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INFLUENCE OF BRIEF IMMERSION IN AN AQUEOUS SOLUTION OF SODIUM CHLORIDE AND/OR COPPER SULPHATE ON PIKEPERCH (*SANDER LUCIOPERCA* (L.)) BLOOD PARAMETERS*

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Abstract

Research focusing on the effects of chemotherapeutics on the physiological state of fish determines their application in aquaculture. The aim of the study was to determine the impact of sodium chloride (NaCl) and/or copper sulphate ($CuSO_4$) on the hematological and biochemical parameters of the blood plasma of pikeperch, *Sander lucioperca* (body weight – 346.42 ± 44.82 g; body length – 29.8 ± 1.3 cm) during rearing in recirculating aquaculture systems (RAS). Three groups of fish were subjected to treatment with NaCl (5 g/L), $CuSO_4$ (1.5 mg/L), and NaCl+$CuSO_4$ (5 g/L + 1.5 mg/L). Blood was drawn 0, 24 and 48 h following immersion. Significant differences were noted immediately following the conclusion of immersion: mean corpuscular volume (MCV; elevated values in the NaCl+$CuSO_4$ group) and mean corpuscular hemoglobin concentration (MCHC; elevated values in the NaCl group). Immediately following fish exposure in all three experimental groups (0 h), significantly elevated glucose and lactic acid levels were recorded as were lowered levels of chloride ions (Cl^-). After 24 and 48 h, significant differences were observed in Cl^-. In the NaCl group lowered levels of this parameter were sustained after 48 h, while in the other two groups this was sustained for 24 h. It was confirmed that the physiological impact on the pikeperch of the prophylactic bath in sodium chloride and/or copper sulphate was short and that the treatments did not have a significant impact on the health condition of pikeperch.

Key words: pikeperch, copper sulphate, sodium chloride, blood biochemistry, hematological indicators

Aquaculture has developed dynamically over the past two decades, and it is enjoying increasing success in replacing traditional capture fisheries conducted in open waters (FAO, 2018). Increasingly effective procedures for rearing fish in recirculating aquaculture systems (RAS) are being developed (e.g., Bregnballe, 2015). One such rearing procedure that facilitates increasing production efficiency by eliminating pathogenic agents is the prophylactic bath, which fights pathogenic agents and prevents various diseases and contributes substantially to the improved health condition of fish reared in RAS (Noga, 2010; Bregnballe, 2015). Table salt (sodium chloride; NaCl) and copper sulphate ($CuSO_4$) are the two therapeutic agents that are used most widely in aquaculture (Noga, 2010). Sodium chloride is usually used to fight ectoparasites such as those of the genus *Ichthybodo* sp./*Chilodonella* sp./*Trichodina* sp. (García-Magaña et al., 2019), while other agents, such as copper sulphate, are more effective in combating bacteria and fungi (Straus et al., 2009; Moshtaghi et al., 2016). These agents are used separately, but NaCl and $CuSO_4$ are also applied simultaneously in one bath (Antychowicz, 1996; E. Terech-Majewska, unpublished data). The application of a combined bath in which two or more agents are used simultaneously reduces the number of manipulations, which are significant stressors. Knowledge regarding the effects of applying various agents in either prophylactic and/or therapeutic procedures in fish is far from complete. In fact, little is known regarding how prophylactic procedures such as these impact fish health and their physiological state (Mutlu et al., 2016).

The development of RAS technology permits rearing fish species that, until recently, were impossible to produce on larger scales for consumption or stock enhancement (Badiola et al., 2012; Meriç, 2017). One of these species is pikeperch, *Sander lucioperca*, and its production using RAS technology has piqued increasing interest in recent years (FAO, 2012; Blecha et al., 2016; Steinberg

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et al., 2019). Pikeperch has very specific environmental requirements (oxygen concentration, temperature, ammonia, nitrites) and is considered to be a difficult species to rear (Bregnballe, 2015). Additionally, pikeperch is very susceptible to infectious diseases (Rónyai et al., 2003; FAO, 2012; Németh et al., 2013). Fish disease vectors in RAS include stocking material originating from outside RAS, e.g., from earthen ponds. Pikeperch can be cultured, for example, in a combined system (earthen ponds → RAS) (Zakęś and Demska-Zakęś, 1996; FAO, 2012). Juvenile pikeperch (BW – 0.2–0.7 g) produced in earthen ponds are transferred to RAS (Zakęś, 2012; Policar et al., 2013; Blecha et al., 2016). Inherent in this procedure is the great risk of transferring pathogens (e.g., ectoparasites) from the pond environment to RAS (e.g., Németh et al., 2013). Pikeperch reproduction is also conducted in RAS using spawners from earthen ponds (FAO, 2012; Policar et al., 2019). This is why it is crucial to develop safe, effective prophylactic bathing procedures for this species, and this includes determining the physiological responses of pikeperch to these procedures.

