

Influence of brief immersion in an aqueous solution of sodium chloride and/or copper sulphate on the physiological state of pikeperch (*Sander lucioperca* (L.)) selects

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Received – 21 March 2022/Accepted – 11 June 2022. Published online: 30 June 2022; ©Inland Fisheries Institute in Olsztyn, Poland

Citation: Rożyński, M., Demska-Zakęś, K., Zakęś, Z. (2022). Influence of brief immersion in an aqueous solution of sodium chloride and/or copper sulphate on the physiological state of pikeperch (*Sander lucioperca* (L.)) selects. Fisheries & Aquatic Life 30, 104-111.


Abstract. The study was conducted with the aim of determining the influence of brief immersions in aqueous solutions of sodium chloride (NaCl) and/or copper sulphate (CuSO₄) on the physiological state (hematological and blood plasma biochemical indicators) of pikeperch selects (body weight 509.89 ± 99.56 g; body length 33.9 ± 2.0 cm). Immersion was conducted in a water temperature similar to that of spawning (15.1°C). The fish were divided into three experimental groups and one control group: NaCl group (NaCl concentration – 5 g l⁻¹), CuSO₄ group (CuSO₄ concentration – 1.5 mg l⁻¹), and NaCl+CuSO₄ group (NaCl concentration – 5 g l⁻¹; CuSO₄ concentration – 1.5 mg l⁻¹). Blood was drawn immediately after immersion (0 h), 24, and 48 h following the conclusion of immersion. Immediately after immersion, elevated values were noted in white and red blood cell counts, hematocrit, and hemoglobin, while mean corpuscular hemoglobin concentration values were elevated only in the NaCl group 24 and 48 h following the conclusion of immersion. Changes in blood plasma biochemical indicators were noted only immediately after the conclusion of immersion with elevated values of glucose (NaCl, CuSO₄, and NaCl+CuSO₄ groups), lactate (NaCl and CuSO₄ groups), and

ammonia, sodium, and chlorine ions (CuSO₄ group). Greater aspartate aminotransferase activity was noted in the fish from the CuSO₄ group immediately after immersion, while the least significant changes following immersion were noted in specimens from the NaCl+CuSO₄ group.

Keywords: welfare, prophylaxis, *Sander lucioperca*, table salt, hematological indicators, blood plasma biochemical indicators

Introduction

Percidae, which includes pikeperch, *Sander lucioperca* (L.), is one of the families of fish that could potentially lead to dynamic development in aquaculture in the future. Currently, significant progress is being made in the production of species from this group of fish at various stages of culture. The main factor still limiting rapid increases in production is small scale hatch production (Fontaine et al. 2012). Only a few farms or hatcheries maintain their own spawning stocks in recirculating aquaculture systems (RAS), and their aim is to ensure an optimal quantity of larvae for their own uses. Most farms obtain material for stocking or culture from spawners maintained in ponds or caught in the

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wild (Křistan et al. 2012). When obtaining sex products from percids, the primary focus is on minimizing the negative effects of procedures on the health of the spawners so, after spawning, they can either be transferred to ponds or released back into the wild in good condition. It must be mentioned here that spawning requires of fish significant energy expenditures that leave fish in a weakened condition thus increasing their susceptibility to various kinds of infections.

With the aim of eliminating epizootic risks, pikeperch spawners that are ready for spawning are treated primarily with prophylactic immersions in aqueous solutions, for example, of sodium chloride or copper sulphate. Sodium chloride is primarily effective in eliminating ectoparasites such as *Ichthybodo* sp., *Chilodonella* sp., and *Trichodina* sp. (García-Magaña et al. 2019), while copper sulphate is effective against bacterial, fungal, and viral pathogens (Straus et al. 2009, Moshtaghi et al. 2016). While applied separately, combining NaCl and CuSO₄ in one immersion is also practiced (Antychowicz 1996, E. Terech-Majewska, unpublished materials). Applying various prophylactic agents (two or more simultaneously) in a single immersion reduces the amount of manipulation a significant stress factor to which the fish are subjected. The available literature currently reports few data on this topic; thus, the pool of knowledge regarding the effects of using various prophylactic and/or therapeutic procedures on fish is far from complete (Mutlu et al. 2016). The reactions of fish to given agents are influenced not only by the properties of the agents but also immersion temperatures (Noga 2010, Overton et al. 2018). Since water temperature during the spawning period differs from that normally applied during the grow-out phase (22–23°C), it is important to determine the reaction of fish when they are exposed to the most commonly used agents of sodium chloride and copper sulphate under the thermal conditions typical of the spawning period. Hematological and blood plasma biochemical indicators are unquestionably good, reliable markers of health and physiological status (Blaxhall 1972, Rożyński et al. 2019). Qualitative and quantitative changes in hematological and biochemical

