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Integrating Physiological and Reflex Biomarkers of Fishing Capture Stress in Coastal Shark Species

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UNIVERSITY OF MIAMI

INTEGRATING PHYSIOLOGICAL AND REFLEX BIOMARKERS OF FISHING
CAPTURE STRESS IN COASTAL SHARK SPECIES

By

Jacob M. Jerome

A THESIS

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Master of Science

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Integrating Physiological and Reflex
Biomarkers of Fishing Capture Stress in
Coastal Shark Species

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In both commercial and recreational fisheries, many sharks are captured and released alive due to harvest regulations, the capture of non-target species, or conservation ethics. Nevertheless, released individuals may suffer post-release fitness loss or even mortality due to capture stress. Additionally, some of these species that would otherwise be intended for release are found dead upon capture. Understanding physiological and behavioral responses of sharks to capture stress is important for determining best fishing practices and for establishing effective management strategies. In this study, I investigated sub-lethal effects of capture through monitoring blood glucose, lactate, hematocrit and reflex impairment on four species of coastal sharks: blacktip (*Carcharhinus limbatus*), great hammerhead (*Sphyrna mokarran*), nurse (*Ginglymostoma cirratum*) and sandbar (*Carcharhinus plumbeus*). A probability of impairment score was given to each individual based off reflexes developed for this study. I further evaluated inter- and intra- specific relationships between these parameters and fight time, season, and shark size. Of the physiological parameters accessed, lactate emerged as the most significant with increases associated with fight time, shark species,

and reflex impairments. Reflex indices showed significant impairment with increasing fight time with the “Jaw” reflex being most significant in all evaluations. Species-specific differences were detected in all parameters with nurse sharks consistently having the lowest values and impairments while great hammerheads had the highest. These relative differences in species-specific stress responses is consistent with relative difference in fighting behavior exhibited for these species on a fishing line as well as reported at-vessel and post-release mortality rates for these species. Collectively, these results indicate that lactate can be used as a measure of shark capture stress, jaw reflex impairments can be utilized as significant indicators of shark capture stress on a species specific basis, and that species’ ecology likely contributes to these responses both physiologically and in terms of reflex impairment. This work connects species-specific reported at-vessel and post-release mortality rates with their responses to capture by revealing trends in physiological changes and reflex impairments. The drumline capture method used likely represents a more benign fishing method compared to typical recreational and commercial fishing gears and therefore these responses are likely subdued when compared to other fishing techniques. Therefore, continued investigation is warranted to better understand the varied responses among species and different capture techniques.

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Chapter 1: General Introduction and Scope of Work

Overexploitation of Marine Fish

In many marine environments, the overexploitation of some species of fish and their habitats has caused varying levels of population declines (Hutchings 2000, Myers et al. 2007, Dulvy et al. 2014). This overexploitation is driven by fishing efforts that have depleted wild fish stocks (Worm et al. 2005) and caused overall landings of fish to fall, despite increased fishing efforts (Christensen et al. 2007). While commercial practices have long been known to be a major cause of declining fish stocks through the retention of fish for profit as well as bycatch, recreational fishing has recently been shown to contribute to large population declines of some marine fish (Cooke and Cowx 2006). In many situations catches are not always retained and are released because they are not the intended catch (bycatch) or due to harvest regulations (Arlinghaus et al. 2007, Cooke and Cowx 2006, Worm et al. 2013). In addition, anglers often voluntarily release fishes they don't intend to consume because of conservation ethics (Arlinghaus et al. 2007, Cooke and Cowx 2006, Hall et al. 2000). Extrapolating from Canadian recreational fisheries, Cooke and Cowx (2004) suggested that 47.1 billion fish are landed globally each year, of which 30 billion are released. While fish released alive without obvious signs of injury are often thought to survive, they can succumb to post release fitness loss through behavior impairments that can impede growth, reproduction or disease resistance, all of which can lead to mortality. The culmination of these effects can cause significant impacts on populations, especially on a regional scale (Arlinghaus et al. 2007, Cooke and Cowx 2006). It is difficult to monitor the fate of many fishes post-release and their maintained fitness and survival are not guaranteed. These impacts are especially

detrimental when the species involved play key roles in their environment; as is the case with many sharks that are top predators (Stevens et al. 2000, Baum and Worm 2009). Moreover, impacts on sharks are exacerbated due to *k*-selected features such as slow growth rates and low fecundity (Musick et al. 2000, Stevens et al. 2000, Worm et al. 2013). As a result, several elasmobranch species face population declines globally due to overfishing (Dulvy et al. 2008, Dulvy et al. 2014, Lucifora et al. 2011) and the cryptic mortality that can occur because of capture related fitness loss. While fishing effort and subsequently shark retention can be quantified by managers, the effects of capture and release on shark populations is more difficult to analyze. Therefore there is a need to better understand the physiological and behavioral consequences of capture and release on sharks (Gallagher et al. 2012).

Catch and Release Fishing

In many tuna, swordfish, or trawling commercial fisheries, sharks caught as by-catch are often discarded back into the ocean. In the year 2000 alone, it was estimated that 227,000 metric tons of sharks were released alive by commercial fishers (Worm et al. 2013). Even vessels that target sharks often have to relinquish their catch due to size, species or harvest regulations. In addition to commercial fisherman, recreational anglers often encounter and release sharks either due to ethical reasons or species regulations. In 2013 and 2014, NOAA reported that an estimated ~11.5 and 11 million cartilaginous fish were released, respectively, without being retained by recreational fisherman in the US (NMFS 2013). While the notion of releasing a shark after capture seems to satisfy ethical or conservation mindsets, there can be cryptic lethal, as well as sub-lethal, consequences of capture not seen by anglers (Gallagher et al. 2015).

While the physiological disruptions teleost fish experience from capture have been studied extensively (Muoneke and Childress 1994, Diodati and Richards 1996, Tomasso et al. 1996, Bartholomew and Bohnsack 2005), there has been comparatively less research focusing on the physiological stress induced from catch and release fishing on sharks (Moyes et al. 2006, Mandelman and Skomal 2009, Gallagher et al. 2014b). Examining the effects of capture stress on shark survival is important given that these species are encountered in both commercial and recreational fisheries worldwide, and in many situations are released to an unknown fate (Cooke and Cowx 2006, Arlinghaus et al. 2007, Worm et al. 2013).

Fish escaping from gear or released by fishers may die as a direct result of physical damage and physiological stress or indirectly due to reduced capacity to escape predators or resist disease (Chopin and Arimoto 1995). The process of capture is known to impact shark health by altering levels of plasma electrolytes, serum constituents, acid-base levels, and blood constituents (Cliff and Thurman 1984, Manire et al. 2001, Hoffmayer and Parsons 2001, Mandelman and Skomal 2009, Marshall et al. 2012, Kneebone et al. 2013, Gallagher et al. 2014b). Measuring these parameters give researchers information on the extent of stress experienced by each shark.

Behavioral indices and reflex impairments integrate the effects of capture-related stressors by reflecting the status of physiological systems and predatory avoidance mechanisms (Davis 2005, Davis and Ottmar 2006). Both behavior and reflex impairment have been used to indirectly measure delayed mortality in fishes (Chopin and Arimoto 1995, Davis and Parker 2004, Davis 2005, Davis and Ottmar 2006, Hueter et al. 2006, Braccini et al. 2012). In sharks, behavior and reflex impairments have emerged as a new

tool to study the condition of sharks after capture but their relationships with known blood measures have yet to be explored. While there has been information provided on what happens to sharks after release from fisheries in terms of behavior and reflex impairment (Hueter and Manire 1994, Manire et al. 2001, Hueter et al. 2006, Braccini et al. 2012, Afonso and Hazin 2014, Gallagher et al. 2014b, Danylchuk et al. 2014), there is still much more to understand about whether reflex impairments can be used as a relative proxy for understanding health, morbidity, and the physiological status in sharks when they are captured and after they are released.

Physiological Stress Response

During their daily lives, fish are challenged by a suite of biotic and abiotic elements in their environment, many of which can trigger stress responses. These stressors can come in many forms (thermal, physical, etc.) and ultimately result in both a physical and metabolic response. In reaction to stressors, fish generally undergo a stress response classified into three main categories: primary, secondary, and tertiary (Barton and Iwama 1991). The primary response is characterized by the release of corticosteroids and catecholamines, which subsequently lead to an assortment of physiological or metabolic changes, classified as the secondary stress response (Barton and Iwama 1991, Mazeaud et al. 1977). A variety of responses are considered to be secondary, but generally they are described as metabolic, hematological, and structural (Barton and Iwama 1991). Within the secondary stress response, three noteworthy metabolic disturbances occur: impacting levels of blood glucose, hematocrit, and lactate. These three metabolites have widely been used as a measure of the secondary stress response (see below) and as a proxy for shark stress as a whole. The tertiary response results from

the physiological disturbances prior which affect homeostasis after the stressor and subsequently features such as growth, metabolic rate, reproductive capacity, foraging ability, and disease resistance (Barton and Iwama 1991). While fish have a natural ability to respond physiologically to stress on these three levels, extreme disturbances can push them past this normal capacity, resulting in allostasis, the loss of health and even death (Barton and Iwama 1991). Post-release mortality is widely recognized as a confounding factor to fishing practices where animals are released, as it can cryptically threaten the ability to retain productive fish stocks (Muoneke and Childress 1994, Cooke and Cowx 2004, Gallagher et al. 2014b).

When either physical or environmental stressors are introduced to an organism, energy is needed to maintain homeostasis. Oxygen is necessary for aerobic metabolism to produce the organisms' required energy in an efficient manner (Mazeaud et al. 1977). However, there is a threshold for which aerobic metabolism can occur in the muscles due to the high oxygen demand from the oxygen dependent process (Heath and Pritchard 1965). Therefore, under challenging conditions, anaerobic metabolism takes suite. While some energy is produced through this method without the requirement of oxygen, it is comparatively less efficient than aerobic metabolism and produces harmful lactic acid as a byproduct (Cliff and Thurman 1984).

Blood Glucose

Glucose is a monosaccharide that is used for energy in living organisms, and when grouped together, form a readily mobilized structure known as glycogen. Organisms take up glucose mainly through food consumption at which point the metabolite is either utilized immediately or stored in the form of glycogen in the skeletal

muscles and liver (Heath and Pritchard 1965). Glycogen stored in the muscle is generally used for that particular muscle whereas glycogen in the liver can be recruited to the bloodstream when needed (Mazeaud et al. 1977). When the aforementioned stress response begins, the release of catecholamines can prompt the recruitment of liver glycogen. The glycogen now present in the bloodstream can be distributed to the muscles for energy use. Through this process, glucose found in the blood stream elevates as glycogen stores in the liver likely decrease (Heath and Pritchard 1965).

