THE EFFECT OF CAUDAL STIMULATION ON HEMATOLOGICAL INDICATORS OF STRESS IN BLACKNOSE SHARKS, *CARCHARHINUS ACRONOTUS*

by
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EXECUTIVE SUMMARY

I conducted this study with the mentorship of Field School, particularly its Director, Dr. Catherine Macdonald, and Director of Program Development, Dr. Julia Wester. In 2017, shortly after realizing I wanted to find work in the environmental field, I took an Introduction to Shark Research Skills course with Field School, located in Miami, Florida. Soon after, I entered into an internship with Field School and have continued working with them. Much of their research centers around ecology, physiology, and the human dimensions of conservation of elasmobranchs, the group composed of sharks and rays. I have always gravitated toward the human dimensions of environmental problems because of my background in psychology. Most of the research I have assisted with has been in that realm. However, I wanted to use the Capstone research project as an opportunity to delve into new skills and topics I had not had the opportunity to explore as much.

As I neared the end of my time in the Johns Hopkins Environmental Science and Policy (ESP) program, I realized I wanted to use the Capstone to both take advantage of what I have learned and fill the gaps in my education and experience from being a remote student by getting more hands-on field experience. I chose to take on a physiology-focused project in order to gain new skills and explore physiological concepts in a deeper way than I had previously understood them.

This project enabled me to tie in my interest in ecology and policy gained from the ESP courses. When I started the program, I deliberated about which track to follow, and ultimately chose not to opt-in to a track, choosing instead to take a wide range of classes from each. By researching this one aspect of shark stress physiology, I've acquired new skills while simultaneously touching on a wide variety of topics relevant to my ESP courses. Sound management regulations for sharks and other recreationally and commercially targeted species necessitate a clear understanding of their biology and physiology, especially as it relates to impacts of their harvest on individuals and overall populations. As predators, sharks occupy important niches within their ecosystem, and overexploitation can lead to impacts across all trophic levels within an ecosystem. Due to their ecological, commercial, and cultural importance, it is imperative that efforts be made to understand the scope of and the causes underlying the decline of numerous species, and identify ways to more effectively conserve them. Successful conservation is informed by understanding the interactions between all of these seemingly diverse topics. I was happy this project afforded me the opportunity to synthesize these topics into a single study.

Mentors: Dr. Catherine Macdonald and Dr. Julia Wester of Field School

TABLE OF CONTENTS

LIST OF FIGURES

1. INTRODUCTION

Shark fishing is practiced worldwide in both a commercial and recreational capacity, with practices ranging from small artisanal and subsistence-based fisheries to massive commercial longline operations to recreational land-based catch-and-release angling (Worm et al., 2013; United States National Marine Fisheries Service, 2014). In recent years, recreational shark fishing has increased in popularity to the extent that, in 2013, more sharks were landed by recreational anglers in the United States than were landed by commercial shark fishing operations (United States National Marine Fisheries Service, 2014). In recent years, recreational shark anglers and clubs worldwide caught hundreds of thousands of sharks yearly and released over 70% of their catch (Babcock, 2008). Though much of the recreational shark fishery is catch-and-release, many targeted species such as great hammerheads (*Sphyrna mokarran*) are highly susceptible to capture stress and post-release mortality, also known as cryptic mortality (Gallagher et al., 2014). A likely result is that people who intend to release their catch alive may unknowingly contribute to morbidity and mortality post-release (Whitney et al., 2017). Even when sharks are released alive and seem to be in good condition, there is a risk of mortality within subsequent hours, with certain often targeted species particularly at risk (Gallagher et al., 2014).

Catch-and-release fishing is becoming an increasingly popular practice, with recreational anglers commonly releasing caught sharks to follow management regulations, because of personally-held conservation ethics, or when they have caught a non-targeted species (Gallagher, Cooke & Hammerschlag, 2015; Arlinghaus et al., 2007). Scientists may release sharks for similar reasons, and also as a way to potentially gather mark-recapture data on populations as well as long-term data from individuals. In order to achieve these goals, it is important to minimize the risk of mortality for individual sharks, including through the exploration of potential interventions that might reduce stress and facilitate sharks' healthy release (Cooke & Suski, 2005). In the interest of science and conservation, it is important to consider modifying fishing practices and gear to minimize post-release mortality for more sensitive species (Marshall et al., 2012).

