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RESEARCH ARTICLE

Effects of chloramine-T exposure on oxidative stress biomarkers and liver biochemistry of rainbow trout, *Oncorhynchus mykiss* (Walbaum), brown trout, *Salmo trutta* (L.), and grayling, *Thymallus thymallus* (L.)

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Abstract. Oxidative stress, biochemical and enzymological biomarkers were compared among rainbow trout, *Oncorhynchus mykiss* (Walbaum), brown trout, *Salmo trutta* (L.), and grayling, *Thymallus thymallus* (L.) to determine the mechanisms of hepatotoxicity caused by chloramine-T bath treatment. The fish were exposed to chloramine-T at a concentration of 9 g m^{-3} for 20 min three times daily every three days. The control groups of fish, which were not exposed, were handled in the same way as the chloramine-T treatment groups. Chloramine-T treatment varies among fish species; for example, such treatment baths markedly decrease the carbonyl derivate levels of oxidative protein and aminotransferase activities, the elevation of which is a compensatory mechanism for impaired metabolism, but only in rainbow trout livers. No significant changes were noted in oxidative stress biomarkers between the control and chloramine-treated brown trout groups. Grayling chloramine-T exposure resulted in significant elevation in severe oxidative stress biomarker levels. Increased carbonyl derivates of oxidative protein can modify aminotransferase and lactate dehydrogenase activity and lactate and pyruvate levels, all of which cause primarily increased enzyme activity because of oxidative stress in the livers of the chloramine-exposed fish. The present study indicates that chloramine-T at a dose of 9 g m^{-3} can partially attenuate oxidative stress, and can be used for the prophylactic treatment of rainbow and brown trout.

Keywords: chloramine-T, oxidative stress, aminotransferases, lactate dehydrogenase, liver

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Introduction

Chloramine-T is a widel-used therapeutic agent for the treatment of bacterial and parasitic gill and skin diseases in intensive salmonid aquaculture (Powell et al. 1994, 1998, Leef et al. 2007). Chloramine-T is used extensively as a prophylactic or disinfective treatment in a variety of doses; however, treatment doses in the range of 5-20 mg l^{-1} appear to be the most common (From 1980, Bullock et al. 1991, Powell et al. 1994). Chloramine-T in known to increase freshwater bathing efficacy and reduce amoeba survival (Powell and Clark 2003). Recent

studies also suggest that chloramine-T is as effective in seawater as in fresh water (Harris et al. 2004, 2005). Concentrations of between 8.5 and 12 mg l^{-1} have been demonstrated to be successful in controling bacterial gill diseases in hatcheries (From 1980, Bullock et al. 1991). However, Powell et al. (1994) suggest that juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum), exposed to 10 and 20 mg chloramine-T l^{-1} exhibit a significant predisposition for erosive dermatitis of the caudal fin, which appears to be caused by opportunistic pathogens of the genera *Pseudomonas* spp. and *Flavobacter* spp. These authors recommend prophylactic doses of chloramine-T of less than 10 mg l^{-1}. Repeated rainbow trout exposure to chloramine-T has also been demonstrated to decrease growth rates (Powell et al. 1994).

Changes in enzyme activity are used widely in to assess fish health (Gul et al. 2004, Saravanan et al. 2012). Enzymes like aminotransferases and lactate dehydrogenase are good bioindicators of exposure to xenobiotics (Talas et al. 2009, Kavitha et al. 2010, Lavanya et al. 2011). Among these enzymes, aminotransferases are used widely to detect tissue damage and as relevant stress indicators in aquatic biomonitoring (Saravanan et al. 2012). Determinations of these enzymes in tissues are sensitive indicators used to assess and monitor the degree of liver cell inflammation and necrosis, which results in the release of these enzymes into circulation through increased permeability of cell membranes or breakdown (Nanji 1983, Jayarama et al. 2010).

