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Hematological parameters of three freshwater stingray species (Chondrichthyes: Potamotrygonidae) in the middle Rio Negro, Amazonas state

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ABSTRACT

This paper aimed to study and compare the hematology of newborns, young, subadults, adult males, adult females and pregnant females of *Potamotrygon wallacei* (cururu stingray), *Potamotrygon motoro* and *Paratrygon aiereba*. Newborn cururu stingrays had lower red blood parameters than those of other development stages. Thrombograms and leukograms showed a conservative pattern between development stage, sexual dimorphism and pregnancy. In *P. motoro* and *P. aiereba*, variables relating to red blood parameters, biochemistry and leukograms showed little variation between the species' biological characteristics, thus showing that these variables are not good criteria for differentiating them within the same species. In conclusion, the development stage is an important factor for differentiating hematological properties in the cururu stingray, while this has not been observed in *P. motoro* and *P. aiereba* stingrays.

1. Introduction

Traditionally, blood parameter investigation has been used among fish to determine stress and pathological conditions (Tavares-Dias and Moraes, 2006; Pavlidis et al., 2007). One of the blood constituents, erythrocytes, can be used to diagnose anemia, as well as to characterize different strategies in fish populations, regarding their metabolic demand for oxygen (Wilhelm Filho et al., 1992). In addition, erythrocytes are the main indicator for different adaptive physiological strategies

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relating to environmental variations (Val et al., 1992). Another important contribution towards hematological parameter analysis comes from leukogram and thrombogram evaluations, which mainly indicate infections (Oliveira et al., 2015a), although these evaluations can also be used as a tool in determining systematic relationships between species (Pavlidis et al., 2007). The plasma biochemical profile is also an important biological tool for assessing stress, diet and tangible ion regulation among potamotrygonids (Brinn et al., 2012).

One notable characteristic of fish is the wide variation in their blood parameters (Kori-Siakpere et al., 2005). These variations are generally attributed to genetic variation, nutritional status, age, sex, capture stress, handling procedures and blood sampling (Kori-Siakpere et al., 2005; Svobodová et al., 2008). Although hematology is a useful tool, it has been little used for species in their natural environment (Kori-Siakpere et al., 2005), especially for freshwater stingrays (Brito et al., 2015; Oliveira et al., 2015a).

Potamotrygonidae is the only taxon within Chondrichthyes that is adapted to live exclusively in freshwater (Compagno and Cook, 1995). Currently, this family consists of four genera (Potamotrygon, Plesiotrygon, Paratrygon and Heliotrygon), with approximately 25 species distributed among these genera (Carvalho and Lovejoy, 2011; Loboda and Carvalho, 2013; Fontenelle et al., 2014; Silva and Carvalho, 2015). Freshwater stingrays have high ecological importance within the Amazon ichthyofauna, since they take on the ecological role of apex predators, thus contributing to the balance and dynamics of their natural environments. In addition to the ecological importance of freshwater stingrays, they are also economically valuable for the state of Amazonas, as they are marketed as ornamental fish (Duncan et al., 2010). They are one of the most important fishery resources in this state.

In this context, this paper aimed to study and compare the hematology of *Potamotrygon wallacei*, *Potamotrygon motoro* and *Paratrygon aiereba* at different development stages (newborns, young, subadults, adult males, adult females and pregnant females) in the middle Rio Negro, Amazonas, Brazil.

The results from this paper may help to guide regulatory agencies in their decision-making. For example, the two-year ban on the stingray trade, which was enacted due to lack of information on the biology of these elasmobranchs (IN No. 118/06-IBAMA), hindered sustainable use of this resource in this region. However, despite the quota system that was introduced, which was established according to species/year (IN No. 204/08-IBAMA), and the growing concern of organs responsible for maintenance of the resources that constitute Amazon biodiversity, destructive fishing and illegal transfers of stingrays to the international market have not been prevented.

2. Materials and methods

2.1. Study area

The middle Rio Negro contains one of the most important groups of islands in the Amazon region, called the Mariuá archipelago. This has approximately 1600 islands and is home to a rich biodiversity of ornamental fish, including *Potamotrygon wallacei*, *Potamotrygon motoro* and *Paratrygon aiereba* stingrays. In this archipelago, located in the middle Rio Negro, the waters are black and have low pH and low concentrations of dissolved oxygen and ions. Therefore, the middle Rio Negro waters have low electrical conductivity and scarcity of nitrogen compounds and alkaline and alkaline earth elements (Souza et al., 2006; Duncan et al., 2009). Stingrays were caught in various habitats in this archipelago, which is located near the municipality of Barcelos, Amazonas, Brazil. All of these fish were caught with prior authorization (license No. 15116-1) from the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA).

2.2. Target species

Three freshwater stingray species were chosen for investigation in this study, as follows: *Potamotrygon wallacei* (cururu stingray), *P. motoro* and *P. aiereba*. The *Potamotrygon wallacei* stingray is the main ornamental potamotrygonid species, accounting for approximately 50% of exports (IBAMA, 2008). This stingray is small and can reach a maximum disc width of 45 cm. The species is still being scientifically described, although it has a well-defined identity. Cururu stingrays exhibit sexual segregation and their annual reproductive cycle is regulated by the river level. Copulation takes place during the ebb tide and the offspring are born during the drought season (Araújo, 1998). The distribution of this species is endemic and restricted to the middle Rio Negro. Stingrays are found in plant litter environments with low water flow and current, typical of marginal creek (“igapó”) areas (Araújo, 1998).

