










## Artículo original / Original article

# Effects of acute and chronic exposure to different water salinities in juvenile *Piaractus brachypomus*: Hematology, blood biochemistry, liver and gill performance and histology

Efectos de la exposición aguda y crónica a diferentes salinidades del agua en juveniles de *Piaractus brachypomus*: hematología, bioquímica sanguínea, desempeño e histología hepática y branquial

André de Sena-Souza <sup>1</sup>; Fabil Aremil Costa-Santos <sup>1</sup>; Gustavo Soares da Costa-Júlio <sup>1</sup>; Sidney dos Santos-Silva <sup>1</sup>; Imaculada de Moraes Carvalho-Ananias <sup>1</sup>; Camila Gomes-de Oliveira <sup>1</sup>; Pedro Paulo Cortezzi-Pedras <sup>1</sup>; Gisele Cristina Favero <sup>1</sup>; Ronald Kennedy Luz <sup>1\*</sup>

<sup>1</sup>Universidade Federal de Minas Gerais, Belo Horizonte, Brasil

## ABSTRACT

The present study assessed the effects of acute and chronic exposure to varying salinity levels on juvenile red-bellied pacu (*Piaractus brachypomus*). Experiment 1 investigated acute salt exposure over 96 hours, while Experiment 2 examined chronic exposure to different salinities over 60 days. Both experiments revealed alterations in hematological and histological parameters. In Experiment 1, a salinity of 12 g salt L<sup>-1</sup> was lethal, whereas salinities near 3 g salt L<sup>-1</sup> were more appropriate. In Experiment 2, optimal performance after 60 days was observed at salinities up to 2 g L<sup>-1</sup>. More pronounced disturbances in blood parameters and tissue structure occurred at 4 and 6 g salt L<sup>-1</sup>. Consequently, salinities up to 2 g salt L<sup>-1</sup> are recommended for the cultivation of *P. brachypomus*.

**Keywords:** neotropical species; physiology; growth; osmotic shock

## RESUMEN

Se evaluó el efecto de la exposición aguda y crónica a diferentes salinidades en juveniles de paco (*Piaractus brachypomus*). En el experimento 1, se analizó la exposición aguda a la sal durante 96 horas, mientras que el experimento 2 evaluó la exposición crónica a distintas salinidades durante 60 días. En ambos experimentos se identificaron alteraciones en los parámetros sanguíneos y en la estructura histológica de los animales. En el experimento 1, la salinidad de 12 g de sal L<sup>-1</sup> resultó letal, siendo más adecuadas salinidades cercanas a 3 g de sal L<sup>-1</sup>. En el experimento 2, los mejores resultados de desempeño, tras 60 días, se obtuvieron con salinidades de hasta 2 g de sal L<sup>-1</sup>. Los mayores desequilibrios en los parámetros sanguíneos y las alteraciones tisulares más severas se registraron a 4 y 6 g de sal L<sup>-1</sup>. Por lo tanto, salinidades de hasta 2 g de sal L<sup>-1</sup> se consideran más adecuadas para el cultivo de *P. brachypomus*.

**Palabras clave:** especie neotropical; fisiología; crecimiento; choque osmótico.

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\* luzrk@yahoo.com (corresponding author)



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## 1. INTRODUCTION

Aquaculture has become established as a rapidly expanding sector within the productive industry, reaching levels surpassing those of capture fisheries (FAO, 2024). According to this report, the growth in aquatic organism production is driven by increasing market demand and global per capita fish consumption.

Within this context of intensified production, the adoption of low-cost inputs is prevalent and contributes to disease prevention (Khor et al., 2024), prophylactic treatments (Tavares-Dias, 2022), animal welfare (Huang et al., 2025), and enhanced zootechnical performance (Abdel-Latif et al., 2023) throughout various segments of the production chain.

Among these inputs, common salt (NaCl) is particularly notable. At appropriate concentrations, it demonstrates low toxicity, broad accessibility, and cost-effectiveness. Salt is utilized in aquaculture for multiple purposes: adjusting water salinity at various cultivation stages to enhance productive performance (Abdel-Latif et al., 2023; Araújo et al., 2020; Ferreira et al., 2023; Jomori et al., 2013), implementing prophylactic treatments (Oladosu & Oladosu, 2019), and mitigating stress during routine practices such as transport (Luz & Favero, 2024), biometric handling, and slaughter (Uehara et al., 2021).

Nevertheless, excessive use of salt can result in structural and physiological damage to aquatic organisms. Alterations in blood biochemistry have been documented in various freshwater fish species exposed to saline water baths for different durations (Demska-Zakęś et al., 2021; dos Santos Silva et al., 2026; Mattioli et al., 2017). Comparable effects have also been observed during prolonged exposure to saline water (Fiúza et al., 2015; Mohamed et al., 2021; Zidan et al., 2022).

Beyond impacts on blood biochemistry, inappropriate salinity levels during cultivation can induce histopathological alterations in organs such as the liver and gills (dos Santos Silva et al., 2026; Mohamed et al., 2021; Takata et al., 2021; Zidan et al., 2022).

Red-bellied pacu (*Piaractus brachypomus*) is a South American species endemic to the Amazon and Orinoco river basins (Escobar L. et al., 2019). In addition to being among the most widely produced native species in Latin America, it is now extensively cultivated in Asia (Prathib, 2025; Seshagiri et al., 2022). This widespread cultivation is attributed to its resilience, adaptability to diverse farming systems, and significant commercial value (Angeles-Escobar et al., 2022; de Morais Carvalho Ananias et al., 2025).

Given the increasing production of this species and the potential application of common salt in aquaculture, this study aimed to evaluate the effects of acute and chronic exposure to varying salinity levels on blood parameters, productive performance indicators, and histological characteristics in juvenile *P. brachypomus*.

## 2. MATERIALS AND METHODS

### Experimental site and facilities

This study was conducted at the Universidade Federal de Minas Gerais (UFMG, Brazil), in the larviculture sector of the Aquaculture Laboratory (LAQUA). All procedures were approved by the Animal Ethics Committee (CEUA-UFMG; No. 102/2024 and No. 150/2023).

### Experiment 1. Acute exposure to water salinity for 96 hours

A total of 120 juvenile *Piaractus brachypomus* were used, with an average weight of  $32.45 \pm 6.52$  g and a length of  $13.26 \pm 0.97$  cm. The juveniles were distributed into five recirculating aquaculture systems (RAS). Each RAS consisted of a 200 L water reservoir equipped with mechanical and biological filtration, and four 28 L rearing tanks (replicates), following the model described by Ferreira et al. (2021). Six juveniles were stocked per tank, for a total of 24 individuals per treatment.

During the first 14 days, the fish were acclimated to experimental conditions in freshwater. Feeding was provided twice daily (08:00 and 16:00 h) to apparent satiation using a commercial extruded diet (Aquos Starter, 2–3 mm), containing 45% crude protein, 4% crude fiber, 15% mineral matter, 8% ether extract, 2% calcium, and 0.8% phosphorus.

After the acclimation period, the fish were transferred to other RAS under similar conditions but previously adjusted to different salinity levels: freshwater (control); S3 – 3 g salt L<sup>-1</sup>; S6 – 6 g salt L<sup>-1</sup>; S9 – 9 g salt L<sup>-1</sup>; and S12 – 12 g salt L<sup>-1</sup>. Salinity solutions were prepared using commercial sodium chloride (salt) from Refinaria Sal Garça LTFA (Mossoró, Rio Grande do Norte, Brazil). The fish were maintained under these conditions for 96 h.

During this period, the first feeding was carried out after 24 h, followed by two daily feedings at 08:00 and 16:00 h.

Water quality parameters—temperature, salinity, electrical conductivity, and pH—were measured using a HI9146 probe (Hanna Instruments); dissolved oxygen was determined using a YSI 6920VZ2 multiparameter probe; and total ammonia was measured using a colorimetric kit (LabconTest, Alcon®, Camboriú, SC, Brazil) at 24, 48, and 96 h of exposure. The average temperature was  $27.49 \pm 0.32$  °C; pH remained at  $7.79 \pm 0.34$ ; dissolved oxygen was  $6.68 \pm 0.71$  mg L<sup>-1</sup>; and total ammonia remained below 0.5 mg L<sup>-1</sup> in all treatments. Salinity and electrical conductivity varied according to treatments.

Survival was assessed daily through direct counting of individuals, and mortality was recorded.

### Experiment 2. Performance and physiology of juveniles under different salinity levels

A total of 224 juvenile *P. brachypomus* were used in this experiment, with an average weight of  $5.74 \pm 0.90$  g and a length of  $5.63 \pm 0.22$  cm. The lower weight in Experiment 2 compared to Experiment 1 was due to the longer experimental duration (60 days), which was intended to evaluate productive performance. The juveniles were distributed into four RAS units, as previously described. Each system was prepared with the following salinity levels, based on the results of Experiment 1: S0 – freshwater (control); S2 – 2 g salt L<sup>-1</sup>; S4 – 4 g salt L<sup>-1</sup>; and S6 – 6 g salt L<sup>-1</sup>.

The juveniles were directly stocked into the different salinity treatments. The experiment was divided into two phases: in phase 1 (0–30 days), a stocking density of 0.5 juveniles L<sup>-1</sup> (14 fish per tank) was used; in phase 2 (31–60 days), the density was reduced to 0.25 juveniles L<sup>-1</sup> (7 fish per tank), due to the growth observed during the initial period.

Water conditions were as follows: average temperature of 28.34 ± 0.27 °C; pH of 6.42 ± 0.69; dissolved oxygen of 4.28 ± 0.31 mg L<sup>-1</sup>; and total ammonia below 0.5 mg L<sup>-1</sup> in all treatments. Salinity and electrical conductivity varied across treatments.

Fish were fed *ad libitum* in two daily meals (08:00 and 15:00 h) using a commercial extruded diet (Nanolis 45% – Socil, 0.8 mm), containing 45% crude protein, 8–9% ether extract, 15% mineral matter, 3.5–4% crude fiber, 2–3% calcium, and 1% phosphorus (manufacturer's specifications). Once apparent satiation was reached (approximately 30 min after feeding), feed leftovers were collected and dried in an oven (Nova Ética/Ethink) at 55 °C to estimate feed intake. Daily tank cleaning was performed to remove feces, and partial water exchange (50% of tank volume) was carried out every 15 days, replacing water with the same salinity and temperature as each RAS system.

### Survival and zootechnical performance

In Experiment 2, growth was determined by weight measurements using a semi-analytical balance (Ay-220, 220 g × 0.0001 g, Marte, Brazil) and length measurements with a digital caliper (Starrett Electronic, Massachusetts, USA). Biometric measurements were performed at 15, 30, 45, and 60 days of cultivation. Based on these data, the following parameters were calculated:

- Final weight (FW) (g) = Biomass (g) / number of fish per tank;
- Final length (FL) (cm) = total length of the fish;
- Weight gain (WG) (g) = Final weight (FW) – Initial weight (IW);
- Daily weight gain (DWG) (g day<sup>-1</sup>) = (Final weight – Initial weight) / number of days;
- Daily feed intake (DFI) (g) = Total feed intake (g) / number of fish per tank / number of days;
- Apparent feed conversion ratio (FCR) = Total feed intake (g) / weight gain (g);
- Specific growth rate (SGR) (% day<sup>-1</sup>) = 100 × (lnPf – lnPi) / time interval between samplings (days), where Pi is the initial weight, and Pf is the final weight;

Survival (%) was determined at each sampling point through direct counting of individuals.