Alanine aminotransferase (AlAT) and aspartate aminotransferase (AsAT) are intracellular aminotransferases which catalyze the transfer of amino groups from α-aminoacids to α-ketoacids. AlAT is an enzyme involved in amino acid metabolism and is, therefore, found in many tissues. The highest levels of AlAT are found in liver and kidney tissues. Tissue destruction leads to the release of the intracellular enzyme into the circulating blood. Markedly elevated serum AlAT levels are a symptom of a variety of liver diseases (Henderson 1986); thus, AlAT is a reasonably specific indicator of liver disease. The level of AlAT activity reflects damage to hepatocytes and is considered to be a highly sensitive and fairly specific preclinical and clinical biomarker of hepatotoxicity (Ozer et al. 2008, 2010). AsAT is an enzyme that catalyzes the transfer of an amino group between L-aspartate and α-ketoglutarate to form oxaloacetate and L-glutamate. Although not specific for liver disease, it can be used in combination with other enzymes to monitor the course of various liver disorders (Huang et al. 2006). Lactate dehydrogenase (LDH) is an oxidoreductase that catalyzes the interconversion of lactate and pyruvate. When disease or injury affects tissues containing LDH, the cells release LDH into the bloodstream, where it occurs in higher than normal levels. Therefore, LDH is most frequently measured to evaluate the presence of tissue or cell damage (Henderson 1986).

Lactate is considered to be a central player in cellular, regional, and whole body metabolism (Gladden 2004). It now appears that lactate is an anaerobic metabolite in the presence of anoxia, a hypoxic metabolite in the presence of dysoxia, and an aerobic metabolite in the presence of an adequate oxygen supply (Gladden 2004). When tissue hypoxia is present, pyruvate oxidation in the Krebs cycle is decreased. Lactate production is increased, and ATP formation continues via glycolysis. Because lactic acid is a product of anaerobic metabolism, elevated lactate levels represent inadequate tissue oxygen delivery (Shinde et al. 2005). Hence in this study, instead of measuring only lactate levels, it was also decided to evaluate pyruvate levels to predict the effects of the disinfectant treatment.

The aim of the present study was to examine the effects of chloramine-T exposure on rainbow trout, *O. mykiss* (Walbaum), brown trout, *Salmo trutta* (L.), and grayling, *Thymallus thymallus* (L.), using oxidative stress biomarkers (thiobarbituric acid reactive substances levels and oxidatively modified protein products), biochemical and enzymological biomarkers (aminotransferases and lactate dehydrogenase activity, lactate and pyruvate levels) to observe toxic effects. The results obtained from this study will be useful in monitoring the effects of bathing these fish species with chloramine-T.

Materials and Methods

Experimental Fish

Twenty two rainbow trout, twenty one brown trout, and twenty clinically healthy grayling, aged 1+, were used in the experiments. The average body weights of the fish were: rainbow trout – 44.3±0.06 g; brown trout – 29.4±0.08 g; grayling – 28.6±0.18 g. The study was conducted at the Department of Salmonid Research in Rutki, Inland Fisheries Institute in Olsztyn, Poland. The experiments were performed at a water temperature of 16±2°C and pH of 7.5. The dissolved oxygen contents were appriximately 12 ppm, and additional oxygen was supplied. All of the biochemical assays were conducted at the Department of Animal Physiology, Institute of Biology and Environmental Protection, Pomeranian University (Slupsk, Poland).

The fish from each species were divided into two groups and stocked into 6 square tanks (70 individuals tank^{-1}) with volumes of 250 l each, supplied with the same water that was used during the two-day acclimation period. The water supply to each tank was turned off on alternate days, and the rainbow trout (n=11), brown trout (n=11), and grayling (n=10) were exposed to chloramine-T in concentrations of 9 g m^{-3}. The control groups of rainbow trout (n=11), brown trout (n=10), and grayling (n=10) were handled in the same way as those exposed to chloramine-T. The fish were bathed in the disinfectant for 20 min three times every three days. The fish were sampled two days after the last bath, but they were not anesthetized before tissue sampling.

The livers were removed from the trout after decapitation, and one trout was used for the preparation of each homogenate. In brief, the livers were excised, weighed, and washed in ice-cold buffer. Minced tissue was rinsed clear of blood with cold isolation buffer, and then homogenized on ice in a glass Potter-Elvehjem homogenizing vessel with a motor-driven Teflon pestle. The isolation buffer contained 100 mM tris-HCl, and pH was adjusted to 7.2 with HCl.

