the TBARS level following Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm [Galaktionova et al., 1998]. The level of TAC in the sample (%) was calculated with respect to the absorbance of the blank.

Statistical analysis. The mean ± S.E.M. values was calculated for each group to determine the significance of inter group difference. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors test ($p>0.05$). Significance of differences between the oxidative stress biomarkers level (significance level, $p<0.05$) was examined using Mann-Whitney *U* test. Correlations between parameters at the set significance level were evaluated using Spearman's correlation analysis [Zar, 1999]. All statistical calculation was performed on separate data from each individual with STATISTICA 10.0.

Results.

Influence of Chloramine-T on lipid peroxidation biomarker, measured as 2-thiobarbituric acid reactive substances in the gill tissue of grayling are presented in Fig. 1A. A non-significantly higher

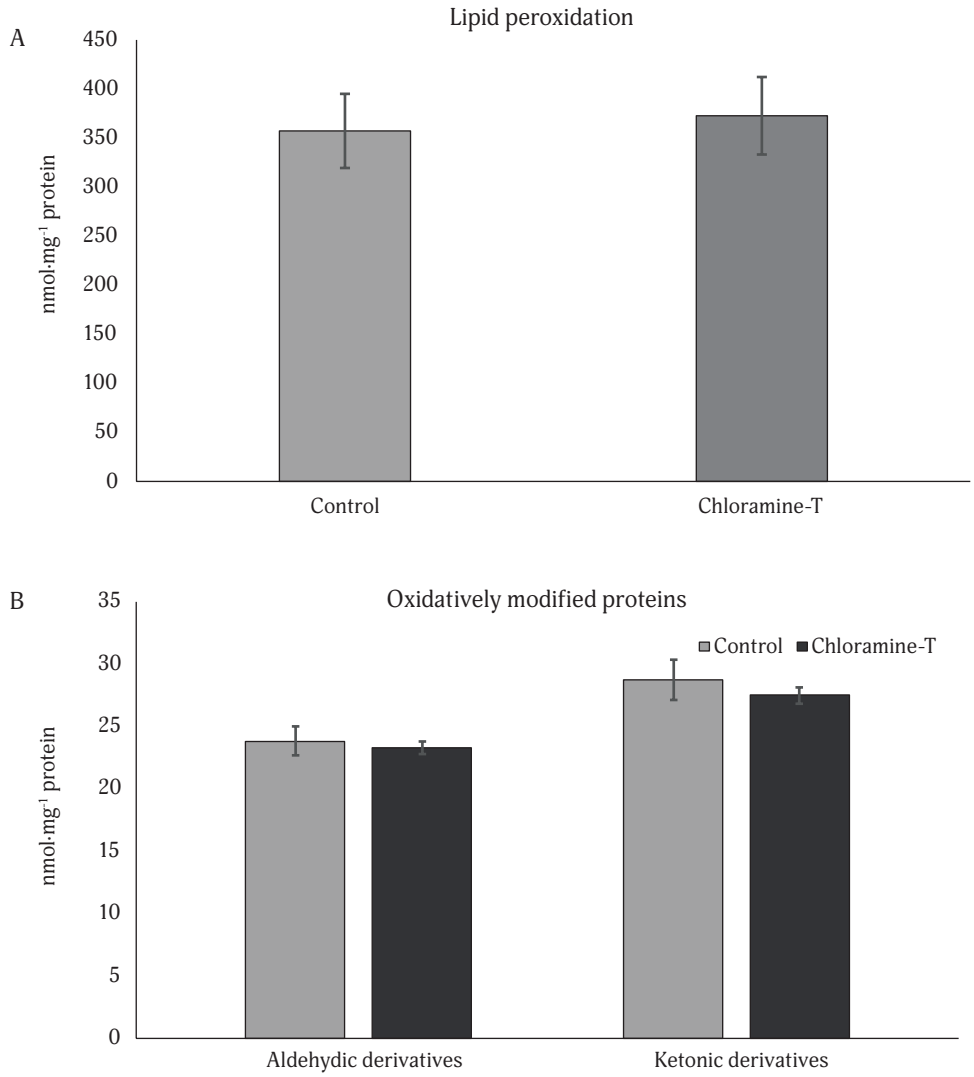


Fig. 1. Influence of Chloramine-T on lipid peroxidation biomarker, measured as 2-thiobarbituric acid reactive substances (A), as well as aldehydic and ketonic derivatives of oxidatively modified proteins (B) in the gill tissue of grayling (*T. thymallus*).

Data are represented as mean ± S.E.M.

TBARS level (by 4.5%, $p>0.05$) in the grayling disinfected by Chloramine-T compared to control group was observed (Fig. 1A).

The aldehydic and ketonic derivatives of oxidatively modified proteins in the gill tissue of the grayling disinfected by Chloramine-T were non-significantly lower compared to controls (Fig. 1B).

The main enzymatic antioxidants include superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). Each of these enzymes is responsible for the reduction of different reactive oxygen species (ROS), and they are located in different cellular compartments [Nunes-Silva, Freitas-Lima, 2015]. Antioxidant defense in the gill tissue of grayling disinfected by Chloramine-T are shown in Table 1.

