Animal 18 (2024) 101347

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

Effects of oxygen levels and temperature on growth and physiology of pikeperch juveniles cultured in a recirculating aquaculture system

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ARTICLE INFO

Article history:
Received 17 April 2024
Revised 19 September 2024
Accepted 23 September 2024
Available online 2 October 2024

Keywords:
Environment
Intensive fish culture
Percid fish
Production
Welfare

ABSTRACT

This study aimed to understand how environmental factors, specifically water temperature and oxygen saturation, affect the growth performance and physiology of pikeperch (*Sander lucioperca*) juveniles in recirculating aquaculture systems (**RASs**). Given the importance of optimising growth conditions in aquaculture to maximise efficiency, it aims to assess whether different combinations of oxygen levels and temperatures can enhance growth while maintaining the physiological health and welfare of the fish. The experimental design included the culturing pikeperch juveniles (22.7 ± 7.1 g) were exposed to hypoxia (78 ± 14%), normoxia (105 ± 12%), and hyperoxia (140 ± 18%) conditions for 72 days. This was conducted at two temperatures, 20 °C and 23 °C, each in a separate but identical RAS. The level of oxygen supply was controlled with micro bubble diffusers on the bottom of each tank. The hyperoxia at 23 °C positively affected total length, BW, specific growth rate, feed intake and feed conservation rate (**FCR**). The slowest growth and feed intake, along with the highest FCR, were observed in hypoxia at 20 °C. Fish reared under 23 °C exhibited significantly higher visceral-somatic index (3.54 ± 0.83 at 23 °C and 2.76 ± 0.73 at 20 °C) regardless of oxygen levels. It was primarily responsible for the observed growth difference (Final BW: 58.3 ± 18.8 g at 23 °C and 53.0 ± 18.3 g at 20 °C). The water temperature also affected haematocrit, haemoglobin, leucocyte count, mean corpuscular haemoglobin, mean corpuscular volume (**MCV**) of the blood cells; the concentration of lymphocytes, neutrophile granulocyte bands and segments. Among biochemical markers, temperature affected cytoplasmic and mitochondrial enzymes, ammonia and triglyceride levels in blood plasma. Elevated antioxidant activity was observed in muscle, intestine and liver tissues. Oxygen levels demonstrated significant effects on growth, feed intake and conversion, the MCV of the blood cells, the concentration of the glucose, lactate and ammonia in blood plasma, and antioxidant biomarkers in the liver tissue. The analysis indicated a significant effect of oxygen on energy metabolism. The results showed hyperoxia under 23 °C create conditions for the highest growth and feed intake, high feed utilisation. There are, however, concerns about the physiological conditions and welfare of intensively cultured pikeperch juveniles, as higher feed intake led to increased visceral fat content in the body, elevated antioxidant activity in the liver, muscle and intestine tissues, morphology of blood cell, and energy metabolism.

Implications

Pikeperch aquaculture production in a recirculating aquaculture system stands as a promising tool for diversifying European freshwater aquaculture. To achieve stable recirculating aquaculture system operation, optimal oxygen levels and water temperature are crucial. We found that hyperoxia at 23 °C improved growth, feed intake, and feed conversion, while hypoxia at 20 °C led to poorer outcomes. However, the growth benefits of hyperoxia also raised concerns about fish welfare, with increased visceral fat and physiological stress. These findings suggest that while hyperoxia conditions at warmer temperatures can optimise growth in aquaculture, careful monitoring of fish health is needed to prevent negative impacts.

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https://doi.org/10.1016/j.animal.2024.101347
1751-7311/

Introduction

Pikeperch (*Sander lucioperca* L.) is effectively used for the diversification of intensive freshwater aquaculture in Europe. Nevertheless, accounting overall share of the production, most market-sized pikeperch are still caught in open waters, as aquaculture production has a niche character and upscaling is limited (Policar et al., 2019). The most recent progress in pikeperch culture is vastly related to the zoo-technological aspects as the broodstock management (Kucharczyk et al., 2022; Malinovskyi et al., 2021), reproduction (Knowles et al., 2022; Kristan et al., 2018), larval (Imentai et al., 2019; Yanes-Roca et al., 2020), juvenile (Penka et al., 2021) and grow-out culture (Ende et al., 2021). More complex approaches such as induction out of season spawning (Özgür et al., 2021), domestication (Khendek et al., 2018), use of alternative proteins (Tran et al., 2021), genetic strain selection (Tsaparis et al., 2022) and ploidy manipulation (Dadras et al., 2021; Jenő et al., 2021) have potential to further contribute to the production. Nevertheless, the importance of technological innovations cannot be overstated, as the longest production phase – the grow-out; has the highest cost input, playing a crucial role in cost-effectiveness (Ende et al., 2021; Kolarova et al., 2021; Steinberg et al., 2019, 2017).

Within the controlled environment of recirculating aquaculture systems (**RASs**), dissolved oxygen concentration and water temperature are critical factors influencing fish survival, growth, feed consumption, and health status (Schäfer et al., 2021b; Stejskal et al., 2009). Oxygen levels are particularly vital in intensive aquaculture, and its inadequate levels can compromise the physiology of fish (Schäfer et al., 2021b). Adequate oxygen saturation contributes to enhanced feed intake, efficient feed utilisation, and shortened production cycles (Elbialy et al., 2020; Schäfer et al., 2021a). Furthermore, it directly impacts physiological parameters, metabolic rates, influencing their growth performance. There is also a certain risk of high level of oxygen−inducing oxidative stress and changes within antioxidant defence systems (McArley et al., 2021). Similarly, the combined effects of temperature and oxygen saturation can affect the growth and stress fish performances (Mock et al., 2022). Elevated oxygen saturation levels also enhance the respiratory efficiency, a factor that becomes particularly crucial during bacterial and parasite disease outbreaks (Rupp et al., 2019). Such outbreaks can lead to damage to the gill tissue, diminishing its capacity for oxygen extraction and ammonia excretion (Kolarova et al., 2021; Meinelt et al., 2007). Hence, maintaining heightened levels of oxygen saturation is not only essential for routine metabolic processes but also becomes imperative in mitigating the adverse effects of disease-induced gill damage, ensuring the respiratory well-being of pikeperch in intensive aquaculture settings.

Pikeperch temperature optimum is in the range of 21–27 °C, depending on the age, and is highest for juveniles (Frisk et al., 2012). Maintaining optimal oxygen levels within this temperature range in RAS can be challenging due to the lower solubility of oxygen in warmer water (Jiang et al., 2021). Adjusting the RAS to higher water temperatures would impact the general physiology and metabolic rate of the fish, alongside influencing oxygen consumption rates and gill ventilation (Schäfer et al., 2021b). Such modifications necessitate alterations to the system's design, including adjustments to filtration, oxygen saturation and water flow rate, to maintain stability. That makes the interplay between dissolved oxygen and temperature determining, especially during the grow-out phase, which is the longest and most cost-intensive phase of pikeperch aquaculture. Optimising this interplay can significantly enhance production efficiency and cost-effectiveness of the grow-out. Therefore, targeted research addressing these factors is essential for improving the sustainability and profitability of pikeperch production in RAS.

The study aimed to determine the interactive effect of dissolved oxygen and water temperature on the growth and physiological status of pikeperch juveniles cultured in RAS. By exploring their interaction in context of the physiological status of the fish, the study aims to provide practical insights for the enhanced growth, and physiology of pikeperch juveniles. Additionally, the research aims to contribute to sustainable practices in freshwater aquaculture, addressing the specific needs and challenges of pikeperch production.

Material and methods

Ethical statement

This study was performed in accordance with national and international guidelines for the protection of animal welfare (EU-harmonized Animal Welfare Act of the Czech Republic) and to the Czech National Directive (Law against Animal Cruelty, No. 246/1992).