Analytical procedures

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

Thiobarbituric acid reactive substances (TBARS) assay

Thiobarbituric acid reactive substances assay was used for lipid peroxidation, which was estimated by the amount of TBARS in the homogenate using the method by Kamyshnikov (2004). In the TBARS assay, 2.1 ml of tissue homogenate was heated together with 20% trichloracetic acid and 0.8% 2-thiobarbituric acid solution in boiling water for 10 min. Then the tubes were cooled, and after centrifugation (3,000 g for 10 min), absorbance was measured at 540 nm. The concentration (nmol) of malondialdehyde (MDA) was calculated using 1.56 $\times 10^5$ mM^{-1} cm^{-1} as the extinction coefficient, and lipid peroxide levels in liver tissue was expressed in nmol of MDA mg^{-1} of protein.

Content of oxidative protein modification (OMP) product assay

The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of amino acid reactions with 2.4-dinitrophenyl hydrazine (DNFH) as described by Levine et al. (1990) and modified by Dubinina et al. (1995). The carbonyl content was calculated from absorbance measurements at 370 nm and 430 nm and molar absorption coefficient of 22,000 M^{-1}cm^{-1}. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehyde derivates, OMP_{370}) and 430 nm (ketonic

derivates, OMP_{430}) and expressed in nmol of mg protein^{-1}.

Alanine aminotransferase (AlAT, E.C. 2.6.1.2), aspartate aminotransferase (AsAT, E.C. 2.6.1.1) activities assay

Aminotransferase activities were analyzed spectrophotometrically with standard enzymatic methods (Reitman and Frankel 1957). The ketoacids produced by the enzyme action reacts with DNFH producing a hydrazone complex measured calorimetrically at 530 nm. AlAT and AsAT activities were expressed as μmol pyruvate h^{-1} mg of protein^{-1}.

Lactate dehydrogenase (LDH, E.C. 1.1.1.27) activity assay

The colorimetric method by Sevela and Tovarek (1959) was used for determinations of LDH level. LDH activity was expressed as μmol pyruvate h^{-1} mg of protein^{-1}.

Lactate and pyruvate concentrations assay

Lactate and pyruvate concentrations were measured according to the procedure described by Herasimov and Plaksina (2000). One ml of tissue homogenate sample was added to 6 ml distilled water and 1 ml metaphosphoric acid (10%). The mixture was centrifuged at 800 g for 5 min to separate the supernatant. One ml of $CuSO_4$ (25%) and 0.5 g $Ca(OH)_2$ were added to the supernatant, which was then mixed for 30 min. The mixture was centrifuged at 1,000 g for 10 min. The resulting supernatant was resuspended in 3 ml *p*-dimethylaminobenzaldehyde and 1 ml NaOH (25%) for the lactate concentration assay. The solutions were heated in a water bath at 37°C for 45 min, and then centrifuged at 1000 g for 10 min. Absorbance was measured at 420 nm. A solution of *p*-dimethylaminobenzaldehyde and NaOH (25%) was used as the blank. The pyruvate concentration assay was performed by resuspending the resulting supernatant in 0.1 ml $CuSO_4$ (10%), 4 ml H_2SO_4, and 0.1 ml hydroquinone, which was then heated in a water bath at 100°C for 15 min. Absorbance was measured at 430 nm. A calibration curve for lactate (0-5 mM) and pyruvate (0-5 mM) was used, and the results were expressed in μmol lactate mg protein^{-1} and nmol pyruvate mg of protein^{-1}, respectively.

Statistical analysis

The results are expressed as means ± S.E.M. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors tests ($P > 0.05$). The significance of differences in lipid peroxidation levels, carbonyl derivative levels of amino acid reactions, metabolite and enzyme activities in livers ($P < 0.05$) were examined with the Mann-Whitney U test according to Zar (1999). Additionally, relationships between levels of TBARS, protein oxidative destruction products, and the enzyme activities of all individuals were evaluated using Spearman's correlation analysis. All statistical calculations were performed on separate data from each individual with Statistica 8.0 (StatSoft Inc., Poland).

Results

In the control groups, the lipid peroxidation level measured by TBARS content was higher in brown trout livers as compared to that in rainbow trout and grayling (Fig. 1). No effect was found in the liver MDA content of the chloramine-treated fish.

Chloramine-T significantly decreased the aldehyde and ketonic derivates of oxidative protein modification in rainbow trout livers only (Fig. 2). In contrast, the rate of protein oxidative destruction in chloramine-treated grayling livers was significantly increased by 10.6% ($P = 0.023$) and 8.4% ($P = 0.049$) for aldehyde and ketonic derivates, respectively. There were no significant differences in the oxidative protein modification contents between the chloramine-treated brown trout and the controls (Fig. 2).