parameters are important criteria for assessing health, making diagnoses, and formulating therapeutic recommendations (Movahed et al. 2016). Moreover, changes in hematological and biochemical profiles can be good indicators of physiological responses to environmental changes (Tavares-Dias et al. 2002).

The aim of the present study was to investigate and identify the effects prophylactic immersion in aqueous solutions of sodium chloride, copper sulphate, or the two simultaneously in one immersion at a water temperature similar to that of spawning (15°C) had on the hematological and blood plasma biochemical indicators of pikeperch selects.

Materials and Methods

The study material was pikeperch selects with a mean body weight (BW) of 509.89 ± 99.56 g and a mean body length (SL) of 33.9 ± 2.0 cm. The fish were divided into three experimental groups and one control group. The experimental groups were exposed to three immersion variants in sodium chloride (NaCl) (Chempur; Piekary Śląskie, Poland) and/or copper sulphate (CuSO₄) (Chempur, Piekary Śląskie, Poland) (Table 1). The concentrations applied and immersion time were chosen based on, inter alia, recommendations in Noga (2010). In each immersion group, the first seven specimens were treated separately, and the exposure time of each fish to the solutions tested began only after the handling of the previous fish was finished. The subsequent 42 specimens were immersed as a group. The control groups were immersed in pure water without sodium chloride or copper sulphate using the same procedures as those of the experimental groups. The exposure time was 30 min, and immersion was done in either 40 l (individual immersion) or 100 l (group immersion) tanks filled with solutions made with water from the rearing tank inflows of the RAS in which the fish were kept. The physicochemical parameters of the water used to make the solutions were as follows: oxygen concentration – $8.31 \text{ mg O}_2 \text{ l}^{-1}$; water pH – 8.03; electrolytic

Table 1

Experimental setup for immersing pikeperch in an aqueous solutions of sodium chloride (NaCl), copper sulphate (CuSO₄) or both simultaneously (NaCl+CuSO₄). Blood sampled: 0 h (immediately after immersion), 24, and 48 h after immersion. Details in Materials and Methods

	Unit	Control group	Experimental groups								
			NaCl group			CuSO ₄ group			NaCl+CuSO ₄ group		
Sodium chloride concentration	g l ⁻¹	0	5.0			-			5.0		
Copper sulphate concentration	mg l ⁻¹	0	-			1.5			1.5		
Sample collection	h	0	0	24	48	0	24	48	0	24	48
N	specimens	7	7	7	7	7	7	7	7	7	7

conductivity – 446 $\mu\text{S cm}^{-1}$; total hardness – 295.9 mg CaCO₃ l⁻¹; water temperature during immersion – 15.1°C. Throughout immersion the water in the tanks was aerated (Hailea ACO-9610; Guangdong Hailea Group Co., Ltd, Chaozhou, China).

Blood samples were collected from the caudal vein with heparinized syringes of specimens subjected to immersion separately immediately after exposure concluded. The specimens that were immersed as a group were transferred to a rearing tank after treatment. Blood was collected from them 24 and 48 h after the conclusion of immersion (n = 7; Table 1). Blood was sampled from all specimens after they had been anesthetized in an aqueous solution of the anesthetic MS-222 (Tricaine methanesulfonate) (Sigma-Aldrich, St. Louis, MO, USA) at a dose of 100 mg l⁻¹ (Rożyński et al. 2019). The fish were not fed before immersion (24 h) or after the conclusion of it until the moment blood was collected. The blood samples were analyzed in a BC2800Vet semi-automatic hematological analyzer (Mindray, Shenzhen, China) and the following hematological parameters were determined: white blood cell count (WBC); red blood cell count (RBC); hemoglobin (HGB); hematocrit (HCT); mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC). The remaining portions of the blood samples were centrifuged to isolate the plasma, which was analyzed in a BS120 automatic biochemical analyzer (Mindray, Shenzhen, China) to determine the following biochemical indicators: glucose

(GLU), lactic acid (LACT), potassium (K⁺), sodium (Na⁺), chlorine (Cl⁻) ions, total protein (TP), albumin (ALB), globulin (GLB), ammonia ions (NH₃). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities were also determined.