Wild animals perceive capture and restraint as a threat to their survival and in response, exhibit various behavioral, physiological, and biochemical responses that vary across and within taxa (Romero, 2004). A shark's natural response to hooking (e.g., mobilizing energetic resources for escape to increase survival likelihood) has negative concomitant impacts, including increased physiological stress (Mohan et al., 2020). The physiological impact of capture on all species of shark can be profound, and depending upon the species and its stress response, may push the individual past the threshold for recovery, even if it is released alive (Marshall et al., 2012; Marshall et al., 2015). For scientists releasing tagged sharks with the goal of gathering long-term data from individuals, this outcome is problematic.

Even when sharks are caught and handled by shark scientists attempting to minimize risk to the animal, sharks undergoing scientific workup have been shown to exhibit stress during capture and handling, leading to cryptic mortality (Gallagher et al., 2014). This mortality can be difficult to measure and the impact on a species and its population often goes unnoticed, complicating the process of creating effective conservation-minded recreational fishing regulations. Regulations must take into account the fact that release alone will not ensure that a shark survives capture. It is important to accurately measure cryptic mortality, but even more so to identify interventions which can reduce it. This study aims to test the feasibility of one such intervention for the blacknose shark, *Carcharhinus acronotus*.

1.1 Shark Conservation

Marine populations of apex predators are diminishing worldwide (Myers & Worm, 2005), with evidence of decreases in shark populations documented in south Florida, including in the Keys and Gulf of Mexico (Heithaus et al., 2007). Sharks' ecological niche can vary considerably across species and size classes, but they are generally known to be higher level tropic predators and many species exhibit K-selected reproductive strategies, with slower growth rates and lower fecundity (Renshaw et al., 2012). As such even small amounts of pressure from fishing can cause the populations of vulnerable species to decline, with declining populations of some species inducing trophic cascades as a result of changes to predator and prey abundance (Baum & Worm, 2009; Ferretti et al., 2010). Though teleosts are also susceptible to pressure from recreational and commercial fishing, the stress on elasmobranch populations is more extreme due to their life history traits, including smaller populations and an inability to rebound quickly from overfishing (Renshaw et al., 2012).

The approximate number of sharks landed yearly is difficult to quantify due to the scope of the practice, the existence of substantial unreported or illegal catches, and the fact that many fisheries are artisanal and recreational, and therefore are not reporting their catch in the same way (Worm et al., 2013). Researchers working to estimate the mortality of sharks from fishing each year estimate that in 2000, approximately 100 million sharks were landed (Worm et al., 2013). This was, they concluded, a conservative estimate, with the upper estimate limit of yearly mortality ranging to 273 million sharks per year. Many shark species are being harvested at a rate which exceeds the average population rebound rate, with exploitation rates ranging from 6.4% to 7.9% and the average rebound rate measured at 4.9% (Worm et al., 2013). As a result, sharks are being harvested faster than they are able to repopulate.

1.2 Stress Physiology

Blacknose sharks are in the Carcharhinidae family and are a ram ventilating species that must constantly move forward in order to pass oxygenated water over their gills (Carlson, Palmer & Parsons, 1999; Driggers et al., 2004). Interference with the forward motion through capture will therefore decrease their ability to adequately respirate. Capture initiates a shark's primary stress response, during which the neuroendocrine system produces corticosteroids to maintain performance and oxygenation (Anderson, 2012; Skomal, 2007). Capture stress can be lethal for all species, though the relationship between capture stress and mortality varies by species as well as differences in gear and handling of individuals (Dapp et al., 2016a; Dapp et al., 2016b).