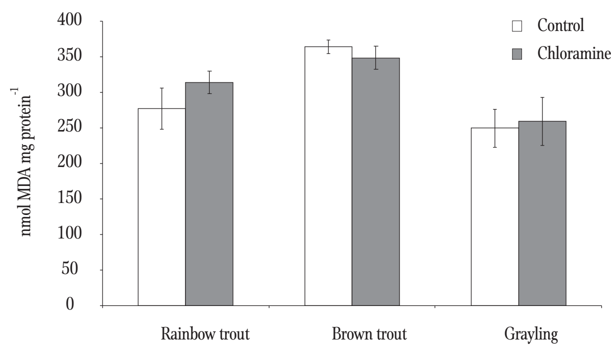

Figure 1. Lipid peroxidation, measured with thiobarbituric acid reactive substances (TBARS) levels (nmol MDA mg protein^{-1}) in the livers of rainbow trout (*O. mykiss*), brown trout (*S. trutta*), and grayling (*T. thymallus*) exposed to chloramine-T bathing. Values are means ± S.E.M.

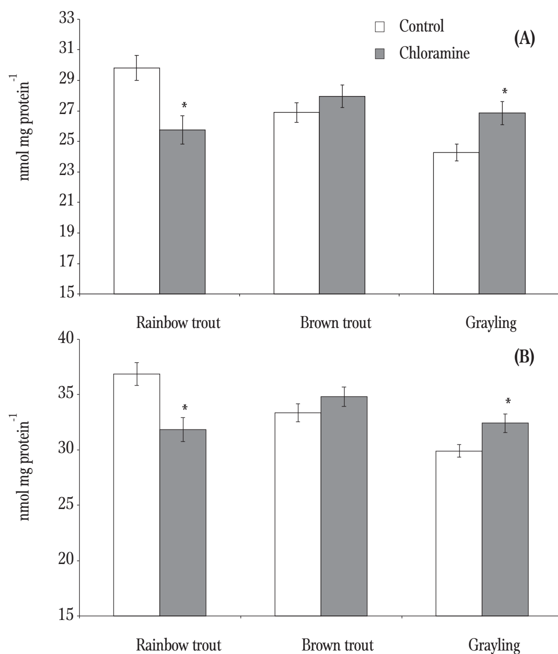

Figure 2. Aldehyde derivates (A, OMP_{370}) and ketonic derivates (B, OMP_{430}) of oxidative protein modification (nmol mg protein^{-1}) in the livers of rainbow trout (*O. mykiss*), brown trout (*S. trutta*), and grayling (*T. thymallus*) exposed to chloramine-T bathing. Values are means ± S.E.M. The asterisk indicates a statistically significant difference (P < 0.05) compared with the control group.

A significant increase in liver levels of AlAT and AsAT was observed in chloramine-treated brown trout and grayling in comparison to the controls