Experimental fish and environmental conditions

Pikeperch juveniles used in this study were produced at the experimental aquaculture hall of the Laboratory of Intensive Aquaculture, Faculty of Fisheries and Protection of Waters, University of South Bohemia (FFPW USB) from hatched larvae after the nest spawning (Malinovskyi et al., 2018) and by the following larval culture using the combined system of pond and RAS aquaculture according to Policar et al. (2016). The experimental fish were distributed randomly into 18 tanks (1.5 m^3 volume each) within two identical RAS in the mentioned experimental hall. Each tank was stocked with 1 300 pcs. of high-quality experimental pikeperch (age 5 months) with initial BW = 22.7 ± 7.1 g (mean ± SD) and total length = 132.7 ± 13.0 mm without any morphological deformities with initial fish density 0.87 fish/L and biomass 19.7 kg/m^3. Before the start of the experiment, the fish were given 10 days to acclimate on the tank, fish density and other environmental conditions, such as water temperature 20 ± 0.7 °C (18.6–21.4 °C) or 23 ± 0.8 °C (21.4–24.6 °C), photoperiod regime 12L/12D (light = L/dark = D) with light intensity 75 ± 10 lux (55–95 lux), pH 6.8 ± 0.2 (pH 6.6–7.0), total ammonium (NH_4^+) 0.27 ± 0.15 mg/L (0–0.57 mg/L) and nitrite (NO_2^-) 0.19 ± 0.07 mg/L (0.05–0.33 mg/L) concentrations, biological oxygen demand 7.8 ± 2.1 mg/L (3.6–12 mg/L) and chemical oxygen demand 10.2 ± 2.5 mg/L (5.2–15.2 mg/L). The parameters of water quality in both RAS are summarised in Table 1. To maintain water quality, 30% of the water flow in each system was treated with an ozone generator, a disinfection unit producing ozone at a dose of 10 g/h (ozone generator model OT 10, Ozontech, Czech Republic; Kolarova et al., 2021).

Each experimental RAS attributed one water temperature regime (20 and 23 °C) and comprised nine circular tanks (each volume 1.5 m^3) with a total water volume 30 m^3 including volume for pikeperch culture (13.5 m^3) and water treatment management (16.5 m^3) with mechanical and biological filtration. Three tanks within each RAS provided hypoxia (78 ± 14%), normoxia (105 ± 12%) and hyperoxia (141 ± 18%) conditions in three repetitions, occupying all 18 tanks. The general modulation of dissolved oxygen was done using oxygen cone C-0 (Aquacultur Fischtechnik, Germany) incorporated within the RAS, which was set to maintain the hypoxia levels of the oxygen saturation for the whole recirculating system. This general oxygen saturation of water was then supplemented by different intensities of pure oxygen supply to achieve normoxia and hyperoxia levels of saturation. The intensity

Table 1
Summary of water quality parameters from two identical recirculating aquaculture systems (RAS) during 72 days of pikeperch (*Sander lucioperca*) culture under different oxygen saturation levels and water temperatures.

Parameters	RAS 1	RAS 2	F-statistics	One-way ANOVA p-value
Water temperature, °C	20.0 ± 0.68	23.0 ± 0.8	F(1, 142) = 693.0	<0.005
pH	6.78 ± 0.15	6.72 ± 0.11	F(1, 142) = 1.027	0.312
NH_4^+, mg/L	0.26 ± 0.14	0.28 ± 0.10	F(1, 142) = 0.972	0.320
NO^{2-}, mg/L	0.19 ± 0.08	0.20 ± 0.06	F(1, 142) = 0.018	0.900
BOD, mg/L	7.94 ± 1.94	7.59 ± 1.94	F(1, 142) = 1.174	0.276
COD, mg/L	10.3 ± 1.72	10.2 ± 1.70	F(1, 142) = 0.008	0.983

Abbreviations: NH_4^+ = Total ammonium; NO^{2-} = Nitrite; BOD = Biological oxygen demand; COD = Chemical oxygen demand.

of the additional oxygen supply was managed with micro bubble diffusers (Pentair Aquatic Eco-Systems, USA) placed on the bottom of each tank. The oxygen was sourced of the 600-litre pressure tank with liquid oxygen (Linde gas s.r.o., Czech Republic) placed outside of the experimental hall. The selected temperature and oxygen saturation ranges align with the biological optimum for pikeperch, thus not presenting any welfare concerns. However, they remain important variables that can be adjusted during the intensive culture of this species.

Monitoring of water quality and light intensity

The oxygen saturation and water temperature were measured continuously in each tank with an automatic sensor (SC 1000, HACH Lange, Düsseldorf Germany). The pH, light intensity, NH_4^+, NO^{2-} and residual ozone concentrations were measured once per day at 0800 h. in each system. The pH was measured with a portable probe (WTW 3310 meter, Tepec, s.r.o., Prague, Czech Republic). The light intensity was measured with a UNITEST 93514 digital luxmeter (Beha-Amprobe GmbH, Glottertal,. Germany). The concentration of NH_4^+ was determined using a simple titration and colorimetric reference kit using Nessler's reagent and Seignett salt. Nitrite concentration was analysed with a handheld titration and colorimetric kit using sulphanilic acid (C6H7NO3S) and NED solution (N-(1-naphthyl) ethylenediamine dihydrochloride). Using these kits, the approximate concentration of ammoniacal nitrogen (NH_4^+–N) in mg/l and nitrite nitrogen (NO_2^-–N) in mg/l was determined. Subsequently, both analysed concentrations were recalculated using coefficients for the final concentration of NH_4^+ and NO^{2-} ions. Any residual ozone was not evident in fish tanks during the experiment, and this parameter was measured using an ozone test kit (0–2.3 mg/l, Model Oz-2; HACH Company, Loveland, Colorado, USA) according to Kolářová et al. (2021). Parameters of biological oxygen demand and chemical oxygen demand were measured in the hydrochemical laboratory of FFPW USB at 2-week intervals during the whole experiment. The biological oxygen demand was determined monometrically on an OxiTop® OC 100 instrument (Tepec, s.r.o., Prague, Czech Republic) according to DIN 38409. The chemical oxygen demand was determined with potassium permanganate according to the Kubel method – direct heating with a hot plate.

Fish feeding and calculation of growth and survival performance, feed utilisation and fish body condition parameters

The duration of the experiment was 72 days. At both the start and the end of the trial a total length, standard length (mm) and BW of 100 randomly selected experimental fish per tank were measured. Before each biometric measurement, the fish were anesthetised in clove oil with a concentration of 33 mg/L.

During the experiment, experimental fish were fed with floating pellets R-2 Europa 15F (Skretting, France) currently used for successful pikeperch intensive culture (Penka et al., 2021) with the size of pellets 2 mm, containing 55.0% CP, 16.0% crude fat, 16.5% carbohydrates, 0.6% fibre, 10% crude ash and straight energy 19.4 MJ/kg. The feeding was done by combining the automatic belt feeders set for the 8 h duration, and manual feeding through the light phase of the day. Fish were fed *ad libitum* until the uneaten feed was present on the water's surface. The feed delivered each day was weighed, and finally, uneaten pellets were deducted for the calculation of the total feed intake (g) presenting the total dry feed consumption per final survived fish. The Fultońs coefficient (**FC**) of stocked and finally produced pikeperch was calculated in each tank at the beginning and at the end of the experiment. The specific growth rate (**SGR**), survival rate (**S**) and feed conversion ratio (**FCR**) were calculated at the end of the experiment also in each tank. All mentioned parameters were calculated as follows:

$$FC = BW \times 10^5/TL^3$$

$$SGR\,(\%/d) = [(\ln FBW - \ln IBW)/d] \times 100$$

$$Survival\,(\%) = (FNSF/INSF) * 100$$

$$FCR\,(g/g) = FI/(FBW - BWI)$$

where:BW – body weight (g),TL – total length (cm),lnFBW – natural logarithm for the final BW of fish at the end of the experiment, lnIBW – natural logarithm for the initial BW of fish,d – duration of the experiment (days),FNSF – final number of survived fish (pcs.),INSF – initial number of stocked fish (pcs.),FI– the total feed intake = the total dry feed consumption per final survived fish, FBW (g) – the final BW of fish at the end of the experiment,IBW (g) – the initial BW of fish at the beginning of the experiment (g).

Assessment of blood haematological and plasma biochemical parameters

Six randomly selected fish individuals from each experimental group were used for following blood sampling, resulting in a total of 36 samples. Blood was drawn from the caudal vessels of anesthetised pikeperch with clove oil (33 mg/L) at the end of the experiment. Blood samples were individually collected using heparinised (0.01 ml Heparin inj. sol., Leciva, Zentiva Group, Czech Republic) syringes to stabilise of 1 ml blood.

Immediately after the sampling, the blood samples were analysed to determine haematocrit, haemoglobin, erythrocyte count (**RBC**), leucocyte count, mean corpuscular haemoglobin, mean corpuscular volume and differential leucocyte count as: lymphocytes, monocytes, neutrophile granulocytes (bands and segments) according to Svobodová et al. (2012).