The second most important stingray species on the international market is *P. motoro*, accounting for 30% of exports (IBAMA, 2008). It is widely distributed across the Amazon basin (Sanchez-Duarte et al., 2014), reaches a disc width of 70 cm and has sexual segregation. The species has an annual reproductive cycle with a mating period during the drought season, pregnancy during the flood tide and birth in the early flood. Its preferred habitat comprises areas with a muddy bottom, where there is more influence from water flow (personal observation).

In addition to these species, *P. aiereba* is also commercially important in the state of Amazonas. It is large, reaching a disc width of up to 130 cm and weighing more than 60 kg. This species is widely distributed throughout the Amazon basin, and is predominantly exploited by commercial fishing (Lasso et al., 1996). Its preferred habitat is composed of beach regions with little water flow intensity (personal observation). In the Tapajós river basin, reduction of the natural stock of this species has been observed due to exploitation on a large scale, in order to obtain fillets (Araújo, personal communication). In the state of

Amazonas, some slaughterhouses located in the municipality of Iranduba have also intensified their purchases of these fish for processing and marketing frozen fillets, without any control over this exploitation.

2.3. Capture, animal age classification and blood collection

From December 2006 to October 2010, 10 field samples were taken with the aid of a hand net, head torch and paddle (Oliveira et al., 2012). A total of 357 *Potamotrygon wallacei* stingrays (28 newborns, 10 young, 76 subadults and 243 adults, with 129 adult males and 114 adult females, of which 29 were pregnant), 65 *P. motoro* stingrays (3 newborns, 48 young, 3 subadults and 11 adults, with 8 adult males and 3 adult females, of which one was pregnant) and 48 *P. aiereba* stingrays (9 newborns, 16 young, 20 subadults and 3 adults, with one adult male and two adult females, of which one was pregnant) were caught.

After the stingrays had been caught, they were immediately anesthetized with eugenol (0.2 g/L). The handling and blood collection procedures followed the recommendations of Oliveira et al. (2012). The anticoagulant used for blood collection was 10% EDTA, in accordance with the recommendations of Oliveira et al. (2015b). The fish were classified according to sexual dimorphism, and females were distinguished according to presence or absence of pregnancy. The size class was determined according to the disc width (DW). For *Potamotrygon wallacei*, the classification (newborns, DW $\leq$ 9.0 cm; young, 9.0 < DW $\leq$ 12.0 cm; subadults, 12.0 < DW < 16.0 cm; and adults, DW $\geq$ 16.0 cm) followed the recommendations of Araújo (1998). For *P. motoro* stingrays (newborns, DW $\leq$ 14.0 cm; young, 14.1 < DW $\leq$ 35.0 cm; subadults 35 < DW $\leq$ 40; and adults, DW > 40.0 cm), the classification followed the recommendations of Araújo (1999). For *P. aiereba* stingrays (newborns, DW $\leq$ 22.0 cm; young, 22.0 < DW $\leq$ 30.0 cm; subadults, 30.0 < DW $\leq$ 51.0 cm; and adults, DW > 51.0 cm), the classification followed the recommendations of Araújo (2011).

The life stage classification (newborns, young, subadults and adults) was determined from the stage of maturation of the reproductive organs (male and female), in association with the disc width. For this assessment, on *Potamotrygon wallacei* (Araújo, 1998), *P. motoro* (Araújo, 1999) and *P. aiereba* (Araújo, 2011), several fish were sacrificed for morphological determinations and for classification of the reproductive characteristics.

Pregnant females were identified in accordance with the recommendations of Araújo (1998). Sexual dimorphism was established through macroscopic observation, using clasper presence (males) or absence (females). After all the procedures had been completed and recovery from the anesthesia had occurred, all the stingrays were returned to their capture sites.

2.4. Blood parameters and analysis methods

The blood collected was divided into two aliquots: one for determining red blood parameters, leukocyte counts and thrombocyte counts and the other for obtaining plasma and subsequently performing assays on biochemical constituents. Erythrocyte counts (RBC) were conducted in a Neubauer chamber after dilution in formalin-citrate solution; hematocrit (Ht) was determined using the microhematocrit method; and the hemoglobin (Hb) concentration was found using the cyanmethemoglobin method. Through these data, the following red cell indexes were calculated: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Blood smears were prepared and stained following the recommendations of Oliveira et al. (2015a). Subsequently, these were used for leukocyte and total thrombocyte counts (Tavares-Dias and Moraes, 2006), and for leukocyte differential counts, which were based on the counts of 200 leukocyte types of interest.

Plasma was obtained after centrifugation at 750 G. It was then frozen in liquid nitrogen (−86 °C) until the time of the biochemical analyses. Glucose, triglycerides, total cholesterol, total protein and urea concentrations were determined by means of enzyme-colorimetric methods, with quantification using commercial kits (Doles, GO, Brazil) that were specific for each parameter. Sodium (Na^+) and potassium (K^+) ion assays were analyzed by means of flame photometry (Micronal B462, Brazil) and chloride levels (Cl^-) were analyzed by means of a colorimetric method using a commercial kit (Doles, GO, Brazil).

