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Research Article

Comparative study of serum biochemical and hematological parameters of *Andinoacara rivulatus* and *Ichthyoelephas humeralis* in Los Ríos Province, Ecuador

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ABSTRACT. A comparative study of serum biochemical and hematological parameters from *Andinoacara rivulatus* and *Ichthyoelephas humeralis* in Los Ríos Province, Ecuador, was carried out. Two of the native species that are the most commercialized in Quevedo, Mocache and Fumisa, were identified; 60 specimens were captured in each area (180 total). A factorial design (3×2) was used: three habitat zones (Fumisa, Quevedo, and Mocache) and two species (*A. rivulatus* and *I. humeralis*). Sex, weight, length, serum biochemistry (total protein, albumin, globulin, albumin/globulin ratio, aspartate aminotransferase (AST), alanine aminotransferase (ALT), calcium, phosphorus, sodium, magnesium, and potassium), hematological parameters (hemoglobin, hematocrits, erythrocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total leukocytes, granulocytes leukocytes, and agranulocytes leukocytes) in fishes were determined. There was zone-species interaction for all indicators except MCH and MCHC ($P < 0.05$). For total proteins, albumins and ALT were for *I. humeralis* in Fumisa and Mocache zones (2.81, 1.57, and 326.67 g dL^{-1}, respectively). In comparison, *A. rivulatus* was higher in Fumisa and Quevedo in globulins and the albumins/globulins ratio with 2.28 g dL^{-1} and 2.14, respectively. Electrolytes were found in low concentrations in the two species. For the hematological parameters, all presented significant differences ($P < 0.05$) except MCH and MCHC. For *I. humeralis*, its highest values were presented in hemoglobin with 13.37 g dL^{-1} in Fumisa, for MCV and total leukocytes (93.11 fL^{-1} and 8.03×10^{3} μL^{-1}) respectively, in the Mocache area. This study will provide essential tools in monitoring the health status of these fish species in particular and fish species in general.

Keywords: *Andinoacara rivulatus*; *Ichthyoelephas humeralis*; commercial freshwater fishes; aminotransferases; hemoglobin; leukocytes; albumin; electrolytes

INTRODUCTION

Fish has become an important resource to meet a rapidly expanding human population's food and nutrition security needs. In the world, a total of 18,253 freshwater fish species have been reported, and together with the species of marine fish, they add up to 36,105, representing approximately 50% of all vertebrates (Fricke et al. 2022). South America is the continent with the greatest diversity, approximately 8000 registered species, although information is still scarce.

In Los Ríos Province (Ecuador), 951 freshwater fish species have been registered, several of which have the potential for cultivation (Nugra et al. 2018). Between these species, including "bocachico" (*Ichthyoelephas humeralis*) and "vieja azul" (*Andinoacara rivulatus*), constitute an important resource as a source of food and trade for rural and urban populations (Revelo & Laaz

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2012). Both species are distributed in along the Pacific slope of South America; they have been reported in the basins of the Guayas, Santiago, and Esmeralda rivers in Ecuador, Tumbes, Zarumilla, and Piura in Peru, have an omnivorous diet and can reach a size of 184 and 195 cm, respectively (Jiménez-Prado et al. 2015, Méndez-Martínez et al. 2022a). Revelo & Elías (2004) and Revelo (2010) investigated these species' biological and fishing aspects on the water systems of Los Ríos and Guayas Province in Ecuador. However, concerning blood biochemical and hematological indicators, there is no information.

The total bodyweight of fish comprises about 1.3-7% blood, and this is considered a vital active component, bringing about an exchange of gases between the organism and the environment (Fazio et al. 2013). Fish being poikilothermic animals, are under the influence of environmental changes. Any physical or chemical change in the habitat of fish is quickly reflected in their blood cell components (Enayat-Gholampour et al. 2020, Medina-Robles et al. 2020). Blood biochemistry and hematology are indicators that have proven to be a very useful tool in the evaluation and monitoring of the health status of both wild and captive organisms (Méndez-Martínez et al. 2021, 2022b). These variables of blood biochemistry and hematology are dependent on endogenous and exogenous factors (Ahmed et al. 2020, Enayat-Gholampour et al. 2020) and provide important information on metabolic disorders, deficiencies, chronic stress, and knowledge of the health status of fish populations before they present clinical signs (Vargas 2019, Méndez-Martínez et al. 2022a).

