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Hematological Changes in *Orthopristis rubra* (Cuvier 1830) (Haemulidae: Haemulinae) During the Transition from Natural Environment to Confinement

Cambios Hematológicos en *Orthopristis rubra* (Cuvier 1830) (Haemulidae: Haemulinae) durante la Transición del Entorno Natural al Confinamiento

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Keywords
Blood parameters, stress, acclimatization, fish physiology, environmental transitions,

ABSTRACT | In this research, the dynamics of blood responses in *Orthopristis rubra* are analyzed addressing the impacts during capture, transport, and confinement-induced stress. Spanning 504 hours, the study captures the changes in hematological variables during the acclimatization of this species in artificial environments. The results obtained from two groups of fish, captured and examined with a four-month difference, support the reproducibility of the observed hematological changes. Notable fluctuations in parameters such as total hemoglobin concentration, hematocrit, red blood cell count, mean corpuscular hemoglobin concentration and white blood cell count highlight acute stress responses in the initial hours, followed by stabilization, indicative of a successful adjustment to the challenges posed by captivity. These reliable indicators of acclimatization are crucial for assessing the well-being of fish in artificial environments. Critical hydrological variations, including oxygen levels and temperature disparities, significantly impact hematological parameters. The fish's capacity to respond and maintain elevated values post-acclimatization emphasizes their ability to fine-tune physiological mechanisms. The study not only enhances our understanding of *O. rubra*'s responses but also lays a foundation for future investigations into the underlying mechanisms governing stress and acclimatization in fish.

Palabras clave
Parámetros sanguíneos, estrés, aclimatación, fisiología de peces, transiciones ambientales,

RESUMEN | En esta investigación, se analiza la dinámica de las respuestas hematológicas en *Orthopristis rubra* abordando los impactos durante la captura, el transporte y el estrés inducido por el confinamiento. Abarcando 504 horas, el estudio captura los cambios en las variables hematológicas durante la aclimatación de esta especie en ambientes artificiales. Los resultados obtenidos de dos grupos de peces, capturados y examinados con cuatro meses de diferencia, respaldan la reproducibilidad de los cambios hematológicos observados. Las notables fluctuaciones de parámetros como la concentración total de hemoglobina, el hematocrito, el recuento de glóbulos rojos, la concentración media de hemoglobina corpuscular y el recuento de glóbulos blancos ponen de manifiesto respuestas de estrés agudo en las primeras horas, seguidas de una estabilización, indicativa de una adaptación satisfactoria a los retos que plantea el cautiverio. Estos indicadores fiables de aclimatación son cruciales para evaluar el bienestar de los peces en entornos artificiales. Las variaciones hidrológicas críticas, incluidos los niveles de oxígeno y las disparidades de temperatura, afectan significativamente a los parámetros hematológicos. La capacidad de los peces para responder y mantener valores elevados tras la aclimatación pone de relieve su habilidad para afinar los mecanismos fisiológicos. El estudio no sólo mejora nuestra comprensión de las respuestas de *O. rubra*, sino que también sienta las bases para futuras investigaciones sobre los mecanismos subyacentes que rigen el estrés y la aclimatación en los peces.

INTRODUCTION

Fish demonstrate remarkable sensitivity to environmental fluctuations, and their physiological responses provide valuable insights into the impacts of environmental stressors within aquatic ecosystems (Davison *et al*. 2023, Schulte 2014, Sopinka *et al*. 2016, Witeska *et al*. 2022a). Hematology has proven to be a valuable approach for assessing this physiological response, contributing to the optimization of their growth, health, and productivity

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in aquaculture settings (Bagheri and Imanpour 2011, Chen and Luo 2023, Nabi *et al*. 2022, Pinto *et al*. 2019). Hematological indices are therefore widely used by fish biologists and researchers globally (Akinrotimi *et al*. 2010, Fazio, 2019, Witeska *et al*. 2023). However, hematological values may undergo considerable alterations after capture, and the stress produced by different capture methods can significantly affect blood parameters. Therefore, acclimating fish for laboratory studies is crucial to allow gradual adjustments to their immediate non-natural environment, improve health, and reduce variability in physiological responses during experiments (Hickey 1982, Akinrotimi *et al*. 2010, Fazio 2019,).