There were no statistically significant changes in the activities of antioxidant defenses instead CAT and SOD activity in the gill tissue of grayling disinfected by Chloramine-T (Table 1). The CAT activity was lower by 64% ($p=0.002$) after Chloramine-T influence. The SOD activity in the gill tissue of grayling disinfected by Chloramine-T was also decreased by 37% ($p=0.001$) compared to the controls. SOD acts on superoxide radicals to form oxygen and the lesser reactive nonradical species, hydrogen peroxide, while GR can regen-

Table 1. Antioxidant enzymes' activities in the gill tissue of grayling disinfected by Chloramine-T

Enzymes	Control group (n=10)	Disinfected group (n=10)
SOD, U·mg^{-1} protein	232.98±17.62*	146.02±15.59*
CAT, μmol H_2O_2·min^{-1}·mg^{-1} protein	12.07±1.93*	4.37±0.62*
GR, nmol NADPH$_2$·min^{-1}·mg^{-1} protein	4.23±0.80	3.15±0.45
GPx, μmol GSH·min^{-1}·mg^{-1} protein	110.60±9.0	128.69±16.31

Values expressed as mean ± S.E.M.

* the significant change was shown as $p<0.05$ when compared values of control and disinfected groups.

erate oxidized glutathione (GSSG, glutathione disulfide) to reduced glutathione (GSH). The CAT is located mainly in peroxisomes and mitochondria and also removes H_2O_2. CAT requires iron as a cofactor, and similar to GPx and SOD, its activity is highest in highly oxidative muscle fibers [Gomes et al., 2012].

A non-significant decrease of TAC level (by 14%, $p>0.05$) in the gill tissue of grayling as a consequence of bathing with Chloramine-T was found (Fig. 2).

Several correlations between checked parameters were found (Fig. 3). Gill ketonic derivatives correlated positively with aldehydic derivatives of oxidatively modified proteins ($r=0.839$, $p=0.002$) and TBARS level ($r=0.782$, $p=0.008$) (Fig. 3A). Correlations between SOD activity, aldehydic derivatives of oxidatively modified proteins ($r= -0.668$, $p=0.035$) and TAC ($r= -0.730$, $p=0.017$) were inverse (Fig. 3B).

Discussion.

Our results showed that Chloramine-T bathing not alter the lipid peroxidation with non-significant decrease of aldehydic and ketonic derivatives of oxidative proteins (Figs 1A and 1B). However, stability of oxidative stress biomarkers resulted in decrease of SOD and CAT activity (Table 1). Moreover, decreased SOD activity can be resulted to increase of aldehydic derivatives of oxidatively modified proteins ($r= -0.668$, $p=0.035$) (Fig. 3B).