The data were analyzed statistically with Statistica 12 (StatSoft, Inc., USA). The data were verified for normal distribution (Shapiro-Wilk's test) and homogeneity of variance (Levene's test). Two-factor analysis of variance (Two-way ANOVA) was used for statistical comparisons of the data (type of immersion [G] × time after immersion [T]). Further statistical analysis was done with Tukey's test. The factors tested were significant at $P \leq 0.05$.

Results and Discussion

The values of all the basic hematological indices analyzed (WBC, RBC, HGB, HCT) in pikeperch selects were determined by the amount of time elapsed following the conclusion of immersion (T) ($P \leq 0.05$; Table 2). Immediately after immersion significant increases in these parameters were noted in fish subjected to all immersion variants tested, but after 24 and 48 h these returned to the initial values. Elevated values of hematological parameters were noted in comparison to those of the initial samples in juvenile pikeperch specimens subjected to various concentrations of sodium chloride (BW – 94 g; NaCl concentrations of 10–20 g l⁻¹) (Demska-Zakeś et al. 2021).

Table 2

Effects of immersion in an aqueous solution of sodium chloride (NaCl) and/or copper sulphate (CuSO₄) and time following conclusion of exposure on hematological indicators of pikeperch selects (mean values (\pm SD); n = 7). Details in Materials and Methods and Table 1

Parameter	Experimental groups	Control group	Time following conclusion of exposure			ANOVA (P value)		
			0 h	24 h	48 h	G	T	G \times T
WBC	NaCl		68.10 \pm 29.21	46.49 \pm 8.76	37.64 \pm 13.90			
	CuSO ₄	54.33 \pm 8.97	70.83 \pm 25.08	56.50 \pm 20.29	51.30 \pm 13.42	0.3230	0.0010	0.7976
	NaCl+CuSO ₄		62.10 \pm 14.10	53.74 \pm 11.27	49.51 \pm 16.63			
RBC	NaCl		1.78 \pm 0.35	1.47 \pm 0.16	1.29 \pm 0.21			
	CuSO ₄	1.69 \pm 0.16	1.84 \pm 0.31	1.67 \pm 0.36	1.56 \pm 0.21	0.1114	0.0001	0.6961
	NaCl+CuSO ₄		1.77 \pm 0.19	1.66 \pm 0.17	1.50 \pm 0.29			
HGB	NaCl		25.71 \pm 6.37	22.57 \pm 2.77	19.17 \pm 4.80			
	CuSO ₄	23.88 \pm 2.32	27.10 \pm 5.41	23.27 \pm 4.20	23.01 \pm 3.46	0.3104	0.0121	0.7514
	NaCl+CuSO ₄		25.45 \pm 2.43	24.75 \pm 4.00	23.09 \pm 5.95			
HCT	NaCl		31.34 \pm 7.13	25.11 \pm 2.80	21.41 \pm 5.03			
	CuSO ₄	28.37 \pm 2.69	32.75 \pm 7.02	27.73 \pm 5.22	26.44 \pm 3.86	0.1343	0.0002	0.5534
	NaCl+CuSO ₄		30.57 \pm 3.27	29.26 \pm 4.06	26.40 \pm 5.87			
MCV	NaCl		130.26 \pm 9.46	126.71 \pm 4.83	122.04 \pm 12.19			
	CuSO ₄	124.66 \pm 4.73	131.16 \pm 9.08	124.00 \pm 5.73	125.69 \pm 7.82	0.4419	0.1400	0.5103
	NaCl+CuSO ₄		128.41 \pm 4.72	130.10 \pm 7.72	130.20 \pm 9.42			
MCH	NaCl		23.51 \pm 2.39	25.06 \pm 1.51	24.00 \pm 2.88			
	CuSO ₄	23.13 \pm 1.08	23.96 \pm 1.68	23.00 \pm 1.58	24.13 \pm 2.17	0.6459	0.1658	0.5799
	NaCl+CuSO ₄		23.60 \pm 1.05	24.24 \pm 2.21	24.99 \pm 2.55			
MCHC	NaCl		180.57 \pm 7.00	198.43 \pm 7.68	197.14 \pm 7.76			
	CuSO ₄	185.71 \pm 1.89	183.29 \pm 4.31	185.57 \pm 5.91	192.14 \pm 6.04	0.0297	0.0000	0.0065
	NaCl+CuSO ₄		184.00 \pm 2.94	186.29 \pm 6.65	192.29 \pm 8.52			