### Blood analyses

In Experiment 1, after 24 h and 96 h of exposure (E) to different salinity levels, three fish per tank (n = 12 fish per treatment) were anesthetized with 50 mg L<sup>-1</sup> eugenol (Ferreira et al., 2021) and subjected to blood collection by caudal venous puncture using heparinized syringes. In Experiment 2, blood samples were collected at 30 and 60 days of cultivation (n = 12 juveniles per treatment at each sampling). After blood collection, fish were euthanized with a solution containing 285 mg L<sup>-1</sup> eugenol (Mattioli et al., 2017) for tissue sampling.

Blood samples were placed in microtubes containing anticoagulant (10% sodium heparin) for hemoglobin determination using a commercial colorimetric kit (Quibasa-Bioclin, Belo Horizonte, MG, Brazil) and hematocrit determination using the microhematocrit method (Goldenfarb et al., 1971), with capillary tubes. Total plasma protein (TPP) was measured using an analogical refractometer (0–90% Brix; RHB0-90) after breaking the microhematocrit tube. Based on erythrocyte count, hemoglobin, and hematocrit values, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated according to the formulas described by Wintrobe (1934).

The remaining whole blood was centrifuged at 4000 rpm for 10 min to obtain plasma for biochemical analysis. Glucose, triglycerides, cholesterol, lactate, chlorides, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were determined using colorimetric methods with commercial kits (Quibasa-Bioclin, Belo Horizonte, MG, Brazil), with readings performed in a spectrophotometer (semi-automatic analyzer Bioclin 100®). Lactate was measured only in the sample corresponding to 60 days of Experiment 2.

### Indices

In Experiment 2, after euthanasia, liver and visceral weights were recorded to calculate the hepatosomatic index (HSI) =  $100 \times (\text{liver weight/body weight})$  and the viscerosomatic index (VSI) =  $100 \times (\text{viscera weight/body weight})$ .

### Histological analyses

In Experiment 1, after blood collection and euthanasia, gill and liver samples were collected. Gill and liver tissues were fixed in Bouin's solution for subsequent histological analysis, following the methodology described by Usman et al. (2021). After 12 h, the tissues were removed from the fixative, washed in 70% ethanol, and subsequently embedded in paraffin. Samples were sectioned into 5  $\mu\text{m}$  thick slices using a microtome (Leica RM2125 RTS). Histological slides were prepared using hematoxylin and eosin (H&E) staining. The analysis was performed using a light microscope (Nikon Eclipse 50i) to identify and record the frequency of morphological alterations. Slides were examined for abnormalities, and the number of affected samples was recorded. Slides were numerically coded and randomly analyzed. For each replicate, two non-consecutive histological sections were evaluated. The assessment considered the presence or absence of each histological alteration at the individual level, and lesion frequency was calculated as the proportion of affected individuals relative to the total number of individuals per treatment. Data were then expressed as percentages to classify histological alterations: less than 5% was considered absent (–), 5–25% mild (+), 25–50% moderate (++), and above 50% severe (+++). Tissue impairment was evaluated according to the severity criteria proposed by Abdel-Moneim et al. (2012), Jahan et al. (2024) and de Oliveira et al. (2025).

The same procedure was applied after 60 days of cultivation in Experiment 2.

### Statistical analysis

Data from both experiments were tested for homogeneity of variances and normality using Levene's and Shapiro–Wilk tests, respectively. Subsequently, data from Experiment 1 were analyzed using a two-way analysis of variance (ANOVA) with salinity levels (S0, S3, S6, S9, S12) and sampling times

(24hE and 96hE), followed by Tukey's post hoc test at the 5% of significance level. In Experiment 2, hematological data and somatic indices were analyzed using a two-way ANOVA with salinity levels (S0, S2, S4, S6) and cultivation times (30 and 60 days), followed by Tukey's test ( $p < 0.05$ ). Zootechnical performance data were analyzed using a one-way ANOVA, followed by Tukey's test at the 5% significance level.

### 3. RESULTS

#### Experiment 1.

During the experimental period, total mortality was recorded in treatment S12, occurring between 24 and 48 h of salinity exposure. No mortality was observed in the other treatments.

Hemoglobin concentrations showed significant differences as a function of salinity ( $P = 0.0039$ ), sampling time ( $P < 0.0001$ ), and their interaction ( $P = 0.0001$ ) (**Table 1**). At 24 h of exposure (24hE), the highest concentrations were observed in S9. At 96 h (96hE), the lowest values were recorded in S3, while the highest were observed in S0. When comparing sampling times, a decrease in hemoglobin concentrations was observed in S9 between 24hE and 96hE.

Hematocrit levels did not differ significantly among salinities ( $P = 0.1133$ ), but did differ significantly with respect to sampling time ( $P = 0.0002$ ) and the interaction between factors ( $P = 0.0058$ ) (**Table 1**). At 24hE, the highest values were observed in S9 and the lowest in S0. At 96hE, the highest values were recorded in S6 and the lowest in S3. A reduction in hematocrit values between 24hE and 96hE was also observed in S3 and S9.

The number of erythrocytes showed significant differences only as a function of salinity ( $P < 0.0001$ ), with the lowest values recorded in S9 (**Table 1**).

Mean corpuscular volume (MCV) showed significant differences with respect to salinity ( $P = 0.0009$ ) and sampling time ( $P = 0.0057$ ), with no significant interaction between factors ( $P = 0.099$ ) (**Table 1**). The highest value was observed in S9, and a decrease was noted between 24hE and 96hE.

Mean corpuscular hemoglobin (MCH) followed a pattern similar to that of MCV, showing differences among salinities ( $P = 0.0002$ ) and sampling times ( $P = 0.002$ ), with no interaction effect ( $P = 0.1874$ ) (**Table 1**). The highest value was recorded in S9, with a decrease from 24hE to 96hE. In contrast, mean corpuscular hemoglobin concentration (MCHC) did not show significant differences among salinities ( $P = 0.1284$ ), sampling times ( $P = 0.1675$ ), or their interaction ( $P = 0.5075$ ) (**Table 1**).

**Table 1.**

*Hematological parameters (mean ± standard error) of juvenile *Piaractus brachypomus* subjected to acute exposure to different salinity levels for 96 hours.*

Time	Salinity (g de salt L <sup>-1</sup> )					Mean	P-value Salinity	P-value Time	P-value Interaction
	0	3	6	9	12				
<b>Hemoglobin (g dL<sup>-1</sup>)</b>									
24hE	6.35 ± 0.21 Ba	6.43 ± 0.18 Ba	6.84 ± 0.16 Ba	8.04 ± 0.32 Aa	6.55 ± 0.25 B	6.87 ± 0.13	0.0039	<0.0001	0.0001
96hE	6.45 ± 0.17 Aa	5.75 ± 0.18 Ba	6.40 ± 0.28 Aba	5.92 ± 0.30 ABb		6.14 ± 0.12			
Mean	6.41 ± 0.13	6.10 ± 0.14	6.62 ± 0.16	6.98 ± 0.31					
<b>Hematocrit (%)</b>									
24hE	22.58 ± 0.98 Ca	24.0 ± 0.89 BCa	23.75 ± 0.76 BCa	27.16 ± 1.14 Aa	25.83 ± 1.33 AB	24.67 ± 0.60	0.1133	0.0002	0.0058
96hE	22.09 ± 0.73 ABa	20.36 ± 1.13 Bb	23.58 ± 0.67 Aa	21.00 ± 0.75 ABb		21.78 ± 0.44			
Mean	22.34 ± 0.60	22.18 ± 0.80	23.66 ± 0.49	24.08 ± 0.92					
<b>Erythrocytes (x 10<sup>6</sup> µL<sup>-1</sup>)</b>									
24hE	0.39 ± 0.02	0.35 ± 0.02	0.37 ± 0.01	0.31 ± 0.01	0.29 ± 0.03	0.34 ± 0.01 A	<0.0001	0.1264	0.8226
96hE	0.39 ± 0.01	0.38 ± 0.01	0.41 ± 0.01	0.32 ± 0.01		0.37 ± 0.01 A			
Mean	0.39 ± 0.01 a	0.37 ± 0.01 a	0.39 ± 0.01 a	0.32 ± 0.01 b					
<b>MCV (fL<sup>-1</sup>)</b>									
24hE	555.7 ± 29.33	684.0 ± 46.14	639.3 ± 24.47	817.8 ± 55.92	885.0 ± 66.70	709.9 ± 25.36 A	0.0009	0.0057	0.099
96hE	579.2 ± 13.43	534.7 ± 37.91	585.6 ± 31.37	643.6 ± 31.56		587.0 ± 18.19 B			
Mean	561.9 ± 21.65 b	609.3 ± 33.38 b	612.5 ± 20.25 b	726.5 ± 36.19 a					
<b>MCH (pg<sup>-1</sup>)</b>									
24hE	165.98 ± 10.91	185.85 ± 12.49	184.73 ± 6.65	239.30 ± 12.33	216.51 ± 21.79	196.19 ± 6.44 A	0.0002	0.002	0.1874
96hE	172.74 ± 15.28	151.78 ± 11.26	158.77 ± 9.37	184.71 ± 9.02		165.91 ± 5.57 B			
Mean	168.10 ± 8.4 b	169.56 ± 9.02 b	171.75 ± 6.23 b	210.71 ± 9.54 a					
<b>MCHC (g dL<sup>-1</sup>)</b>									
24hE	29.98 ± 1.27	26.92 ± 0.96	29.17 ± 1.16	29.99 ± 1.36	26.53 ± 1.11	28.55 ± 0.55 A	0.1284	0.1675	0.5075
96hE	29.40 ± 1.43	27.54 ± 0.59	27.24 ± 1.06	27.11 ± 1.54		27.81 ± 0.61 A			
Mean	29.69 ± 0.94	27.21 ± 0.56	28.20 ± 0.79	28.61 ± 1.04					

\* Uppercase letters in the same row compare sampling times within each salinity level, and lowercase letters in the same column compare salinity levels within each sampling time, according to Tukey's test ( $p < 0.05$ ). When the interaction was not significant, letters in the marginal means indicate comparisons of main effects: uppercase letters for sampling times and lowercase letters for salinity levels. MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

**Table 2.**

*Blood biochemical parameters (mean ± standard error) of juvenile *Piaractus brachypomus* subjected to acute exposure to different salinity levels for 96 hours.*