In our previous study [Tkachenko et al., 2012], Chloramine-T bathing markedly decrease aldehydic and ketonic derivatives of oxidative protein, and aminotransferases activity only in rainbow trout liver, and their elevation is a compensatory mechanism to impaired metabolism. No significant changes were found in oxidative stress biomarkers between control and chloramine-treated brown trout. For grayling, Chloramine-T ex-

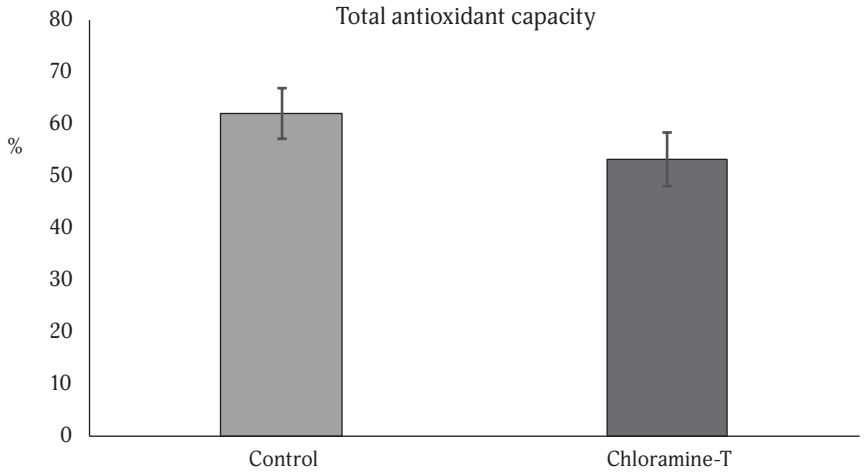


Fig. 2. Influence of Chloramine-T on total antioxidant capacity in the gill tissue of grayling (*T. thymallus*).
Data are represented as mean ± S.E.M.