2.5. Statistical analysis

In order to exclude outliers, the steam and life statistical test was used. Subsequently, a normality test was also used to investigate whether the data showed normal distribution. When the data showed normality, analysis of variance (ANOVA) was applied to compare the following groups: newborns, young, subadults, adult males, adult females and pregnant females. When data did not show normality, the Mann-Whitney test was used. After division according to sexual dimorphism, pregnancy and development stage, these parameters were statistically compared between the three species investigated using ANOVA or the Kruskal-Wallis (KW) test. Statistical results were expressed as mean and standard deviation (SD), and all statistical analyses were considered significant when $p < 0.05$.

3. Results

The mean and standard deviation values from biometric measurements on *Potamotrygon wallacei*, *P. motoro* and *P. aiereba* newborns, young, subadults, adult males, adult females and pregnant females are shown in Table 1.

Table 1
Mean ± standard deviation values for biometric parameters of *P. wallacei* (cururu stingray), *P. motoro* and *P. aiereba*, among newborn, young, subadult and adult (male, female and pregnant) specimens in the middle Rio Negro, Amazonas, Brazil.

Biometric parameters of *P. wallacei*	Newborns n = 28	Young n = 10	Subadults n = 76	Adults n = 243		
				Males n = 129	Females n = 85	Pregnant females n = 29
Disc width (cm)	8.1 ± 0.8	11.2 ± 0.7	14.4 ± 0.9	19.5 ± 2.5	20.3 ± 3.1	22.6 ± 3.1
Total length (cm)	15.8 ± 1.9	19.4 ± 1.4	25.0 ± 2.1	31.8 ± 3.7	32.6 ± 5.2	35.6 ± 3.8
Weight (g)	35.3 ± 10.8	79.1 ± 13.2	154.7 ± 52.3	418.6 ± 157.9	493.7 ± 268.2	625.3 ± 311.8
Biometric parameters of *P. motoro*	Newborns n = 3	Young n = 48	Subadults n = 3	Adults n = 11 Males n = 8	Females n = 2	Pregnant females n = 1
Disc width (cm)	12.9 ± 1.6	23.9 ± 4.8	38.0 ± 1.7	48.3 ± 2.8	46.5 ± 2.1	48.0
Total length (cm)	25.7 ± 4.0	41.3 ± 8.4	57.7 ± 11.6	79.0 ± 4.5	76.0 ± 8.5	82.0
Weight (g)	131.7 ± 57.7	689.0 ± 444.5	2693.3 ± 633.2	5852.5 ± 744.7	4950.0 ± 777.8	5500.0
Biometric parameters of *P. aiereba*	Newborns n = 9	Young n = 16	Subadults n = 20	Adults n = 3 Males n = 1	Females n = 1	Pregnant females n = 1
Disc width (cm)	19.2 ± 2.3	26.0 ± 2.3	35.7 ± 5.4	52.0	82.5	75.0
Total length (cm)	31.9 ± 6.3	41.5 ± 5.6	99.6 ± 226.5	72.0	105.0	105.0
Weight (g)	428.6 ± 298.6	760.8 ± 209.4	1885.2 ± 1824.5	8.0	30.0	18 760.0

The hematological parameters of *Potamotrygon wallacei* stingrays are shown in Table 2. Lower Ht values were observed in newborns, while higher Ht values were observed in young individuals. Hb, MCV and MCHC values were lower in newborns, while glucose and triglyceride values were lower in young individuals. For cholesterol, values were higher in newborn specimens, and urea values were high in young individuals. For ionic compounds (Cl^-, Na^+ and K^+), there was similarity in all stages. Leukograms had lower values for newborns, while thrombograms showed similarity between stages.

The hematological parameters of *P. motoro* and *P. aiereba* are shown in Table 3 and Table 4, respectively. It could be seen that there was similarity in most of the parameters analyzed. Through these results, further *P. motoro* and *P. aiereba* studies should examine whether the size of the fish is what promotes hematological changes.

For *P. motoro* (Table 3) and *P. aiereba* (Table 4), blood counts and blood chemistry showed little variation between different fish development stages, thus indicating that these variables are not appropriate for distinguishing these life stages. The results from hematological comparisons between species are shown in Table 5, in which statistical differences can be seen between newborn, young, subadult and adult male individuals, especially regarding red blood parameters.

Table 2
Hematological comparison of development stage, sexual dimorphism and pregnancy of *P. wallacei* stingrays in the middle Rio Negro, Amazonas, Brazil (ANOVA).

