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Effects of elevated carbon dioxide on growth performance and physiology of juvenile RAS- based Eurasian perch (Perca fluviatilis)

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Effects of elevated carbon dioxide on growth performance and physiology of juvenile RAS-based Eurasian perch (*Perca fluviatilis*)

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Abstract

The present 105-day experiment studied the influence of exposure to carbon dioxide (CO_2) in its dissolved form on the growth performance, somatic condition, blood plasma biochemistry, and fin integrity of Eurasian perch (*Perca fluviatilis*) juveniles reared in recirculating aquaculture systems (RAS). Eurasian perch juveniles (mean initial body weight of 117.7 ± 28.2 g) were divided into three CO_2 groups in triplicate: Low (5 mg L^{-1}, control), Medium (15 mg L^{-1}), and High (30 mg L^{-1}). The exposure to dissolved CO_2 had a significant effect on total length, whereby the juveniles exposed to Low CO_2 achieved significantly greater length as compared to Medium and High treatments (p = 0.009). Fulton's condition factor (K) was significantly higher in Medium (2.35 ± 0.32) and High (2.34 ± 0.22) groups in comparison with the Low group (2.11 ± 0.24; $p \leq 0.001$). Mean final body weight, standard length, specific growth rate (SGR), thermal growth coefficient (TGC), feed conversion ratio (FCR), and survival rate (as high as 99.33%) were not significantly different among the treatments ($p >$ 0.05). Analysis of the somatic indices showed the trade-off between gonadosomatic index (GSI) and kidney/fat somatic indices, while no significant differences in blood plasma biochemical parameters were found ($p > 0.05$). The fin condition was very high, with an intact fin percentage ranging from 84% to 100%. Results indicate that chronic exposure to dissolved CO_2 concentrations up to 30 mg L^{-1} does not compromise the welfare or production performance of juvenile Eurasian perch in RAS but may induce subtle physiological adjustments associated with altered energy partitioning and somatic development, reflected in increased condition factors and shifts among somatic indices.

Keywords: Carbon dioxide; Growth performance; Hypercapnia; Fin condition; Perch; Intensive aquaculture

1. Introduction

Recirculating aquaculture systems (RAS) support sustainable inland aquaculture by enabling high-density fish production with reduced water use and effluent discharge. However, respiration in fish and biological processes in RAS usually cause the accumulation of dissolved carbon dioxide (CO_2) leading to hypercapnia that adversely affects fish growth, feed efficiency, and welfare (Fivelstad, 2013; Mota et al., 2019; Skov, 2019) (Fivelstad et al., 2015; Steinberg et al., 2017). Excess CO_2 leads to disturbances of acid-base balance, suppression of feeding, changes in nitrogen excretion, and increased risks of nephrocalcinosis in farmed teleost fish (Colt et al., 2012; Fivelstad et al., 1999; Pěnka et al., 2026; Takvam et al., 2023). Although operational practices generally recommend keeping levels of dissolved CO_2 below 15-20 mg L^{-1}, physiological tolerances are highly specific to each species and stage of life, and lab-derived tolerance values do not always accurately represent physiological risks on the farm scale (Fivelstad et al., 2015; Pěnka et al., 2026; Steinberg et al., 2017).

Eurasian perch (*Perca fluviatilis*) is a member of the family Percidae with rising popularity in land-based aquaculture in Europe due to their high market demand and suitability for RAS production systems (Pěnka et al., 2025; Policar et al., 2019). Previous studies involving other percids have reported that high exposure to CO_2 results in linear or threshold dependent reduction in growth performance, energy metabolism, and changes in hematological parameters for values between 4-30 mg L^{-1} (Pěnka et al., 2026; Steinberg et al., 2017). However, most of the research carried out until now has been limited by the shorter duration of experiments, smaller-scale laboratory systems, or focusing on mature individuals (Pourhosein-Sarameh and Falahatkar, 2024). Therefore, the critical question of CO_2 tolerance in young Eurasian perch remains unanswered.

Hypercapnia induces respiratory acidosis in fish. In teleosts, compensation for hypercapnic respiratory acidosis relies on branchial carbonic anhydrase (CA) to catalyze CO_2 hydration,

generating protons (H^+) and bicarbonate ions (HCO_3^-). Gill ionocytes actively excrete H^+ via apical sodium-hydrogen exchanger 3 (NHE3) or V-type H^+-ATPase while retaining HCO_3^- basolateral Na^+/HCO_3^- cotransporters to restore intracellular and plasma pH (Dymowska et al., 2012; Evans et al., 2005; Gilmour and Perry, 2009; Perry and Gilmour, 2006) . However, this continuous active ion transport imposes a substantial ATP demand, elevating standard metabolic rate and compressing the scope for growth (Heuer and Grosell, 2014; Kovac and Goss, 2024). Although these ion regulatory adjustments help restore intracellular pH homeostasis, compensation of extracellular pH is often incomplete under elevated CO_2 conditions. This persistent acidosis increases energetic demands and can reduce aerobic performance (Brauner et al., 2019; Brauner and Richards, 2020). Prolonged exposure to high CO_2 concentrations can also alter branchial ammonia excretion (NH_3/NH_4^+), thus increasing reliance on renal excretory pathways. The resulting physiological burden places greater demands on kidney function, stimulates activation of the hypothalamic-pituitary-internal (HPI) axis, increases circulating cortisol levels, and alters plasma protein profiles (Stejskal et al., 2009; Takvam et al., 2023).

Across teleost species, uncompensated hypercapnia consistently reduces feed intake and worsens feed conversion ratio, with important economic and welfare consequences for aquaculture (Fivelstad, 2013; Fivelstad et al., 2018, 2015; Jafari et al., 2024). To define evidence-based carbon dioxide thresholds for commercial production, the present study evaluated juvenile Eurasian perch during a 105-day on-growing period in production-scale RAS. By examining growth performance, feed utilization, somatic indices, and plasma biochemical responses under three different CO_2 exposure regimes (5 mg L^{-1}, 15 mg L^{-1}, 30 mg L^{-1}), the study provides valuable information on the physiological tolerance limits of perch during the early stages and contributes to the development of welfare-orientated management strategies for RAS operations.

2. Materials and methods

2.1 Ethical approval

Experiments were performed strictly according to Czech law regarding animal care (Act No. 166/1996 Coll. on Veterinary Care and Act No. 246/1992 Coll. on Protection of Animals against Cruelty). Approval of protocol was granted by the Animal Welfare Committee at Faculty of Fisheries and Protection of Waters (FFPW), University of South Bohemia in České Budějovice (Czech Republic) and Ethical Commission at FFPW (Ref. No. MZE-39723/2025-13143 and MZE-61035/2024).

2.2 Design of experimental system

The 105-days feeding experiment was carried out on three separate RAS at the Laboratory of Intensive Aquaculture (LIA), which is part of FFPW (Vodňany, Czech Republic). The overall layout and set-up of the rearing system was done based on the design methodology used previously (Pěnka et al., 2026; Rahimnejad et al., 2023). Each individual RAS system comprised three light-grey cylindrical plastic tanks (diameter: 885 mm; height: 620 mm; operating volume: 380 L), a mechanical filter (Ratz Aqua & Polymer Technik GmbH, Remscheid, Germany), and a two-step biological filtration (Figure 1). After the fish-carrying tanks, the first step to treating the water consisted of a home-made moving bed biofilter (890 L) and the second step of a commercial Nexus 310 biofilter (100 L; Evolution Aqua Ltd., Wigan, UK). Both biofilters were aeration continuously through the use of a Secoh EL-S 250 W air blower (Secoh Shanghai Mec. Ltd., Shanghai, China) with air-stones fixed to the filters for keeping the effectiveness of the biofilters. Depending on increasing fish biomass and biological oxygen demand during the trial, the aeration rates varied from 1.50 to 4.00 L min^{-1} (Figure 1).

