JACK N. AVERITT COLLEGE of GRADUATE STUDIES GEORGIA SOUTHERN UNIVERSITY

Georgia Southern University

Georgia Southern Commons

Electronic Theses and Dissertations

Jack N. Averitt College of Graduate Studies

Fall 2023

Use of Hematological Markers to Assess Physiological Condition and Health Status in Free-Ranging Sand Tiger Sharks (Carcharius taurus)

Chestina N. Craig

Follow this and additional works at: https://digitalcommons.georgiasouthern.edu/etd

 Part of the Comparative and Evolutionary Physiology Commons, Marine Biology Commons, Other Physiology Commons, and the Zoology Commons

Recommended Citation

Craig, Chestina N., "Use of Hematological Markers to Assess Physiological Condition and Health Status in Free-Ranging Sand Tiger Sharks (Carcharius taurus)" (2023). *Electronic Theses and Dissertations*. 2660.
https://digitalcommons.georgiasouthern.edu/etd/2660

This thesis (open access) is brought to you for free and open access by the Jack N. Averitt College of Graduate Studies at Georgia Southern Commons. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of Georgia Southern Commons. For more information, please contact digitalcommons@georgiasouthern.edu.

USE OF HEMATOLOGICAL MARKERS TO ASSESS PHYSIOLOGICAL CONDITION AND HEALTH STATUS IN FREE-RANGING SAND TIGER SHARKS (*CARCHARIUS TAURUS)*

BY

CHESTINA NICOLE CRAIG

(Under the direction of Johanne M. Lewis)

ABSTRACT

The contents of blood can provide information about the physiological condition and health of vertebrates. This study seeks to better understand the stress physiology and blood bacteria presence of the sand tiger shark (*Carcharius taurus)*, as sharks are known to have unique physiology and immune systems. In this study the blood metabolites glucose, lactate, and ketones (3-hydroxybuteric acid and acetoacetate), were used to understand how biotic and abiotic factors affect the acute stress response to capture and handling. Metabolite concentrations from blood plasma were analyzed using colorimetric assays. Glucose and ketones showed no significant responses to capture and handling stressors, while lactate increased with longline soak time. There was an interactive effect of fork length and sex on the ketone acetoacetate at the time of longline capture. Bacteria in the blood of sand tiger sharks was quantified using blood culture methods. Sharks that were positive for blood bacterial growth had near significant lower levels of lactate during capture compared to sharks negative for bacterial growth. Blood bacteria presence did not differ between sexes or across fork lengths. The results from this study demonstrate that while sand tiger sharks respond metabolically to longline capture, they may be physiologically robust to capture. While none of the bacterial results were statistically significant, they represent potentially interesting trends that should be investigated further in future studies. More studies are necessary to better understand the implications of abiotic and biotic factors on the stress response of sand tiger sharks.

INDEX WORDS: Stress physiology, Elasmobranchs, fuel, Capture stress, Anaerobic metabolism, Fishing impacts, Bacteremia, Microbiome, Glucose, Lactate, Ketones, Blood bacteria

USE OF HEMATOLOGICAL MARKERS TO ASSESS PHYSIOLOGICAL CONDITION AND HEALTH STATUS IN FREE-RANGING SAND TIGER SHARKS (*CARCHARIUS TAURUS)*

BY

CHESTINA NICOLE CRAIG

B.S., Marine Biology, California State University Long Beach 2017

A Thesis Submitted to the Graduate Faculty of Georgia Southern University

in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

STATESBORO, GEORGIA

© 2023

CHESTINA N. CRAIG

All Rights Reserved

USE OF HEMATOLOGICAL MARKERS TO ASSESS PHYSIOLOGICAL CONDITION AND HEALTH STATUS IN FREE-RANGING SAND TIGER SHARKS (*CARCHARIUS TAURUS)*

BY

CHESTINA NICOLE CRAIG

Major Professor:	Johanne M. Lewis
Committee:	Lisa D. Brown
	Stephen P. Vives

Electronic Version Approved:
December 2023

DEDICATION

For Dad.

ACKNOWLEDGMENTS

This thesis would not have been possible without the guidance, patience, and kindness of my advisor Dr. Johanne Lewis. I would also like to thank the members of my thesis committee Dr's Lisa Brown and Stephen Vives for their knowledge and assistance with this project. This project would not have been possible without the help of Dr. Kady Lyons, and Bryan Frazier, and the rest of the crew at Georgia Aquarium and South Carolina Department of Natural Resources, thank you for helping me obtain samples. Huge thank you to Georgia Sea Grant and their research trainee program for the funding which made this project possible. Thank you to Tonya Wiley for your help with my Sea Grant application. Dr. Catherine Macdonald, Jake Jerome, and the rest of the crew at Field School, thank you for your hospitality and letting me join you in the field for some experimental sampling. Thank you, Dr. Christine Bedore, for teaching me key shark handling and blood draw skills among other impactful things, you are so deeply appreciated. Thank you to Bronc Rice and Paul Roberts for helping me do the sampling that got my bacteria methods off the ground. Thank you to my undergraduate assistant Eleanor Greenburg for all your help. Huge thanks to Dr. Kelly Young, my undergraduate lab advisor, who I would not have found my way scientifically without. I also would never have gotten into graduate school without the guidance of Dr's Gwen Goodmanlowe, Kristene Parsons, and Joe Bizzaro. Thank you to Dr. Michelle Guidone for your statistical advice. A huge appreciation for Georgia Southern Professors Dr's John Carroll, Danny Gleason, Checo Colon-Gaud, and Risa Cohen. My lab mates Lauren, Bailey, Emily S., and Kennedy, thank you for always being there with assistance and advice. Thank you to GSU graduate students Patrick H., Sidney, Emily K., Sam, Sammi, Josh, Conner, Alex, Mike, Patrick L., Molly, Renee, Luke, Raymond, and Abbie for all your support. Thank you to my family: Mom, Will, and auntie Pam, the fellow family scientist, for letting me cry about lab mistakes with you. Thank you to my fiancée Jay for reading every single word I wrote during grad school and being my most steadfast supporter and editor. I would also like to thank my chosen family who carried me through this degree even from afar: Cole, Sydney, Alison, Megan, Leigh, Zoe, Brooke, Farah, Julia, Lawrence, Eliann, Marc, Kaytie, Gabe, Soli, Chelsey, Nicole, Tyus and so many others, thank you for being people I can come home to. Finally, eternal gratitude for

my dear sweet Wilton, who tried so hard to help me write this document with every paw pace across my keyboard.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

THE PHYSIOLOGICAL RESPONSE OF SAND TIGER SHARKS (*C. TAURUS*) TO LONGLINE CAPTURE, HANDLING, AND SAMPLING

INTRODUCTION

The negative effects of capture and handling on elasmobranchs

Elasmobranchs captured on fishing gear and handled by humans will incur a stress response due to the nature of these interactions. Sharks are often caught and released by recreational anglers (Cooke and Schram, 2007) and make up a large portion of bycatch in commercial fisheries (Oliver et al., 2015). Recreational angling and commercial fishing impacts may be similar as they both involve restriction of movement during capture (due to hooking, restraint, etc.), and air exposure during handling which causes an inability to uptake oxygen if not ventilated with seawater (Cooke and Suski, 2005). Even if the organism survives the capture and handling stress event and is subsequently released, they may incur a decrease of fitness as a result. For elasmobranchs, the consequences of encountering these stressors can vary from brief changes in blood biochemistry, reduced reproductive success, increased chance of predation, or delayed mortality (Skomal and Mandelman, 2012; Cooke and Schram, 2007; Butcher et al., 2015). Populations of species with high fishing mortality levels can be very impacted by capture and handling, but even species with low fishing mortality rates can suffer negative population effects if the species is slow growing with low reproductive output (Shroeder and Love, 2002). Most elasmobranchs are slow growing, with low reproductive output and can be in danger of overexploitation from fishing. Because of the impact that stressors from capture and handling may have on elasmobranch species, it is essential to have a comprehensive understanding of their physiological response to capture and handling to understand the implications fishing pressures can have on individual health, which can impact population health.

The stress response to capture as well as the magnitude of stress, varies across elasmobranch species (Mandelman and Skomal, 2009; Skomal and Mandelman, 2012). Within species the stress response can vary based on sex and age class, female sharks have been seen to display lower concentrations of stress metabolites than that of male sharks, and smaller sharks more frequently experience mortality or take longer to recover from capture stress than their larger conspecifics (Diaz and Serafy, 2005; Ellis et al., 2017; Mandelman and Skomal, 2009; Whitney et al., 2017). The type of handling sharks encounter has been shown to elicit different magnitudes of stress as well, for example being handled in the water alongside the boat elicited a larger stress response in black tip sharks (*Carcharhinus limbatus*) than being handled on deck and ventilated with seawater (Weber et al., 2020). Gear type can also exert stress parameter influence, prior studies have demonstrated that sharks caught in gillnets display a greater biochemical stress response than those caught with longline gear (Hyatt et al., 2012). The duration of the shark's interaction with the gear can also influence stress parameters, as angling time increases, stress parameters often do too (Kneebone et al., 2013). Abiotic environmental factors, such as temperature, dissolved oxygen, and salinity may also influence the stress response of captured sharks, in Hyatt, 2016 sharks caught in warmer waters had higher values of plasma stress parameters. Due to the high variability in the stress response to capture across elasmobranch species, their unique metabolism, and their vulnerability to exploitation by fisheries, it is important to characterize species specific responses to handling and capture across different variables such as handling time, sex, length, health, sampling methods, and abiotic variability to get a full picture of how capture and handling will affect shark species across multiple variables.

The vertebrate stress response

Stress in vertebrates occurs across two different defined timescales: acute and chronic. Acute stress is rapid and short term (typically across minutes to hours), while chronic stress is more long term (days to weeks). Acute stress will affect short term survival while chronic stress can affect feeding ability, reproduction, and longer-term survival. This thesis primarily focuses on the acute stress response.

The stress response is broken down into three core steps: the primary stress response, the secondary stress response, and the tertiary stress response (Figure 1) (Wendelaar Bonga, 1997). The primary stress response involves the release of stress hormones from the HPA (Hypothalamic-Pituitary-Adrenal) axis, then followed by the secondary response which involves changes in blood biochemistry triggered by the stress hormones and anaerobic metabolism if entered (Figure 1). Finally, the tertiary stress response includes behavioral changes and a potential reduction in fitness (Figure 1) (Skomal and Mandleman, 2012). In the vertebrate primary stress response, the first set of hormones secreted are the catecholamines, norepinephrine and epinephrine. These hormones arise within seconds to minutes and are responsible for what is considered the "flight or fight response" (Romero and Butler, 2007). These hormones aid the body in coping with stress by stimulating increased blood flow, increased heart rate, and increased alertness (Romero and Butler, 2007). The second class of hormones involved in the vertebrate stress response are corticosteroids, which include the glucocorticoids (Romero and Butler, 2007). These hormones are released less rapidly than the catecholamines, and their concentrations will increase within minutes to hours of an encounter with a stressor (Romero and Butler, 2007). Catecholamines and corticosteroids can both trigger an increase in the rate of glycogenolysis, which is the process that transforms stored glycogen from liver and muscle tissues into glucose, with the result being an increased concentration of circulating plasma glucose (Figure 2) (Romero and Butler, 2007; DeRoos and DeRoos, 1978). This increase in circulating fuel is characteristic of the secondary stress response in vertebrates. This increased available fuel is intended to aid in withstanding increased energy demands triggered by the stressor. The release of catecholamines also primes the body for exhaustive exercise. If exhaustive exercise is needed to overcome the stressor, the body's oxygen demand may exceed what is available and can result in the accumulation of lactate, a byproduct of anaerobic metabolism. While lactate is not a direct product of the secondary stress response in vertebrates, and is more indicative of metabolic state, it often accompanies the secondary stress response.

