Ecophysiological interactions and water-related physicochemical parameters among freshwater stingrays


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(With 5 figures)

Abstract

The objective of this study was to compare and correlate the ecology of neonates and young individuals of Potamotrygon wallacei, Potamotrygon motoro and Paratrygon aiereba with regard to their hematological profile and the physicochemical parameters of the water that they inhabit. Principal component analysis (PCA) on the complete blood count revealed total variation of 72.92%, thus demonstrating a differentiation system for oxygen demand. On the other hand, P. motoro was considered to be an intermediate species, given that its complete blood count characteristics interacted with both P. wallacei and with P. aiereba. The interaction among the biochemical variables was shown to total 64.67% of the factors. This allowed differentiation of P. wallacei from P. aiereba, while P. motoro maintained an intermediate position. These characteristics of differentiation within the preferred environment corroborate the PCA of the present study and confirm that these species can be differentiated through considering the complete blood count and biochemical parameters. The PCA on water properties showed 68.57% differentiation, mainly comprising the x axis (49.44%). It can be affirmed that P. motoro has the capacity to inhabit the preferential areas of P. wallacei and P. aiereba, as well as occupying localities in which other stingrays are not found. In conclusion, P. wallacei presents patterns differentiating it from P. aiereba, while P. motoro is a species that presents intermediate characteristics. The latter can be considered to be a more broadly distributed species regarding its ecophysiological characteristics.

Keywords: hematology, potamotrygonids, physiology, differences, ecology.

Interações ecofisiológicas e dos parâmetros físico-químico da água em arraias de água doce

Resumo

Este trabalho tem por objetivo investigar o perfil hematológico e os parâmetros físico-químicos da água, comparando e correlacionando ecologicamente entre neonatos e jovens de Potamotrygon wallacei (arraia cururu), Potamotrygon motoro e Paratrygon aiereba. A análise de componentes principais (PCA) do hemograma revelou um total 72,92% de variação, constituindo-se em um sistema de diferenciação na demanda por oxigênio. P. wallacei apresenta diferenciação no eixo X quando comparada a P. aiereba, por outro lado P. motoro constitui-se como uma espécie intermediária que apresenta as características do hemograma interagindo tanto com P. wallacei quanto com P. aiereba. A interação entre as variáveis bioquímica demonstram um total de 64,67% dos fatores, no qual foi possível diferenciar, a arraia P. wallacei
de *P. aiereba*, tending *P. motoro* a different aspect of species intermediate among the others. These aspects of differentiation of environment of preference corroborated a PCA obtained in the present study and confirm that these species can be differentiated when considering the variables referent to the hemogram and biochemistry. No ions, in the trombogram and in the leucogram, was found to be possible to differentiate the species. The PCA of the water properties constituted by 69.57% of differentiation that constituted principally in the eixo x (49.44%). It is possible to confirm that *P. motoro* has the capacity to habituate such areas of preference of *P. wallacei* and *P. aiereba*, although not the latter is found in the same area. On the other hand, male and female adults are isolated in different areas (Charvet-Almeida et al., 2005). This species presents widespread distribution across the Amazon basin and is predominantly exploited by commercial fisheries. The preferential habitat of this species is beach regions, with low intensity of water currents (personal observation). Moreover, its preferential feeding item is small teleosts (Shibuya et al., 2009).

Despite these differences in most of the ecological and biological characteristics, there have been no investigations on the interactions and differences in physiological traits of these species, or on the physicochemical characteristics of the water in which they naturally occur. The objective of the present study was to compare and correlate the ecology of neonates and young individuals of *Potamotrygon wallacei*, *Potamotrygon motoro* and *Paratrygon aiereba* regarding their physiological profile (complete blood count, plasma biochemistry, white blood cell count and trombogram) and the physicochemical parameters of the water that they inhabit.

2. Material and Methods

The Mariuá archipelago is considered to be the largest group of freshwater islands in the world, with a total of approximately 1,600 islands. They provide shelter for rich biodiversity of ornamental fish, including the stingray species *P. wallacei*, *P. motoro* and *P. aiereba*. Between the years 2006 and 2011, a total of 114 specimens were captured with the aid of a hand net (rapiche) and a head torch, with previous authorization (IBAMA License No. 15116-1). After the fish had been caught, they were anesthetized with eugenol (0.2 g/L). The handling and blood sampling procedures followed the recommendations of Oliveira et al. (2012, 2015b).

