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Effects of ozonation on water quality and pikeperch (*Sander lucioperca*) performance in a recirculating aquaculture system

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Abstract: The aim of this study was to examine the effects of ozonation on the water quality, and growth, blood biochemistry, antioxidant capacity and survival of pikeperch (*Sander lucioperca*) reared in a recirculation aquaculture system for 30 weeks. A group without ozone treatment was used as a control. The ozone application led to a significant reduction of the water chemical oxygen demand, biological oxygen demand and unsuspended solids concentration. The results revealed that an ozone treatment as a water treatment method has a positive influence on the intensive culture of pikeperch ensuring a higher survival rate (77%) compared to the non-treated control group (67.2%). Moreover, the ozonation prevented fin damage to a large extent and reduced the prevalence of an *Ichthyophthirius multifiliis* infection. Furthermore, the ozone application led to a reduction in the thiobarbituric acid reactive substance level and enhanced the superoxide dismutase activity in the fish gills. However, the effect of ozonation was null on the plasma biochemical parameters. Overall, these findings suggest that an ozone treatment, using adequate technological equipment to destroy the residual ozone, improves the water quality and protects pikeperch against any possible infection and fin damage in a recirculation aquaculture system.

Keywords: blood biochemistry; oxidative stress; survival; body condition; health condition

Ozone is an effective oxidising agent which is often used in intensive aquaculture systems for water disinfection including the removal of bacterial, viral, fungal and protozoan pathogens, and for improvement in the water quality by oxidation of the organic matter (mainly carbon), turbidity and odour. Ozone applications in an aquaculture system also beneficially influence the water quality indirectly through mechanical and biological filtration, oxygen saturation, and sedimentation of undissolved substances, etc. (Bullock et al. 1997; Kasai et al. 2002; Wietz et al. 2009; Goncalves and

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Gagnon 2011; Li et al. 2015; Skowron et al. 2019). Solid particles (including faeces, feed residue, algae, etc.) can cause several technological and fish health problems in intensive farming using a recirculation aquaculture system (RAS). Moreover, pathogenic agents can cause lesions, diseases and mortality, and upon decomposition, release ammonia and consume oxygen. The efficacy of the ozone treatment depends on the ozone concentration, duration of the ozone exposure, pathogenic load and level of the organic matter (Goncalves and Gagnon 2011). Usually, treatment systems use high-efficiency mechanical filters to clarify the water before any ozone treatment (Kasai et al. 2002). The use of ozone in the RAS is mostly limited by the selection of the fish species, physiological parameters, and their health condition rather than by the nitrification performance of the biofilter.

An excessive ozone concentration is harmful to cultured fish causing gross tissue damage and mortality and can also damage the nitrification bacteria in the biofilter (Goncalves and Gagnon 2011). Ozone toxicity was recognised by Wedemeyer et al. (1979a, b), who reported a 96-h 50% lethal concentration of 9.3 mg/l for rainbow trout (*Oncorhynchus mykiss*) [total length (TL) 100 mm to 130 mm]. Ozone has been reported to be toxic to a wide range of fresh and saltwater organisms at residual concentrations between 0.01 mg/l and 0.1 mg/l. Direct treatment which may cause fish lesions and mortality is not recommended for a rearing tank with fish (Goncalves and Gagnon 2011). Ozone toxicity could be normally induced by its casual overdose, however, residual ozone at low concentrations is also highly toxic to fish (Bullock et al. 1997). The maximum safe level of chronic ozone exposure for salmonids is 0.002 mg/l (Wedemeyer et al. 1979a, b). Exposure to ozone concentrations greater than 0.008–0.06 mg/l leads to severe gill damage in most fish species which can result in serum osmolality imbalances (Bullock et al. 1997). These ozone concentrations can also cause immediate mortality or make the fish more susceptible to microbial infections. Wedemeyer et al. (1979a) reported gill epithelial damage and the death of rainbow trout exposed to 0.009 3 mg/l of ozone.

Oxidation-reduction reactions occur during the ozone disinfection process and considerations about the residual oxidants should be taken into account in RAS (Goncalves and Gagnon 2011). Yan et al. (2014) showed that ozonated secondary effluents from municipal wastewater treatment plants produced a total aldehyde (mixture of formaldehyde, acetaldehyde, propionaldehyde and glyoxal) which were responsible for the body deformities in Japanese medaka (*Oryzias latipes*) embryos. This deformity could be reduced by post-treatment biofiltration through the removal of the ozonated by-products including aldehydes.