Biochemical analyses were determined according to Kolářová and Velíšek (2012). Fish blood was centrifuged (4 °C, 1 073 × g, 10 min) and blood plasma was separated. The plasma samples

were stored at –80 °C until analysis. The biochemical blood profile was determined using the VETTEST 8008 analyzer (IDEXX Laboratories, USA). The VETTEST 8008 is self-testing and self-calibrating device. Periodic quality control analysis is carried out according to the device manufacturer's instructions. Sample analysis was carried out on selective testing discs (Multilayer film slides, Kodak) by means of a laser reading the bar codes. The methodology followed the procedures outlined by Kolářová and Velíšek (2012).

The plasma biochemical parameters including the aspartate transaminase, alanine aminotransferase, creatine kinase, lactate dehydrogenase, alkaline phosphatase, lactate, total protein, albumin, globulin, glucose, ammonia, triglyceride, creatinine, magnesium, calcium and phosphorus were analysed during the measuring. QA/QC measures were consistently applied according to validated standard operation procedures.

Calculation of fish body condition parameters

After the blood sampling, all sampled fish from each group/tank were killed by severing the spinal cord in accordance with guidelines of animal welfare. BW of each fish as the whole, also visceral fat, liver and spleen in each fish were weighted for the calculation of the visceral-somatic (**VSI**) and hepato-somatic (**HSI**) and the spleen-somatic (**SSI**) indices according to formulas:

$$\mathrm{VSI}\,(\%) = (\mathrm{VW}/\mathrm{BW}) \times 100$$

$$\mathrm{HSI}\,(\%) = (\mathrm{LW}/\mathrm{BW}) \times 100$$

$$\mathrm{SSI}\,(\%) = (\mathrm{SW}/\mathrm{BW}) \times 100$$

where:VW (g) is the weight of the visceral fat,LW (g) is the weight of the liver,SW (g) is spleen weight,BW (g) is the body weight.

After weighing the mentioned tissues, the liver, muscle and intestine samples were quickly collected and frozen from each killed fish and stored at –80 °C for 28 days pending analysis of the oxidative stress and antioxidant activity parameters.

Oxidative stress and antioxidant activity parameters of selected tissues

Before the analysis of oxidative stress and antioxidant activity parameters, the frozen samples of liver, muscle and intestine were weighed and homogenised (1:10 w/v) by the ball homogenizer (TissueLyser II QIAGEN) in 50 mM of a cooled potassium phosphate buffer, pH 7.0, containing 0.5 mM ethylenediaminetetraacetic acid. Ice was used for cooling during the homogenisation. The homogenate was halved, with a portion for thiobarbituric acid reactive substances and another centrifuged (Micro 200 R, Hettich, Germany) (at 12 000 × g) for 30 min at 4 °C to obtain the postmitochondrial supernatant for the antioxidant enzyme analyses.

Reactive oxygen species were measured using 2,7-dichlorofluorescin diacetate. The oxidative damage was evaluated by lipid peroxidation, calculated from the thiobarbituric acid reactive substances assay. The total superoxide dismutase activity was determined spectrophotometrically at 420 nm. The catalase activity was measured spectrophotometrically at 240 nm. The glutathione reductase activity was determined spectrophotometrically at 340 nm by measuring the oxidation of nicotinamide adenine dinucleotide phosphate. The glutathione peroxidase activity (**GPx**) was derived from the rate of NADPH oxidation at 340 nm by coupled reaction with Glutathione reductase. The amount of reduced glutathione (**GSH**) was determined according to Ferrari et al. (2007). The enzymatic activity of glutathione-S-transferase (**GST**) was determined spectrophotometrically at 340 nm. The protein levels were estimated spectrophotometrically using bovine serum albumin as a standard.

Statistical analysis

All data were analysed by Statistica v. 13 (StatSoft, Czech Republic). Prior to statistical analysis, the normality of the residuals was checked with Shapiro-Wilk's test and the data were log transformed if data did not exhibit normality (West, 2021). The group-to-group statistical comparison was made by analysis of variance (one-way ANOVA), followed by Tukey's posthoc comparison test. Two-way factorial ANOVA was used for the determination of the effects of oxygen levels and water temperature on the growth (total length, standard length, BW and SGR), feed intake and utilisation (IF and FCR) and fish body condition parameters (FC, VSI, HSI and SSI), haematological and biochemical parameters, oxidative stress and antioxidant activity parameters. Statistical significance was set at $P < 0.05$. All values are presented as mean ± SD.

Results

Growth and survival, feed utilisation and fish body condition parameters

The oxygen level significantly affected all growth and feed utilisation parameters ($P < 0.005$; Table 2) and did not affect S, FC, VSI, HSI and SSI (Table 3). The temperature had a significant influence on the total length, BW, SGR, feed intake ($P < 0.005$; Table 2), FCR ($P = 0.013$) and VSI ($P = 0.006$; Table 3). The total length was greater in hyperoxia (199–192 mm) and normoxia (193–196 mm) under both tested temperatures. The total length was lower at hypoxia under 20 °C (183 mm). The standard length was higher at hyperoxia and normoxia under both temperatures (163–167 mm), and lower at hypoxia under both temperatures (155–156 mm). The BW was higher at hyperoxia under 23 °C (62.9 g) and lower at hypoxia under 20 °C (47.1 g). The SGR varied in a similar pattern as BW and total length displaying higher values at hyperoxia under 23 °C (1.51%/d) and the lower at hypoxia under both temperatures (1.04–1.16%/d). The survival rate ranged from 95.0 to 97.8% without significant differences among groups (Table 2). The increase of feed intake was evident at hyperoxia under 23 °C (39.4 g per fish) and was significantly higher compared to hypoxia under 20 °C (27.6 g per fish). The better FCR was observed at hyperoxia (0.98 g/g) and normoxia (0.97 g/g) under 23 °C and was higher at hypoxia under 20 °C (1.13 g/g). Generally, higher VSI values were under 23 °C (VSI = 3.22–3.78%) compared to 20 °C (VSI = 2.54–3.10%) without significant effect of temperature. The only parameter significantly ($P = 0.005$) affected by the interaction between oxygen level, and temperature was HSI with the higher values observed in 23 normoxia and 20 hypoxia (2.12 and 2.06, respectively). There was no significant effect of temperature and oxygen level on SR, FC and SSI.

Blood haematological parameters

There was a prevailing effect of water temperature on the studied haematological parameters of blood (Table 4). The sole effect of oxygen was only evident for values of mean corpuscular volume. The effect of temperature was significant for all of the haematological parameters with the exception of RBC and monocytes. The oxygen level and temperature combination significantly affected leucocyte count and mean corpuscular haemoglobin parameters.

The values haematocrit, haemoglobin, mean corpuscular haemoglobin, mean corpuscular volume and neutrophile granulocyte bands were higher at hyperoxia under 23 °C and at hyperoxia and

Table 2
Growth performance, survival, feed utilisation, and body condition parameters of pikeperch (*Sander lucioperca*) intensively cultured at different oxygen levels (hypoxia = 78 ± 14%, normoxia = 105 ± 12%, and hyperoxia = 141 ± 18%) under two water temperatures (20 °C and 23 °C) for 72 days.

Parameters	Groups						F-statistics	One-way ANOVA *P*-value
	20-Hypoxia	20-Normoxia	20-Hyperoxia	23-Hypoxia	23-Normoxia	23-Hyperoxia		
Survival, %	97.1 ± 0.38	96.3 ± 1.15	97.7 ± 1.40	96.1 ± 1.10	96.9 ± 0.66	95.9 ± 0.70	F(5,12) = 0.7628	0.593
TL	183 ± 15.5b	193 ± 22.3a	192 ± 22.4a	189 ± 16.0ab	196 ± 19.6a	199 ± 21.9a	F(5, 624) = 8.4371	<0.005
SL	155 ± 14.6b	167 ± 17.7a	166 ± 19.8a	156 ± 14.6b	163 ± 16.8a	165 ± 15.9a	F(5, 624) = 10.927	<0.005
BW	47.1 ± 14.5c	55.2 ± 18.2b	56.7 ± 20.4b	52.1 ± 15.4bc	59.8 ± 18.8b	62.9 ± 20.5a	F(5, 624) = 10.041	<0.005
CF	0.75 ± 0.09	0.75 ± 0.09	0.80 ± 0.39	0.76 ± 0.10	0.77 ± 0.08	0.80 ± 0.28	F(5, 624) = 1.2277	0.294
SGR	1.04 ± 0.66b	1.24 ± 0.65b	1.28 ± 0.75b	1.16 ± 0.64a	1.40 ± 0.63ab	1.51 ± 0.63b	F(5, 624) = 6.8631	<0.005
FCR	1.12 ± 0.03a	1.02 ± 0.03ab	1.04 ± 0.04ab	1.07 ± 0.01ab	0.97 ± 0.05c	0.98 ± 0.03bc	F(4, 13) = 5.9702	0.006
FI	26.1 ± 0.93b	28.6 ± 0.44ab	29.6 ± 1.20a	28.2 ± 1.56ab	29.2 ± 0.67a	30.5 ± 0.74a	F(4, 13) = 8.3108	0.002