Fazio et al. (2013) compared the hematological profile of four teleost fish species (*Gobius niger*, *Mugil cephalus*, *Sparus aurata*, *Dicentrarchus labrax*) and established the similarities and differences between these species, and suggested that the differences found in the study could be attributed to the feeding behavior, lifestyle, and adaptation of the different fish species to the habitat. It is also noteworthy that these fish species are subject to overexploitation, contamination of aquatic ecosystems as a result of urbanization, industrialization, indiscriminate use of chemicals in the agricultural sector; residues of agricultural inputs, to this are added the effects of climate change, and increase of diseases (González et al. 2016, Malachy et al. 2017, Méndez-Martínez et al. 2022b).

An increase or decrease in blood parameters is considered the symbol of an unhealthy state, environmental stress, or tissue injury (Groff & Zinkl 1999). Evidence indicates that pollutants upon reaching bodies of water can trigger the oxidative stress process in organisms that live there and are responsible for effects on cells and tissues associated with mutation and carcinogenesis.

Among the body's physiological processes, proteins stand out for having a significant level of participation, acting as enzymes, antibodies, and hormones necessary for the growth and repair of tissues (Puello-Caballero et al. 2018). Both plasma and cellular proteins are part of a general circulating product reservoir for the maintenance of all tissues. It has been found that total protein levels may be decreased due to kidney damage, starvation, liver damage (Crivelenti et al. 2011, Aguirre-Guzman et al. 2016), septicemia, such as occurs in *Aeromonas* infection (Abd-Allah et al. 2019). Serum albumin can decrease its levels in liver disease, which also affects calcium (Ca^{2+}), probably because albumin acts as a serum calcium transporter (Kulkarni & Barad 2015).

The blood and its constituents may reflect many diseases. The abnormalities of erythrocytes, leukocytes, thrombocytes, and clotting factors are considered primary blood disorders. Such aberrations in the function or structure of the blood cells may result in anemia, leukopenia, leukocytosis, neutropenia, thrombocytopenia, and other blood cell abnormalities (Fazio et al. 2013, Yanuhar et al. 2021). The objective of this research was to carry out a comparative study of the serum biochemical and hematological parameters of *A. rivulatus* and *I. humeralis* in the province of Los Ríos, Ecuador. Then, to have a basic knowledge of hematology represents a valuable guide to assess the condition within the fish long before there is an outward manifestation of diseases, once reference values are established under standardized conditions.

MATERIALS AND METHODS

Study area

This investigation was carried out in three areas of the Los Ríos Province: Fumisa, Camarones area (0º43'16'S, 79º27'W); Pajarito precinct belonging to Mocache canton (1º08'3.92"S, 79º29'7.8"W), and Quevedo, La Ruta del Río (1º02'30'S, 79º28'30'W) (Fig. 1). The climatic conditions for these areas are shown (Table 1).

Samples collections

The native freshwater species (*Ichthyoelephas humeralis* and *Andinoacara rivulatus*) are most commercialized in Quevedo, Mocache, and Fumisa in Los Ríos Province. Sixty individuals were caught for each zone (180 in total). Subsequently, sexing was performed based on the morphological characteristics according to the criteria of Nugra et al. (2018) and Rodríguez-Pulido et al. (2018). A digital scale ± 0.01 g