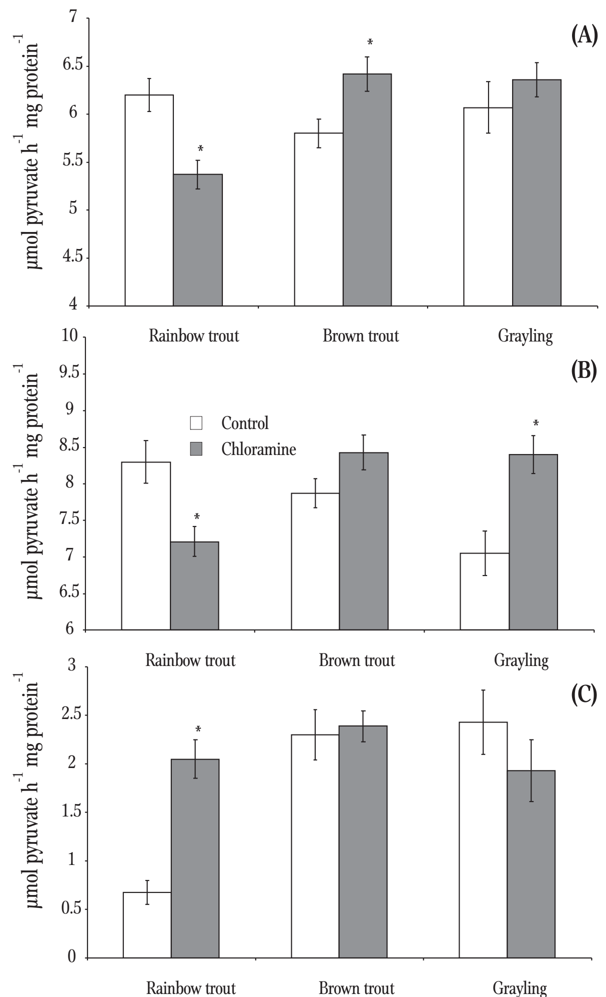

Figure 3. Alanine aminotransferase (A), aspartate aminotransferase (B), and lactate dehydrogenase (C) activity (µmol pyruvate h^{-1} mg protein^{-1}) in the livers of rainbow trout (*O. mykiss*), brown trout (*S. trutta*), and grayling (*T. thymallus*) exposed to chloramine-T bathing. Values are means ± S.E.M. The asterisk indicates a statistically significant difference (P < 0.05) compared with the control group.

(Fig. 3). Chloramine-T treatment caused a significant increase of hepatic AlAT activity in brown trout by 10.7% (P = 0.014) in comparison with the controls (Fig. 3a). Similarly, chloramine-T treatment caused a significant increase in hepatic AsAT activity in grayling by 19.1% (P = 0.008) in comparison with the controls (Fig. 3b). However, the hepatic aminotransferase activities of the chloramine-treated rainbow trout were significantly reduced by 13.4% (P = 0.002) and 13.1% (P = 0.005), respectively, in comparison with those in the control groups. The LDH activity in rainbow trout livers was

increased three times ($P = 0.000$) after the chloramine-T baths (Fig. 3c).

A significant decrease in liver lactate levels (85%, $P = 0.000$) was observed in chloramine-treated brown trout in comparison to the control group (Fig. 4a). Chloramine-T administration caused a significant increase in liver pyruvate levels in brown trout and grayling by 10% ($P = 0.041$) and 25.3% ($P = 0.023$), respectively (Fig. 4b).

Correlation analysis data among lipid peroxidation, oxidatively modified protein levels, and selected biochemical enzyme activity and substrate levels are presented in Tables 1 and 2. The OMP_{370} level in rainbow trout livers correlated positively with both AlAT ($r = 0.855$, $P = 0.001$) and AsAT activity ($r = 0.745$, $P = 0.008$), while OMP_{430} levels correlated with aminotransferase activities AlAT ($r = 0.945$, $P = 0.001$) and AsAT ($r = 0.845$, $P = 0.000$), respectively. Lipid peroxidation biomarker (TBARS) levels correlated with aldehyde ($r = 0.770$, $P = 0.009$) and ketonic derivates ($r = 0.806$, $P = 0.005$) of oxidatively modified protein contents, and with AlAT ($r = 0.782$, $P = 0.013$) and AsAT ($r = 0.745$, $P = 0.000$) activities in brown trout livers. AsAT activity was linked with the lipid peroxidation biomarker ($r = 0.770$, $P = 0.009$) and oxidatively modified protein content ($P = 0.758$, $P = 0.011$) in grayling livers (Table 1). Several correlations between the parameters investigated were found in chloramine-treated rainbow trout livers (Table 2). Aminotransferase activities correlated with oxidative stress biomarkers in chloramine-exposed grayling. The relationships between aldehyde ($r = 0.879$, $P = 0.001$) and ketonic derivates ($r = 0.915$, $P = 0.000$) of protein oxidative modification contents and AsAT activity were positive in brown trout (Table 2).

