**Introduction**

- The morphology and function of leukocytes is not well studied in elasmobranchs.
- Sharks and other cartilaginous fishes are the most primitive type of vertebrates to have both innate and adaptive immunity comparable to that observed in mammals[1].
- Understanding the role of leukocytes in a greater variety of elasmobranchs furthers research in comparative immunology.
- Understanding the makeup of blacktip shark’s blood facilitates comparisons to other organisms and determination of conserved traits between species.
- The findings of this study will further research in husbandry, and aid in the care of captive species and in clinical assessments of wild sharks.
- This study focuses on the morphological characteristics of blacktip shark leukocytes using light, SEM, and TEM microscopy.
- The goal of this study was to determine a baseline set of reference intervals for healthy wild blacktip shark peripheral blood leukocytes, and to determine the range of the granulocyte-lymphocyte ratio in blacktip sharks.

**Methods**

- 22 blacktip sharks were caught off of Biscayne Bay in Miami, Florida, using circle-hook drumline system[21].
- Whole blood was collected for fixation in light, SEM and TEM microscopy.
- Counts of 100 were conducted for WBC in light microscopy.
- Counts of 100 were conducted for WBC in light microscopy.
- Cell morphology was characterized in light, SEM, and TEM.
- Measurements were taken in light microscopy using ZEN.
- Minitab and Excel were used for statistical analysis.

**Results**

**Lymphocyte and Thrombocyte Morphology in SEM**

**Lymphocyte Morphology in TEM and Light Microscopy**

**Monocyte Morphology in TEM and Light Microscopy**

**Neutrophil Morphology in TEM and Light Microscopy**

**Thrombocyte Morphology in TEM and Light Microscopy**

**Comparison of Gender and Age**

**Discussion**

- Average blood cell type percentages per 100 cells include lymphocytes (68.08%), thrombocytes (17.31%), and CEG type 2 (14.99%), CEG type 1 (14.66%), immature leukocytes (13.97%), neutrophils (1.69%), FEG (0.55%), and monocytes (0.25%).
- Lymphocytes were the second most abundant cell type after erythrocytes.
- Vertebrate lymphocytes are highly conserved across species[25].
- Four granulocytic cell types were found. A neutrophil and three morphologically distinct eosinophilic granulocytes: (1) fine eosinophilic granulocyte (FEG), (2) coarse eosinophilic granulocyte (CEG) type 1, and (3) coarse eosinophilic granulocyte (CEG) type 2.
- Classification of specialized granulocyte subtypes is commonly observed in many shark species[25].
- The GLR is an indicator of stress in fish. Acute stress can cause a shift in peripheral blood leukocyte distributions and increase the GLR[35].
- Larger sample sizes of wild blacktip sharks should be used.

**Table 3**: Average count with standard deviation and minimum and maximum count for each cell type.

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Minimum</th>
<th>Mean</th>
<th>Maximum</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEG1</td>
<td>51</td>
<td>14.66</td>
<td>19.1</td>
<td>0.80</td>
</tr>
<tr>
<td>CEG2</td>
<td>27</td>
<td>14.98</td>
<td>19.1</td>
<td>0.89</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>8</td>
<td>1.493</td>
<td>9.99</td>
<td>0.03</td>
</tr>
<tr>
<td>Monocyte</td>
<td>42</td>
<td>0.238</td>
<td>4.99</td>
<td>0.10</td>
</tr>
<tr>
<td>Thrombocyte</td>
<td>87</td>
<td>4.99</td>
<td>19.1</td>
<td>0.89</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>6</td>
<td>17.31</td>
<td>29.99</td>
<td>0.89</td>
</tr>
<tr>
<td>Immature</td>
<td>59</td>
<td>13.97</td>
<td>39.34</td>
<td>0.30</td>
</tr>
</tbody>
</table>

**Acknowledgments**

Special thanks to the UM Shark Research and Conservation Program, Dr. Liza Merly, Dr. Patricia Blackwelder and Dr. Neil Hammerschlag.

**References**

1. Smith et al. 2015
2. Tavarea, 2008
3. Dičić et al. 2013