Sodium chloride is probably the most commonly applied agent in pikeperch aquaculture (e.g., FAO, 2012). In turn, information on the application of copper sulphate in this species is scant and far from complete (E. Terech-Majewska, unpublished materials). Good indicators of the responses of animals to prophylactic procedures are indicators of their physiological state, e.g., hematological and blood biochemical indicators. These indicators are applied as tools to study the state of fish health (Blaxhall, 1972; Rożyński et al., 2019). Qualitative and quantitative changes in hematological and biochemical parameters are important criteria that serve to evaluate the health of an animal and to make diagnoses (Movahed et al., 2016). Additionally, analyses of changes in hematological (e.g., red blood cell count (RBC), white blood cell count (WBC)) and biochemical profiles (e.g., alanine transaminase (ALT), aspartate aminotransferase (AST)) are often used in studies of the toxicity of various agents to which fish are exposed. Changes in these parameters can provide information on disrupted organ function, e.g, in the liver or the kidneys, etc. (Fazio, 2019; Bojarski and Witeska, 2020).

The aim of the present study was to determine the impact of exposure to aqueous solutions of sodium chloride, copper sulphate, or a combination of sodium chloride and copper sulphate on hematological and biochemical indicators of pikeperch health condition.

Material and methods

Fish and rearing conditions

The study fish were obtained from out-of-season spawning conducted at the Department of Sturgeon Fish Breeding in Pieczarki, Inland Fisheries Institute in Olsztyn (DSFB, IFI Olsztyn, Poland) (Zakęś, 2007). Larval and juvenile pikeperch were reared in a recirculating aquaculture system (RAS) according to methods developed at the Department of Aquaculture (DA IFI Olsztyn, Poland; FAO, 2012). The RAS was fitted with an SDK CN 3.2 biofilter with a volume of 3.2 m^3 (SDK, Ostróda, Poland), which was filled with synthetic RK BioElements with a combined volume of 1.5 m^3 (RK Plast, Skive, Denmark). The filter surface area proper was 750 m^2/m^3. The fish were reared in square tanks with volumes of 1 m^3 at a stocking density of 8 individuals/L. When the fish had attained a body weight (BW) of approximately 1.7 g (age – 80 days post hatch), they were transported to the DA IFI Olsztyn in polyethylene bags (20 L water + 20 L oxygen; 1.0 kg fish/bag); water temperature – 20°C; transport time – 2 h). Further rearing of juvenile pikeperch was conducted in a prototype RAS equipped with, *inter alia*, rearing tanks with volumes of 0.2 m^3 and a biological filter filled with RK BioElements (RK Plast) (Z. Zakęś, unpublished data). Optimal environmental conditions for pikeperch were maintained during rearing: stocking density 10 kg/m^3, water temperature 22.0 ± 0.1°C, pH 7.78–8.07, dissolved oxygen at the tank outflows was above 7.5 mg O_2/L (Zakęś et al., 2015). The concentration of total ammonia nitrogen (TAN = NH_4^+-N + NH_3-N) measured at the rearing tank outflows did not exceed 0.2 mg TAN/L, while nitrites (NO_2-N) did not exceed 0.1 mg NO_2-N/L. The photoperiod applied during rearing was 24L:0D, and the light intensity measured at the water surface in the rearing tanks was 30–40 lx. Commercial feed was delivered with an automatic band feeder (Fischtechnik GmbH, Nienburg, Germany) continually for 19 h/day (10:00–05:00). During this period of rearing, the fish were fed Aller Aqua Performa EX feed, and then Aller Aqua Ivory with recommended granulation sizes for given pikeperch developmental stages (Aller Aqua, Christiansfeld, Denmark) and of the following composition: protein – 54.0%, carbohydrates – 13.0%, crude fat – 15.0%, crude fiber – 1.5%, gross energy – 21.1 MJ/kg (Zakęś, 2017). The fish were reared until they reached a BW of approximately 350 g (fish age – 10 months after hatching).