Liltved et al. (2006) pointed out that the ozone treatment of water is useful for freshwater, but may not be suitable for seawater. In a marine-based aquaculture, compounds like bromate and bromoform, which are regarded as potential hazardous compounds, are formed when the ozone is in contact with the seawater (Sugita et al. 1996; Tango and Gagnon 2003; Lee et al. 2008; Goncalves and Gagnon 2011; Silva et al. 2011). Schroeder et al. (2015) showed that long-term exposure to ozone-produced oxidants has no adverse effects on the nitrification performance of the relevant biofilter-bacteria in a marine RAS.

Pikeperch (*Sander lucioperca*) is a promising and high-quality fish species for inland intensive aquaculture systems and its diversification quickly developed in Europe during the last fifteen years ensuring a well-balanced and high-quality year-round marketable production. The main technological problems for growing pikeperch are maintaining a high water quality in the RAS, and keeping the water free of organic matter, bacterial and other pathogens (Policar et al. 2019). Therefore, the aim of this study was to evaluate the effects of a long-term ozone treatment (30 weeks) in a RAS on the water quality, growth performance, survival, blood biochemistry and antioxidant capacity of the pikeperch.

MATERIAL AND METHODS

Experimental fish

In total, 4 000 juvenile pikeperch [TL: 45.5 ± 5.5 mm, mean body weight (W): 0.6 ± 0.12 g] were harvested from a production pond of a fish farm in Nove Hrady (Czech Republic) and transported to the RAS of the Faculty of Fisheries and Protection of Waters at the University of South Bohemia (FFPW USB; Czech Republic). The fish were adapted to a commercial feed (Inicio Plus 1.1 mm; Biomar Group A/S, Brande, Denmark) and the RAS conditions for 12 days according to Policar et al. (2013; 2016). Then, the fish were cultured for 55 days under

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intensive culture conditions in the RAS as described by Policar et al. (2019) to reach the targeted size used for this study (TL: 102.5 ± 8.1, W: 9.2 ± 2.0 g).

Experimental design

The experiment was carried out in two identical recirculation aquaculture systems in the experimental facilities of the Faculty of Fisheries and Protection of Waters, the University of South Bohemia. The technological equipment of the RAS, which has a total volume of 30 m^3 water (15 m^3 for the filtration system and 15 m^3 for 10 tanks), has been described in detail by Policar et al. (2018). In total, four culture tanks with identical water volumes of 1 500 litres were used in both recirculation aquaculture systems. Two experimental groups (Oz+: cultured in the RAS with ozonated water; Oz–: cultured in the RAS without any ozone treatment) were tested in each RAS with four replicates. In each replicate, 500 pikeperch were stocked and cultured at an initial density of 0.33 fish/l and 3.03 kg/m^3. The group Oz+ was treated with a continuous ozone application at a dose of 2.5 g ozone/h into 30% of the inflow water during the first 15 weeks, and a 6 h application of ozone at a dose of 10 g/h into the same water flow during the following 15 weeks. An OT 10 ozone generator model (Ozontech s.r.o., Zlín, Czech Republic) was used under a pressure of a maximum 40 kPa, a maximal temperature of 40 °C, relative air humidity of 80% and a maximal ozone production of 10 g/h). The produced ozone from the generator was injected to a chamber where most of the ozone was spent. Afterwards, treated water flowed through a Kripsol sand filter (KD-FILTER, Hulín, Czech Republic) with activated coal and afterwards through an oxygen saturation column (model C-1 from the company Aquacultur Fischtechnik GmbH, Nienburg, Germany), where the degradation of the residual ozone was undertaken by increasing the water pressure to about 2 bar.

