Blood Cytochemical Staining of Freshwater Stingray (*Potamotrygon* spp.)

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**Introduction**

Freshwater Stingray (*Potamotrygon* spp.) family live exclusively in fresh water restricted to South America. One of the peculiarities of the species that make up this family is the immense variability of their marking. Each individual fish is unique in coloration and pattern, which make them one of the most popular ornamental fish in the world (1). Cytochemistry for this stingray has never been reported. The aim of this work is to demonstrate the cytochemical nature of the blood cells in the Freshwater Stingray, *Potamotrygon* spp. for future health monitoring purposes.

**Materials and Methods**

Peripheral blood from 4 healthy freshwater stingrays (*Potamotrygon* spp.) was collected from the caudal vein and anti-coagulated with lithium heparin. Cytological details were observed by light microscopic using Wright's-Giemsa stain (WG) and cytochemical staining of leukocytes were determined using commercial kit (Sigma) for leukocyte alkaline phosphatase (ALP), sudan black B (SBB), myeloperoxidase (PO), acid phosphatase (AcP), alpha-naphthyl acetate esterase (ANAE) and periodic acid Schiff (PAS). The dog leukocytes used as a control, were stained in parallel.

**Results and Discussion**

The results indicated that there were 7 types of blood cells in freshwater stingrays, namely, red blood cells, thrombocytes, eosinophils, heterophils, basophils, lymphocytes and monocytes. The morphological characteristics of erythrocytes and thrombocyte were agreed to those reported in other elasmobranch, with larger erythrocyte compared to sharks, teleosts and mammals (2, 3). The granulocytes could not be differentiated by cytochemical stains. The blood cells were best classified by nuclear morphology and cytoplasmic characteristics. The cytochemical staining patterns of blood cell types were shown in Table 1.

It was also noted that despite immediate fixation, all stains that were done 24 hours post-collection, would turn negative. This indicated the rapid intracellular enzymatic degeneration and the inability of stingray cellular fixation by standard procedures. This study provided blood cell information for future reference of this species.

**Table 1. Cytochemical staining patterns of blood cells from *Potamotrygon* spp. (n=4)**

<table>
<thead>
<tr>
<th></th>
<th>SBB</th>
<th>PO</th>
<th>AcP</th>
<th>ALP</th>
<th>ANAE</th>
<th>PAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Thrombocyte</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>C,ir</td>
<td>++</td>
<td>C</td>
</tr>
<tr>
<td>Granulocyte</td>
<td>-</td>
<td>+</td>
<td>C,ir</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>C,ir</td>
<td>C,ir</td>
<td>I</td>
</tr>
<tr>
<td>Monocyte</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++ C</td>
<td>+ C</td>
</tr>
</tbody>
</table>

*Granulocyte: can not differentiate heterophil, eosinophil and basophil by cytochemical staining
+++: strong, ++: medium, +: week, ir: irregular, C: cytoplasm and I: intergranular staining

**References**