Experimental procedures

The experiment was conducted using fish with a mean BW of 346.42 ± 44.82 g and a standard length (SL) of 29.8 ± 1.3 cm. Three experimental groups that were subjected to the bath treatments were created with 7 individuals each netted at random from the tank (n = 7; Table 1). The control group (group C) consisted of seven fish (n = 7). Each of the groups was stocked into a separate tank (0.2 m^3) in the RAS in which the fish were kept before creating the experimental groups. The treatment solutions used were as follows: sodium chloride (Chempur®, Piekary Śląskie, Poland) at a concentration of 5 g/L (NaCl group), copper sulphate (Chempur®, Piekary Śląskie, Poland) at a concentration of 1.5 mg/L ($CuSO_4$ group), and sodium chloride and copper sulphate at concentrations of 5 g/L and 1.5 mg/L (NaCl+$CuSO_4$ group; Table 1). The fish bath concentrations used were

based on the recommendations of, *inter alia*, Noga (2010). The manipulations the control group were subjected to were analogous to those experienced by the fish in the experimental groups. The control group was immersed in water from the RAS without any added sodium chloride or copper sulphate. The baths were conducted in 40 L tanks filled with solutions mixed using water from the RAS (inflows to the rearing tanks) in which the fish were held. The physical and chemical parameters of the water used to mix the solutions were as follows: dissolved oxygen – 8.31 mg O_2/L, water pH – 8.03, electrolytic conductivity – 446 µS/cm, total alkalinity – 186.0 mg $CaCO_3$/L, total hardness – 295.9 mg $CaCO_3$/L). Immersion time was 30 min at a water temperature of 22.0°C (Antychowicz, 1996; E. Terech-Majewska, unpublished data). Throughout the bath treatment the water was oxygenated (LP-100 air-pump, Shenzhen Xing Risheng Industrial Co., Ltd, Shenzhen, China). Each of the seven individuals in all of the groups was netted at random and immersed individually, while the exposure time of a given individual only began after the conclusion of the manipulation of the preceding individual. Immediately following the conclusion of immersion each individual was anesthetized (MS-222 buffered with sodium bicarbonate, 100 mg/L, 2 min, Sigma-Aldrich, St. Louis, MO, USA). Then blood was drawn for further analysis from the caudal vein of each individual using a lithium-heparin syringe (Smiths Medical, Minneapolis, MN, USA; n = 7). The remaining individuals from the NaCl, $CuSO_4$, and NaCl+$CuSO_4$ groups were immersed as a group (of 14 fish) following an analogous regime (a tank with a volume of 100 L with continuous oxygenation with an LP-100 air-pump was used), and following its conclusion, these fish were placed in RAS tanks with volumes of 0.2 m^3 each (in total, three tanks were stocked (3 groups)). Blood from these fish was drawn at 24 or 48 h following the conclusion of immersion (n = 7; Table 1). Blood was drawn from all of the fish after they had been anesthetized in an aqueous solution of the anesthetic MS-222 at a concentration of 100 mg/L for 2 min (Rożyński et al., 2019). The fish were not fed for 24 h before immersion or from the conclusion of immersion until blood was drawn.

After drawing the blood, transferring it to the test tubes (Micro tube 1.3 mL LH, Sarstedt AG and Co., Nümbrecht, Germany), and mixing it thoroughly (each sample separately), the blood was tested in a BC2800Vet semi-automatic hematology analyzer (Mindray, Shenzhen, China). The analyzer readings were calibrated to the pikeperch blood based on traditional methods for fish blood hematological determinations (Dacie and Lewis, 2001). The analyzer was calibrated by Stamar (Dąbrowa Górnicza, Poland). Then the blood was centrifuged (6500 rpm, 20°C, 3 min; Fresco 17, Thermo Scientific, Waltham, MA) and subjected to biochemical and ionic determinations with an automatic BS120 biochemistry analyzer (Mindray, Shenzhen, China). The following hematological parameters were analyzed: white blood cell count (WBC); red blood cell count (RBC); hemoglobin (HGB); hematocrit (HCT); mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); and mean corpuscular hemoglobin concentration (MCHC). The following biochemical indicators were determined: glucose (GLU); triglycerides (TG); cholesterol (CHOL); total protein (TP); albumin (ALB); globulin (GLB); lactic acid (LACT); ammonia (NH_3); lactate dehydrogenase (LDH); alanine transaminase (ALT); aspartate aminotransferase (AST); and alkaline phosphatase (ALP). The levels of the following ions were also determined: potassium (K^+); sodium (Na^+); chloride (Cl^-).

