STANDARDIZING PERIPHERAL BLOOD LEUKOCYTE COUNTS
IN SEVERAL SHARK SPECIES
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Introduction

• Blood samples from wild sharks can provide an efficient way to gain insight into an animal’s health status
• Peripheral blood leukocyte (PBL) counts are one way to assess the immune status or health condition of an animal
• Measures of shark health are scarce in the literature and have mainly involved their care in an aquarium setting; information on hematological assessments in wild sharks is rare and is related to the difficulty of in-field testing.
• Standardized methods for assessing peripheral blood counts in wild sharks are needed to provide a comprehensive picture of normal health and physiology in a variety of species that have not been studied extensively.
• Due to the nucleation of erythrocytes in sharks (and most non-mammalian vertebrates) the automated cell count method does not work.
• Obtaining accurate total leukocyte counts from peripheral blood smears that can be obtained in the field, fixed, stained, and evaluated later is a possible solution to some of these challenges.
• To obtain estimation of complete blood counts (CBC) from peripheral blood smears, it is necessary to determine the calculated count factor or CCF that yields the total count based on the number of leukocytes counted in 40x field under light microscopy.
• The first specific aim was to determine whether a peripheral blood smear could be used to conduct a total white blood cell count (CBC) and establish the CCF appropriate for use with shark blood.
• The second specific aim was to evaluate PBL in the Lemon shark (N. brevirostris) as a specific sample to determine what leukocyte types are present and conduct differential counts.
• Establishing methodologies to conduct effective complete blood counts and differential counts can provide critical information for studies assessing the health condition of wild sharks.

Methods

• Sharks whole blood was collected by caudal venipuncture by the Shark Research and Conservation team at the University of Miami during regular shark tagging trips between September 2019 and February 2020.
• For the CBC estimation project:
  - 5mL of anticoagulated blood was mixed with 995µl of Natt & Herrick solution.
  - WBC’s obtained by solution were counted on a hemocytometer immediately following collection to determine CBC count.
  - For the same shark sample, multiple counts of stained leukocytes were counted on the blood smear at 40x.
  - The calculated count factor or CCF was determined using the equations developed in Figure 5.
• For Lemon shark differential counts:
  - Leukocytes were observed under light microscopy under oil immersion at 100x and the cell types were compared to other known leukocytes to identify cells present.
  - For each blood smear, replicate counts of 100 leukocytes were made for the different leukocyte observed; the number of thrombocytes and immature cells seen for every 100 leukocytes was recorded.
  - Cell diameters of each cell type (min. 10 cells) were determined using Zen software program.
  - Grandmean-to-Lymphocyte ratios were recorded for each shark.

Table 1: Reference intervals for differential counts of the lemon shark (Negaprion brevirostris) peripheral blood leukocytes

Table 2: Leukocyte measurements (in diameter, cm) for Lemon Shark (N. brevirostris)

Results

• In comparison to values in the literature for other sharks, the lymphocyte percentage for Lemon sharks is higher.
• The granulocytes and monocytes overall had a much larger diameter, which corresponds to the fact that they are likely the main phagocytotic cells in peripheral blood; both coarse eosinophilic and fine eosinophilic granulocytes were present in lemon shark blood.
• Hematological reference intervals for lemon sharks in this population are established here for the first time.
• Results of the estimation of CBC experiment were mixed; the initial goal was to establish a single number (the CCF) for use as a proxy, facilitating the use of blood smears instead of complete blood counts for wild sharks.
• Successfully establishing the CCF would necessitate it having both precision and accuracy.
• Results indicate that more research in this area is needed to establish an appropriate CCF for sharks; substantial variation in counts did not yield a CCF that could reliably estimate the CBC from blood smears.
• An important drawback to the application of these methods are related to irregularities in counts when identifying cells.

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