Parameters	Newborns	Young	Subadults	Adults		
				Males	Females	Pregnant females
Ht (%)	14.6 ± 3.7^{a}	29.0 ± 1.9^{b}	23.7 ± 5.3^{c}	23.2 ± 5.2^{c}	23.2 ± 4.5^{c}	23.9 ± 5.5^{bc}
Hb (g/dL)	2.2 ± 0.7^{a}	4.0 ± 0.9^{b}	4.3 ± 0.8^{b}	4.5 ± 1.0^{b}	4.4 ± 1.1^{b}	4.7 ± 0.7^{b}
RBC (million/μL)	0.3 ± 0.1^{a}	0.4 ± 0.1^{ab}	0.4 ± 0.1^{bc}	0.4 ± 0.1^{bc}	0.4 ± 0.1^{ac}	0.4 ± 0.1^{ac}
MCV (fL)	420.4 ± 83.1^{a}	501.0 ± 197.5^{ab}	552.1 ± 153.7^{b}	573.6 ± 170.9^{b}	583.8 ± 139.0^{b}	548.2 ± 122.7^{b}
MCH (pg)	62.8 ± 14.9^{a}	73.0 ± 12.4^{ac}	97.8 ± 24.4^{bc}	102.1 ± 24.6^{b}	108.8 ± 31.7^{b}	104.1 ± 26.6^{b}
MCHC (g/dL)	15.4 ± 4.3^{a}	21.1 ± 9.5^{b}	18.4 ± 4.1^{b}	19.6 ± 4.6^{b}	19.1 ± 4.5^{b}	18.4 ± 2.6^{ab}
Glucose (g/dL)	41.2 ± 9.0^{a}	20.4 ± 5.4^{b}	32.4 ± 9.6^{c}	31.7 ± 9.8^{c}	30.0 ± 13.1^{bc}	30.0 ± 10.6^{bc}
Triglycerides (mM/L)	62.0 ± 21.1^{a}	30.8 ± 13.4^{b}	56.8 ± 17.0^{a}	60.5 ± 19.9^{a}	63.7 ± 27.5^{a}	67.5 ± 20.7^{a}
Cholesterol (mM/L)	76.6 ± 22.8^{a}	32.6 ± 6.6^{b}	50.9 ± 21.6^{bc}	57.4 ± 23.5^{c}	49.2 ± 22.6^{bc}	48.7 ± 19.6^{bc}
Total protein (g/dL)	0.8 ± 0.3^{a}	1.0 ± 0.1^{abc}	1.0 ± 0.3^{ac}	1.2 ± 0.3^{b}	1.1 ± 0.4^{bc}	1.4 ± 0.4^{b}
Urea (mM/L)	1.6 ± 0.6^{a}	3.1 ± 0.2^{b}	1.4 ± 0.7^{a}	1.3 ± 0.4^{a}	1.4 ± 0.6^{a}	1.1 ± 0.2^{a}
Cl^- (mM/L)	125.5 ± 10.6^{a}	139.6 ± 2.5^{a}	126.6 ± 20.3^{a}	124.4 ± 19.6^{a}	126.8 ± 21.6^{a}	122.0 ± 16.8^{a}
Na^+ (mEq/L)	135.7 ± 14.2^{a}	137.6 ± 9.3^{a}	139.9 ± 16.8^{a}	143.1 ± 19.8^{a}	144.6 ± 22.8^{a}	148.1 ± 20.0^{a}
K^+ (mEq/L)	8.8 ± 1.4^{a}	7.8 ± 0.2^{a}	10.1 ± 1.2^{a}	9.4 ± 2.2^{a}	9.0 ± 2.9^{a}	9.0 ± 1.7^{a}
Leukocytes (μL)	2763.0 ± 526.7^{a}	3112.7 ± 231.6^{ab}	5925.0 ± 3515.5^{b}	3459.2 ± 1630.2^{a}	3114.3 ± 5041.6^{a}	3823.9 ± 3041.7^{ab}
Thrombocytes (μL)	564.0 ± 288.4^{a}	1406.5 ± 60.8^{a}	791.3 ± 637.6^{a}	822.8 ± 669.1^{a}	863.0 ± 523.8^{a}	774.3 ± 694.7^{a}
Lymphocytes (%)	45.8 ± 9.8^{a}	44.5 ± 3.9^{a}	43.4 ± 15.0^{a}	44.1 ± 15.8^{a}	41.0 ± 16.9^{a}	37.3 ± 12.7^{a}
Lymphocytes (μL)	1240.8 ± 425.4^{a}	1386.7 ± 358.0^{a}	2342.9 ± 1662.4^{a}	1688.6 ± 985.7^{a}	2119.5 ± 1757.6^{a}	1452.6 ± 1279.5^{a}
Monocytes (%)	27.1 ± 3.5^{a}	22.4 ± 8.3^{a}	30.3 ± 8.8^{a}	29.7 ± 14.7^{a}	30.4 ± 11.6^{a}	36.9 ± 9.2^{a}
Monocytes (μL)	748.3 ± 221.2^{a}	894.7 ± 14.1^{ab}	1882.8 ± 1163.4^{b}	1205.2 ± 847.7^{a}	698.6 ± 379.5^{a}	1538.0 ± 1214.5^{ab}
Heterophils (%)	23.8 ± 12.9^{a}	28.5 ± 7.5^{a}	21.0 ± 11.9^{a}	19.1 ± 9.8^{a}	20.0 ± 10.7^{a}	23.8 ± 9.9^{a}
Heterophils (μL)	761.1 ± 152.3^{ab}	656.6 ± 15.7^{ab}	1028.5 ± 516.5^{b}	688.2 ± 363.9^{a}	615.8 ± 277.3^{a}	908.2 ± 731.7^{ab}
Basophils (%)	3.0 ± 0.8^{ab}	4.6 ± 2.0^{ab}	4.2 ± 3.4^{a}	2.4 ± 2.2^{b}	2.6 ± 2.7^{ab}	3.0 ± 2.0^{ab}
Basophils (μL)	136.1 ± 131.0^{ab}	114.2 ± 17.2^{ab}	244.1 ± 216.2^{a}	111.1 ± 117.3^{a}	79.3 ± 95.8^{a}	124.8 ± 126.9^{ab}

Different letters in the same line mean statistical differences.