Water quality

The following physical and chemical water quality parameters were measured twice a day (7.00 and 15.00): temperature, dissolved oxygen, pH, oxidation-reduction potential (ORP) using an Oximeter 3205 (WTW, měřicí a analytická technika, s.r.o., Prague, Czech Republic), a 3310 pH meter with the included pH electrode or with an ORP electrode (WTW, měřicí a analytická technika, s.r.o., Prague, Czech Republic). The water's total ammonia nitrogen, nitrite (NO_2^-) and nitrate (NO_3^-) concentrations were measured in the hydrochemical laboratory of FFPW USB. The residual ozone was measured using an ozone test kit (0–2.3 mg/l, Model Oz-2; HACH Company, Loveland, Colorado, USA). In addition, the optical density of the unsuspended substances ($ODUS_{720}$) was measured at 720 nm, characterising the water turbidity using a handheld AquaPen-C AP-C 100 from Photon Systems Instruments s.r.o. (Drásov, Czech Republic).

The total nitrogen, total phosphorus, chemical oxygen demand (COD), biological oxygen demand (BOD) and unsuspended solids (US) were analysed in both groups (Oz+ and Oz–) at two-week intervals during the whole experiment at the hydrochemical laboratory of FFPW USB. The COD was determined with potassium permanganate according to Kubel – direct heating with a hot plate. The BOD was determined manometrically on an OxiTop® OC 100 instrument (WTW, Weilheim, Germany) according to DIN 38409. The unsuspended substances (US_{105}) were determined using filtration (filter was dried at 105 °C) and the filter weight difference (before and after the filtration) and was calculated in mg/l.

Assessment of fish conditions and survival rate

At the beginning and at the end of the experiment, the total length and weight of 150 pikeperch from each group (Oz+ and Oz–) were measured using a classical meter with an accuracy of 1 mm and a Mettler AE 2000 scale (Mettler - Toledo, s.r.o., Prague, Czech Republic) with an accuracy of 0.01 g.

All the biometrical measurements were performed after anesthetising with clove oil (0.33 ml/l) according to Kristan et al. (2014). Fulton's condition factor was calculated from the obtained biometric data as:

$$CF = (W/TL^3) \times 100 \quad (1)$$

where:
CF – Fulton's condition factor;

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W – mean body weight in g;
TL – total length in cm.

At the end of the experiment, the survival rate of the experimental groups was determined as the percentage of the surviving fish to the total number of stocked fish.

Monitoring of health status and welfare of fish

Health condition. The visual monitoring of the fish's health condition was performed daily. Detailed examination of the health state during the long-term test was performed ten times in total (at the start, in approximately one-month intervals during the experiment and at the end). The fish were sacrificed by stunning them and cutting the spinal cord in accordance with national and international guidelines for the protection of animal welfare (EU-harmonised Animal Welfare Act of the Czech Republic). Pathological and parasitological examinations were performed for three fish from each group per sampling, 60 fish in total during the experiment.

The body surface, body colour, fin integrity, eyes, gills and internal organs were evaluated macroscopically. The body cavity was examined with a focus on the condition of the internal organs and fat content. Microscopically, smears from one half of the body and from four gill arches from one side were examined.

At the end of the experiment, 75 fish from each experimental group were evaluated focusing on the fin damage: right and left pectoral fins, right and left ventral fins, small and large dorsal fins, anal and caudal fins. The fins' condition was evaluated according to Policar et al. (2016): degree of damage: 0 = minimal (< 5%); 1 = small (5–30%), 2 = mean (30–70%); 3 = complete (> 70%). Subsequently, the average degree of fin damage was determined.

At the end of the experiment, 30 fish per group were sacrificed as described earlier and their liver and spleen weights were recorded to calculate the hepatosomatic index, spleen somatic index and fat somatic index as follows:

$$HSI\ (\%) = (W_{liver}/W_{body}) \times 100 \quad (2)$$

$$SSI\ (\%) = (W_{spleen}/W_{body}) \times 100 \quad (3)$$

$$FSI\ (\%) = (W_{fat}/W_{body}) \times 100 \quad (4)$$

where:
HSI – hepatosomatic index;
W – mean weight;
SSI – spleen somatic index;
FSI – fat somatic index.

Biochemical analyses. At the end of the experiment, blood was withdrawn from the caudal vessels of the fish using heparinised needles, syringes and tubes (0.01 ml of Heparin Pharmaceuticals Injection Solution 1 × 10 ml from Zentiva Group, a.s., Prague, Czech Republic, was used to stabilise 1 ml of fish blood). The samples were centrifuged immediately after collection at 4 000 rpm for 10 min, and the plasma was separated and stored at –80 °C. The plasma biochemical parameters including the total protein, albumin, lactate, cortisol and ammonia (NH_3) concentrations, and the activities of the alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), lactate dehydrogenase (LDH) were analysed using a VETTEST 8008 analyser (IDEXX Laboratories Inc., Westbrook, ME, USA).