While the switch from aerobic to anaerobic glycolysis allows organisms to continue to process glucose for energy, and most fishes can naturally clear the negative byproducts that result from anaerobic states, the prolonged excitation of this response can have negative consequences. If enough glucose is not released into the bloodstream to satisfy the energy demand from a stressor or glycogen stores deplete, an organism will not be able to provide energy to its muscles, including those needed to function such as the brain and heart, resulting in cellular death.

In teleost fishes, this disturbance has been studied extensively through varying physical stressors. Cech et al. (1996) determined that glucose increased significantly for striped bass (*Morone saxatilis*) exercised in a swim tunnel. When monitoring the stress response of rainbow trout (*Oncorhynchus mykiss*) to rod and reel angling as well as handling, Meka and McCormick (2005) saw no significant relationship with glucose to either of these stressors. The study did, however, produce results that showed a correlation between higher glucose values and higher water temperatures as well as larger fish (Meka and McCormick 2005). In largemouth bass (*Micropterus salmoides*) White et al. (2008) found no significant increase in glucose levels after exercise and air exposure

whereas Vanlandeghem et al. (2010) saw that both cold and heat shock, as well as hypoxic shock increased glucose levels significantly. While studying the response of saithe (*Pollachius virens*) to longline fisheries, Roth and Rotabakk (2012) saw an increase in glucose levels during the 6-12 hour soak time. In rod and reel angling, Lennox et al. (2015) saw a significant increase of glucose for fat snook (*Centropomus parallelus*). While the disturbance of glucose varies among teleost fishes and type of stressor, it is typically associated with an increase in blood glucose levels.

Similar work on sharks has shown that glucose is, in general, useful for studying the effects of capture stress. Cliff and Thurman (1984) found that in juvenile dusky sharks (*Carcharhinus obscurus*), blood glucose rose significantly with increasing stress events (capture, fight, confinement). Hoffmayer and Parsons (2001) serially sampled Atlantic sharpnose (*Rhizoprionodon terraenovae*) over a one hour time period and found a significant increase in blood glucose levels. Using pop-up satellite archival tags (PSAT) on longline caught blue sharks (*Prionace glauca*) to delineate survival versus morbid animals, Moyes et al. (2006) found that glucose levels did not vary significantly between the two groups. Comparing exercised-stressed and recovered juvenile sandbar sharks (*Carcharhinus plumbeus*), Brill et al. (2008) found that glucose levels did not differ between the two groups. Brooks et al. (2011) observed a parabolic trend with blood glucose in relation to longline hook time in Caribbean reef sharks (*Carcharhinus prezeii*), suggesting that glucose peaked around 80 minutes of fight time but then individuals were able to recover. Kneebone et al. (2013) exercised juvenile sand tigers (*Carcharias taurus*) with rod and reel and found that glucose showed no significant relationship with angling time. In this study, however, most animals were angled for less than 3 minutes,

potentially not allowing enough time for a response in glucose. These studies collectively suggest that glucose can be used as an indicator of the secondary stress response in sharks however, more work is needed to develop an understanding of to what degree glucose increases in response to capture stress and how the response differs between species that commonly interact with fisheries.

Lactate

Lactate is produced in the body as a byproduct of anaerobic metabolism, a process that generally occurs when the oxygen supply is insufficient for aerobic metabolism to take place (Heath and Pritchard 1965). The high concentration of lactate in the muscles causes rapid diffusion into the blood stream, resulting in a hypoxic state (Heath and Pritchard 1965). This influx of lactate in the bloodstream has been associated with the physiological stress response for both teleost and elasmobranch fishes.

In teleost fishes, the relationship between lactate levels and capture response has been widely studied. In striped bass (*Morone saxatilis*), lactate values increased from control values and were proportional to fight time (Tomasso et al. 1996). Just 15 minutes after capture, coral trout (*Plectropomus leopardus*) experienced significantly higher lactate levels than at the moment of capture (Frisch and Anderson 2000). Similarly, Meka and McCormick (2005) found that with increasing angling duration, lactate values rose significantly in rainbow trout (*Oncorhynchus mykiss*). In recreationally angled bonefish (*Albula* spp.), blood lactate values were positively correlated with angling duration (Brownscombe et al. 2015). These studies have shown that, in general, there is a positive linear relationship between the length of a capture event and the level of lactate in the blood, indicating the state of anaerobic glycolysis.

Similar studies have examined the relationship between lactate and angling in sharks. Cliff and Thurman (1984) saw a significant increase in lactate values over a 70-minute stress period in juvenile sandbar sharks (*Carcharhinus obscurus*). Lactate concentrations doubled in bull sharks (*Carcharhinus leucas*) and tripled in bonnethead (*Sphyrna tiburo*) and blacktip (*Carcharhinus limbatus*) sharks with increasing relative effects of capture and restraint (Manire et al. 2001). In Atlantic sharpnose (*Rhizoprionodon terraenovae*), lactate rose in a linear fashion through a 60-minute stress period of angling (Hoffmayer and Parsons 2001). Brill et al. (2008) found that exercise-stressed juvenile sandbar sharks had over an order of magnitude higher lactate concentrations than control animals. Additionally, the interaction between the duration of exercise induced by capture (referred here after as ‘fight time’) and animal size had a positive significant effect on lactate concentrations for great hammerhead, blacktip, bull, lemon, and tiger sharks (Gallagher et al. 2014b). This subset of studies accurately represents the literature on the relationship between lactate concentrations and exercise induced stress resulting from fight time in sharks, suggesting that lactate can be an informative biomarker for monitoring stress in sharks.

Hematocrit

Hematocrit is measured as the proportion by volume of whole blood that contains red blood cells. Red blood cells, or erythrocytes, are an organism’s fundamental means of transporting oxygen to tissues. As stated above, oxygen is needed to efficiently convert glycogen stores through aerobic glycolysis into usable energy for muscles. A higher red blood cell count would allow for oxygen to be more easily delivered to tissues in the body (Wells and Davie 1985). As a stress event occurs and catecholamines are released,

blood hematocrit can be affected by means of swelling red blood cells, loss of plasma fluid, or mobilization of red blood cells from the spleen (Turner et al. 1983). Hematocrit measurements are often used as a proxy for blood oxygen carrying capacity in fishes (Wells et al. 1986). It is expected that in fish, large active pelagic species have higher baseline hematocrit values to satisfy daily energy expenditure needs compared to benthic or less active species (Wells et al. 1986, Wilhelm et al. 1992). Nevertheless, it has been noted that catecholamines released during a stress event can cause swelling of red blood cells, increasing hematocrit values (Nikinmaa and Huestis 1984, Railo et al. 1985).

In teleost fishes, hematological changes associated with stress have been extensively studied. These studies have shown that for the most part, an increase in hematocrit is typically associated with both physical and environmental stressors among species. Turner et al. (1983) saw a 10-15% increase in hematocrit immediately following severe exercise in flathead sole (*Hippoglossoides elassodon*). After an extensive study on rainbow trout (*Oncorhynchus mykiss*) exercise, Wells and Weber (1990) saw an increase in hematocrit following exercise with both red blood cell swelling and erythrocyte recruitment from the spleen. In coral trout (*Plectropomus leopardus*) acutely stressed from capture, handling and transport, Frisch and Anderson (2000) viewed an increase in hematocrit over a 30 to 60 minute period with levels returning to normal thereafter. Wells et al. (2003) saw a significant increase in hematocrit after angling exercise in tarpon (*Megalops cyprinoides*). Similarly, Roth and Rotabakk (2012) showed that hematocrit increased significantly for saithe (*Pollachius virens*) after longline capture.

In sharks, the relationship between hematocrit values and stress seem to be more varied. Arterial O² content has been shown to be markedly higher in exercise-stressed

lemon sharks (*Negaprion brevirostris*) (Bushnell et al. 1982), and Wells and Davie (1985) suggest that a plausible mechanism for this could be increased hematocrit levels. Wells and Davie (1985) found markedly high hematocrit values in capture-stressed shortfin mako sharks (*Isurus oxyrinchus*). Similarly, Brill et al. (2008) found that hematocrit significantly increased in juvenile sandbar sharks after capture. Conflicting results have also been seen in other species. Hematocrit levels showed no significant change in capture-stressed sharpnose sharks (*Rhizoprionodon terraenovae*) (Hoffmayer and Parsons 2001), bonnethead (*Sphyrna tiburo*), blacktip (*Carcharhinus limbatus*), bull (*Carcharhinus leucas*) (Manire et al. 2001), or between alive and morbid blue sharks (Moyes et al. 2006). While an increase in hematocrit may show some evidence of a coping mechanism for some elasmobranchs, it is important to consider the diversity of life histories associated with the species under investigation as a means for explaining hematocrit's role in the stress response of sharks.

Reflex Impairment

Reflex impairment is an ecologically significant measure that is capable of responding proportionally to the severity of many types of stressors (Davis 2005). Reflex impairments have been correlated with delayed mortality that results from capture-related stressors by reflecting the status of physiological systems and predatory avoidance mechanisms (Davis and Ottmar 2006). Davis (2005) presented that reflex impairment not only integrates the effects of capture related stressors, but that they can be predictive of delayed mortality by means of both physical and physiological injury. Changes in fish condition have also been linked to delayed mortality (Davis 2005), including in elasmobranchs (Hueter et al. 2006).

In teleost fish, research on reflex impairment has varied across multiple species and stressor types and revealed that both physical and environmental stressors can impair behaviors and lead to delayed mortality. It should be noted that ‘behavior impairment’ and ‘reflex impairment’ appear to be used interchangeably within the literature. Olla and Davis (1989) saw that coho salmon (*Oncorhynchus kisutch*) showed a lower affinity to avoiding predators, characterized as behavior impairment, following handling. Similar results were found for Chinook salmon (*Oncorhynchus tshawytscha*) (Olla et al., 1995). In regards to fish discards, Ryer (2002) and Ryer et al. (2004) showed that both sablefish and walleye Pollock swim slower and allow predators to approach closer distances when stressed from towing and escape experiments. In sablefish, 10 minutes of air exposure causes behavior impairment in the form of reduced visual and physical reflexes associated with predator avoidance (Davis and Parker 2004). While these findings show that stressors do in fact cause behavioral impairment, Davis (2005) correlated behavioral impairment with delayed mortality by monitoring sablefish during a 24 hour recovery period. By developing a reflex action mortality predictor (RAMP) score, Davis and Ottmar (2006) were able to show that walleye Pollock, sablefish, northern rock sole (*Lepidopsetta polyxystra*) and pacific halibut (*Hippoglossus stenolepis*) all had reflex impairment that was significantly related to mortality. A similar result found that an increasing RAMP score was correlated with mortality for walleye Pollock, coho salmon, rock sole and halibut (Davis 2007). Raby et al. (2012) validated the use of RAMP scores outside laboratory experiments by monitoring migration success of coho salmon in the wild after seine net capture. The authors found that fish with higher RAMP scores, indicating increased impairment, had significantly higher failure rates of migration.

McArley and Herbert (2014) discovered that snapper (*auratus*) may be able to recover from angling, despite a reflex impairment that is correlated with duration and air exposure. In bonefish (*Albula vulpes*) Brownscombe et al. (2015) showed that angling along with air exposure increases reflex impairment.