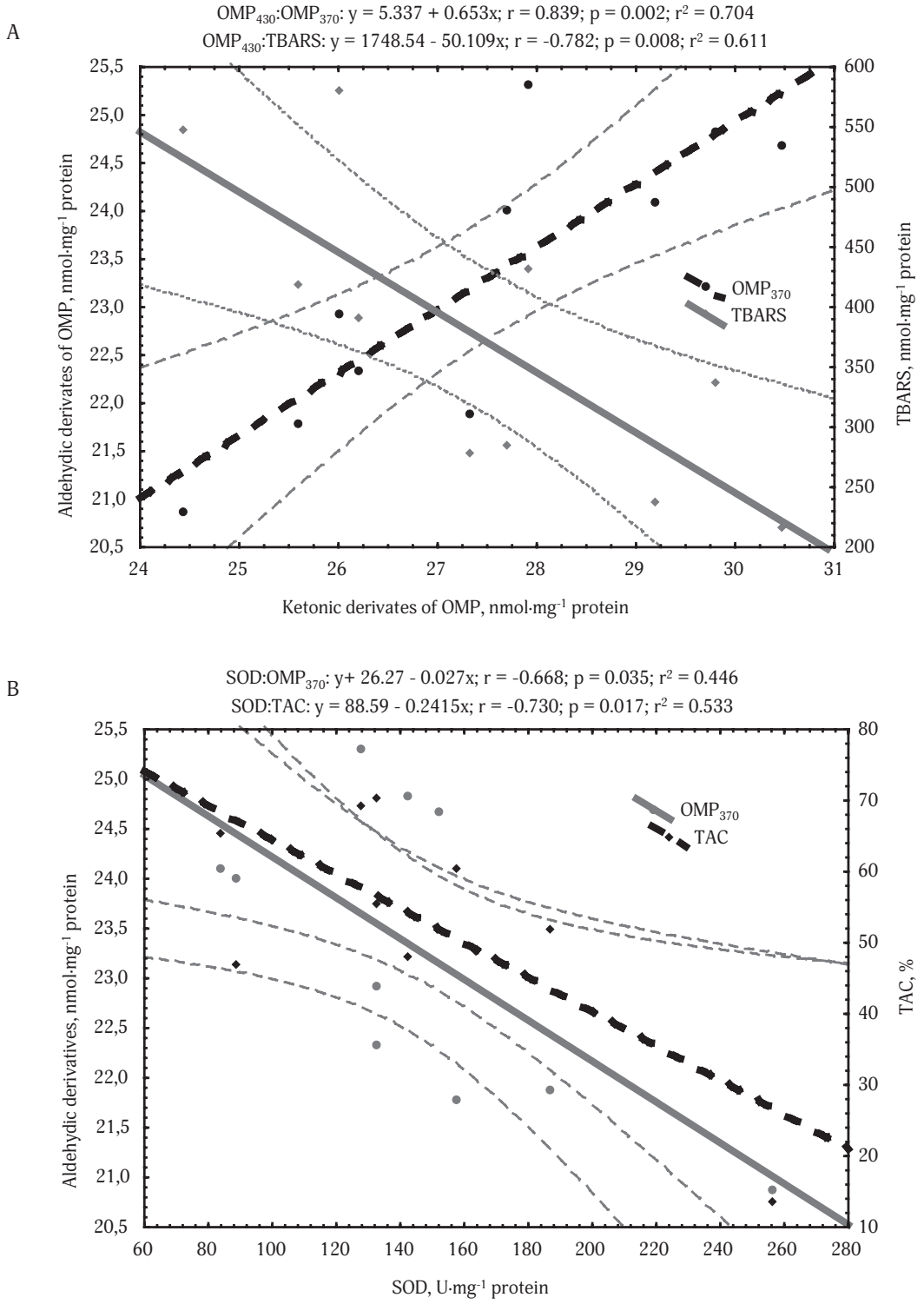


Fig. 3. Correlations between TBARS, aldehydic and ketonic derivatives of oxidatively modified proteins (A) as well as between SOD activity, aldehydic derivatives of oxidatively modified proteins and TAC (B) in the gill tissue of grayling disinfected by chloramine-T.

posure caused significantly elevation in the levels of severe oxidative stress biomarkers in the liver. Increased aldehydic and ketonic derivatives of oxidative protein could modify lactate and pyruvate levels, aminotransferases and lactate dehydrogenase activities, principally causing increased enzymes activity due to oxidative stress in the liver of chloramine-exposed fish.

Accumulating evidence of other researchers has shown that Chloramine-T causes oxidative stress by inducing the generation of reactive oxygen species (ROS) [Stanley et al., 2010]. The data suggest that HOCl and monochloramine can increase endothelial permeability by causing very rapid cytoskeletal shortening and cell retraction, possibly as a result of the oxidation of intracellular sulfhydryls [Tatsumi, Fliss, 1994]. Sakuma and co-workers [2009] assessed the influence of monochloramine on the conversion of xanthine dehydrogenase into xanthine oxidase in rat liver *in vitro*. When incubated with the partially purified cytosolic fraction from rat liver, monochloramine (2.5—20 microM) dose-dependently enhanced xanthine oxidase activity concomitant with a decrease in xanthine dehydrogenase activity, implying that monochloramine can convert xanthine dehydrogenase into the ROS producing form xanthine oxidase. It was found that monochloramine could increase ROS generation in the cytoplasm of rat primary hepatocyte cultures, and that this increase might be reversed by an xanthine oxidase inhibitor, allopurinol. These results suggest that monochloramine has the potential to convert xanthine dehydrogenase into xanthine oxidase in the liver, which in turn may induce the ROS generation in this region [Sakuma et al., 2009].