Elasmobranch Stress Response

While the physiological stress response is well conserved across most vertebrate species, there are differences between higher vertebrates, teleosts, and sharks (Terrien and Prunet, 2013). The primary stress response in elasmobranchs, like all other vertebrates, involves the release of stress hormones; catecholamines and corticosteroids. Fishes and other vertebrates use cortisol as their primary corticosteroid, while elasmobranchs utilize 1-alphahydroxycortocosterone (1α-OHB), as well as corticosterone at much lower circulating levels (Anderson et al., 2012). In prior studies it was demonstrated that the rise in plasma glucose is proportional to the amount of epinephrine or norepinephrine administered in spiny dogfish (*Squalus acanthias)* and nursehound sharks *(Scyliorhinus stellaris*) (DeRoos and DeRoos, 1978). This elevated circulating plasma glucose can provide locomotive muscles in elasmobranchs with energy to sustain increased movement during stress events (Richards et al., 2003; Speers-Roesch and Treberg, 2010). With some exceptions, rises in plasma glucose have been characterized in many elasmobranch species after encounter with a stressor. In Caribbean reef sharks (*Carcharinus perezi)* caught on midwater longlines, circulating glucose concentrations rose with time on the line, and maxed out at around 120 – 180 minutes (Brooks et al., 2012). In serially sampled lemon sharks (*Negaprion brevirostis*), individuals held in tonic immobility between blood samples had higher blood glucose levels than sharks that were allowed to free swim in between samples (Brooks et al., 2011).

While the use of glucose as an energy source for day-to-day functions and during stress is shared across vertebrate groups, elasmobranchs also utilize ketone bodies as a primary source of energy (Ballantyne, 1997; Speers-Roesh and Treeburg, 2010; Zammit and Newsholme, 1979). This is in contrast with all other vertebrate predator species that rely upon glucose and carbohydrates for most of their aerobic energy and utilize ketone bodies as emergency energy supplies during starvation when glycogen stores become depleted (Veech, 2004). In mammals' ketones are sourced from the liver and adipose tissue, but elasmobranchs do not have adipose tissue and therefore ketone bodies are mostly sourced from their liver (Figure 2) (Ballentyne, 1997). There are three different ketones present in elasmobranchs: 3-hyrdoxybuteric acid or beta-hydroxybutyrate (BHB), acetoacetate (AcAc), and acetone. Elasmobranchs

have capacity for fatty acid oxidation and ketogenesis in their liver but have limited capacity for fatty acid oxidation in most extrahepatic tissues, including cardiac and skeletal muscle, instead opting for ketone body oxidation in this tissue, indicating the importance of ketone bodies as an oxidative fuel in elasmobranchs (Watson and Dickson, 2001; Speers-Roesh and Treeburg, 2010). How ketones are used by different elasmobranch species is not entirely clear, but it is thought that ketone usage is species specific (Speers-Roesch et al., 2006), and there has been high interspecies variation of plasma and serum ketone bodies seen across shark species, including both BHB and AcAc (Moorhead, 2019; Watson and Dickson, 2001).

While it is known that ketones are a source of aerobic fuel in sharks, their potential role as a mobilized fuel source in the acute stress response is less understood than the response of glucose. During acute air exposure stress events BHB levels remained unchanged in the plasma of the Atlantic stingray (*Hypanus sabinus*) (Lambert et al., 2018). In white sharks (*Carcharodon carcharias*), BHB levels did not change with capture duration, but were significantly different across shark size, with larger sharks having less circulating BHB (Gallager et al., 2019). However, results from Richards et al., 2003 do suggest that bodies may play a part in the metabolic recovery, and are an important source of ATP, after exhaustive exercise in spiny dogfish *(Squalus acanthias*). To date there have been no studies exploring the use of AcAc during acute stress in elasmobranchs. Further investigations into the role of ketone bodies (specifically BHB and AcAc) during acute stress in elasmobranch species are needed to understand if they play a role in the stress response.

When sharks encounter capture or handling they will employ escape tactics such as burst swimming and thrashing. During exhaustive exercise the body's oxygen demands can exceed the rate at which oxygen can be gained from the surrounding environment. When oxygen demand exceeds oxygen supply, cells switch to anaerobic means of energy production; in vertebrates muscle tissue this occurs primarily via lactic acid fermentation. In lactic acid fermentation cells are limited to only carbohydrate fuel stores, such as glycogen, and will accumulate the pathway's final product, lactate, if anaerobic conditions persist. Plasma lactate has been seen to increase with angling time in Caribbean reef sharks

(Carcharhinus perezi) (Bouyuocos et al., 2018), black tip sharks (*Carcharinus limbatus*) (Whitney et al., 2017), the common thresher shark (*Alopias vulpinus)* (Herberer et al., 2010*)* and shortfin mako sharks (*Isurus oxyrinchus)* (French et al., 2015). Increases in plasma lactate have also been noted in laboratory air exposure experiments in the Atlantic stingray (*Hypanus sabinus*) (Lambert et al., 2018). Typically, peak lactate levels are not seen until hours after the initial stress event has occurred, in Richards et al. (2003) sustained plasma lactate accumulation in the spiny dogfish (*Squalus acanthias*) occurred 12 hours after the initial stress event, and in Frick et al., (2012) peak lactate levels were not seen until 3 hours after the simulated capture stressor. Lactate is typically considered the metabolite that is the best predictor of mortality; moribund sharks in Marshall et al., (2012) had higher levels of lactate after longline capture, than those that were released alive.

Rises in plasma glucose and lactate after exhaustive exercise, or interaction with a stressor such as capture and handling has been reported in many shark species such as the dusky shark (*Carcharhinus obscurus*, Cliff and Thurman, 1984), bonnethead sharks (*Sphyrna tiburo*, Hyatt, 2016), and the common thresher sharks (*Alopias vulpinus*, Herberer et al., 2010). In most cases elevated levels of plasma glucose and lactate can be used as indicators that the stress response has been initiated, glucose sometimes as a proxy for stress hormones, and lactate as an indicator that the organism has switched to anaerobic metabolism due to insufficient oxygen delivery. While lactate is not directly a product of the acute stress hormones, it and glucose are linked within the stress response given the fact that anaerobic energy production relies on carbohydrate fuel stores and an increased availability of fuel is needed to continue energy production. The role of ketones in this process is much less understood, and as ketones are such an important fuel source in elasmobranchs it is important to classify their potential role as fuel in the acute stress response.

Study Species - Sand Tiger Sharks

Sand tiger sharks (*Carcharias taurus*) (figure 4) are a large, nearshore, piscivorous shark species and member of the order *Lamniformes* and family *Odontaspidaidae*. They are listed as critically

endangered worldwide according to the IUCN red list, however there are distinct populations that have different conservation statuses around the world (IUCN 2020). In the United States, sand tiger sharks are currently listed by the National Marine Fisheries Survey as a species of concern. Sand tiger sharks are slow lumbering swimmers that exhibit seasonal migrations, traveling south into the waters off South Carolina, Georgia, and Florida during the winter months, and then returning to waters off the coast of New England during the summer months (Kneebone et al., 2014). These sharks are slow growing with female sand tigers maturing at a length of 2200 -2300 mm TL and males at 1900 - 1950 mm TL (Gilmore et al., 1983). Sand tiger sharks have low fecundity and only produce offspring bi or tri-annually and are considered to have one of the lower reproduction rates in the elasmobranch subclass (Goldman et al., 2006). Males and females have distinctively different reproductive cycles in which males display a distinct yearly reproductive hormone cycle, and females exhibit a two year long reproductive cycle (Goldman et al., 2006; Henningsen et al., 2008; Lucifora, 2002; Wyffels et al., 2020). Their status as a species of concern and slow reproduction makes them a species of interest to conservationists and scientists, who study them in the wild and at captive institutions. Their success in captivity and frightening appearance has made them a popular shark species in aquaria and are frequently used as an ambassador species for other sharks. Additionally, as a large nearshore shark species, they can may be caught by recreational shark anglers, even though they are illegal to take and keep (Kilfoil et al., 2017). All these instances: research, aquaria, and capture, sharks may fight on a line, be removed from the water, handled, and from this, incur a stress response.

Only a few studies have analyzed secondary stress metabolites in sand tiger sharks after capture (Hoopes et al., 2022; Kneebone et al., 2013; Otway, 2010). Only one of these studies used longline capture. Understanding sand tiger shark response to longline capture across different seasons and locations is of particular importance considering longline fisheries tend to have the largest elasmobranch bycatch of all commercial fisheries (Oliver et al., 2015). Additionally, the physiological stress response of sand tiger sharks to duration of handling, and type of sampling, has not yet been investigated. Previous catch and release tagging studies have estimated post release mortality to be relatively low (5% - 6%,

Haulsee et al., 2016; Kilfoil et al., 2017) and therefore sand tigers may be robust to capture stress. Considering that sand tiger sharks have such a slow growth rate and reproductive output they still may be vulnerable to over exploitation even if they are robust to capture. Understanding which factors attenuate the stress response is important information that can inform angling and research best practices.

The objectives of this study were to investigate if metabolites (glucose, lactate, and ketones) are affected by biotic (length, sex) and abiotic factors (temperature, dissolved oxygen) during longline capture. As well as understanding the effect of longline soak time on plasma metabolites. Another goal of this study was to understand if the plasma metabolites are affected by length of handling and the occurrence of acoustic tagging surgeries and if there an interactive effect of handling and surgery on the metabolites of. It is expected that stress events like duration of capture, handling, and surgery will all have a measurable effect on the stress response through secondary metabolites. Abiotic factors will have an influence on plasma metabolites during the stressor, and there will be length differences in some stress metabolite concentrations.

Prior studies on sand tiger sharks noted significant differences in plasma glucose concentrations between juveniles and adults (Hoopes et al., 2022). Therefore, it is predicted that there will be a negative relationship between fork length and glucose concentration; however, it is not predicted that there will be sex differences in glucose concentration. In experimental angling studies of sand tiger sharks, glucose concentration did not increase with angling time, but lactate did (Kneebone et al., 2013). For this reason, glucose concentrations are not predicted to increase with longline soak time, but lactate concentrations are expected to increase with soak time. It is expected that increasing temperatures and decreasing dissolved oxygen concentrations will be connected to increasing plasma lactate concentrations. In previous studies on acute stress and ketones bodies, in most cases ketones did not significantly change in response to acute stressors (Lambert et al., 2018). It is not expected that either ketone body (AcAc or BHB) will respond to acute stressors like capture, handling, and surgery. However, sex differences have been seen in BHB concentrations in other shark species, therefore it is predicted that ketone concentrations may differ across sexes during longline capture (Valls et al., 2016).

MATERIALS AND METHODS

Standard Sampling Procedure

To characterize the stress response to capture and handling, sand tiger sharks were sampled from Saint Helena Sound in South Carolina in late March – early April in 2021, 2022, and 2023.

Sampling was conducted by Georgia Aquarium and South Carolina DNR on the R/V Silver Crescent. Sharks were sampled via bottom longline gear with ~2-meter gangions that include a bite bar and baited 16/0-18/0 circle hooks. A bite bar is a length of PVC pipe affixed perpendicularly to the gangions of the longline to prevent internal hooking of captured sharks. Water temperature and dissolved oxygen levels were recorded for each longline set using a standard CTD sensor (conductivity, temperature, depth). Soak time of these longlines ranged from a minimum of 54 minutes to a maximum of 313 minutes. Sharks captured on the long line were reeled to the boat and brought on board for sampling where a hose pumping seawater was placed in their mouth to aid in ventilation. Immediately a blood sample was taken from each shark via caudal venipuncture using 18-gauge needles with 10 ml heparinized syringes and this blood sample was then immediately placed on ice until testing. Measurements of total length (TL mm) and fork length (FL mm) were taken. Sharks were sexed and all sharks had a fin clip, gill clip, and muscle biopsy taken. Sharks were tagged with a dart tag, a second and final blood sample taken, and then released. Whole blood was tested, either immediately after collection or once the boat was back at the dock, on several point of care meters, more information on the meters is presented later in this section and in Appendix I. Within one minute of the blood draw, whole blood samples intended for laboratory biochemical testing were then placed in tubes with heparin or lithium heparin, and placed on ice or refrigerated until whole blood was able to be spun down to separate plasma from the red blood cells (RBCs). Spinning down the blood typically occurred at the end of the sampling day, or in between longline sets depending on the time available to complete this task. Plasma was then stored at -20 °C for a maximum of 7 days while still in the field and then stored at -80 °C in the laboratory

until analysis. Due to differences in research goals across sampling years, there are slight differences in sampling protocols across all three years.