Photoperiod was maintained under a continuous 12:12 h light:dark cycle at a low surface irradiance of 7 lux, measured at the water surface using a UNITEST 93514 lux meter (Beha-

Amprobe GmbH, Glottertal, Germany). Daily system water loss was replenished by replacing 5% of total system volume with municipal tap water. A continuous system flow rate of 3 m^3 h^{-1} ensured complete water turnover in each experimental tank at least twice per hour. Water temperature and dissolved oxygen (DO) concentration were recorded three times daily (at 07:00, 14:30, and 18:00 h) using a portable oxygen meter (YSI ProODO, YSI Inc., Yellow Springs, OH, USA). Dissolved CO_2 was measured at the same sampling intervals using a portable OxyGuard CO2 analyzer (OxyGuard International A/S, Farum, Denmark). Water pH was recorded daily using a WTW 3310 pH meter (Xylem Česká republika, spol. s r.o., Prague, Czech Republic) following the procedure outlined by Policar et al. (2024). Concentrations of total ammonia nitrogen (NH_4^+-N) and nitrite nitrogen (NO_2^--N) were determined via handheld titration and colorimetric kits utilizing sulfanilic acid ($C_6H_7NO_3S$) and N-(1-naphthyl)ethylenediamine dihydrochloride, with raw values subsequently recalculated using standard conversion coefficients supplied by the Laboratory of Aquatic Toxicology and Ichthyopathology (FFPW, Vodňany, Czech Republic). Mean water quality parameters were measured during the 105-day (15-week) experimental period (Table 1).

At the start of the trial, 50 fish (initial mean body weight: 115.65 ± 31.20 g) were assigned randomly to each of nine tanks (three tanks per RAS unit), resulting in an initial stocking density of 17.1 kg m^{-3}. Increased stocking densities were not possible due to the system design, which did not have pure oxygen addition capacity. Particulate settled solids were manually siphoned off from the bottom of each tank before feeding. A commercial floating feed (BioMar EFICO 7170F, 3 mm; BioMar A/S, Brande, Denmark; proximate composition: crude protein 56%, crude fat 12%, ash 9.2%, nitrogen-free extractives 16.5%, gross energy 20.8 MJ kg^{-1}) was administered via automatic belt feeders. An initial daily feeding ration (DFR) of 1.0% of total biomass in the tanks (Pěnka et al., 2024a) was evaluated and adjusted every 28 days after batch

weighing due to biomass increase. Feed was offered to apparent satiation; any remaining feed was siphoned off daily, weighed, and used to calculate net feed consumption.

2.3 CO_2 dosing

The 105-day (15-week) trial was conducted across three independent RAS, each comprising three replicate tanks (n = 9 tanks total). Three different levels of dissolved CO_2 were applied to the systems: Low (5 mg L^{-1}), Medium (15 mg L^{-1}), and High (30 mg L^{-1}). Desired levels of CO_2 in Medium and High groups were achieved by addition of food-grade CO_2 (Linde Gas a.s., Prague, Czech Republic) into the systems using modular Wedge Lock diffusers (Pentair Aquatic Eco-Systems, Apopka, FL, USA) installed inside the sump of pumps (Figure 1). The Low RAS system was supplied without additional CO_2, which made it the control group.

2.4 Fish study groups

The juveniles of perch used in the current experiment were bred in an intensive aquaculture system combining ponds in the LIA (Vodňany, Czech Republic), using procedures outlined by (Policar et al., 2015; Stejskal et al., 2020, 2009). At the beginning of the experiment, the age of all juveniles was four months, and they had equal initial body weights. Altogether 450 juveniles were randomly distributed in three experimental RAS in triplicates (50 juveniles per tank). The values of biometric traits at the beginning of the experiment (mean ± standard deviation) were: initial total length (iTL) = 250.9 ± 20.7 mm, initial standard length (iSL) = 218.9 ± 16.9 mm, and initial body weight (iBW) = 117.7 ± 28.2 g.

2.5 Biometric parameters

Prior to biometric measurements and other handling procedures fish were anesthetized with tricaine mesylate (MS-222; Sigma-Aldrich, St. Louis, MO, USA) in 80 mg L^{-1} concentration to minimize stress Rożyński et al. (2018). Blood sampling was performed without anesthesia to avoid possible effects on biochemical. For biometrics, measurements were made at the start and at the end of the experiment for 50 randomly chosen fish from each experimental group to

evaluate average body characteristics. Total length (TL) and standard length (SL) were measured to the closest millimeter using a fish ruler, and body weight (BW) was assessed using precise scale (KERN PCB 1000-2; Kern & Sohn GmbH, Balingen, Germany). Also, number of fish and average body weight of each tank were measured at baseline, at 28-day intervals and at the end of the experiment to measure actual biomass of tanks and adjust daily feeding ration (DFR).

The performances in growth, survival, and production were analyzed based on the beginning and final biometric measurements of the experimental groups (Low, Medium, High) using the following indices:

- Fulton´s condition factor (K) was calculated as:

$$K = \frac{BW}{TL^3} \times 100$$

- The coefficient of variation in body weight (CV) was calculated as:

$$CV = \frac{SD_{BW}}{Mean_{BW}}$$

where SD_{BW} is standard deviation of body weight in grams.

- The specific heterogeneity rate (SHR, ‰ day^{-1}) per tank was calculated as:

$$SHR = \frac{\ln(CV_{final}) - \ln(CV_{initial})}{\text{experimental duration (days)}} \times 100$$

where BW is individual body weight in grams and TL is total length in centimeters.

- The specific growth rate (SGR, % day^{-1}) per tank was calculated as:

$$SGR = \frac{\ln(\text{final biomass}) - \ln(\text{initial biomass})}{\text{feeding days}} \times 100$$

- The thermal growth coefficient (TGC) per tank was calculated as:

$$TGC = \frac{(\text{final biomass})^{1/3} - (\text{initial biomass})^{1/3}}{\sum(\text{mean daily temperature [°C]} \times \text{experimental duration [days]})} \times 100$$

- The feed conversion ratio (FCR) was calculated as:

$$\text{FCR} = \frac{\text{total feed consumed (g)}}{\text{final biomass (g)} - \text{initial biomass (g)}}$$

- The survival rate (SR, %) per tank was calculated as:

$$\text{SR} = \frac{\text{final number of fish}}{\text{initial number of fish}} \times 100$$

2.6 Somatic condition indices

After collecting blood samples during both the beginning and the end of trials, the fish were euthanized through mechanical stunning and dissection of the spinal cord. Specified tissues were isolated and weighed with an accuracy of 0.01 g using an electronic balance (KERN PCB 1000-2; Kern & Sohn GmbH, Balingen, Germany). Tissue-somatic relative indices were expressed as the percentages of total body weight using the following formulae:

- The hepatosomatic index (HSI, %):

$$\text{HSI} = \frac{\text{liver weight (g)}}{\text{body weight (g)}} \times 100$$

- The kidney somatic index (KSI, %):

$$\text{KSI} = \frac{\text{kidney weight (g)}}{\text{body weight (g)}} \times 100$$

- The fat somatic index (FSI, %):

$$\text{FSI} = \frac{\text{visceral fat weight (g)}}{\text{body weight (g)}} \times 100$$

- The gonadosomatic index (GSI, %):

$$\text{GSI} = \frac{\text{gonad weight (g)}}{\text{body weight (g)}} \times 100$$

2.7 Biochemical examination of plasma

Blood samples were collected from two individuals chosen randomly per tank at the end of the experiment (n = 6 per experimental group). The blood collection was performed from the (*vena* and *arteria caudalis*) using the using sampling material (needles, syringes, test tubes) treated with anticoagulant heparin (Heparin Léčiva, Zentiva, Prague, Czech Republic). The plasma

separation was immediately performed by centrifugation at 4 000 rpm for 15 min., carefully separated and frozen at −80°C for further processing. The plasma biochemical parameters were measured by a FUJI DRI-CHEM NX500i autoanalyzer (FUJIFILM Europe GmbH, Düsseldorf, Germany) using reagent slides requiring 10 µL of sample. The assayed parameters were total protein (TP), albumin (ALB), lipase (LIPA), amylase (AMY), glucose (GLU), alanine aminostransferase (ALT); aspartate amonotransferaze (AST), ammonia (NH_3), cortisol (CORT) and globulin (GLOB).