Acclimation periods, lasting a minimum of several days, are crucial to enable fish to adapt to the impacts of capture, handling, and transportation (Witeska *et al*. 2016, Ahmed *et al*. 2020). The specific duration of acclimation may vary depending on factors such as species, size, and the fish's origin, but it must be adequate to allow the fish to effectively compensate for the challenges associated with capture, handling, and transport (Faggio *et al*. 2014, Ahmed and Sheikh, 2020).

The analysis of fundamental and easily determined blood parameters in fish holds significant importance for various reasons. These parameters, including hematocrit, hemoglobin concentration, red blood cell count, and leukocyte count, act as crucial indicators of overall well-being (Esmaeili 2021, Yanuhar *et al*. 2021). Hematological parameter changes serve as physiological markers of stress in fish (Fazio *et al*. 2015, Simide *et al*. 2016), and are relatively straightforward to measure, rendering them suitable for both field and laboratory studies (Lawrence *et al*., 2020; Witeska *et al*., 2022b), providing a comprehensive overview of fish health and physiology (Fazio 2019, Seibel *et al*. 2021, Chen and Luo, 2023).

Ensuring a stress-free environment during the acclimatization phase is crucial, as variations in blood parameters induced by stress can potentially distort results and lead to misinterpretations of the effects of experimental treatments. This precaution not only enhances the reliability of the findings but also enables a more accurate interpretation of the actual impact of the treatments (Fazio 2019). Minimizing the stress from capture and confinement is not only essential for obtaining precise results but is also vital for establishing reference values that genuinely reflect the fish's physiological status. Neglecting this step may introduce confounding variables, complicating the exclusive attribution of observed changes to the experimental treatments. Therefore, a meticulous and well-planned acclimatization process stands as a fundamental prerequisite before embarking on any experiment, ensuring a robust foundation for research and an accurate interpretation of the obtained results.

The grunt *Orthopristis rubra* is a prevalent demersal fish species with a broad geographic range from the Caribbean Sea to the South Atlantic Ocean, making it an ideal candidate for studying responses to varied ecological settings (Marceniuk *et al*. 2019, Froese and Pauly 2023). Indeed, *O. rubra* has been established as a valuable model organism for monitoring mercury contamination in coastal marine ecosystems, underscoring its significance in environmental research (Santos *et al*. 2021, Seixas *et al*. 2021).

A previous study on *O. rubra* aimed to identify potential differences in blood parameters between sexes and establish correlations with size, weight, and sexual maturity over an 8-month captivity period (Nirchio *et al*. 1987). The study comprehensively investigated hematocrit values, hemoglobin concentration, erythrocyte counts, leukocyte counts, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and plasma protein concentration. Remarkably, no significant differences in any blood parameter were observed between males and females. However, it's essential to note that prolonged containment may elevate research costs due to maintenance demands, and there is also the potential for disease outbreaks or equipment failures. Striking the right balance between acclimatization duration and result accuracy is crucial to ensure meaningful findings and optimize research efforts.

The primary objective of this study was to monitor the dynamic changes in hematological parameters of *O. rubra*, spanning from its capture in the natural habitat, through transportation, to an extended period of confinement. In essence, the overarching goal was to trace the trajectory of these hematological values until they reached stabilization. This process seeks to establish robust benchmarks that can serve as reference values for evaluating the species' acclimatization process.

MATERIALS AND METHODS

Capture, transport and confinement

The study involved two groups of fish collected with a 4-month interval between them (February and May), aiming to assess result reproducibility in the experimental design. Fish procurement was carried out using trawling nets during commercial fishing operations near Cubagua Island, Nueva Esparta State, Venezuela (10°49'43.7” N 64°09'57.6" W). Sampling occurred between 10 am and 12 pm, ensuring daylight conditions. Notably, the fishermen's nets were deployed for no more than 3 hours, emphasizing a targeted and brief capture period. Utilizing an ichthyometer with a precision of 1 mm, a total of 150 individuals falling within the size range of 190 to 210 mm (measured as furca length) were selected. The number of fish collected and their size range were standardized across each independently analyzed group. Subsequently, the fish were transported during 2 h from the capture site to the premises of the Institute of Scientific Research at Universidad de Oriente. The transportation was facilitated within a live-well equipped boat, wherein a continuous flow of seawater ensured optimal conditions for the fish well-being.