Means of main effects

Parameters	Water temperature		Oxygen level			Factorial ANOVA p-value		
	20 °C	23 °C	Hypoxia	Normoxia	Hyperoxia	Temperature	Oxygen	Temperature*Oxygen
Survival, %	96.7 ± 1.00	96.3 ± 0.86	96.6 ± 0.95	96.6 ± 0.90	96.3 ± 1.07	0.383	0.817	0.310
TL	189 ± 20.7	195 ± 19.8	186 ± 16.0	194 ± 21.0	195 ± 22.4	<0.005	<0.005	0.566
SL	163 ± 18.3	161 ± 16.2	155 ± 14.6	165 ± 17.4	166 ± 17.9	<0.005	0.370	0.233
BW	53.0 ± 18.3	58.3 ± 18.8	49.6 ± 15.1	57.5 ± 18.6	59.8 ± 20.6	<0.005	<0.005	0.896
CF	0.77 ± 0.24	0.78 ± 0.18	0.75 ± 0.10	0.76 ± 0.09	0.80 ± 0.34	0.067	0.536	0.844
SGR	1.19 ± 0.69	1.36 ± 0.65	1.10 ± 0.65	1.32 ± 0.64	1.40 ± 0.70	<0.005	<0.005	0.708
FCR	1.06 ± 0.05	1.01 ± 0.06	1.09 ± 0.03	1.00 ± 0.05	1.01 ± 0.05	0.013	<0.005	0.877
FI	28.1 ± 1.77	29.3 ± 1.35	27.1 ± 1.64	28.9 ± 0.60	30.0 ± 1.02	0.024	<0.005	0.385

Values are presented as mean ± SD with different letters indicating significant differences at $P < 0.05$. Abbreviations: TL = Total length (mm); SL = Standart length (mm); CF = Condition factor = [Body weight (g) / Standard length^3] × 100; SGR = Specific growth rate = [ln final body weight − ln initial body weight) / days] × 100; FCR = Feed conversion ratio = dry feed fed / weight gain; FI = Feed intake (g fish-1) = dry feed consumed (g) / fish.

Table 3
Body mass indices of pikeperch (*Sander lucioperca*) intensively cultured at different oxygen levels (hypoxia = 78 ± 14%, normoxia = 105 ± 12%, and hyperoxia = 141 ± 18%) under two water temperatures (20 °C and 23 °C) for 72 days.

Parameters	Groups						F-statistics	One-way ANOVA *P*-value
	20-Hypoxia	20-Normoxia	20-Hyperoxia	23-Hypoxia	23-Normoxia	23-Hyperoxia		
VSI	3.62 ± 0.43	3.22 ± 0.88	3.78 ± 1.10	3.10 ± 0.72	2.54 ± 0.51	2.64 ± 0.89	F(5, 30) = 2.3981	0.061
HIS	2.06 ± 0.46a	1.74 ± 0.40ab	1.91 ± 0.31ab	1.32 ± 0.23b	2.12 ± 0.58a	1.83 ± 0.15ab	F(5, 30) = 3.3485	0.016
SSI	0.04 ± 0.03	0.08 ± 0.04	0.04 ± 0.03	0.05 ± 0.02	0.05 ± 0.03	0.04 ± 0.04	F(5, 30) = 1.3442	0.273

Means of main effects

Parameters	Water temperature		Oxygen level			Factorial ANOVA p-value		
	20 °C	23 °C	Hypoxia	Normoxia	Hyperoxia	Temperature	Oxygen	Temperature*Oxygen
VSI	3.54 ± 0.83	2.76 ± 0.73	3.36 ± 0.63	2.88 ± 0.77	3.21 ± 1.12	0.006	0.325	0.609
HIS	1.90 ± 0.40	1.76 ± 0.49	1.69 ± 0.52	1.93 ± 0.52	1.87 ± 0.24	0.261	0.311	0.005
SSI	0.05 ± 0.04	0.05 ± 0.03	0.05 ± 0.03	0.06 ± 0.04	0.04 ± 0.03	0.735	0.209	0.208

Values are presented as mean ± SD with different letters indicating significant differences at $P < 0.05$. Abbreviations: VSI = Visceral-somatic index (%) [VW/BW] × 100; HIS = Hepato-somatic index (%) [LW/BW] × 100; SSI = Spleen-somatic index (%) [SW/BW] × 100; VW = Visceral fat weight, g; LW = Liver weight, g; SW = Spleen weight, g.

normoxia 23 °C, and were significantly lower at 20 °C. Vice versa, under 23 °C, the significantly lower leucocyte count and lymphocytes were observed at all oxygen levels. The neutrophile granulocyte segments were significantly higher at hypoxia under 23 °C compared to 20 °C. The levels of RBC and monocyte parameters were without significant differences in all tested groups (Table 3).

Blood plasma biochemical parameters

Similarly, to haematological parameters, there was a prevailing effect of temperature on biochemical markers of blood plasma. The effect of oxygen level was evident in lactate, glucose and ammonia (Table 5). The temperature had a significant effect on all studied cytoplasmatic and mitochondrial enzymes and also on ammonia and triglyceride. The interaction between temperature and oxygen significantly affected only creatine kinase. All analysed blood proteins, minerals and creatinine were not affected, neither oxygen nor temperature without a significant difference among groups.

The highest values of aspartate transaminase, alanine aminotransferase, creatine kinase, lactate dehydrogenase and alkaline phosphatase were at all oxygen levels under 23 °C. The lowest aspartate transaminase, alanine aminotransferase, creatine kinase, lactate dehydrogenase parameters were found at all oxygen levels under 20 °C. The alkaline phosphatase was the lowest only at hypoxia under 20 °C. The lactate was the highest at hypoxia under 23 °C and the lowest at normoxia and hyperoxia under 20 °C.

The highest glucose was evident at hypoxia under both temperatures. The lowest glucose were at normoxia and hyperoxia under both temperatures. The highest ammonia was found at hypoxia under 23 °C and the lowest at normoxia and hyperoxia under 20 °C. The fluctuation of triglyceride values corresponded to the water temperature, regardless of oxygen level (Table 5).

Oxidative stress and antioxidant biomarkers

The antioxidant activity was significantly affected by the water temperature. The oxygen levels and temperature did not affect

Table 4
Haematological profile of pikeperch (*Sander lucioperca*) intensively cultured at different oxygen levels (hypoxia = 78 ± 14%, normoxia = 105 ± 12%, and hyperoxia = 141 ± 18%) under two water temperatures (20 °C and 23 °C) for 72 days.