2.8 Fin erosion

Fin condition was assessed both before and after the experiment in all the tested perch by one observer to avoid any inter-observer bias. As described by Stejskal et al. (2011), visual examination was done on the caudal fin, anal fin, first dorsal fin, second dorsal fin, pectoral fins and ventral fins. The extent of fin damage was determined using a four-grade ordinal scale where Grade 0 meant very little damage (< 5%), Grade 1 meant mild erosion (5-30%), Grade 2 indicated moderate damage (30-70%) and Grade 3 implied severe erosion (>70%).

2.9 Statistical analysis

All values are shown as mean ± standard deviation (SD). Statistical analyses were performed with the Statistica program (v.14; TIBCO Software Inc., Palo Alto, CA, USA) and R Studio (v.4.5.1; R Core Team, 2022) using the package with significance level $p < 0.05$. Normal distribution of data was checked using Shapiro-Wilk test. Homogeneity of parameters among replicate tanks was tested by two-way ANOVA and did not show any significant effect of tank. Comparative analyses of production and physiological traits among three experimental groups (Low, Medium, High) were conducted through one-way or two-way ANOVA in case of parametric data, then Tukey´s HSD post hoc analysis of means for pairwise comparisons. In case of non-parametric data sets, non-parametric tests were carried out: global effect of groups on data was tested through Kruskal-Wallis test, while Dunn's test was employed to conduct

pairwise comparisons. Non-parametric analyses were used in case of mean percentage of square root transformed data depicting spatial distribution of fish. The dose-response relationship between CO_2 concentrations and mean body weight was tested through linear regression analysis. Statistical significance was expressed as $p < 0.05$ for values ranging from 0.01 to 0.05, and $p < 0.01$ for values below 0.01.

Furthermore, to determine the multivariate relationships between variables and the possible clustering of samples across groups, principal component analysis (PCA) (with Pearson correlation heatmap) was conducted separately for datasets "Somatic condition indices" and "Blood plasma parameters". Before conducting the PCA, continuous variables were standardized using z-scores; ordinal analyses and biplot visualizations were performed using Canoco 5 software (v.5; ter Braak and Šmilauer, 2012) and heatmaps were created using Statistica (v.14; TIBCO Software Inc., Palo Alto, CA, USA).

3. Results

3.1 Biometric parameters

There was no statistically significant differences between the experimental groups prior to the beginning of the experiment for total length (TL), standard length (SL), body weight (BW), initial Fulton's condition factor (K), or the initial coefficient of body weight (CV) ($p > 0.05$)..

Following the conclusion of experimental period, dissolved CO_2 concentration had a significant impact on the final total length of Eurasian perch ($F_{(2,312)} = 4.77$, $p = 0.009$). Fish maintained under the Low CO_2 group recorded the highest final TL (254.11 ± 20.62 mm) significantly greater than that seen in the Medium (244.63 ± 22.86 mm) and High CO_2 (248.10 ± 23.92 mm) groups.

Despite the noted disparity in length, there were no significant effects on the final standard length as a result of the varying levels of dissolved CO_2 ($F_{(2,312)} = 1.60$, $p > 0.05$). In a similar

trend, body weight across groups also maintained its statistical insignificance ($F_{(2,312)} = 1.161$, $p > 0.05$) (Table 2).

There were statistically significant differences in the Fulton's condition factor of the final fish among grouts ($F_{(2,312)} = 27.34$, $p \geq 0.001$). The condition factor of fish exposed to groups Medium (2.35 ± 0.32) and High (2.34 ± 0.22) CO_2 was found to be significantly higher than that of the Low CO_2 group (2.11 ± 0.24) (Table 2). This indicates that the fish exposed to higher levels of dissolved CO_2 had higher body mass for the same length.

No effects of any significance were found on the final coefficient of variation of body weight (CV; $F_{(2,6)} = 0.138$, $p > 0.05$) and specific heterogeneity rate (SHR; $F_{(2,6)} = 0.114$, $p > 0.05$), which means that the level of variation in size in the tested populations was similar regardless of dissolved CO_2 concentration.

Similarly, there were no effects of group on the growth parameters. Neither specific growth rate (SGR; $F_{(2,6)} = 0.621$, $p > 0.05$), thermal growth coefficient (TGC; $F_{(2,6)} = 0.489$, $p > 0.05$), feed conversion ratio (FCR; $F_{(2,6)} = 0.748$, $p > 0.05$), nor survival rate (SR; $F_{(2,6)} = 0.571$, $p > 0.05$) (Table 2). In numerical values, fish in the medium CO_2 concentration had the lowest feed conversion ratio and the highest feed efficiency, while the survival rate in the high CO_2 concentration group was the highest (99.33 ± 0.94%). However, none of these differences have reached statistical significance.

3.2 Somatic condition indices

The results of PCA confirmed the existence of multivariate group patterns. The first principal component (PC1) explained 50.0% of total variance and clearly separated the control fish (Initial group) from the rest of dissected groups (Low, Medium, High). These groups differed by growth factors, namely BW, TL, and SL, thus implying that these morphological features accounted for the differences between the groups (Figure 2).

The second principal component (PC2), which accounted for 19.2% of total variance, showed variability in energy storage and organ allocation. Along this axis, the GSI was opposed to both KSI and FSI (Figure 2).

Correlation analysis also showed a significant association between growth factors. Specifically, body weight was strongly positively correlated with standard length (r = 0.93) and total length (r = 0.95), thereby confirming the association between fish size and body weight (Figure 2). Moreover, body weight was also moderately positively correlated with HSI (r = 0.50).

Several organosomatic trade-offs were also evident. GSI was negatively correlated with both the KSI (r = -0.46) and HSI (r = -0.34) (Figure 2).

3.3 Biochemical examination of plasma

Analysis of blood biochemical parameters regarding multiple parameters revealed specific trends related to protein metabolism, digestive enzymes activity, and stress after 105 days of experiment. The first principal component (PC1) described 53.7% of total variance and was mainly explained by the fractions of plasma proteins such as total protein (TP), albumin (ALB), and globulin (GLOB), along with the activity of digestive enzymes: amylase (AMY) and lipase (LIPA). Parameters describing protein status were grouped in the inverse polarity to the activity of lipase; therefore, high levels of lipase were associated with low values of total protein, albumin, and activity of amylase (Figure 3).

The second principal component (PC2) was responsible for 16.4% of total variance and was mostly characterized by the physiological stress and metabolic activity of an individual (Figure 3). This axis was characterized by strong positive loading of cortisol (CORT) and negative loading of aspartate aminotransferase (AST), which meant that differences in physiological conditions of individuals were related to stress endocrine reactions and metabolic state (Figure 3).

Correlation analysis showed high values of biochemical parameters. Total protein had almost perfect positive correlation with albumin (r = 0.97) and globulin (r = 1.00). On the other hand, there was strong negative correlation between amylase and lipase activity (r = -0.90) (Figure 3).

Grouping of the fish samples based on the PCA ordination showed further patterns linked to the tested groups. The fish of the Medium CO_2 group had grouped centrally in the ordination plot and had fairly balanced protein and enzyme levels. On the other hand, the fish that belonged to the Low and High CO_2 groups had exhibited higher spread in the ordination, specifically along the PC2 axis that is linked to cortisol and the PC1 axis related to the lipase/AST (Figure 3).