Table 3
Hematological comparison of development stage, sexual dimorphism and pregnancy of *P. motoro* stingrays in the middle Rio Negro, Amazonas, Brazil (Kruskal-Wallis test).

Parameters	Newborns	Young	Subadults	Adults		
				Males	Females	Pregnant females
Ht (%)	18.0 ± 0.0[a]	21.3 ± 2.0[a]	20.0 ± 5.3[a]	21.2 ± 4.2[a]	20.0 ± 2.8[a]	18.0[a]
Hb (g/dL)	2.6 ± 0.5[a]	3.9 ± 0.7[a]	3.7 ± 1.5[a]	3.5 ± 1.0[a]	4.1 ± 1.8[a]	2.9[a]
RBC (million/μL)	0.3 ± 0.5[ade]	0.4 ± 0.1[bc]	0.3 ± 0.3[ace]	0.3 ± 0.3[de]	0.3[e]	0.3[e]
MCV (fL)	709.1 ± 94.5[a]	484.9 ± 98.2[a]	569.7 ± 141.0[a]	757.5 ± 179.6[a]	474.2 ± 124.8[a]	562.5[a]
MCH (pg)	101.5 ± 6.3[ac]	88.6 ± 16.3[a]	104.3 ± 39.9[ac]	121.4 ± 26.5[c]	91.8 ± 3.4[ac]	89.4[ac]
MCHC (g/dL)	14.5 ± 2.6[a]	18.6 ± 2.8[a]	17.9 ± 3.8[a]	16.4 ± 3.6[a]	20.1 ± 6.0[a]	15.9[a]
Glucose (g/dL)	31.9 ± 16.2[a]	34.6 ± 9.6[a]	32.0 ± 10.1[a]	28.9 ± 10.2[a]	20.1 ± 8.7[a]	13.9[a]
Triglycerides (mM/L)	58.7 ± 4.8[a]	60.8 ± 20.7[a]	82.0 ± 37.0[a]	81.4 ± 20.5[a]	75.9 ± 8.1[a]	70.2[a]
Cholesterol (mM/L)	69.8 ± 6.3[a]	51.6 ± 23.5[a]	37.8 ± 5.6[a]	69.0 ± 40.4[a]	84.8 ± 7.7[a]	79.4[a]
Total protein (g/dL)	0.8 ± 0.4[a]	1.0 ± 0.3[a]	0.7 ± 0.1[a]	1.3 ± 0.4[a]	1.5 ± 0.4[a]	1.2[a]
Urea (mM/L)	6.7 ± 1.7[a]	1.6 ± 0.5[b]	1.0 ± 0.5[a]	2.1 ± 0.6	0.9 ± 0.8	0.4
Cl^- (mM/L)	122.2 ± 13.0[a]	119.1 ± 10.7[a]	124.3 ± 0.2	113.7 ± 4.0	128.8 ± 3.2	125.0
Na^+ (mEq/L)	126.1 ± 22.3[a]	137.3 ± 17.2[a]	134.0 ± 7.7[a]	141.4 ± 7.0[a]	134.0[a]	116.3[a]
K^+ (mEq/L)	11.6[a]	9.6 ± 1.6[a]	9.9 ± 0.9[b]	8.5 ± 0.9[b]	8.0[b]	7.6[b]
Leukocytes (μL)	3055.0[a]	2908.2 ± 617.3[a]	8140.0 ± 8923.7[a]	2520.0 ± 499.9[a]	2500.0[a]	4000.0[a]
Thrombocytes (μL)	940.0[a]	816.0 ± 621.2[a]	850.0 ± 1202.1[a]	800.0 ± 507.8[a]	1200.0[a]	500.0[a]
Lymphocytes (%)	50.0[a]	42.5 ± 11.8[a]	43.2 ± 25.8[a]	45.6 ± 10.9[a]	59.0[a]	50.0[a]
Lymphocytes (μL)	1527.5[a]	1700.2 ± 1262.1[a]	457.5[a]	1078.4 ± 399.5[a]	1475.0[a]	2000.0[a]
Monocytes (%)	28.0[a]	27.8 ± 8.0[a]	26.5 ± 2.1[a]	26.2 ± 4.3[a]	30.0[a]	20.0[a]
Monocytes (μL)	855.4[a]	1367.8 ± 872.1[a]	2251.7 ± 2537.4[a]	649.6 ± 224.7[a]	750.0[a]	800.0[a]
Heterophils (%)	20.0[a]	23.7 ± 10.0[a]	28.0 ± 31.1[a]	26.6 ± 15.5[a]	8.0[a]	35.0[a]
Heterophils (μL)	611.0[a]	860.9 ± 416.6[a]	891.0 ± 33.9[a]	742.1 ± 128.2[a]	200.0[a]	1400.0[a]
Basophils (%)	2.0[a]	4.2 ± 3.0[a]	2.2 ± 3.2[a]	2.7 ± 0.6[a]	3.0[a]	5.0[a]
Basophils (μL)	61.1[a]	181.0 ± 178.0[a]	325.1 ± 459.8[a]	98.4 ± 143.0[a]	75.0[a]	200.0[a]

Different letters in the same line mean statistical differences.