This style of research in sharks has evolved differently. Hueter and Manire (1994) developed a behavior release condition score (BRCS) to monitor shark behavior at release and to use as a proxy for stress. BRCS consists of visually monitoring released sharks and assigning individuals to a category that reflects their release. Conditions range from “Good”, “Fair”, “Poor”, “Very Poor”, and “Dead”. They require examination of tail beat, orientation in the water, and swimming vigor upon release. Using tag return data from a marine species survey, (Hueter and Manire 1994) estimated that as delayed mortality increases, the BRCS decreases, indicating the potential for the score to be used as an indicator of stress. Building off this work, Manire et al. (2001) used BRCS as an indicator of stress level and compared it to different serum constituents in blacktip (*Carcharhinus limbatus*), bonnethead (*Sphyrna tiburo*), and bull sharks (*Carcharhinus leucas*). While intraspecific differences were present, several blood serum constituents changed significantly with increasing stress levels (Manire et al. 2001). Notably, serum lactate increased significantly in all three species (Manire et al. 2001). Using a tag-recapture study, Hueter et al. (2006) estimated the relative survival of fish released in different conditions based off tag returns. Their model was applied to an example from field research to predict mortality (Hueter et al. 2006). Going further, a BRCS was assigned to tiger sharks (*Galeocerdo cuvier*) which were then monitored for post-release mortality via satellite tags, however, zero of the individuals displayed post-release

mortality, despite having varying BRCS (Afonso and Hazin 2014). Braccini et al. (2012) used an “Activity” behavior index, among other techniques, to categorize and predict post capture survival in gummy (*Mustelus antarcticus*) and port-jackson sharks (*Heterodontus portusjacksoni*). Utilizing reflex impairments developed for teleost fish, Gallagher et al. (2014b) saw that impairments of one reflex aligned with species that experienced greater delayed mortality after an angling event. Similarly, Danylchuk et al. (2014) saw that post-release mortality was positively related to RAMP scores for recreationally angled juvenile lemon (*Negaprion brevirostris*) sharks. Finally, Hyatt et al. (2015) investigated the efficacy of using BRCS as an estimate for physiological stress. The authors found a coarse relationship between the two with intraspecific differences and suggesting for further research in the area (Hyatt et al. 2015). For sharks, causal relationships between stress and reflex impairments are starting to emerge but further research is needed to develop a greater scope of impairments to measure across multiple species and to test possible relationships between physiological stress and reflex impairments.

Chapter 2: Evaluation of Physiological and Reflex Impairments for Coastal Sharks in Relation to Capture Stress

Background

This study utilized assessments of both physiological indicators and reflex impairments to investigate the sublethal consequences associated with capture stress in sharks. The study focused primarily on four coastal shark species while also incorporating additional data collected for other species encountered. Blacktip (*Carcharhinus limbatus*), nurse (*Ginglymostoma cirratum*), great hammerhead (*Sphyrna mokarran*) and sandbar (*Carcharhinus plumbeus*) were the primary species used in this study; each species varies in their regional and global threat status (as assessed through the International Union for the Conservation of Nature Red List of Threatened Species, IUCN) as well as their life history. Nonetheless, these four species are often encountered by commercial and recreational anglers near and offshore and as a result may experience varying degrees of capture induced stress. The specific objectives of this study were to: (1) characterize the physiological capture stress response in sharks by measuring blood glucose, lactate, and hematocrit upon capture and evaluating the relationships of these parameters with fight time, species, and shark size; (2) characterize potential shark reflex impairments in response to capture and evaluate their relationships with fight time, species, shark size, and physiological parameters (glucose, lactate, hematocrit); and (3) test for species specific differences in both the physiological and reflex impairment responses to capture stress.

Methods

Study Site and Species

This study was conducted inside Florida state waters between latitudes N 25.791 and N 25.597. Sampling was conducted from June 2015 to May 2016, across the wet and dry seasons (wet = June to November; dry = December to April). Species encountered whose data was used for this project include: blacknose (*Carcharhinus acronotus*), blacktip (*Carcharhinus limbatus*), bull (*Carcharhinus leucas*), great hammerhead (*Sphyrna mokarran*), lemon (*Negaprion brevirostris*), nurse (*Ginglymostoma cirratum*), sandbar (*Carcharhinus plumbeus*), scalloped hammerhead (*Sphyrna lewini*), and tiger (*Galeocerdo cuvier*). Species rank on the IUCN Red List of Threatened Species as follows: “Near Threatened” – blacknose, blacktip, bull, lemon, tiger; “Vulnerable” – sandbar; “Endangered” – great hammerhead, scalloped hammerhead; and “Data Deficient” – nurse.

Fishing and Capture Methods

Sharks were caught using a standardized circle-hook drumline system as described by Gallagher et al. (2014b). In short, gear consists of a submerged weight with two attachment points: 1) a line running to the surface with buoy floats and 2) a swivel connecting a 23 m monofilament gangion line (~400 kg test) that terminates with a baited 16/0 5°-offset circle hook (Figure 1). The proximal end of the monofilament line is connected to the weight via a hook timer (Lindgren Pitman HT600) that consists of a magnetic release timer to display the time each animal has been on the line. Gear was allowed to soak for up to 1 hour until retrieval began. Upon capture of a shark, the animal was secured either alongside the boat or on a semi submerged platform, depending on

size and species. A water pump was placed in the mouth of each individual to allow for continued respiration. All data collected for this project, including reflex evaluation (described below), occurred within one minute of capture and securing of individuals. Following blood draw (described below), each individual was subjected to a standardized workup procedure that allowed for the collection of data for additional projects. This procedure did not impact my ability to assess capture stress as all handling and air exposure occurred after the data was collected for this study (i.e. there was little to no additive impacts of handling stress or air exposure).

Reflexes

Following the approach of Davis and Ottmar (2006), Gallagher et al. (2014b) and Danylchuk et al. (2014), each reflex was graded with either a 0 (zero, non-impaired) or a 1 (one, impaired). All reflexes were evaluated within a thirty-second-time frame to standardize the procedure. The first reflex evaluated was the animal's vigor when the fishing line was initially retrieved (herein termed 'retrieval'). For this test, the ability of the captured individual to resist being reeled in on the fishing line was determined. A grade of 0 required individuals to actively resist against being reeled to the boat by myself, which was often accompanied with bouts of burst swimming. Individuals graded with a 1 did not show burst swimming upon retrieval with minimal resistance. Once individuals were brought to the boat, a nictitating membrane test was conducted (herein termed 'nictitating') following Gallagher et al. (2014b). The Nictitating test consisted of a small burst of seawater at a distance of 4 cm to the eye of the shark from a 10 ml plastic needleless syringe. A grade of 0 required the firing of the nictitating membrane within 2 seconds of contact. A grade of 1 was given to individuals whose nictitating membrane

did not appear or was delayed past 2 seconds. Next, a PVC pipe attached to a submerged pump was placed in each individual's mouth to allow water to continuously flow over their gills. While some individuals willingly allowed their mouths to be opened, others maintain downward pressure forcing the jaws closed in a clenching manner (herein termed 'jaw'). A grade of 0 was assigned if individuals maintained downward pressure as I attempted to open their mouth with upward pressure on the snout. Individuals whose mouths were opened easily without the presence of downward pressure were given a grade of 1. The fourth reflex was monitored visually by closely examining the gills of the animal (herein termed 'gill') and watching for the contraction and relaxation of the gill musculature. A grade of 0 resulted in movement of the gill muscles, an attempt by the animal to move water for respiration. A grade of 1 was given to individuals who did not contraction or relaxation of their gill musculature. Finally, each animal was monitored for a restrained body flex (herein termed 'flex'). A grade of 0 resulted from the tension of muscles in response to restraint or tail grab. A grade of 1 was given to individuals who did not show muscle tension while being restrained. Both the gill and flex indicators were monitored on the whole animal for the entire 30 seconds. Due to the strict guidelines of evaluating impairment and subsequent training, I alone evaluated each individual to retain consistent results. From the reflex results from each shark, a probability of impairment score was calculated as a simple proportion of the measured reflexes that were impaired in an individual shark (probability of impairment score of 0 = no reflexes impaired; 1 = all reflexes impaired) (Davis 2007, Raby et al. 2012).

Blood Draw/Processing

Whole blood (5-7 ml) was drawn from each individual via caudal venipuncture and immediately processed for glucose and hematocrit levels. Glucose was measured on whole blood by adding 10 μ l of blood to a glucose meter (ACCU-CHEK glucose meter; Roche Diagnostics, Basel, Switzerland; see Cooke et al. 2008 for validation study with fish). Hematocrit was determined through centrifugation (13,000 g x 3 minutes) of microhematocrit tubes on board the vessel and measuring the packed red blood volume (% Hct) with a crito-caps card reader in duplicates. Four ml of whole blood was separated into two vials and spun via centrifugation (13,000 g for 5 minutes) for plasma extraction. Vials containing plasma were stored on ice until frozen at -80° C. Remaining whole blood and plasma were stored for use in other projects. Lactate values were measured on plasma by adding 10 μ l of plasma to a lactate meter (Lactate Pro LT-1710 portable lactate analyzer; Arkray Inc., Kyoto, Japan; see Cooke et al. 2008 for validation study with fish). Though the lactate meter is designed for use with whole blood, plasma can also be utilized by fitting values to an equation ($y = 0.8202x + 0.1292$, $R^2 = 0.9874$) to obtain corrected numbers (authors unpublished data).

Statistical Analysis

Lactate values were log transformed while glucose values were transformed by ranking fractions then taking the arc sin of the square root of values to obtain normality and homogeneity for statistical analysis. Generalized linear models (GLMs) were used to explore potential relationships among blood parameters (glucose, lactate, and hematocrit) and the independent variables fight time, shark species, and shark length. A stepwise approach was taken starting with fully saturated models and then removing factors with p-values > 0.05. Final models included all significant factors and second order

interactions. Mean ranks were generated to examine for mean species-specific differences (if any) after controlling for fight time and/or size effects. An ordinal logistic regression was used to explore potential relationships among probability of impairment scores and the independent variables fight time, shark species, and shark length. Parameter estimates were produced from dummy variables and log odds were examined for differences among categorical variables. Binomial logistic regressions were used to explore potential relationships between individual reflexes (retrieval, nictitating, jaw, gill, flex) and glucose, lactate, hematocrit, and fight time. Kruskal-Wallis H tests were used to explore differences among species type for glucose, lactate, hematocrit, and probability of impairment scores. First and second order regression analysis was employed to test for possible effects of fight time on the four parameters. Only species with $n \geq 5$ were included for each statistical test. All statistical analysis was performed using SPSS (Ver. 24, 2016) and statistical significance was declared at $p < 0.05$.