However, statistical analysis via one-way ANOVA and Kruskal-Wallis tests failed to show any significant differences ($p > 0.05$) between the Low, Medium, and High CO_2 groups in any of the blood biochemical characteristics investigated. The absence of significant results of tested groups was probably due to high within-group variability observed specifically in the Low and High CO_2 groups (Table 3).

3.4 Fin erosion

Fin erosion occurrence rate was relatively low in all experimental conditions. The largest number of the examined fishes had intact fins (Grade 0) regardless of the concentration of dissolved CO_2, and the percentages varied from 84 to 100 depending on the fin type (Table 4). Pectoral fins of the fishes were not significantly affected by the experiment. For the high CO_2 condition, 96 and 93% of the examined fishes had no pectoral fin damage for the left and right fins, respectively. Mild erosion (Grade 1) occurred in 4-5% of the fishes. One fish (1%) from the high CO_2 group had moderate erosion (Grade 2) of the right pectoral fin. There were no moderate or severe lesions found for the medium and low CO_2 conditions (Table 4).

Likewise, ventral fins had a very high incidence rate of fins that were intact. Those fish that did not have any visible lesions constituted 93-97% of the total number of fish, while mild erosion

was found in only 3-7% of the fish (Table 4). The condition known as moderate erosion (Grade 2) occurred only in 1% of the fish in the high CO_2 group on the right ventral fin (Table 4). Similarly, both first and second dorsal fins remained in a good state during the course of the experiment. Intact dorsal fins could be seen in 93-97% of the fish in all tested groups. While mild erosion (Grade 1) could be seen in 3-6% of fish, moderate erosion (Grade 2) was found in the first dorsal fin of fish from the high CO_2 group (2%) (Table 4).

Compared to all other fins analyzed, the caudal fin demonstrated the highest frequency of erosion. The percentage of fish possessing the intact caudal fins varied from 84% for the medium CO_2 level to 90% and 87% for low and high CO_2 levels respectively (Table 4). Grade 1 (mild erosion) was noted in 10-16% of fish and had the highest rate of fin erosion among all fins. Only Grade 2 (moderate erosion) occurred in 1% of fish subjected to the high CO_2 level. The lowest rate of erosion was characteristic for the anal fin. The presence of the intact fins was determined for 97-100% of fish while mild erosion (Grade 1) was found in 2-3% of fish. No cases of moderate or severe lesions were registered.

4. Discussion

4.1 Biometric parameters

This study shows that juvenile Eurasian perch can maintain a relatively stable production performance for 105 days under the exposure to dissolved CO_2 levels (5, 15 and 30 mg L^{-1}). However, high CO_2 had an impact on body shape, since the total length of perch was significantly reduced in the Medium and High groups when compared to the Low treatment. At the same time, standard length, body weight, specific growth rate (SGR), thermal growth coefficient (TGC), feed conversion ratio (FCR), survival, coefficient of variation (CV) and specific heterogeneity rate (SHR) were not significantly different. Such a pattern of results suggests that the effect of chronic hypercapnia on Eurasian perch is relatively mild with respect to body shape and does not affect the general growth and production. Such results are different

from those obtained for highly sensitive salmonids, e.g. Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) whose exposure to levels of dissolved CO_2 above 10-15 mg L^{-1} usually results in chronic growth impairment, reduced efficiency of feed conversion efficiency, and nephrocalcinosis (Fivelstad, 2013; Fivelstad et al., 2018, 2015; Jafari et al., 2024; Mota et al., 2019). The performance of Eurasian perch in the current research corresponds to the results previously observed for pikeperch (*Sander lucioperca*) whose CO_2 tolerances are higher compared to cold-water fish in RAS (Pěnka et al., 2026; Policar et al., 2013, 2024; Steinberg et al., 2017).

The reduction in total length of the fish supports the findings of previous pikeperch studies that have shown that chronic exposure to high concentrations of CO_2 affects fish growth. Steinberg et al. (2017) investigated the effects of CO_2 concentrations of approximately 4, 15, and 30 mg L^{-1} on the growth and physiology of adult pikeperch reared in RAS for 58 days. The authors reported a linear decrease in body weight of approximately 6% between Low and High CO_2 groups. Increased CO_2 concentrations also reduced feed intake during the exposure period and negatively affected hematocrit and oxygen consumption rates. They concluded that although pikeperch were able to survive at 30 mg L^{-1} CO_2, physiological alterations were already evident at 15 mg L^{-1}.

Similarly, Pěnka et al. (2026) evaluated the effects of CO_2 concentrations of approximately 5, 15, and 30 mg L^{-1} on juvenile pikeperch held in RAS for 105 days. Their results showed a comparable linear decline in body weight, with fish from the highest CO_2 treatment exhibiting approximately 6% lower body weight than those in the lowest treatment. Elevated CO_2 concentrations also led to reduced feed intake and adversely affected hematocrit and oxygen consumption. The authors concluded that while juvenile pikeperch tolerated CO_2 concentrations up to 30 mg L^{-1}, measurable physiological effects were already present at 15 mg L^{-1}.

383 Moreover, the difference could be related to the length of the experiment and the size of fish.
384 In Steinberg et al. (2017), much bigger pikeperch with an average weight of approximately 252
385 g was used compared to approximately 118 g Eurasian perch in the current experiment. Stejskal
386 et al. (2009) observed size-dependent changes in oxygen consumption and ammonia excretion
387 in Eurasian perch under RAS conditions, stating the importance of changing metabolic needs
388 with changes in body mass. Thus, comparisons between different species should be interpreted
389 carefully in case of different sizes and developmental states.
390 Another reason for the difference may be related to the feeding conditions. According to
391 Steinberg et al. (2017), reduced feeding under elevated CO_2 concentrations was stated within
392 the first weeks of acclimation and its subsequent attenuation, which were considered as
393 habituation. In the current experiment, fish were fed approximately 1% biomass with no
394 significant differences in FCR and SGR. The lack of a significant effect of elevated CO_2 on
395 FCR suggests that there was sufficient effective food consumption in the event of increased
396 CO_2 concentration. Thus, if hypercapnia affected fish appetite and metabolism, this effect was
397 not enough to show a difference in whole-animal food conversion in 105 days of exposure.
398 This finding contradicts the results obtained in a number of salmonid research works. Good et
399 al. (2018) studied the effect of long-term exposure of Atlantic salmon post-smolts to a level of
400 approximately 20 mg L^{-1} CO_2 in RAS over 384 days. This study is much longer than the current
401 105-day trial and was conducted to assess the effects of CO_2 on health and production during
402 growth towards harvest size. The authors found that elevated CO_2 resulted in production and
403 health problems, indicating the relevance of relatively moderate hypercapnia in RAS
404 production. Likewise, molecular and physiological responses of Atlantic salmon to 5–40 mg L^{-1}
405 CO_2 for 12 weeks revealed dose-dependent effects on blood and differential gene expression
406 in the gills, making Mota et al. (2020) question the safety of CO_2 levels above 15 mg L^{-1}

Thus, the discrepancy between the findings of the present study and the findings in salmonids highlights the importance of establishing species-specific levels of CO_2 that can lead to production problems. The Atlantic salmon responds to relatively moderate chronic exposure to CO_2 at several physiological levels, while the Eurasian perch maintained its growth, feed conversion, and survival over 105 days These findings do not necessarily indicate a lack of sensitivity to CO_2 in Eurasian perch, but rather suggest the existence of physiological compensation mechanisms that mitigate the effects of elevated CO_2 exposure.

The absence of significant differences in body weight and total length between groups suggests that growth performance and overall biomass production were not adversely affected. It means that the effects of CO_2 can alter the body proportionality. This idea is reinforced by the significantly high Fulton's condition factor (K) seen in the Medium and High group. Those fish reared in Medium and High CO_2 groups showed a significantly higher K compared to those fish grown at 5 mg L^{-1} CO_2, which means that the body mass to total length ratio is greater.