2021 Sampling Season

Sharks in 2021 were sampled between April 5th and 6th from St. Helena sound South Carolina. Eleven sharks were captured during this sampling season, a total of five females and six males. During the 2021 sampling season the standard sampling procedure was followed except for the fact that only a single initial blood sample was taken.

2022 Sampling Season

During the 2022 sampling trips, sharks were sampled between March 28th and April 5th from St. Helena sound South Carolina. A total of 19 sharks were captured with 8 females and 11 males. The shark workup then followed the same procedure as the standard sampling procedure, with the addition of acoustic tagging surgeries for some sand tiger sharks. The duration of handling of the animal was recorded from the time the animal reached the boat from the longline to the time the animal was released.

2023 Sampling Season

Sharks were sampled between March 28th and April 4th, with a total of 22 sharks captured with 8 males and 14 females. During the 2023 field sampling season standard sampling protocol was followed, with the addition of internal acoustic tagging of every animal. Duration of handling time and acoustic tagging surgeries were recorded. Handling time was recorded as the number of minutes between the time the shark reached the boat, and when the shark was released. The time it took to surgically implant acoustic transmitters was recorded as the time from the initial incision to the final suture.

Field Blood Testing

During the 2021 and 2022 sampling seasons the initial blood sample was tested for circulating plasma glucose, lactate, and ketone levels on several point of care blood meters including The iPet Pro glucose meter (Ultimed Inc. Excelsior, MN, USA), Contour next glucose meter (Senseonics, Inc. Parsippany, NJ, USA) , Ketosense ketone meter (i-Sens Inc, Torrance, CA, USA) , and the Lactate Plus lactate meter (Nova Biomedical Inc. Waltham, MA, USA) (for more information on the use of point of care meters see Appendix I)

Laboratory Assays

Plasma glucose, lactate and ketone concentrations in the plasma were determined using standard spectrophotometric procedures using a Spectra Max M3 (Molecular Devices, San Jose, CA, USA). All plasma samples were thawed on ice, diluted appropriately to remain in the linear portion of the assay, and analyzed in triplicate. All assays were read for absorbance at 340nm.

To assess the concentration of glucose samples were analyzed using the commercial glucose kit (GAHK20) from Sigma Aldrich (Raleigh, NC, USA). Samples were diluted 10x with diH_2O. Standards of a known concentration, including an assay blank, were made up through a serial dilution of glucose standard to create a standard curve. During the assay a series of reactions take place in the wells that transform glucose into 6-phospho-gluconate using the enzyme hexokinase. During these reactions an equivalent amount of NAD+ is reduced to NADH, this concentration of NADH is read by the spectrophotometer and used to calculate the concentration of glucose in the sample.

The concentration of lactate in plasma samples was analyzed using a colorimetric assay. Prior to conducting the assay, plasma samples were deproteinated by adding 1 part sample to 2 parts 6% perchloric acid. These were then centrifuged for 10 minutes at 4 °C at 10,000X. The supernatant was pipetted off the top and stored at -80 °C until assayed. Deproteinated samples were then diluted 1:2 in 6% perchloric acid (PCA), to create a total dilution factor of 9x. Standards are made up with lactic acid (Sigma Aldrich, L1750) to create the standard curve. The enzyme lactate dehydrogenase (Sigma Aldrich)

was used in this assay to transform the lactate present in the sample into pyruvate while simultaneously reducing NAD+ (Sigma Aldrich) to NADH. The amount of reduced NADH is equivalent to the amount of lactate present in the plasma sample, and the amount of NADH in the well after the reaction takes place is picked up by the spectrophotometer (protocol modified from Clow et al. 2017).

Ketone body assays were performed directly on shark plasma, using two separate assays to determine both acetoacetate (AcAc) and 3-hydroxybuteric acid (beta-hydroxybutyrate, or BHB) concentrations. AcAc concentrations were determined using a commercial assay kit (Sigma Aldrich, Raleigh, NC, USA. The reaction used to quantify the amount of AcAc in the sample was the oxidation of AcAc and NADH to BHB and NAD+ using the enzyme beta-hydroxybutyrate dehydrogenase. The change between NADH to NAD+ is proportional to the amount of AcAc in the sample. The change of NADH to NAD+ is measured by the spectrophotometer and is compared between a sample and sample blank to assess AcAc concentration in the sample using the following equation:

$$AcAc\ mM = [(sample\ blank - sample) / (water - 8mM\ standard)] * 8$$

BHB concentrations were determined using a commercial assay kit (Sigma Aldrich, Raleigh, NC, USA. The reaction used to quantify the amount of BHB in the sample was the reduction of BHB and NAD+ to AcAc and NADH using the enzyme beta-hydroxybutyrate dehydrogenase. The change between NAD+ to NADH is proportional to the amount of BHB in the sample. The change of NADH to NAD+ is measured by the spectrophotometer and is compared between a sample and sample blank to assess BHB concentration in the sample using the following equation:

$$BHB\ mM = [(sample - sample\ blank) / (8mM\ standard - water)] * 8$$

Data Analysis

For all statistical testing, $p < 0.05$ is defined as significance. All statistical analyses were conducted in JMP version 16. For data used in t-tests, ANOVAs and ANCOVAs, normality testing was conducted using the Shapiro-Wilk goodness of fit test. All data were either normal or had a sample size large enough (>30) where the violation of normality could be ignored. In the case of t-tests and ANOVA, if variances were unequal, nonparametric methods were used. For regression and multiple regression

analysis Q-Q plots were generated to check for outliers, only one outlier from the dataset was removed, a male shark caught in 2021, due to its large size. Once this individual was removed, the data set was able to meet assumptions. An additional individual was removed from the pooled dataset, one pregnant female from 2022 not because she was an outlier but to control for the physiological effects of pregnancy as she was noted to be pregnant during sampling.

Fork length differences between males and females were compared using a pooled t-test, variances between the two samples were equal. Water temperature, and dissolved oxygen were compared across years using Welch's ANOVA, a non-parametric statistical test, and the Steel Dwas post hoc test. Soak time, plasma glucose, and plasma lactate were compared across years with an ANOVA and Tukey Kramer post hoc test. AcAc and BHB levels were compared across years with pooled t-tests. As the objective of the study was to compare metabolite concentrations during longline capture and what environmental factors may have an influence over their concentrations, metabolite data was pooled for further analyses with the idea that differences in years may be a result of differences in temperature across years.

Differences in plasma metabolite concentrations due to sex or fork length were analyzed using an analysis of covariance (ANCOVA). Multiple linear regression was used to compare the effect of soak time, dissolved oxygen, and water temperature on plasma metabolites. Using single factor linear regression, handling time and surgery duration were compared to the change in plasma metabolite concentrations for glucose, lactate, and AcAc.

To analyze the effect of fork length and sex on plasma metabolites, data from 2021, 2022, and 2023 were pooled to ensure a relatively even sex ratio in the data, and because fork lengths did not significantly differ across sampling years. One-way ANCOVA analysis was performed to elucidate the effect of sex on circulating plasma metabolite levels while controlling for any covariation across fork lengths.

The analysis of the effect of handling and acoustic tagging surgery was limited to data from 2022 and 2023 due to the fact that post handling blood samples were not obtained during the 2021 sampling season. To analyze the effect of acoustic tagging surgery, handling time, and if there is an interactive

effect of these two factors on the change in plasma metabolites across handling time, ANCOVA analysis was used. To obtain change across handling time values for the plasma metabolites the metabolites value from the post handling blood sample was subtracted from the metabolite value from the initial blood sample so that an increase in the metabolite concentration across handling would be represented as a positive value, and any decrease in the metabolite concentration across handling would be represented as a negative value. Sharks from just 2023 were then used to explore if were any sex differences in how each of the metabolites changed from the initial blood sample to the post handling blood sample.

Sample size differences across statistical testing is largely due to the inability to obtain data for BHB and AcAc levels in different individuals as a result of unexpected laboratory methodological difficulties. Additionally, fork length data was missing for two individual sharks in 2022 (one male, one female).

All means in the results section are presented with standard deviation noted after the ±.

RESULTS

Sex differences across length

Shark fork length varied significantly by sex, with females being significantly larger than males ($t46 = -3.96$, $p = 0.0003$). Mean female fork length was 2187±165 mm and mean male fork length was 2010±145 mm.

Environmental and metabolite differences across years

Water temperature was significantly different across sampling years (Welch's ANOVA, $F_2 = 102$, $p < 0.0001$), with 2021 being significantly lower than both 2022 and 2023, but no significant difference between 2022 and 2023 (Steel-Dwass, 2021 & 2022 $p = 0.0024$, 2021 & 2023 $p < 0.0001$). Dissolved oxygen was significantly different across years (Welch's ANOVA, $F_2 = 23.2$, $p < 0.0001$), with 2021 having the highest dissolved oxygen levels, and 2023 having the lowest (Steel-Dwass, 2021 & 2022 $p = 0.0251$, 2021 & 2023 $p < 0.0001$, 2022 and 2023 $p = 0.0138$). Longline soak duration was not significantly different across sampling years. Plasma glucose was significantly different across years (ANOVA, $F_{(2, 48)} = 4.60$, $p = 0.0149$), with plasma glucose from 2021 being significantly higher than plasma glucose from 2023 (Tukey Kramer, $p = 0.0168$), and 2022 not being significantly different from either 2021 and 2023. Plasma lactate was significantly different across years (ANOVA, $F_{(2,46)} = 17.60$, $p < 0.0001$), with 2021 being significantly higher than both 2022 and 2023 (Tukey Kramer, $p < 0.0001$ for both), but 2022 and 2023 not being significantly different from each other. AcAc and BHB plasma concentrations were not significantly different between years. Regardless of these significant differences in metabolites across years, metabolite data was still pooled for the subsequent analyses of the effect of temperature on plasma metabolite concentrations.

The relationship between biotic factors and plasma metabolites

There was no interaction between fork length and sex on circulating glucose levels calculated from the initial blood sample. Additionally, initial plasma glucose did not differ between sexes or have a relationship with fork length (Figure 4, graph A). All sharks combined displayed a mean initial plasma glucose level of 2.98 ± 0.76 mM, (Table 1). Similarly, there was no interaction between fork length and sex on initial plasma lactate levels. Initial lactate did not differ across sexes or display a relationship with fork length (Figure 4, graph B). Mean concentration of initial plasma lactate in all sharks was 3.12 ± 2.31 mM (Table 1). There was no interaction between fork length and sex on circulating BHB levels. Additionally, initial BHB did not differ between sexes and did not have a significant relationship with fork length (Figure 4, graph D). BHB for all sharks had a mean concentration of 0.22 ± 0.17 mM (Table 1). Alternatively, there was an interactive effect of fork length and sex on circulating AcAc levels (ANCOVA, $F_{(3,29)} = 4.58$, $p = 0.04$), where female sharks had a positive relationship between fork length and circulating AcAc levels and male sharks had a negative relationship between fork length and circulating AcAc levels (Figure 4, graph C). Mean concentration of AcAc was 0.74 ± 0.74 mM (Table 1). Sharks consistently displayed higher AcAc values than BHB (Table 1).