Upon arrival at the research center, the fish were placed in a 4x4 m concrete pond with a depth of 0.40 m, situated outdoors to preserve the natural photoperiod. The pond received a continuous flow of seawater, ensuring optimal conditions for the fish throughout the experiment. The density, approximately 23 indiv.m^{-3}, was consistently maintained in both groups of fish studied. To accomplish this, a nylon net was employed as a dynamic regulator of fish density within the pond. Initially anchored at one end of the enclosure, the net was progressively repositioned as specimens were removed for experimental purposes, gradually nearing the central axis of the pond. Throughout the experiment, fish were fed once a day with fresh sardines (*Sardinella* sp.) *ad libitum*, following the collection of blood samples (see below).

Environmental variables

Temperature, salinity, dissolved oxygen (DO), and pH, were assessed once daily at noon in both the natural habitat and the confinement pond. The measurements were conducted using a thermometer with 0.1°C precision, a refractometer with a measurement range of 0-100% and a resolution of 0.1%, a YSI Model 51B portable oxygen meter, and a portable Hanna pH/mV Meter (HI8424), respectively. DO levels in the pond were continuously monitored every 4 hours throughout a 24-hour cycle.

Blood collection and hematological parameters

Blood samples were collected at various time points throughout the 21-day trial, following a chronological sequence. The first sampling occurred immediately after capture, followed by a second collection at the confinement point (2 h after capture and transport from the collection site). Subsequent samplings were conducted at regular intervals: every 24 h up to 168 h, every 48 h from 216 to 336 h, and finally at 504 h. All blood samples were collected before any food supply to standardize the conditions. For each sampling event, specimens for blood extraction were randomly selected from groups of 10 individuals. The fish underwent anesthesia using a benzocaine solution at a concentration of 150 mg/L before blood collection. Blood samples, each measuring 1 mL, were directly collected from the caudal hemal arch at the base of the caudal peduncle using disposable syringes equipped with a 21-gauge needle.

Following collection, the blood was deposited into vacutainer tubes with adequately heparinized inner surfaces, ensuring proper preservation and facilitating subsequent analysis. After the blood extraction procedure, the fish were transferred to a container filled with seawater and aeration to aid in their rapid recovery. Once fully recovered, the fish were returned to their natural habitat.

The quantification of hematological parameters was carried out following the protocol outlined by Blaxhall and Daisley (1973). This encompassed the determination of red blood cell count (RBC), packed cell volume or hematocrit (PCV), hemoglobin concentration (Hb), white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCHb), and mean corpuscular hemoglobin concentration (MCHbC).

Statistical analysis

Statistical analyses were performed following the recommendations described by Sokal and Rohlf (2011). To compare the data of the parameters determined in the two groups and to compare the values over time (hours), a two-way analysis of variance was used. In cases where the difference between groups or between hours was significant, the SNK *a posteriori* test between hours and a Student's t-test between groups were applied to determine the different mean values. When required, the data were transformed to meet the assumptions of normality and homoscedasticity, which were verified by the Kolmogorov-Smirnov and Levene's tests, respectively. PCV was transformed using the relation $\theta = \text{arcsine} \sqrt{p}$, where p is a percentage. For RBC, WBC, Hb, MCV, MCHb, and MCHbC, the square root transformation was applied.

RESULTS

Table I provides information on salinity, temperature, pH and DO levels, showing the environmental conditions that the fish encountered during the experimental phase. The term "pond" designates the controlled confinement environment, while "Cubagua" denotes the capture site in the natural habitat. In particular, salinity and temperature exhibited higher values in the confinement pond compared to Cubagua. pH levels remained relatively similar in both locations. Conversely, there was a notable disparity in dissolved oxygen levels, with higher concentrations observed in the pond than in Cubagua. Figure 1 illustrates the daily variation in DO in the confinement pond.