However, this finding is the opposite of research in which elevated CO_2 led to a decrease in the condition factor. In Atlantic salmon and other species of cultured fish, chronic hypercapnia has been associated with reduced feed intake, impaired growth performance, and poorer condition, leading to a decrease in condition factor under intensive recirculating aquaculture conditions. Thus, the increased condition factor in Eurasian perch is probably a specific reaction of the species or a consequence of the peculiar growth pattern. A higher K factor should not be interpreted as evidence of improved health or welfare, as it merely describes the relationship between body mass and length and does not provide information on the composition of the additional mass.

The lack of significant differences in CV and SHR is also consistent with the absence of effects on the growth. Elevated CO_2 did not result in greater variability in the size of the populations. The issue is relevant for commercial production, as higher size variability would increase the

complexity of grading and harvesting (Ak, 2023; Kestemont et al., 2003; Policar et al., 2019) . Furthermore, survival rates were high and were not significantly affected by groups. According to Steinberg et al. (2017), pikeperch were able to survive 30 mg L^{-1} CO_2 if other water quality parameters were kept at acceptable levels. Therefore, both articles indicate that 30 mg L^{-1} CO_2 should be considered a sublethal dose for percids.

Therefore, a significant difference in the present results from previous studies is the level of production caused by elevated CO_2. Earlier works on pikeperch and salmonids showed relatively evident reductions in growth rate or feeding efficiency while Eurasian perch maintained their traditional production parameters. Such observations point to the relatively high tolerance of this species to elevated CO_2. However, fish significant decrease in length means that elevated CO_2 had some physiological effects. Longer experiments, higher stocking density, more fish biomass, and the combination with other RAS stressors could show stronger impact on the physiology of perch.

4.2 Somatic condition indices

The absence of statistically significant differences in the hepatosomatic index (HSI), fat somatic index (FSI), kidney somatic index (KSI), and gonadosomatic index (GSI) indicate that prolonged exposure to 15 and 30 mg L^{-1} CO_2 did not affect relative organ size or visceral energy reserves in Eurasian perch.

These findings are consistent with a recent study conducted in RAS on pikeperch (Pěnka et al., 2026). Similarly, after 105 days of CO_2 exposure, juvenile pikeperch showed no significant differences in organosomatic indices despite the negative effects of elevated CO_2 on growth performance, feed efficiency, and several plasma parameters. This suggests that HSI, FSI, KSI, and GSI may be relatively insensitive indicators of sublethal CO_2 exposure in percid culture, even when other physiological parameters are affected.

In contrast, studies on salmonids have demonstrated that chronic hypercapnia can induce broader physiological and pathological responses. Furthermore, Mota et al. (2020) reported concentration-dependent changes in blood parameters and gill gene expression in Atlantic salmon exposed to 5-40 mg L^{-1} CO_2. These findings indicate that physiological responses at the cellular or tissue level may occur without corresponding changes in organ-to-body mass ratios. The principal component analysis (PCA) revealed that the primary factors distinguishing the initial fish from the three experimental groups were BW, TL, and SL. This observation should be considered when interpreting organosomatic indices, as differences between the initial and final sampling points cannot be automatically attributed to CO_2 exposure. The fish exhibited substantial growth throughout the experiment, and ontogenetic changes were therefore expected.

The positive association between BW and HSI observed in the PCA is biologically plausible, as the liver plays a central role in nutrient processing, lipid metabolism, glycogen storage, and detoxification (Mommsen et al., 1999). However, because no significant differences were detected among treatment groups, this relationship cannot be attributed to CO_2 exposure. Likewise, the negative correlation between GSI or KSI and HSI may reflect natural patterns of resource allocation among tissues rather than a direct effect of elevated CO_2 on reproductive or renal investment.

This interpretation is particularly relevant because chronic hypercapnia imposes additional demands on acid-base regulation (Steinberg et al., 2017). Elevated environmental CO_2 increases blood CO_2 and initially causes respiratory acidosis. Fish compensate through active branchial and renal ion transport processes that increase bicarbonate retention and proton excretion, thereby restoring blood pH (Claiborne et al., 2002; Evans et al., 2005). Evidence from salmonids shows that such compensatory mechanisms can successfully maintain acid-base

480 homeostasis over prolonged exposures, although not without detectable physiological costs
481 (Fivelstad et al., 2018, 2015; Jafari et al., 2024; Mota et al., 2020, 2019) .
482 If these compensatory mechanisms were energetically demanding or physiologically limiting,
483 structural or functional changes in the gills and kidneys might be expected. For example,
484 chronic CO_2 exposure in Atlantic salmon has been associated with disturbances in ion
485 regulation, altered plasma chemistry, reduced growth, and increased incidence of
486 nephrocalcinosis at higher concentrations (Fivelstad et al., 1999, 2007, 2018). However, no
487 such responses were evident in the present study. This result may indicate either that
488 compensation occurred within the physiological capacity of the fish or that organosomatic
489 indices were not sufficiently sensitive to detect underlying alterations.
490 Consequently, future studies should complement HSI, FSI, KSI, and GSI measurements with
491 histological, biochemical, and molecular analyses. Such approaches would provide a more
492 comprehensive assessment of physiological responses to chronic hypercapnia. This
493 recommendation is supported by studies of Atlantic salmon demonstrating significant
494 physiological and tissue-level responses to elevated CO_2 despite minimal changes in traditional
495 morphological welfare indicators. Mota et al. (2020) documented persistent molecular and
496 physiological responses to hypercapnia, while Höglund et al. (2023) found suppressed growth
497 and altered stress-coping mechanisms under chronic hypercapnia despite an absence of
498 morphological welfare responses.
499 Overall, the present study supports the view that organosomatic indices are relatively
500 insensitive indicators of CO_2-induced stress in Eurasian perch. The stability of HSI, FSI, KSI,
501 and GSI, together with high survival rates and unaffected growth performance, suggests a
502 substantial capacity for acclimation to the tested levels of environmental CO_2. Similar
503 conclusions have been proposed for other species in RAS, where physiological disturbances
504 were detectable before morphological indices responded (Fivelstad, 2013; Mota et al., 2020).

505 ***4.3 Biochemical examination of plasma***

506 Plasma biochemical data presents an interesting contrast to the data from the literature on
507 studies of chronic CO_2 exposure, leading to significant changes in blood biochemistry. In this
508 present study, there were no significant differences between Low, Medium and High CO_2
509 exposure groups for all measured plasma biochemical parameters including TP, ALB, GLOB,
510 LIPA, AMY, GLU, ALT, AST, NH_3 and cortisol.

511 Quite different results have recently been obtained in pikeperch. Namely, the results of 105-day
512 RAS study of pikeperch reported by Pěnka et al. (2026) present the impairment of protein
513 metabolism (significant decrease in plasma total protein, albumin and globulin levels and
514 increase in ammonia level) and activation of stress response (increase in cortisol level) in fish
515 exposed to high CO_2. It is interesting to note that CO_2 exposures and the duration of experiments
516 were almost identical in both studies (Pěnka et al., 2026; Steinberg et al., 2017).

517 Furthermore, according to Steinberg et al. (2017), the physiological effect of higher levels of
518 CO_2 on pikeperch involves hematocrit and oxygen consumption. In general, the literature on
519 pikeperch shows that percid species have the potential to survive at 30 mg L^{-1} CO_2 while being
520 able to show several physiological modifications. Accordingly, the absence of any
521 corresponding biochemical response of Eurasian perch increases the probability that can be
522 expected that this species has more physiological resistance to the current environment.