Table 4
Hematological comparison of development stage, sexual dimorphism and pregnancy of *P. aiereba* stingrays in the middle Rio Negro, Amazonas, Brazil (Kruskal-Wallis test).

Parameters	Newborns	Young	Subadults	Adults		
				Males	Females	Pregnant females
Ht (%)	23.0 ± 4.4[a]	27.4 ± 3.1[a]	25.7 ± 4.9[a]	32.0[a]	18.0[a]	22.0[a]
Hb (g/dL)	4.5 ± 1.9[a]	3.8 ± 0.8[a]	3.8 ± 0.6[a]	4.9[a]	2.8[a]	5.4[a]
RBC (million/μL)	0.4 ± 0.1[a]	0.4 ± 0.1[a]	0.4 ± 0.1[a]	0.39[a]	0.4[a]	0.5[a]
MCV (fL)	642.8 ± 93.2[a]	753.0 ± 193.3[a]	734.5 ± 167.1[a]	820.5[a]	439.0[a]	458.3[a]
MCH (pg)	110.3 ± 53.1[a]	110.9 ± 29.5[a]	107.6 ± 36.6[a]	125.6[a]	67.6[a]	112.5[a]
MCHC (g/dL)	19.4 ± 6.3[a]	14.3 ± 3.0[b]	14.0 ± 2.6[b]	15.3[ab]	15.4[ab]	24.5[ab]
Glucose (g/dL)	13.0 ± 3.7[a]	17.2 ± 6.5[a]	17.6 ± 3.7[a]	27.6[a]	13.5[a]	26.9[a]
Triglycerides (mM/L)	87.5 ± 43.8[a]	105.4 ± 57.6[a]	104.9 ± 45.8[a]	118.0[a]	55.0[a]	126.6[a]
Cholesterol (mM/L)	72.6 ± 34.4[a]	63.6 ± 15.8[a]	56.7 ± 21.5[a]	72.1[a]	25.8[a]	56.3[a]
Total protein (g/dL)	1.4 ± 0.6[a]	1.3 ± 0.2[a]	1.5 ± 0.1[a]	2.0[ac]	1.3[a]	2.7[c]
Urea (mM/L)	4.8 ± 2.5[a]	4.3 ± 2.9[a]	4.3 ± 3.3[a]	1.6[a]	1.4[a]	2.8[a]
Cl^- (mM/L)	124.2 ± 10.0[a]	122.4 ± 0.7[a]	114.5 ± 15.6[a]	136.3[a]	130.1[a]	149.0[a]
Na^+ (mEq/L)	135.1 ± 14.1[a]	144.3 ± 2.9[a]	136.4 ± 16.3[a]	119.8[a]	132.0[a]	117.1[a]
K^+ (mEq/L)	8.7 ± 1.2[a]	9.2 ± 1.7[a]	9.6 ± 1.5[a]	9.9[a]	7.0[a]	7.9[a]
Leukocytes (μL)	2970.0 ± 479.9[a]	2696.7 ± 545.7[a]	2859.5 ± 765.3[a]	4515.0[a]	3000.0[a]	2228.0[a]
Thrombocytes (μL)	636.0 ± 280.9[a]	610.0 ± 367.6[a]	700.0 ± 461.0[a]	2150.0[b]	1040.0[ab]	1418.0[ab]
Lymphocytes (%)	48.1 ± 10.2[a]	47.0 ± 9.8[a]	44.5 ± 8.8[a]	45.0[a]	52.0[a]	51.0[a]
Lymphocytes (μL)	1447.3 ± 138.8[a]	1228.8 ± 482.0[a]	1220.9 ± 442.0[a]	2031.7[a]	1560.0[a]	1136.3[a]
Monocytes (%)	29.0 ± 1.1[a]	28.3 ± 4.6[a]	26.8 ± 4.5[a]	30.5[a]	26.0[a]	17.0[a]
Monocytes (μL)	878.3 ± 39.6[a]	733.3 ± 243.1[a]	789.9 ± 2877.4[a]	1377.1[a]	780.0[a]	378.8[a]
Heterophils (%)	20.0 ± 11.9[a]	21.7 ± 13.6[a]	23.2 ± 13.2[a]	16.5[a]	20.0[a]	26.0[a]
Heterophils (μL)	652.4 ± 39.7[a]	899.4 ± 1.2[a]	760.1 ± 212.0[a]	745.0[a]	600.0[a]	579.3[a]
Basophils (%)	2.8 ± 0.7[a]	3.4 ± 0.8[a]	3.5 ± 1.1[a]	8.0[b]	2.0[a]	6.0[ab]
Basophils (μL)	159.9 ± 141.2[a]	190.9 ± 147.5[a]	177.7 ± 179.8[a]	361.2[a]	60.0[a]	133.7[a]

Different letters in the same line mean statistical differences.