Statistical analysis

The data obtained were analyzed statistically with Statistica 12 (StatSoft, Inc., Tulsa, OK, USA). The data were tested for normal distribution with the Shapiro-Wilk test and for homogeneity of variance with Levene's test. Statistical comparisons of the data were done with two-way analysis of variance (ANOVA in factorial system). Further statistical analysis was performed using Tukey's test. Values of $P \leq 0.05$ were recognized as statistically significant.

Table 1. Experimental set up during pikeperch exposure to aqueous solutions of sodium chloride (NaCl), copper sulphate ($CuSO_4$), or a solution containing both chemicals tested (NaCl+$CuSO_4$). Blood sampling time: 0 h (immediately following immersion), 24 and 48 h after the conclusion of the procedure. For details see Material and methods section

	Units	Control group	Experimental groups								
			group NaCl			group $CuSO_4$			group NaCl+$CuSO_4$		
Sodium chloride concentration	g/L	0		5.0			–			5.0	
Copper sulphate concentration	mg/L	0		–			1.5			1.5	
Sampling	h	0	0	24	48	0	24	48	0	24	48
N	indiv.	7	7	7	7	7	7	7	7	7	7

Results

The type of bath (G) and the time after bathing (T) were not noted to have had significant impacts on the WBC, RBC, or HGB indicators (P>0.05; Table 2). However, the HCT value was determined significantly by the period of time following the conclusion of the procedure (P≤0.05; Table 2). Immediately following immersion (0 h), the HCT values in all groups increased significantly, but after 24 and 48 h these values retuned to those noted prior to bathing. The impact of time (T) on MCV and MCHC values was significant, and a significant impact was also noted on MCHC values and the interactions between the tested factors of G × T (P≤0.05; Table 2).

Table 2. Impact of exposure to aqueous solutions of sodium chloride (NaCl) and/or copper sulphate ($CuSO_4$) and time after bathing on the hematological parameters of pikeperch (mean values; n = 7). For details see Material and methods section and Table 1

Indices	Bath group	Control group	Time after bathing			ANOVA (P-value)		
			0 h	24 h	48 h	G	T	G × T
WBC	NaCl	77.29	77.09	84.66	78.13	0.72	0.64	0.68
	$CuSO_4$		87.70	85.27	80.44			
	NaCl+$CuSO_4$		88.08	71.50	80.93			
RBC	NaCl	1.82	1.83	1.82	1.76	0.46	0.70	0.87
	$CuSO_4$		2.01	1.92	1.83			
	NaCl+$CuSO_4$		1.88	1.78	1.91			
HGB	NaCl	25.71	25.01	27.35	25.53	0.40	0.40	0.53
	$CuSO_4$		29.99	27.36	26.67			
	NaCl+$CuSO_4$		29.08	25.53	27.86			
HCT	NaCl	30.36	33.00	31.70	29.49	0.41	**<0.01**	0.77
	$CuSO_4$		36.92	32.38	31.11			
	NaCl+$CuSO_4$		36.27	30.57	33.08			
MCV	NaCl	123.50	134.99	129.18	123.97	0.31	**<0.001**	0.58
	$CuSO_4$		136.65	125.00	126.49			
	NaCl+$CuSO_4$		143.28	126.99	128.70			
MCH	NaCl	23.06	22.30	24.76	23.50	0.63	0.42	0.13
	$CuSO_4$		24.47	23.30	23.89			
	NaCl+$CuSO_4$		25.28	23.37	23.95			
MCHC	NaCl	187.00	165.00	192.00	189.43	0.60	**<0.001**	**<0.05**
	$CuSO_4$		179.50	186.57	189.00			
	NaCl+$CuSO_4$		177.17	184.29	185.83			

G – bath group, T – time , G × T – interaction.