Time	Salinity (g de salt L <sup>-1</sup> )					Mean	P-value Salinity	P-value Time	P-value Interaction
	0	3	6	9	12				
<b>Glucose (mg dL<sup>-1</sup>)</b>									
24hE	68.07 ± 2.50 Bb	63.34 ± 2.74 Bb	83.66 ± 3.25 Ab	88.26 ± 3.08 6 Ab	28.42 ± 3.94 C	66.53 ± 3.05	<0.0001	<0.0001	<0.0001
96hE	110.6 ± 0.41 Aa	112.2 ± 0.53 Aa	111.0 ± 0.57 Aa	108.5 ± 0.64 Aa		110.6 ± 0.33			
Mean	89.33 ± 4.60	87.78 ± 5.27	97.32 ± 3.27	99.81 ± 2.60					
<b>Cholesterol (mg dL<sup>-1</sup>)</b>									
24hE	90.18 ± 2.33 Bb	100.5 ± 2.74 ABb	106.9 ± 4.17 Ab	99.72 ± 3.79 ABa	59.64 ± 3.88 C	91.39 ± 2.64	<0.0001	<0.0001	0.0039
96hE	117.5 ± 4.71 Ba	133.3 ± 3.95 Aa	124.0 ± 3.98 ABa	101.8 ± 7.93 Ca		119.1 ± 3.13			
Mean	103.2 ± 3.83	116.2 ± 4.18	115.1 ± 3.36	100.7 ± 4.18					
<b>Triglycerides (mg dL<sup>-1</sup>)</b>									
24hE	148.4 ± 14.86	102.6 ± 21.39	138.0 ± 23.43	111.7 ± 13.37	144.4 ± 28.30	129.1 ± 3.60 A	<0.0001	<0.0001	0.3791
96hE	102.1 ± 18.16	73.72 ± 21.68	97.42 ± 25.72	84.31 ± 21.81		88.94 ± 3.54 B			
Mean	125.3 ± 6.12 a	87.52 ± 5.35 b	118.6 ± 6.60 a	98.00 ± 4.60 b					
<b>Proteins (g dL<sup>-1</sup>)</b>									
24hE	4.30 ± 0.06	4.40 ± 0.06	4.53 ± 0.06	4.63 ± 0.06	4.16 ± 0.09	4.40 ± 0.03 A	0.0194	0.7333	0.1911
96hE	4.35 ± 0.05	4.46 ± 0.06	4.58 ± 0.10	4.40 ± 0.11		4.45 ± 0.04 A			
Mean	4.33 ± 0.03 b	4.42 ± 0.04 ab	4.55 ± 0.05 a	4.52 ± 0.06 ab					
<b>Lactate (mg dL<sup>-1</sup>)</b>									
24hE	34.33 ± 1.53 Ba	29.16 ± 205 Ca	30.33 ± 2.52 BCa	25.91 ± 1.62 Ca	176.4 ± 2.19 A	41.41 ± 5.63	<0.0001	<0.0001	0.0059
96hE	9.46 ± 1.89 Ab	10.38 ± 1.88 Ab	11.89 ± 1.50 Ab	14.90 ± 2.09 Ab		11.76 ± 0.96			
Mean	23.67 ± 2.98	20.17 ± 2.42	21.55 ± 2.52	20.64 ± 1.73					
<b>Chlorides (mEq L<sup>-1</sup>)</b>									
24hE	166.9 ± 4.11	160.5 ± 2.54	160.2 ± 5.05	167.4 ± 7.11	220.9 ± 2.57	176.0 ± 3.73 A	<0.0001	0.0022	0.6311
96hE	154.4 ± 4.82	150.7 ± 5.28	147.1 ± 3.12	164.6 ± 3.28		154.1 ± 2.25 B			
Mean	160.4 ± 3.39 ab	155.8 ± 2.97 ab	153.4 ± 3.16 b	165.9 ± 3.57 a					
<b>ALT (U L<sup>-1</sup>)</b>									
24hE	9.18 ± 0.69 Aa	7.90 ± 0.85 Aa	8.33 ± 0.46 Aa	9.27 ± 0.72 Aa	8.66 ± 0.62 A	8.66 ± 0.30	0.7787	<0.0001	0.0021
96hE	5.36 ± 0.60 Bb	7.54 ± 0.60 Aa	6.00 ± 0.21 ABb	5.40 ± 0.49 Bb		6.09 ± 0.27			
Mean	7.27 ± 0.61	7.72 ± 0.51	7.16 ± 0.34	7.42 ± 0.61					
<b>AST (U L<sup>-1</sup>)</b>									

24hE	172.8 ± 19.88	169.9 ± 34.09	170.6 ± 30.49	169.5 ± 23.84	191.1 ± 8.43	174.6 ± 12.56 A	0.6647	0.4309	0.765
96hE	177.9 ± 31.57	160.5 ± 28.84	185.2 ± 17.65	217.5 ± 19.40		185.4 ± 13.67 A			
Mean	175.0 ± 17.19 a	165.2 ± 21.76 a	178.9 ± 16.12 a	191.3 ± 20.25 a					

\* Uppercase letters in the same row compare sampling times within each salinity level, whereas lowercase letters in the same column compare salinity levels within each sampling time, according to Tukey's test ( $p < 0.05$ ). When the interaction was not significant, letters in the marginal means indicate comparisons of the main effects: uppercase letters for sampling times and lowercase letters for salinity levels, according to Tukey's test ( $p < 0.05$ ). ALT: alanine aminotransferase; AST: aspartate aminotransferase.

Blood glucose levels showed significant differences as a function of salinity ( $P < 0.0001$ ), sampling time ( $P < 0.0001$ ), and their interaction ( $P < 0.0001$ ) (**Table 2**). At 24 h of exposure (24hE), the highest values were observed in S6 and S9, while the lowest were recorded in S12. At 96 h of exposure (96hE), an increase in glucose levels was observed compared to 24hE across all treatments.

Cholesterol levels showed significant differences among salinities ( $P < 0.0001$ ), sampling times ( $P < 0.0001$ ), and their interaction ( $P = 0.0039$ ) (**Table 2**). At 24hE, the highest values were recorded in S6 and the lowest in S12. At 96hE, the highest values were observed in S3 and the lowest in S9. Regarding sampling time, increases were observed in S0, S3, and S6 at 96hE.

Triglyceride levels showed significant differences as a function of salinity ( $P < 0.0001$ ) and sampling time ( $P < 0.0001$ ), with no significant interaction ( $P = 0.3791$ ) (**Table 2**). The highest values were recorded in S0 and S6. Regarding sampling time, higher levels were observed at 24hE.

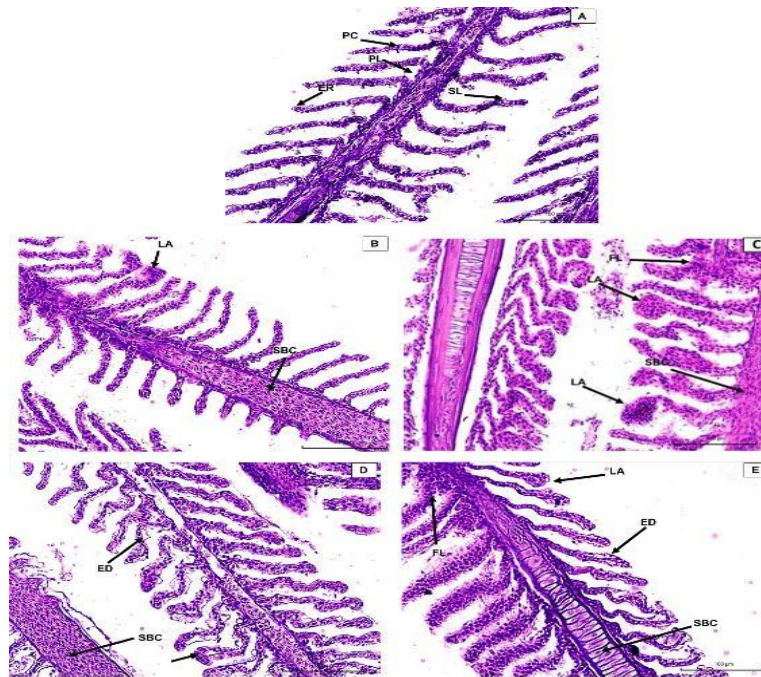
Total protein showed significant differences among salinities ( $P = 0.0194$ ), but not with respect to sampling time ( $P = 0.7333$ ) or their interaction ( $P = 0.1911$ ). The lowest values were observed in S0.

Lactate levels showed significant differences as a function of salinity ( $P < 0.0001$ ), sampling time ( $P < 0.0001$ ), and their interaction ( $P = 0.0059$ ) (**Table 2**). At 24hE, the lowest values were observed in S9 and S3, while the highest were recorded in S12. At 96hE, no differences among treatments were observed. When comparing sampling times, lactate levels were lower at 96hE across all salinities.

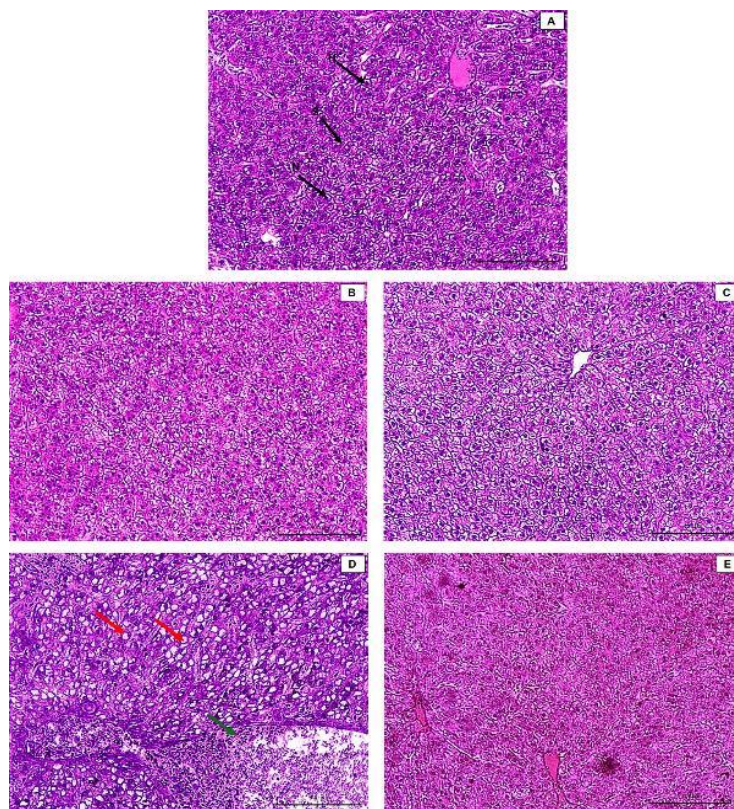
Chloride levels showed significant differences among salinities ( $P < 0.0001$ ) and sampling times ( $P = 0.0022$ ), with no interaction effect ( $P = 0.6311$ ) (**Table 2**). In terms of time, higher values were observed at 24hE. Among salinities, the highest value was recorded in S9 and the lowest in S6.