Moreover, HOCl and related oxidants such as N-chloramines may damage DNA. There is a strong link between chronic inflammation and the incidence of many cancers caused by HOCl and related oxidants such as N-chloramines. Stanley and co-workers [2010] examined the ability of HOCl and various N-chloramines to form chlorinated base products on nucleosides, nucleotides, DNA, and in cellular systems. Experiments were performed with N-chloramines formed on Nα-acetyl-histidine (His-C), Nα-acetyl-lysine (Lys-C), glycine (Gly-C), taurine (Tau-C), and ammonia (Mono-C). Treatment of DNA and related materials with HOCl and Nα-acetyl-histidine resulted in the formation of 5-chloro-2'-deoxycytidine, 8-chloro-2'-deoxyadenosine and 8-chloro-2'-deoxyguanosine. Cellular RNA was also a target for HOCl and His-C, with evidence for the formation of 5-chloro-cytidine. HOCl and the model N-chloramine, His-C, are able to chlorinate cellular genetic material, which may play a role in the development of various inflammatory cancers [Stanley et al., 2010].

Cellular oxidative stress occurs when pro-oxidant forces overwhelm antioxidant defenses. These antioxidant defenses comprise enzymatic and non-enzymatic mechanisms [Xia et al., 2013]. SOD is important in the disproportionation of superoxide anions into hydrogen peroxide (H_2O_2) and dioxygen [Filipak Neto et al., 2008]. The SOD-CAT system provides the first line of defense against oxygen toxicity and is usually used as a biomarker of ROS production. Results of the present investigation indicated that Chloramine-T significantly decrease the SOD activity at the lowest concentration. The alteration of SOD activity revealed that gill tissue might suffer from oxidative stress. This result is consistent with a previous study performed with fish exposed to carbamazepine [Li et al., 2011]. Similarly, Zhang and co-workers [2004] found that SOD activity decreased gradually as the concentration of 2,4-dichlorophenol increased. In addition, the decrease of SOD may be the result of adaptation or loss in compensatory mechanisms [Jifa et al., 2006].

CAT is mainly located in the peroxisomes and, along with glutathione peroxidase, is responsible for the reduction of H_2O_2 produced from the metabolism of long chain fatty acids in peroxisomes [Filipak Neto et al., 2008]. CAT has one of the highest turnover rates of all enzymes: one molecule of CAT can convert millions of molecules of hydrogen peroxide to water and oxygen per second [El-Gendy et al., 2009]. In the current study, gill CAT activity in grayling was significantly inhibited by Chloramine-T. This reduction demonstrates that Chloramine-T induces peroxidative damage in the gill tissue by altering the levels of CAT. Such disruption of antioxidant systems would enhance the generation of ROS and produce more serious oxidative damage to tissues [Ferreira et al., 2010].

Our data are in agreement with data obtained by other researchers. For instance, Boran and Alti-

nok [2014] have assessed the impacts of Chloramine-T treatment on antioxidant enzyme activities and genotoxicity in rainbow trout, *O. mykiss*. Juvenile rainbow trout were exposed to therapeutic, and higher concentrations of Chloramine-T. Red blood cells acetylcholinesterase, Δ-aminolevulinic acid dehydratase, paraoxonase and liver glutathione S-transferase activity were increased at 10 and 20 mg L^{-1} Chloramine-T-exposed fish, while they were decreased at 30 mg L^{-1} Chloramine-T-exposed fish. On the other hand, liver CAT activity and liver protein levels were increased at 10 mg L^{-1} and decreased at 20 and 30 mg L^{-1} concentrations of Chloramine-T. Liver SOD activity decreased at 10 mg L^{-1} and 20 mg L^{-1} Chloramine-T and increased at 30 mg L^{-1} of Chloramine-T. Compared to control, comet assay indicated that Chloramine-T did not cause significant DNA damage to red blood cells of the fish. Results indicated that 10 or 20 mg L^{-1} Chloramine-T can be safely used to prevent or treat external parasitic and bacterial infection of rainbow trout.

