Dorsal Fin Morphometric Analysis for Species Identification and its Application to Forensic Science

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Rapidly declining shark populations are having a negative effect on the overall marine ecosystem. Shark populations are exploited for their fins, which are used for shark fin soup, a Chinese delicacy. The United States government enacted several laws including the Magnuson-Stevens Fishery Conservation Act and the Shark Conservation Act of 2011. However, these laws are difficult to enforce due to the lack of an inexpensive and discriminatory method of differentiating the shark species after the fins have been processed. To address this issue, a federal agency's forensic science laboratory has requested research to be done in this field. The reason that shark fins are difficult to differentiate is because some sharks, such as the Short-fin Mako (*Isurus oxyrinchus)* and the Porbeagle shark (*Lamna nasus*), have a similar appearance to their fins, especially in the processed form. Many sharks are morphologically similar due to comparable feeding habits, migratory patterns, and the pelagic zone they inhabit. This can inevitably lead to misidentification of the fins. A method of differentiating the species is imperative because there are different levels of protection for the assorted species. For example, the Great White Shark (*Carcharodon carcharias*) is listed as an endangered species under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) with the highest level of protection in Appendix I. However, the Great (*Sphyrna mokarran)* and Scalloped Hammerhead (*Sphyrna lewini)* sharks are listed under Appendix II. In order to determine if there are diagnostic differences between various shark species, this research project involved investigating the cross-sectional morphology of dorsal shark fins. The three shark species used were the Common Thresher shark (*Alopias vulpinus*), Short-fin Mako, and Blue shark (*Prionace glauca*), which were obtained from previous shark research done with the University of New Haven. When studying the cross-sectional morphology, each fin was sliced into five pieces. Measurements that were considered to possibly have diagnostic differences included proximal-distal lengths, caudal-cephalic lengths, thickness of platelet cartilage, fibrous cartilage, and platelet count at percentages of fifteen, fifty, and eighty-five of each slice. Platelet count proved to be the most promising morphometric method in differentiating the shark species.

Introduction

It has been predicted that approximately twenty species of shark will become extinct by 2017 due to unregulated fishing techniques allowing the killing of over 100 million sharks every year from shark finning (Vercler, 2007). Shark finning is the act of cutting off shark fins and discarding the carcass back into the ocean while the shark is usually still alive. The reason the rest of the body is unwanted and only the fins are kept is because the fins are considered the only valuable part of the shark at a cost of approximately $700/kg in Asia (Vercler, 2007). Shark fins are used frequently in the Chinese culture to make shark fin soup, which is considered a delicacy and served at banquets and dinner parties, such as weddings, to show respect for the guests. The collagenous fibers of the fins are used in the soup (Musick, 2004). The Chinese consider it to be "one of the eight treasured foods from the sea" (Vannuccini, 1999). Rapidly declining shark numbers have caused a ripple effect on the balance of the global marine ecosystem (Fairclough).

The high demand of shark fins has created an entire industry dedicated to shark finning. The demand has caused an estimated decline of all the shark species, except Mako (*Isurus oxyrinchus)*, of more than 50% in the past eight to fifteen years (Baum, 2003). In an attempt to minimize the prevalent problem of shark finning, the United States government enacted several laws including the Shark Finning Prohibition Act of 2000, Magnuson-Stevens Fishery Conservation Act, and the Shark Conservation Act of 2011, which states that sharks that have been caught in United States waters must be brought to shore with the fins still naturally attached to the body. The Shark Finning Prohibition Act of 2000 made shark finning illegal by anyone under U.S. Jurisdiction (Shark Finning Prohibition Act of 2000). The states included under U.S. Jurisdiction thus far are Hawaii, Oregon, Washington, California, Illinois, Maryland, Delaware, New York, Massachusetts and Texas. However bills have been initiated in 2015 to ban the sale, trade, and possession of shark fin and such products in Pennsylvania, Florida, Nebraska, New Jersey, and Rhode Island (Vorphol, 2015). In addition, the act requires the National Oceanic and Atmospheric Administration's (NOAA) National Marine Fisheries Service (NMFS) to publicize regulations and also to improve data collection, establish research programs, and work with other nations on agreeing upon set regulations and data collection methods. Since that the Magnuson-Stevens Fishery Act and the Shark Conservation Act have also been legislated (2010 Shark Finning Report to Congress). However, these laws are difficult to enforce due to the lack of an inexpensive and discriminatory method of differentiating the shark species after the fins have been processed.