The effect of abiotic factors on plasma metabolites

For the following results data from 2021, 2022, and 2023 were pooled. Initial plasma metabolite levels were compared to long line soak duration, dissolved oxygen, and water temperature using a multiple regression to establish any significant relationships between the variables. Mean values and ranges for water temperature, dissolved oxygen, and long line soak time are available in Table 2.

In the multiple regression model initial glucose had a significant negative relationship with water temperature (Multiple Linear Regression, $p = 0.0116$, Figure 5, Figure 6) but displayed no relationship with dissolved oxygen or long line soak time (Table 3).

In the multiple regression model circulating initial lactate had a significant negative relationship with water temperature (Multiple Linear Regression, $p = 0.0423$, Figure 7, Figure 8), a significant

positive relationship with long line soak time (Multiple Linear Regression, $p = 0.0015$, Figure 9) and had no relationship with dissolved oxygen. Long line soak time (standard beta = 0.43) had a greater influence on initial plasma lactate concentration than water temperature (standard beta = -0.37) (Table 4). Soak time, water temperature, and dissolved oxygen had no relationship with both ketone bodies (BHB and AcAc) (AcAc: Table 5, BHB: Table 6).

The effect of handling time and surgery duration on plasma metabolites

Mean handling time was 22 ± 5.5 minutes, with a range of times from 15 to 35 minutes. There was no significant relationship between change in glucose and handling time, there were no significant differences in glucose changes between sharks that had surgery and those that did not, and there was no interactive effect of handling time and surgery on changes in glucose across handling (Figure 10). Mean plasma glucose change was -0.18 ± 0.78 mM.There was no significant relationship between change in lactate and handling time, there were no significant differences in lactate changes between sharks that had surgery and those that did not, and there was no interactive effect of handling time and surgery on changes in lactate across handling (Figure 11). Mean lactate change was 0.58 ± 0.44 mM.

There was no significant relationship between change in AcAc and handling time, there were no significant differences in AcAc changes between sharks that had surgery and those that did not, and there was no interactive effect of handling time and surgery on changes in AcAc across handling (Figure 12). The change in AcAc averaged -0.1 ± 0.87mM.

The effect of sex on metabolite changes during handling and surgery

For the following results only data from 2023 were used. T-tests were used to look for differences in metabolite changes across sexes, for all tests variances were equal. Female and male sharks did not significantly differ in how circulating glucose levels changed in response to handling and surgery (Pooled T, $t_{14} = 0.305$, $p = 0.76$).

Female and male sharks did display a significant difference in how circulating lactate levels changed in response to handling. Female sharks had a significantly higher increase in circulating plasma lactate in response to handling and surgery than males (Pooled T, $t_{14} = -2.62$, $p = 0.02$, Figure 13). In fact, some males displayed a decrease in circulating lactate levels in response to handling, while all females had an increase in lactate in response to handling. Mean lactate change of male sharks was 0.262 mM, and mean glucose change in females was 0.768 mM. AcAc change across handling and surgery did not differ significantly across sexes. There were no significant differences in how long females or males were handled for, or surgery duration.

DISCUSSION

The objectives of this study were to assess if the physiological response that occurs during longline capture is affected by biotic (fork length, sex) and abiotic factors (temperature dissolved oxygen), and longline soak time. This research also aimed to understand the effect of handling time and duration of acoustic tagging surgeries on the plasma metabolites glucose, lactate, and ketones.

The plasma glucose values from this study fall within the range of previously published values. Mean plasma glucose levels at capture in this study (3.0 ± 0.76 mM) are higher than those published for captive sand tiger sharks (1.8 ± 0.08 mM), values published for sharks captured through purportedly less stressful methods (2.5 ± 0.20 mM), but lower than sharks caught in Delaware Bay via longline. Considering that aquarium sharks are more acclimated to physical restraint than wild sharks, they possibly experience a lower magnitude of stress response than wild sharks when captured and handled for a blood draw. Therefore, it is reasonable to consider the potential that longline capture induces an increase in circulating glucose in sand tiger sharks as the glucose concentrations from this study are higher than those in aquarium sharks. Longline capture may trigger an initial release of catecholamines and corticosteroids that then trigger a release of glycogen from storage tissue, which in turn raises plasma glucose.

Mean lactate levels in this study are lower compared to previously published values (Hoopes et al., 2022). In both these studies sharks were captured by longline, however soak times were marginally longer (mean 174 minutes) in Hoopes et al., (2022), than they were in this current study, which may explain the difference in mean plasma lactate between the studies. Sharks in that study were also caught during a different time of the year, August, and a different location, Delaware Bay, Delaware, then the current study.

AcAc values were consistently higher than that of BHB in the sand tiger sharks from this study. A similar trend was noted in nurse sharks (*G. cirratum*) (Moorhead, 2019). Prior to the ketone work on nurse sharks, it had been hypothesized that BHB would always be higher than AcAc in elasmobranchs

due to the thought that AcAc rapidly transforms into BHB. Prior work on the small spotted catshark and spiny dogfish revealed that those species had higher circulating values of plasma BHB than AcAc (Zammit and Newsholme, 1979). Ratios of AcAc to BHB have been used in small mammals to determine cellular energy status, and as markers of health in human medicine and perhaps these differing AcAc and BHB ratios are a result of different energetic needs or energy utilization patterns differing across elasmobranch species (Tanaka et al., 1979; Inaba et al., 2020). Further research into ketone body concentrations in different shark species is needed to understand why some species have higher levels of AcAc over BHB and vice versa.

To the best of our knowledge these are the first reported ketone body values for sand tiger sharks and may prove useful in sand tiger shark husbandry and nutrition. However, caution should be taken in using these values as a reference point considering they come from animals exposed to stressors, with unknown nutritional histories, and should not be considered "baseline values".

Biotic factors effect on plasma metabolites

Glucose and lactate both did not have a relationship with fork length or differ between sexes. The lack of relationship between plasma glucose and fork length differs from results in Hoopes et al., (2022), where juvenile sharks exhibited significantly higher glucose concentrations than adult sand tiger sharks, this would potentially indicate that shark fork length should have a negative relationship with plasma glucose. This difference in statistical results may be because Hoopes et al., (2022) used maturity status as the predictor variable and our current study instead used fork length. The lack of sex differences across glucose and lactate is consistent with previous metabolite data published for sand tiger sharks (Hoopes et al., 2022).

There was no significant interaction between sex and fork length, and plasma BHB concentrations. BHB concentrations were not significantly different between sexes, and there was no significant relationship between fork length and plasma BHB concentration. This lack of relationship between length and BHB concentration contrasts with published data on other Lamniform sharks, a

previous study on juvenile white sharks *(Carcharodon carcharias*) noted a significant negative relationship between shark length and plasma BHB concentrations (Gallagher et al., 2019). The authors of the study stated that this relationship was indicative of sharks using different energy sources as they age (Gallagher et al., 2019). White sharks have an ontogenetic diet shift as they age, shifting from a piscivorous diet as a juvenile, to feeding on marine mammals as an adult (Kim et al., 2012; Estrada et al., 2006). Diet studies on the sand tiger shark revealed that their diets mostly consisted of teleosts and elasmobranchs across their lifetimes (Lucifora et al., 2009). Perhaps these differences in diets across ontogeny may account for the difference in the relationship with BHB and fork length in the two species.

As male shark fork length increased, plasma concentrations of AcAc decreased. In contrast as female shark fork length increased, plasma AcAc concentrations increased. There may be an ontological shift in the type of energy utilized between sexes, with male sand tiger sharks using a different energy source such as amino acids or fatty acids after maturity, and female sharks relying more on AcAc as they mature. It is known that male and female sand tiger sharks have distinct reproductive cycles that have been tracked by hormones in the wild and captivity (Henningsen et al., 2008; Lucifora, 2002; Wyffels et al., 2020). These reproductive cycles occur across different time periods, with males exhibiting a yearly hormone cycle, and females exhibiting a biannual hormone cycle as well as biennial reproduction (Henningsen et al., 2008; Lucifora et al., 2002). Importantly, the plasma samples in this current study were taken in late March and early April across all sampling years. April is around the start of the breeding season in sand tiger sharks (Wyffels et al., 2020). In a previous study, juvenile nurse sharks with higher AcAc levels had higher body condition scores (Moorhead et al., 2019) and it may be possible that this higher level of AcAc in female sand tiger sharks at reproductive maturity sizes may indicate female energetic investment for reproduction. If this AcAc trend is due to energy mobilization and investment for pregnancy it would be interesting to track AcAc plasma concentrations across the breeding season as AcAc is a ketone body produced by the liver and in previous studies in female sand tiger sharks it has been seen that as gonadosomatic index increases (eg. larger ovarian follicles), hepatosomatic index decreases (Lucifora, 2002).

This AcAc trend could possibly represent an ontological and sexual shift in reliance on ketone bodies in general, but this same interaction with sex and fork length was not seen in this study in BHB. This is not the first-time sex differences in a ketone body have been noted in a shark species. In Valls et al., 2016, BHB changed with seasonality in male small spotted catsharks (*S. canicula)*, with adult males having higher plasma BHB in the winter. BHB is one of the end products of ketogenesis and is what is stored as energy in the liver (Fukao et al., 2004). BHB and AcAc are both transportable in the bloodstream as mobilized energy, and BHB must be transformed into AcAc before it can again return to the form of Acety-CoA to be utilized to create cellular energy (Fukao et al., 2004). Therefore, AcAc plasma concentrations may provide a snapshot of ketone body utilization as an energy source at the point when the blood sample was taken. In the spiny dogfish, while BHB values were influenced by feeding status, AcAc remained unchanged. It is possible that these differences in AcAc across sexes may not be due to sex differences in feeding (Wood et al., 2010).

Due to the nature of this study, it is difficult to determine whether these AcAc concentration trends are a chronic state in sand tiger sharks, or if this is simply an acute stress response that is different between sexes and across lengths. Perhaps male sharks with shorter fork lengths and females with longer fork lengths respond to a stressor by increasing their circulating AcAc to use as an energy source to aid in the resistance of that stressor.

Abiotic factor effect on plasma metabolites

Plasma glucose did not exhibit a significant relationship with dissolved oxygen but did have a significant negative relationship with water temperature. The lack of relationship between dissolved oxygen and plasma glucose is not unexpected as dissolved oxygen in this study never fell below 7.5mg/L, and therefore sharks were never exposed to hypoxic conditions. It is worth noting that the temperature range of 16 – 19 °C in this study is not a very broad one. This temperature range falls well within the typical temperature range of sand tiger sharks (Kneebone et al., 2014). Temperature seems to be an

important environmental variable to sand tiger sharks as sea surface temperature was one of the most important predictors of shark location in previous studies that used sand tiger shark acoustic tracking data in their models (Haulsee et al., 2018). Another telemetry study noted that the mean temperature juvenile sand tiger sharks occupied in their Delaware Bay nursery grounds was 19.97 °C (Kneebone et al., 2018). Another study that tracked adult sand tiger sharks on their fall southward migration noted that sharks spent 95% of their time in water that ranged from 17 to 23 °C (Teter et al., 2014). Because the temperature range in this study is so small, and the shark were caught within a temperature range that sand tigers have are known to behaviorally select for, it is likely that this significant trend is an artifact of the data and not actually biologically relevant.

The change in glucose across temperatures is not very large at a range of 1.37 mM to 5.7mM, a 4.3mM shift. This glucose concentration difference across temperatures may be biologically negligible to these sharks. Similar ranges of glucose have been seen in other studies, in sand tiger sharks subjected to an angling stressor had a glucose range of 3.38mM to 5.72mM, a 2.34mM range, however there was not a significant relationship between glucose and time exposed to the angling stressor (Kneebone et al., 2013). However, in French et al., (2015), where shortfin mako shark plasma glucose had a significant positive relationship with water temperature, plasma glucose only had a small difference of 4mM between the smallest and largest value.