Chloramine T was found to increase freshwater bathing efficacy and reduced amoeba survival [Powell, Clark, 2003]. Recent studies also suggest that Chloramine-T in seawater is as effective in seawater as in fresh water [Harris et al., 2004, 2005]. Concentrations of between 8.5 and 12 mg per L have been demonstrated to be successful for the control of bacterial gill diseases in hatcheries [Bullock et al., 1991]. However, Powell and co-workers [1994] suggest that juvenile rainbow trout exposed to 10 and 20 mg Chloramine-T per L showed significant predisposition to an erosive dermatitis of the caudal fin which appeared to be caused by opportunistic pathogens of the genus *Pseudomonas* spp. and *Flavobacter* spp. They recommend that a prophylactic dose of Chloramine-T must be less than 10 mg per L.Also repeated exposures of rainbow trout to chloramine T resulted in decreased growth rates.

To estimate a Chloramine-T margin of safety, defined as the highest dosing regimen above the proposed maximum therapeutic regimen at which no adverse effects are observed, Bowker and co-workers [2011] conducted seven experiments with fry, fingerling, and juvenile rainbow trout that examined mortality and an eighth experiment that examined mortality, gross pathology, and histopathology after Chloramine-T exposure at different concentration. Across experiments, 92% of all mortalities occurred within 20 h of the first exposure to Chloramine-T. The histopathological changes of most concern were associated with gill tissues, but these were evident only in moribund fish exposed to doses of 60 mg L^{-1} or higher. Based on analysis of the survival data, the margin-of-safety estimates were approximately 100 mg/L for rainbow trout fry, at least 60 mg L^{-1} for fingerlings, and 50—60 mg L^{-1} for juveniles. Tissue responses to Chloramine-T at these concentrations were minor and did not warrant decreasing these estimates.

Powell and Harris [2004] examined the acute (within 12 h) toxicity of freshwater- and seawater-acclimated Atlantic salmon *Salmo salar* smolts under aerated (100% air saturation with O_2) and oxygen supersaturation conditions (200% air saturation with O_2). Chloramine-T was more acutely toxic to salmon in seawater than to those in freshwater, and oxygen supersaturation enhanced the toxicity in both sea- and freshwater. Freshwater Atlantic salmon appear to be as sensitive as rainbow trout and more sensitive than channel catfish *Ictalurus punctatus* to Chloramine-T toxicity. Seawater-acclimated salmon, however, appear to be more sensitive to Chloramine-T than are trout and catfish. The primary mechanism of toxicity in both seawater and freshwater appears to be extensive oxidative necrosis of the gill filament and lamellar epithelium, causing acute osmoregulatory dysfunction.

Moreover, Sanchez and co-workers [1998] have concluded that although Chloramine-T and formalin may continue to be useful in the aquaculture industry they cause potentially harmful alterations to fish skin. Juvenile rainbow trout were exposed to therapeutic concentrations of formalin or Chloramine-T to assess the effects of these chemicals on the morphology of the piscine epidermis and its mucous coat. Repeated treatment, once weekly for 4 weeks, with either chemical did not affect the mucous coat of the epithelium or the degree of folding of the basal lamina. However, treated fish had increased numbers of highly dense vesicles within the apical portions of epithelial cells. The epidermal mucous cells of chloramine-T-treated fish were significantly smaller than in controls. Treatment with either chemical resulted in a significantly thinned epidermis.

Very interesting data were obtained by Kuklina and co-workers [2014]. They investigated the Chloramine-T impact on crayfish *Astacus leptodactylus* (Esch., 1823) cardiac activity. To assess whether narrow-clawed crayfish *Astacus leptodactylus* can respond by heart rate changes to presence in water of such biocide as Chloramine-T, adult males were exposed to its low (2 and 5 mg L^{-1}), moderate (10 mg L^{-1}, commonly used in industry and aquaculture) and exceeded (20 and 50 mg L^{-1}) concentrations. After short-term exposure to both Chloramine-T concentrations, crayfish were found to respond rapidly, within 2–5 min. According to heart rate changes, the 1-h exposure did not adversely affect crayfish at either concentration, as well as during daily exposure to 10 mg L^{-1}. As assessed by the heart rate, the 24-h exposure to 50 mg L^{-1} of Chloramine-T was toxic for crayfish and led to substantial loss of energy that became apparent during subsequently conducted physical stress. Crayfish were tolerant to short-term Chloramine-T exposure, while rapid crayfish reaction to an increased chemical level indicated their high sensitivity.