Oxidative stress. The fish liver and gill samples, for determining the antioxidant capacity and oxidative stress markers, were collected at four different time points (days 0, 7, 28 and 210) and stored at –80 °C until analysis. Prior to the analysis, the samples were weighed and homogenised in a 50 mM phosphate buffer [pH 7, containing 0.5 mM Ethylenediaminetetraacetic acid (EDTA)] (1 : 10 wt : vol) using an Ultra Turrax homogeniser (IKA®-Werke GmbH & Co. KG, Staufen, Germany). The homogenates were divided into two parts, the first part was used to determine the oxidative stress biomarkers without centrifugation of the thiobarbituric acid reactive substance (TBARS) test and the second part was used to determine the antioxidant capacity biomarkers, where the homogenate was centrifuged as follows: the samples were centrifuged for 30 min at 30 000 rpm and 4 °C for the superoxide dismutase (SOD) activity and the samples were centrifuged for 15 min at 10 000 rpm and 4 °C for measurement of the rest of antioxidant parameters. The oxidative damage was evaluated by lipid peroxidation which was determined through the TBARS assay according to the method of Lushchak et al. (2005). The total superoxide dismutase activity (ES 1.15.1.1) was determined spec-

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trophotometrically at 420 nm by measuring nitro blue tetrazolium (Marklund and Marklund 1974). The catalase activity (CAT, 1.11.1.6 ES) was measured spectrophotometrically at 240 nm according to Beers and Sizer (1952). The glutathione reductase activity (GR, ES 1.6.4.2) was determined spectrophotometrically by measuring the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm (Carlberg and Mannervik 1975). The amount of reduced glutathione (GSH) was determined as described by Ferrari et al. (2007). The enzymatic activity of glutathione-S-transferase (GST) was determined spectrophotometrically at 340 nm (Habig et al. 1974).

Statistical analyses

All the data are presented as the mean ± standard deviation. The statistical analysis of the data was performed using Statistica v12 (StatSoft CR s.r.o., Prague, Czech Republic). The percentage data were arcsine transformed prior to the statistical analysis. For the data that did not have a normal distribution, the Kruskal-Wallis test was used (significance $P < 0.05$).

Ethics statement

All the laboratory experimental procedures complied with valid legislative regulations in the Czech Republic (law No. 166/1996 and No. 246/1992); the permit No. 2293/2015-MZE-17214 and No. 55187/2016-MZE-17214. All the samplings were carried out with the relevant permission from the Departmental Expert Committee for Authorisation of Experimental Projects of the Ministry of Education, Youth and Sports of the Czech Republic (permit No. MSMT 4394/2017-2).

RESULTS

Water quality

Table 1 lists the average values of the water quality parameters analysed with 14-day intervals during the whole experiment. The basic parameters including the temperature, dissolved oxygen and pH were very similar for both groups (Oz+; Oz–) and estimated at 22.1 ± 0.47 °C, 108 ± 15%, and 7.1 ± 0.5, respectively. The ozonation of the water led to a significant reduction of the COD, BOD, US_{105} and $ODUS_{720}$ values compared to the Oz group. However, the water total ammonia nitrogen, nitrite, nitrate, total nitrogen and total protein concentrations were not influenced by the ozonation. No residual ozone was measured during the test. The water in both systems (Oz+ and Oz–) had a similar oxidative reduction potential (230.5 ± 35.2 mV).