Table 3. Impact of exposure to aqueous solutions of sodium chloride (NaCl) and/or copper sulphate ($CuSO_4$) and time after bathing on the pikeperch biochemical parameters of blood plasma (mean values; n = 7). For details see Material and methods section and Table 1

Indices	Bath group	Control group	Time after bathing			ANOVA (P-value)		
			0 h	24 h	48 h	G	T	G × T
1	2	3	4	5	6	7	8	9
GLU	NaCl	64.20	221.29	59.00	67.60	0.61	**<0.001**	0.38
	$CuSO_4$		206.83	76.40	105.00			
	NaCl+$CuSO_4$		248.33	66.00	71.20			
TG	NaCl	454.20	457.14	485.00	488.20	0.28	0.90	0.79
	$CuSO_4$		405.00	416.40	409.60			
	NaCl+$CuSO_4$		477.33	385.60	453.00			
CHOL	NaCl	265.60	243.57	288.00	279.40	0.14	0.19	0.59
	$CuSO_4$		219.33	235.60	268.80			
	NaCl+$CuSO_4$		270.00	261.40	278.60			

Table 3 – contd.

1	2	3	4	5	6	7	8	9
	NaCl		3.31	3.55	3.48			
TP	$CuSO_4$	3.59	2.97	3.70	3.67	0.57	**<0.01**	0.27
	NaCl+$CuSO_4$		3.46	3.50	3.75			
	NaCl		1.35	1.54	1.62			
ALB	$CuSO_4$	1.68	1.22	1.56	1.35	0.11	**<0.001**	0.33
	NaCl+$CuSO_4$		1.37	1.54	1.70			
	NaCl		1.95	2.01	1.86			
GLOB	$CuSO_4$	1.91	1.75	2.14	2.32	0.53	0.28	0.12
	NaCl+$CuSO_4$		2.09	1.96	2.05			
	NaCl		39.11	2.62	2.74			
LACT	$CuSO_4$	1.54	26.32	3.02	2.74	0.21	0.38	0.10
	NaCl+$CuSO_4$		35.72	1.62	3.14			
	NaCl		472.63	183.30	206.74			
NH_3	$CuSO_4$	209.62	334.23	295.46	391.14	0.11	**<0.001**	**<0.001**
	NaCl+$CuSO_4$		401.18	176.18	244.24			
	NaCl		1046.14	428.00	360.20			
LDH	$CuSO_4$	306.80	233.83	607.00	371.80	0.21	0.38	0.10
	NaCl+$CuSO_4$		382.83	207.60	378.40			
	NaCl		30.71	12.80	51.80			
ALT	$CuSO_4$	30.80	27.00	13.20	12.00	0.46	0.06	0.58
	NaCl+$CuSO_4$		33.67	3.80	44.20			
	NaCl		113.43	40.00	36.20			
AST	$CuSO_4$	32.40	62.17	60.20	39.20	0.63	**<0.05**	0.52
	NaCl+$CuSO_4$		71.83	28.00	40.40			
	NaCl		120.57	123.80	132.20			
ALP	$CuSO_4$	124.60	113.83	146.00	135.60	0.84	0.28	0.64
	NaCl+$CuSO_4$		118.00	110.20	148.00			

G – bath group, T – time , G × T – interaction.

Table 4. Impact of exposure to aqueous solutions of sodium chloride (NaCl) and/or copper sulphate ($CuSO_4$) and time after bathing on the concentrations of selected ions (mmol/L) in pikeperch blood plasma (mean values; n = 7). For details see Material and methods section and Table 1

Indices	Bath group	Control group	Time after bathing			ANOVA (P value)		
			0 h	24 h	48 h	G	T	G x T
K^+	NaCl	3.81	4.25	4.59	4.02	0.86	0.28	**<0.001**
	$CuSO_4$		3.01	4.71	4.79			
	NaCl+$CuSO_4$		5.39	3.80	3.87			
Na^+	NaCl	122.00	124.66	123.43	120.45	**<0.004**	0.12	**<0.03**
	$CuSO_4$		91.74	120.42	109.64			
	NaCl+$CuSO_4$		122.60	122.19	122.52			
Cl^-	NaCl	214.20	121.87	118.48	119.42	**<0.02**	**<0.001**	**<0.001**
	$CuSO_4$		87.55	120.64	210.30			
	NaCl+$CuSO_4$		104.85	119.64	203.16			

G – bath group, T – time, G × T – interaction.