Alanine aminotransferase (ALT) showed significant differences as a function of sampling time ( $P < 0.0001$ ) and the interaction between factors ( $P = 0.0021$ ) (**Table 2**). At 24hE, ALT levels did not vary among salinities; however, at 96hE, the highest value was recorded in S3. In contrast, aspartate aminotransferase (AST) did not show significant differences (**Table 2**).

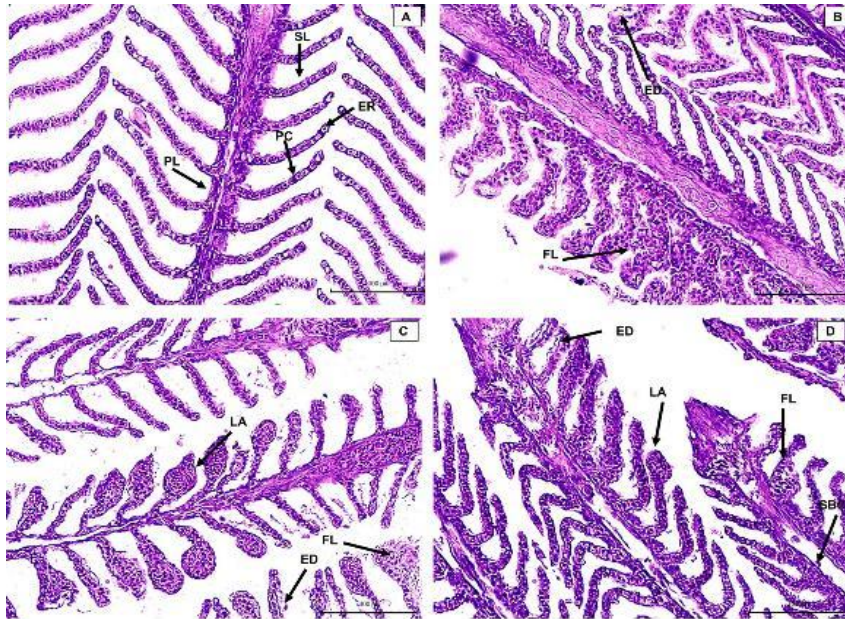
## Histological analyses



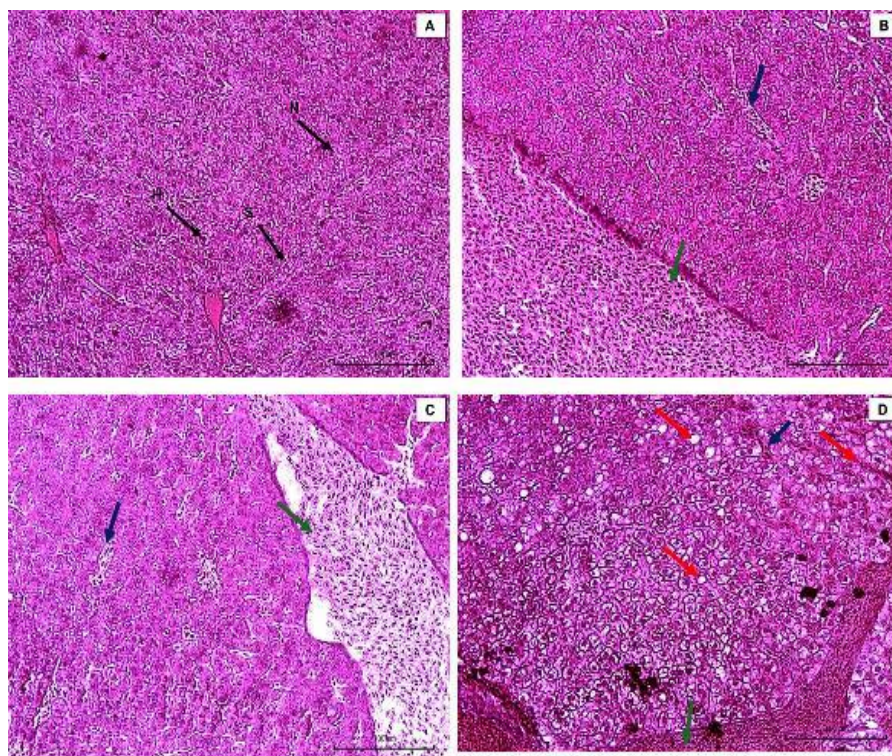
**Figure 1.** Histopathological alterations in the gills of juvenile *Piaraactus brachypomus* after 24 h of exposure (24hE) to different salinity levels. [A] Control; [B] 3 g salt L<sup>-1</sup>; [C] 6 g salt L<sup>-1</sup>; [D] 9 g salt L<sup>-1</sup>; [E] 12 g salt L<sup>-1</sup>. Primary lamellae (PL); secondary lamellae (SL); pillar cells (PC); erythrocytes (ER); epithelial lifting (EL); lamellar aneurysm (LA); severe blood congestion (SBC); lamellar fusion (LF)



**Figure 2.** Histopathological alterations in the liver of juvenile *Piaraactus brachypomus* after 24hE of exposure to different salinity levels. [A] Control; [B] 3 g salt L<sup>-1</sup>; [C] 6 g salt L<sup>-1</sup>; [D] 9 g salt L<sup>-1</sup>; [E] 12 g salt L<sup>-1</sup>. Hepatocytes (H); nuclei (N); sinusoids (S); congested blood vessel (green arrow); vacuolization (red arrow)



**Figure 3.** Histopathological alterations in the gills of juvenile *Piaraactus brachypomus* after 96 h of exposure (96hE) to different salinity levels. [A] Control; [B] 3 g salt L<sup>-1</sup>; [C] 6 g salt L<sup>-1</sup>; [D] 9 g salt L<sup>-1</sup>. Primary lamellae (PL); secondary lamellae (SL); pillar cells (PC); erythrocytes (ER); epithelial lifting (EL); lamellar aneurysm (LA); severe blood congestion (SBC); lamellar fusion (LF)



**Figure 4.** Histopathological alterations in the liver of juvenile *Piaraactus brachypomus* after 96hE of exposure to different salinity levels. [A] Control; [B] 3 g salt L<sup>-1</sup>; [C] 6 g salt L<sup>-1</sup>; [D] 9 g salt L<sup>-1</sup>. Hepatocytes (H); nuclei (N); sinusoids (S); congested blood vessel (green arrow); sinusoidal dilation (blue arrow); vacuolization (red arrow)

**Table 3.**

*Histopathological alterations in juvenile *Piaractus brachipomus* after acute exposure to different salinity levels for 96 hours.*

Organs	Alterations	Salinity (g of salt L <sup>-1</sup> )						
		0	3	6	9	12		
24hE	Liver	Sinusoidal dilation	-	-	-	-	-	
		Vacuolization	-	-	-	++	-	
		Congested blood vessel	-	-	-	+	-	
		Sinusoidal congestion	-	-	-	-	-	
		Pyknotic nuclei	-	-	-	-	-	
		Degenerated hepatocytes	-	-	-	-	-	
		Necrosis	-	-	-	-	-	
24hE	Gills	Branchial epithelial hyperplasia	-	-	-	-	-	
		Epithelial displacement	-	-	-	+	+++	
		Lamellar fusion	-	+	+	++	+++	
		Severe blood congestion	-	++	++	++	+++	
		Lamellar aneurysm	-	++	++	++	+++	
		Rupture of epithelial cells with hemorrhage	-	-	-	-	-	
		Rupture of the lamellar epithelium	-	-	-	-	-	
96hE	Liver	Necrosis	-	-	-	-	-	
		Sinusoidal dilation	-	++	++	++	*	
		Vacuolization	-	-	-	++	*	
		Congested blood vessel	-	+	++	++	*	
		Sinusoidal congestion	-	-	-	-	*	
		Pyknotic nuclei	-	-	-	-	*	
		Degenerated hepatocytes	-	-	-	-	*	
	Necrosis	-	-	-	-	*		
	96hE	Gills	Branchial epithelial hyperplasia	-	-	-	-	*
			Epithelial displacement	-	+	+	+++	*
			Lamellar fusion	-	++	+++	+++	*
			Severe blood congestion	-	++	+++	+++	*
			Lamellar aneurysm	-	-	++	+++	*
Rupture of epithelial cells with hemorrhage			-	-	-	-	*	
96hE	Gills	Rupture of the lamellar epithelium	-	-	-	-	*	
		Necrosis	-	-	-	-	*	

\* Histopathological alterations recorded in <5% of cases were considered absent (-), 5–25% as mild (+), >25–50% as moderate (++), and >50% as severe (+++). \* Animals did not survive the exposure period.

The intensity of histopathological alterations is presented in **Table 3**. Regarding gill tissue, no alterations were observed in the control group (S0) at any of the sampling times.

At 24 h of exposure (24hE), epithelial lifting was observed with mild intensity in S9 (**Figure 1D**) and severe intensity in S12 (**Figure 1E**). This alteration was also observed at 96 h (96hE) in S3 (**Figure 3B**), and S6 (**Figure 3C**), with mild intensity, and in S9 (**Figure 3D**), with severe intensity.

Lamellar fusion was observed at 24hE in S3 (**Figure 1B**), S6 with mild intensity (**Figure 1C**), S9 with moderate intensity (**Figure 1D**) and S12 with severe intensity (**Figure 1E**). At 96hE, this alteration occurred with moderate intensity in S3 (**Figure 3B**) and severe intensity in S6 (**Figure 3C**) and S9 (**Figure 3D**).

Severe blood congestion was observed at 24hE in S3 (**Figure 1B**), S6 (**Figure 1C**) and S9 with moderate intensity (**Figure 1D**), while in S12 it was classified as severe (**Figure 1E**). At 96hE, this

alteration occurred with moderate intensity in S3 (**Figure 3B**) and severe intensity in S6 (**Figure 3C**) and S9 (**Figure 3D**).

At 24hE, lamellar aneurysms were identified in S3 (**Figure 1B**) and S6 (**Figure 1C**), with moderate intensity in S9 (**Figure 1D**) and severe intensity in S12 (**Figure 1E**). At 96hE, a lamellar aneurysm was observed with moderate intensity in S6 (**Figure 3C**) and severe intensity in S9 (**Figure 3D**).

The remaining alterations evaluated in the gill tissue were not observed or showed very low intensity across the different salinity levels at both sampling times.

Regarding liver tissue, no histopathological alterations were observed in the control treatment (S0) at any sampling time.

At 24hE, no sinusoidal dilation was observed in the liver; however, at 96hE, treatments S3 (**Figure 4B**), S6 (**Figure 4C**) and S9 (**Figure 4D**) showed this alteration with moderate intensity.

Hepatic tissue vacuolization was observed in S9 at both 24hE (**Figure 2D**) and 96hE (**Figure 4D**), with a similar degree of intensity in both cases.

Blood vessel congestion was observed at 24hE in S9 (**Figure 2D**) with mild intensity. At 96hE, this alteration was present with mild intensity in S3 (**Figure 4B**) and S6 (**Figure 4C**) and with moderate intensity in S9 (**Figure 4D**).