There is a possibility that this glucose and temperature trend is an artifact due to the statistical difference of both glucose and temperature across sampling years. There may have been something different across the three sampling years, like food availability or reproductive state, affecting sand tiger shark glucose concentrations that was not measured, and this difference is being shown through temperature as a proxy.

There was no significant relationship between longline soak time and initial plasma glucose. The lack of a significant relationship between soak time and plasma glucose in sand tiger sharks is consistent with prior studies. In Kneebone et al., (2013) juvenile sand tiger sharks did not display a significant

relationship between angling time and plasma glucose concentrations (Kneebone et al., 2013). A similar trend was seen in Mako sharks, where glucose was not significantly affected by angling time (French et al., 2015). While an increase in plasma glucose was not seen in this current study or the two mentioned, this does not necessarily mean that during these stressors there is not elevated production of glucose. Glucose is produced during stress events to fuel locomotory muscles, but for these locomotory muscles to obtain this energy they must use the extra glucose in the glycolysis process. So, while it is possible that these organisms are producing extra plasma glucose in response to the stressor, they may also be using this plasma glucose up, and therefore this process would not be captured by just blood samples. To say definitively whether duration on a longline has an effect on plasma glucose or not, it would be necessary to use hook timers or experimental methods. For example, a shark may be caught and brought to the boat for an initial blood draw, and then left on the line to continue fighting for a predetermined length of time, after witch a second blood sample is taken to compare the change in metabolites across hooking time. Testing for stress hormones like catecholamines at the same time would also be helpful to establish the relationship between stress hormones and glucose mobilization in sand tiger sharks.

The negative relationship between water temperature and lactate differs from most other published literature on capture stress and water temperature in fishes, the common trend is a positive relationship between plasma lactate and water temperature (French et al., 2015; Kieffer et al., 1994). Warmer water has lower oxygen concentrations and so organisms experiencing stressors in warmer waters would be expected to enter anaerobic metabolism more rapidly and accumulate more lactate. However, the dissolved oxygen levels experienced by the sharks in this current study never fell below 7.5mg/L, so sharks were not exposed to hypoxic conditions linked to temperature. This result may be an artifact of the very small range of temperatures within the current data set, as well as the statistical difference of both lactate and temperature across sampling years. There may have been something different across the three sampling years, as stated above for glucose, affecting sand tiger shark lactate concentrations that was not measured, and this difference is being shown through temperature as a proxy.

As expected, lactate had a significant positive relationship with longline soak time; as soak time increased, initial plasma lactate at capture increased. As there was no significant relationship between dissolved oxygen and lactate, and because dissolved oxygen never dipped below 7.5mg/L in this study, we can conclude that any changes seen in lactate concentrations are due to endogenous hypoxia from increased physical activity. This finding is consistent with prior literature, plasma lactate concentrations increased 21-fold (0.4 to 8.6 mM within 3 minutes) across angling time in juvenile sand tiger sharks (Kneebone et al., 2013). This same pattern was also seen in shortfin mako sharks, a member of the same taxonomic order as sand tigers (French et al., 2015). Longline soak time is only a coarse estimate of the amount of time spent on the line by each shark, and a greater longline soak time duration would not necessarily mean that the sharks caught on it were on the line longer than sharks caught on a longline with a shorter soak time. However, the longer a longline is deployed in the water the greater the possibility that a shark caught on that line has been hooked for longer. It would be expected that the longer a shark spent fighting on the line, the more anaerobic glycolysis can take place leading to an increase in plasma lactate. To confirm this theory about sand tiger sharks' response to longline capture, hook timers or other experimental methods like those mentioned previously would need to be used.

Neither AcAc nor BHB were affected by longline soak duration, dissolved oxygen, or water temperature during longline capture. In prior studies on other species BHB has been affected by water temperature in other shark species. In juvenile blacktip reef sharks, those held at a temperature 2 °C higher than the baseline temperature had elevated plasma BHB concentrations (Schoen et al., 2021b). However, this observation of temperature effect on BHB values was seen across a 7-day acclimation period, and the effect of temperature on BHB concentration may take course across a longer timescale (Schoen et al., 2021b). The lack of relationship between BHB and longline soak time is consistent with other literature where BHB had no relationship with angling time in the shortfin mako shark (French et al., 2015). Even though there is no statistical relationship between BHB and soak time does not necessarily mean that there was no increase in BHB production. It is still possible that BHB was being

mobilized, but was also being used up at the same time for energy. In spiny dogfish it was seen that BHB is up taken by white muscle at the same rate that it is released from the liver (Richards et al., 2003).

The effect of handling time and surgery on the change in plasma metabolites

Shark handling time had no significant effect on any of the plasma metabolites (glucose, lactate, and AcAc). There was no difference in plasma metabolite changes between sharks that did or did not undergo acoustic tagging surgeries. The robustness of sand tiger sharks to acoustic tagging surgery is not unexpected considering that in a previous acoustic tagging study, sharks released after surgery only had an estimated mortality rate of 5% (or 1/20 sharks from the study) (Teter et al., 2014). Other stress hormones like catecholamines may have been elevated during handling and acoustic tagging surgeries, however these stress hormones were not measured during this study.

In prior studies BHB was shown to increase significantly after cannulation surgery in spiny dogfish and remained significantly elevated from baseline levels through 48 hours post cannulation surgery but unfortunately in my study BHB was unable to be measured in the 2023 sharks (Schoen et al., 2021a). Possibly AcAc and BHB are involved in a less immediate rapid response to stress. During acute stress events BHB levels remained unchanged in the plasma of the Atlantic stingray, while other markers of stress (glucose and lactate) demonstrated that a stress response was mounted (Lambert et al., 2018). In white sharks, BHB levels did not change with capture duration (Gallager et al., 2019). However, results from Richards et al., (2003) do suggest that ketone bodies may play a part in the metabolic recovery, and be an important source of ATP, after exhaustive exercise in spiny dogfish (*Squalus acanthias),* and so it may be more useful to measure ketone bodies across more chronic timescales. To truly investigate BHB and AcAc as measures of stress these animals may need to be exposed to more extreme levels of acute stress or sampled for longer after an encounter with a stressor.

. There were no significant differences in the duration of handling or surgery between the sexes. Interestingly female sharks displayed a significantly different change in lactate when compared to male sharks. All female sharks displayed an increase in plasma lactate across handling, while male sharks

generally displayed a small increase in lactate or a decrease Male sharks may be more robust to this type of sampling than females are. This could be due to the fact that these samples were collected at around the beginning of the sand tiger shark breeding season and the difference in physiology across sexes reproductive hormones could cause (Wyffels et al., 2020).

CONCLUSIONS AND FUTURE DIRECTIONS

Plasma glucose and lactate levels within this study were consistent with prior literature, and these are the first published ketone body values for sand tiger sharks. As glucose did not appear to be affected by acute stressors in this study future work should be done to measure the stress response of sand tiger sharks to longline capture by quantifying the concentration of both glucose and stress hormones like catecholamines. There may be a sex determined ontogenetic shift in the use of AcAc, as shown by the trend between fork length, sex, and AcAc. Further investigation should be conducted on plasma AcAc in sand tiger sharks, and other elasmobranch species to understand the possible ontogenetic shift by sex seen in the AcAc plasma levels of sand tiger sharks. Measurement of AcAc at different points in the breeding cycle and across ontogeny should be taken to explore if concentrations of AcAc fluctuate with reproductive state, development, or season, and the corresponding changes in physiology across these variables.

As noted by this study, longline capture seems to elicit a metabolic stress response in sand tiger sharks, and this stress increases with the length of possible time the animal could be hooked on the line, as shown by the increase in lactate with increased longline soak time. Like prior studies, sand tiger sharks appear to be robust to handling and acoustic tagging surgery. Even so it is important for future studies to focus on not just post release survival as a consequence of this capture stress but also organism health, potential behavioral changes post stress, and the impact of capture stress on reproductive output.

CHAPTER 2:

BLOOD CULTURE SURVEY IN FREE-RANGING SAND TIGER SHARKS (*C. TAURUS)*

INTRODUCTION

Most animals have a collection of microorganisms that naturally live on multiple tissues throughout the body, which are collectively referred to as a microbiome. Microbiomes are often determined by bacterial residency in each tissue, and the function to the animal it provides (Hammer et al., 2019). Animal microbiomes are known to influence an organism's physiology and behavior, and some animals are very dependent on these host – microbiome interactions to function properly (Hammer et al. 2019; Perry et al., 2021). One phylogenetic group with well-known microbiomes are the fishes. Microbes are found on their epidermis, within their GI tract, in their mouth, and within their gills (Perry et al., 2021). In most cases, these microbial communities are beneficial to the host animal, for example bacteria with antibiotic properties have been found on the skin of California bat rays (*Mylobatis californica*), suggesting that these bacteria may help fight infection from injuries (Ritche et al., 2014). It is thought that some gut bacteria in the bonnethead shark (*Sphyrna tiburo)* help with digestion of marine plants, which have been found in bonnethead stomachs (Leigh et al., 2021). In contrast to the established understanding of the microbes of the epidermis, gills, GI tract, and mouth, the presence of bacteria in fish blood is much less researched and blood represents a potentially new tissue with a distinct microbial community (Perry et al., 2021).

In vertebrates, it is generally accepted that blood is sterile and the presence of bacteria in the blood indicates infection. Additionally, vertebrate blood may contain transient bacteria, but it is thought that this is quickly eliminated by the immune system, or the bacteria proliferates resulting in sepsis. However, bacteriemia (the presence of bacteria in the bloodstream) has been observed in healthy humans, Greenland halibut (*Reinhardtius hippoglossoides*), Atlantic halibut (*Hippoglossus hippoglossus),* and wild caught red snapper (*Lutjanus campechanus*) (McLaughlin et al., 2002; Tarnecki et al., 2016; Fronton et

al., 2023). Bacteria have been isolated from the blood of seemingly healthy elasmobranchs (Mylniczenko et al., 2007; Tao et al., 2014). Approximately 27% of the sharks sampled (healthy captive and free swimming *in situ*) had bacteria present in their blood, several species which are known to be pathogenic in fishes (*Vibrio spp., Pseudomonas spp.,* etc) (Mylniczenko et al., 2007). Notably, pelagic elasmobranch species produced more positive blood cultures than benthic individuals (Mylniczenko et al., 2007). In another study, nine of ten sampled lesser electric rays (*Narcine bancroftii*) had bacteria positive blood cultures (Tao et al., 2014). It is unclear whether non-sterile blood is a baseline condition in elasmobranch fishes, and whether these bacteria could become pathogenic during times of chronic or even acute stress. It is also important to know if bacteria in the blood has the potential to hinder elasmobranchs ability to biochemically and metabolically cope with stressors (eg. capture and handling). Physiological stress has been known to affect microbiome communities, e.g. elevated cortisol levels caused compositional changes in the gut microbiome of the Atlantic salmon (*Salmo salar*) (Uren and Webster et al., 2020).

Not much is known about how the immune system may affect the stress response during capture and handling in elasmobranchs, as this is a difficult metric to measure and compare - particularly in wild sharks. Blood bacterial presence may be one health factor with the potential to attenuate or depress the stress response. For example, it has been observed in the round stingray (*Urobatis halleri)* that legacy chemical contamination does affect the stress response (Lyons and Wynne-Edwards, 2019), specifically an inability to produce plasma glucose, however this is an abiotic interaction, and it is unclear if blood bacterial presence could cause a similar effect, if any effect at all. Investigating the physiological link between bacteremia and the acute stress of capture and handling has important implications for further understanding how the elasmobranch immune system and stress response interact. In addition to understanding how bacteremia may interact with the stress response, understanding what variables, like animal size and sex, drive bacterial presence or absence is important to better understand elasmobranch health across all species.