Table I. Hydrological parameters of the fish capture site in Cubagua Island and confinement pond.
Tabla I. Parámetros hidrológicos del sitio de captura de los peces en la Isla de Cubagua y en el estanque de confinamiento.

Source	Salinity (g/L)		Temperature (°C)		pH		O_2 (mg/L)	
	Min	Max	Min	Max	Min	Max	Min	Max
Pond (n=22)	35.6	40.5	24.5	32.5	7.3	7.7	4.9	8.5
Cubagua (n=22)	36.1	37.8	21.8	27.4	7.4	7.8	3.3	4.7
			Mean value					
Pond (n=22)	38.1		27.5		7.5		6.8	
Cubagua (n=22)	37.8		25.0		7.6		4.0	
t-Student	3.2**		6.1**		0.12ns		10.5**	

**, P<0,01

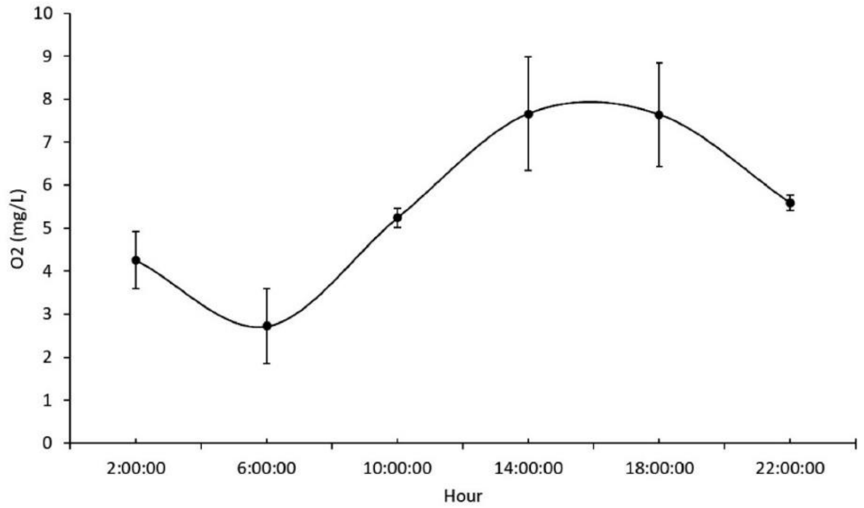

Figure1. Daily variation in dissolved oxygen (DO) in the confinement pond.
Figura 1. Variación diaria de oxígeno disuelto (OD) en el estanque de confinamiento.

Figure 2 illustrates the distinctive pattern of blood parameters in *O. rubra* during the experimental period. From the time of capture to 168 h, a significant increase in RBC, HB, and PCV was observed. Subsequently, these indicators showed a tendency to stabilize and remained so until 504 h of confinement. WBC exhibited fluctuating values up to 168 h, after which they stabilized. Regarding the hematimetric indices MCHb and MCHbC, fluctuating behavior was evident from the time of capture to 168 h, followed by stabilization. An increase in MCV was recorded during the first 48 h, followed by a pronounced decrease between 96 and 120 h, and stabilization from 168 to 504 h.

ANOVA results showed remarkable temporal differences in *O. rubra* blood parameters, emphasizing significant variations over time, while no significant differences were observed between groups (Tables II). Table III displays the results of the Post Hoc Analysis applied to parameters exhibiting significant differences (P<0.05) among various time points in both groups.