Results

Fight times ranged from 1-128 minutes (mean \pm SD = 45.4 ± 29.1) for 116 individual sharks of 9 species. An additional 107 individuals were sampled without hook timers for a total of 223 blood and reflex-sampled sharks (Table 1). This range represents a robust distribution of fight times and thus levels of capture stress for the sharks in this study. Sharks sampled ranged from small to large body plans with species mean sizes (total length, cm \pm SD) as follows: blacknose (121.0 ± 4.0), blacktip (157.1 ± 16.2), sandbar (202.2 ± 10), nurse (220.8 ± 38.2), tiger (225.3 ± 53.6), lemon (228.4 ± 33.2), bull (240.8 ± 25.6), scalloped hammerhead (267.6 ± 28.8), and great hammerhead (269.2 ± 44.7). Six juvenile sharks were sampled (lemon (2), nurse (3), tiger (1)) while the

remaining in the study were either sub-adults or adults based on published data on length versus maturity state (Compagno et al. 2005).

Modeling Parameter Effects on Physiology

The influence of fight time, species, size, and their interactions on each blood parameter was assessed through stepwise generalized linear models (GLM). Only species with ≥ 8 individuals that exhibited values for all independent and dependent variables were included in the analysis. As a result, each blood parameter was assessed with a different grouping of species; glucose: blacktip (45), bull (12), great hammerhead (12), and nurse (21); lactate: blacktip (17) and nurse (14); hematocrit: blacktip (30), great hammerhead (8), and nurse (12; Table 2). Species effects were significant in the final models for all three blood parameters ($p < 0.05$), whereas size was not ($p > 0.05$; Table 3). Glucose significantly increased with fight time ($p < 0.05$), but no such relationship was detected for hematocrit ($p > 0.05$; Table 3). The interaction between fight time and species had a significant effect on the final model for lactate values ($p < 0.05$), but non-significant effects for glucose and hematocrit ($p > 0.05$; Table 3).

Modeling Parameter Effects on Reflex Behavior

The effects of fight time, species, size and their interactions on the probability of impairment were assessed through ordinal logistic regression. Two variations of the model were produced to alleviate the discrepancies between the probability of impairment values for nurse sharks and the remaining species. Nurse sharks lack a nictitating membrane and therefore formed dissimilar impairment probabilities than other species (see Table 4 for clarification). For nurse sharks, the final model could not significantly predict the probability of impairment ($X^2(2) = .396, p > 0.05$), therefore,

fight time and size had no significant effect on the probability of impairment (Wald $X^2(1) = .198, p > .05$ & Wald $X^2(1) = .235, p > 0.05$ respectively). For blacktip, bull, great hammerhead, and sandbar sharks, the final model could significantly predict the probability of impairment ($X^2(5) = 19.144, p < .005$), indicating that at least one of the independent variables was significant (Table 5). An increase in fight time (expressed in minutes) was associated with an increase in the odds of a higher probability of impairment, with an odds ratio of 1.018 (95% CI, 1.000 to 1.036), Wald $X^2(1) 4.028, p < 0.05$ (i.e. with every minute on the line, the probability of impairment increases 1.018 times).

Furthermore, species effects significantly influenced the probability of impairment with log ratios indicating sandbar sharks have a significantly lower probability of impairment when compared to blacktip, bull, and great hammerhead sharks (Figure 2). As a result, sandbar sharks were used as the reference category in the model. Model log ratios revealed that bull, blacktip, and great hammerhead sharks are more likely to have a higher probability of impairment (19.71, 26.51, and 48.02 times respectively) when compared to sandbar sharks (Figure 2). Shark size and interaction terms were non-significant ($p > 0.05$).

Parameter Effects on Individual Reflexes

Each reflex was evaluated against fight time and blood parameters through binomial logistic regressions. All species with available data were pooled together to test each reflexes ability to reflect the physiological status of sharks in general and to satisfy minimum sample size requirements for binomial logistic regressions. With the independent variables fight time, glucose, lactate, and hematocrit in the model, lactate

was statistically significant, indicating that an increase in lactate levels significantly predicted the reflex impairment for 4 of the 5 reflexes (all except for nictitating membrane; $p < 0.05$). Fight time, glucose, and hematocrit were all non-predictive for impairment for the entire set of assessed reflexes (Table 6).

Each reflex was evaluated against variables independently for a one versus one model. This was done to examine the relative predictability of each variable without the influence of others in the model. Fight time significantly predicted the impairment of jaw and gill reflexes, while hematocrit significantly predicted only the jaw reflex. Glucose significantly predicted the impairment of jaw and flex reflexes. Finally, lactate significantly predicted the impairment of the retrieval, jaw, gill, and flex reflexes. (Table 7).

Comparing Physiology and Reflex Impairment

Mean values (mmol/L \pm SD) of glucose for species included in analyses were: scalloped hammerhead (10.7 ± 1.4), great hammerhead (10.2 ± 1.4), tiger (8.9 ± 2), blacknose (6.6 ± 1.5), blacktip (5.2 ± 1), lemon (5.1 ± 0.8), sandbar (4.6 ± 0.9), bull (3.4 ± 1.1) and nurse (1.9 ± 0.4 ; Table 8). Mean ranks of glucose were statistically significantly different between species ($X^2(8) = 172.148, p < 0.001$). Pairwise comparisons were performed using Dunn's (1964) procedure with Bonferroni correction for multiple comparisons. Values below in parenthesis are mean ranks unless otherwise stated. Post hoc analyses revealed statistically significant differences in glucose mean rank between bull (93.29) and tiger (192.83), great hammerhead (199.53), scalloped hammerhead (204.40), and sandbar (111.06) sharks, as well as between blacktip (133.93)

and great hammerhead (Figure 3). Nurse sharks (34.61) were significantly lower compared to all other species (Figure 3).

Mean values (mmol/L \pm SD) of lactate for species included in analyses were: blacktip (6.3 \pm 3.4), great hammerhead (5.6 \pm 2.8), sandbar (3.5 \pm 2.2) and nurse (1.2 \pm 0.5; Table 9). Mean values of lactate were statistically significantly different between species ($X^2(3) = 42.082, p < 0.001$). Post hoc analyses revealed statistically significant differences in lactate mean rank between nurse (13.66) versus sandbar (34.91), great hammerhead (44.70), and blacktip (45.61; Figure 4). Nurse sharks had significantly lower lactate values than all other species while blacktip sharks had the highest.

Mean values (percent \pm SD) of hematocrit for species included in analyses were: great hammerhead (26.8 \pm 5.2), bull (26.4 \pm 4.8), blacktip (25.5 \pm 4.1), sandbar (24.3 \pm 5.4) and nurse (20 \pm 3.9; Table 10). Mean ranks of hematocrit were statistically significantly different between species ($X^2(4) = 30.642, p < 0.001$). Post hoc analyses revealed statistically significant differences in hematocrit mean rank between nurse (30) versus blacktip (63.59), bull (68.06), and great hammerhead (72.75; Figure 5). Great hammerhead sharks had the highest hematocrit values while nurse sharks had the lowest.

Mean scores (proportion \pm SD) of the probability of impairment for species included were: great hammerhead (0.45 \pm 0.17), blacktip (0.43 \pm 0.26), bull (0.32 \pm 0.28), sandbar (0.17 \pm 0.22), and nurse (0.06 \pm 0.13; Table 11). Mean values for the probability of impairment were statistically significantly different between species ($X^2(4) = 37.807, p < 0.001$). Post hoc analyses revealed statistically significant differences in the probability of impairment mean rank between nurse (25.86) versus blacktip (62.47) and great hammerhead (65.17), and sandbar (36.82) versus blacktip (Figure 6). Nurse and

sandbar sharks had the lowest probabilities of impairment while blacktip and great hammerhead had the highest.

Effect of Fight Time on Physiology and Reflex Behavior

Species-specific comparisons

Species with sample sizes ≥ 8 individuals were examined for potential species-specific differences in the effect of fight time on the blood and reflex parameters. For blacktip sharks, regression analyses established a significant second order quadratic relationship for both glucose and lactate ($F(2,42) = 8.758, p = 0.001, R^2 = 0.294$ and $F(2,14) = 13.956, p < 0.000, R^2 = 0.666$ respectively; Figures 7,8). There was no relationship between fight time and hematocrit nor the probability of impairment for blacktip sharks (Table 12). In great hammerhead sharks, probability of reflex impairment significantly increased with fight time, $F(1,7) = 5.789, p = .047, R^2 = 0.453$ (Figure 9). There were no significant relationships between fight time and glucose (Table 13). I did not detect a significant relationship between any of the blood or reflex parameters and fight time for nurse sharks.

Discussion

Capture elicits a complex set of responses that can alter both an animal's physiology and behavior. Although these responses allow animals to survive day to day activities, their continuation can impede normal function and have the potential to lead to mortality. The responses involved span several systems (circulatory, respiratory, and muscular) and their severity seems inherently linked with the duration of capture (Cliff and Thurman 1984, Manire et al. 2001, Gallagher et al. 2014b). By examining both the physiological and reflex responses to a standardized experimental fishing technique, I

documented differences in the stress response to capture among multiple shark species as well as the potential factors that affect these responses.

Physiological and reflex impairment differences among species emerged as a primary finding in this study. Interspecific trends in the physiological response of sharks has been well documented (Manire et al. 2001, Mandelman and Skomal 2009, Frick et al. 2010, Marshall et al. 2012, Gallagher et al. 2014b), and this work is consistent with these patterns. The final models for glucose, lactate, hematocrit and the probability of impairment all revealed significant species effects. These results along with the comparison of mean values among species further highlights these interspecific differences and revealed a general pattern in regards to species overall sensitivity to capture (from lowest to highest) with exceptions: nurse, bull, sandbar, blacktip, scalloped, and great hammerhead (Tables 8-11). Nurse sharks routinely revealed significantly low values that are representative of a negligible physiological stress response to capture. This species often did not trigger hook timers (data not included in analysis) which suggests reduced behavior once hooked and consequently a smaller stress response. Even with fight times of up to 70 minutes, blood and reflex parameters did not increase significantly and remained the lowest of any species reported. This is consistent with recent data revealing that this species does not behaviorally respond by fighting hard when captured, but rather settles on the bottom (Gallagher et al. 2016). This is likely because nurse sharks are not an obligate ram ventilator and also have a low metabolic rate (Whitney et al. 2016). Additionally, nurse sharks had the lowest values for their probability of impairment. This is again likely related to their biology which influences their behavior during capture. Bull sharks showed significantly lower glucose levels