The reason a new method of differentiating species is necessary is because there are different levels of protection for the various shark species. Furthermore, some species of shark fins have a similar appearance especially in the processed form such as Short-fin Mako (*Isurus oxyrinchus)* and the Porbeagle shark (*Lamna nasus*). The reasons they appear so similar is due to feeding habits, migratory patterns, and the pelagic region that they

populate. The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is the agreement that was made by 180 nations ensuring that international trade does not threaten species of wild plants or animals. This treaty has been in place since 1975 (NOAA). Various species of sharks are listed in different in their appropriate appendices based on their conservation status. For example, Appendix I is the greatest level of protection and includes species that are in danger of becoming extinct. Appendix II is the level below and includes species that are not yet threatened with extinction, but are expected to become so unless trade controls become more strict. Finally, Appendix III of CITES includes shark species for which countries have asked other CITES Parties for help in controlling their trade and export permits must be obtained (NOAA). One of the species listed under Appendix I, highest level of protection is the Great White Shark (*Carcharodon carcharias*). Some of the sharks included in Appendix II include Oceanic Whitetip shark (*Carcharhinus longimanus)*, Hammerhead sharks (*Sphyrna lewini, S. mokarran, S. zygaena*), and Porbeagle sharks *(Lamna nasus)* (CITES Secretariat). As of right now, there are no law enforcement agencies that are solely responsible for implementing the shark fin ban laws. It is the responsibility of the members of CITES to ensure the laws are being upheld.

The main method of differentiating species thus far has been the use of DNA tests. The created method uses species-specific primers, which are based on consistent nucleotide sequence differences among species in a particular locus, the Internal Transcribed Spacer 2 locus. The primers are used in a multiplex PCR format to produce diagnostic amplicons (Pinhal, 2012). The method was used to discriminate between six shark species common in fisheries around the world. Testing for the six sharks can be done in one tube with six tests that focus on a species-specific portion of shark DNA, which is used as that species identification tag (Verlecar, 2007). The issue with this method is that it is expensive and time consuming.

This research aims to differentiate various shark species by examining the dorsal shark fins' cross-sectional morphology for diagnostic characteristics. The most distinguishing factor observed during this research project was the platelet count at four different slices of three species of shark, Mako (*Isurus oxyrinchus)*, Blue sharks (*Prionace glauca*), and Thresher (*Alopias vulpinus*) sharks.

Methods and Materials

Samples used in this research were obtained through previous shark fin research done at the University of New Haven. Three different shark species were used in this research project, which included the Common Thresher shark (*A. vulpinus*), Shorfin Mako shark (*I. oxyrinchus)*, and Blue shark (*P. glauca*). Three samples of each of the three shark species were used, giving a total of nine samples. A method involving salting, sun drying, and then using variable hot and cold treatments was used to process the samples obtained at shark tournaments by previous students. Those samples were sliced using a band saw in order to get a smooth cut that would allow examination of the various components of a shark fin. The inferior part of the fin was measured from free tail to leading edge for consistency purposes. The overall length of the dorsal fin was measured from the middle of the inferior to the tip of the superior part of the fin. The dorsal fin was sliced at percentages of 20,40, 60, and 80 as seen in the figure below. After slicing, the inferior part of each slice was examined and the platelets were counted along with measurements of the thickness of the skin, thickness of the fibrous cartilage, thickness of the platelet cartilage and the lengths of each component. The measurements listed were taken at each of the caudal-cephalic percentages (20%, 40%, 60%, and 80%) and for each of those slices at the proximal distal percentages of 15%, 50%, and 85% going from the bottom of the fin to the tip. The variables measured included the lengths and widths of the trailing edge, leading edge, free tail, platelet, platelet count, thickness of skin, platelet cartilage, and fibrous cartilage. The factors used in the statistics were the species, weight, girth, total length, fork length, state of the dorsal (dry), and the sex of the shark. Platelet counts were examined at 20%, 40%, 60%, and 80% of each of the dorsal fin samples and documented.