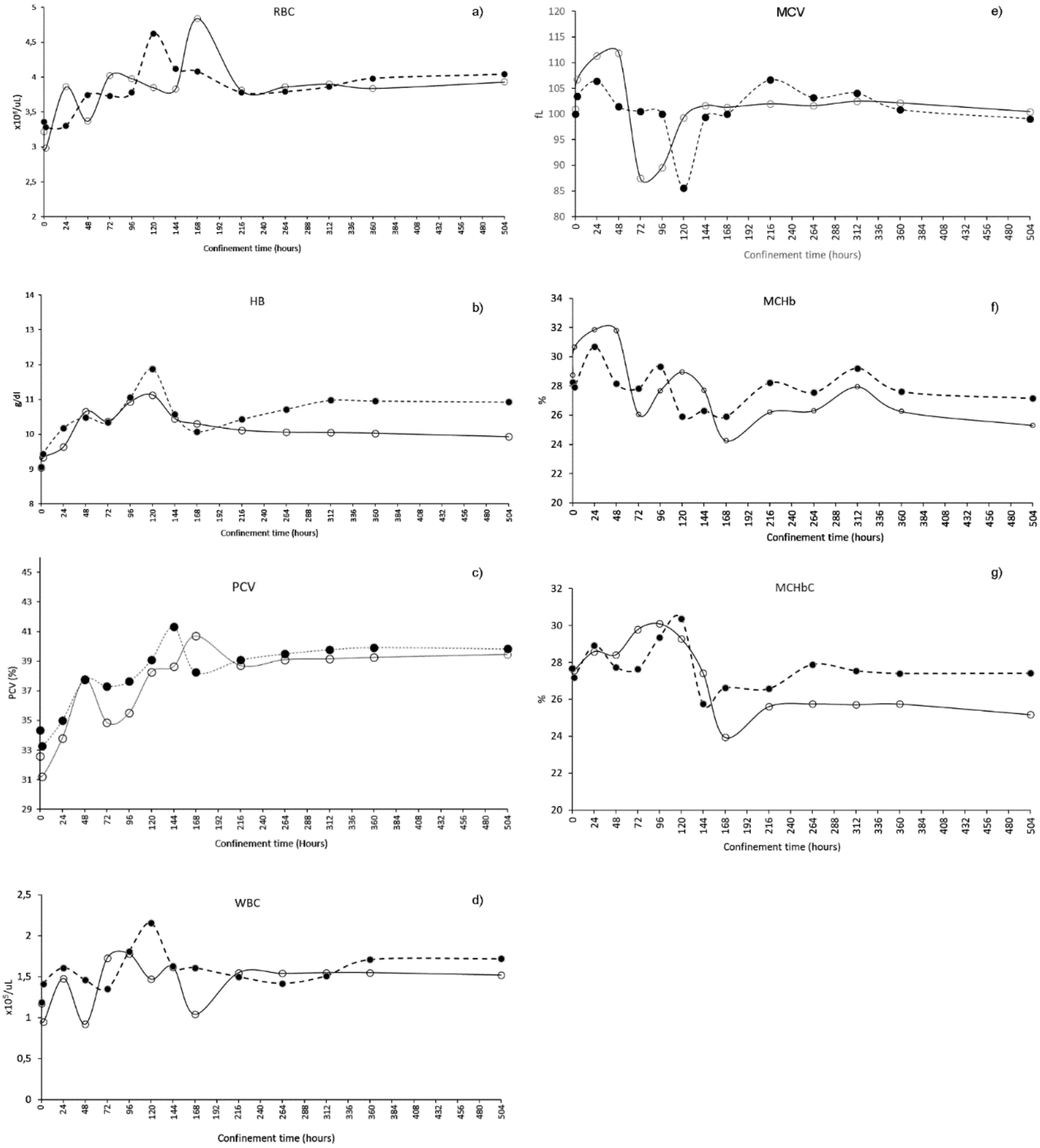

Figure 2: Temporal variation in hematological parameters of *Orthopristis rubra* over 21 days post-capture. a) RBC, b) Hb, c) PCV, d) WBC, e) MCV, f) MCHbC. Continuous line, group captured in February; dotted line May group.

Figura 2: Variación temporal en los parámetros hematológicos de *Orthopristis rubra* durante 21 días después de la captura. a) RBC, b) Hb, c) PCV, d) WBC, e) MCV, f) MCHbC. Línea continua, grupo capturado en febrero; línea punteada, grupo de mayo.

Table II. Two-Factor ANOVA for Hematological Parameters in the Two Analyzed Groups. Red blood cell count (RBC), packed cell volume or hematocrit (PCV), hemoglobin concentration (Hb), white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Factor A (Groups); Factor B (Hours); Interaction (AxB). Values highlighted in bold indicate statistically significant differences, with their respective probability values.