All the bath variants tested significantly increased blood glucose levels. However, significantly higher GLU levels compared to those in the control group were only recorded following the completion of bathing (0 h) ($P \leq 0.05$; Table 3). After 24 and 48 h, the value of this indicator did not differ significantly from that observed in the control group. Similar dependencies were observed for TP and ALB, but decreases were noted in the values of these parameters at 0 h. Bathing time had a significant impact on lactate (LACT) and ammonia (NH_3) levels. After a dynamic increase in LACT values in all groups immediately after bathing (0 h), the values of this indicator after 24 and 48 h did not differ from those in the control group, while the NH_3 values in the $CuSO_4$ group remained at high levels 24 and 48 h after bathing (Table 3). Consequently, a significant impact was also noted in the interactions between the factors tested, i.e., G × T ($P \leq 0.05$; Table 3). The analysis of the level of enzymes such as LDH, ALT, AST, and ALP showed that only in the case of AST did the time after bathing (T) have a significant impact on the values of this indicator ($P \leq 0.05$; Table 3). In all the groups, 48 h after the procedure the value of this indicator did not differ significantly from the level determined in the control group.

The bath treatments had a significant impact on the concentration of all the electrolytes determined, i.e., K^+, Na^+, and Cl^- ($P \leq 0.05$; Table 4). Statistical analysis indicated that the concentrations of K^+ ions were not affected by the bath type (G) or the time after the procedure (T); however, when these factors were combined (G × T), they had a significant impact on the levels of this electrolyte that were determined (Table 4). Na^+ concentrations were significantly lower in the $CuSO_4$ bath group immediately after the end of the procedure (0 h), but after 24 and 48 h they did not differ from the level observed in the control group. In turn, no significant influence of bath type on the level of this electrolyte was noted in the NaCl and NaCl+$CuSO_4$ groups. Decreases in Cl^- concentrations were noted in all the agents and groups tested at 0 and 24 h ($P \leq 0.05$; Table 4), but after 48 h levels in the $CuSO_4$ and NaCl+$CuSO_4$ groups returned to those observed in the control group, while in the NaCl group they were still significantly lower than in the control group ($P \leq 0.05$; Table 4). Consequently, the statistical analysis indicated that Cl^- concentrations were determined by the type of bath (G), the period following the procedure (T), and both of these factors combined (G × T) ($P \leq 0.05$; Table 4).

Discussion

Biosecurity, including prophylactic measures, is one of the most significant factors for preventing the occurrence and/or spread of diseases during fish production (Faruk et al., 2012). Sodium chloride and copper sulphate are popular therapeutic agents used in aquaculture (Noga, 2010; Hosseini and Hoseini, 2012). Sodium chloride offers a variety of benefits. It is readily available, relatively inexpensive, and safe for the environment (Mifsud and Rowland, 2008). Despite its toxic properties in waters with low alkalinity, copper sulphate is one of the most popular chemical substances used to combat fish diseases (Reardon and Harrell, 1990; Klesius and Rogers, 1995; Noga, 2010). There is speculation that $CuSO_4$ could have a degenerative effect on the gills, liver, and kidneys, e.g., in rosy barb, *Pethia conchonius* (Kumar and Pant, 1981). It can negatively influence blood morphological and red blood cell parameters, e.g., in rainbow trout, *Oncorhynchus mykiss* (Dick and Dixon, 1985), and spotted snakehead, *Channa punctata* (Singh, 1995). It has also been confirmed that it can be a source of chronic stress in different species of fish, e.g., in carp, *Cyprinus carpio*, and *Rutilus rutilus caspicus* it causes changes in glucose levels and other stress indicators (Tóth et al., 1996; Hoseini et al., 2016).