523 Especially worth mentioning is the lack of cortisol response. Cortisol is one of the key endocrine
524 hormones of the fish stress response, but its chronic action makes it difficult to interpret due to
525 temporal issues (Carbajal et al., 2019; Laberge et al., 2019; Lemos et al., 2023). Thus, acute
526 exposure will cause a considerable increase in cortisol level, but long-term exposure can result
527 in acclimation and the normalization of baseline cortisol levels, although the stressor is still
528 present. Chronic hypercapnia experiments in Atlantic salmon proved that the physiological
529 stress response can vary with time, and the recent welfare studies emphasize that baseline

cortisol cannot reflect chronic hypercapnia-induced stress (Fivelstad et al., 2018, 2015; Jafari et al., 2024; Mota et al., 2020, 2019).

Consequently, there are no significant differences between the groups in terms of the terminal level of plasma cortisol.

The results of the PCA analysis suggested that the plasma-related variables demonstrate specific physiological connections. TP, ALB, and GLOB were highly positively correlated, while LIPA was negatively correlated with protein-related variables. Cortisol and AST were important for the construction of a second axis related to stress and metabolic state. These connections seem logical and reasonable, but due to the insignificant differences in CO_2 groups, they can only be considered as physiological characteristics of individuals.

The relatively large variability detected in the Low and High groups could have been one reason for the absence of statistically significant results. It is particularly relevant regarding cortisol and enzymes, since their levels can be influenced by handling, fasting/feeding status, sampling time, and individual state (Folmar, 1993; McDonald and Milligan, 1992). Although only two fish per tank were sampled for blood biochemical analyses, resulting in a total sample size of six fish per experimental group ($n = 6$), this sample size should be considered when interpreting the results.

Therefore, the result can be considered as an indication of biochemical resistance rather than the absence of physiological effects. It should be noted that there is evidence of a physiological reaction of Atlantic salmon at lower concentrations of CO_2. Mota et al. (2020) report linear changes in blood parameters and up-regulation / down-regulation of 88 genes in the gills at concentrations of 5 to 40 mg L^{-1} CO_2. The difference indicates that biochemical blood analysis may not always be sensitive enough. Biochemical blood profiling is nevertheless widely recognized as a sensitive tool for detecting physiological disturbances, stress, tissue damage,

554 and metabolic alterations in fish (Folmar, 1993; Kolářová and Velíšek, 2012; Wagner and
555 Congleton, 2004).

556 ***4.4 Fin erosion***

557 The condition of the fish fin tended to be generally quite good in all groups. There does not
558 appear to be any clear concentration-related effect of CO_2 on fin erosion. Most of the fish had
559 unscathed fins and, where fin erosion was detected, it appeared to be mostly mild erosion. The
560 caudal fin was more prone to fin erosion, while the anal fin had the least number of cases of fin
561 damage. Moderate fin erosion did not occur at a high incidence but could be found in the high
562 CO_2 group.

563 This lack of reaction from fin health is consistent with past research, which states that fish tend
564 to maintain a normal morphology in chronic hypercapnia conditions (Pěnka et al., 2026).
565 Studies in Atlantic salmon have observed physiological and cellular effects of CO_2 elevation
566 without the corresponding morphological damage. A recent experiment done on the effect on
567 welfare observed stunted growth and altered physiological stress response, but no
568 morphological changes related to welfare.

569 This current result also agrees with the recent pikeperch experiment in which no differences
570 were observed in fin erosion rate between the 5-30 mg L^{-1} CO_2 treatments. Therefore, there is
571 now some evidence from two closely related percid species that fin erosion is quite resistant to
572 chronic CO_2 levels of up to 30 mg L^{-1}, at least for 105 days.

573 The increased number of cases of caudal fin erosion is most likely associated with mechanical
574 rather than toxicological aspects of rearing conditions. Caudal fins are used actively in
575 movement and are therefore most prone to abrasions, contacts with tank walls, and collisions
576 with other fish. Therefore, fin health in RAS should be seen as an integrative welfare parameter
577 affected by several environmental factors and not as a specific biomarker of hypercapnia
578 (Policar et al., 2016; Stejskal et al., 2011).

However, occasional cases of caudal fin erosion in the High group can be biologically significant. Although frequency was not sufficient to show statistically any treatment effects, concentration-dependent development may hint at a possible interaction between CO_2 exposure and tissue integrity. Increased CO_2 levels can affect acid-based balance and increase the cost of maintaining homeostasis. Thus, it is possible that if energy is spent on homeostasis, then tissue integrity and resistance to mechanical damage can be affected. However, this explanation is speculative and cannot be proven on current results.

The significance of the above distinction is emphasized by the fact that according to histological findings, external morphology tends to underestimate the effect of chronic exposure to CO_2. Transcriptional responses to increased CO_2 were identified in Atlantic salmon, even in cases of relatively weak external manifestations of the condition. Mota et al. (2020) identified a widespread transcriptional response in gill tissue at CO_2 levels between 5- 40 mg L^{-1}. Therefore, the extremely rare cases of fin erosion found in this study can be considered to be proof of intact gross fin structure rather than an indication of no changes in the integumentary system.

4.5 Implications for Eurasian perch production and future research

In general, compared to the results found in previous research, it can be concluded that Eurasian perch displayed high tolerance to chronic exposure to CO_2. Unlike pikeperch, where 15-30 mg L^{-1} CO_2 have been associated with a lower body weight, SGR, feed intake, metabolic oxygen consumption and hematological parameters (Pěnka et al., 2026; Steinberg et al., 2017), Eurasian perch showed intact body weight, SGR, FCR, survival, organosomatic indices and plasma biochemical parameters under the same CO_2 conditions. Thus, the most apparent response in the current experiment could be the decrease in total length along with the increase in the Fulton condition factor at CO_2 exposure. This response differs from the decrease in condition factor found in several salmonid fish.

5. Conclusions

The results obtained from this study show that juvenile Eurasian perch can withstand the presence of high CO_2 concentration of 15-30 mg L^{-1} in intensive aquaculture with no effects on growth parameters, survival rate, feed conversion, organosomatic indices, and plasma biochemical composition. While there was a decrease in final total length due to the high CO_2, it does not mean that elevated CO_2 has no biological impact, as almost all other production and physiological parameters were not affected. This shows that fish have high levels of tolerance to high CO_2 during the ongrowing stage. Therefore, it is still recommended to keep the CO_2 level below 15 mg L^{-1}.

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Declaration of competing interest

The authors affirm that they have no competing financial interests or personal relationships that could in any way have influenced the research presented in this paper.

Author contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by all the authors. The first draft of the manuscript was written by Tomáš Pěnka and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Table 1: Mean values of the monitored water chemistry parameters in each RAS unit under CO_2 treatment of: Low (5 mg L^{-1}), Medium (15 mg L^{-1}), and High (30 mg L^{-1}) for 105 days (15 weeks).

Parameter	Low	Medium	High
DCO_2 (mg L^{-1})	4.97 ± 2.08	14.67 ± 4.02	29.68 ± 3.02
pH	7.01 ± 0.22	6.90 ± 0.42	6.78 ± 0.20
DO (%)	105.16 ± 6.25	104.56 ± 7.08	108.50 ± 8.04
Temperature (°C)	22.11 ± 0.38	22.20 ± 0.42	22.08 ± 0.47
NH_4^+ (mg L^{-1})	0.53 ± 0.17	0.71 ± 0.27	0.71 ± 0.33
NO_2^- (mg L^{-1})	0.64 ± 0.24	0.74 ± 0.13	0.92 ± 0.41

Values expressed as mean ± SD.

NH_4^+ = ammonium cation; DCO_2 = dissolved carbon dioxide concentration; DO = dissolved oxygen concentration; NO_2^- = nitrite anion; pH = logarithmic and inversely indicates the activity of hydrogen cations in the solution: $pH = -\log_{10}[H^+]$.