G – immersion variant, T – time following conclusion of exposure, G \times T – interaction. Abbreviations: WBC – white blood cell count ($10^3 \mu\text{l}^{-1}$), RBC – red blood cell count ($10^6 \mu\text{l}^{-1}$), HGB – hemoglobin (g l^{-1}), HCT – hematocrit (%), MCV – mean corpuscular volume (fl), MCH – mean corpuscular hemoglobin (pg), MCHC – mean corpuscular hemoglobin concentration (g l^{-1}).

Changes in RBC, HGB, and HCT were noted in this study for up to 24 h following the conclusion of immersion; however, higher WBC values persist even longer than 48 h following the conclusion of exposure (Demska-Zakęś et al. 2021). A common reaction in fish to increased water salinity is erythrocyte swelling, which is caused primarily by the effect, via adrenergic receptors, of catecholamine released as a result of the stress response (Jensen et al. 1993). In the present study, MCHC values were significantly affected by the immersion variant applied, the time elapsed following its conclusion, and by the interactions of the factors tested (G \times T) ($P \leq 0.05$; Table 2).

Immediately after the conclusion of immersion, stress responses of varying degrees were confirmed in the pikeperch selects from all groups. These were indicated by elevated concentrations of glucose, which is released to provide additional energy during the stress response, and lactate, which accumulates as a product of anaerobic glycolysis (Wendelaar Bonga 1997, Mushtaq et al. 2014). Analyzing the changes in these parameters during the experiment indicated they depended on the immersion variant applied and the time elapsed following its conclusion. The mildest course of changes in the values of these parameters was noted in the group immersed

Table 3

Effects of immersion in an aqueous solution of sodium chloride (NaCl) and/or copper sulphate (CuSO₄) and time following conclusion of exposure on blood plasma biochemical indicators of pikeperch selects (mean values (\pm SD); n = 7). Details in Materials and methods and Table 1