Many covariates have been identified that can interact with and impact hematological indicators. Different species vary in how they respond to being hooked, with species like blacktip sharks (*Carcharhinus limbatus*) exhibiting more fight behavior than nurse sharks (*Ginglymostoma cirratum*) and tiger sharks (*Galeocerdo cuvier*) and exhibiting greater increases in lactate (Gallagher et al., 2017). Some species demonstrate resilience in spite of longer capture and fight times, whereas others are at risk of mortality even after short fight times and rapid release. Other covariates that are well documented to alter hematological indicators are fight time and handling time. Hematological indicators of stress are significantly related to capture time and handling time, with increased time fighting or being handled leading to increased evidence of stress (Dapp et al., 2016a; Mohan et al., 2020). These physiological changes do not guarantee mortality, but increase its likelihood (Whitney et al., 2017). Another covariate with an impact on physiological condition is water temperature. Increased water temperature correlated with increased lactate and mortality in a study of recreationally caught blacktip sharks (Whitney et al., 2017). Biscayne Bay exhibits a seasonal sea surface temperature difference of 6°C between the winter median (23°C) and summer median (29°C) (Caccia & Boyer, 2005) with a high temperature of 32.9°C observed between 1994 and 2003. Sharks caught during the warmer summer temperatures will be more likely to exhibit increased stress and risk of mortality.

Ram ventilating species may be particularly susceptible to capture stress because, depending upon the method of capture, their ability to extract oxygen from the water might be limited by restraint (Dapp et al., 2016b; Manire et al., 2001). Ram ventilators may also, compared to buccal pumping species, be susceptible to additional stress during scientific workups from being held immobile. It has been suggested that there is a positive influence on circulation caused by the lateral movement of a fish's caudal fin, with tail movement supporting blood circulation into the liver and kidneys (Johansen, 1971). However, during capture and workup, sharks are often restrained and held immobile, thereby unable to move their tail and receive secondary circulatory support that motion might provide. It is possible, therefore, that restraint might limit the caudal fin's ability to provide circulatory support and clear the body of lactate and other by-products of stress.

Catch-and-release fishing is becoming an increasingly popular practice, with recreational anglers commonly releasing caught sharks to follow management regulations, because of personally-held conservation ethics, or when they have caught a non-targeted species (Arlinghaus et al., 2007; Gallagher, Cooke & Hammerschlag, 2015). Scientists may release sharks for similar reasons, and also as a way to potentially gather mark-recapture data on populations as well as long-term data from individuals. In order to achieve these goals, it is important to minimize the risk of mortality for individual sharks, including through the exploration of potential interventions that might reduce stress and facilitate sharks' healthy release (Cooke & Suski, 2005). It is important to consider modifying fishing practices and gear to minimize post-release mortality for more sensitive species (Marshall et al., 2012).

1.3 Experimental Interventions to Reduce Mortality

This study investigates the feasibility of manually manipulating the tail during scientific workup as a way to impact a shark's circulation and blood chemistry potentially. Improved circulation during work-up may improve shark condition by supporting blood flow through the liver and kidneys, where the majority of clearance of the by-products of stress and exertion is likely taking place (Barrera et al., 2013).

Several studies document the value of hematological indicators in assessing shark health. Both lactate and glucose levels tend to elevate when sharks experience stress, though the scale of the response can vary by species (Gallagher et al., 2014; Jerome et al., 2018; Marshall et al., 2012). Comparing the lactate and glucose values of individual sharks at different times during a workup can provide insight into how each individual responds to stressors. Lactate may increase or decrease from the beginning to the end of the workup depending upon how sharks respond to stressors as well as efforts by researchers to reduce physiological stress. Lactate has been demonstrated to be a valuable measurement for predicting mortality, however lactate levels and specific impacts of increased levels vary to such a degree that lactate values cannot be used to predict poor outcomes across species (Manire et al., 2001; Marshall et al., 2012). There is ongoing scientific interest in improving practices to reduce negative effects on sharks, combining an understanding of shark physiology and ecology with the human aspects of conservation (Arlinghaus, Cooke & Potts, 2013).