Increases in the MCV index can be caused by hypoxia (Nussey et al., 1995). When using agents like these, hypoxia can be caused by excessive mucus production and gill epithelial hyperplasia, which impairs gas and ion exchange in the gills and causes respiratory stress syndrome (Tavares-Dias et al., 2002). Similar reactions to immersion in an aqueous solution of $CuSO_4$ and significantly elevated MCV values were noted in pacu, *Piaractus mesopotamicus* (Tavares-Dias et al., 2002). This phenomenon was confirmed in juvenile individuals of this species (BW – 169.47 g, SL – 20.5 cm) immediately following the conclusion of immersion (at concentrations of 0.5 and 1.0 mg $CuSO_4$/L; Tavares-Dias et al., 2002). Immersion in $CuSO_4$ also induced elevated MCV values in spotted snakehead (Singh, 1995). In the present study of juvenile pikeperch, higher values of MCV were also noted especially immediately following the prophylactic baths. The values of this indicator were determined by the time following the conclusion of the procedure, but these values were not influenced by the type of bath or the agents applied. In contrast to our observations, elevated MCV values resulting from immersion in $CuSO_4$ were not noted in carp (BW – 64.5 g; total body length (TL) – 17.6 cm) that were subjected to baths in an aqueous solution at a concentration of 2.0 mg/L (Mottahari et al., 2013). In pikeperch, the time that elapsed from bathing impacted not only the values of MCV but also those of MCHC. Importantly, the values of this red blood cell indicator were significantly determined by the interaction of T × G. Lower MCHC values in comparison to the control group were noted in all experimental groups immediately following exposure, and these were short-term in nature. Decreases in this parameter were most likely the result of erythrocyte edema resulting from exposure to catecholamines (Brown et al., 2001). In the days following exposure, increased plasma osmolality can lead to the value of the MCHC parameter to return to the initial value (control group; current study). Increases in this parameter were observed in a study of juvenile pikeperch (BW – 150.0–450.0 g) by Brown et al. (2001) that examined

the effects of pikeperch acclimatizing to abrupt increases in salinity. After 24 h of exposure to a NaCl solution (16 g/L) a significant decrease in MCHC values was noted in this species, which most likely resulted from erythrocyte swelling caused by increased catecholamine activity. However, after holding the fish for six days in these conditions, as a result of increased plasma osmolality the value of this parameter increased significantly to values in excess of those confirmed in the control group (Brown et al., 2001). Differences in the reactions of pikeperch (decreases and increases in MCHC values) most probably result mainly from fish size or fish exposition time to the aqueous solution of NaCl, which, in the case of the present study, was 30 min, while in Brown et al. (2001) it was 24 h or 6 days.

The increased glucose levels in blood plasma observed in the present study in all groups of pikeperch immediately following the conclusion of immersion is a commonly occurring body response to stressors (Hoseini and Tarkhani, 2013; Sopinka et al., 2016; Rożyński et al., 2018). Increased levels of this compound in the blood in response to stress occurs to provide the body with additional energy that permits it to contend with disruptions associated with the stress response (Wendelaar Bonga, 1997). Significantly increased levels of glucose were confirmed in carp (BW – 64.5 g; TL – 17.6 cm) subjected to prophylactic baths in an aqueous solution of copper sulphate at a concentration of 2 mg $CuSO_4$/L for 2 weeks. Hyperglycemia in this species is caused by the stress response associated with higher concentrations of ammonia in the blood plasma induced by disruptions in the removal of this nitrogen compound from the body. Under stressful conditions, bodies adapt physiologically by mobilizing additional energy resources with the aim of increasing the chance of survival. The activation of these processes, i.e., glycogenolysis and gluconeogenesis, can result in increased blood plasma glucose levels (Sadok et al., 2004; Mottahari et al., 2013; Maltez et al., 2018). On the other hand, some studies suggest that ammonia, by participating in counter-ion exchange with Na^+, facilitates osmoregulatory processes while fish acclimatize to changes in water salinity (Knoph and Thorud, 1996). Elevated ammonia levels in the blood plasma of pikeperch have also been observed, which could have a direct impact on glucose levels in the bodies of the fish studied. Similar body responses to stress and elevated levels of glucose caused by prophylactic baths in a NaCl solution have also been observed in other fish species, e.g., tambaqui, *Colossoma macropomum* (Chagas et al., 2012). Immediately following the conclusion of immersion, elevated levels of glucose were noted in this species in the groups immersed for periods of 60 min at concentrations of 6 and 8 g NaCl/L and 120 min at concentrations of 4, 6, and 8 g NaCl/L; these were longer than those to which the pikeperch were subjected in the present study. However, for baths that were as long as the immersion time of 30 min in the present study, no elevated values of this parameter were noted at any of the concentrations tested (2, 4, 6, and 8 g NaCl/L), this even included the concentrations that were higher than those applied to the pikeperch (Chagas et al., 2012). Glucose levels were also not affected in Atlantic salmon, *Salmo salar*, that were subjected to prophylactic baths in an aqueous solution of sodium chloride (BW – 110 g, sodium chloride concentration – 15 g/L, immersion time – 120 min; González et al., 2016). In turn, elevated blood plasma glucose levels in juvenile pacu (BW – 174.95 g, SL – 20.9 cm) 24 h following immersion in an aqueous solution of copper sulphate at a concentration of 1.0 mg $CuSO_4$/L is explained by the body's stress response of increased corticosteroid and catecholamine secretion (Diouf et al., 2000; Tavares-Dias et al., 2002). Hyperglycemia has also been observed in Indian catfish, *Heteropneustes fossilis*, 24 h following immersion in an aqueous solution of copper sulphate at a concentration of 0.25 mg $CuSO_4$/L for 30 days (Singh and Reddy, 1990). In turn, Carneiro et al. (2009) confirmed that sodium chloride added at low concentrations to water, e.g., during transport, can limit the stress phenomenon in fish. In their study, the aqueous solution of sodium chloride (6 g NaCl/L) contributed to lowered levels of cortisol and glucose in the blood plasma of juvenile South American catfish, *Rhamdia quelen* (BW – 24.7 g; TL – 13.4 cm) during transport. Elevated levels of lactic acid in all groups subjected to prophylactic baths also attests to the stress response of pikeperch caused by the presence of NaCl and $CuSO_4$ in the water. In response to the presence of stress stimuli, the body mobilizes to induce increased energy requirements that significantly increase oxygen demand. Because the oxygen distribution system (red blood cells) is not able to meet this demand, anaerobic glycolysis occurs in the cells, which results in the release of lactic acid in the muscles (Hvas and Oppedal, 2019). What is significant in the present study is that the increased levels of glucose and lactic acid detected in the fish immediately following immersion (0 h) returned to levels comparable to those of the control group after 24 and 48 h.