Table 1. Average values of the water quality parameters (mg/l) in the Oz+ and Oz– groups during a long-term (30 weeks) intensive pikeperch (*S. lucioperca*) culture in an RAS

Parameter	Oz+	Oz–
TAN (NH_4–N)	0.31 ± 0.1[a]	0.37 ± 0.1[a]
Nitrate (NO_3–N)	23.6 ± 9.0[a]	26.4 ± 10.2[a]
Nitrite (NO_2–N)	0.06 ± 0.02[a]	0.09 ± 0.03[a]
Total nitrogen	29.4 ± 8.9[a]	34.6 ± 10.9[a]
Total phosphorus	1.2 ± 0.6[a]	1.4 ± 0.6[a]
COD	6.4 ± 1.2[b]	10.7 ± 1.6[a]
BOD	5.3 ± 1.8[b]	8.1 ± 1.3[a]
US_{105}	4.3 ± 2.8[b]	8.2 ± 6.2[a]
$ODUS_{720}$	0.004 1 ± 0.000 5[b]	0.008 5 ± 0.004 1[a]

Data are presented as the mean ± SD (n = 15)
BOD = biological oxygen demand; COD = chemical oxygen demand; $ODUS_{720}$ = optical density of the unsuspended solids measured at 720 nm; TAN = total ammonia nitrogen; US_{105} = unsuspended substances, determined using filtration (filter was dried at 105 °C)
[a,b]Values in the same row with different superscript letters are statistically different ($P < 0.05$)

Growth, survival and organosomatic indices

The fish survival in the long-term test with the water ozonation in the RAS is an important factor which was evaluated at the end of the test. The group of pikeperch kept in the water with the ozonation (Oz+) showed a significantly higher survival rate (77.0%) than the group reared without the ozonation (Oz– 67.2%).

The fish reared in the ozonated water exhibited a slightly higher W and TL than the Oz– group, but no significant differences could be found (W: Oz+ = 130.0 ± 26.2 g; Oz– =123.5 ± 21.3 g; TL: Oz+ = 258.0 ± 16.3 mm; Oz– = 238 ± 15.0 mm). Fulton's condi-

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tion coefficient for both groups ranged from 0.76 to 0.91 indicating optimal conditions for the fish. The fat somatic index reported a high fat content in the body cavity of the pikeperch, but was the same in both tested groups (Oz– group = 3.3 ± 0.86%; Oz+ = 3.3 ± 0.79%). The hepatosomatic index was significantly higher in the Oz– group (1.77 ± 0.27) than the Oz+ group (1.34 ± 0.31). In contrast, the fish in the Oz+ (0.09 ± 0.03) group exhibited a significantly higher spleen somatic index than the Oz– group (0.04 ± 0.05).

Assessment of eye damage

The fish macroscopical investigation showed that the eyes of the fish were damaged irregularly in both groups (Oz+ and Oz–). Pathological changes included exophthalmos, enophthalmos, cataract and complete loss of the eye bulb.

Assessment of fin damage

Larger and more frequent fin damage was observed in all the fins of the pikeperch reared in the water without ozonation (Oz–). As shown in Figure 1, significantly lower grades of pectoral, dorsal and caudal fins damage were observed in the Oz+ group compared to Oz–. None of the cases were classified as grade 3 damage. The prevalence of stage 1 and stage 2 (together) damage were seen more often in all the fins (with the exception of the dorsal fin) of the group reared without ozonation (Oz–) (Figure 2).

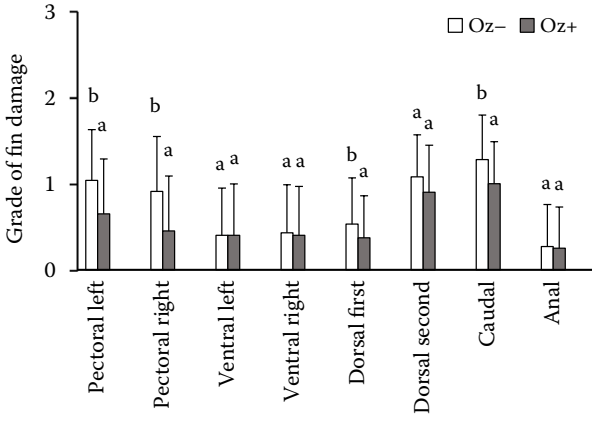

Figure 1. Average grade of the fin damage in the pikeperch (*S. lucioperca*) cultured under the recirculation aquaculture system with and without water ozonation (Oz+ and Oz–) after three weeks

Values marked by a different index for the individual fins are statistically different ($P < 0.05$)

Parasitological examination

Parasitic infections with *Ichthyophthirius multifiliis* were repeatedly found on the skin and gills of the fish at very low intensities (sporadically to mild) at a prevalence of 33–66%. An *I. multifiliis* infection was diagnosed seven times in the Oz– group and only four times the Oz+ group. Due to the infection with *I. multifiliis*, all the fish in both groups were treated with a long-term therapeutic bath with 0.015 ml/l of formaldehyde three times during the whole culture period to reduce the fish mortality. Once (24th week of rearing), an infection with *Trichodina* sp. in both groups (Oz+ and Oz–) in a moderate to strong intensity with a prevalence of 33% was found.