Parameter	Experimental groups	Control group	Time following conclusion of exposure			ANOVA (P value)		
			0 h	24 h	48 h	G	T	G \times T
GLU	NaCl		198.14 \pm 28.49	62.83 \pm 4.37	74.14 \pm 9.92			
	CuSO ₄	60.14 \pm 7.38	186.50 \pm 31.93	71.86 \pm 11.61	86.29 \pm 27.07	0.0059	0.0000	0.0210
	NaCl+CuSO ₄		149.43 \pm 40.80	63.43 \pm 7.63	66.00 \pm 10.52			
LACT	NaCl		36.39 \pm 18.99	1.75 \pm 0.84	2.94 \pm 2.47			
	CuSO ₄	1.39 \pm 1.03	37.95 \pm 17.21	1.51 \pm 0.91	2.16 \pm 1.28	0.0905	0.0000	0.0111
	NaCl+CuSO ₄		17.19 \pm 16.84	3.47 \pm 5.62	2.00 \pm 0.68			
K ⁺	NaCl		3.46 \pm 0.60	3.22 \pm 0.68	3.57 \pm 0.64			
	CuSO ₄	3.89 \pm 1.30	4.97 \pm 1.25	3.98 \pm 1.59	3.23 \pm 0.64	0.1343	0.1761	0.2914
	NaCl+CuSO ₄		3.46 \pm 1.24	3.51 \pm 0.56	3.09 \pm 1.16			
Na ⁺	NaCl		151.77 \pm 5.11	149.83 \pm 1.64	145.14 \pm 4.32			
	CuSO ₄	149.9 \pm 4.91	147.73 \pm 5.11	156.75 \pm 5.59	146.73 \pm 4.72	0.5320	0.0175	0.1184
	NaCl+CuSO ₄		151.76 \pm 9.37	151.39 \pm 6.48	149.88 \pm 4.04			
Cl ⁻	NaCl		219.47 \pm 49.24	188.87 \pm 33.38	199.10 \pm 18.45			
	CuSO ₄	197.46 \pm 40.90	277.05 \pm 30.04	213.90 \pm 35.36	192.23 \pm 50.14	0.0906	0.0005	0.2657
	NaCl+CuSO ₄		220.09 \pm 39.82	191.26 \pm 20.88	192.11 \pm 38.46			
TP	NaCl		3.62 \pm 0.26	3.75 \pm 0.19	3.57 \pm 0.19			
	CuSO ₄	3.57 \pm 0.46	3.76 \pm 0.17	3.91 \pm 0.35	3.53 \pm 0.27	0.4556	0.2153	0.6168
	NaCl+CuSO ₄		3.55 \pm 0.24	3.59 \pm 0.15	3.65 \pm 0.25			
ALB	NaCl		1.39 \pm 0.26	1.25 \pm 0.34	1.48 \pm 0.17			
	CuSO ₄	1.23 \pm 0.40	1.40 \pm 0.09	1.47 \pm 0.10	1.46 \pm 0.21	0.7550	0.0168	0.7497
	NaCl+CuSO ₄		1.29 \pm 0.14	1.46 \pm 0.17	1.54 \pm 0.23			
GLB	NaCl		2.23 \pm 0.27	2.49 \pm 0.27	2.09 \pm 0.20			
	CuSO ₄	2.34 \pm 0.46	2.36 \pm 0.13	2.44 \pm 0.34	2.07 \pm 0.21	0.4614	0.0181	0.5126
	NaCl+CuSO ₄		2.27 \pm 0.21	2.13 \pm 0.14	2.10 \pm 0.15			
NH ₃	NaCl		135.36 \pm 87.57	107.75 \pm 23.10	108.74 \pm 71.54			
	CuSO ₄	90.39 \pm 47.88	315.10 \pm 117.42	118.34 \pm 40.91	118.03 \pm 36.91	0.0017	0.0000	0.0002
	NaCl+CuSO ₄		130.16 \pm 52.04	94.77 \pm 30.63	116.99 \pm 39.68			
ALT	NaCl		35.00 \pm 58.00	41.83 \pm 32.53	43.43 \pm 41.65			
	CuSO ₄	4.29 \pm 3.5	20.67 \pm 13.60	56.86 \pm 101.19	26.14 \pm 40.99	0.5893	0.0271	0.5259
	NaCl+CuSO ₄		8.86 \pm 11.82	14.29 \pm 17.25	51.00 \pm 52.68			
AST	NaCl		27.00 \pm 25.68 ^a	56.5 \pm 70.66 ^a	18.86 \pm 16.93 ^a			
	CuSO ₄	25.71 \pm 18.22	129.50 \pm 122.53 ^b	18.00 \pm 17.39 ^a	21.14 \pm 10.42 ^a	0.1381	0.0272	0.0010
	NaCl+CuSO ₄		27.00 \pm 12.19 ^a	14.14 \pm 8.4 ^a	35.29 \pm 27.57 ^a			
ALP	NaCl		135.00 \pm 38.84	115.00 \pm 20.64	130.00 \pm 40.02			
	CuSO ₄	122.14 \pm 33.59	129.67 \pm 19.70	137.29 \pm 23.54	136.43 \pm 35.00	0.7879	0.4957	0.9071
	NaCl+CuSO ₄		126.29 \pm 27.02	123.71 \pm 31.51	142.57 \pm 34.82			

G – immersion variant, T – time following conclusion of exposure, G \times T – interaction. Abbreviations: GLU – glucose (mg dl⁻¹); LACT – lactic acid (mg dl⁻¹); TP – total protein (g dl⁻¹); ALB – albumin (g dl⁻¹); GLB – globulin (g dl⁻¹); NH₃ – ammonia (μ g dl⁻¹); ALT – alanine aminotransferase (U l⁻¹); AST – aspartate aminotransferase (U l⁻¹); ALP – alkaline phosphatase (U l⁻¹).