when compared to tiger, great hammerhead, and scalloped hammerhead sharks with values similar to that of Manire et al. (2001). Bull sharks are a coastal species compared to the semi-pelagic nature of tiger, great, and scalloped hammerheads which may attribute to their lower glucose levels due to a lower metabolic rate as a result of a reduced need to sustain long distance swimming. Their potentially lower metabolic rate would not require as much energy for daily activities and thus may be an explanation of their lower glucose levels compared to the semi-pelagic species. Similarly, Marshall et al. (2012) found significantly lower glucose values in coastal species when compared to pelagic. While research on the effects of capture stress on the physiological status of great hammerhead sharks is limited (Gallagher et al. 2014b, this study), this species consistently has among the most extreme metabolite values reported and were the only species in the current study where fight time significantly affected their probability of reflex impairment. The increased probability of impairment is likely a result of a heightened capture stress response. Indeed, this species has been shown to exhibit pronounced “fighting” reactions when on a fishing line, characterized by repeated burst accelerations (Gallagher et al. 2016). This data along with their high metabolite values agrees with the available data on at-vessel mortality rates for large hammerheads, which can be upwards of 90% (Morgan and Burgess 2007, Gallagher et al. 2014a). Interestingly, blacktip sharks had a significantly higher probability of reflex impairment compared to sandbar sharks despite no significant differences in their blood parameters. The higher probability of impairment may be due to the behavioral actions this species exhibits during capture. Blacktip sharks showed the highest forces (in g’s) for their maximum acceleration during capture compared to nurse and tiger sharks (Gallagher et

al. 2016). The authors also found a positive and significant relationship between maximum acceleration and plasma lactate, suggesting that shark behavior during capture likely results in physiological stress reflected through lactate. This response may have had carry-over effects on their reflex impairment assessments. While there are no current published studies on sandbar behavior during capture, Brill et al. (2008) saw that oxygen delivery following exercise was likely not compromised, suggesting that hook and line capture and release do not increase mortality rates. This supports Gallagher et al. (2014a) estimate that over 75% of sandbars caught and released in tuna and swordfish commercial longlines survive. Additionally, (Morgan and Burgess 2007) reported a large difference in at-vessel mortality for these two species with blacktips showing 88% mortality compared to 36.1% for sandbars. The combination of this data suggest that reflex impairments for these species may be a better predictor of mortality than the physiological parameters used. Danylchuk et al. (2014) saw that mortality was higher for individuals with higher impairment. While reflex impairments have not been significantly related to mortality in sharks, these studies show promising results that point to the potential use of reflex impairments as indicators of mortality.

Due to the complex nature of the capture stress response, it is important to consider multiple factors in analyzing blood and reflex behavior, including their interactions with one another. The final model for glucose distinguished a significant relationship between glucose and fight time, revealing that glucose levels apparently peaked between 50 and 80 minutes of fight time before decreasing. The initial increase is likely attributed to the hormonal component of the stress response wherein glucocorticoid stress hormones are released that activate glycogen stores to be dispersed from the liver

to satisfy the increased energetic demand that a stressor causes (Cliff and Thurman 1984, Hoffmayer and Parsons 2001, Frick et al. 2010). The following period of hypoglycemia is consistent with other findings (Manire et al. 2001, Frick et al. 2010, Brooks et al. 2011) and may suggest a complete utilization of the glucose mobilized through the stress response or a total depletion of hepatic glycogen stores, or a combination of the two. The final model for lactate detected a significant interaction between fight time and species, of which blacktip and nurse sharks were included. This interaction reflects the relationship that each species exhibits between lactate and fight time; lactate increased with fight time in blacktips while it does not in nurse sharks. An increase in lactate due to continued anaerobic respiration as capture stress prolongs has been well documented across multiple species of shark and capture techniques (Cliff and Thurman 1984, Hoffmayer and Parsons 2001, Manire et al. 2001, Brill et al. 2008, Gallagher et al. 2014b). In the current study, regression analyses for blacktip sharks revealed significant second order quadratic relationships between lactate and fight time. Previous work on capture stressed blacktips have revealed more linear trends in the relationship between fight time and lactate as well as significantly higher mean values. Manire et al. (2001) saw a steady increase in lactate for blacktip sharks subjected to gill net capture, however this capture technique fully restrains animal movement which prevents respiration in the obligate ram ventilating blacktip, ultimately leading to the production of lactate immediately upon capture. Additionally, mean lactate values for blacktip sharks captured on commercial longline gear was 36.8 mmol/L compared to 6.3 mmol/L found in this study (Marshall et al. 2012). These results, however, reflect soak times ranging from 4 to 12 hours without delineation. Similar to this study, Brooks et al. (2011) saw a parabolic

trend with lactate values in longline captured Caribbean reef sharks (*Carcharhinus perezii*) with peak values attained at 120 – 180 minutes of hooking. In the present study, max fight time for any individual where lactate was collected was 96 minutes which may suggest that given longer fight times, a stronger parabolic trend would be seen. The parabolic trend seen in this study may be attributed to the capture method used. The experimental drumline system was designed to reduce stress in sharks and promote recovery compared to other fishing methods such as gill nets and longlines. The sharks in this study may have had a chance to recover after bouts of burst swimming that initiated the increase in lactate levels and subsequently the following decline.

Fight time and shark size were non-significant effects for hematocrit. Hematocrit values tend to increase in response to capture stress in teleost fish and have been used as an indicator of the stress response (Wells and Weber 1990, Frisch and Anderson 2000, Roth and Rotabakk 2012). In sharks, hematocrit values rarely correlate with fight times although Brill et al. (2008) did find that hematocrit levels rose significantly in exercise stressed juvenile sandbar sharks. Body size can impact physiological responses in terms of available energy such as glycogen stores. In both teleost and elasmobranchs, size has been significantly correlated with glucose levels and suggests that larger fish are at an advantage due to higher volumes of energy available in the form of glycogen. (Ferguson et al. 1993, Meka and McCormick 2005, Gingerich and Suski 2012, Barkley et al. 2016). However, shark size was non-significant for glucose, lactate, hematocrit and the probability of impairment in this study. The final model for the probability of impairment index revealed that fight time was significant, indicating that longer fight times were associated with more impaired reflexes. Throughout this study and others (Cliff and

Thurman 1984, Manire et al. 2001, Brill et al. 2008, Brooks et al. 2011, Gallagher et al. 2014b, Danylchuk et al. 2014, French et al. 2015), the duration of capture continues to emerge as a significant factor in dictating the severity of key physiological stress response indicators and now shows promise for predicting probability of impairment in sharks. The relatively weak relationship found in the current study, however, may be attributed to a relatively small sample size with required pooling of all species in analysis. It has been well demonstrated that there are species specific differences in the capture stress response in sharks (Manire et al. 2001, Mandelman and Skomal 2009, Frick et al. 2010, Marshall et al. 2012, Gallagher et al. 2014b) and it is therefore likely that reflex impairments also differ. Evaluating these reflex indicators on a species level is needed to fully understand their response to capture stress.

When probing reflex impairments further, I saw that increases in lactate were associated with an increased likelihood of impaired retrieval, jaw, gill and flex reflexes. While Manire et al. (2001) was able to demonstrate that lactate values increased as release condition decreased for blacktip and bonnethead sharks, this is the first study to date that successfully links increases in blood lactate with increased reflex impairment for any shark species. In order to get a better understanding of each reflex, fight time and blood parameters were assessed to reflexes on an individual basis. As a result, the jaw reflex yielded the greatest number of significant effects with all blood parameters and fight time increasing impairment. In juvenile lemon sharks, Danylchuk et al. (2014) found reflex indices to be positively correlated with post-release mortality with the congruent “BITE” reflex impaired in three of their four post-release mortality occurrences. Together, this data suggests that reflex indices involving the mouth of

sharks may be most suitable at reflecting both physiological disruptions associated with the stress response and delayed mortality. Although interspecific differences likely exist in reflex impairments, statistical test sample size requirements and in some cases lack of variation among reflex impairment levels impeded species-specific examination.

However, visually inspecting the data revealed interesting trends. Nurse shark jaw and gill reflexes were never impaired despite there being a significant relationship between these reflexes and fight time in the pooled analysis. During prolonged capture, physiological strains require sharks to switch to anaerobic respiration which doesn't require oxygen. Impaired jaw and gill reflexes with higher fight times coincide with the inability to respire. The lack of this relationship in nurse sharks is congruent with the previously described behavior during capture and metabolism for this species. Blacktip, great hammerhead, and sandbar sharks retrieval reflexes were rarely impaired in this study. In fact, the retrieval reflex was the second least impaired reflex (14.1% overall impairment), just behind nictitating (11.5%). For the retrieval reflex to be impaired, sharks must display little movement upon retrieval with no bouts of burst swimming. The relatively small number of impaired individuals suggests that glucose levels remained elevated without depleting hepatic stores as glucose is primarily used for muscle activation. This aligns with the previous results that indicated increases in fight time are correlated with higher glucose levels. In this study, sharks likely did not reach complete depletion of glycogen stores because of the relatively short fight times and benign capture method used. Additionally, the only parameter that significantly affected the retrieval reflex was lactate, with elevated lactate predicting higher impairment. Since capture time was non-significant for the retrieval reflex, the high lactate levels associated with

impaired vigor could point to either individual shark differences in physiology or possible compounding parameters that exacerbate the stress response such as water contamination or previous interactions with fisheries.

Chapter 3: Limitations, Future Directions, and Conclusions

This study is not without limitations as sample sizes for certain species precluded an in-depth analysis for some of the relationships tested here. In addition, the absence of control data to represent a state of resting or minimally stressed animals makes it difficult to infer the extent to which disturbances were actually affected by the capture event. Moreover, without directly linking these physiological and reflex statuses to mortality, I can only infer based on previous literature and the results from this study that increases in lactate and reflex impairment relate to post-release mortality. Lastly, the capture method used for this study (experimental drumline) does not fully reflect the capture methods used in recreational and commercial fisheries (rod and reel, gill net, longline, trawl, etc.) and therefore likely produces a more benign capture stress response as it was shown in this study and others that fight time significantly effects the capture stress response in sharks (Cliff and Thurman 1984, Manire et al. 2001, Gallagher et al. 2014b). This fishing method is designed to reduce stress on captured animals through the use of circle hooks that reduce instances of foul hooking as well as a 23 meter main line connected to the drum with a swivel that promotes respiration for obligate ram ventilators throughout the capture duration. In addition, this gear was allowed to soak for a maximum of 1.5 hours, a considerably less amount of time compared to other fishing techniques that can range from 1-12 hours.

Future research should focus on understanding the relationship between mortality and these factors as well as the thresholds of these responses. Additionally, effort should be directed at observing shark behavior at the onset of hooking to better understand its effects on physiological status and reflex impairments. Gallagher et al. (2016) has begun

to unveil species-specific differences in these capture behaviors that are compatible to the species-specific results found in the physiology and reflex impairments in this study.

Future work should look at not only max acceleration during capture but should consider video monitoring as hidden behaviors may be revealed that effect an individual's physiology. Lastly, future work should examine potential recovery mechanisms and times for these species, especially for the reflex impairments. Increased time to recover for these reflexes may point to increased susceptibility to disease, lack of migratory movements, or predation, all of which can lead to increased mortality.