Table 1
Correlation analysis between lipid peroxidation, oxidative modified protein levels, and enzymatic activity in the livers of rainbow trout (*O. mykiss*), brown trout (*S. trutta*), and grayling (*T. thymallus*)

Species	Parameters	Correlative coefficient	P value
Rainbow trout	OMP_{370}-AlAT	0.855	0.001
	OMP_{370}-AsAT	0.745	0.008
	OMP_{430}-AlAT	0.945	0.000
	OMP_{430}-AsAT	0.845	0.001
	OMP_{370}-OMP_{430}	0.927	0.000
	OMP_{430}-Pyruvate	0.655	0.029
	AlAT-AsAT	0.927	0.000
	Lactate-Pyruvate	0.772	0.005
Brown trout	TBARS-OMP_{370}	0.770	0.009
	TBARS-OMP_{430}	0.806	0.005
	TBARS-AlAT	0.782	0.008
	TBARS-AsAT	0.745	0.013
	OMP_{370}-OMP_{430}	0.976	0.000
	OMP_{370}-AlAT	0.685	0.029
	OMP_{370}-AsAT	0.745	0.013
	OMP_{430}-AlAT	0.758	0.011
	OMP_{430}-AsAT	0.867	0.001
	AlAT-AsAT	0.83	0.003
	AlAT-Lactate	0.685	0.029
Grayling	TBARS-AsAT	0.770	0.009
	OMP_{370}-OMP_{430}	0.976	0.000
	OMP_{370}-AsAT	0.758	0.011
	OMP_{430}-AsAT	0.697	0.025
	AlAT-LDH	-0.867	0.001

Discussion

The primary organ site for xenobiotic metabolism is the liver (Liu et al. 2010). In most cases, metabolic processes are accomplished without injury to the liver itself; however, many xenobiotic compounds are toxic and can cause liver damage (Mudipalli 2007). A growing body of evidence suggests that chloramine-T causes oxidative stress by inducing the generation of reactive oxygen species (ROS) (Bilzer and Lauterburg 1991, Tatsumi and Fliss 1994, Stanley et al. 2010). Sakuma et al. (2009) report that monochloramine can increase ROS generation in the cytoplasm of primary hepatocyte cultures in rats. HOCl and model N-chloramine are able to chlorinate cellular genetic material, which could play a role in the development of various inflammatory cancers (Stanley et al. 2010). These authors have also examined the ability of various N-chloramines to form chlorinated base products on nucleosides, nucleotides, DNA, and in cellular systems; experiments performed with N-chloramines inndicated such products formed on Nα-acetyl-histidine (His-C), Nα-acetyl-lysine (Lys-C), glycine (Gly-C), taurine (Tau-C), and ammonia (Mono-C) (Stanley et al. 2010).

Histological changes in fish tissues, including structure damage, necrosis, alterations, and hemorrhage, have been observed in toxic chloramine-treated animals (Powell et al. 1994, Powell and Perry 1996, Sanchez et al. 1998). Sanchez et al. (1998) report that chloramine-T exposure of juvenile rainbow trout

Table 2
Correlation analysis between lipid peroxidation, oxidative modified protein levels, and enzymatic activity in the livers of rainbow trout (*O. mykiss*), brown trout (*S. trutta*), and grayling (*T. thymallus*) exposed to chloramine-T bathing

Species	Parameters	Correlative coefficient	P value
Rainbow trout	TBARS-OMP_{370}	0.800	0.003
	TBARS-OMP_{430}	0.800	0.003
	TBARS-AlAT	0.709	0.015
	TBARS-AsAT	0.800	0.003
	TBARS-Lactate	0.609	0.047
	OMP_{370}-AlAT	0.782	0.004
	OMP_{370}-AsAT	0.936	0.000
	OMP_{370}-Lactate	0.618	0.043
	OMP_{430}-AlAT	0.782	0.004
	OMP_{430}-AsAT	0.936	0.000
	OMP_{430}-Lactate	0.618	0.043
	AlAT-AsAT	0.809	0.003
	AlAT-Lactate	0.618	0.043
	Pyruvate-Lactate	0.645	0.032
Brown trout	OMP_{370}-OMP_{430}	0.936	0.000
	OMP_{370}-AlAT	0.727	0.011
	OMP_{370}-AsAT	0.809	0.003
	OMP_{430}-AlAT	0.845	0.001
	OMP_{430}-AsAT	0.773	0.005
Grayling	TBARS-OMP_{370}	0.733	0.016
	TBARS-OMP_{430}	0.648	0.043
	TBARS-AlAT	-0.697	0.025
	TBARS-AsAT	0.818	0.004
	OMP_{370}-OMP_{430}	0.855	0.002
	OMP_{370}-AsAT	0.879	0.001
	OMP_{430}-AsAT	0.915	0.000
	Pyruvate-Lactate	0.758	0.011