in the variant in which NaCl and CuSO₄ were applied together. Elevated glucose levels were also noted in other studies of juvenile pikeperch immersed in sodium chloride and copper sulphate solutions identical to those applied in the present study (NaCl 5 g l⁻¹, CuSO₄ 1.5 mg l⁻¹) but at a different water temperature (22°C) (Rożyński et al. 2022). Similar reactions were also noted in juvenile pikeperch immersed in sodium chloride solutions of various concentrations (BW – 94 g; NaCl concentrations of 10–20 g l⁻¹), and elevated glucose values were noted in these fish up to 24 h after the conclusion of immersion (Demska-Zakęś et al. 2021). The hyperglycemia noted in all three groups of pikeperch immediately following the conclusion of immersion, and particularly that in the NaCl and CuSO₄ group, could have been caused by elevated blood plasma ammonia concentrations caused by the disrupted elimination of this compound from the fish bodies (Wilson and Taylor 1993, Mottahari et al. 2013). Elevated levels of this nitrogen compound were noted in the blood plasma of all the groups tested. It was confirmed that the values of this parameter were determined by both the immersion variant and the time elapsed following its conclusion, and interactions between these parameters also affected the ammonia values in the pikeperch selects ($P \leq 0.05$; Table 3). Some studies revealed that ammonia participates in ion exchange with Na⁺, and, thus, it supports osmoregulatory processes as fish acclimatize to changes in water salinity (Knoph and Thorud 1996).

Copper has a toxic effect primarily on gills through structural changes and by increasing gill epithelium permeability, which can impair respiratory function and disturb systemic ionic balance (Mottahari et al. 2013). Immediately following the conclusion of immersion, changes were noted in the concentrations of sodium and chlorine ions in the blood plasma of specimens from the CuSO₄ group ($P \leq 0.05$; Table 3). It does, however, appear that the concentrations of these ions could have been caused by the stress response (Perry and Gilmour 2006) since similar changes were not noted in the group immersed in NaCl + CuSO₄ ($P > 0.05$; Table 3).

The current study revealed a link between the time elapsed from the conclusion of immersion and AST activity ($P \leq 0.05$; Table 3). Elevated activity of this enzymes was only observed immediately following the conclusion of immersion in the CuSO₄ group. Similar observations were reported in other studies when juvenile pikeperch specimens were exposed to analogous immersion conditions (NaCl 5 g l⁻¹, CuSO₄ 1.5 mg l⁻¹) at a higher water temperature (22°C) (Rożyński et al. 2022). Fluctuations in AST values could also signal liver dysfunction. Additionally, elevated AST activity stemming from the stress response often results in the inclusion of amino acids in the Krebs cycle, which can elevate ammonia concentrations in blood plasma (Svobodova et al. 1994) as was confirmed in pikeperch, *inter alia*, during the present study. An important observation was that similar changes in AST values were not noted in the group immersed in the solution that included both NaCl and CuSO₄ ($P > 0.05$; Table 3).

Summary

The study provided evidence that sodium chloride and copper sulphate applied both separately and/or together in one immersion do not negatively affect the physiological states of pikeperch selects. Among the hematological parameters, only MCHC values in the groups exposed to sodium chloride remained elevated for up to 48 h following immersion. This, however, did not indicate that this compound is unsafe for use in prophylactic treatments for pikeperch. Furthermore, all changes in biochemical profiles noted immediately following immersion resolved after 24 h. The reactions of pikeperch to immersion in sodium chloride and/or copper sulphate at temperatures similar to those of the spawning period (15.1°C) were very similar to those of immersion at higher temperatures that are applied during pikeperch culture (22°C). One important observation was that the least significant changes were noted when NaCl and CuSO₄ were applied

together. Further research is definitely required into the tidal ability of the brief immersions applied in this study.

Acknowledgments. The authors would like to thank Marek Hopko, Agnieszka Sikora, Konrad Stawecki, and Elżbieta Ziomek for their help in writing this article. This research was funded as part of project number IRS-046-3/1/2018 entitled “The effects of prophylactic immersion in aqueous solutions of sodium chloride and/or copper sulphate on hematological and biochemical indicators in pikeperch (*Sander lucioperca*),” and by statutory topic S-028 at the Inland Fisheries Institute in Olsztyn. The study was conducted in accordance with ethical requirements (Resolution of the Local Ethical Committee for Animal Experimentation in Olsztyn, No. 51/2018 of June 26, 2018).

Author contributions. M.R. and Z.Z. conceived/designed the study with contributions from K.D.Z.; M.R. and Z.Z. collected the data; M.R., K.D.Z., and Z.Z. analyzed the data; Z.Z. and M.R. coordinated the study and led the writing and revision to which all authors contributed. All authors gave final approval for publication.

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