In summary, I demonstrated that both physiological and reflex differences were evident across species in response to capture stress. These differences correspond to the varying at-vessel and post-release mortality rates found in previous studies. Here, sandbar sharks were found to have a low probability of reflex impairment compared to other species which aligns with the estimated 75% survival rate of this species (Gallagher et al. 2014a). Similarly, blacktip sharks showed an elevated response in both physiological and reflex responses compared to the other species. These results correspond with Morgan and Burgess (2007) who found that 88% of blacktip sharks experience at-vessel mortality from bottom longlines. As one of the few studies that have evaluated the response of nurse sharks to capture, I revealed that this species exhibits a relatively low physiological and reflex impairment response compared to the other species examined. In contrast, great hammerhead sharks consistently displayed the most extreme values in both the physiological and reflex impairment parameters accessed. With their high at-vessel mortality rates in commercial fisheries (Morgan and Burgess 2007) and their potential for post-release mortality (Gallagher et al. 2014b), this species is especially vulnerable to

capture stress and should be avoided by recreational and commercial fishers when possible. Capture duration continues to be a significant factor when evaluating the extent of the stress response and this study provides a further link to its effect on reflex impairments. Finally, the use of a reflex index has shown potential for reflecting shark physiological status with a direct relationship with lactate values. Reflex impairments can be ecologically significant measures that are capable of responding proportionally to the severity of many types of stressors and have been correlated with delayed mortality in teleost fishes (Davis 2005, Davis and Ottmar 2006). Although few studies have evaluated reflex impairments in sharks (Danylchuk et al. 2014, Gallagher et al. 2014b), data shows promising results that these indices, particularly the jaw response, may be able to predict mortality in some species. Future research should be directed at exploring this relationship further as it pertains to individual species and concurrently to mortality of those individuals.

TABLES

Table 1: Summary of replication for physiological samples and reflex assessments and fight time range for all of the species included in the present study.

Species (N)	Glucose (mmol/L)	Lactate (mmol/L)	Hematocrit (%)	Reflexes	Fight Time Range (min)
Blacknose (5)	5	0	2	4	6 - 58
Blacktip (74)	71	18	41	31	8 - 106
Bull (17)	17	1	8	8	3 - 128
Great Hammerhead (17)	16	5	10	12	4 - 100
Lemon (10)	10	1	3	2	4 - 100
Nurse (67)	66	25	34	28	1 - 100
Sandbar (22)	17	11	12	14	34 - 106
Scalloped Hammerhead (5)	5	4	4	4	29 - 51
Tiger (6)	6	1	2	3	22 - 83

Table 2: Species included for each dependent variable in the stepwise univariate GLMs.

	Lactate (mmol)	Glucose (mmol)	Hematocrit (%)
Species included (N)	blacktip (17) nurse (14)	blacktip (45) bull (12) great hammerhead (12) nurse (21)	blacktip (30) great hammerhead (8) nurse (12)

Table 3: Summary of significant results from stepwise univariate GLMs. Significance (*) declared at $p < 0.05$. Non-significant terms are indicated by "ns".

	Lactate (mmol)	Glucose (mmol)	Hematocrit
Fight time	*	*	ns
Species	*	*	*
Size	ns	ns	ns
Fight time x Species	*	ns	ns

Table 4: Summary of potential impairment probabilities for blacktip, bull, great hammerhead, sandbar, and nurse sharks.

	Blacktip, Bull, Great Hammerhead, Sandbar	Nurse
Potential Impairment Probabilities	0	0
	0.2	0.25
	0.4	0.5
	0.6	0.75
	0.8	1
	1	-

Table 5: Summary of significant results from ordinal logistic regression including blacktip, bull, great hammerhead, and sandbar sharks. Significance (*) declared at $p < 0.05$. Non-significant terms are indicated by "ns".

	Probability of Impairment
Fight time	*
Species	*
Size	ns

Table 6: Summary of significant results from binomial logistic regressions including blacktip, bull, great hammerhead, lemon, nurse, sandbar, scalloped hammerhead, and tiger sharks. Regressions were run for each variable independently. Significance (*) declared at $p < 0.05$. Non-significant terms are indicated by "ns".

	Retrieval	Nictitating	Jaw	Gill	Flex
Fight time	ns	ns	ns	ns	ns
Glucose	ns	ns	ns	ns	ns
Lactate	*	ns	*	*	*
Hematocrit	ns	ns	ns	ns	ns

Table 7. Summary of significant results from binomial logistic regressions including blacktip, bull, great hammerhead, lemon, nurse, sandbar, scalloped hammerhead, and tiger sharks. Regressions were run for each variable independently. Significance (*) declared at $p < 0.05$. Non-significant terms are indicated by "ns".

	Retrieval	Nictitating	Jaw	Gill	Flex
Fight time	ns	ns	*	*	ns
Glucose	ns	ns	*	ns	*
Lactate	*	ns	*	*	*
Hematocrit	ns	ns	*	ns	ns

Table 8: Summary of species sampled for glucose with mean \pm SD values.

Species (N)	Glucose (mmol/L) mean \pm SD
Scalloped Hammerhead (10)	10.7 \pm 1.4
Great Hammerhead (16)	10.2 \pm 1.4
Tiger (6)	8.9 \pm 2
Blacknose (5)	6.6 \pm 1.5
Blacktip (71)	5.2 \pm 1
Lemon (10)	5.1 \pm 0.8
Sandbar (17)	4.6 \pm 0.9
Bull (17)	3.4 \pm 1.1
Nurse (66)	1.9 \pm 0.4

Table 9: Summary of species sampled for lactate with mean \pm SD values.

Species (N)	Lactate (mmol/L) mean \pm SD
Blacktip (18)	6.3 \pm 3.4
Great Hammerhead (5)	5.6 \pm 2.8
Sandbar (11)	3.5 \pm 2.2
Nurse (25)	1.2 \pm 0.5

Table 10: Summary of species sampled for hematocrit with mean \pm SD values.

Species (N)	Hematocrit (%) mean \pm SD
Great Hammerhead (10)	26.8 \pm 5.2
Bull (8)	26.4 \pm 4.8
Blacktip (41)	25.5 \pm 4.1
Sandbar (12)	24.3 \pm 5.4
Nurse (34)	20 \pm 3.9

Table 11: Summary of species sampled for probability of impairment with mean \pm SD values.

Species (N)	Reflexes (proportion) mean \pm SD
Great Hammerhead (12)	0.45 \pm 0.17
Blacktip (31)	0.43 \pm 0.26
Bull (8)	0.32 \pm 0.28
Sandbar (14)	0.17 \pm 0.22
Nurse (28)	0.06 \pm 0.13

Table 12: Summary of significant results from linear regressions testing fight time effects for blacktip sharks. Significance declared at $p < 0.05$ for first order (1*) and second order quadratic (2*) regressions. Non-significant terms are indicated by "ns".

	Lactate (mmol)	Glucose (mmol)	Prob. of Impairment	Hematocrit (%)
Fight time	2*	2*	ns	ns

Table 13: Summary of significant results from linear regressions testing for fight time effects for great hammerhead sharks. Significance declared at $p < 0.05$ for first order (1*) and second order quadratic (2*) regressions. Non-significant terms are indicated by "ns". N/A indicates insufficient sample size.

	Lactate (mmol)	Glucose (mmol)	Prob. of Impairment	Hematocrit (%)
Fight time	N/A	ns	1*	ns

FIGURES

Figure 1: Drumline fishing apparatus: (a) bullet floats; (b) large floating poly-ball; (c) rope attaching float to submerged weight; (d) ~18kg cement weight; (e) hook timer; (f) main line of ~410 kg test monofilament; (g) 4 m double-stranded leader of ~410 kg test monofilament; (h) 16/0 5°-offset circle hook. The distance between (e) and (h) is 23 m, and the length between (a) and (d) ranged from 30 to 60m. (Gallagher et al. 2014b)

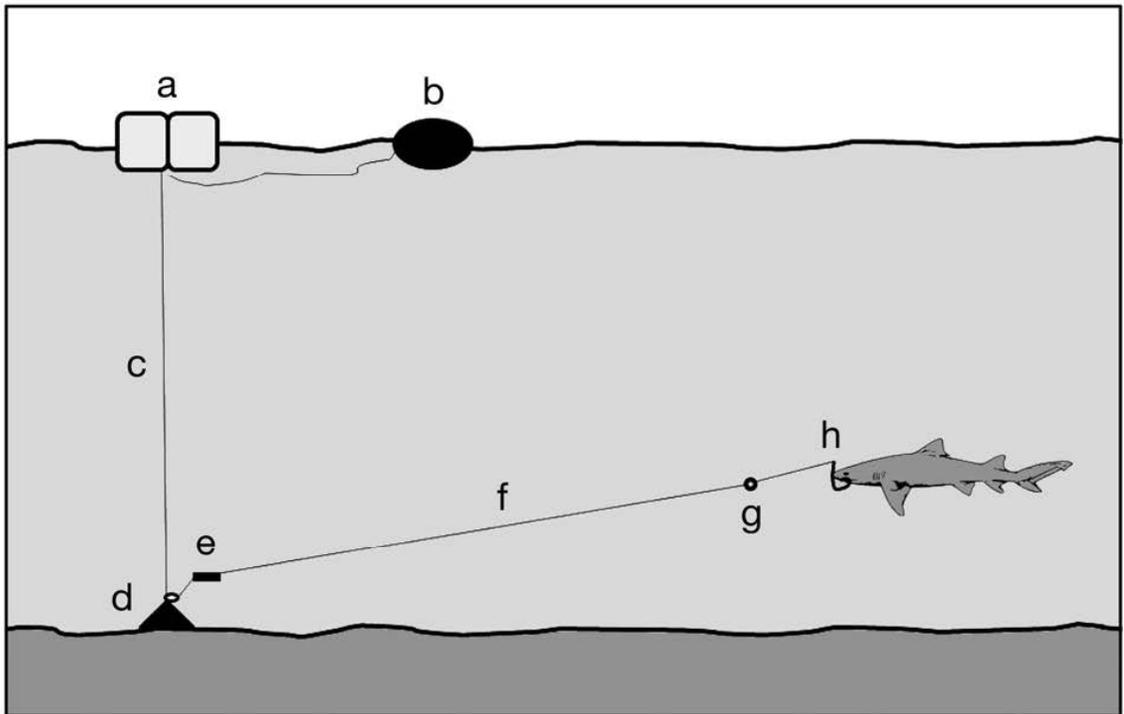


Figure 2: Log ratio estimates of the probability of impairment with sandbar sharks as the reference category. * indicates significant difference from reference category.