This study provides preliminary data on whether manual manipulation of the caudal fin during shark restraint is feasible without disrupting the workup and leads to reduced lactate levels in the blood relative to control animals at release.

2. METHODS

2.1 Fishing Site

Fishing for this project took place in Biscayne Bay, a shallow, oligotrophic bay in southeastern Florida. Biscayne Bay is within Florida waters and is bounded to the west by the developed coastline of Miami as well as mangrove forests, and to the east by multiple barrier islands separated by channels. Within the bay, there are seagrass beds, coral reefs, and sandy bottom habitats. Biscayne Bay is a culturally and economically significant resource in south Florida, providing habitat for more than 30 endangered species or species of special concern (Cantillo et al., 2000), and more than 100 species that are important to local recreational and commercial fisheries (Stoa, 2016).

2.2 Case Study Species

The blacknose shark *(Carcharhinus acronotus)* is an ideal study species because we commonly encounter them on scientific surveys in the waters of the nearshore Atlantic and Biscayne Bay, Florida inhabiting seagrass, sandy, and coral bottom habitats.The blacknose shark is a small coastal species found in tropical and warmer temperate waters in the Western Atlantic Ocean, Gulf of Mexico, and Caribbean Sea from Brazil to Virginia, USA (Castro, 2011). Blacknose sharks closely resemble and share similar habitats and morphological characteristics with blacktips (*Carcharhinus limbatus*), however, blacknose can be differentiated by the black smudge present on their snout (Castro, 2011). Blacknose sharks grow to a maximum size of 140 cm total length (Castro, 2011). Males reach maturity at a total length between 97 and 106 cm and females reach maturity around 103 cm (Castro, 2011; Compagno, 2005; Driggers et. al., 2004). Blacknose sharks tend to inhabit shallow sandy and coral bottoms on continental shelves (Castro, 2011; Compagno, 2005).

As a result of the 2007 Stock Assessment of Small Coastal Sharks in the US Atlantic and Gulf, the National Marine Fisheries Service determined that blacknose were overfished and that overfishing was still occuring (SEDAR, 2007). The 2007 Stock Assessment was regulated as part of the Small Coastal Shark Complex, but they are now managed separately and reduced species-specific quotas have been assigned. Blacknose sharks were last assessed by the International Union for the Conservation of Nature (IUCN) Red List in 2008, when they were evaluated as "Near Threatened" and identified as showing decreasing population trends (Morgan, et al. 2009).

2.3 Sampling

Fieldwork for this project was conducted with scientists from Field School, located in Miami, Florida. Field School conducts scientific sampling of coastal waters in southeast Florida using longline and drumline fishing methods as part of ongoing research projects. Field School routinely employs techniques such as modified gear including circle hooks and bilge pump hoses inserted into the shark's mouth during workup to decrease shark mortality. They also rely on short "soak times" (i.e., the length of time fishing gear is in the water is kept under 90 minutes) and brief scientific workups (i.e., length of time sharks are restrained is kept under 5 minutes) to improve animal outcomes.

From July 2018 to November 2020, over 100 blacknose sharks were captured, and 81 yielded data relevant to this study via scientific longlines and drumlines in Biscayne Bay, Florida. As part of ongoing research conducted by Field School, researchers deploy both longlines and drumlines designed to minimize the risk of mortality.

Longline gear consisted of a 15-meter line sitting horizontally in the water column with a danforth anchor and polyform ball affixed to both ends to keep the line stationary and mark it visually. At regular lengths along the longline, gangions, short lengths of wire and monofilament, ending in a either a 13/0 or 15/0 non-offset non-stainless steel baited circle hook, were attached with tuna clips. Knots in the horizontal rope at regular intervals allowed for the tuna clips to slide back and forth if pulled by a fish, without tangling. Two such lines, each with twenty-two 13/0 or 15/0 circle hooks, were set at a time.

Drumline gear consisted of a 40-pound weight attached to a vertical length of rope topped with a buoy. At the top of the weight, a swivel was attached to a gangion ending in a 15/0 or 16/0 circle hook. Drumlines were set at regular intervals, spaced apart so fish caught on different drums were unlikely to tangle.