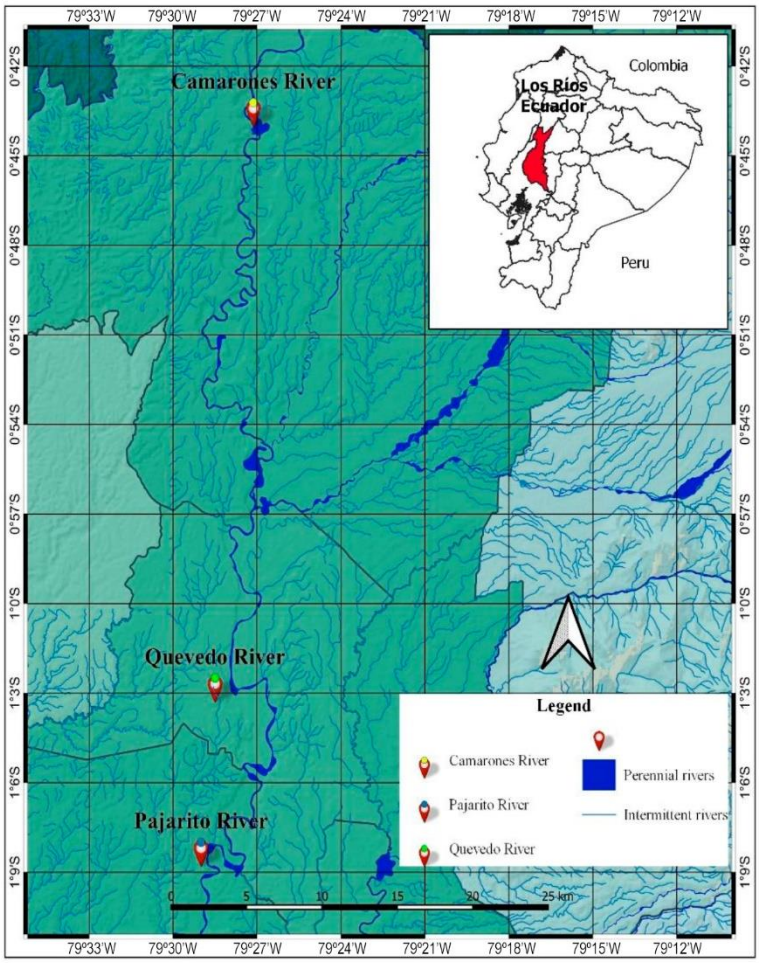

Figure 1. Map of sampling areas.

Table 1. Annual climatic characteristics of three areas of the Los Ríos Province, Ecuador.

Zone	Temperature (°C)	Precipitation (mm)	Weather
Fumisa	26 (21-31)	2750 (2000-3500)	Tropical
Mocache	25 (20-30)	2563 (1626-3500)	Tropical semi-humid
Quevedo	27 (23-31)	2125 (1750-2500)	Tropical humid

(PE 3600 Mettler-Toledo, Columbus, OH, USA) was employed to measure individual live weight for both sexes. The total length (TL) was determined with the help of a tape measure (Truper, 3m-Fh, Distrito Federal, MX), measuring the tip of the snout (October-December 2020) to the tip of the tail.

Blood sampling, biochemical and hematological analysis

For the study, 10 individuals were taken for each species and zone (n = 30 specimens) from which four milliliter of blood was extracted by puncture of the caudal artery at the level of the hemal arch, using 3 mL disposable syringes (Bio-In, Guayaquil, EC), deposited in vacutainer tubes (Vacuette, Laborgeräte GmbH, Eschau, DE) with heparinized inner surfaces. They were then centrifuged (Gemmy, PLC-05, Taipei, TW) at 161 g-forces for 10 min to separate blood components (Chang-Jung et al. 2011, Banaee et al. 2019, Méndez-Martínez et al. 2021).

Then kit commercial reagents (Diagnostics worldwide, Wiesbaden, DE) were applied for serum biochemical analysis. Samples were incubated 10 min at 37ºC for proteins, 5 min at 25ºC for albumins, 15 min

at 37ºC for aspartate aminotransferase (AST), and 5 min at 37ºC for alanine aminotransferase (ALT) (Aguirre-Guzman et al. 2016, Hung-Sheng et al. 2018). Absorbance (ABS) readings were performed with a SunostIk Plus, Kunshan Road, CHN spectrophotometer at 546 nm (protein), 578 nm (albumin), and 340 nm (AST and ALT), respectively (Chang-Jung et al. 2011, Hung-Sheng et al. 2018, Karatas & Albayrak 2018). Globulins and albumins/globulins ratio were determined using the formulas described by Conroy (1998). Electrolytes were incubated for 20 min at 37°C for sodium (Na^+) and magnesium (Mg^{2+}), 30 min at 25°C for phosphorus (P^-) and Ca^{2+}, and 10 min at 37°C for potassium (K^+). Samples were analyzed in a spectrophotometer with ABS reading at 405 nm for Na^+ and Mg^{2+}, 570, 340, and 380 nm for Ca^{2+}, P^-, and K^+, respectively (Memaster et al. 1991, Prakash & Verma 2020).

Hemoglobin was determined with Drabkin's reagent following the cyanmethemoglobin methodology and spectrophotometer reading at 546 nm. The total count of erythrocytes and leukocytes was performed in a Neubauer chamber of 0.0025 mm^2 (Optik Labor, DE), for the count of erythrocytes, Hayem solution was used as a diluent, and for leukocytes, the acetic acid reagent was used (Conroy 1998), in both cases the dilution was 1:200. Hematocrit was determined in a microcentrifuge. The erythrocyte indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were determined applying the formulas described by Conroy (1998). blood smears were stained with Romanowsky (Wright) to perform granular and non-granular leukocytes count (Conroy 1998, Karatas & Albayrak 2018).