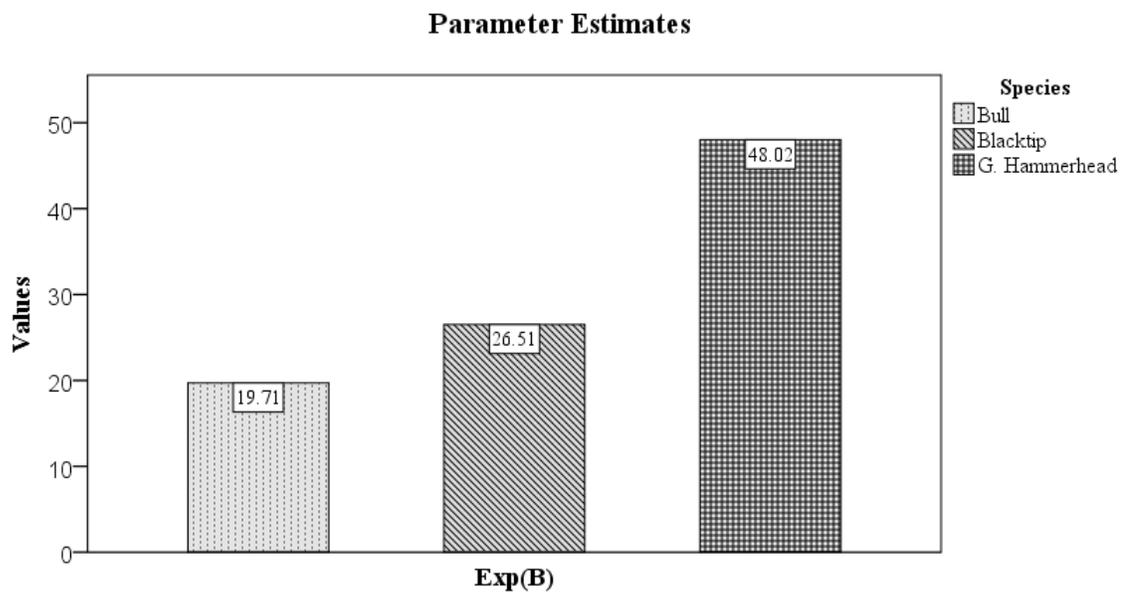


Figure 3: Mean rank values for glucose for each species of shark. Solid and dashed lines represent significant differences between groups. * indicates significant difference between all other groups.

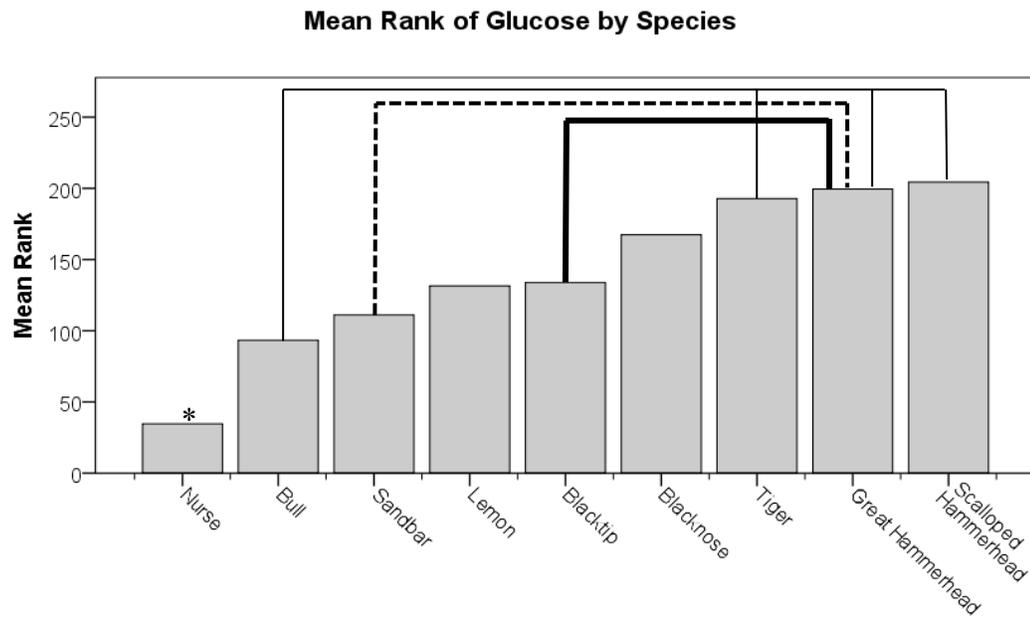


Figure 4: Mean rank values for lactate for each species of shark. * indicates significant difference between all other groups.

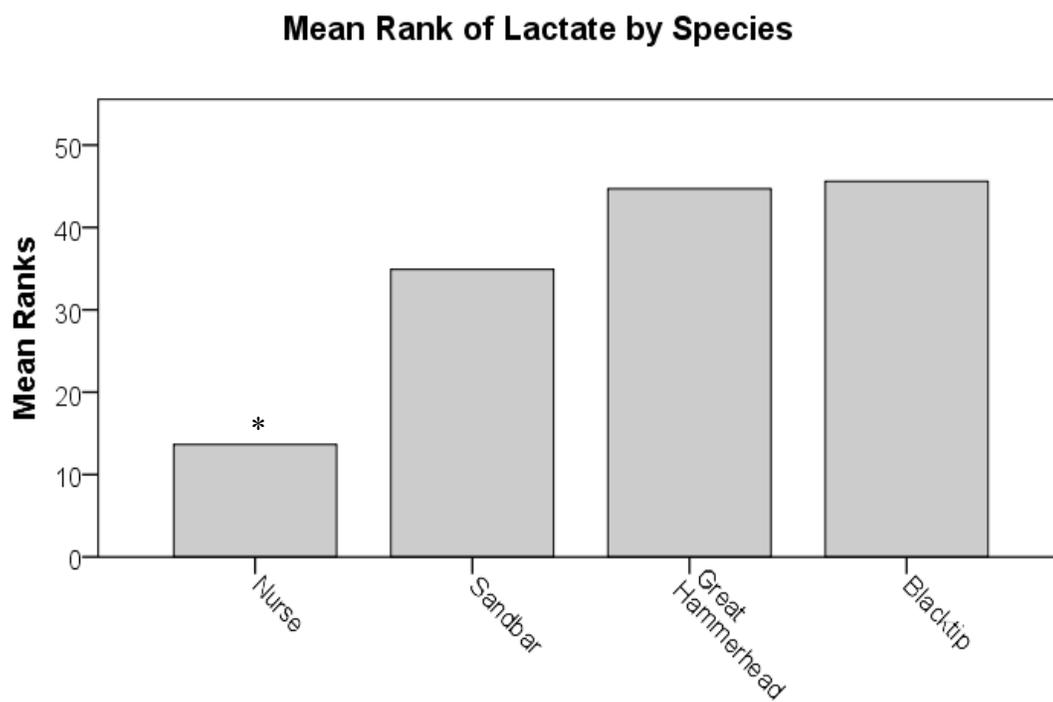


Figure 5: Mean rank values for hematocrit for each species of shark. Solid lines represent significant differences between groups.

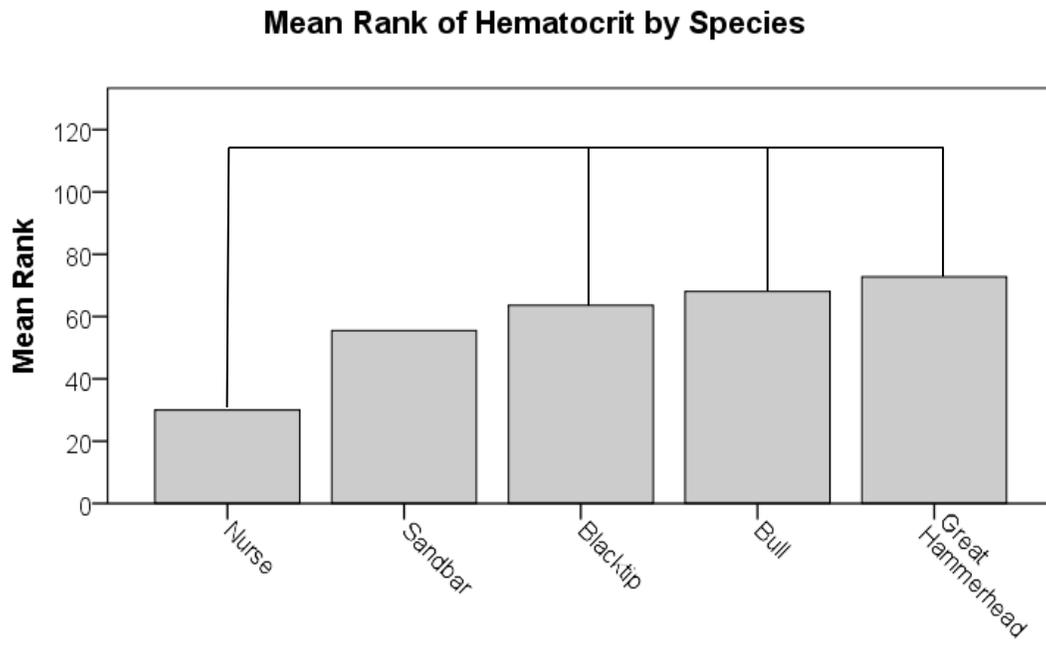


Figure 6: Mean rank values for the probability of impairment for each species of shark.

Solid lines represent significant differences between groups.

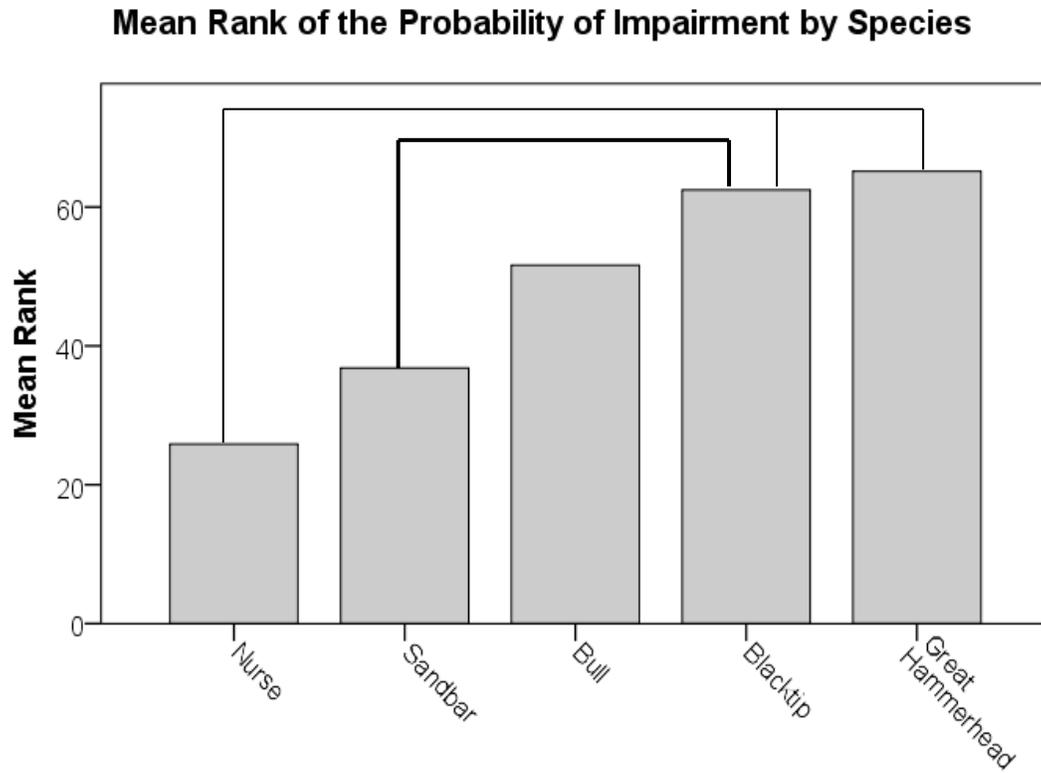


Figure 7: Data represents the relationship between fight time and glucose for blacktip sharks. Curve represents a significant second order polynomial. To promote comparability to other studies, data plotted represents untransformed values in contrast to the transformed values on which statistical analysis was conducted.