Parameters	Groups						F-statistics	One-way ANOVA *P*-value
	20-Hypoxia	20-Normoxia	20-Hyperoxia	23-Hypoxia	23-Normoxia	23-Hyperoxia		
PCV (l/l)	0.29 ± 0.05bc	0.30 ± 0.10b	0.30 ± 0.06b	0.37 ± 0.03ab	0.40 ± 0.07ab	0.42 ± 0.04a	F(5, 30) = 5.197	0.001
Hb (g/l)	40.2 ± 5.16ab	44.9 ± 16.1ab	38.1 ± 4.62b	44.3 ± 2.87ab	51.7 ± 4.98ab	53.9 ± 5.65a	F(5, 30) = 3.766	0.009
RBC (T/l)	2.00 ± 0.29	2.06 ± 0.53	2.03 ± 0.36	2.12 ± 0.37	2.32 ± 0.40	1.98 ± 0.23	F(5, 30) = 0.672	0.647
WBC (G/l)	8.27 ± 1.90ab	10.6 ± 4.93ab	14.9 ± 3.58a	10.5 ± 9.35ab	6.62 ± 2.33ab	6.43 ± 1.34b	F(5, 30) = 2.699	0.040
MCH (pg)	20.5 ± 3.99b	21.3 ± 3.56ab	19.4 ± 4.55b	21.3 ± 3.27ab	22.6 ± 2.61ab	27.5 ± 3.59a	F(5, 30) = 3.684	0.010
MCV (fl)	144 ± 26.9b	142 ± 15.5b	149 ± 25.3b	176 ± 30.0ab	173 ± 18.1ab	214 ± 25.5a	F(5, 30) = 7.879	<0.005
MCHC (g/l)	142 ± 9.29	151 ± 23.1	131 ± 31.0	122 ± 15.9	132 ± 19.1	129 ± 15.1	F(5, 30) = 1.527	0.211
Lymphocytes (%)	96.1 ± 1.65	96.0 ± 1.79	96.5 ± 2.10	93.2 ± 4.45	92.0 ± 5.19	92.0 ± 4.44	F(5, 30) = 2.136	0.088
Monocytes (%)	1.17 ± 0.91	1.47 ± 0.99	2.12 ± 1.95	1.03 ± 1.04	3.42 ± 2.96	2.00 ± 0.95	F(5, 30) = 1.684	0.169
NGB (%)	0.53 ± 0.48	1.32 ± 0.39	0.25 ± 0.27	1.05 ± 0.60	2.08 ± 2.50	2.67 ± 2.32	F(5, 30) = 2.453	0.056
NGS (%)	2.25 ± 0.83ab	1.18 ± 1.59ab	1.12 ± 0.62b	4.72 ± 3.67a	2.50 ± 1.84ab	3.33 ± 1.99ab	F(5, 30) = 2.748	0.037

Means of main effects

Parameters	Water temperature		Oxygen level			Factorial ANOVA p-value		
	20 °C	23 °C	Hypoxia	Normoxia	Hyperoxia	Temperature	Oxygen	Temperature*Oxygen
PCV (l/l)	0.29	0.39	0.33	0.35	0.36	<0.005	0.395	0.74
Hb (g/l)	41.1	50.0	42.2	48.3	46.0	<0.005	0.174	0.182
RBC (T/l)	2.03	2.14	2.06	2.19	2.00	0.391	0.479	0.584
WBC (G/l)	11.3	7.83	9.37	8.62	10.7	0.038	0.568	0.033
MCH (pg)	20.4	23.8	20.9	22.0	23.4	0.008	0.247	0.036
MCV (fl)	145	187	160	157	181	<0.005	0.041	0.158
MCHC (g/l)	141	128	132	142	130	0.054	0.352	0.494
Lymphocytes (%)	96.2	92.4	94.6	94.0	94.3	0.003	0.916	0.842
Monocytes (%)	1.58	2.15	1.10	2.44	2.06	0.311	0.140	0.223
NGB (%)	0.70	1.93	0.79	1.70	1.46	0.015	0.292	0.230
NGS (%)	1.52	3.52	3.48	1.84	2.23	0.006	0.131	0.765

Values are presented as mean ± SD with different letters indicating significant differences at $P < 0.05$. Abbreviations: PCV = haematocrit (l/l); Hb = haemoglobin (g/l); RBC = erythrocyte count (T/l); WBC = leucocyte count (G/l); MCH = mean corpuscular haemoglobin (pg); MCV = mean corpuscular volume (fl); MCHC = mean corpuscular haemoglobin concentration (g/l); NGB = Neutrophile granulocytes bands (%); NGS = Neutrophile granulocytes segments (%).

Table 5
Biochemical blood plasma profile of pikeperch (*Sander lucioperca*) intensively cultured at different oxygen levels (hypoxia = 78 ± 14%, normoxia = 105 ± 12%, and hyperoxia = 141 ± 18%) under two water temperatures (20 °C and 23 °C) for 72 days.

Parameters	Groups						F-statistics	One-way ANOVA *P*-value
	20-Hypoxia	20-Normoxia	20-Hyperoxia	23-Hypoxia	23-Normoxia	23-Hyperoxia		
ALP (μkat/l)	1.14 ± 0.27	1.28 ± 0.58	1.24 ± 0.38	1.39 ± 0.36	1.56 ± 0.33	1.60 ± 0.25	F(5, 30) = 1.402	0.252
ALT (μkat/l)	1.29 ± 0.17b	1.25 ± 0.22ab	1.27 ± 0.19ab	1.74 ± 0.34ab	1.59 ± 0.29ab	1.55 ± 0.15a	F(5, 30) = 4.601	0.003
AST (μkat/l)	3.00 ± 0.39	3.07 ± 0.35	2.96 ± 0.53	3.32 ± 0.38	3.38 ± 0.34	3.51 ± 0.45	F(5, 30) = 1.801	0.143
Ca (mmol/l)	2.66 ± 0.17	2.60 ± 0.29	2.66 ± 0.13	2.69 ± 0.50	2.61 ± 0.45	2.67 ± 0.71	F(5, 30) = 0.042	0.999
P (mmol/l)	3.77 ± 0.35	3.70 ± 0.43	3.67 ± 0.85	3.54 ± 0.55	3.68 ± 0.38	3.74 ± 0.16	F(5, 30) = 0.152	0.978
GLU (mmol/l)	7.09 ± 0.75a	4.59 ± 0.81b	5.09 ± 0.39b	8.06 ± 1.16a	4.48 ± 0.63b	5.21 ± 0.60b	F(5, 30) = 22.34	<0.005
NH3 (μmol/l)	577 ± 41.3bc	492 ± 14.5c	456 ± 39.4c	795 ± 63.9a	609 ± 97.7b	636 ± 60.5b	F(5, 30) = 25.05	<0.005
TRIG (mmol/l)	2.22 ± 0.24c	2.21 ± 0.12c	2.34 ± 0.33bc	2.78 ± 0.37b	2.80 ± 0.17b	2.89 ± 0.42ab	F(5, 30) = 6.919	<0.005
CK (μkat/l)	13.7 ± 0.58c	15.1 ± 0.83b	14.6 ± 0.81b	17.4 ± 0.69a	15.1 ± 0.79b	16.5 ± 0.46a	F(5, 30) = 21.67	<0.005
LDH (μkat/l)	23.0 ± 1.36b	22.3 ± 0.96b	22.5 ± 1.85b	25.6 ± 0.99a	26.1 ± 0.77a	25.9 ± 0.71a	F(5, 30) = 14.23	<0.005
LACT (mmol/l)	5.59 ± 0.46b	4.10 ± 0.57c	4.60 ± 0.58c	6.60 ± 0.73a	4.80 ± 0.61bc	4.95 ± 0.21bc	F(5, 30) = 15.20	<0.005

Means of main effects

Parameters	Water temperature		Oxygen level			Factorial ANOVA p-value		
	20 °C	23 °C	Hypoxia	Normoxia	Hyperoxia	Temperature	Oxygen	Temperature*Oxygen
ALP (μkat/l)	1.22	1.52	1.26	1.42	1.42	0.026	0.514	0.940
ALT (μkat/l)	1.27	1.63	1.52	1.42	1.41	<0.005	0.471	0.683
AST (μkat/l)	3.01	3.40	3.16	3.22	3.23	0.008	0.902	0.733
Ca (mmol/l)	2.64	2.66	2.67	2.60	2.66	0.898	0.912	0.995
P (mmol/l)	3.71	3.65	3.66	3.69	3.70	0.721	0.976	0.749
GLU (mmol/l)	5.59	5.91	7.57	4.53	5.15	0.210	<0.005	0.210
NH3 (μmol/l)	508	680	686	551	546	<0.005	<0.005	0.118
TRIG (mmol/l)	2.26	2.82	2.50	2.50	2.62	<0.005	0.533	0.987
CK (μkat/l)	14.5	16.3	15.6	15.1	15.5	<0.005	0.187	<0.005
LDH (μkat/l)	22.6	25.9	24.3	24.2	24.2	<0.005	0.947	0.455
LACT (mmol/l)	4.77	5.45	6.10	4.45	4.78	<0.005	<0.005	0.354

Values are means of three replicates and presented as mean ± SD. Significance level was set at $P < 0.05$. Values with different letters indicate significant differences. Abbreviations: GLU = Glucose; TRIG = Triglyceride; ALT = Alanine transaminase; AST = Aspartate aminotransferase; ALP = Alkaline phosphatase; LDH = Lactate dehydrogenase; LACT = Lactate; NH3 = Ammonia; CK = creatinine kinase; Ca = Calcium; P = Phosphorus.