Statistical analysis

A factorial analysis (3×2) with three habitat zones (Quevedo, Mocache, and Fumisa) and two species of fish (*I. humeralis* and *A. rivulatus*) was used. Double classification analysis of variance was applied to the results, considering the habitat zone and species as sources of variation. The difference between the means was quantified using the Tukey with significance level of $P < 0.05$. The Kolmogorov-Smirnov ($P < 0.05$) test was used for the normal distribution of the data, and the Bartlett ($P < 0.05$) was used for the variances, respectively. All statistical tests were performed with the IBM SPSS statistical program. 22.0. for $P < 0.05$. The results are presented as means ± SE (standard error).

RESULTS

For sexing (Table 2), the highest percentages were found on females in the three habitat zones with differences of 13.34, 10, and 20% concerning males on Fumisa, Mocache, and Quevedo zones, respectively. When was analyze the species/habitat relationship, the prevalence of females over males continues with the greatest differences on *Ichthyoelephas humeralis* with 20, 13.34, and 40% for Fumisa, Mocache, and Quevedo zones, respectively. On *Andinoacara rivulatus* a similar behavior was maintained except for Quevedo, where 50% was found for each sex. In weight and length (Table 3), there was species-habitat zone interaction ($P < 0.05$) with the highest values for *I. humeralis* with 297.97 g and 28.73 cm.

The indicators of blood biochemistry (Table 4) presented highly significant differences ($P < 0.05$) with species-zone interaction with the highest values for *I. humeralis* for total protein, albumins, ALT and AST, the Fumisa zones (2.81 and 1.57 g dL^{-1}, for proteins and albumins) and Quevedo (326.67 and 326.33 UL^{-1}, for ALT and AST). While *A. rivulatus* was superior for globulins in the Fumisa zone and the albumins/globulins ratio in Quevedo with 2.28 g dL^{-1} and 2.14; protein in this species 2.24 g dL^{-1} was highest in the Fumisa zone. The highest values ($P < 0.05$) in hemoglobin (13.37 g dL^{-1}) were for *I. humeralis* in Fumisa.

Regarding the content of Na^+, P^-, Ca^{2+} and K^+ (Table 5), they presented interaction ($P < 0.05$) zone-species, the highest results for *I. humeralis* in the Quevedo zone, with 3.50, 2.44, 136.33 and 2.77 mmol L^{-1} for P^-, Ca^{2+}, $Na^{+,}$ and K^+, respectively. However, for Mg^{2+} with the highest or Mocache with 0.60 mmol L^{-1} in *A. rivulatus*. This species presented values lower than *I. humeralis* for K^+ (0.49, 0.59 and 0.37 mmol L^{-1}) and Ca^{2+} (1.52, 0.21 and 0.33 mmol L^{-1}) in Fumisa, Mocache, and Quevedo; for P^- and Na^+, there were no differences in Mocache, but it was lower in Fumisa and

Table 2. Sexing of species.

Relationship		% Males	% Females
Zone			
Fumisa		43.33	56.67
Mocache		45.00	55.00
Quevedo		40.00	60.00
Zona/species			
Fumisa	*A. rivulatus*	46.68	53.32
	I. humeralis	40.00	60.00
Mocache	*A. rivulatus*	46.67	53.33
	I. humeralis	43.33	56.67
Quevedo	*A. rivulatus*	50.00	50.00
	I. humeralis	30.00	70.00

Table 3. Weight and length of *Andinoacara rivulatus* and *Ichthyoelephas humeralis* according to the zone of habitat. Different superscript letters denote significant differences ($P < 0.05$). Mean values ± standard error.

Species	Habitat zone			*P*-value
	Fumisa	Quevedo	Mocache	
		Weight (g)		
A. rivulatus	213.97 ± 5.01^{c}	197.30 ± 5.05^{d}	231.70 ± 4.35^{b}	0.0010
I. humeralis	226.87 ± 5.32^{b}	297.97 ± 5.72^{a}	225.90 ± 4.56^{b}	
		Length (cm)		
A. rivulatus	23.89 ± 0.54^{d}	24.69 ± 0.44^{d}	26.00 ± 0.36^{c}	0.0001
I. humeralis	26.95 ± 0.63^{c}	28.73 ± 0.56^{a}	27.79 ± 0.55^{b}	

Table 4. Serum proteins and transaminase of *Andinoacara rivulatus* and *Ichthyoelephas humeralis* according to habitat zone: Different superscript letters denote significant differences ($P < 0.05$). Mean values ± standard error. ALT: alanine aminotransferase, AST: aspartate aminotransferase.