The size classes were determined based on the disc width (DW). For *P. wallacei*, the classification followed...
the recommendations of Araújo (1998); for *P. motoro*, those of Araújo (1999); and for *P. aiereba*, those of Araújo (2011). After body mass and total length (TL) had been determined, all the stingrays were returned to the locations where they had been caught. The animals were classified as either neonates or young individuals. A total of 38 specimens of *P. wallacei* were caught, 51 of *P. motoro* and 25 of *P. aiereba*.

The blood sampled was divided into two aliquots, one for determining the complete blood count, white blood cell count and thrombogram, and another for obtaining plasma and subsequent assaying of the biochemical components and plasma ions. In the complete blood count, the erythrocyte count (RBC) was determined in a Neubauer chamber, while the hematocrit (Ht) was determined through the microhematocrit method and the hemoglobin concentration (Hb) through the cyanmethemoglobin method. The following hematimetric indices were calculated based on these data: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Blood extensions were prepared and stained in accordance with the recommendations of Oliveira et al. (2015b), and these was used to determine the thrombogram and white blood cell count, from the morphological descriptions of Oliveira et al. (2015b). The extensions were used for the total leukocyte and thrombocyte count (Tavares-Dias and Moraes, 2006), and also for the differential leukocyte count, which was based on counting 200 white blood cell types and then determining the percentage of each cell type that was present.

Plasma was obtained through centrifugation at 750 g, and was then frozen in liquid nitrogen (-86°C) until the time of performing the biochemical analyses. The plasma biochemical variables, such as glucose, triglycerides, total cholesterol, total proteins and urea, were determined through enzymatic-colorimetric methods that were quantified using commercial kits (Doles, GO, Brazil) that were specific for each parameter. Sodium (Na⁺) and potassium (K⁺) ions were assayed through flame photometry (Micronal b462, Brazil). Chloride (Cl⁻) levels were determined through the colorimetric method, using a commercial kit (Doles, GO, Brazil).

The physicochemical properties of the water, such as temperature (°C), pH, conductivity (µS/cm) and dissolved oxygen (mg/L), were determined at the location where the each specimen was caught, using a multiparameter digital device (Orion 5-Star Plus). Water samples were collected, stored in ice and then transported to the laboratory for analyses of other parameters, such as hardness (mg/L), alkalinity (mg/L), total ammonia (mg/L) and nitrite (mg/L), following the methodology described by Boyd and Tucker (1992). Sodium and potassium levels (mEq/L) were analyzed through flame photometry on water samples brought in from the field that had been preserved and refrigerated (Boyd and Tucker, 1992), using a Micronal B 462 device.

To assess the interactions or differences among the hematological characteristics and the physicochemical properties of the water, multivariate exploratory statistics were applied, consisting of principal component analysis (PCA). These analyses were divided into complete blood count (6 variables), plasma biochemistry (5 variables), plasma ions (3 variables), thrombogram and white blood cell count (10 variables) and physicochemical properties of the water (10 variables). Interactions were considered significant when the sum of the X and Y axes was greater than or equal to 60%.

### 3. Results

The DW and body mass results are shown in Table 1 and demonstrate that the *P. wallacei* stingrays were the smallest among the stingrays studied. The PCA on the complete blood counts (Figure 1) of the three species of stingrays studied revealed a total variation of 72.92%. It is possible to observe that *P. wallacei* has a lower index of the red series than the *P. aiereba* stingray. On the other hand *P. motoro* is a kind of intermediate patterns of red series, varied between the two species, but with greater similarity to the species *P. aiereba*.

We can observe in the PCA of Figure 2 (biochemistry plasma) the interaction between species *P. wallacei* and

### Table 1. Mean values ± standard deviation for the biometry of neonate and young *Potamotrygon wallacei*, *Potamotrygon motoro* and *Paratrygon aiereba* stingrays from the midsection of the Negro River, Amazonas, Brazil.