No other relevant histopathological alterations were observed in the liver tissue of animals subjected to the different treatments at any of the sampling times.

## Experiment 2.

Hemoglobin concentrations showed significant differences only as a function of cultivation days ( $P < 0.0001$ ), with higher values recorded at 60 days (**Table 4**).

Hematocrit levels showed significant differences among salinities ( $P < 0.0001$ ), cultivation days ( $P < 0.0001$ ), and their interaction ( $P = 0.0001$ ) (**Table 4**). At 30 days, the highest values were observed in S2, whereas at 60 days, the highest values were recorded in S0. Additionally, an increase in hematocrit levels was observed in S0, S4, and S6 at 60 days.

The number of erythrocytes followed a similar pattern, with significant differences among salinities ( $P < 0.0001$ ), cultivation days ( $P < 0.0001$ ), and their interaction ( $P < 0.0001$ ) (**Table 4**). The lowest values were observed in S0 at 30 days. At 60 days, the lowest values were recorded in S6 and the highest in S4. An increase in erythrocyte count was also observed at 60 days in S0, S2, and S4.

Mean corpuscular hemoglobin (MCH) showed a significant effect only for the interaction between factors ( $P = 0.0016$ ) (**Table 4**). No differences among salinities were observed at 30 days; however, at 60 days, the highest values were recorded in S6. Additionally, an increase in MCH values was observed in S6 and a decrease in S2 at 60 days.

Mean corpuscular hemoglobin concentration (MCHC) showed significant differences only as a function of salinity ( $P = 0.0065$ ), with higher values observed in S4 (**Table 4**).

Total protein showed significant differences among salinities ( $P < 0.0001$ ) and cultivation days ( $P < 0.0001$ ) (**Table 5**), with higher values observed in S6 and at 30 days of cultivation.

Glucose levels followed a similar pattern, showing differences among salinities ( $P < 0.0001$ ) and cultivation days ( $P = 0.0028$ ) (**Table 5**), with higher values recorded in S6 and at 60 days.

Cholesterol showed significant differences among salinities ( $P < 0.0001$ ) and in the interaction between factors ( $P < 0.0001$ ) (**Table 5**). The highest values were observed in S6 at both sampling times, while at 30 days, the lowest value was recorded in S2. A decrease was observed in S4, whereas an increase was observed in S6 at 60 days of cultivation.

Triglycerides showed significant differences between sampling times ( $P < 0.0001$ ) and salinities ( $P < 0.0001$ ) (**Table 5**), with higher values observed in S0 and at 30 days.

Chloride concentrations showed significant differences among salinities ( $P < 0.0001$ ), cultivation times ( $P < 0.0001$ ), and their interaction ( $P < 0.0001$ ) (**Table 5**). At 30 days, the highest values were observed in S6, whereas at 60 days, the highest values were recorded in S0. Additionally, a decrease in chloride levels was observed in all salinities at 60 days.

Alanine aminotransferase (ALT) showed significant differences only between cultivation days ( $P < 0.0001$ ), with higher values observed at 30 days (**Table 5**).

Aspartate aminotransferase (AST) showed significant differences among salinities ( $P = 0.0031$ ), cultivation days ( $P < 0.0001$ ), and their interaction ( $P = 0.0105$ ) (**Table 5**). The highest values were observed in S2 and S6 at 30 days, whereas no differences were observed at 60 days. A decrease in AST activity was observed across all salinities after 30 days.

The viscerosomatic index (VSI) did not show differences as a function of cultivation days ( $P = 0.0842$ ), but significant differences were observed among salinities ( $P < 0.0001$ ) and in the interaction between factors ( $P = 0.0426$ ) (**Table 6**). The highest values were observed in S4 and S6 at 30 days, whereas at 60 days, the highest value was recorded in S6. An increase in VSI was observed only in S6 at 60 days.

The hepatosomatic index (HSI) showed significant differences among salinities ( $P < 0.0001$ ) and cultivation days ( $P < 0.0001$ ) (**Table 6**), with higher values observed in S6 at 30 days.

**Table 4.**

*Hematological parameters (mean ± standard error) of juvenile *Piaractus brachypomus* maintained under different salinity levels after 30 and 60 days of cultivation.*

Days of cultivation	Salinity (g of salt L <sup>-1</sup> )				Mean	P-value Salinity	P-value Days of cultivation	P-value Interaction
	0	2	4	6				
<b>Hemoglobin (g dL<sup>-1</sup>)</b>						0.253	<0.0001	0.0501
30 days	6.67 ± 0.56	7.98 ± 1.34	7.36 ± 1.17	6.76 ± 1.12	7.22 ± 1.19 B			
60 days	8.50 ± 1.28	8.09 ± 0.36	8.54 ± 0.87	8.26 ± 1.21	8.36 ± 1.00 A			
Mean	7.62 ± 0.28 a	8.03 ± 0.21 a	7.95 ± 0.23 a	7.54 ± 0.28 a				
<b>Hematocrit (%)</b>						<0.0001	<0.0001	0.0001
30 days	23.27 ± 3.10 Bb	27.09 ± 2.58 Aa	22.45 ± 1.36 Bb	22.30 ± 2.05 Bb	23.81 ± 3.02			
60 days	30.25 ± 1.13 Aa	27.72 ± 2.49 Ba	26.09 ± 2.34 Ba	26.25 ± 1.38 Ba	27.73 ± 2.55			
Mean	26.91 ± 0.87	27.40 ± 0.53	24.27 ± 0.56	24.05 ± 0.62				
<b>Erythrocytes (x 10<sup>6</sup> µL<sup>-1</sup>)</b>						<0.0001	<0.0001	<0.0001
30 days	0.97 ± 0.11 Bb	1.22 ± 0.10 Ab	1.23 ± 0.11 Ab	1.14 ± 0.09 Aa	1.14 ± 0.14			
60 days	1.40 ± 0.08 Ba	1.43 ± 0.12 ABa	1.50 ± 0.11 Aa	1.17 ± 0.09 Ca	1.38 ± 0.16			
Mean	1.18 ± 0.05	1.33 ± 0.03	1.37 ± 0.03	1.16 ± 0.02				
<b>MCV (fL<sup>-1</sup>)</b>						<0.0001	0.5933	<0.0001
30 days	229.7 ± 31.96 Aa	225.1 ± 29.76 Aa	186.5 ± 21.66 Ba	181.1 ± 9.40 Bb	207.6 ± 32.91			
60 days	215.8 ± 15.59 Aa	193.5 ± 22.21 Bb	172.6 ± 20.05 Ca	229.0 ± 0.09 Aa	200.6 ± 28.04			
Mean	222.4 ± 5.74	209.3 ± 6.77	179.2 ± 4.92	205.0 ± 7.52				
<b>MCH (pg<sup>-1</sup>)</b>						0.1246	0.3424	0.0016
30 days	68.02 ± 6.87 Aa	67.03 ± 13.26 Aa	59.28 ± 11.33 Aa	58.79 ± 12.39 Ab	63.26 ± 11.76			
60 days	60.03 ± 7.19 Ba	54.96 ± 5.36 Bb	57.12 ± 8.53 Ba	72.08 ± 12.93 Aa	61.26 ± 10.99			
Mean	63.81 ± 1.83	61.95 ± 2.77	58.15 ± 2.13	65.44 ± 3.14				
<b>MCHC (g dL<sup>-1</sup>)</b>						0.0065	0.6266	0.7877
30 days	29.95 ± 4.39	29.66 ± 5.05	33.82 ± 5.17	30.94 ± 4.78	31.10 ± 4.98			
60 days	28.07 ± 3.91	29.16 ± 3.24	33.10 ± 5.50	32.06 ± 3.92	30.47 ± 4.62			
Mean	28.97 ± 0.86 b	29.42 ± 0.91 b	33.46 ± 1.11 a	31.47 ± 1.04 ab				

\* Uppercase letters in the same row compare sampling times within each salinity level, and lowercase letters in the same column compare salinity levels within each sampling time, according to Tukey's test ( $p < 0.05$ ). When the interaction was not significant, letters in the marginal means indicate comparisons of the main effects: uppercase letters for sampling times and lowercase letters for salinity levels. MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

**Table 5.**

*Blood biochemical parameters (mean ± standard error) of juvenile *Piaractus brachypomus* maintained under different salinity levels after 30 and 60 days of cultivation.*

Days of cultivation	Salinity (g of salt L <sup>-1</sup> )				Mean	P-value Salinity	P-value Days of cultivation	P-value Interaction
	0	2	4	6				
<b>Total protein (g dL<sup>-1</sup>)</b>						<0.0001	<0.0001	0.0641
30 days	5.02 ± 0.19	4.69 ± 0.16	4.91 ± 0.28	5.32 ± 0.26	4.98 ± 0.32 A			
60 days	4.53 ± 0.35	4.30 ± 0.27	4.36 ± 0.36	5.24 ± 0.34	4.56 ± 0.47 B			
Mean	4.75 ± 0.08 b	4.48 ± 0.06 c	4.60 ± 0.09 bc	5.28 ± 0.06 a				
<b>Glucose (mg dL<sup>-1</sup>)</b>						<0.0001	0.0028	0.7802
30 days	65.01 ± 7.38	64.31 ± 6.09	63.60 ± 9.37	71.80 ± 6.97	66.42 ± 8.08 B			
60 days	68.48 ± 6.93	67.71 ± 4.25	67.85 ± 5.98	78.85 ± 6.09	70.98 ± 7.52 A			
Mean	66.92 ± 1.60 b	66.01 ± 1.20 b	65.73 ± 1.70 b	75.32 ± 1.49 a				
<b>Cholesterol (mg dL<sup>-1</sup>)</b>						<0.0001	0.3861	<0.0001
30 days	83.55 ± 8.52 Ca	70.86 ± 14.12 Da	104.12 ± 13.63 Ba	112.66 ± 15.52 Ab	92.56 ± 21.08			
60 days	77.92 ± 10.84 Ba	78.76 ± 10.40 Ba	82.74 ± 10.24 Bb	140.74 ± 7.83 Aa	92.16 ± 26.48			
Mean	81.14 ± 2.12	74.64 ± 2.68	92.97 ± 3.33	123.89 ± 4.25				
<b>Triglycerides (mg dL<sup>-1</sup>)</b>						<0.0001	<0.0001	0.1809
30 days	319.95 ± 75.65	217.73 ± 84.50	247.92 ± 61.14	260.31 ± 49.21	261.48 ± 76.39 A			
60 days	206.07 ± 52.88	145.98 ± 32.22	140.69 ± 20.75	211.09 ± 32.55	175.18 ± 48.54 B			
Mean	260.53 ± 17.91 a	180.30 ± 14.90 c	194.31 ± 15.07 bc	236.87 ± 10.51 ab				
<b>Chlorides (mEq L<sup>-1</sup>)</b>						<0.0001	<0.0001	<0.0001
30 days	159.2 ± 17.55 Ca	171.4 ± 7.94 Ba	175.1 ± 17.97 Ba	199.1 ± 8.16 Aa	173.5 ± 19.27			
60 days	142.1 ± 9.53 Ab	126.9 ± 11.09 Bb	133.6 ± 11.62 ABb	135.8 ± 12.86 ABb	134.6 ± 12.25			
Mean	151.0 ± 3.43	149.1 ± 5.25	151.4 ± 5.54	159.1 ± 7.63				
<b>ALT (U L<sup>-1</sup>)</b>						0.0855	<0.0001	0.1138
30 days	17.16 ± 4.91	17.63 ± 4.03	13.66 ± 4.22	18.70 ± 2.31	16.68 ± 4.35 A			
60 days	9.30 ± 3.09	10.27 ± 2.53	9.16 ± 4.60	8.81 ± 2.18	9.38 ± 3.21 B			
Mean	13.59 ± 1.22 a	13.95 ± 1.06 a	11.41 ± 0.99 a	13.52 ± 1.20 a				
<b>AST (U L<sup>-1</sup>)</b>						0.0031	<0.0001	0.0105
30 days	57.25 ± 24.13 Ba	98.25 ± 26.48 Aa	65.75 ± 24.77 Ba	91.45 ± 22.46 Aa	79.81 ± 29.11			
60 days	52.62 ± 22.02 Aa	50.37 ± 10.91 Ab	35.66 ± 6.65 Ab	45.54 ± 11.07 Ab	46.63 ± 14.58			
Mean	54.93 ± 5.61	79.10 ± 7.17	55.72 ± 5.88	68.50 ± 6.21				