Species	Habitat zone			*P*-value
	Fumisa	Quevedo	Mocache	
		Total protein (g dL^{-1})		
A. rivulatus	2.54 ± 0.07^{b}	0.86 ± 0.02^{e}	2.12 ± 0.07^{c}	0.0001
I. humeralis	2.81 ± 0.08^{a}	1.98 ± 0.04^{d}	0.89 ± 0.01^{e}	
		Globulins (g dL^{-1})		
A. rivulatus	2.28 ± 0.05^{a}	0.51 ± 0.03^{d}	1.79 ± 0.04^{b}	0.0001
I. humeralis	0.26 ± 0.02^{e}	0.81 ± 0.02^{c}	0.46 ± 0.04^{d}	
		Albumins (g dL^{-1})		
A. rivulatus	0.25 ± 0.02^{e}	0.55 ± 0.03^{c}	0.33 ± 0.03^{d}	0.0001
I. humeralis	1.57 ± 0.06^{a}	1.18 ± 0.04^{b}	0.37 ± 0.04^{d}	
		Albumins/globulins ratio		
A. rivulatus	0.11 ± 0.01^{e}	2.14 ± 0.05^{a}	0.19 ± 0.012^{e}	0.0001
I. humeralis	1.27 ± 0.04^{c}	1.58 ± 0.03^{b}	0.71 ± 0.01^{d}	
		ALT (U L^{-1})		
A. rivulatus	72.00 ± 1.34^{c}	58.67 ± 0.37^{e}	71.6 ± 1.457^{c}	0.0001
I. humeralis	68.00 ± 0.45^{d}	246.33 ± 5.23^{b}	326.67 ± 4.76^{a}	
		AST (U L^{-1})		
A. rivulatus	71.67 ± 2.45^{d}	60.00 ± 2.45^{e}	71.33 ± 1.33^{d}	0.0001
I. humeralis	102.33 ± 4.23^{c}	245.33 ± 6.78^{b}	326.33 ± 6.34^{a}	

Quevedo in 0.54, 1.48 mmol L^{-1} for P^{-} and 8.67, 16.66 mmol L^{-1} for Na^{+}, it was only higher in Mg^{2+} in 0.08, 0.30 mmol L^{-1} in Quevedo and Mocache. Hematological parameters presented significant differences ($P < 0.05$) except MCH and MCHC. For *I. humeralis*, its highest values were presented for MCV (93.11 fL^{-1}) and total leukocytes (8.03×10^{3} μL^{-1}) in the Mocache area. Whereas, hematocrits, erythrocytes, granulocytes leukocytes, and agranulocytes leukocytes were higher for *A. rivulatus* in Quevedo with 39.33%, 4.56×10^{6} μL^{-1} and 58.33% for hematocrits, erythrocytes, and granulocytes leukocytes, respectively; and 52% for agranulocytes leukocytes in Mocache (Table 6).

DISCUSSION

In the reproductive biology of fish, complexity and plasticity establish a population structure in which the proportion of the sexes (Table 2) is not always one to one but depends on the allocation of resources and natural selection (Rodríguez-Pulido et al. 2018). The introduction and crossbreeding of a species of fish (especially wild ones) leads to a high adaptation to a wide range of geographical locations, which leads to phenotypic variations concerning the pure stock (strains) of the reproducers (Kelley et al. 2017).

Pineda-Santis et al. (2004), in studies of *Colossoma macropomum*, reported 73.71% females and 26.09% males. Alvarez-Leon (2019) found in the Bogotá River in the species *Andinoacara rivulatus*, *H. microlepis*,

Table 5. According to the habitat zone, electrolytes content in the blood of *Andinoacara rivulatus* and *Ichthyoelephas humeralis*. Different superscript letters denote significant differences ($P < 0.05$). Mean values ± standard error.