After a period between 60 and 90 minutes, gear was hauled and checked for sharks. Upon capture of a blacknose shark, larger individuals were secured on a semi-submerged platform while the majority were small enough to bring on deck where they were similarly secured. A bilge pump hose was positioned into the mouth to allow saltwater to continuously flow over the shark's gills for respiration during sampling. Immediately after the shark was restrained, the first blood draw (<4ml blood) occurred through caudal venipuncture and blood was immediately processed using commercial point-of-care meters for glucose and lactate levels.

Immediately after the first blood draw, the scientific workup began during which the person securing the caudal fin of the shark moved the tail back and forth in a prescribed arc to simulate the movement of swimming. For the duration of the work-up, the person securing the tail continued to move it back and forth except when it needed to be stationary for the length measurements and blood draws. The workup process was timed to establish the time difference between the first and second blood draws. A researcher took measurements of each shark's precaudal length, caudal fork length, total length, and girth, measured to the nearest centimeter. Pre-caudal length (PCL) was measured from the tip of the rostrum to the precaudal pit. Fork length (FL) was measured from the tip of the rostrum to the fork in the caudal fin. Total length (TL) was measured from the tip of the rostrum to the tip of the upper lobe of the caudal fin. Girth is a measurement of the shark's circumference, taken from just behind the pectoral fins and in front of the dorsal and extending over the top of the body from pectoral to pectoral. Additionally, each shark was sexed based on an external visual survey of the cloaca. Researchers used scissors to collect a small sample of the trailing edge of the dorsal fin (<5mm) and put it into a vial of dimethyl sulfoxide. A 4mm biopsy punch was used to collect a white muscle sample from the shark's flank below the dorsal fin. Each shark was tagged with a unique mark-recapture identification tag (M-type dart tag) at the base of the dorsal fin. The hook was removed or cut and a second sample of blood was drawn and processed for glucose and lactate levels, and then the shark was released. Time was recorded upon the identification of a shark on the line, at the moment the shark was removed from the water, at first and second blood draw, and at release. Sea surface temperature was recorded for each subject. The only way this workup differed from others was in the manual manipulation of the caudal fin.

Glucose was measured on whole blood using a commercially available point-of-care glucose metre (ACCU-CHEK glucose metre; Roche Diagnostics, Basel, Switzerland; see Cooke et al. 2008 for validation study with teleost fish). Lactate was measured on whole blood using a lactate metre (Lactate Pro LT-1710 portable lactate analyser; Arkray Inc., Kyoto, Japan; see

Cooke et al. 2008 for validation study with teleost fish).

Variables used in analysis included what group the shark was assigned to (treatment vs. control), change in lactate from first to second blood draw, blood draw (initial vs. final), and change in time from the beginning to the end of the workup. Results were analyzed using a linear regression to investigate whether being assigned to either treatment or control predicted a difference in how lactate changed from the beginning to the end of the workup. Subsequent model iterations also included temperature, shark total length, and time between blood draws as predictors of change in lactate.

3. RESULTS

A total of 20 blacknose sharks were subjected to the manual manipulation of the tail from March to November 2020. Of those 20, six yielded either error messages or "hi" values on the lactate metre, with no corresponding numerical value for either or both blood draws. Fourteen yielded numerical values for both blood draws, and these were included in the data. The glucose metre proved more capricious, with error messages occurring in one or both blood draws for ten individuals. With such a small sample size yielding numerical values for both glucose and lactate, and because lactate has been found to be a more reliable indicator of stress and cryptic mortality (Marshall et al., 2012), I have decided to focus the preliminary data for this project on lactate. Sixty-seven sharks subjected to a normal workup (control group) yielded lactate values for both their initial and final blood draws from July 2018 to June 2020.

Of the fourteen sharks in the treatment group, six (42.9%) experienced declines in lactate from the beginning to end of the work-up and eight (57.1%) experienced increases. Of the control group, the lactate values of three did not change (44.8%), 25 decreased (37.3%), and 39 (58.2%) increased from the beginning to the end of the work-up.

