

EFFECTS OF DIFFERENT SALINITY LEVELS ON ERYTHROCYTE COUNT, LEUKOCYTE COUNT, AND BLOOD GLUCOSE OF STRIPED CATFISH (*Pangasianodon hypophthalmus*)

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ABSTRACT

Pangasianodon hypophthalmus is a stenohaline freshwater catfish that is increasingly exposed to salinity fluctuations due to tidal intrusion and the expansion of brackish water aquaculture. This study aimed to analyze the erythrocyte and leukocyte counts, as well as blood glucose levels, in *P. hypophthalmus* reared at different salinities. This study used a completely randomized design (CRD) with four treatments: 0 ppt salinity, 5 ppt salinity, 7 ppt salinity, and 9 ppt salinity, with three replicates. The hematological parameters analyzed included total erythrocytes, total leukocytes, and blood glucose. The results showed that salinity had a significant effect ($p < 0.05$) on total erythrocytes, total leukocytes, and blood glucose. A salinity of 5 ppt produced an optimal hematological profile, with a total erythrocyte count of 2.25×10^6 cells/mm³, a total leukocyte count of 7.65×10^4 cells/mm³, and blood glucose of 52.33 mg/dL. These findings indicate that a salinity of 5 ppt is the optimal salinity threshold for *P. hypophthalmus* aquaculture in brackish water environments and provide a physiological basis for adaptive aquaculture management amid changing environmental conditions.

Keywords: *Pangasianodon hypophthalmus*, Salinity, Erythrocytes, Leukocytes, Blood glucose

1. INTRODUCTION

Pangasianodon hypophthalmus is a freshwater aquaculture species with high economic value in Southeast Asia, known for its rapid growth rate, efficient feed conversion capacity, and high market demand¹. The intensification of aquaculture activities, coupled with changes in freshwater availability due to climate impacts, has further increased the species' exposure to salinity variations triggered by tidal intrusion and brackishwater pond

practices. These conditions pose significant physiological challenges, given that *P. hypophthalmus* is a stenohaline species²⁻³. Salinity is a major abiotic factor that can disrupt the osmotic and ionic balance in freshwater teleost fish, triggering an integrated stress response controlled by the hypothalamic–pituitary–interrenal (HPI) axis⁴⁻⁵. Activation of the HPI axis results in increased plasma cortisol concentrations, which then trigger gluconeogenesis and glycogenolysis, resulting in hyperglycemia,

a secondary stress indicator⁶⁻⁷. Simultaneously, osmotic stress also triggers changes in hematological parameters, including erythrocyte dynamics, which reflect oxygen transport capacity, and leukocyte mobilization, which indicates activation of the nonspecific immune response⁸. The number of erythrocytes, the number of leukocytes, and blood glucose levels are physiological biomarkers that are sensitive and integrative in fish^{9,10}.

Previous studies have reported that salinity exposure in *P. hypophthalmus* elicits physiological responses, including increased numbers of granulocytes, a subset of leukocytes, at higher salinities¹¹, increased blood glucose and cortisol at salinities of 6–24 ppt¹², and a progressive increase in glucose at salinities of 0–16 ppt over 56 days¹³. However, the temporal dynamics of hematological and glycemic responses at moderate salinity ranges relevant for aquaculture during prolonged rearing periods remain inadequately documented¹⁴. Determining the salinity threshold capable of triggering measurable physiological stress responses, as well as characterizing changes in these responses over time, is a crucial step towards formulating evidence-based management guidelines for *P. hypophthalmus* cultivation in brackish water environments.

Therefore, this study was designed to assess the effect of different salinity levels on erythrocyte count, leukocyte count, and blood glucose concentration in *P. hypophthalmus*, with the aim of identifying physiological stress thresholds and adaptive response patterns that support sustainable aquaculture practices.

2. RESEARCH METHOD

Time and Place

This research was conducted in February 2024 at the Marine Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau.

Method

The method used was a Completely Randomized Design (CRD) experimental method with four salinity treatments: 0 ppt, 5 ppt, 7 ppt, and 9 ppt, with three replications. The treatments used refer to the research of Fitriani et al.¹⁵, which found the best salinity was 7 ppt.