Species	Habitat zone			*P*-value
	Fumisa	Quevedo	Mocache	
		Phosphorus (mmol L^{-1})		
A. rivulatus	2.65 ± 1,33^{d}	2.02 ± 1.29^{e}	2.92 ± 0.38^{c}	0.0001
I. humeralis	3.19 ± 0.26^{b}	3.50 ± 0.10^{a}	2.90 ± 0.66^{c}	
		Potassium (mmol L^{-1})		
A. rivulatus	1.60 ± 1.04^{d}	2.18 ± 0.40^{c}	2.30 ± 0.44^{b}	0.0001
I. humeralis	2.09 ± 0.13^{c}	2.77 ± 0.42^{a}	2.67 ± 0.51^{a}	
		Calcium (mmol L^{-1})		
A. rivulatus	0.72 ± 1.24^{d}	2.23 ± 0.31^{a}	1.84 ± 0.29^{c}	0.0110
I. humeralis	2.24 ± 0.23^{a}	2.44 ± 0.37^{a}	2.17 ± 0.75^{b}	
		Sodium (mmol L^{-1})		
A. rivulatus	114.33 ± 9.81^{c}	119.67 ± 10.07^{bc}	127.33 ± 8.02^{b}	0.0320
I. humeralis	123.00 ± 6.08^{b}	136.33 ± 11.6^{a}	127.68 ± 6.81^{b}	
		Magnesium (mmol L^{-1})		
A. rivulatus	0.23 ± 0.05^{c}	0.56 ± 0.11^{a}	0.60 ± 0.10^{a}	0.0200
I. humeralis	0.43 ± 0.23^{b}	0.48 ± 0.16^{b}	0.30 ± 0.10^{c}	

Table 6. According to the habitat zone, the hematological parameters of *Andinoacara rivulatus* and *Ichthyoelephas humeralis*. Different superscript letters denote significant differences ($P < 0.05$). Mean values ± standard error. MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration.

Species	Habitat zone			*P*-value
	Fumisa	Quevedo	Mocache	
		Hemoglobin (g dL^{-1})		
A. rivulatus	11.50 ± 0.85^{b}	13.07 ± 1.56^{a}	10.10 ± 2.47^{c}	0.0350
I. humeralis	13.37 ± 1.20^{a}	12.97 ± 1.23^{a}	8.80 ± 0.99^{d}	
		Hematocrits (%)		
A. rivulatus	29.00 ± 0.93^{d}	39.33 ± 1.62^{a}	33.33 ± 0.86^{c}	0.0300
I. humeralis	37.67 ± 1.62^{b}	39.00 ± 1.60^{a}	36.00 ± 1.20^{b}	
		Erythrocytes ($\times 10^{6}$ μL^{-1})		
A. rivulatus	3.66 ± 0.15^{c}	4.56 ± 0.85^{a}	4.53 ± 0.58^{a}	0.0020
I. humeralis	3.23 ± 0.81^{d}	4.33 ± 0.15^{b}	4.36 ± 0.23^{b}	
		MCV (fL^{-1})		
A. rivulatus	90.16 ± 2.57^{c}	92.85 ± 0.84^{ab}	91.23 ± 1.80^{b}	0.0360
I. humeralis	91.54 ± 2.04^{b}	92.82 ± 0.63^{ab}	93.11 ± 0.37^{a}	
		MCH (Pg)		
A. rivulatus	29.84 ± 0.99	30.84 ± 0.28	29.56 ± 0.58	0.3300
I. humeralis	30.55 ± 1.45	30.85 ± 0.29	30.94 ± 0.19	
		MCHC (g dL^{-1})		
A. rivulatus	33.09 ± 0.07	33.22 ± 0.04	32.42 ± 0.80	0.1100
I. humeralis	33.15 ± 0.13	33.24 ± 0.09	33.23 ± 0.08	
		Leukocytes ($\times 10^{3}$ μL^{-1})		
A. rivulatus	5.33 ± 0.57^{c}	5.37 ± 0.55^{c}	5.50 ± 0.70^{c}	0.0030
I. humeralis	7.20 ± 0.40^{b}	7.67 ± 0.50^{ab}	8.03 ± 0.35^{a}	
		Agranulocytes Leukocytes (%)		
A. rivulatus	50.00 ± 0.78^{b}	41.67 ± 0.50^{e}	52.00 ± 0.70^{a}	0.0170
I. humeralis	48.33 ± 0.63^{c}	46.67 ± 0.63^{d}	41.66 ± 0.63^{e}	
		Granulocytes Leukocytes (%)		
A. rivulatus	50.00 ± 0.66^{b}	58.33 ± 0.61^{a}	48.00 ± 1.03^{c}	0.0180
I. humeralis	51.67 ± 0.64^{b}	58.32 ± 0.74^{a}	58.30 ± 0.63^{a}	

Ichthyoelephas humeralis, and *Eremophilus mutisii* in female-male percentages of 49.30-50.70; 58.40-41.60; 73.80-26.20; 61.40-38.60 and 81.82-18.18%, respectively. The variability that could be given the reproductive and spawning season in the species of the current investigation occurs in the winter season; other factors such as distribution, mortality, feeding, pH, and water temperature. In this way, embryonic gonads starting from a common rudiment, made up of somatic cells of the gonadal crest and primordial gonadal cells, can develop in two different adult organs, ovaries or testes, affecting the demographic structure of the population (Pacheco-Bedoya 2018, Castro & Polas 2020).