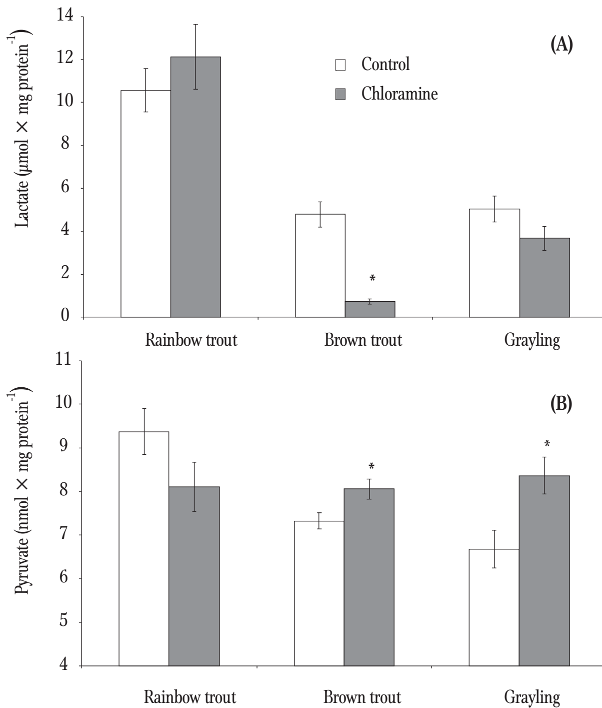

Figure 4. Lactate (µmol × mg protein^{-1}) and pyruvate (nmol × mg protein^{-1}) levels in the livers of rainbow trout (*O. mykiss*), brown trout (*S. trutta*), and grayling (*T. thymallus*) exposed to chloramine-T bathing. Values are means ± S.E.M. The asterisk indicates a statistically significant difference (P < 0.05) compared with the control group.

caused potentially harmful alterations to fish skin and resulted in a significantly thinned epidermis. Treatment with chloramine-T repeated once weekly for four weeks did not affect the epithelial mucous coat or the degree of folding of the basal lamina. Fish treated with chloramine-T had increased numbers of highly dense vesicles in the apical portions of the epithelial cells, and the epidermal mucous cells were significantly smaller than those in the controls (Sanchez et al. 1998). Powell et al. (1994) demonstrate that repeated intermittent exposure of adult trout to therapeutic doses of chloramine-T (9 g m^{-3}) caused mucous cell hyperplasia that was partially compensated for by elevated ventilation rates and lamellar epithelium thinning. They also suggest that, although there was mucous cell hyperplasia in response to repeated chloramine-T exposure, the lamellar epithelium thinning is sufficient to offset any diffusive limitations, thus ensuring gas exchange was not adversely affected. Chloramine-T can be used for repeated prophylactic treatment of adult trout with no apparent detrimental effects to gas exchange or metabolic rate (Powell et al. 1994, Powell and Perry 1996).

The results of the resent study indicate that chloramine-T bathing markedly decreases aldehyde and ketonic derivates of oxidative protein, as well as aminotransferase activity only in rainbow trout livers (Figs. 2, 3). Exposing grayling to chloramine-T caused significantly elevated levels in severe oxidative stress biomarkers (carbonyl derivates of protein oxidative destruction, AsAT activity). Liver injury following chloramine-T exposure is characterized by elevated levels of hepatic marker enzymes, which indicate cellular leakage and the loss of the functional integrity of hepatic membranes (Bilzer and Lauterburg 1991) and increased endothelial permeability (Tatsumi and Fliss 1994). High aminotransferase (AlAT and AsAT) and lactate dehydrogenase (LDH) activities are crucial parameters in detecting liver damage (Henderson 1986). Changes in the activities of aminotransferases are liver specific, and are tools used to study varying cell viability and cell membrane permeability (Dasgupta et al. 1996). In the present study, liver aminotransferase activities were increased throughout the study period in brown trout and grayling, which indicates disruption in the Krebs cycle caused by chloramine-T bathing. Higher levels of aldehyde and ketonic derivates of oxidative protein during chloramine-T exposure of grayling also led to elevated AsAT levels, which indicated increased transaminase activities. This was also indicated by the high correlation between aldehyde and ketonic derivates and AsAT activity (Table 2).