There are no current published studies on the presence of bacteria in the blood of sand tiger sharks, or how this presence correlates with the acute stress response. It is thought that elasmobranch to

microbiome relationships could be changed under human care (Perry et al., 2021). Considering sand tiger sharks conservation status and common usage in public aquariums, understanding how bacterial presence in the blood interacts with physiological stress parameters can fill an important knowledge gap in a very preliminary area of research.

The presence of bacteria in the blood of healthy vertebrates is a relatively new concept and while investigation of this has been undertaken in mammals as well as bony fishes, even less work on this subject has been done in elasmobranchs. The objectives of this study are to explore blood bacterial presence in sand tiger sharks, evaluate if there are length and sex differences in blood bacterial presence, and understand if there is a possible interaction between blood bacterial presence and stress metabolites in the sand tiger shark.

MATERIALS AND METHODS

Field Capture of Elasmobranchs

Twenty-two sand tiger sharks were sampled via baited longline from St. Helena sound, South Carolina during the months of March and April 2023, for further information on these sampling methods see Chapter 1.

Blood Sampling

To prevent cross contamination from bacteria on the shark's epidermis, the skin at the blood collection site was disinfected with isopropyl alcohol (Mylniczenko et al, 2007). Blood for the bacteria cultures was drawn via caudal venipuncture with sterile 18 or 20-gauge needles and 3 ml syringes, at least 2mL of blood was collected from each animal. To assess for both anaerobic and aerobic growth two culture tubes were made up of 1mL of blood combined with 10mL of tryptic soy broth (Soybean-Casein Digest Medium) (BD Difco, Franklin Lakes, NJ, USA). Field control tubes were created by uncapping the culture tube and holding it uncapped in the same location and for the same amount of time as the blood cultures. Culture tubes for blood bacteria and controls sat at room temperature for approximately 48 hours before being placed in an incubator at 35 °C for 72 hours, previous studies using elasmobranch blood demonstrated that almost all bacterial growth is completed within 72 hours (Tao et al. 2014).

Laboratory culture plates

After the 72 hour of incubation period, culture tubes were removed from the incubator and a streak plate was made from each culture on marine agar and blood agar to determine if each sample was positive or negative for bacterial growth (Sanders, 2012). Marine agar was prepared in house by adding 44.08g of marine agar base (BD Difco Marine Agar 2216) to 800ml of diH_2O). These plates were then once again incubated at 35 °C for 72 hours and subsequently inspected for bacterial growth by observing

the presence or absence of any bacterial colonies. Any plate with colonies was considered positive for growth, any plates with no visible colonies were considered negative for growth.

Statistical Analysis

The relationship between sand tiger shark fork length and blood bacterial presence or absence was investigated using a logistic regression. Blood bacterial presence or absence was compared to shark sex using a contingency table and Fishers Exact Test. Initial metabolite levels were compared between individuals with blood bacterial presence or absence using pooled t-tests. In all cases the assumption of normality and equal variances was met. Due to high contamination of the aerobic field controls, and the fact that all but one shark were positive for aerobic bacterial growth, statistics were not performed for aerobic cultures.

RESULTS

Bacterial presence in sand tiger sharks

Blood from 16 out of the 22 sharks sampled were positive for anaerobic bacterial growth on marine agar. Only blood from one individual was negative for aerobic bacterial growth on marine agar, this individual was also negative for anaerobic growth on marine agar as well. No anaerobic field controls were positive for bacterial growth, and 2 out of 5 aerobic field cultures were positive for bacterial growth.

Comparison between blood bacterial presence and biological characteristics in sand tiger sharks

There was no significant relationship between fork length and anaerobic bacterial presence streaked on marine agar. There were no significant differences in bacterial presence from anaerobic marine agar cultures between sexes.

Comparison of blood bacterial presence and initial stress parameters in sand tiger sharks

There was no significant difference in plasma glucose and AcAc levels between sharks that were positive for anaerobic bacteria and sharks negative for anaerobic bacteria. However, there was a near significant difference in initial lactate between those that were positive or negative for anaerobic bacteria streaked on marine agar (Pooled T, $t_{20} = -2.013$, $p = 0.057$). Initial lactate was higher in individuals that were negative for anaerobic bacterial growth on marine agar (mean = 2.9 ± 1.1 mM lactate) versus those that were positive for growth (mean = 1.89 ± 1.0 mM lactate).

DISCUSSION

Microbiomes are found in the mouths, gills, GI tracts, and on the skin of elasmobranch species (Perry et al., 2021). These microbial communities serve important functions for the organisms that house them and have provided information to scientists about the ecology and physiology of their hosts (Perry et al., 2021; Ritche et al., 2014). In recent years the existence of a blood microbiome has been suggested as recent studies have cultured bacteria from the blood of sharks with no apparent illness. For these reasons the objectives of this study were to explore blood bacterial presence in sand tiger sharks, evaluate if there are length and sex differences in blood bacterial presence and understand if there is a possible interaction between blood bacterial presence and stress metabolites in the sand tiger shark.

Blood culture survey of sand tiger sharks

Contrary to study predictions, sand tiger shark fork length did not have a significant effect on blood bacterial presence, and there was no significant difference in blood bacteria presence between sexes.

There were high rates of positive cultures across all four culture types evaluated (marine agar aerobic, marine agar anaerobic). In marine agar cultures, 72% of the anaerobic and 95% of the aerobic cultures were positive for bacterial growth. None of the anaerobic marine agar field control cultures were positive, however a few aerobic marine agar field control cultures were positive, introducing the possibility that some aerobic marine agar cultures were perhaps contaminated. In a previous study on the bacterial presence in the blood of the lesser electric ray (*Narcine bancroftii*), 90% of the rays sampled had positive blood cultures (Tao et al., 2014). Lesser electric ray blood cultures were plated on both marine and blood agar. These positivity rates are much higher than the 27% positive culture rate seen in a study that was pooled across a large variety of elasmobranch species some of which were housed in aquaria and some from the wild (Mylniczenko et al., 2007). The Mylniczenko study also used blood agar and agar specially formulated to culture *Vibrio* species. Here, only marine agar was used to culture bacteria, each of the different culture media (marine agar, blood agar, vibrio agar) used across this study and the two

previously mentioned, have different nutritional compositions that promote the growth of different bacterial species, which may be the cause of the different blood culture positivity rates. If elasmobranchs have a blood microbiome it is likely that these microbiomes are species specific, which may also explain the differences in blood culture positivity rates across studies. Pelagic elasmobranchs had higher rates of positive cultures (38.7%) as compared to more benthic species (13.9%) (Mylniczenko et al., 2007). Considering that sand tiger sharks are pelagic sharks, the relatively high positivity rate in the current study, could be considered consistent with the prior findings that pelagic elasmobranchs had higher rates of blood bacterial presence.

While the rates of bacterial growth are high in this study, they are consistent with the rest of the limited literature on this subject. It cannot be negated that contamination is always a possible factor, especially with the aerobic cultures where several controls were positive.

Blood bacterial presence and its relationship with blood metabolites

There were no significant differences in the plasma metabolites glucose and AcAc between sharks with positive or negative anaerobic cultures. However, a near significant difference was seen in plasma lactate levels between individuals positive or negative anaerobic cultures. Until recently, the presence of bacteria in the bloodstream would be considered a negative health event and would be assumed to have deleterious effects. As evidence of the presence of bacteria in the blood of healthy vertebrates, including elasmobranchs, continues to build it suggests that bacterial presence in blood may not always indicate disease. Possibly the bacteria found in blood of sand tiger sharks may play a role in helping cope with the metabolic switch to an anaerobic state during the capture and exhaustive exercise. It has been observed that a specific genus of bacteria (*Veillonella)* increases in runners after marathons (Scheiman et al., 2019). This genus of bacteria utilizes lactate as its sole carbon source, and when mice were inoculated with the species isolated from marathon runners, it significantly increased their treadmill performance time most likely through the prevention of lactate build-up (Scheiman et al., 2019). This genus of bacteria resides mostly in the gut, however, acute stress has been seen to increased intestinal

permeability, which in turn promotes the translocation of gut bacterial, potentially into the blood (reviewed in Kelly et al., 2015). Additionally, in vertebrates it is known that microbial presence can influence the stress response and that inversely stress hormones can influence microbiome composition (Uren Webster et al., 2020; Vodička et al., 2018). It is thought that the gut microbiome of higher vertebrates plays a large role in the development of the hypothalamic-pituitary-adrenal (HPA) axis and stress signaling, however this has yet to be explored in other vertebrates (Ortega et al., 2021).

It is important to note that one major limitation of the current study is that there are many species of bacteria that are unable to be cultured under laboratory conditions and so other bacteria may have been missed by our methods (Amann et al., 1995). Another limitation important to consider is the possibility of contamination from seawater, sample transport, and laboratory equipment. In the future genetic analysis may be used to identify sources and microbes whose origins are not from the elasmobranch blood samples.

CONCLUSIONS AND FUTURE DIRECTIONS

Bacteria was able to be cultured from the blood of sand tiger sharks. These observations can be added to the other current literature as potential evidence that elasmobranch blood may not be sterile at baseline. It appears that bacteria in the blood of sand tiger sharks may potentially influence plasma lactate concentration at the time of longline capture. This is an interesting trend worth investigating in the future using larger sample sizes and more extensive laboratory techniques such as 16s rDNA sequencing to determine the types of bacteria present in the bloodstream of these animals and help understand the effect of the presence of these microorganisms. It is pertinent to investigate blood bacterial presence alongside markers of immune status such as white blood cell counts and complete blood cell counts to begin to evaluate the effect of bacteria in the bloodstreams on the immune system.

REFERENCES

Amann, R. I., Ludwig, W., & Schleifer, K. H. (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological reviews*, *59*(1), 143–169. https://doi.org/10.1128/mr.59.1.143-169.1995

Anderson, P. A., Huber, D. R., & Berzins, I. K. (2012). Correlations of capture, transport, and nutrition with spinal deformities in sandtiger sharks, *Carcharias taurus*, in public aquaria. *Journal of Zoo and Wildlife medicine: official publication of the American Association of Zoo Veterinarians*, *43*(4), 750–758. https://doi.org/10.1638/2011-0066R1.1

Ballantyne, J.S. *(1997).* Jaws: the inside story. The metabolism of elasmobranch fishes. *Comparative Biochemistry and Physiology B, 118, 703-742. DOI:10.1016/S0305-0491(97)00272-1*

Bouyoucos, I. A., Talwar, B. S., Brooks, E. J., Brownscombe, J. W., Cooke, S. J., Suski, C. D., & Mandelman, J. W. (2018). Exercise intensity while hooked is associated with physiological status of longline-captured sharks. *Conservation Physiology*, *6*(1). https://doi.org/10.1093/conphys/coy074

Brooks, E. J., Sloman, K. A., Liss, S., Hassan-Hassanein, L., Danylchuk, A. J., Cooke, S. J., Mandelman, J. W., Skomal, G. B., Sims, D. W., & Suski, C. D. (2011). The stress physiology of extended duration tonic immobility in the juvenile lemon shark, *Negaprion brevirostris* (Poey 1868). *Journal of Experimental Marine Biology and Ecology*, *409*(1), 351–360. https://doi.org/10.1016/j.jembe.2011.09.017

Brooks, E. J., Mandelman, J. W., Sloman, K. A., Liss, S., Danylchuk, A. J., Cooke, S. J., Skomal, G. B., Philipp, D. P., Sims, D. W., & Suski, C. D. (2012). The physiological response of the Caribbean reef shark (*Carcharhinus perezi*) to longline capture. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology*, *162*(2), 94–100. https://doi.org/10.1016/j.cbpa.2011.04.012