Statistical analysis was done by using the program VSN International, GenStat, Version 16. Summary stats were run on the raw data, which included the means of the platelet counts and their standard deviations. A two way Analysis of Variance (ANOVA) was conducted to compare the platelet counts by species at varying slice percentages (20%, 40%, 60%, and 80%).

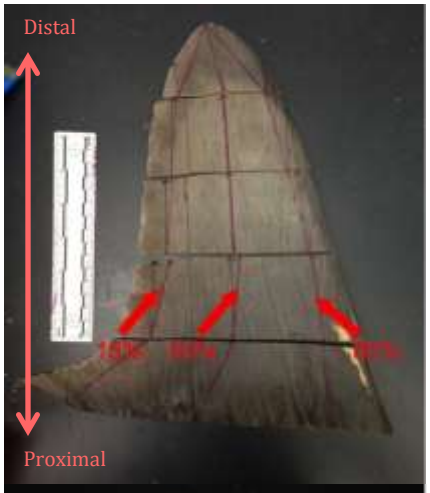

Figure 1: Each slice was also examined at percentages of 15, 50, and 85 (proximal-distal) for the thicknesses of the platelet cartilage, skin, and fibrous cartilage.

Figure 2: Slice Percentages (Caudal-Cephalic) at 20%, 40%, 60% and 80%.

Results

The results displayed in Figure 3 below represent the 9 sharks used and the average platelet counts for each shark with two standard deviations. According to these results, the Thresher sharks have a mean of 0 platelets at 80%, while Mako sharks have a mean of approximately 1.67 platelets at 80%. The graph also shows the disappearance of platelets after the 20% slice in Blue shark.