Table 2: Growth and condition parameters of juvenile Eurasian perch (*Perca fluviatilis*) exposed to different CO_2 concentrations: Low (5 mg L^{-1}), Medium (15 mg L^{-1}), High (30 mg L^{-1}) for 105 days (15 weeks).

Parameter	Low	Medium	High	F-statistic	*p*-value
Initial					
TL (mm)	203.29 ± 18.31	202.87 ± 19.13	203.90 ± 18.25	$F_{(2,447)} = 2.640$	0.212
SL (mm)	172.42 ± 15.86	173.25 ± 15.95	177.26 ± 17.72	$F_{(2,447)} = 2.525$	0.194
BW (g)	112.28 ± 29.46	114.93 ± 29.38	119.74 ± 34.03	$F_{(2,447)} = 2.210$	0.201
K	2.14 ± 0.21	2.13 ± 0.26	2.14 ± 0.20	$F_{(2,447)} = 3.527$	0.178
CV (%)	26.18 ± 0.01	25.37 ± 0.01	28.64 ± 0.03	$F_{(2,6)} = 1.263$	0.349
Final					
TL (mm)	254.11 ± 20.62[a]	248.10 ± 23.92[b]	244.63 ± 22.86[b]	$F_{(2,312)} = 4.77$	0.009
SL (mm)	221.14 ± 19.53	216.04 ± 21.85	217.93 ± 21.25	$F_{(2,312)} = 1.60$	0.203
BW (g)	250.89 ± 79.67	243.86 ± 81.57	234.57 ± 71.92	$F_{(2,312)} = 1.161$	0.314
K	2.11 ± 0.24[b]	2.35 ± 0.32[a]	2.34 ± 0.22[a]	$F_{(2,312)} = 27.34$	≥0.001
CV (%)	33.3 ± 0.46	33.1 ± 0.27	31.7 ± 0.14	$F_{(2,6)} = 0.138$	0.873
SHR	118.13 ± 13.84	118.08 ± 8.21	114.24 ± 4.67	$F_{(2,6)} = 0.114$	0.894
SGR (% day^{-1})	0.42 ± 0.05	0.43 ± 0.05	0.37 ± 0.08	$F_{(2,6)} = 0.621$	0.569
TGC (°C day^{-1})	0.11 ± 0.02	0.13 ± 0.01	0.20 ± 0.08	$F_{(2,6)} = 0.489$	0.636
FCR	1.55 ± 0.20	1.41 ± 0.10	1.42 ± 0.04	$F_{(2,6)} = 0.748$	0.513
SR (%)	98.00 ± 1.63	98.00 ± 1.63	99.33 ± 0.94	$F_{(2,6)} = 0.571$	0.593

[a-b]Values in the same row sharing different uppercase letters differ significantly ($p < 0.05$).

Values expressed as mean ± SD

BW = body weight; CV = coefficient of variation of body weight; FCR = feed conversion ratio; K = Fulton's condition factor; SGR = specific growth rate; SHR = specific heterogeneity rate; SL = standard length; SR = survival rate; TL = total length; TGC = thermal coefficient rate

Table 3: Plasma biochemical parameters in blood plasma of juvenile pikeperch (*Sander lucioperca*) at the end of the experiment exposed to different CO_2 concentrations: high (30 mg L^{-1}), medium (15 mg L^{-1}) and low (5 mg L^{-1}) for 105 days (15 weeks).

Parameter	Low	Medium	High	F-statistic	*p*-value
TP (g L^{-1})	47.17 ± 12.91	53.83 ± 8.47	40.83 ± 13.32	$F_{(2,15)} = 1.830$	0.195
ALB (g L^{-1})	9.33 ± 3.72	10.50 ± 2.26	8.00 ± 3.52	$F_{(2,15)} = 0.898$	0.428
LIPA (µkat L^{-1})	0.85 ± 0.77	0.54 ± 0.04	0.84 ± 0.76	$F_{(2,15)} = 0.483$	0.626
AMY (µkat L^{-1})	27.70 ± 14.64	39.10 ± 4.96	33.41 ± 16.48	$F_{(2,15)} = 1.147$	0.344
GLU (mmol L^{-1})	4.50 ± 0.80	3.73 ± 0.23	3.92 ± 0.78	$F_{(2,15)} = 2.22$	0.143
ALT (µkat L^{-1})	11.70 ± 7.24	15.22 ± 2.99	6.23 ± 8.16	$F_{(2,15)} = 2.885$	0.087
AST (µkat L^{-1})	5.78 ± 5.77	2.93 ± 1.53	3.72 ± 3.61	$F_{(2,15)} = 0.802$	0.467
NH_3 (µmol L^{-1})	1232.80 ± 186.75	1227.50 ± 166.88	1755.60 ± 155.86	$F_{(2,15)} = 1.016$	0.386
CORT (nmol L^{-1})	156.83 ± 140.87	118.50 ± 122.73	193.98 ± 147.26	$F_{(2,15)} = 0.453$	0.644
GLOB (g L^{-1})	36.00 ± 4.52	43.33 ± 6.35	32.83 ± 9.85	$F_{(2,15)} = 2.22$	0.143

[a-b]Values within the same row sharing different uppercase letters differ significantly ($p < 0.05$).

Values expressed as mean ± SD

ALB = albumin; ALT = alanine aminotransferase; AMYL = amylase; AST = aspartate aminotransferase; CORT = cortisol; GLOB = globulin; GLU = glucose; LIPA = lipase; NH_3 = ammonia; TP = total protein

816 Table 4: Percentage distribution of fin erosion scores in different fish fins across CO_2 groups
817 (Initial, Low: 5 mg L^{-1}, Medium: 15 mg L^{-1}, and High: 30 mg L^{-1}) after 105 days. Fin erosion
818 was classified as Grade 0 (< 5%), Grade 1 (5-30%), Grade 2 (30-70%), and Grade 3 (> 70%)

Fish fin	Grade	Initial (%)	Low (%)	Medium (%)	High (%)
Pectoral left	0	100	97	100	96
	1	0	3	0	4
	2	0	0	0	0
	3	0	0	0	0
Pectoral right	0	98	97	99	93
	1	2	3	1	5
	2	0	0	0	1
	3	0	0	0	1
Ventral left	0	100	93	97	97
	1	0	7	3	3
	2	0	0	0	0
	3	0	0	0	0
Ventral right	0	100	96	95	95
	1	0	4	5	4
	2	0	0	0	1
	3	0	0	0	0
Dorsal first	0	100	95	94	93
	1	0	5	6	5
	2	0	0	0	2
	3	0	0	0	0
Dorsal second	0	99	97	95	96
	1	1	3	5	4
	2	0	0	0	0
	3	0	0	0	0
Caudal	0	89	90	84	87
	1	11	10	16	13
	2	0	0	0	1
	3	0	0	0	0
Anal	0	100	100	97	100
	1	0	0	3	2
	2	0	0	0	0
	3	0	0	0	0