Sirri and co-workers [2013] tested two concentrations of water disinfectants, Chloramine T and peracetic acid, on *Garra rufa* to ascertain possible exposure damage to the epidermis and gills. Fish were exposed to 2 mg L^{-1} and 10 mg L^{-1} of Chloramine T in a 40-minute static bath up to six times a day for one week. No mortality or severe histological changes were found in treated or control fish. Histochemical evaluation of mucous cells did not reveal changes in mucin type in fish exposed to the two disinfectants. The results have suggested a good tolerability of *Garra rufa* to the two disinfectants at the concentrations tested.

Conclusions.

The current study investigated the biological effects of Chloramine-T on the oxidative stress biomarkers (levels of 2-thiobarbituric acid reactive substances and oxidative modified protein derivatives) and antioxidant defense (superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, and total antioxidant capacity) in the gill tissue of grayling. Disinfective procedure by Chloramine-T in in final concentration 9 mg per L has no significant effect on lipid peroxidation and carbonyl content of protein damage, as well as total antioxidant capacity. No statistically significant alterations in the activities of antioxidant defenses instead catalase and SOD activity in the gill tissue of grayling disinfected by Chloramine-T were noted. Our studies indicated that Chloramine-T in dose 9 mg per L could at least partly attenuate oxidative stress and can be used for prophylactic treatment of grayling. However, more detailed studies on using of these specific biomarkers to monitor the disinfectant treatment in aquaculture are needed.

This work was supported by grant of the Pomeranian University for Young Scientists.

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Поступила в редакцию 14.04.16 г.
Принята после рецензии 25.07.16 г.

Влияние дезинфекции хлорамином-Т на содержание биомаркеров окислительного стресса в жаберной ткани хариуса (*Thymallus thymallus*)

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Хлорамин-Т является широко используемым дезинфицирующим средством для лечения жаберных заболеваний рыб в пресной воде, а в последнее время стали обращать внимание и на его использование в морской воде. Однако, несмотря на широкое применение хлорамина-Т, остаются до конца не изученными физиологические ответы рыб после дезинфицирующих мероприятий с хлорамином-Т. Целью настоящего исследования было изучение эффектов хлорамина-Т на жаберную ткань хариуса (*Thymallus thymallus*) с использованием биомаркеров окислительного стресса (содержание продуктов, которые реагируют с 2-тиобарбитуровой кислотой и окислительно модифицированные производные белков) и антиоксидантной защиты (супероксиддисмутаза, каталаза, глутатионредуктаза, глутатионпероксидаза, общая антиокислительная активность). Наши результаты показали, что дезинфекция хлорамином-Т сопровождалась незначительным снижением интенсивности процессов перекисного окисления липидов и содержания альдегидных и кетоновых производных окислительно модифицированных белков на фоне ингибирования активности каталазы и супероксиддисмутазы. Наши исследования показали, что хлорамин-Т в дозе 9 мг на литр может, по крайней мере, частично ослабить окислительный стресс в жаберной ткани хариуса. Тем не менее, необходимы более детальные исследования по использованию этих специфических биомаркеров для мониторинга дезинфицирующих мероприятий в аквакультуре.

Ключевые слова: хлорамин-Т, дезинфекция, хариус *Thymallus thymallus*, жаберная ткань, перекисное окисление липидов, окислительно модифицированные белки, антиоксидантная защита, общая антиокислительная активность