Procedures

Preparation of Containers and Test Fish

A 60 x 30 x 30 cm aquarium was used as the rearing tank. The container was cleaned, filled with water, then treated with a 25 ppm KMnO₄ solution and aerated for 24 hours. Afterward, the aquarium was rinsed and dried for one day. The water came from a drilled well and was allowed to settle for 2 x 24 hours. Each aquarium was filled to a height of 25 cm (±40 L). The test fish were striped catfish, measuring 8 cm long and weighing 4 g, obtained from Nasir Fish Seedlings, Pekanbaru.

Salinity Media Preparation

The maintenance medium consisted of a mixture of freshwater and seawater. The seawater came from the Pakning River, Bengkalis, with a salinity of 30 ppt. Salinities of 5, 7, and 9 ppt were obtained by diluting the seawater with freshwater at specific ratios. Salinity values were measured using a hand refractometer. The media preparation followed the dilution formula of Arrokhman et al.¹⁶, as follows:

$$V_1 \times M_1 = V_2 \times M_2$$

Description:

- V_1 : Volume of seawater to be diluted (L)
- M_1 : Salinity of seawater to be diluted (ppt)
- V_2 : Volume of water with the desired salinity (L)
- M_2 : Desired salinity (ppt)

Test Fish Maintenance

The test fish were reared for 45 days, fed commercial feed (FF 999) three times daily at satiation, at 7:00 AM, 12:00 PM, and 5:00 PM WIB¹⁷. To maintain water quality

in the rearing tank, siphoning was performed every morning using a siphon hose. Furthermore, 30% of the total water volume was changed daily.

Collecting Fish Blood

Three fish from each treatment were used as blood samples. Blood samples were drawn three times: at the beginning of the treatment period, then on the 23rd day, and finally on the 45th day. Prior to blood collection, the fish were anesthetized using cold temperatures ($\pm 8^{\circ}\text{C}$)¹⁸. The syringe and microtube used were rinsed with 10% EDTA solution¹⁹. Blood was then drawn from the caudal vein using a 1 mL syringe and placed into an Eppendorf tube²⁰⁻²¹. The collected blood was then used to measure total erythrocyte count, total leukocyte count, and blood glucose levels.

Total Erythrocytes

Anticoagulated blood was aspirated into an erythrocyte hemocytometer pipette (identified by the red bead) up to the 0.5 graduation mark. Hayem's solution was subsequently added until the fluid level reached the 101 mark. The contents were mixed thoroughly by gently rotating the pipette in a figure-eight pattern for approximately 3–5 minutes to ensure uniform dilution. Prior to loading the counting chamber, the first two drops were discarded to eliminate any air bubbles. A small volume of the diluted blood was then transferred into the hemocytometer chamber, covered with a coverslip, and observed under a microscope using 100 $\times$ total magnification (10 $\times$ objective $\times$ 10 $\times$ eyepiece). The total erythrocyte count was obtained from five small squares of the hemocytometer using the formula²².

Number of Erythrocytes = $\sum n \times 10^6 \text{ cells/mm}^3$
n : number of the calculated erythrocyte

Total Leukocytes

The procedure for calculating total leukocytes refers to Blaxhall & Daisley²². The blood sample is drawn from a microtube using a leukocyte pipette to the 0.5 mark,

and Turk's solution is added to the 11 mark. The sample is then homogenized by shaking the leukocyte pipette in a figure-of-eight motion for 5 minutes. Once homogenized, two drops of blood are removed to remove air. The blood is then placed on a hemocytometer and covered with a coverslip. The blood is then observed under a microscope at 10 $\times$ 40 magnification. Total leukocytes are counted using a microscope in four large hemocytometer squares using the following formula:

$$\sum \text{Leukocytes} = \sum n \times 50 \text{ sel/mm}^3$$

$\sum n$ = Total number of leukocytes in 4 large boxes

50 = Dilution factor

Blood Glucose

Blood glucose concentrations were determined using a GlucoDr (Allmedicus) glucometer, which operates within a measurement range of 20–600 mg/dL. Glucose measurements were conducted in the morning prior to the fish being fed²³.