4. Discussion

In the biometric analysis on the development stages of *P. wallacei*, weight gains (doubling of weight) from newborn to young and from young to subadult were observed. Although the disc width values were similar between adult males and adult females of *P. wallacei*, body mass was greater in females. In addition, pregnant females had a mean disc width of

Table 5
Hematological comparison of *P. wallacei*, *P. motoro* and *P. aiereba* stingrays caught in the middle Rio Negro, Amazonas, Brazil (ANOVA).

Parameters	Newborns			Young			Subadults			Male adults			Female adults			Pregnant females		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Ht (%)	a	ab	b	a	b	a	a	a	a	a	a	a	a	a	a	a	a	a
Hb (g/dL)	a	a	b	a	a	a	a	ab	b	a	b	ab	a	a	a	a	a	a
RBC (million/μL)	a	a	a	a	a	a	a	ab	b	a	b	ab	a	a	a	a	a	a
MCV (fL)	a	b	b	a	a	b	a	ab	b	a	b	ab	a	a	a	a	a	a
MCH (g/dL)	a	ab	a	a	b	c	a	a	a	a	a	a	a	a	a	a	a	a
MCHC (%)	a	a	a	a	a	b	a	ab	b	a	a	a	a	a	a	a	a	a
Glucose (g/dL)	a	a	b	a	b	a	a	ab	b	a	a	a	a	a	a	a	a	a
Triglycerides (mM/L)	a	a	a	a	a	b	a	ab	b	a	b	b	a	a	a	a	ab	b
Cholesterol (mM/L)	a	a	a	a	ab	b	a	a	a	a	a	a	a	a	a	a	a	a
Total protein (g/dL)	a	ab	b	ab	a	b	a	a	b	a	ab	b	a	a	a	a	a	b
Urea (mM/L)	a	b	b	ab	a	b	a	a	b	a	b	ab	a	a	a	a	b	c
Cl^- (mM/L)	a	a	a	a	b	b	a	a	a	a	a	a	a	a	a	a	a	a
Na^+ (mEq/L)	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
K^+ (mEq/L)	a	a	a	a	b	ab	a	a	a	a	a	a	a	a	a	a	a	a
Leukocytes (μL)	a	a	a	a	a	a	a	ab	b	a	a	a	a	a	a	a	a	a
Thrombocytes (μL)	a	a	a	a	b	b	a	a	a	a	a	a	a	a	a	a	a	a
Lymphocytes (%)	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Lymphocytes (μL)	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Monocytes (%)	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Monocytes (μL)	a	a	a	a	a	a	a	ab	b	a	a	a	a	a	a	a	a	a
Heterophils (%)	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Heterophils (μL)	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Basophils (%)	a	a	a	a	a	a	a	a	a	a	ab	b	a	a	a	a	a	a
Basophils (μL)	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a

1: *Potamotrygon wallacei*; 2: *Potamotrygon motoro;* 3: *Paratrygon aiereba.*
Different letters in the same line mean statistical differences.

22.57 cm and the highest mean weight (625.35 g) of all the classes studied. The values for the biometric data on this species were much lower than that those of the four freshwater stingray species studied by Brito et al. (2015).

In *P. motoro*, there was considerable differentiation of the biometric data during the development stages, such that young individuals had twice the disc width of newborns. In a study conducted by Brito et al. (2015), biometric comparisons was made between *Potamotrygon scobina, Potamotrygon orbignyi, Potamotrygon falkneri* and *Potamotrygon motoro*. There was a statistical difference in that study, such that biometrics could differentiate between adult individuals of *P. scobina* and *P. orbignyi*. On the other hand, *P. falkneri* and *P. motoro* showed similar values to those of subadults in the present study.

In *P. aiereba*, newborn individuals had the same biometrics as adult *P. wallacei* individuals, and *P. aiereba* adults had biometrics two to five time higher than those of adults of *P. motoro* in the present study. It is important to highlight the low number of newborn, subadult, adult male, adult female and pregnant individuals of *P. motoro* in the sample, and also the low number of adult individuals of *P. aiereba*, which can be attributed to the limitations of the fishing gear used in this study.

Some elasmobranchs such as freshwater stingrays have lower erythrocyte counts in their blood than do teleost fish. However, these cells are two to three times larger than those of teleost fish (Saunders, 1966; Wilhelm Filho et al., 1992; Luer et al., 2004). Moreover, the hemoglobin levels of *Potamotrygon* species are higher than those of marine stingrays (Wilhelm Filho et al., 1992; Brito et al., 2015). This pattern was also observed in the present study, thus corroborating other studies that reported that the stingray species studied had fewer total erythrocytes and lower Hb concentrations and hemoglobin in red blood cells (MCHC), compared with teleosts, thus resulting in low blood viscosity (hemodilution). Furthermore, the higher erythrocyte counts in teleosts are indicated by elevated MCV. These results can be attributed to the fact that freshwater stingrays are more sedentary than sharks and teleost fish (Wilhelm Filho et al., 1992) and thus present a lower metabolic rate.