Tabla II. ANOVA de dos factores para los parámetros hematológicos en los dos grupos analizados. Recuento de glóbulos rojos (RBC), volumen de células empaquetadas o hematocrito (PCV), concentración de hemoglobina (Hb), recuento de glóbulos blancos (WBC), volumen corpuscular medio (MCV), hemoglobina corpuscular media (MCH) y concentración media de hemoglobina corpuscular (MCHC). Factor A (Grupos); Factor B (Horas); Interacción (AxB). Los valores resaltados en negrita indican diferencias estadísticamente significativas, con sus respectivos valores de probabilidad.

Parameter	Source	F-value	P
HB	A	2.07	0.148
	B	**2.86**	0.002
	AxB	1.97	0.075
PCV	A	3.59	0.056
	B	**10.35**	0.000
	AxB	1.02	0.430
RBC	A	0.35	0.563
	B	**4.45**	0.000
	AxB	1.90	0.352
WBC	A	1.22	0.269
	B	**2.06**	0.021
	AxB	0.99	0.4513
MCV	A	0.01	0.8824
	B	**5.65**	0.0000
	AxB	3.43	0.0002
MCHb	A	0.41	0.503
	B	**11.87**	0.000
	AxB	**8.85**	0.000
MCHbC	A	1.74	0.185
	B	**20.51**	0.000
	AxB	**13.90**	0.000

Table III. Post Hoc Analysis Results (Least Significant Difference) Applied to Parameters with Significant Differences (P<0.05) Between Different Time Points in Both Groups. Displayed are the Time Points Responsible for the Differences in Each Sampling.

Tabla III. Resultados del Análisis Post Hoc (Diferencia Mínima Significativa) aplicado a los parámetros con diferencias significativas (P<0.05) entre diferentes puntos temporales en ambos grupos. Se muestran los puntos temporales responsables de las diferencias en cada muestreo.

Parameter	Group I	Group II
PCV	24, 72, 96, 168	0, 24, 168
Hb	0-120, 168	0, 24, 120
RBC	24-96, 168	0, 24, 120, 144, 168
WBC	0, 48,168	0, 96, 120
MCHb	24, 48, 120, 168	24, 120, 144, 168
MCV	0, 24, 48, 72, 96	120

DISCUSSION

This study explores variations in hematological parameters of two groups of *O. rubra*, collected four months apart, from the moment of their capture in the wild to 21 days of confinement under non-natural conditions. The identified patterns, within the conditions experienced by both groups of fish, proved to be reproducible and revealed fluctuations in blood parameters during the initial 146-168 h, after which stabilization occurred, indicating effective acclimatization to captive conditions.

In the realm of fish hematological studies, our observations align with stress-induced alterations that transcend species boundaries. In *Ictalurus punctatus*, handling and capture resulted in elevated levels of key hematological parameters, including RBC, PCV, and Hb (Aguirre-Guzman *et al*., 2016). A similar trend was noted in *Sparus aurata*, where acute stress due to manipulation led to elevated levels of various blood parameters, encompassing RBC, PCV, Hb, and WBC, when compared to the non-stressed control group (Fazio *et al*. 2015). These stress-associated changes have also been documented in other species like the spotted dogfish, *Scyliorhinus canicula* (Torres *et al*. 1986), silver tilapia, *Oreochromis niloticus* (Rocha et al. 2018) and juveniles of *Oncorhynchus kisutch* (Aldrin *et al*., 1979).

It is essential to note that, despite the stress experienced by the fish during the approximately three hours of capture at the collection site, the initial values (at the time of capture) should closely align with the levels expected under natural conditions. Therefore, regardless of whether the values of hematological parameters stabilized, they did not return to levels similar to those recorded at the time of capture but rather remained elevated after stabilization. Exploring the potential relationship between this post-acclimatization elevation and the conditions of confinement is crucial for a comprehensive understanding of the fish's physiological response to capture and extended confinement stress.