<table>
<thead>
<tr>
<th>Variables</th>
<th><em>P. wallacei</em></th>
<th><em>P. motoro</em></th>
<th><em>P. aiereba</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Disc width (cm)</td>
<td>8.88 ± 1.60</td>
<td>23.30 ± 5.40</td>
<td>23.54 ± 3.99</td>
</tr>
<tr>
<td>Total length (cm)</td>
<td>16.78 ± 2.37</td>
<td>40.37 ± 9.02</td>
<td>38.05 ± 7.41</td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>46.80 ± 22.57</td>
<td>654.90 ± 451.21</td>
<td>641.20 ± 289.19</td>
</tr>
</tbody>
</table>
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*P. motoro*, which differ considerably from *P. aiereba* to the axis X. The ions are represented in Figure 3, for effective examination, it is necessary that the sum of X and Y-axes are higher than 60%. For the ions, these values have not reached the minimum level for the analysis (50.62%). The same applies to figures for the PCA thrombogram and white blood cells (57.43%) (Figure 4).

The data presented in Figure 5 demonstrate the PCA of water properties of the places where the stingrays were caught, showing that there was differentiation of 68.57%, mainly along the X-axis (49.44%). These values indicate that the species *P. motoro* is distributed broadly across the environment, while the species *P. aiereba* interacts more narrowly over the positive portion of the X-axis.

### 4. Discussion

The PCA on the complete blood counts (Figure 1) constitutes a system of differentiated demand for oxygen, given that studies that investigated the complete blood count in freshwater stingrays have correlated the red series with respiratory activity and capacity (Brito et al., 2015; Oliveira et al., 2015c). *P. wallacei* presented clear differentiation in the X axis, in relation to *P. aiereba*. In turn, *P. motoro* was shown to be an intermediate species that presented complete blood count characteristics that interacted both with *P. wallacei* and with *P. aiereba*. Oliveira (2013) reported differences in habitats among these three species, such that *P. wallacei* presented preference for areas of igapós (typical Amazon submerged vegetation), *P. aiereba* preferred beaches and *P. motoro* inhabited areas that were intermediate between the igapós and beaches, which are classified as muddy-bottom areas. These characteristics of differentiation of the preferred environment corroborate the PCA obtained in the present study and confirm that these species can be differentiated with regard to the variables of the complete blood count.
The interaction among the biochemical variables (Figure 2) demonstrated that a total of 64.67% of the factors could be differentiated, with regard to the X axis. The greatest differentiation was between *P. wallacei* and *P. arieeba*, while *P. motoro* demonstrated characteristics closer to those of *P. wallacei*. However, *P. motoro* still presented an intermediate position between the other two species, similar to the results found with the complete blood count, which was associated with the preferential habitat and also the feeding preferences (Shibuya et al., 2009; Oliveira et al., 2015c). Regarding the ion values, the three species presented similarity to each other. This was also reported by Oliveira (2013) and was corroborated by the study by Duncan et al. (2009), considering that the waters of the Negro River present low ion concentrations (Duncan and Fernandes, 2010).

Thus, the immunological systems of the *P. wallacei*, *P. motoro* and *P. arieeba* stingrays cannot be considered to be different. This characteristic was also described by Oliveira et al. (2015c), who affirmed that the systems were conservative in nature. However, according to the data presented (Figure 4), it is clear that although *P. motoro* presents a pattern that is similar to that of the other species, there are also some different features that are not found in the other species. This could be due to adjustments to the immunological system of this species, considering that *P. motoro* is frequently caught in the areas that are preferred by *P. wallacei* and *P. arieeba*.

According to the data obtained (Figure 5), it can be confirmed that *P. motoro* has the capacity to inhabit the areas of *P. wallacei* and *P. arieeba*. This plasticity demonstrates that *P. motoro* tolerates different physicochemical properties in water. The *P. arieeba* species has distribution restriction on the favorable properties of water being present such as sandy soil areas (beaches). The species *P. wallacei* presents distributed areas in which the properties of water are considered unfavorable, this species occurs mainly in areas with litter (biomass).

The present study demonstrates the integration among the biological, ecological and hematological characteristics and water properties of the localities that freshwater stingrays inhabit in the Amazon basin. This makes it possible to conclude that *P. wallacei* presents distribution patterns that are differentiated from those of *P. arieeba*. Moreover, *P. motoro* presents intermediate characteristics between those described for the other species. Thus, *P. motoro* can be considered as a more broadly distributed species regarding its ecophysiological characteristics. This information can be used in investigations regarding species management and conservation.

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**References**


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