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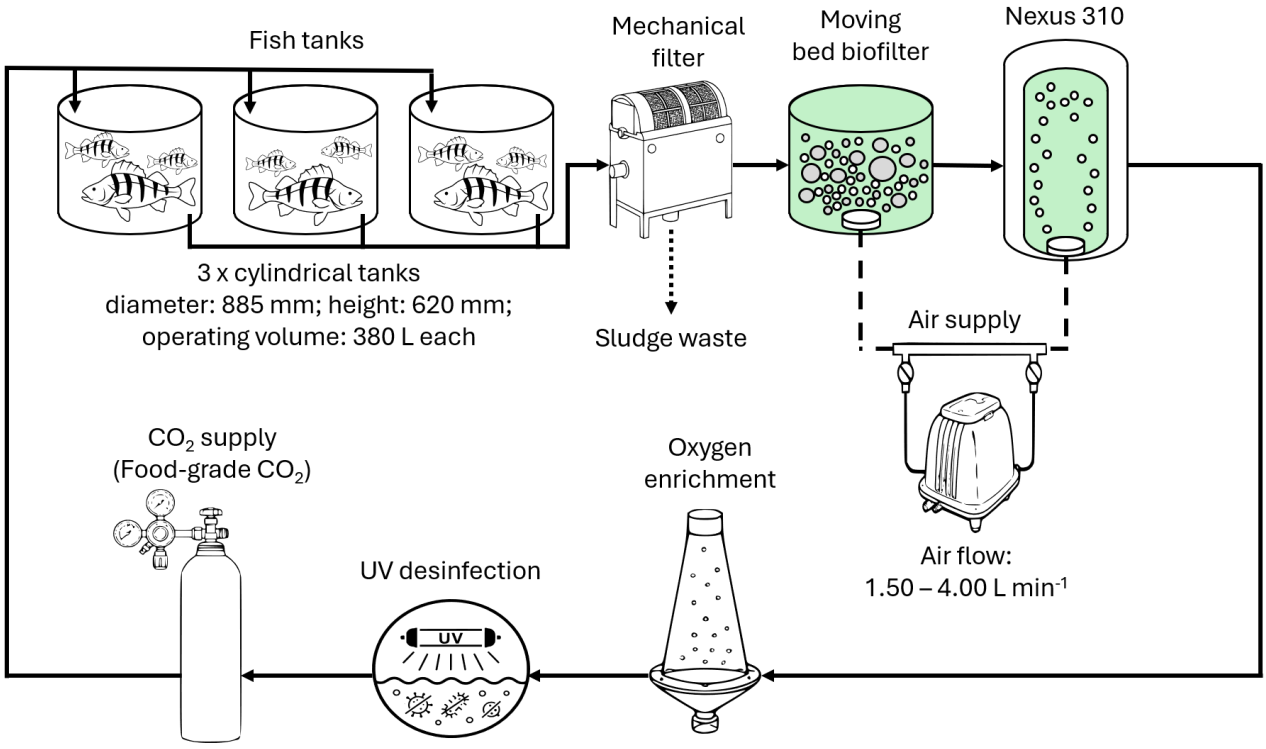
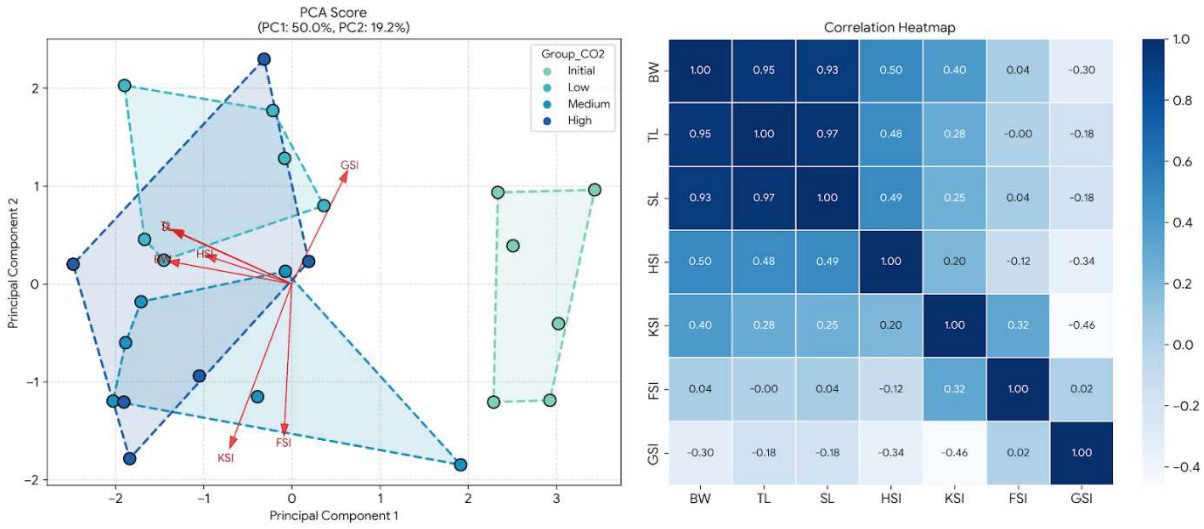


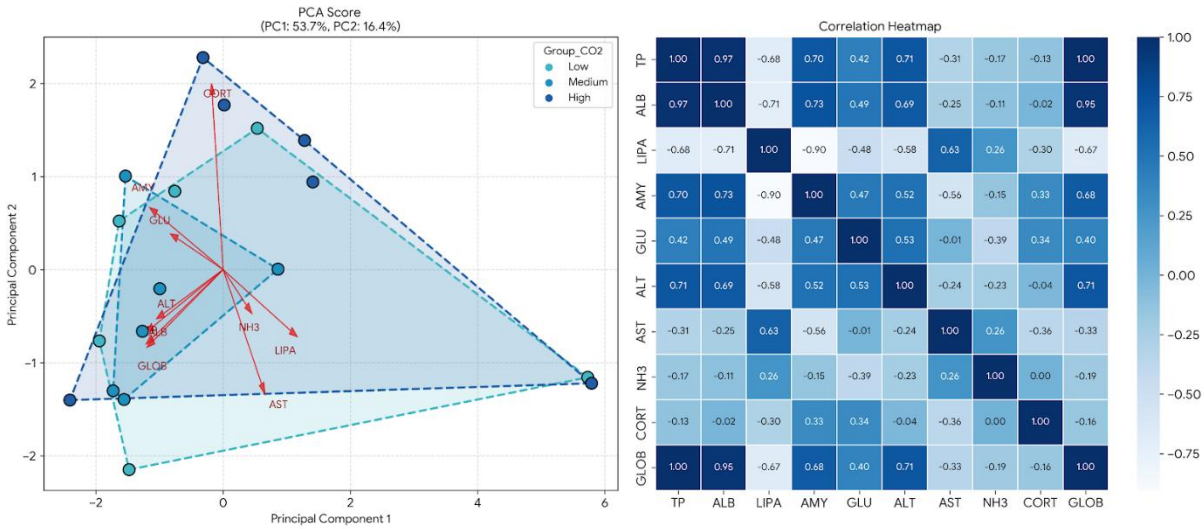
Figure 1: Schematic design of recirculating aquaculture system (RAS) illustrating the water treatment loop, including mechanical filtration, two-stage biofiltration with aeration, oxygen enrichment, UV disinfection, and CO_2 supplementation supplying three 380 L cylindrical fish tanks.



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827 Figure 2: Principal component analysis (PCA; PC1: 50.0%, PC2: 19.2%) biplot and Pearson
828 correlation heatmap for somatic indices (BW = body weight; TL = total length; SL = standard
829 length; HSI = hepatosomatic index; KSI = kidney somatic index; FSI = fat somatic index; GSI-
830 gonad somatic index) of juvenile Eurasian perch (*Perca fluviatilis*) after 105 days of experiment
831 for groups: Initial (n = 6), Low (5 mg L^{-1}; n = 6), Medium (15 mg L^{-1}; n = 6), High (30 mg L^{-1};
832 n = 6).

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835 Figure 3: Principal component analysis (PCA; PC1: 53.7%, PC2: 16.4%) biplot and Pearson
836 correlation heatmap for blood biochemical parameters (ALB = albumin; ALT = alanine
837 aminotransferase; AMYL = amylase; AST = aspartate aminotransferase; CORT = cortisol;
838 GLOB = globulin; GLU = glucose; LIPA = lipase; NH3 = ammonia; TP = total protein) of
839 juvenile Eurasian perch (*Perca fluviatilis*) after 105 days of experiment for groups: Initial (n =
840 6), Low (5 mg L^{-1}; n = 6), Medium (15 mg L^{-1}; n = 6), High (30 mg L^{-1}; n = 6).