Studies in various fish species have revealed the impact of different salinity, temperature, and oxygen conditions on blood parameters. For example, a study by Ali *et al*. (2024) on juvenile *Oreochromis niloticus* showed that RBC decreased with increasing salinity up to 10‰, followed by an abrupt fluctuation until reaching a significantly higher value at 25‰. Another investigation focused on *Tilapia guineensis*, examining both adult and juvenile sizes, demonstrated that fish transferred from a natural environment with a salinity of 15‰ to a laboratory and exposed to salinities of 10‰, 5‰, and 0‰ for 7 days exhibited a significant reduction in Hb, RBC, PCV, MCHbC, MCV, and MCHb (Akinrotimi *et al*., 2012). In *Mugil cephalus*, a noticeable decrease in RBC, PCV, and Hb was observed in fishes exposed to 25‰ compared to those at 45‰, attributed to salinity-induced osmoregulatory dysfunction. Conversely, at 45‰, a significant reduction in the number of WBC was recorded (Fazio *et al*., 2013). In the Red Spotted Grouper, *Epinephelus akaara*, previously maintained at a water temperature of 15°C and transferred to 15, 20, and 25°C during an experimental test of 7 days, PCV values were significantly higher at 15°C compared to 20 and 25°C, while Hb values also increased at 15°C, albeit without significant differences (Cho *et al*. 2015).

When comparing hydrological parameters between the fish collection site and the confinement pond (Table I), significant differences ($P<0.05$) were observed in salinity, temperature, and DO, while pH differences did not reach statistical significance.

DO stands as a paramount indicator of water quality, playing a crucial role in the survival of fish and other aquatic organisms. The stress induced by hypoxia is well-documented, with levels below 1-2 mg/L for a few hours having detrimental impacts on fish growth and potential consequences of death (Abdel-Tawwab *et al*. 2019).

While the hematological response observed in *O. rubra* initially seemed unrelated to the oxygen saturation in the pond, given that the mean DO levels consistently surpassed those of the natural environment, continuous monitoring of DO levels revealed a different situation. Concentrations of 7.65 ml/L observed between 2 p.m. and 6 p.m. were followed by substantial reductions during the night, reaching as low as 2.72 ml/L at 6:00 a.m., coinciding with the onset of dawn in the local area (Fig. 1). The decline in nocturnal DO levels could be attributed to various factors, including oxygen consumption by the fish, the biological activity of associated microorganisms in the pond, and the absence of photosynthesis (D'Autilia *et al*. 2004).

Although fish here studied did not reach lethal DO thresholds, exposure to near-critical levels could elucidate why the fish stabilized their hematological values above those detected in natural conditions. Indeed, hematological responses in diverse fish species under hypoxic conditions indicate an array of reactions, including increased PCV, Hb, and the release of young erythrocytes (Moraes *et al*. 2002, Mudiganti *et al*. 2014, Scott and Rogers 1981, Swift 1981, Yamamoto *et al*. 1983). Consequently, the initial elevation of RBC, Hb, and PCV values *in O. rubra* can be attributed to immediate reactions to the stress of capture and confinement. Despite acclimatization, these metrics persistently remain elevated, signifying a responsive physiological mechanism. In reaction to the daily hypoxic conditions in the pond, fish demonstrate an adaptive response by increasing RBC and Hb production to enhance oxygen-carrying capacity.

Moreover, it is crucial to consider that the average temperature in the pond was consistently 2.5 °C higher than the temperature in the capture area. Given that fish are naturally cold-blooded, their metabolic rate increases at higher temperatures, implying greater oxygen consumption as the temperature rises (Ali *et al*. 2024). This temperature elevation may not only have influenced oxygen consumption but also could have contributed to maintaining the blood parameter values of the fish related to respiration above the levels initially detected in their natural habitat after reaching acclimatization. Therefore, the higher levels of these stabilized parameters after acclimatization demonstrate a successful adjustment to environmental pressure that may involve mechanisms such as the release of young red blood cells and alterations in cellular morphology, optimizing oxygen uptake. This adjustment allows the fish to maintain a greater oxygenation capacity even in difficult and prolonged pond conditions.