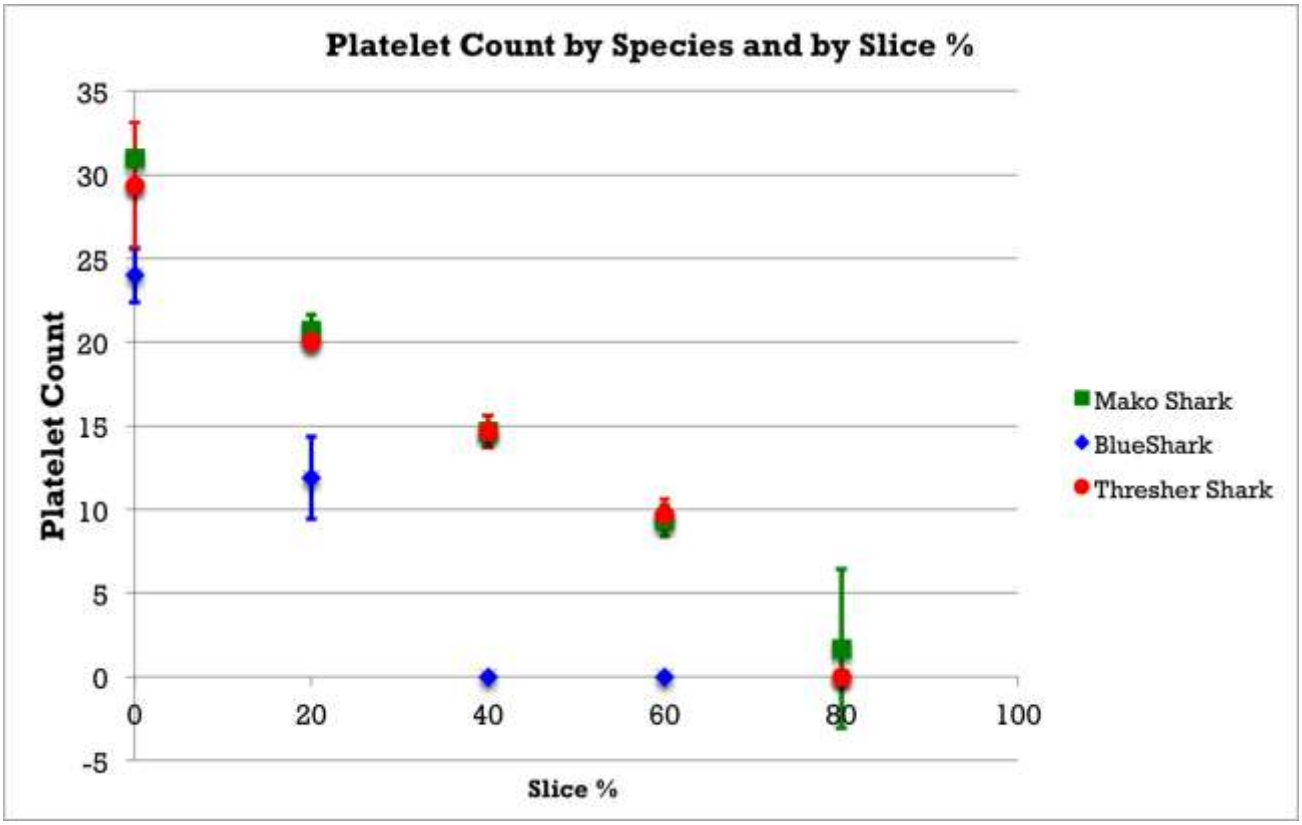

Figure 3: Platelet count by Slice % and Species of Thresher sharks (*A. vulpinus*), Shorfin Mako sharks (*I. oxyrinchus)*, and Blue sharks (*P. glauca*).

The results obtained show an obvious difference between Blue sharks compared to Mako and Thresher sharks. By looking at the 40% slice of a dorsal fin, one can determine if it is a blue shark. If it has 0 platelets visible, it must be a Blue. If there are platelets present at the 80% slice, it is most likely a Mako or Thresher shark because the platelets are no longer visible after the 20% slice in Blue shark dorsal fins. These results make it easy to determine the species by looking at the platelet cartilage alone.

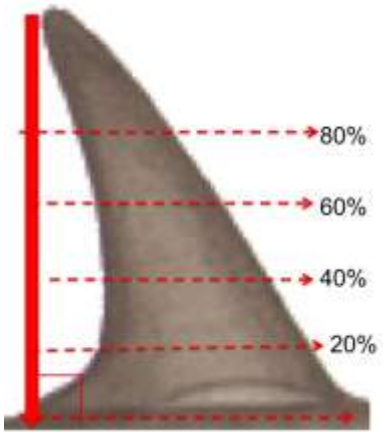

Figure 4: Standardization of Slicing

Discussion

To confirm the results, more samples of each species of shark will should be measured and platelets should be counted at the same percentages. Additional platelet counts should be documented at percentages of 10, 30, 50, 70, and 90 as well for further validation. Doing the additional platelet counts may also strengthen the currently small difference between the Mako shark and the Thresher shark at 80%. Observing the two sharks at percentages such as 70 and 90 may determine a more significant difference and establish a concrete point of variance between the two shark species.

One of the limitations in this project was time. Due to the time constraint, only three samples were observed in the three different species. Other limitations such as the species of sharks obtained, played a role in the restricted amount of data used to do the analysis.

In order to ensure the methodology is reproducible and precise, the fins' proximal-distal length should be measured from the tip of the whole fin, straight to the free tail at a 90° angle from the base line. The caudal-cephalic slices should be cut across the fin at a 90° angle from the proximal-distal measurement and go to the leading edge's point of attachment. Each of the slices (20, 40, 60, 80) should be at a right angle as shown in Figure 4.

Conclusions

From the analysis observed through conducting a two-way ANOVA, it is clear that Blue sharks show significant diagnostic variation at the 40% slice with no platelets. This was very consistent in all three of the samples. However, the Mako shark and Thresher shark are both too similar based off of only three samples and the percentages at which the platelets were counted. Observing and recording the platelet counts for more samples of each species may yield a better result, especially if combined with additional percent slices such as 10, 30, 50, 70, and 90 percent.

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Biography

I am from Rockaway, New Jersey and I am a senior at the University of New Haven. I will be graduating in May 2016 with a double major in Forensic Science and Biology. I am involved with Alpha Lambda Delta National Honor Society, the Forensic Science and Chemistry Club, and Rotaract Club. I have presented at the Northeastern Association of Forensic Scientists and plan to present at other conferences. After graduation, I hope to go on with my education and get a PhD in Biochemistry.