Data Analysis

The data obtained from erythrocyte counts, leukocyte counts, and blood glucose measurements were organized into tables before statistical analysis. Statistical processing was carried out using SPSS version 26, with differences among treatments assessed through a one-way analysis of variance (ANOVA). When significant effects were detected, mean comparisons were subsequently performed using the Student Newman Keuls (SNK) multiple comparison test

3. RESULT AND DISCUSSION

The observation results showed that the total erythrocytes of striped catfish (*P. hypophthalmus*) experienced significant changes ($p < 0.05$) along with differences in media salinity (Table 1). At day 0, the highest total erythrocytes were obtained at 0 ppt salinity, which was $1.92 \times 10^6 \text{ cells/mm}^3$, and decreased with increasing salinity to reach the lowest value of $1.66 \times 10^6 \text{ cells/mm}^3$ at 9 ppt salinity. This condition reflects the

initial physiological response of the fish to osmotic stress due to exposure to saline media.

At day 23 and day 45, the total erythrocytes in all treatments increased compared to day 0, with the highest value at 5 ppt salinity (2.23×10^6 cells/mm³ at day 45) and the lowest at 9 ppt salinity (1.85×10^6 cells/mm³ at day 23). The increase in erythrocytes on day 23 and day 45 is thought to be an adaptive response of the fish to maintain oxygen transport capacity through erythropoiesis stimulated by changes in osmotic pressure. This is in line with reports in other euryhaline fish that exposure to

sublethal salinity can stimulate red blood cell production as a physiological compensatory mechanism²⁴⁻²⁵. The erythrocyte counts of striped catfish in all treatments were still within the normal range for freshwater teleost fish, namely $1.05\text{--}3.0 \times 10^6$ cells/mm³, indicating that the fish were still able to adapt to the tested salinity without experiencing pathological anemia²². Furthermore, Manna et al.²⁶ stated that the total erythrocyte count of healthy striped catfish ranges from $1.4\text{--}2.8 \times 10^6$ cells/mm³, and the values in this study remained within this range throughout the observation period.

Table 1. Total erythrocytes, leukocytes, and blood glucose of striped catfish (*P. hypophthalmus*) at various salinity levels

Observation Time	Salinity (ppt)	Erythrocytes count (x10 ⁶ cells /mm ³)	Leukocytes count (x10 ⁴ cells /mm ³)	Blood glucose (mg/dL)
Day 0	0	1.92 ± 0.15 ^d	7.23 ± 0.09 ^a	44.66 ± 2.51 ^a
	5	1.84 ± 0.15 ^c	7.44 ± 0.07 ^b	45.00 ± 0.01 ^a
	7	1.75 ± 0.23 ^b	7.87 ± 0.01 ^c	47.33 ± 0.57 ^a
	9	1.66 ± 0.20 ^a	7.98 ± 0.02 ^c	49.00 ± 1.00 ^a
Day 23	0	2.20 ± 0.01 ^c	7.45 ± 0.03 ^a	46.33 ± 5.03 ^a
	5	2.23 ± 0.02 ^d	7.94 ± 0.03 ^b	57.33 ± 2.08 ^b
	7	2.14 ± 0.01 ^b	8.13 ± 0.04 ^c	64.66 ± 2.51 ^c
	9	1.85 ± 0.01 ^a	9.11 ± 0.07 ^d	74.66 ± 2.51 ^d
Day 45	0	2.21 ± 0.01 ^c	7.58 ± 0.09 ^a	49.00 ± 1.03 ^a
	5	2.25 ± 0.01 ^d	7.65 ± 0.12 ^a	52.33 ± 2.51 ^a
	7	2.18 ± 0.01 ^b	7.88 ± 0.02 ^b	57.00 ± 2.00 ^b
	9	2.12 ± 0.02 ^a	7.93 ± 0.05 ^b	63.66 ± 3.03 ^c

Description: Superscripts within the same row denote significant differences among treatments ($p < 0.05$)