On the other hand, the values of MCV, MCHb, and MCHbC, known as hematimetric indices and directly related to the size and hemoglobin content of red blood cells, experienced an initial increase during the first 72 hours. This was followed by a noticeable decrease between 96 and 168 h, eventually returning to levels similar to those recorded at the time of capture. This temporal pattern suggests a dynamic response to the stress of capture and confinement. The reduction in these mean values, particularly after 96 h, may indicate a strategic adjustment to increase the oxygen capture surface area. This adjustment, facilitated by an increase in RBC and a simultaneous decrease in MCV, optimizes oxygen consumption. It allows the fish to maintain a greater oxygenation capacity during the daily and prolonged hypoxic conditions that occur in the pond at night. Moreover, the observed fluctuations in these hematimetric indices align with the changes in temperature and oxygen levels in the pond, further emphasizing the intricate interplay between environmental factors and the fish's hematological responses during acclimatization. This dynamic adjustment in blood parameters would reflects the ability of fish to fine-tune its physiological mechanisms to cope with the varying conditions of its captive environment.

Studies on tilapias (Rocha *et al*. 2018) and *Clarias gariepinus* (Achilike and Wusu, 2019) have demonstrated variations in WBC counts linked to captivity and environmental conditions, suggesting a correlation with elevated stress levels influenced by various factors. In the present study, we observed significant initial variability in WBC, potentially indicating an acute response to the stress of capture and confinement. Although short-term stress in *O. rubra* led to a transient and variable increase in WBC compared to the initial values at the time of capture, the subsequent stabilization at a higher level over an extended period suggests an ongoing immune response in an altered environment. The initial variability might be attributed to agitation and sudden environmental shifts, but the successful acclimatization and possible enhancement of the immune state are evident.

Importantly, throughout the entire experimental period, no observable signs of pathological conditions were evident in the fish. Behaviors associated with illness, such as lack of appetite, flashing, open sores, or abnormal surface behaviors like diving or gasping, were not observed. Similarly, physical signs indicating parasitic, fungal, or bacterial infections, such as white spots, cottony appearance, or hyperemia of fins or body, were absent, reinforcing the overall well-being and health of the specimens during the study.

Shifting the focus back to hematological variables, the two-factor analysis of variance applied to PCV, Hb, and RBC did not reveal a significant interaction between the factor group and time. In contrast, a significant interaction between these factors was observed for MCV, MCHb, and MCHbC. This difference in results can probably be attributed to the unique nature of each hematological parameter and their responses to the factors evaluated. PCV, Hb, and RBC represent more general properties of blood physiology, such as blood cell volume, Hb amount, and RBC count, respectively. On the other hand, VCM, MCHb, and MCHbC are more directly related to specific characteristics of RBC, such as their size and Hb content. Therefore, the sensitivity of the latter parameters to rapid or specific changes induced by the stress of capture and confinement could explain the observation of a significant interaction, highlighting the complexity of hematological responses. It emphasizes the need to consider the uniqueness of each index when interpreting the results, as each measurement represents different aspects of blood physiology and may respond uniquely to time and group factors.

CONCLUSIONS

The findings of the study, in particular the stabilization of hematological parameters after 168 h, demonstrate the acclimatization of *O. rubra* to the artificial environment and show the fish's capacity to face the initial stress phase, achieving homeostasis in blood indicators in approximately one week (7 days). This knowledge not only lays the foundation for delving into the underlying mechanisms of hematological responses, but also provides crucial reference points. These reference points could serve as comparison values and offer important information to evaluate and improve the acclimatization processes of the species. Furthermore, understanding the connections between hematological changes and other physiological and behavioral aspects would contribute to a comprehensive approach, providing essential information for the effective management and conservation of fish that are extirpated from the wild and that face environmental changes under confinement conditions.

Declaration of conflict of interest of the authors

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of good practices in the use of animals

The experiment and handling of the fish strictly adhered to the ethical guidelines established by the Ethics Committee on Animal Experimentation at Universidad Técnica de Machala (process number UTMACH-CEEA-003-2023).

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