Table 6
Antioxidant activity in the liver of pikeperch (*Sander lucioperca*) exposed to different oxygen levels (hypoxia = 78 ± 14%, normoxia = 105 ± 12%, and hyperoxia = 141 ± 18%) and two water temperatures (20 °C and 23 °C) for 72 days.

Parameters	Groups						F-statistics	One-way ANOVA *P*-value
	20-Hypoxia	20-Normoxia	20-Hyperoxia	23-Hypoxia	23-Normoxia	23-Hyperoxia		
ROS	103 ± 0.83	101 ± 0.90	102 ± 0.76	102 ± 1.92	102 ± 1.35	102 ± 1.26	F(5, 30) = 0.977	0.448
TBARS	0.24 ± 0.04	0.23 ± 0.05	0.23 ± 0.10	0.24 ± 0.13	0.25 ± 0.06	0.28 ± 0.07	F(5, 30) = 0.335	0.888
SOD	0.71 ± 0.08a	0.36 ± 0.08ab	0.41 ± 0.11ab	0.46 ± 0.11ab	0.49 ± 0.07ab	0.51 ± 0.03b	F(5, 30) = 11.81	<0.005
CAT	1.37 ± 0.05c	1.25 ± 0.19b	1.24 ± 0.19b	1.60 ± 0.30ab	1.78 ± 0.45a	1.85 ± 0.25a	F(5, 30) = 6.088	<0.005
GPx	4.21 ± 0.70b	4.36 ± 0.58ab	4.77 ± 0.75ab	5.02 ± 0.45a	5.28 ± 0.40a	5.60 ± 0.45a	F(5, 30) = 5.302	<0.005
GR	0.37 ± 0.09	0.34 ± 0.18	0.39 ± 0.05	0.44 ± 0.10	0.45 ± 0.11	0.45 ± 0.11	F(5, 30) = 0.995	0.438
GSH	7.22 ± 1.36	7.01 ± 0.82	7.19 ± 1.17	8.07 ± 0.74	8.35 ± 0.70	8.52 ± 0.76	F(5, 30) = 2.871	0.031
GST	3.96 ± 0.54b	4.66 ± 0.73ab	4.92 ± 0.91ab	5.05 ± 0.43ab	5.36 ± 0.38a	5.53 ± 0.74a	F(5, 30) = 4.507	0.004

Means of main effects

Parameters	Water temperature		Oxygen level			Factorial ANOVA p-value		
	20 °C	23 °C	Hypoxia	Normoxia	Hyperoxia	Temperature	Oxygen	Temperature*Oxygen
ROS	102	102	102	102	102	0.664	0.333	0.131
TBARS	0.23	0.26	0.24	0.24	0.25	0.382	0.878	0.733
SOD	0.50	0.48	0.59	0.42	0.46	0.695	<0.005	<0.005
CAT	1.29	1.75	1.49	1.52	1.55	<0.005	0.870	0.195
GPx	4.45	5.30	4.62	4.82	5.18	<0.005	0.062	0.966
GR	0.37	0.45	0.40	0.40	0.42	0.044	0.870	0.882
GSH	7.14	8.31	7.64	7.68	7.86	<0.005	0.848	0.779
GST	4.51	5.31	4.50	5.01	5.23	<0.005	0.031	0.635

Values are means of three replicates and presented as mean ± SD. Significance level was set at $P < 0.05$. Values with different letters indicate significant differences. Abbreviations: ROS = Reactive oxygen species (μM/L); TBARS = Thiobarbituric acid (nmol/mg protein); SOD = Superoxide dismutase activity (nmol NBT/min/mg protein); NBT = Nitroblue tetrazolium; CAT = Catalase activity (μmol H_2O_2/min/mg protein); GPx = Glutathione peroxidase activity (mU/mg protein); GR = Glutathione reductase (nmol NADPH/min/mg protein); GSH = Reduced glutathione (nmol GSH/mg protein); GST = Glutathione S-transferase (nmol/min/mg protein).

reactive oxygen species in the liver, muscle and intestine, thiobarbituric acid reactive substances in liver, catalase activity in intestine, GPx, Glutathione reductase and GSH in muscle and GST in intestine. Oxygen level had the effect on superoxide dismutase activity and GST in liver only (Table 6). The temperature had a significant effect on antioxidant activity in liver (catalase activity, GPx, Glutathione reductase, GSH and GST), muscle (thiobarbituric acid reactive substances, superoxide dismutase activity, CT and GST; Table 7) and intestine (thiobarbituric acid reactive substances, GPx, Glutathione reductase and GSH; Table 8) tissues. The combination of oxygen level and temperature affected only superoxide dismutase activity in liver and intestine tissues.

The highest thiobarbituric acid reactive substances in muscle and intestine was found in normoxia and hyperoxia and all oxygen levels under 23 °C, respectively. The lowest thiobarbituric acid reactive substances in muscle was measured at hyperoxia under 20 °C and in intestine at all oxygen levels under 20 °C. The activity of superoxide dismutase activity in liver was significantly higher at hypoxia under 20 °C, in muscle at hyperoxia under 23 °C and in intestine at hyperoxia under 20 °C. Generally, the higher activity

Table 7
Antioxidant activity in the muscle of pikeperch (*Sander lucioperca*) exposed to different oxygen levels (hypoxia = 78 ± 14%, normoxia = 105 ± 12%, and hyperoxia = 141 ± 18%) and two water temperatures (20 °C and 23 °C) for 72 days.

Parameters	Groups						F-statistics	One-way ANOVA *P*-value
	20-Hypoxia	20-Normoxia	20-Hyperoxia	23-Hypoxia	23-Normoxia	23-Hyperoxia		
ROS	102 ± 1.52	101 ± 1.12	102 ± 1.19	102 ± 1.43	101 ± 1.69	102 ± 1.69	F(5, 30) = 0.447	0.812
TBARS	0.28 ± 0.11	0.28 ± 0.09	0.26 ± 0.05	0.30 ± 0.08	0.35 ± 0.09	0.39 ± 0.11	F(5, 30) = 1.758	0.152
SOD	0.30 ± 0.05 cd	0.27 ± 0.06bc	0.25 ± 0.07c	0.38 ± 0.11b	0.42 ± 0.05ab	0.46 ± 0.07a	F(5, 30) = 8.786	0.003
CAT	0.10 ± 0.02	0.10 ± 0.01	0.10 ± 0.03	0.12 ± 0.03	0.14 ± 0.05	0.15 ± 0.04	F(5, 30) = 3.470	<0.005
GPx	3.13 ± 0.70	3.41 ± 0.98	3.23 ± 0.55	3.32 ± 0.48	3.45 ± 0.26	3.44 ± 0.35	F(5, 30) = 0.278	0.922
GR	0.21 ± 0.04	0.22 ± 0.06	0.26 ± 0.10	0.24 ± 0.07	0.26 ± 0.08	0.27 ± 0.10	F(5, 30) = 0.524	0.756
GSH	3.99 ± 0.28	4.32 ± 0.35	4.02 ± 0.74	4.28 ± 0.46	4.33 ± 0.46	4.45 ± 0.50	F(5, 30) = 0.894	0.498
GST	1.23 ± 0.11	1.06 ± 0.09	1.19 ± 0.41	1.31 ± 0.10	1.42 ± 0.40	1.48 ± 0.15	F(5, 30) = 2.225	0.078

Means of main effects

Parameters	Water temperature		Oxygen level			Factorial ANOVA p-value		
	20 °C	23 °C	Hypoxia	Normoxia	Hyperoxia	Temperature	Oxygen	Temperature*Oxygen
ROS	102	102	102	101	102	0.942	0.366	0.929
TBARS	0.28	0.35	0.29	0.32	0.33	0.023	0.593	0.375
SOD	0.27	0.42	0.34	0.35	0.35	<0.005	0.952	0.126
CAT	0.10	0.14	0.11	0.12	0.12	<0.005	0.388	0.409
GPx	3.26	3.41	3.22	3.43	3.34	0.460	0.715	0.928
GR	0.23	0.26	0.22	0.24	0.26	0.298	0.533	0.900
GSH	4.11	4.36	4.13	4.33	4.23	0.137	0.629	0.556
GST	1.16	1.40	1.27	1.24	1.33	0.007	0.655	0.391

Values are means of three replicates and presented as means ± SD. Significance level was set at $P < 0.05$. Values with different letters indicate significant differences. Abbreviations: ROS = Reactive oxygen species (μM/L); TBARS = Thiobarbituric acid (nmol/mg protein); SOD = Superoxide dismutase activity (nmol NBT/min/mg protein); NBT = Nitroblue tetrazolium; CAT = Catalase activity (μmol H_2O_2/min/mg protein); GPx = Glutathione peroxidase activity (mU/mg protein); GR = Glutathione reductase (nmol NADPH/min/mg protein); GSH = Reduced glutathione (nmol GSH/mg protein); GST = Glutathione S-transferase (nmol/min/mg protein).