\* Uppercase letters in the same row compare sampling times within each salinity level, and lowercase letters in the same column compare salinity levels within each sampling time, according to Tukey's test ( $p < 0.05$ ). When the interaction was not significant, letters in the marginal means indicate comparisons of the main effects: uppercase letters for sampling times and lowercase letters for salinity levels. ALT: alanine aminotransferase; AST: aspartate aminotransferase.

**Table 6.**

*Somatic indices (mean ± standard error) of juvenile *Piaractus brachypomus* maintained under different salinity levels after 30 and 60 days of cultivation.*

Days of cultivation	Salinity (g of salt L <sup>-1</sup> )				Mean	P-value Salinity	P-value Days of cultivation	P-value Interaction
	0	2	4	6				
<b>IVS (%)</b>						<0.0001	0.0842	0.0426
30 days	6.78 ± 0.69 Ba	6.81 ± 0.62 Ba	7.78 ± 0.84 Aa	7.88 ± 0.50 Ab	7.31 ± 0.84			
60 days	7.00 ± 1.05 Ba	6.88 ± 1.11 Ba	7.57 ± 0.81 Ba	9.11 ± 1.19 Aa	7.65 ± 1.36			
Mean	6.89 ± 0.18	6.84 ± 0.18	7.68 ± 0.17	7.68 ± 0.17				
<b>IHS (%)</b>						<0.0001	<0.0001	0.1916
30 days	1.76 ± 0.19	1.53 ± 0.23	1.93 ± 0.33	2.10 ± 0.36	1.83 ± 0.35 A			
60 days	1.24 ± 0.28	1.27 ± 0.22	1.39 ± 0.30	1.78 ± 0.17	1.43 ± 0.32 B			
Mean	1,51 ± 0,07 bc	1,40 ± 0,05 c	1,65 ± 0,08 b	1,94 ± 0,06 a				

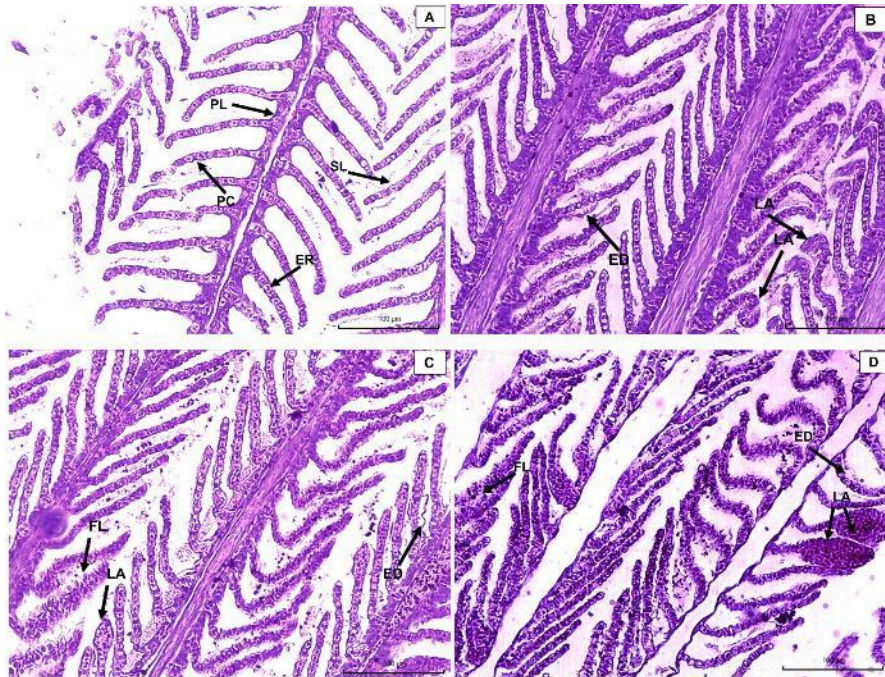
\* Uppercase letters in the same row compare sampling times within each salinity level, and lowercase letters in the same column compare salinity levels within each sampling time, according to Tukey's test ( $p < 0.05$ ). When the interaction was not significant, letters in the marginal means indicate comparisons of the main effects: uppercase letters for sampling times and lowercase letters for salinity levels. VSI: viscerosomatic index; HSI: hepatosomatic index.

The intensity of histopathological alterations is presented in **Table 7**. Histopathological alterations in the liver and gills of juvenile *P. brachypomus* were observed from salinity level S2 onwards. In gill tissue, fish cultivated at S2 (**Figure 5B**), S4 (**Figure 5C**), and S6 (**Figure 5D**) showed epithelial lifting, the greatest severity in S6. Lamellar fusion was also observed in S4 with mild intensity (**Figure 5C**), and in S6 with severe intensity (**Figure 5D**). Treatment S2 presented a lamellar aneurysm of moderate intensity (**Figure 5B**), whereas in S4 (**Figure 5C**) and S6 (**Figure 5D**) this alteration was classified as severe.

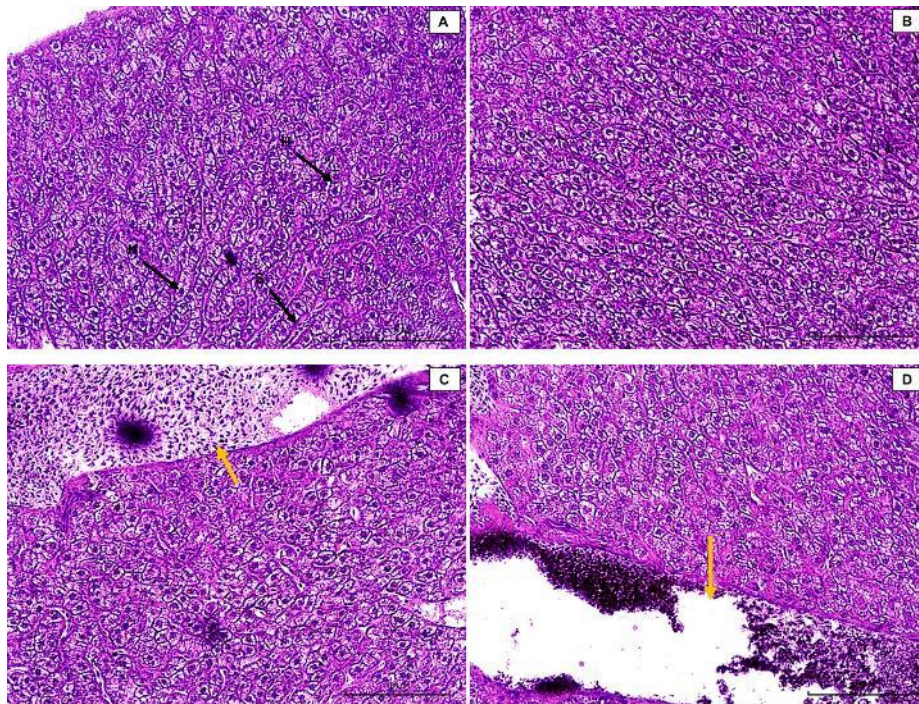
In liver tissue, blood vessel congestion was observed, with a mild incidence in S4 (**Figure 6C**) and S6 (**Figure 6D**).

The remaining evaluated alterations were not observed or did not show sufficient incidence for classification.

## Histological analyses



**Figure 5.** Histological alterations in the gills of juvenile *Piaractus brachypomus* after 60 days of cultivation under different salinity levels. [A] Control; [B] 2 g salt  $L^{-1}$ ; [C] 4 g salt  $L^{-1}$ ; [D] 6 g salt  $L^{-1}$ . Primary lamellae (PL); secondary lamellae (SL); pillar cells (PC); erythrocytes (ER); epithelial lifting (EL); lamellar aneurysm (LA); lamellar fusion (LF)



**Figure 6.** Histological alterations in the liver of juvenile *Piaractus brachypomus* after 60 days of cultivation under different salinity levels. [A] Control; [B] 2 g salt  $L^{-1}$ ; [C] 4 g salt  $L^{-1}$ ; [D] 6 g salt  $L^{-1}$ . Hepatocytes (H); nuclei (N); sinusoids (S); congested blood vessel (yellow arrow)

**Table 7.**

*Histopathological alterations in juvenile *Piaractus brachypomus* after 60 days of cultivation under different salinity levels.*

Organs	Alterations	Salinity (g of salt L <sup>-1</sup> )			
		0	2	4	6
Liver	Sinusoidal dilation	-	-	-	-
	Vacuolization	-	-	-	-
	Congested blood vessel	-	-	+	+
	Sinusoidal congestion	-	-	-	-
	Pyknotic nuclei	-	-	-	-
	Degenerated hepatocytes	-	-	-	-
	Necrosis	-	-	-	-
Gills	Branchial epithelial hyperplasia	-	-	-	-
	Epithelial displacement	-	++	+	+++
	Lamellar fusion	-	-	+	+++
	Severe blood congestion	-	-	-	-
	Lamellar aneurysm	-	++	+++	+++
	Rupture of epithelial cells with hemorrhage	-	-	-	-
	Rupture of the lamellar epithelium	-	-	-	-
	Necrosis	-	-	-	-

\* Histopathological alterations recorded in <5% of cases were considered absent (-), 5–25% as mild (+), >25–50% as moderate (++), and >50% as severe (+++).