Prophylactic baths conducted in aqueous solutions of NaCl and/or $CuSO_4$ can significantly impact physiological processes in fish, e.g., metabolism or osmoregulation (Burgdorf-Moisuk et al., 2011). Changes in the levels of K^+, Na^+, and Cl^- ions in the blood plasma are the body's response to disruptions in respiration and the regulation of the acid-base balance that result from immersion in aqueous solutions of NaCl or $CuSO_4$. Lowering the concentration of ions in the plasma, such as those of Na^+ and Cl^-, is generally observed during the occurrence of stress factors, e.g., prophylactic baths (Perry and Gilmour, 2006). Elevated catecholamine levels cause increased gill capacity, which results in disruptions in the body's water and electrolyte balance. Additionally, decreased levels of Cl^- ions in all the experimental groups could have also stemmed from the body's response to changes in environmental pH. Fish employ blood buffering properties by increasing

the exchange of chloride ions from the blood plasma with bicarbonate ions (HCO_3^-), which lowers the level of chloride ions in the blood plasma (Fivelstad et al., 2015; González et al., 2016). For example, salmonids adapt to changes in pH by increasing levels of HCO_3^- in the blood (Foss et al., 2007).

Summary

The current study indicated that immersing pikeperch in aqueous solutions of sodium chloride and/or copper sulphate had no negative impact on hematological and biochemical profiles, which are reliable reflections of the health and physiological status of the fish. As soon as 24 h after immersion, all hematological parameters in all three groups of pikeperch tested returned to values comparable to those in the control group. However, with regard to biochemical determinations, significant changes 48 h following the conclusion of immersion were sustained only in the group subjected to a bath in an aqueous solution of NaCl in which, 48 h later, the reduced value of the chloride ion was sustained. Lowered values of this parameter do not, however, exclude sodium chloride as an agent for use in prophylactic baths for pikeperch. Other studies confirm that the level of Cl^- in pikeperch stabilizes (i.e., it does not differ from that in the control group) several days after applying a 24 h immersion in an NaCl aqueous solution (Zakęś et al., unpublished data). The present study indicated that both of the agents at the concentrations and exposure times tested either separately or together can potentially be applied as prophylactic baths with juvenile pikeperch reared in a RAS. Future studies should focus on tests of the susceptibility of specific pathogenic organisms to various bath variants. This would permit developing detailed prophylactic bath protocols for pikeperch.

Conflict of interest

The authors declare that they have no competing interests. The authors certify that they have no affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in the manuscript.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors. The study was conducted in accordance with ethical requirements (Resolution of the Local Ethical Committee for Animal Experimentation in Olsztyn, Resolution No. 51/2018 of June 26, 2018).

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