Table 8
Antioxidant activity in the intestine of pikeperch (*Sander lucioperca*) exposed to different oxygen levels (hypoxia = 78 ± 14%, normoxia = 105 ± 12%, and hyperoxia = 141 ± 18%) and two water temperatures (20 °C and 23 °C) for 72 days.

Parameters	Groups						F-statistics	One-way ANOVA *P*-value
	20-Hypoxia	20-Normoxia	20-Hyperoxia	23-Hypoxia	23-Normoxia	23-Hyperoxia		
ROS	102 ± 1.59ab	101 ± 0.82b	103 ± 0.58a	102 ± 1.25ab	102 ± 1.15ab	102 ± 1.30ab	F(5, 30) = 2.274	0.072
TBARS	0.23 ± 0.07	0.25 ± 0.07	0.27 ± 0.06	0.38 ± 0.21	0.37 ± 0.08	0.33 ± 0.08	F(5, 30) = 2.129	0.089
SOD	0.20 ± 0.05ab	0.19 ± 0.05ab	0.34 ± 0.07a	0.24 ± 0.12b	0.25 ± 0.09ab	0.23 ± 0.08ab	F(5, 30) = 2.512	0.052
CAT	0.17 ± 0.05	0.18 ± 0.05	0.16 ± 0.05	0.15 ± 0.13	0.19 ± 0.11	0.19 ± 0.07	F(5, 30) = 0.214	0.954
GPx	1.24 ± 0.20ab	1.18 ± 0.13ab	0.81 ± 0.49b	1.57 ± 0.38a	1.50 ± 0.48a	1.37 ± 0.23ab	F(5, 30) = 3.703	0.010
GR	0.03 ± 0.04	0.03 ± 0.02	0.03 ± 0.01	0.08 ± 0.05	0.08 ± 0.04	0.08 ± 0.03	F(5, 30) = 4.055	0.006
GSH	1.14 ± 0.15	1.20 ± 0.15	1.15 ± 0.12	1.34 ± 0.35	1.50 ± 0.47	1.64 ± 0.36	F(5, 30) = 2.850	0.032
GST	1.08 ± 0.13	1.18 ± 0.22	1.30 ± 0.18	0.99 ± 0.08	1.11 ± 0.61	1.13 ± 0.13	F(5, 30) = 0.810	0.552

Means of main effects

Parameters	Water temperature		Oxygen level			Factorial ANOVA p-value		
	20 °C	23 °C	Hypoxia	Normoxia	Hyperoxia	Temperature	Oxygen	Temperature*Oxygen
ROS	102	102	102	101	103	0.650	0.055	0.109
TBARS	0.25	0.36	0.30	0.31	0.30	<0.005	0.970	0.580
SOD	0.24	0.24	0.22	0.22	0.28	0.867	0.120	0.029
CAT	0.17	0.18	0.16	0.19	0.17	0.941	0.756	0.780
GPx	1.01	1.48	1.41	1.34	1.09	<0.005	0.075	0.640
GR	0.03	0.08	0.05	0.05	0.05	<0.005	0.954	0.997
GSH	1.16	1.49	1.24	1.35	1.40	<0.005	0.420	0.485
GST	1.19	1.08	1.04	1.15	1.22	0.242	0.311	0.905

Values are means of three replicates and presented as means ± SD. Significance level was set at $P < 0.05$. Values with different letters indicate significant differences. Abbreviations: ROS = Reactive oxygen species (μM/L); TBARS = Thiobarbituric acid (nmol/mg protein); SOD = Superoxide dismutase activity (nmol NBT/min/mg protein); NBT = Nitroblue tetrazolium; CAT = Catalase activity (μmol H_2O_2/min/mg protein); GPx = Glutathione peroxidase activity (mU/mg protein); GR = Glutathione reductase (nmol NADPH/min/mg protein); GSH = Reduced glutathione (nmol GSH/mg protein); GST = Glutathione S-transferase (nmol/min/mg protein).

of other parameters such as catalase activity, GPx, Glutathione reductase, GSH, GST in liver, catalase activity and GST in muscle and GPx, Glutathione reductase and GSH in intestine were affected by the temperature without any effect of the oxygen level.

Discussion

Oxygen level and temperature are among the most important factors affecting growth, survival and physiology directly linked to the profitability of the farming (Buentello et al., 2000; Mock et al., 2022; Stejskal et al., 2009; Wang et al., 2009). These factors are also among the easiest to control within the controlled environment of RAS, as the whole concept of water recirculation is built around it; therefore, trials and observations concerning their effect on the farmed fish are gathering attention within the fish farming industry. Despite the importance of such investigations, their number remains scarce (Podduturi et al., 2020), as a scale needed to conduct a trial is a big limitation for research institutions. At the same time, the commercial farms may have little interest in research cooperation, as they follow specific production goals, and are less flexible for controlled trials. Nevertheless, studies concerning production in RAS provided valuable data about the effect of fish density, feeding frequency and bicultural stock on production performance (Liu et al., 2016; Park et al., 2015; Penka et al., 2023, 2021). It implies that there is a potential for further progress achieved by a deeper cooperation between the scientific community and the industry, in a form of the implementation of the industrial elements within the research institutions, and vice versa (Policar et al., 2019). The current study is notable for using RAS that is close to commercial scale, yet small enough to test setups that might be economically unfeasible such as low temperature and insufficient oxygen saturation, which may not be practical in a full-scale operation.

The pikeperch is classified as a species well-suited for moderate climates, with recommended optimal culture temperatures within the range of 22–23 °C (Dadras et al., 2021; Frisk et al., 2012; Policar et al., 2016; Rónyai and Csengeri, 2008). While certain authors propose higher temperatures, specifically 28 °C, as advantageous for intensive juvenile pikeperch aquaculture (Wang et al., 2009), such temperatures are not commonly employed in commercial settings due to the high risk for bacterial infections (Policar et al., 2019). Pikeperch physiological thermal optimum is within the range of 10–27 °C, with the maximum metabolic rate occurring at 26 °C (Frisk et al., 2012). Elevated temperatures in pikeperch have been associated with increased growth rates, feed intake, and feed utilisation, resulting in a shortened production cycle (Wang et al., 2009). These factors have determined commercial pikeperch farm setups targeting water temperature of 22–23 °C, ensuring high efficiency in intensive pikeperch aquaculture and minimising risks of bacterial diseases, resulting in lower fish mortality. The current study conducted tests at 20 and 23 °C, representing the typical temperature range utilised for pikeperch, confirming that higher temperatures support the growth performance of pikeperch juveniles. Through the 72−days experimental trial temperature had positively affected feed intake and utilisation, resulting in significantly better length and weight indices. In the study of Rónyai and Csengeri (2008), the temperature (20 °C and 25 °C) primarily affected the maximally consumable daily ration rather than feed efficiency. In current study, the higher temperature had a positive effect on growth and feed utilisation, however, as well resulted in higher visceral fat levels, being partly responsible for observed increased growth (Table 2). At this stage of culture, such an exchange may not pose any risk for the health status of the fish or its economic value, although it depends on the aims. As an example, higher levels of visceral fat are not favoured for culturing broodstock but may be beneficial when pikeperch juveniles are intended for stocking outdoors, when higher fat storage ensures an increased capacity of energy that would benefit survival (Policar et al., 2016). An option of restricted feed portions at higher water temperature should also be considered (Mattila et al., 2009), and would help to select the most beneficial feeding strategy, prioritising certain production criteria.

A current study confirmed the significance of oxygen saturation for the growth performance of pikeperch juveniles. Even though tested settings included hypoxia conditions, they still had no com-

promising influence on the health status and welfare of the fish, as these values were within the range of tolerance for pikeperch and allowed to display acceptable growth performance (Frisk et al., 2012). Similarly to water temperature, higher levels of oxygen saturation enhanced feed utilisation, which resulted in significantly better growth. In contrast to water temperature, increased oxygen saturation did not affect the VSI, suggesting its modulation to be more suitable for enhancement of pikeperch growth performance if increased fat content of the body is not wanted. Moreover, considering the relatively short duration (72 days) of the experiment compared to the common duration of the grow-out phase (12–16 months), oxygen saturation could have a more prominent effect in commercial farms.