Blood biochemical parameters

The results of the blood plasma biochemical parameters are presented in Table 2. No significant effect of the ozonation could be found on the blood plasma biochemical parameters including the total protein, albumin, cortisol, lactate and NH_3 concentrations, and the activities of the ALP, ALT, AST and LDH.

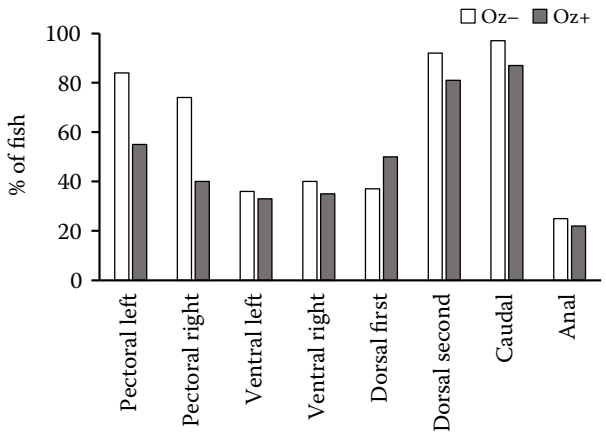

Figure 2. Frequency (%) of the fin damage (grade 1 and grade 2 were taken together) in the pikeperch (*S. lucioperca*) cultured under the recirculation aquaculture system with and without water ozonation (Oz+ and Oz–) after 30 weeks

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Table 2. Plasma biochemical parameters of the pikeperch (*S. lucioperca*) in the Oz+ and Oz– groups at the end of a long-term (30 weeks) intensive culture in a recirculation aquaculture system

	Common published values of pikeperch in good condition	Oz–	Oz+
Cortisol (ng/ml)	not specified	124.5 ± 55.29	151.8 ± 74.50
Albumins (g/l)	1.0–8.00	3.0 ± 1.10	3.5 ± 1.38
Alkaline phosphatase (μkat/l)	0.6–1.20	1.4 ± 0.40	1.3 ± 0.65
Alanine aminotransferase (μkat/l)	0.08–1.43	0.2 ± 0.07	0.2 ± 0.14
Aspartate transaminase (μkat/l)	0.50–4.00	3.0 ± 1.10	3.9 ± 1.45
Total protein (g/l)	23.0–50.00	36.5 ± 4.32	39.0 ± 2.61
Ammonia (μmol/l)	330.0–960.00	931.0 ± 55.81	872.5 ± 191.57
Lactate dehydrogenase (μkat/l)	16.0–25.00	19.6 ± 2.07	20.5 ± 1.55
Lactate (mmol/l)	1.9–5.90	3.5 ± 0.85	4.0 ± 0.48

Data are presented as the mean ± SD (n = 10). No statistically significant differences were found among the treatments

Oxidative stress

The results of the oxidative stress marker and antioxidant enzymes are given in Table 3. A significantly higher TBARS value was detected in the gills of the Oz– group at 35 days after starting the experiment, however, this difference was not found at the end of the experiment. The pikeperch cultured in the ozone treated water showed a significantly higher SOD activity in the gills on day 7 and remained higher in the subsequent samplings throughout the experiment. The other antioxidant enzyme activity including the CAT, GR, GST and GSH activities remained unaffected.

DISCUSSION

It is clear that ozonation helps to remove organic water pollution in the form of various dissolved or undissolved organic substances. Likewise, in this study, the ozonation of the water helped to remove dissolved or undissolved organic substances during the long-term intensive culture of pikeperch. The reduction of the water COD, BOD and undissolved substances (SS) by ozonation is consistent with the results of the study by Davidon et al. (2011). This study showed that water ozonation did not influence the occurrence of oxidising and reducing substances in the water. Also, the nitrogen and phosphorus cycle parameters were not affected by the ozonation in the RAS.