Abdel-Latif et al.¹³ reported that juvenile *P. hypophthalmus* reared for 56 days at a salinity of 0–16 ppt experienced a significant decrease in total erythrocytes at salinities ≥ 12 ppt, while milder decreases were observed at salinities of 4 and 8 ppt. Riaz et al.¹² reported that treatment at a salinity of 6–12 ppt for 90 days in *P. hypophthalmus* decreased erythrocyte values gradually, and the sharpest decrease occurred at salinities ≥ 18 ppt, indicating damage to the erythrocyte membrane due to chronic osmotic stress. Conversely, at low salinities (5–7 ppt), total erythrocytes showed recovery on D-23 and D-45, indicating that erythropoiesis mechanisms can still compensate for osmotic stress in this

salinity range. Zannat et al.²⁷ reported that multi-species probiotics administered to *P. hypophthalmus* treated at 0–12 ppt salinity for 56 days showed increasing structural abnormalities in erythrocytes with increasing salinity, particularly in the form of erythroblast formation and nuclear abnormalities. No signs of severe erythrocyte morphological abnormalities were found, possibly due to the lower salinity range (0–9 ppt) and adequate adaptation duration.

Total leukocytes in *P. hypophthalmus* increased significantly ($p < 0.05$) with increasing salinity at each observation time (Table 1). At day 0, leukocyte values ranged from 7.23 to 7.98×10^4 cells/mm³ (salinity

0–9 ppt), with a similar pattern continuing until day 23 and day 45. The highest value was recorded at 9 ppt salinity on day 23 at 9.11×10^4 cells/mm³. Leukocytosis in high salinity treatments indicates nonspecific immune activation in response to osmotic stress, which triggers leukocyte mobilization into the peripheral circulation²⁸. These findings are in line with Abdel-Latif et al.¹³, who reported an increase in innate immune parameters, including lysozyme activity and leukocyte counts, in juvenile *P. hypophthalmus* treated at 4–10 ppt salinity. Schmitz et al.¹¹ also reported that chronic salinity stimulated increased granulocyte and respiratory burst activity in the same species. Hossain et al.²⁹ also identified leukocyte infiltration in gill and intestinal tissues at salinities ≥ 8 ppt; this study showed a similar pattern even at lower salinities (7–9 ppt), indicating that a measurable immune response occurred within this suboptimal range. However, all leukocyte counts were within the normal physiological range of $2.0\text{--}15.0 \times 10^4$ cells/mm³ for teleosts, thus not indicating pathological inflammation³⁰. The observed mild leukocytosis was a reversible protective response, consistent with other catfish species³¹.

Blood glucose levels in *P. hypophthalmus* increased significantly ($p < 0.05$) with increasing salinity and rearing duration (Table 1). At day 0, glucose levels were not significantly different between treatments. Significant differences began to appear at day 23, with values of 57.33, 64.66, and 74.66 mg/dL at salinities of 5, 7, and 9 ppt, respectively, significantly different from the control (46.33 mg/dL). This pattern continued until day 45, although

some treatments began to show a tendency to stabilize. The observed hyperglycemia is a secondary stress response due to activation of the HPI axis, which triggers cortisol secretion, which in turn stimulates hepatic gluconeogenesis and glycogenolysis to provide energy for osmoregulation^{32–33}. This pattern is consistent with Abdel-Latif et al.¹³, who reported an increase in glucose and lactate in *P. hypophthalmus* treated at 0–16 ppt salinity for 56 days, and Riaz et al.¹², who confirmed a significant increase in glucose and cortisol at 6–24 ppt salinity as an indicator of progressive physiological stress. The relative decrease in glucose at day 45 compared to day 23 in some treatments can be interpreted as an early indication of osmotic acclimatization, as reported by Hieu et al.³⁴ in *P. hypophthalmus* larvae who showed stabilization of stress biomarkers after continuous exposure.

4. CONCLUSION

Maintenance at a salinity of 5 ppt resulted in the most stable physiological conditions of striped catfish (*P. hypophthalmus*), characterized by a total erythrocyte count of 2.25×10^6 cells/mm³, a total leukocyte count of 7.65×10^4 cells/mm³, and blood glucose of 52.33 mg/dL. Increasing salinity above 5 ppt tends to induce osmoregulatory stress as indicated by significant changes in the observed hematological parameters. These results indicate that a salinity of 5 ppt is the most supportive condition for *P. hypophthalmus* cultivation in brackish water environments, and can be used as an initial reference in cultivation management under changing environmental conditions.

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