### Zootechnical performance

No differences in survival rate were observed at any of the evaluated periods (**Table 8**). After 15 days of cultivation, differences were detected only in total length, with the lowest value recorded in S6 ( $P < 0.05$ ). At 30 days, the final weight was higher in S4 and S6 ( $P < 0.05$ ). Similarly, weight gain, daily weight gain, daily feed intake, and specific growth rate were higher in S6 ( $P < 0.05$ ). At 45 days of cultivation, no differences among salinity levels were observed ( $P > 0.05$ ). Finally, at 60 days, daily feed intake was higher in S6.

**Table 8.**

*Zootechnical performance (mean ± standard error) of juvenile *Piaractus brachypomus* maintained under different salinity levels after 15, 30, 45, and 60 days of cultivation.*

Salinity (g of salt L <sup>-1</sup> )	FW (g)	FL (cm)	SGR (% day <sup>-1</sup> )	WG (g)	DWG (g day <sup>-1</sup> )	DFI (g)	FCR	SUR (%)
<b>1 - 15 days</b>								
0	15.51 ± 1.65	9.75 ± 0.23 a	6.61 ± 0.69	9.78 ± 1.65	0.65 ± 0.11	0.72 ± 0.01	1.13 ± 0.20	100.00 ± 0.00
2	16.62 ± 0.59	9.97 ± 0.14 a	7.07 ± 0.26	10.87 ± 0.61	0.72 ± 0.04	0.71 ± 0.02	0.98 ± 0.03	92.85 ± 8.24
4	15.88 ± 1.45	9.82 ± 0.02 a	6.76 ± 0.62	10.14 ± 1.46	0.67 ± 0.09	0.70 ± 0.04	1.05 ± 0.09	96.42 ± 7.14
6	14.72 ± 1.14	9.13 ± 0.20 b	6.26 ± 0.52	8.97 ± 1.16	0.59 ± 0.07	0.67 ± 0.01	1.13 ± 0.12	94.64 ± 6.83
P-value	0.2571	<0.0001	0.2685	0.2651	0.2651	0.1122	0.3362	0.4708
<b>16 - 30 days</b>								
0	25.04 ± 2.51 b	11.25 ± 0.25	3.19 ± 1.18 c	9.52 ± 3.58 c	0.63 ± 0.29 c	0.53 ± 0.10 b	0.89 ± 0.23	85.71 ± 2.91
2	28.27 ± 1.93 ab	11.48 ± 0.21	3.53 ± 0.45 bc	11.65 ± 1.86 bc	0.77 ± 0.12 bc	0.60 ± 0.06 b	0.78 ± 0.13	91.07 ± 5.35
4	30.26 ± 2.37 a	11.70 ± 0.37	4.30 ± 0.59 ab	14.37 ± 2.16 ab	0.95 ± 0.14 ab	0.66 ± 0.05 b	0.69 ± 0.08	94.64 ± 3.41
6	30.17 ± 2.71 a	11.48 ± 0.56	4.77 ± 0.26 a	15.44 ± 1.75 a	1.02 ± 0.11 a	0.81 ± 0.11 a	0.79 ± 0.06	94.64 ± 3.42
P-value	0.0316	0.4579	0.0324	0.0207	0.0207	0.0044	0.3453	0.3599
<b>31 - 45 days</b>								
0	43.42 ± 7.66	12.82 ± 0.73	3.19 ± 0.66	16.70 ± 4.89	0.55 ± 0.16	1.24 ± 0.26	2.31 ± 0.44	100.00 ± 0.00
2	46.38 ± 6.00	12.98 ± 0.40	3.31 ± 0.76	18.18 ± 5.07	0.60 ± 0.16	1.11 ± 0.20	1.89 ± 0.32	100.00 ± 0.00
4	45.64 ± 2.70	13.02 ± 0.16	2.75 ± 0.51	15.44 ± 2.89	0.51 ± 0.09	1.12 ± 0.05	2.24 ± 0.45	100.00 ± 0.00
6	52.52 ± 5.03	13.19 ± 0.59	3.57 ± 0.29	21.83 ± 3.07	0.72 ± 0.10	1.39 ± 0.12	1.93 ± 0.22	100.00 ± 0.00
P-value	0.1823	0.8059	0.3039	0.1992	0.1992	0.1705	0.3189	1.0000
<b>46 - 60 days</b>								
0	59.21 ± 10.35	14.71 ± 0.89	2.07 ± 0.26	15.78 ± 3.40	1.05 ± 0.22	1.20 ± 0.26 b	1.14 ± 0.10	96.42 ± 3.57
2	60.86 ± 10.20	14.76 ± 0.63	1.78 ± 0.25	14.47 ± 4.31	0.96 ± 0.28	1.24 ± 0.13 b	1.33 ± 0.23	100.00 ± 0.00
4	59.36 ± 5.18	14.68 ± 0.27	1.74 ± 0.25	13.72 ± 2.87	0.91 ± 0.19	1.15 ± 0.09 b	1.29 ± 0.25	92.85 ± 7.14
6	70.86 ± 7.76	14.95 ± 0.50	1.99 ± 0.16	18.34 ± 3.00	1.22 ± 0.20	1.65 ± 0.07 a	1.37 ± 0.18	96.42 ± 3.57
P-value	0.2265	0.9275	0.2024	0.2944	0.944	0.003	0.4386	0.7256

\* Means followed by different letters within the same column indicate significant differences according to Tukey's test ( $P < 0.05$ ). FW (g): final weight; FL (cm): final length; SGR (% day<sup>-1</sup>): specific growth rate; WG (g): weight gain; DWG (g day<sup>-1</sup>): daily weight gain; DFI (g): daily feed intake; FCR: feed conversion ratio; SUR (%): survival.

## DISCUSSION

### Experiment 1

Variations in salinity can affect osmoregulation in freshwater fish (Souza-Bastos et al., 2016), increasing energy expenditure as organisms attempt to adapt to these conditions (Griffith, 2016). In the present study, alterations in biochemical and hematological parameters were observed in *Piaractus brachypomus* following exposure to different salinity levels at distinct sampling times. Consistent with our findings, salinities close to 12 g salt L<sup>-1</sup> have also been reported as lethal for other species, such as grass carp (*Ctenopharyngodon idella*) (D. Liu et al., 2023; Zhu et al., 2025), largemouth bass (*Micropterus salmoides*) (Y. Liu et al., 2025), and pacamã (*Lophiosilurus alexandri*) (Mattioli et al., 2017).

Hemoglobin concentrations were higher in S9 during the first 24 h than at lower salinities and in the control. This increase may be associated with higher oxygen demand resulting from a stress condition (Bao et al., 2018). For this salinity level, a decrease in hemoglobin concentrations over time was observed, a pattern also reported in tilapia (*Oreochromis niloticus*) under stress conditions (Bao et al., 2018).

Hematocrit, erythrocyte count, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) followed a similar pattern, with higher values observed in S9. These results indicate a physiological response aimed at meeting increased oxygen and energy demands, likely associated with osmotic stress. This behavior is consistent with findings reported for hybrids of yellow catfish (*Pelteobagrus fulvidraco* × *P. vachellii*) under different stress conditions (Dagoudo et al., 2021, 2023).

An increase in glucose levels is another important stress response in fish (Wells & Pankhurst, 1999). In this study, glucose concentrations were higher in S6 and S9 during the first 24 h, which may be related to glycogenolysis, during which the liver releases glucose into the bloodstream (Mattioli et al., 2017). After 96 h, glucose levels increased across all treatments, suggesting prolonged recovery of *P. brachypomus* from osmotic stress. This pattern has also been reported in hybrids of this species (*Colossoma macropomum* × *P. brachypomus*) (dos Santos Silva et al., 2026).

Cholesterol levels were lower in S12 at 24 h. According to dos Santos Silva et al. (2026), these reduced levels may represent a compensatory response to stabilize cell membranes under osmotic stress. The same authors reported decreases in cholesterol levels in hybrids (*C. macropomum* × *P. brachypomus*) subjected to osmotic shock for 1 and 24 h. In the present study, at 96 h, the lowest cholesterol values were observed in S9, suggesting that lipid reserves serve as a physiological regulatory mechanism and an energy source for the species.

Triglyceride levels were highest during the first 24 h, a pattern also reported in *Lophiosilurus alexandri* (Mattioli et al., 2017). The subsequent decrease in these levels suggests the utilization of lipids as an energy source under conditions of high metabolic demand (dos Santos Silva et al., 2026). Among salinity treatments, the lowest values were recorded in S9, suggesting greater depletion of lipid reserves under this condition, consistent with the patterns observed for cholesterol. Souza et al. (2025) also reported a decrease in triglyceride levels in *P. brachypomus* subjected to thermal stress, which they attributed to increased energy demand resulting from metabolic disturbances.

The lowest total protein values were observed in the control group. According to D. Liu et al. (2023), higher protein concentrations may reflect a reduction in blood water content, suggesting osmotic imbalance in juvenile *P. brachypomus* exposed to saline water.

Lactate levels were higher in S12 at 24 h, indicating increased anaerobic metabolism and oxygen demand (Yousefi et al., 2022). This response has also been reported in common carp (*Cyprinus carpio*) subjected to transport stress (Hoseini et al., 2022) and in *C. macropomum* under hypoxic conditions (do Carmo Neves et al., 2020). At 96 h, a decrease in lactate levels compared to 24 h was observed, suggesting an adaptive response in which fish reduce anaerobic metabolic activity under prolonged stress to optimize energy use (Chen et al., 2025).

Chloride levels were higher across all salinities during the first 24 h, suggesting an initial osmotic imbalance (Perry, 1998). The decrease observed at 96 h suggests an adaptive process of *P. brachypomus* to different salinity conditions. This pattern has also been reported in the same species under thermal stress (Souza et al., 2025). The highest chloride levels at the end of the experimental period were observed in S9, suggesting a greater physiological impact at this salinity.

Alanine aminotransferase (ALT) levels were similar across salinities during the first 24 h of exposure. This transaminase, together with AST, is considered an indicator of hepatic damage or dysfunction (Motamedi-Tehrani et al., 2025). In contrast to the present findings, dos Santos Silva et al. (2026) reported an increase in ALT levels in juveniles of the hybrid (*C. macropomum* × *P. brachypomus*) subjected to osmotic shock for 24 h. Regarding AST, no significant differences among salinities were observed at any sampling time, consistent with findings in *C. macropomum* and its hybrid under osmotic stress conditions (dos Santos Silva et al., 2026).

Hepatic alterations may indicate metabolic dysfunctions and mobilization of energy reserves (Xing et al., 2025), as the liver plays a key role in supplying energy during osmoregulatory processes (Nakadate et al., 2023). In the present study, the most pronounced alterations in juvenile *P. brachypomus* were observed in S6 and S9, consistent with findings reported for other species, such as *Perca fluviatilis* (Xing et al., 2025) and *Pangasianodon hypophthalmus* (Hossain et al., 2022) exposed to higher salinities.

Gill tissue is particularly susceptible to changes in water quality (Strzyzewska et al., 2016). In this study, an increase in the intensity of gill alterations was observed with increasing salinity, a pattern also reported in *L. alexandri* (Takata et al., 2021).