The results of weight and length of our investigation coincide with those obtained by Caez et al. (2019), when evaluating *A. rivulatus* in the Quevedo River under cultivation and wild conditions, where 52 weights were selected for each of the forms of production, weights between 90-228 g were obtained, total length 14.8-21.8 cm. Weight and length are characteristics that have been used to identify adaptation processes in nature and cultivation systems (Ochoa-Ubilla et al. 2016).

González et al. (2016) and González-Martínez et al. (2020), when evaluating the morphometric variations between two populations (wild and cultivated) of the specie *Cichlasoma festae* and *Dormitator latifrons*, respectively, reported for the case of *C. festae* increases on the weight of 22.78 g, and total length of 2.26 cm, while for *D. latifrons* differences were reported for weight and total length with increases of 73.51 g and 3.61 cm. Therefore, differences between both production systems could be explained by the availability of more food for fish in farms than in rivers, latter depending more on climatic conditions.

The disease infection is one of the factors that can reduce fish growth and development. It arises due to the host, pathogen, and environment interactions. The disease causes can be divided into two groups: non-infection (stress, nutritional deficiency, intoxication) and infection (bacteria, viruses, fungi, protozoa, and worms). The cause of the variation in morphometric characters can be attributed to interspecific variability, which is under the influence of environmental parameters where the fish adapt quickly and change the necessary morphological characters (Zhao et al. 2018, Méndez-Martínez et al. 2022a).

To use blood chemistry and hematological parameters as biomarkers, it is important to know the reference and standard values of a given fish species (Fazio 2019, Hernández et al. 2021, Méndez-Martínez et al. 2022b). Blood chemistry studies in South American tropical fish are very scarce; however, it is becoming necessary to carry out diagnostic evaluations with a preventive purpose when referring to the planning of pathology control measures. Moreover, the normal blood parameters of some fish species living in different habitats are still unknown. The total serum protein content is considered the most stable component of blood (Jácome et al. 2019) and acts as an indicator of nutritional condition, physiological status, stress, and the health of fish (Ahmed et al. 2019).

Total protein concentration is a useful tool, both as evidence of inadequate nutrition evidenced by a decrease in serum albumin and as a sign of systemic infections, and can cause an increase in circulating globulins. The globulin plays a major role in the innate immune response of fish (Banaee et al. 2019, Ahmed et al. 2019). Moreover, albumin levels increase due to sexual maturation and help transport lipids in fish blood (Di Marco et al. 2011). During the study, the total protein and albumin levels were recorded as slightly greater in *I. humeralis* in Fumiza, while the globulin and albumin/globulin ratio were slightly greater in *A. rivulatus*. Other workers have found similar results for different fish species (Zhao et al. 2018, Ahmed et al. 2019, Jácome et al. 2019)

Enzymes such as AST and ALT are present in the circulation of normal animals at all times. They are generally synthesized in the liver but are present in equivalent or higher concentrations in the tissue (Kulkarni 2017). Its substrates are also present in the circulation of these functional enzymes, which perform physiological functions in the blood. Stress and starvation can bring about structural changes in the liver, reducing transaminase activity and deamination capacity and affecting fluid balance control (Li et al. 2020). In fish, *I. humeralis* exhibited high values of AST and ALT compared to *A. rivulatus* and significantly higher values for those captured in the Mocache habitat zone, suggesting that the species and habitat reflect the effect on these enzymes' activity.

These enzymes belong to non-plasma-specific enzymes localized within the tissue cells of the liver, heart, gills, kidneys, muscles, and other organs; when present in blood serum or plasma, they may provide specific information on organ dysfunction (Li et al. 2020). Elevation of ALT activity appears to reflect liver disease, and it is more specific for liver disease than AST because of the biological location of these two enzymes. However, the activity of either enzyme, particularly AST, may also be elevated in acute liver necrosis (Fazio 2019, Tiwari & Singh 2020).

The electrolytes are the most important substances that influence body water distribution and retention (osmoregulatory). Regarding electrolytes, the values Na^+, Mg^{2+}, Ca^{2+}, K^+, and P^- are frequently used to

determine fishes' physiological characteristics, toxicity, and health status. In our work, electrolytes were found in low concentrations in the blood serum compared to other works (Percin et al. 2010, Kulkarni & Barad 2015, Fazio et al. 2020). The values of Ca^{2+} and K^{+} reported in the current study are lower than those obtained by Kulkarni & Barad (2015) in the freshwater fish *Notopterus notopterus*; however, regarding Na^{+} values, higher values were obtained in both species of the present study in comparison with those reported for *N. notopterus*.