The linear regression results investigating whether the group predicted lactate indicated treatment group membership did not significantly predict change in lactate ($F(1, 160) = 0.43$, $p = 0.51$). In subsequent models, I incorporated change in time from blood draw one to two as a predictor variable. Similarly, this model indicated there was not a significant effect between the group and change in lactate while controlling for time between blood draws ($t = -0.58$, $p = 0.56$). Time between blood draws was also not a significant ($t = -0.77$, $p = 0.44$; $F(2, 145) = 0.39$, $p = 0.68$).

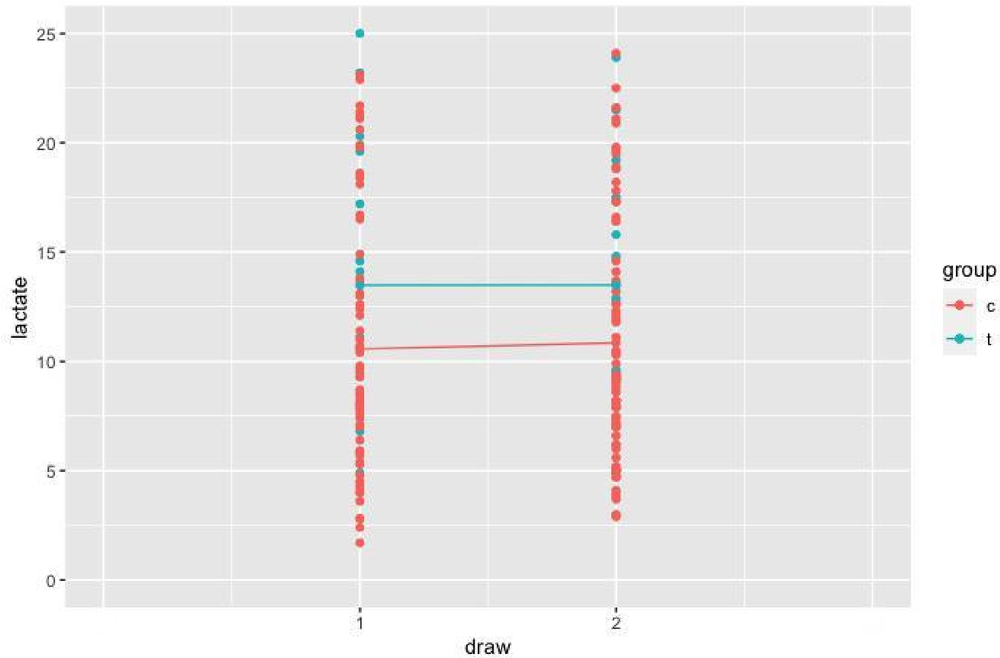

Figure 1. Data visualized as a scatterplot with a regression line showing non-significant relationship between group (control vs. treatment) and change in lactate from draw one to draw two.