## Experiment 2

Chronic exposure to inadequate salinity levels can compromise physiological and hematological functions, impair productive performance, and cause mortality in several freshwater fish species (Fiúza et al., 2015; D. Liu et al., 2023; Mattioli et al., 2017; Takata et al., 2021; Zidan et al., 2022). In the present study, survival did not differ among salinity levels after 60 days of cultivation, indicating high tolerance of *Piaractus brachypomus* to concentrations of up to 6 g L<sup>-1</sup>. Similar results were reported for juvenile *Ctenopharyngodon idella* exposed to salinities up to 6 g salt L<sup>-1</sup> for 8 weeks (D. Liu et al., 2023). This tolerance is also consistent with Experiment 1, in which salinities of up to 9 g salt L<sup>-1</sup> were not lethal to the species during 96 h of acute exposure.

After 15 days of cultivation, a lower final length was observed in S6. However, after 30 days, the final weight was higher in S4 and S6, while specific growth rate, weight gain, daily weight gain, and daily feed intake were higher in S6. Previous studies have shown that higher salinities, up to 10 g salt L<sup>-1</sup>, do not negatively affect the growth of *Oreochromis niloticus* cultivated for 60 days (Dawood et al., 2022).

After 45 days, performance was similar across all salinities, which agrees with findings in juvenile *Clarias gariepinus*, whose growth did not differ from that observed in freshwater when cultivated at salinities of up to 8 g salt L<sup>-1</sup> (Dawood et al., 2022). After 60 days, only daily feed intake was higher in S6. These results suggest that higher salinities may initially affect juvenile *P. brachypomus*, however, after the adaptation period, improved performance was observed, possibly associated with compensatory growth. In conjunction with the physiological results from Experiments 1 and 2, this increased feed intake may reflect a greater metabolic demand under higher salinity conditions.

Hemoglobin concentrations were similar across treatments, consistent with Experiment 1, in which alterations were observed only from S9 during the first 24 h. A progressive increase in hemoglobin was also observed throughout the cultivation period across all salinities. This pattern has also been described in juvenile *Lophiosilurus alexandri* cultivated for 28 days under different salinities (Mattioli et al., 2017). Increased hemoglobin may be associated with an adaptive process in which higher concentrations improve oxygen transport capacity (Abdel-Tawwab et al., 2019).

Hematocrit values were higher in S2 at 30 days, suggesting a faster physiological response to moderate stress than to higher salinities. In Experiment 1, a reduction in hematocrit was observed in S9, a pattern also reported in juvenile *L. alexandri* at 24 and 96 h of exposure (Mattioli et al., 2017). At 60 days, the highest values were recorded in S0, which may reflect more stable physiological conditions and increased erythrocyte production. Nevertheless, increases in hematocrit under higher salinities have also been reported in *L. alexandri* (Mattioli et al., 2017).

The erythrocyte count showed a similar pattern, with lower values in S0 at 30 days. At 60 days, increases were observed in S0, S2, and S4, suggesting progressive metabolic adaptation to intermediate salinities. The lowest values were recorded in S6, where no differences between cultivation times were observed. This pattern is consistent with findings in *Pangasianodon hypophthalmus*, cultivated for 90 days, in which lower erythrocyte concentrations were observed at higher salinities (Riaz et al., 2025).

Mean corpuscular volume (MCV) was lower in S4 and S6 at 30 days, a trend also reported in *Clarias gariepinus* (Okomoda et al., 2024). At 60 days, the highest values were recorded in S6 and S0, with an increase in S6 suggesting a compensatory response.

Mean corpuscular hemoglobin (MCH) did not differ among treatments at 30 days; however, at 60 days, the highest values were recorded in S6. This increase may reflect an adaptive response to the lower erythrocyte concentrations observed at this salinity. Mean corpuscular hemoglobin concentration (MCHC) did not differ among salinities at either cultivation time, although the highest values were generally observed in S4.

Analysis of blood biochemical parameters in juvenile *P. brachypomus* after 60 days of cultivation showed that increased salinity, particularly in S6, promoted significant alterations in glucose, cholesterol, plasma protein, triglyceride, and chloride levels.

The highest glucose values, particularly at 60 days, suggest higher stress levels in *P. brachypomus* under S6. Similar results have been reported in *Pangasianodon hypophthalmus* cultivated for 56 days (Abdel-Latif et al., 2023) and in *Ctenopharyngodon idella* cultivated for 60 days (Chen et al., 2025), in which increased glucose levels were observed at higher salinities. Similarly, cholesterol levels were also higher in S6 at 60 days. In Experiment 1, an increase in cholesterol was observed in S6 when comparing 24 and 96 h of exposure. According to Souza et al. (2025), juveniles subjected to thermal stress exhibit elevated cholesterol levels. Other factors that may explain the higher cholesterol values in S6 include somatic indices and the higher feed intake observed in this treatment.

Total protein values were higher in S6, consistent with Experiment 1. Regarding cultivation time, the highest values were observed at 30 days, suggesting an initial stress response as fish were still adjusting to saline conditions. At 60 days, the decrease in protein levels may reflect its use as an energy source in response to stress-induced metabolic alterations (Monier et al., 2025). Similar reductions in plasma protein have been reported in *C. idella* under increased salinity conditions (Chen et al., 2025).

Overall, triglyceride levels were higher in S0 and at 30 days of cultivation. The lower availability of triglycerides at higher salinities may be related to the greater energy expenditure required to maintain osmoregulatory processes. This pattern was also observed in Experiment 1, where triglyceride levels decreased after 96 h of exposure. Lower triglyceride concentrations with increasing salinity have also been reported in *C. idella*, suggesting that they serve as an additional energy source to maintain homeostasis (D. Liu et al., 2023).

Chloride concentrations were higher in S6 at 30 days, indicating greater osmotic imbalance under this condition. In juvenile *C. idella*, an increase in chloride cells has been reported in response to increasing salinity (Chen et al., 2025). In the present study, chloride levels decreased across all salinities at 60 days, in agreement with Experiment 1 and reinforcing the adaptive capacity of *P. brachypomus* under prolonged exposure to different salinities.

ALT did not differ among salinities and varied only with cultivation time, with higher values at 30 days. This suggests that the evaluated salinities were not sufficient to cause significant hepatic damage during this period. A similar pattern was observed in Experiment 1. The decrease in ALT at 60 days may indicate an initial phase of osmotic stress followed by stabilization of hepatic function. According to Blewett et al. (2022), low ALT levels may be associated with physiological adaptation within tolerable ranges, preventing severe cellular damage or mortality.

Regarding AST, higher concentrations were observed in S6 at 30 days, indicating greater initial stress associated with higher salinity. In juvenile *Aplodinotus grunniens*, a progressive increase in AST with increasing salinity has been reported (Amankwah et al., 2025). At 60 days, no differences among treatments were observed, although a general decrease in this enzyme was observed across all salinities, suggesting physiological adaptation of *P. brachypomus* to chronic exposure.

Somatic indices are related to fish weight, length, and health status (Mazumder et al., 2016). In this study, salinity affected the viscerosomatic index (VSI), with higher values in S4 and S6 at 30 days, possibly associated with higher final weight and feed intake. At 60 days, VSI increased in S6, which showed the highest value among treatments. The hepatosomatic index (HSI) was also higher in S6, particularly at 30 days, which may be related to higher feed intake and productive performance under this salinity. Increased somatic indices under elevated salinity have also been reported in *P. hypophthalmus* (Riaz et al., 2025).

Histologically, juvenile *P. brachypomus* showed vascular congestion in liver tissue in S4 and S6 after 60 days of cultivation, while salinities of up to 2 g salt L<sup>-1</sup> did not cause hepatic alterations. Similarly, juvenile *P. hypophthalmus* tolerated salinities of up to 4 g salt L<sup>-1</sup> for 56 days without liver alterations (Hossain et al., 2022).

Gill tissue showed more severe alterations in S4 and S6. The appearance and intensification of these alterations with increasing salinity have also been reported in *L. alexandri* (Mattioli et al., 2017; Takata et al., 2021), *Clarias gariepinus* (Dawood et al., 2022), and *Perca schrenkii* (Xing et al., 2025).

## CONCLUSIONS

Juvenile *Piaractus brachypomus* tolerate salinity levels of up to 9 g salt L<sup>-1</sup> for 96 hours; however, more severe biochemical, hematological, and histopathological alterations were observed from 6 g salt L<sup>-1</sup> onwards. Under culture conditions, after 30 days, the species showed tolerance to higher salinities with improved productive performance; however, after 60 days, no differences were observed between 0 and 6 g salt L<sup>-1</sup>. Nevertheless, at 60 days, salinities of 4 and 6 g salt L<sup>-1</sup> resulted in greater metabolic and physiological disturbances, as well as more severe histopathological changes. Therefore, salinities of up to 2 g salt L<sup>-1</sup> are considered the most suitable for the cultivation of *P. brachypomus* over a 60-day period, for juveniles with an average initial weight of 5.6 g.

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## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the content of this work.

## AUTHOR CONTRIBUTIONS

Conceptualization: Sena-Souza, A.; Costa-Santos, F. A.; Costa-Júlio, G. S.; Santos-Silva, S.; Carvalho-Ananias, I. M.; Cortezzi-Pedras, P. P.; Oliveira-Gomes, C.; Favero, G. C.; and Luz, R. K.; Methodology: Sena-Souza, A.; Costa-Santos, F. A.; Costa-Júlio, G. S.; Santos-Silva, S.; Carvalho-Ananias, I. M.; Cortezzi-Pedras, P. P.; Oliveira-Gomes, C.; Favero, G. C.; and Luz, R. K.; Validation: Sena-Souza, A.;

Costa-Santos, F. A.; Costa-Júlio, G. S.; Santos-Silva, S.; Carvalho-Ananias, I. M.; Cortezzi-Pedras, P. P.; Favero, G. C.; and Luz, R. K.; Formal analysis: Sena-Souza, A.; Costa-Santos, F. A.; Costa-Júlio, G. S.; Santos-Silva, S.; Carvalho-Ananias, I. M.; Cortezzi-Pedras, P. P.; Oliveira-Gomes, C.; Favero, G. C.; and Luz, R. K.; Investigation: Sena-Souza, A.; Costa-Santos, F. A.; Costa-Júlio, G. S.; Santos-Silva, S.; Carvalho-Ananias, I. M.; Cortezzi-Pedras, P. P.; Favero, G. C.; and Luz, R. K.; Resources: Sena-Souza, A.; and Luz, R. K.; Data curation: Luz, R. K.; Writing—original draft preparation: Sena-Souza, A.; and Luz, R. K.; Writing—review and editing: Sena-Souza, A.; Favero, G. C.; and Luz, R. K.; Visualization: Sena-Souza, A.; and Luz, R. K.; Supervision: Luz, R. K.; Project administration: Luz, R. K.; Funding acquisition: Sena-Souza, A.; and Luz, R. K.; Histological analysis: Oliveira-Gomes, C.

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