The pikeperch cultured in the ozonated water showed a significantly higher survival rate, and a numerically higher body weight and body length than the Oz– group. Similarly, Davidson et al. (2011) reported significantly greater growth rates of rainbow trout (*O. mykiss*) cultured in a RAS with low exchange and ozone treated water. Also, Good et al. (2011) reported a significantly higher body weight of rainbow trout reared in ozonated water in a RAS.

The ozone treatment led to less severe and lower prevalence values of fin damage in the pikeperch, and significant differences were found for the pectoral, first dorsal and caudal fin damage between Oz+ and Oz– groups. This is in parallel with the study by Good et al. (2011), where less fin damage was observed in rainbow trout by the ozone treatment in a RAS.

The water ozonation reduced the occurrence of *I. multifiliis* infections in the pikeperch in this study. However, water ozonation had no effect on the occurrence of *Trichodina* sp., although only one case was observed in both groups (Oz+ and Oz–).

The values of the blood plasma biochemical parameters in this study are comparable with the common reported values of pikeperch in good condition. The lack of difference with the biochemical parameters of the blood plasma between the Oz+ and Oz– groups showed that water ozonation does not influence the blood plasma biochemical indices in pikeperch after 30 weeks of intensive culture. The relatively higher ALP activity in both groups of pikeperch in this study could be indicative of mild liver damage which could be caused by the artificial pellets which may not be optimised for pikeperch intensive culture (Policar et al. 2019). In contrast

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Table 3. Oxidative stress and antioxidant indices of the pikeperch (*S. lucioperca*) in the Oz+ and Oz– groups during a long-term (30 weeks) intensive culture in a recirculation aquaculture system (RAS)

Parameter	Tissue	Day of the test	Group of pikeperch		Parameter	Tissue	Day of the test	Group of pikeperch	
			RAS (Oz–)	RAS (Oz+)				RAS (Oz–)	RAS (Oz+)
TBARS	liver	0.	0.416 ± 0.05	0.416 ± 0.04	GR	liver	0.	0.078 ± 0.05	0.104 ± 0.07
		7.	0.454 ± 0.14	0.356 ± 0.05			7.	0.078 ± 0.04	0.121 ± 0.02
		28.	0.438 ± 0.11	0.430 ± 0.08			28.	0.210 ± 0.09	0.163 ± 0.08
		210.	0.679 ± 0.14	0.709 ± 0.25			189.	0.148 ± 0.08	0.145 ± 0.04
	gills	0.	0.611 ± 0.03^{b}	0.445 ± 0.05^{a}		gills	0.	0.333 ± 0.09	0.330 ± 0.10
		7.	0.541 ± 0.03^{b}	0.328 ± 0.10^{a}			7.	0.307 ± 0.05	0.420 ± 0.08
		28.	0.382 ± 0.10^{b}	0.250 ± 0.02^{a}			28.	0.308 ± 0.07	0.350 ± 0.06
		210.	0.431 ± 0.08	0.488 ± 0.06			210.	0.372 ± 0.03	0.499 ± 0.10
SOD	liver	0.	0.365 ± 0.03	0.353 ± 0.02	GST	liver	0.	1.773 ± 0.49	1.922 ± 0.27
		7.	0.309 ± 0.05	0.311 ± 0.06			7.	1.840 ± 0.58	1.429 ± 0.50
		28.	0.253 ± 0.07	0.270 ± 0.08			28.	2.135 ± 1.28	1.966 ± 0.67
		210.	0.291 ± 0.06	0.325 ± 0.16			210.	2.106 ± 0.65	2.628 ± 0.80
	gills	0.	0.134 ± 0.05	0.144 ± 0.02		gills	0.	0.669 ± 0.17	0.919 ± 0.15
		7.	0.152 ± 0.03^{b}	0.225 ± 0.04^{a}			7.	0.767 ± 0.10	1.007 ± 0.12
		28.	0.137 ± 0.05	0.183 ± 0.04			28.	0.713 ± 0.16	0.756 ± 0.18
		210.	0.462 ± 0.11	0.535 ± 0.14			189.	0.948 ± 0.25	1.271 ± 0.27
CAT	liver	0.	0.824 ± 0.18	0.820 ± 0.05	GSH	liver	0.	23.889 ± 2.19	25.636 ± 3.05
		7.	0.744 ± 0.17	0.637 ± 0.17			7.	24.541 ± 3.83	26.358 ± 4.00
		28.	0.737 ± 0.34	0.783 ± 0.17			28.	23.489 ± 6.04	29.121 ± 1.80
		210.	1.080 ± 0.15	1.354 ± 0.41			210.	33.645 ± 3.54	34.973 ± 4.99
	gills	0.	0.045 ± 0.01	0.032 ± 0.02		gills	0.	6.747 ± 0.58	7.561 ± 0.92
		7.	0.030 ± 0.01	0.029 ± 0.01			7.	6.422 ± 0.8	6.436 ± 0.85
		28.	0.050 ± 0.03	0.025 ± 0.01			28.	7.213 ± 1.46	7.173 ± 0.44
		210.	0.040 ± 0.02	0.056 ± 0.02			210.	10.691 ± 1.71	10.853 ± 1.38