Hematological differences influenced by sex or pregnancy were not observed in the three potamotrygonid species investigated. This characteristic was observed by Oliveira (2008) for *P. wallacei* stingrays and the shark species *Scyliorhinus canicula* (Tudor, 1984). In *P. falkeri* and *P. motoro* stingrays in the Paraná River, municipality of Londrina, Paraná, Brazil, and in *P. orbignyi* and *P. scobina* in the Piririm river, Macapá, Amapá, Brazil, there were no statistical differences between the species studied (Brito et al., 2015).

For *P. wallacei*, the red blood parameters showed that the Ht of newborns was lower than that of other development stages, which was confirmed by the lower number of red blood cells and lower MCV shown in this study. For *P. motoro* and *P. aiereba*, the red blood parameters did not differ between the development stages. The adjustments to cururu stingray red blood parameters are probably due to the habitat that they prefer, i.e. creeks ("igapós") in the floodplain forests, which present low oxygen levels. However, size may be a determining factor in this differentiation, because cururu stingray newborns are smaller than *P. motoro* and *P. aiereba* newborns. In general, subadult and adult *P. wallacei* individuals, as well as *P. motoro* and *P. aiereba* individuals (at all stages), showed similar Ht, RBC and MCV values to those found in studies conducted by Brinn et al. (2012), who investigated the cururu stingray.

In *P. wallacei* newborns, the plasma biochemistry, glucose, triglyceride and total protein levels were higher than those shown by the young, subadults and adults. In this study, these variations were attributed to yolk presence during this development stage (Araújo, 1998; Oliveira, 2008). In *P. motoro* and *P. aiereba*, plasma biochemistry variables did not vary over the course of development, although ontogenetic variation in the diet also occurs in these species. *P. scobina* glucose values (Brito et al., 2015) had the closest values to those found for the species investigated in this study. Cururu stingray triglyceride levels were similar to those of *P. falkneri* (Brito et al., 2015), and the total protein values of the three species in this study were much lower than those of the four species studied by Brito et al. (2015). The ionic compound profiles were similar between the development stages in the three species investigated, thus showing similar results to those from *P. aiereba*, which is also from the middle Rio Negro, Amazonas (Duncan et al., 2009).

From analysis on thrombocyte and leukocyte counts, a very conservative pattern was observed throughout development, in all three species studied. These results were different from those shown by Oliveira (2008), who described changes to some leukogram variables, attributing the differentiation to the loss of parental care at the stage that was described as young. However, there was a low number of samples in that study. In *P. wallacei, P. motoro* and *P. aiereba* in the middle Rio Negro, Amazonas, no neutrophils and eosinophils were found, thus differing from investigations conducted by Brito et al. (2015). Moreover, lymphocytes were the predominant cells in Rio Negro stingrays, although neutrophils predominated in Paraná and Piririm river stingrays (Brito et al., 2015).

In analyzing the blood profile of *P. wallacei* stingrays, it was noteworthy that the development stage should be considered to be a separation criterion, especially between newborn and young individuals. On the other hand, the blood profiles of *P. motoro* and *P. aiereba* during development were not seen to be separation criteria.

In comparative analysis between the three species investigated, newborn individuals of cururu stingrays had lower Ht values than *P. motoro* and *P. aiereba*. This characteristic shows that this variable was different between the species due to MCV variations. Therefore, it is possible to infer that cururu stingray newborns are less active than *P. motoro* and *P. aiereba*, but have a higher capacity for oxygen transport.

In young *P. motoro* individuals, lower Ht values were observed than in *P. wallacei* and *P. aiereba*. However, this change was not explained by RBC and MCV values. In subadults of *P. wallacei*, there were higher Hb, RBC, MCV and MCHC levels, thus demonstrating that cururu stingray adults have higher capacity to carry oxygen than does *P. aiereba*.

Because of the low number of samples of *P. aiereba* individuals, the comparative analysis including them should be interpreted with caution. In this study, it was shown that cururu stingrays have greater capacity to carry oxygen than does *P. motoro*. Among adult females and pregnant females, no red blood series differences were observed between the three stingray species investigated. In general, when adult individuals were compared, the results from this study corroborated the findings of Oliveira (2008), Duncan et al. (2009), Brinn et al. (2012), Oliveira (2013) and Brito et al. (2015), in which various species in the Potamotrygonidae family of stingrays were investigated.

With regard to plasma biochemistry variables, differences were observed in *P. aiereba*, which showed lower glucose levels, higher protein levels and lower urea levels than *P. wallacei* and *P. motoro*. In *P. aiereba* subadults, differences from other stingrays were observed, since the glucose levels were low and triglyceride levels were high. Furthermore, there were differences in total protein and urea levels. In general, similarities between the three species investigated were observed among adults. The possible differences probably originated from different diets between the species, given that *P. wallacei* is a generalist species, *P. motoro* is a species that prefers crustaceans and *P. aiereba* prefers small teleosts (Shibuya et al., 2009).

In newborn and young individuals, the leukograms and thrombograms did not differ between the three species investigated, thus demonstrating a conservative characteristic in relation to the immune system in these development stages. Such results were also described by Oliveira (2008). In subadult individuals, differences between the three stingray species were observed for the leukogram variable, in which the cururu stingray showed higher values than those found in *P. aiereba*. In addition, no differences were observed among *P. wallacei*, *P. motoro* and *P. aiereba* stingrays in the adult stage, including male, female and pregnant individuals.

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