Butcher, P. A., Peddemors, V. M., Mandelman, J. W., McGrath, S. P., & Cullis, B. R. (2015). At-vessel mortality and blood biochemical status of elasmobranchs caught in an Australian commercial longline fishery. *Global Ecology and Conservation*, *3*, 878–889. https://doi.org/10.1016/j.gecco.2015.04.012

Cliff, G., & Thurman, G. D. (1984). Pathological and physiological effects of stress during capture and transport in the juvenile dusky shark, *Carcharhinus obscurus*. *Comparative Biochemistry and Physiology Part A: Physiology*, *78*(1), 167–173. https://doi.org/10.1016/0300-9629(84)90111-7

Clow, K. A., Short, C. E., & Driedzic, W. R. (2017). Low levels of extracellular glucose limit cardiac anaerobic metabolism in some species of fish. *Journal of Experimental Biology*, jeb.159582. https://doi.org/10.1242/jeb.159582

Cooke, S. J., & Schramm, H. L. (2007). Catch-and-release science and its application to conservation and management of recreational fisheries. Fisheries Management and Ecology, 14(2), 73-79. doi:10.1111/j.1365-2400.2007.00527.x

Cooke, S.J., Suski, C.D., 2005. Do we need species-specific guidelines for catch-and-release recreational angling to conserve diverse fishery resources? *Biodiversity and Conservation* 14, 1195–1209. doi:10.1007/s10531-004-7845-0

deRoos, R., & deRoos, C. C. (1978). Elevation of plasma glucose levels by catecholamines in elasmobranch fish. *General and Comparative Endocrinology*, *34*(4), 447–452. https://doi.org/10.1016/0016-6480(78)90285-X

Diaz, G. A., & Serafy, J. E. (2005). Longline-caught blue shark: factors affecting the numbers available for live release. *Fishery Bulletin (Washington, D.C.), 103*(4), 720-724. Retrieved from Aquatic Science & Fisheries Abstracts (ASFA) Professional database. Retrieved from https://search.proquest.com/docview/17417288

Ellis, J. R., McCully Phillips, S. R., & Poisson, F. (2017). A review of capture and post-release mortality of elasmobranchs: Capture mortality of elasmobranchs. *Journal of Fish Biology*, *90*(3), 653–722. https://doi.org/10.1111/jfb.13197

Estrada, J. A., Rice, A. N., Natanson, L. J., & Skomal, G. B. (2006). Use of isotopic analysis of vertebrae in reconstructing ontogenetic feeding ecology in white sharks. *Ecology*, *87*(4), 829–834. https://doi.org/10.1890/0012-9658(2006)87[829:UOIAOV]2.0.CO;2

French, R. P., Lyle, J., Tracey, S., Currie, S., & Semmens, J. M. (2015). High survivorship after catch-and-release fishing suggests physiological resilience in the endothermic shortfin mako shark (*Isurus oxyrinchus*). *Conservation Physiology*, *3*(1), cov044. https://doi.org/10.1093/conphys/cov044

Frick, L. H., Walker, T. I., & Reina, R. D. (2012). Immediate and delayed effects of gill-net capture on acid–base balance and intramuscular lactate concentration of gummy sharks, *Mustelus antarcticus*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *162*(2), 88–93. https://doi.org/10.1016/j.cbpa.2011.02.023

Fronton, F., Ferchiou, S., Caza, F., Villemur, R., Robert, D., & St-Pierre, Y. (2023). Insights into the circulating microbiome of Atlantic and Greenland halibut populations: The role of species-specific and environmental factors. *Scientific Reports*, *13*(1), 5971. https://doi.org/10.1038/s41598-023-32690-6

Fukao, T., Lopaschuk, G. D., & Mitchell, G. A. (2004). Pathways and control of ketone body metabolism: on the fringe of lipid biochemistry. *Prostaglandins, leukotrienes, and essential fatty acids*, *70*(3), 243–251. https://doi.org/10.1016/j.plefa.2003.11.001

Gallagher, A., Meyer, L., Pethybridge, H., Huveneers, C., & Butcher, P. (2019). Effects of short-term capture on the physiology of white sharks *Carcharodon carcharias*: Amino acids and fatty acids. *Endangered Species Research*, *40*, 297–308. https://doi.org/10.3354/esr00997

Gilmore, R. G., Dodrill', J. W., & Linley', P. A. (1983). Reproduction and embryonic development of the sand tiger shark, *Odontaspis taurus* (Rafinesque). *Fishery Bulletin, 81*(2), 201-225.

Goldman, K. J., Branstetter, S., & Musick, J. A. (2006). A re-examination of the age and growth of sand tiger sharks, *Carcharias taurus*, in the western North Atlantic: The importance of ageing protocols and use of multiple back-calculation techniques. *Environmental Biology of Fishes*, *77*(3–4), 241–252. https://doi.org/10.1007/s10641-006-9128-y

Hammer, T. J., Sanders, J. G., & Fierer, N. (2019). Not all animals need a microbiome. *FEMS Microbiology Letters*, *366*(10), fnz117. https://doi.org/10.1093/femsle/fnz117

Haulsee, D., Breece, M., Brown, L., Wetherbee, B., Fox, D., & Oliver, M. (2018). Spatial ecology of *Carcharias taurus* in the northwestern Mid-Atlantic coastal ocean. *Marine Ecology Progress Series*, *597*, 191–206. https://doi.org/10.3354/meps12592

Haulsee, D. E., Fox, D. A., Breece, M. W., Clauss, T. M., & Oliver, M. J. (2016). Implantation and recovery of long-term archival transceivers in a migratory shark with high site fidelity. *PLOS ONE*, *11*(2), e0148617. https://doi.org/10.1371/journal.pone.0148617

Heberer, C., Aalbers, S. A., Bernal, D., Kohin, S., DiFiore, B., & Sepulveda, C. A. (2010). Insights into catch-and-release survivorship and stress-induced blood biochemistry of common thresher sharks (*Alopias vulpinus*) captured in the southern California recreational fishery. *Fisheries Research*, *106*(3), 495–500. https://doi.org/10.1016/j.fishres.2010.09.024

Henningsen, A. D., Murru, F. L., Rasmussen, L. E. L., Whitaker, B. R., & Violetta, G. C. (2008). Serum levels of reproductive steroid hormones in captive sand tiger sharks, *Carcharias taurus* (Rafinesque), and comments on their relation to sexual conflicts. *Fish Physiology and Biochemistry*, *34*(4), 437–446. https://doi.org/10.1007/s10695-008-9202-9

Hoopes, L. A., Clauss, T., Wetherbee, B. M., & Fox, D. A. (2022). Baseline Health and Nutritional Parameters of Wild Sand Tigers Sampled in Delaware Bay. *Journal of Aquatic Animal Health*, AAH.10156. https://doi.org/10.1002/aah.10156

Hyatt, M. W., Anderson, P. A., O'Donnell, P. M., & Berzins, I. K. (2012). Assessment of acid–base derangements among bonnethead (*Sphyrna tiburo*), bull (*Carcharhinus leucas*), and lemon (*Negaprion brevirostris*) sharks from gillnet and longline capture and handling methods. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *162*(2), 113–120. https://doi.org/10.1016/j.cbpa.2011.05.004

Hyatt, M. W. (2016). Behavioral Release Condition Score of Bull and Bonnethead Sharks as a Coarse Indicator of Stress. *Journal of Coastal Research*, *32*(6), 1464. https://doi.org/10.2112/JCOASTRES-D-15-00108.1

Inaba, M., Kumeda, Y., Yamada, S., Toi, N., Hamai, C., Noguchi, K., Yasuda, E., Furumitsu, Y., Emoto, M., & Ohno, Y. (2020). Association of higher arterial ketone body ratio (acetoacetate/β-hydroxybutyrate) with relevant nutritional marker in hemodialysis patients. *BMC Nephrology*, *21*(1), 510. https://doi.org/10.1186/s12882-020-02173-1

IUCN. (2020). *Carcharias taurus: Rigby, C.L., Carlson, J., Derrick, D., Dicken, M., Pacoureau, N. & Simpfendorfer, C.: The IUCN Red List of Threatened Species 2021: e.T3854A2876505* [Data set]. International Union for Conservation of Nature. https://doi.org/10.2305/IUCN.UK.2021-2.RLTS.T3854A2876505.en

Kelly, J. R., Kennedy, P. J., Cryan, J. F., Dinan, T. G., Clarke, G., & Hyland, N. P. (2015). Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. *Frontiers in cellular neuroscience*, *9*, 392. https://doi.org/10.3389/fncel.2015.00392

Kieffer, J. D., Currie, S., & Tufts, B. L. (1994). Effects of Environmental Temperature on the Metabolic and Acid–base Responses of Rainbow Trout to Exhaustive Exercise. *Journal of Experimental Biology*, *194*(1), 299–317. https://doi.org/10.1242/jeb.194.1.299

Kilfoil, J. P., Wetherbee, B. M., Carlson, J. K., & Fox, D. A. (2017). Targeted catch-and-release of prohibited sharks: sand tigers in coastal Delaware waters. *Fisheries*, *42*(5), 281–287. https://doi.org/10.1080/03632415.2017.1306974\

Kim, S. L., Tinker, M. T., Estes, J. A., & Koch, P. L. (2012). Ontogenetic and among-individual variation in foraging strategies of northeast pacific white sharks based on stable isotope analysis. *PLOS ONE*, *7*(9), 1–11. https://doi.org/10.1371/journal.pone.0045068

Kneebone, J., Chisholm, J., Bernal, D., & Skomal, G. (2013). The physiological effects of capture stress, recovery, and post-release survivorship of juvenile sand tigers (*Carcharias taurus*) caught on rod and reel. *Fisheries Research*, *147*, 103–114. https://doi.org/10.1016/j.fishres.2013.04.009

Kneebone, J., Chisholm, J., & Skomal, G. (2014). Movement patterns of juvenile sand tigers (*Carcharias taurus*) along the east coast of the USA. *Marine Biology*, *161*(5), 1149–1163. https://doi.org/10.1007/s00227-014-2407-9

Kneebone, J., Winton, M., Danylchuk, A., Chisholm, J., & Skomal, G. B. (2018). An assessment of juvenile sand tiger (*Carcharias taurus*) activity patterns in a seasonal nursery using accelerometer transmitters. *Environmental Biology of Fishes*, *101*(12), 1739–1756. https://doi.org/10.1007/s10641-018-0821-4

Lambert, F. N., Treberg, J. R., Anderson, W. G., Brandt, C., & Evans, A. N. (2018). The physiological stress response of the Atlantic stingray (*Hypanus sabinus*) to aerial exposure. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *219–220*, 38–43. https://doi.org/10.1016/j.cbpa.2018.02.009

Leigh, S. C., Papastamatiou, Y. P., & German, D. P. (2021). Gut microbial diversity and digestive function of an omnivorous shark. *Marine Biology*, *168*(5), 55. https://doi.org/10.1007/s00227-021-03866-3

Lucifora, L. (2002). Reproductive ecology and abundance of the sand tiger shark, Carcharias taurus, from the southwestern Atlantic. *ICES Journal of Marine Science*, *59*(3), 553–561. https://doi.org/10.1006/jmsc.2002.1183

Lucifora, L. O., García, V. B., & Escalante, A. H. (2009). How can the feeding habits of the sand tiger shark influence the success of conservation programs? *Animal Conservation*, *12*(4), 291–301. https://doi.org/10.1111/j.1469-1795.2009.00247.x

Lyons, K., & Wynne-Edwards, K. E. (2019). Legacy environmental polychlorinated biphenyl contamination attenuates the acute stress response in a cartilaginous fish, the Round Stingray. *Stress*, *22*(3), 395–402. https://doi.org/10.1080/10253890.2019.1570125