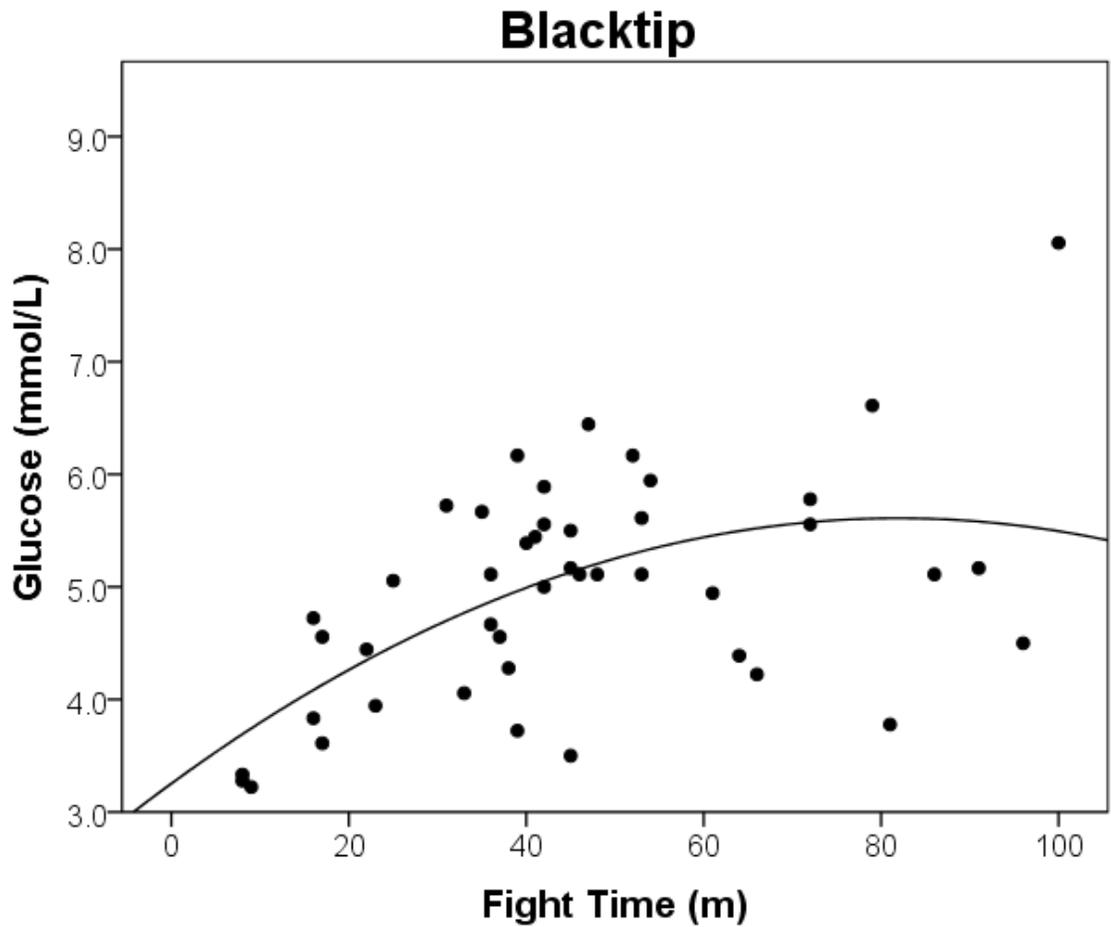


Figure 8: Data represents the relationship between fight time and lactate for blacktip sharks. Curve represents a significant second order polynomial. To promote comparability to other studies, data plotted represents untransformed values in contrast to the transformed values on which statistical analysis was conducted.

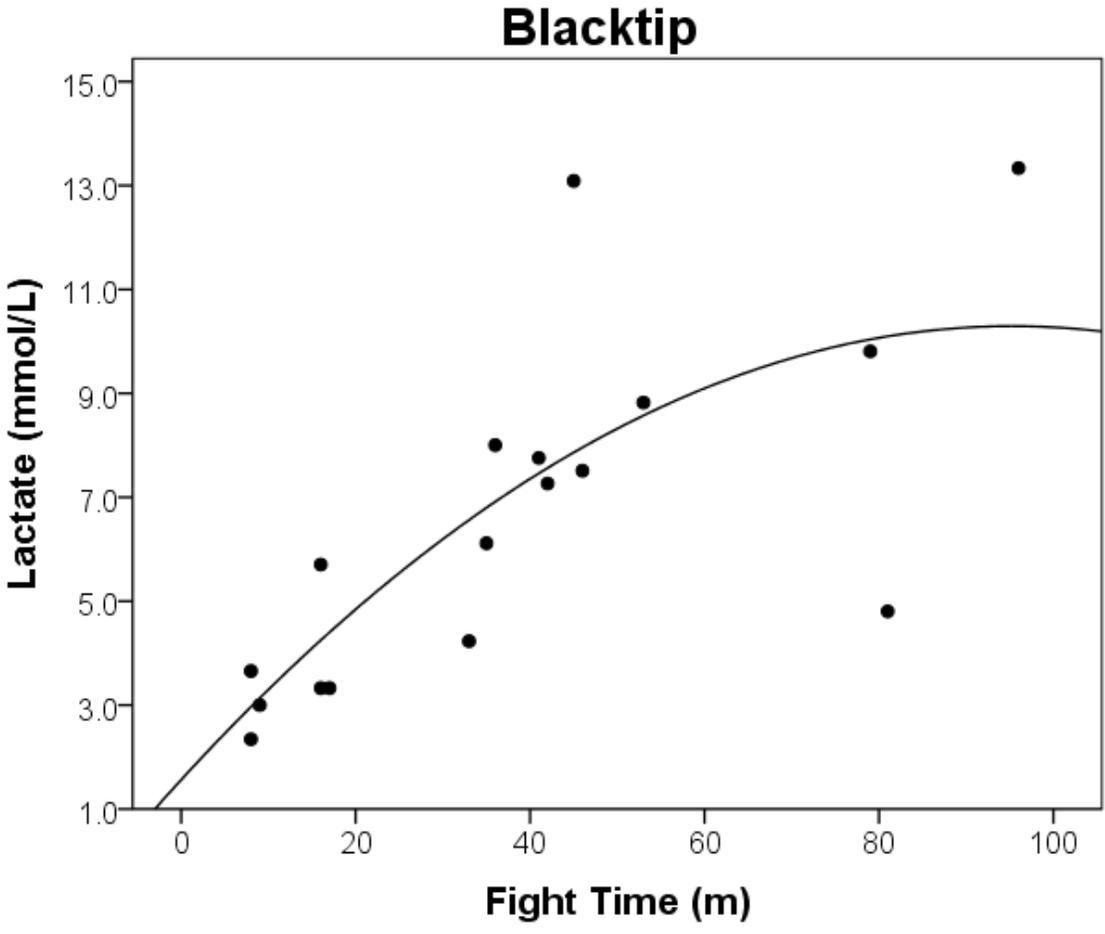
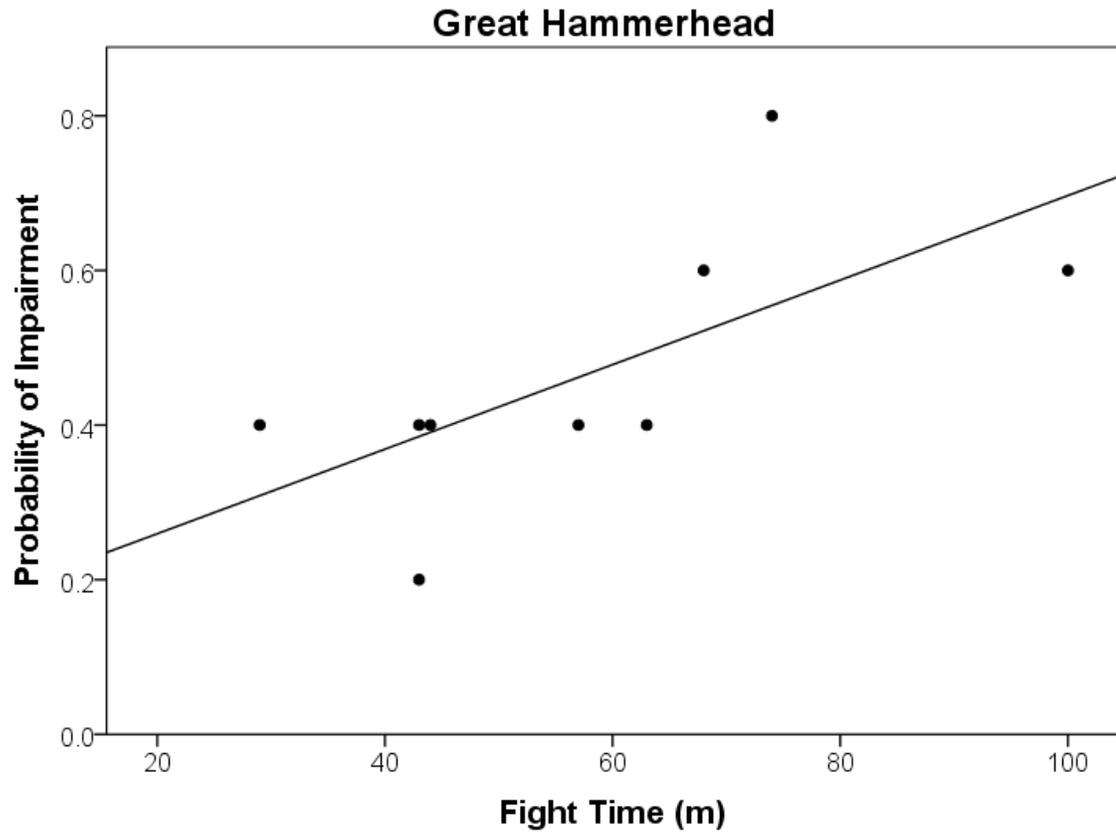


Figure 9: Data represents the relationship between fight time and the probability of impairment for great hammerhead sharks. Fit line represents a significant linear regression.



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