Data are presented as the mean ± SD (n = 10)

CAT = catalase activity (μmol H_2O_2/min/mg of protein); GR = glutathione reductase activity (nmol nicotinamide adenine dinucleotide phosphate/min/mg of protein); GSH = amount of reduced glutathione (nmol GSH/mg of protein); GST = enzymatic activity of glutathione-S-transferase (nmol/min/mg of protein); SOD = superoxide dismutase activity (nmol nitro blue tetrazolium/min/mg of protein); TBARS = thiobarbituric acid reactive substances (nmol/mg of protein)

^{a,b}Values in the same row with different superscript letters are statistically different (P < 0.05)

to our results, Chen et al. (2003) reported a significant enhancement of the liver ALT, AST and ALP activities in Nile tilapia (*Oreochromis niloticus*) reared in a RAS with an ozone treatment.

It is postulated that an ozone treatment may be accompanied with the generation of free radicals which may have negative effects on the fish's health. These effects have been characterised by the induction of lipid peroxidation and suppression of the SOD activity in fish gills (Li et al. 2015). Authors reported the adverse effect of 300–320 mV ORP in a RAS on the feed intake, feed conversion ratio, growth rate, haemoglobin and lower haematocrit concentrations in European seabass (*Dicentrarchus labrax*) reared in a seawater RAS. The authors suggested not to exceed 300 mV ORP for seabass in a RAS. In the current study, the water in both the Oz+ and Oz– groups had a very similar ORP (230.5 ± 35.2 mV). This may indicate that using the ozonation in the RAS did not induce the occurrence of oxidising and reducing substances in the water. This could be rooted in the effectiveness of the mechanical (sand) filter and oxygen saturation column used in the RAS for the residual ozone degradation through which the water passes after ozonation before being impregnated into the breeding tanks.

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The indicators of oxidative stress in the liver and gills of both the Oz+ and Oz– groups were monitored in this study. The elevated TBARS level in the gills indicates a higher lipid peroxidation activity in the gills. Throughout the experiment, higher TBARS values were found in the gills of the Oz– group compared to the Oz+ group at 0, 7, and 28 days after initiation of the experiment. However, this difference was no longer evident at the end of the experiment. Also, the SOD activity in the gills was significantly higher in the Oz+ group on the 7th day of the experiment. Ritola et al. (2000) used ozone concentrations of 0.34 mg/l/min and 0.69 mg/l/min which were high enough to inactivate *Aeromonas* sp. in the water, but not lethal to Arctic charr (*Salvelinus alpinus*). However, ozonation did cause signs of oxidative stress in the blood, but not in the liver during a 30 min exposure time. Arctic charr responded to ozonation quickly by activating a glutathione-dependent defence system in the blood by a high oxidized glutathione level and elevated total glutathione and glutathione peroxidase in the liver.

CONCLUSION

To sum up, using water ozonation in a RAS beneficially affected the survival rate of the pikeperch, lowered the incidence of fin damage and the occurrence of *I. multifiliis*. No effects of ozonation were found in the examined blood plasma biochemical parameters of the pikeperch. The differences in effect of oxidising and reducing substances in the water were observed only on the gills and were not confirmed at the end of the long-term test. These findings suggest that an ozone treatment could be used as an effective and relatively safe disinfection strategy in an intensive pikeperch culture in a RAS.

Conflict of interest

The authors declare no conflict of interest.

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