The haematological parameters can provide valuable information on the physiological status of fish. However, they can exhibit high variations depending on the species, age, sex, health condition and environmental condition such as temperature, pH, and oxygen (De Pedro et al., 2005; Fazio, 2019). The current study indicated a more prominent effect of the temperature, influencing all the studied markers, with the exception of Monocytes and RBC, while the effect of oxygen saturation was significant only for mean corpuscular volume (Table 4). Such a substantial difference can be explained with the increased metabolic rate at higher water temperatures (Currie and Evans, 2020). In our study, hyperoxia at 23 °C, caused a significant ($P < 0.05$) increase of mean corpuscular volume – the average size of red blood cells (RBC), responsible for carrying oxygen through the body (Nabi et al., 2022). The elevated temperatures likely induce physiological responses, such as an accelerated metabolic rate. This heightened metabolism, combined with effects like a reduction of absolute oxygen levels in water at higher temperatures, seems to be reflected in blood cell morphology. Consequently, the observed increase in cell size can be attributed to the interaction between temperature-induced metabolic demands and the resulting adjustments in the oxygen-carrying capacity of the blood. As for short−term effect, these changes indicate that pikeperch juveniles can adjust their physiological parameters to cope with higher metabolic demands and variations in oxygen availability. This is evidenced by the increased mean corpuscular volume and haemoglobin concentrations which support enhanced oxygen transport and metabolic processes. However, the long-term effect of elevated temperatures and high feed intake on haematological profile is hard to predict. It can be speculated that over time, these conditions might lead to chronic stress, potential tissue damage, and impaired organ function due to the constant need for physiological adjustments. Considering the temperature optimum for pikeperch is ontogeny-specific, it can be suggested that pikeperch cannot cope with long−term exposure to high temperatures (Frisk et al., 2012). Another interesting aspect is interconnection of blood cell morphology and immune response. Most veterinary studies in aquaculture predominantly centre around the correlation between water temperature and disease outbreaks (Jørgensen et al., 2009; Rupp et al., 2019). Considering the above-mentioned changes in cell size, studying a link between the cell morphology, water temperature and immune response of the fish would be a promising direction in aquaculture veterinary.

The biochemical profile of plasma in fish provides important information about the organism's internal environment (Fazio, 2019). The hypoxia significantly increased lactate, glucose and NH_4 + levels in our study. Glucose and lactate levels are commonly used as physiological stress indicators. The increase in blood glucose concentration demonstrated the response of fish to metabolic stress caused by hypoxia (Kristan et al., 2014; Malinovskyi et al., 2019). The LAC level is used to observe the occurrence of anaerobic metabolism of carbohydrates and the exploitation of anaerobic reactions to deplete the energy reserves; these factors are associated with stress, hyperventilation, hypoxia and respiratory deficiencies (Zhu et al., 2013). In this study, increased LAC concentrations were caused by hypoxia which is in line with these suggestions. Change in NH_4 + level in the blood indicates alterations in protein catabolism and/or some disturbances in NH_4 + removal (Kolářová and Velíšek, 2012). It was also observed that the interaction between temperature and oxygen significantly affected creatine kinase activity (Table 5). Enzyme activity in blood plasma can be used for the evaluation of stress levels. A significant change in the activity of the cytoplasmic and mitochondrial enzymes indicates tissue damage, which may be induced by stress (Kolářová and Velíšek, 2012). Change activities of these enzymes indicate amplified transamination processes which occur due to amino acid input into the TCA (tricarboxylic acid cycle) cycle to cope with the energy crisis during oxygen level-based stress (Philip et al., 1995). Based on the obtained data, it can be suggested that higher temperatures and higher feed intake exacerbated the physiological stress, as evidenced by increased creatine kinase activity and changes in lactate and glucose levels. These results imply that short-term exposure to higher temperatures and hypoxia can amplify acute stress responses, meaning long-term exposure could result in more pronounced metabolic disturbances and potential organ dysfunction. This underscores the need for careful management of oxygen and temperature conditions in RAS to optimise pikeperch health and growth.

Oxidative stress has been defined as an imbalance of oxidants and antioxidant response, potentially leading to cell damage (Azzi et al., 2004). To cope with oxidative damage, organisms evolved multiple antioxidant defence systems. It was previously reported that within an artificial environment, pikeperch can elevate enzymatic activity, indicating organs and tissue-specific metabolic response (Policar et al., 2016). The liver is often the tissue that is the most responsive, however, that can vary depending on the species and its specific aspects of metabolic activity (Malinovskyi et al., 2022). The antioxidant defence system comprises two main parts: enzymatic, including catalase, superoxide dismutase, and glutathione S-transferase (GST), and nonenzymatic, consisting of glutathione (GSH), vitamins, and carotenoids (Abdel-Latif and Euony, 2016). Superoxide dismutase activity serves as the primary defence mechanism against oxidative stress by facilitating the conversion of two superoxide radicals into molecular oxygen, while catalase activity aids in the breakdown of hydrogen peroxide (Milić et al., 2018). Changes in GST activity represent a crucial aspect of the second phase of biotransformation, playing a role in the disruption of glutathione conjugation with various compounds and the transportation and elimination of reactive compounds, thereby offering additional indirect antioxidant functions (Lushchak, 2010). Antioxidants demonstrate coordinated action, but changes in their levels may tip the balance towards oxidative stress (Milić et al., 2018). In our study, oxygen levels only affected superoxide dismutase activity and GST activity in the liver (Table 6). Temperature significantly affected most parameters of enzymatic activity, particularly in liver, muscle and intestine. The combination of oxygen level and temperature affected superoxide dismutase activity in the liver and intestine (Tables 6 and 8). The different activity of enzymes in fish kept at temperatures of 20 °C and 23 °C, presumably determined by the different intensities of tissue-specific metabolism and indicate a role of various organs in temperature-induced response. The results of the enzymatic activity also align with the growth performance and blood biochemical and haematological profile, indicating significant changes in energy metabolism, presumably driven by feed intake.

In conclusion, this study emphasises the significance of temperature and oxygen saturation in RAS, showcasing their impact on growth performance and physiological parameters in pikeperch juveniles. Higher temperatures positively influence growth but at the expense of elevated visceral fat levels, suggesting considerations for specific production goals. Oxygen saturation alone signif-

icantly contributes to growth performance without compromising fish health under tested conditions, although its effect in this, relatively short, study was less prominent. Results from oxidative stress response, blood biochemistry and haematology, growth performance, and utilisation confirm significant changes in metabolism, blood cell morphology, and organ functions due to varying oxygen and temperature levels. Even within the 72-day study period, it was clear that increased feed intake at higher temperatures has the potential to compromise the physiology of the fish. For future perspectives, exploring the correlation between water temperature and the immune response could unveil valuable insights into disease resistance and enhance health management. Comprehensive metabolic studies would unravel the intricate interactions between temperature, oxygen saturation, and metabolic rates, providing a deeper understanding of pikeperch physiology. Fine-tuning feeding strategies, especially investigating the benefits of restricted feed portions at higher temperatures, presents practical solutions for optimising growth performance.

Ethics approval

This study was performed according to the following national and international guidelines for animal welfare protection (EU-harmonized Animal Welfare Act of the Czech Republic; law No. 166/1996 and no. 246/1992) and was conducted under RTD capacity permits issued to No. 58672/2020-MZE-18134 and No. 33446/2020-MZE-18134. All samples were done with the appropriate permission of the Departmental Expert Committee for the Authorization of Experimental Projects of the Ministry of Education, Youth and Sports of the Czech Republic, permit no. MSMT-8155/2022-4 for project NAZV QK 23020002.

Data and model availability statement

None of the data were deposited in an official repository. The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the corresponding author used Grammarly and DeepL to improve readability of the manuscript. After using these tools, the author reviewed and edited the content as needed and took, full responsibility for the content of the publication.

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Declaration of interest

None.

Acknowledgements

None.

Financial support statement

This study was financially supported by the Ministry of Agriculture of the Czech Republic, project NAZV QK23020002 and by TNA project AE050004 within the EU project Aquaexcel 2020. Part of the work was carried out with the support of VVI CENAKVA Research Infrastructure (ID 90099, MEYS CR, 2019–2022).

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