Na^{+} enters the gill cells from the blood in fish, co-transported with K^{+}. Monovalent ions, like K^{+}, have an important role in osmoregulation and homeostasis. In fishes, Ca^{2+} is used in some functions combined with P^{-} for deposition in bones and the Ca^{2+} repository for plasma, tissues, reproduction, and mitochondrial functions. Ca^{2+} facilitates the linking of excitation and contraction in the skeletal and cardiac muscle by mobilizing the large Ca^{2+} intracellular stores of the sarcoplasmic reticulum. In non-skeletal cells, Ca^{2+} serves as a signal transducer, mediating signaling from activated plasma membrane receptors to carry out various functions such as hormone secretion, neurotransmission, and kinase phosphorylation (Fazio et al. 2020, Verma 2020). Mg^{2+} is important because it helps regulate blood pressure and blood sugar levels, protein building, bone mass, and genetic material present in cells). Mg^{2+} was found in the least amount when compared to other determined electrolytes.

However, it should be noted that quantitative characteristics of electrolytes depend on factors such as the species (Jácome et al. 2019, Ahmed & Sheikh 2020), season (Shahjahan et al. 2013, 2017), feeding habit (Haghighi & Rohani 2013, Chelladurai et al. 2017), stress (Grzelak et al. 2017, Shahjahan et al. 2018) and disease (Sebastiao et al. 2011, Harikrishnan et al. 2012).

In the present study, *A. rivulatus* and *I. humeralis* blood cells were characterized as hematological indices were analyzed. The mature erythrocytes of *A. rivulatus* and *I. humeralis* show an average size and ultrastructural features similar to those described for erythrocytes of other fish species (Groff & Zinkl 1999, Sáez et al. 2018, Vargas 2019). Low erythrocytes levels may indicate anemia. Meanwhile, high levels indicate that the fish is under stress. Also, the low total erythrocytes will cause fish to not take in large amounts of oxygen even though the availability of oxygen in the waters is sufficient. As a result, fish will experience a lack of oxygen (anoxia). Erythrocytes are produced in the spleen and kidneys. Anemia impacts the inhibition of fish growth because the low number of erythrocytes causes a reduced food supply to cells, tissues, and organs so that the metabolic process of fish will be. In our work, the hemoglobin concentrations ranged from 8.80-13.37 g dL^{-1}. Hemoglobin functions to bind oxygen, which will then be used for catabolism to produce energy. The ability to bind oxygen in the blood depends on the amount of hemoglobin (Alamanda et al. 2007). The low feed protein content will cause the low hemoglobin content, which finally will cause fish to get an infection. The decrease in hemoglobin value indicates abnormalities in fish health.

Based on the data obtained, it can be seen that the hematocrit value (37.67-39.33%) of fish from the three research locations is normal compared to other studies (Alamanda et al. 2007, Sáez et al. 2018). However, differences were found significant between them. According to Ahmed & Sheikh (2020), if the fish is affected by disease or their appetite decreases, the blood hematocrit value will be abnormal, and if the hematocrit value is low, the erythrocyte count is low.

Leukocytes are normally lower in healthy fishes than in infected fish and can indicate infectious diseases. In our work, a greater presence (%) of granulocytes leukocytes (basophil, eosinophil, and neutrophil) was found concerning agranulocytes leukocytes (lymphocytes and monocytes), with higher values in fish captured in Quevedo. Sharma et al. (2015) and Fazio (2019) suggested that species with increased values of leukocytes can combat infections more efficiently than other species because of the close association between leukocyte number and immune response, thus regulating different immunological factors functions.

The use and validation of standardized non-lethal and inexpensive methods to monitor fish health are necessary for expanding fish production. The disease diagnosis using blood biochemical and hematological analysis is especially important because it can provide a reliable evaluation via non-lethal means. It is also of interest for nutritional management in captivity; since the interaction between nutrients can be evaluated looking for the best benefits for the species.

In conclusion, there was zone-species interaction for all indicators except for MCH and MCHC. As for biochemical indicators, only globulins and albumins and their ratio were within the literature reports. Electrolytes were found in low concentrations in blood serum. The present study results provide a basic knowledge of the serum biochemical and hematological parameters of two ecologically and economically important fish species to evaluate their physiological and health status for better management.

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