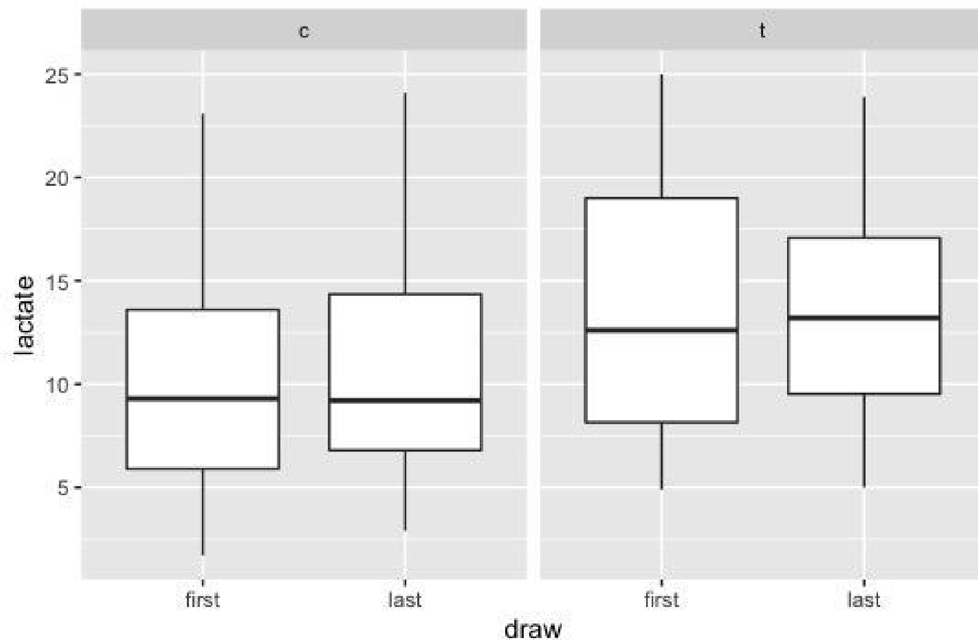

Figure 2. Data visualized as a boxplot with a regression line showing non-significant relationship between group (control vs. treatment) and change in lactate from draw one to draw two.

4. DISCUSSION

The coronavirus pandemic imposed numerous challenges during the 2020 field season. The goal of the project changed over time, as the coronavirus impacted our ability to conduct fieldwork. Initially, I hoped to have a large enough sample size to make a more definitive determination of whether manipulation of the tail had an effect on how lactate in the blood changes from the beginning to the end of the work-up. We previously identified a need for a minimum of thirty blacknose in both the control and treatment group, however, we were only able to sample during a few weeks of the normal field season. I was able to gather some preliminary data, and most importantly, validate that this intervention is possible to apply to a shark undergoing a scientific workup. We considered the possibility that having their tails moved might cause the sharks to tense or thrash and that the desired intervention might actually cause a distraction that prolonged the workup. Fortunately, that did not occur, the movement of the tail did not distract from or prolong the duration of workups. Due to the small effect size and small treatment group sample size, there was no significant difference in lactate change between the control and treatment groups based on this preliminary data. More data should be gathered to further assess if this intervention has an impact on physiological stress, as measured through hematological parameters such as lactate. I plan to continue gathering this data over the next year.

A significant impact on how lactate changes over the course of the work-up would be an important result. This would provide researchers with an intervention that can reduce harm and potentially increase the odds of post-release survival. It would also suggest that the movement of the caudal fin plays an important role in circulation for at least some ram ventilating shark species. A significant difference between the groups would suggest that tail movement assists in blood movement through the liver and kidneys, increasing circulation and lactate clearance.

A non-significant difference, even with a larger sample size does not necessarily mean there is no relationship between the caudal fin movement during swimming and circulation. This type of secondary circulatory support has been demonstrated in teleosts (Johansen, 1971) and therefore, there is no reason to think that cartilaginous fish do not also share this trait. Even so, there can be multiple explanations for why it is not possible to impact how lactate changes during the time of the workup. It is possible that the assistance given by moving the tail is not evident in such a short time, as researchers strive usually to make the process of workup as quick as possible. Perhaps there are greater benefits, but they would not be interpreted through the blood until a point after the shark has already been released. Another possibility represented by a non-significant difference between the groups is that secondary circulatory support necessitates the contraction of muscles associated with a shark voluntarily moving its tail during swimming; manual manipulation might replicate the movement but not the underlying physiological processes leading to increased circulation.

A third possible scenario is that lactate does change in a significant way, but that we see statistically significant increases in lactate in the treatment group. This might be an indication that having their tails manipulated increases their stress more than being held immobile. Hopefully, this would have been evident in a change in behavior in sharks in the treatment group, and as previously mentioned, no difference in behavior was observed.

Although the small sample and effect size of this data does not allow us to draw any conclusions, I believe that further investigation is warranted. Due to the amount of noise inevitable in the data, a larger treatment group is necessary. This project has demonstrated the feasibility of tail movement as a non-disruptive addition to a normal workup. The sharks did not exhibit additional resisting or squirming as a result, and the other researchers were able to continue gathering samples unhindered.