In the present study, changes in protein metabolism during chloramine-T exposure could have affected AlAT, AsAT, and LDH activities in rainbow trout livers, and the elevation of these was a compensatory mechanism to impaired metabolism. The accumulation and binding of xenobiotics in cell membranes, cytoplasm, and mitochondria can cause structural damage and the disintegration of cells, which consequently results in the elevation of aminotransferase activity (Fig. 3). The liver, which is

an important organ for various metabolisms, is highly capable of suppressing ROS generation and promoting ROS elimination (Mori et al. 2007).

The results of the current study are also consistent with the view that the liver is highly resistant to oxidative stress. Consequently, if a measure of chloramine-T exposure occurs, the liver can possibly handle the resulting oxidative stress. These results suggest that chloramine-T at a dose of 9 g m^{-3} can decrease oxidative stress in rainbow trout livers. No significant changes were found in the oxidative stress biomarkers between the control and chloramine-treated brown trout. This study indicates that chloramine-T at a dose of 9 g per m^3 could, at least, partially attenuate oxidative stress, and can be used for the prophylactic treatment of rainbow and brown trout.

The bulk of the evidence suggests that lactate is an important intermediary in numerous metabolic processes; in particular, it is a mobile fuel for aerobic metabolism, and perhaps a mediator of redox states among various compartments both among and between cells. Lactate is considered to be a central player in cellular, regional, and whole body metabolism (Gladden 2004). Recently, it has been proposed that lactate is an additional energy source which supports respiration, maintains ATP levels, and reduces glucose utilization (Gladden 2004, Philp et al. 2005, Sola-Penna 2008). The current findings suggest that lactate levels could, at least, partially attenuate oxidative stress by increasing pyruvate levels in chloramine-treated brown trout livers (Fig. 4).

Glycolysis requires NAD^+ produced, in part, by the conversion of pyruvate to lactate (Bakker 1999, Phypers and Pierce 2006). If the rate of glycolysis rises, concentrations of NADH rise, and lactate production regenerates NAD^+, which raises lactate concentrations. Lactate concentrations directly reflect cellular hypoxia. Under aerobic conditions, pyruvate is converted to acetyl CoA, and it enters the Krebs cycle. With severe shock, the development of intracellular acidosis inhibits gluconeogenesis and reduced liver blood flow delivers less lactate for metabolism. Under anaerobic conditions, glycolysis becomes the predominant mode of hepatic energy production. As such, the liver becomes a lactate-producing organ rather than using lactate for gluconeogenesis (Phypers and Pierce 2006).

In the present study, the lactate level was markedly decreased in chloramine-treated brown trout liver in comparison to the controls (Fig. 4a). Upon exposure to chloramine-T, emergency energy is required to perform the physiological processes that maintain the metabolitic balance in fish tissues. It has been postulated that the Krebs cycle for aerobic respiration in livers is stimulated to produce enormous amounts of energy to maintain homeostasis by supporting pyruvate via lactate conversion. AlAT and AsAT are intracellular aminotransferases that catalyze the transfer of amino groups from α-aminoacids to α-ketoacids. These aminotransferases are present in the cytosol of the hepatocytes (Henderson 1986). The results obtained in the present study indicate significant increases in AlAT and AsAT activities in chloramine-treated brown trout and grayling (Fig. 3). These findings indicate that pyruvate production could enhance aminotransferase activity in brown trout and grayling livers (Fig. 3). The present findings further suggest that pyruvate could maintain intracellular metabolic balance in the livers of chloramine-treated fish; however, the effects of chloramine-T treatment varies among fish species.

In summary, it was concluded that chloramine-T has a profound influence on the biochemical and enzymological profiles of rainbow trout, brown trout, and grayling. Chloramine-T markedly affects protein oxidation levels, aminotransferase and lactate dehydrogenase activities, and lactate and pyruvate levels. These parameters could be used effectively as potential biomarkers of chloramine-T toxicity to fish, and as warning signs when exposing aquatic organisms to pharmaceuticals. In short, increased aldehyde and ketonic derivates of oxidative protein can modify lactate and pyruvate levels and aminotransferase and LDH activities in the livers of chloramine-exposed fish. However, more detailed studies of using of these specific biomarkers to monitor disinfectant treatments in aquaculture are needed.

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Author contributions. H.T. and N.K. performed the experiment, analyzed the data, and wrote the paper, and J.G. designed and performed the experiment.

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