There is a hole in our knowledge of how ram ventilating species' circulation differs from that of buccal pumping species. The fact that all methods of capture rely on impeding the unrestricted forward motion of sharks means that obligate ram ventilators are exposed to reduced respiration, while species that buccal pump can continue to actively respirate. It is not unreasonable to suspect that this might lead to an elevated stress response to capture among ram ventilators (Dapp et al., 2016b). If restraint and immobilization of the tail also limits circulation, ram ventilators might have difficulty clearing lactate as effectively. Increased morbidity and mortality are a possible result. It is important to further study this topic because it is imperative to craft the best species-specific conservation and management strategies.

A suggestion of this difference can be found by looking at how the IUCN has classified shark species. A search of the IUCN Red list database, filtering for sharks present along the US's Atlantic coast, suggests challenges worth exploring to understand how different respiratory strategies might impact a species conservation. When filtering the results to only show species in this geographic area that have been designated as "Least Concern" or "Near Threatened," the search returned twenty sharks, with a mix of species known to use buccal pumping, including the tiger shark (*Galeocerdo cuvier)* and bull shark (*Carcharhinus leucas*) and obligate ram ventilators, including the Bonnethead (*Sphyrna tiburon*) and the Atlantic sharpnose (*Rhizoprionodon terraenovae*) (IUCN, 2020). A search of the same filters altered to show only sharks designated as "Critically Endangered" or "Endangered" resulted in a list of seven species, all of which are obligate ram ventilators, including the scalloped hammerhead (*Sphyrna lewini*) and shortfin mako (*Isurus oxyrinchus*). A complicating factor in this rudimentary analysis is that a large number of sharks and rays that are recognized by the IUCN database are listed as "Data Deficient." As such, while this comparison between the less and more endangered species is incomplete, it does provide an interesting suggestion of where to focus future research questions.

Different reproductive strategies could also help explain the differences between the two groups within the IUCN data, and explain some of the resilience of certain obligate ram ventilating species that are listed as species of "Least Concern." Those species show more r-selected life history traits (e.g. small-bodied, faster growth and maturity, higher levels of natural morality) than their buccal pumping counterparts (Carlson & Parsons, 1997; Parsons, 1983). Diverse life history strategies could make a species more vulnerable or resilient to fishery pressure (Adams, 1980). Further study on this subject could lead to a greater understanding of how the disparate ways circulation functions in species with varying respiratory strategies and how it interacts with their life characteristics.

As researchers, it is imperative to understand and use best practices to mitigate risk of serious injury to species during scientific sampling. Some species with which we interact such as the great and scalloped hammerheads are designated "Endangered" by the IUCN assessments, but this is important for all of our targeted species, because scientific knowledge is enriched by data gained through recapture of tagged animals. In addition to being a tool for use by scientists sampling sharks, this intervention could inform best practices for recreational fishers targeting sharks or who may catch sharks as bycatch. Educational initiatives by government agencies and not-for-profit conservation organizations disseminate information on how to reduce cryptic mortality through proper gear and techniques for reeling fish in and releasing them (Florida Wildlife Commission, 2020; Cooke & Schramm, 2007). Anglers participating in catch-and-release fishing with the goal of releasing the shark alive would likely be willing to follow these, or more species-specific guidelines, when possible.

I will continue gathering data over the next year to expand upon this project and collect a large enough treatment group to assess the efficacy of the intervention more accurately. I will further investigate how covariates such as sea surface temperature, shark's TL, and fight time interact with the shark's assigned groups to impact change in lactate. Understanding what factors predispose a shark to severe capture stress is important for minimizing those factors if possible, or adjusting practices so other stress-inducing factors are minimized. For instance, a strong correlation between sea surface temperature and increase in lactate could be mitigated by shortened gear soak times to reduce fight time. There is value in identifying best practices to reduce mortality as a tool for conservation and more effective scientific sampling.

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