Mandelman, J. W., & Skomal, G. B. (2009). Differential sensitivity to capture stress assessed by blood acid–base status in five carcharhinid sharks. *Journal of Comparative Physiology B*, *179*(3), 267–277. https://doi.org/10.1007/s00360-008-0306-4

Marshall, H., Field, L., Afiadata, A., Sepulveda, C., Skomal, G., & Bernal, D. (2012). Hematological indicators of stress in longline-captured sharks. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology*, *162*(2), 121–129. https://doi.org/10.1016/j.cbpa.2012.02.008

McLaughlin, R. W., Vali, H., Lau, P. C. K., Palfree, R. G. E., Ciccio, A. D., Sirois, M., Ahmad, D., Villemur, R., Desrosiers, M., & Chan, E. C. S. (2002). Are there naturally occurring pleomorphic bacteria in the blood of healthy humans? *Journal of Clinical Microbiology*, *40*(12), 4771–4775. https://doi.org/10.1128/JCM.40.12.4771-4775.2002

Moorhead. (2019). Variation of Body Condition and Plasma Metabolites in a Population of South Florida Nurse Sharks, *Ginglymostoma cirratum* [University of Miami]. https://scholarship.miami.edu/esploro/outputs/graduate/Variation-of-Body-Condition-and-Plasma/991031447105802976#file-0

Mylniczenko, N. D., Harris, B., Wilborn, R. E., & Young, F. A. (2007). Blood culture results from healthy captive and free-ranging elasmobranchs. *Journal of Aquatic Animal Health*, *19*(3), 159–167. https://doi.org/10.1577/H06-039.1

Oliver, S., Braccini, M., Newman, S. J., & Harvey, E. S. (2015). Global patterns in the bycatch of sharks and rays. *Marine Policy*, *54*, 86–97. https://doi.org/10.1016/j.marpol.2014.12.017

Ortega, V. A., Mercer, E. M., Giesbrecht, G. F., & Arrieta, M.-C. (2021). Evolutionary Significance of the Neuroendocrine Stress Axis on Vertebrate Immunity and the Influence of the Microbiome on Early-Life Stress Regulation and Health Outcomes. *Frontiers in Microbiology*, *12*, 634539. https://doi.org/10.3389/fmicb.2021.634539

Otway N. M. (2015). Serum biochemical reference intervals for free-living Sand Tiger sharks (*Carcharias taurus*) from east Australian waters. *Veterinary clinical pathology*, *44*(2), 262–274. https://doi.org/10.1111/vcp.12254

Perry, C. T., Pratte, Z. A., Clavere-Graciette, A., Ritchie, K. B., Hueter, R. E., Newton, A. L., Fischer, G. C., Dinsdale, E. A., Doane, M. P., Wilkinson, K. A., Bassos-Hull, K., Lyons, K., Dove, A. D. M., Hoopes, L. A., & Stewart, F. J. (2021). Elasmobranch microbiomes: Emerging patterns and implications for host health and ecology. *Animal Microbiome*, *3*(1), 61. https://doi.org/10.1186/s42523-021-00121-4

Richards, J. G., Heigenhauser, G. J. F., & Wood, C. M. (2003). Exercise and recovery metabolism in the pacific spiny dogfish (*Squalus acanthias*). *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, *173*(6), 463–474. https://doi.org/10.1007/s00360-003-0354-8

Ritchie, K. B., Schwarz, M., Mueller, J., Lapacek, V. A., Merselis, D., Walsh, C. J., & Luer, C. A. (2017). Survey of Antibiotic-producing Bacteria Associated with the Epidermal Mucus Layers of Rays and Skates. *Frontiers in Microbiology*, *8*, 1050. https://doi.org/10.3389/fmicb.2017.01050

Romero, M. L., & Butler, L. K. (2007). Endocrinology of stress. *International Journal of Comparative Psychology*, *20*(2). https://doi.org/10.46867/IJCP.2007.20.02.15

Sanders, E. R. (2012). Aseptic Laboratory Techniques: Plating Methods. *JoVE*, *63*, e3064. https://doi.org/10.3791/3064

Scheiman, J., Luber, J. M., Chavkin, T. A., MacDonald, T., Tung, A., Pham, L. D., Wibowo, M. C., Wurth, R. C., Punthambaker, S., Tierney, B. T., Yang, Z., Hattab, M. W., Avila-Pacheco, J., Clish, C. B., Lessard, S., Church, G. M., & Kostic, A. D. (2019). Meta-omics analysis of elite athletes

identifies a performance-enhancing microbe that functions via lactate metabolism. *Nature Medicine*, *25*(7), 1104–1109. https://doi.org/10.1038/s41591-019-0485-4

Schoen, A. N., Bouyoucos, I. A., Anderson, W. G., Wheaton, C. J., Planes, S., Mylniczenko, N. D., & Rummer, J. L. (2021a). Simulated heatwave and fishing stressors alter corticosteroid and energy balance in neonate blacktip reef sharks, *Carcharhinus melanopterus* . *Conservation Physiology*, *9*(1), coab067. https://doi.org/10.1093/conphys/coab067

Schoen, A. N., Treberg, J. R., Wheaton, C. J., Mylniczenko, N., & Gary Anderson, W. (2021b). Energy and corticosteroid mobilization following an induced stress response in an elasmobranch fish, the North Pacific spiny dogfish (*Squalus acanthias suckleyi*). *General and Comparative Endocrinology*, *310*, 113799. https://doi.org/10.1016/j.ygcen.2021.113799

Schroeder, D. M., & Love, M. S. (2002). Recreational fishing and marine fish populations in California. *California Cooperative Oceanic Fisheries Investigations Report* 43:182.

Skomal, G. B., & Mandelman, J. W. (2012). The physiological response to anthropogenic stressors in marine elasmobranch fishes: A review with a focus on the secondary response. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *162*(2), 146–155. https://doi.org/10.1016/j.cbpa.2011.10.002

Speers-Roesch, B., Ip, Y. K., & Ballantyne, J. S. (2006). Metabolic organization of freshwater, euryhaline, and marine elasmobranchs: Implications for the evolution of energy metabolism in sharks and rays. *Journal of Experimental Biology*, *209*(13), 2495–2508. https://doi.org/10.1242/jeb.02294

Speers-Roesch, B., & Treberg, J. R. (2010). The unusual energy metabolism of elasmobranch fishes. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *155*(4), 417–434. https://doi.org/10.1016/j.cbpa.2009.09.031

Stoot, L. J., Cairns, N. A., Cull, F., Taylor, J. J., Jeffrey, J. D., Morin, F., Mandelman, J. W., Clark, T. D., & Cooke, S. J. (2014). Use of portable blood physiology point-of-care devices for basic and applied research on vertebrates: A review. *Conservation Physiology*, *2*(1), cou011–cou011. https://doi.org/10.1093/conphys/cou011

Tanaka, J., Ozawa, K., & Tobe, T. (1979). Significance of blood ketone body ratio as an indicator of hepatic cellular energy status in jaundiced rabbits. *Gastroenterology*, *76*(4), 691–696. https://doi.org/10.1016/S0016-5085(79)80167-5

Tao, Z., Bullard, S. A., & Arias, C. R. (2014). Diversity of bacteria cultured from the blood of lesser electric rays caught in the northern gulf of Mexico. *Journal of aquatic animal health*, *26*(4), 225–232. https://doi.org/10.1080/08997659.2014.922513

Tarnecki, A. M., Patterson, William F, & Arias, C. R. (2016). Microbiota of wild-caught Red Snapper *Lutjanus campechanus*. *BMC Microbiology*, *16*(1), 245. doi:10.1186/s12866-016-0864-7

Terrien, X., & Prunet, P. (2013). Crossregulation of the Thyroid Hormone and Corticosteroids in Amphibians and Fish: The Effects of Endocrine Disruption. In R. Payan Carreira (Ed.), *Insights from Veterinary Medicine*. InTech. https://doi.org/10.5772/54314

Teter S. M., Wetherbee B. M., Fox D. A., Lam C. H., Kiefer D. A., Shivji M. (2015) Migratory patterns and habitat use of the sand tiger shark (*Carcharias taurus*) in the western North Atlantic. *Marine and Freshwater Research 66*(2), 158-169. https://doi.org/10.1071/MF14129

Uren Webster, T. M., Rodriguez-Barreto, D., Consuegra, S., & Garcia De Leaniz, C. (2020). Cortisol-Related Signatures of Stress in the Fish Microbiome. *Frontiers in Microbiology*, *11*, 1621. https://doi.org/10.3389/fmicb.2020.01621

Valls, E., Navarro, J., Barría, C., Coll, M., Fernández-Borràs, J., & Rotllant, G. (2016). Seasonal, ontogenetic and sexual changes in lipid metabolism of the small-spotted catshark (*Scyliorhinus canicula*) in deep-sea free-living conditions. *Journal of Experimental Marine Biology and Ecology*, *483*, 59–63. https://doi.org/10.1016/j.jembe.2016.07.001

Veech, R. L. (2004). The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 70(3), 309–319.* doi:10.1016/j.plefa.2003.09.007

Vodička, M., Ergang, P., Hrnčíř, T., Mikulecká, A., Kvapilová, P., Vagnerová, K., Šestáková, B., Fajstová, A., Hermanová, P., Hudcovic, T., Kozáková, H., & Pácha, J. (2018). Microbiota affects the expression of genes involved in HPA axis regulation and local metabolism of glucocorticoids in chronic psychosocial stress. *Brain, Behavior, and Immunity*, *73*, 615–624. https://doi.org/10.1016/j.bbi.2018.07.007

Watson, R. R., & Dickson, K. A. (2001). Enzyme activities support the use of liver lipid–derived ketone bodies as aerobic fuels in muscle tissues of active sharks. *Physiological and Biochemical Zoology*, *74*(2), 273–282. https://doi.org/10.1086/319667

Weber, D. N., Janech, M. G., Burnett, L. E., Sancho, G., & Frazier, B. S. (2021). Insights into the origin and magnitude of capture and handling-related stress in a coastal elasmobranch *Carcharhinus limbatus*. *ICES Journal of Marine Science*, *78*(3), 910–921. https://doi.org/10.1093/icesjms/fsaa223

Wendelaar Bonga, S. E. (1997). *The stress response in fish. Physiological Reviews, 77(3), 591–625.* doi:10.1152/physrev.1997.77.3.59

Whitney, N. M., White, C. F., Anderson, P. A., Hueter, R. E., & Skomal, G. B. (2017). The physiological stress response, postrelease behavior, and mortality of blacktip sharks (*Carcharhinus limbatus*) caught on circle and J-hooks in the Florida recreational fishery. *Fishery Bulletin*, *115*(4), 532–543. https://doi.org/10.7755/FB.115.4.9

Wood, C. M., Walsh, P. J., Kajimura, M., McClelland, G. B., & Chew, S. F. (2010). The influence of feeding and fasting on plasma metabolites in the dogfish shark (*Squalus acanthias*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *155*(4), 435–444. https://doi.org/10.1016/j.cbpa.2009.09.006

Wyffels, J. T., George, R., Adams, L., Adams, C., Clauss, T., Newton, A., Hyatt, M. W., Yach, C., & Penfold, L. M. (2020). Testosterone and semen seasonality for the sand tiger shark *Carcharias taurus*. *Biology of Reproduction*, *102*(4), 876–887. https://doi.org/10.1093/biolre/ioz221

Zammit, V. A., & Newsholme, E. A. (1979). Activities of enzymes of fat and ketone-body metabolism and effects of starvation on blood concentrations of glucose and fat fuels in teleost and elasmobranch fish. *Biochemical Journal*, *184*(2), 313–322. https://doi.org/10.1042/bj1840313

APPENDIX I

VALIDATING POINT OF CARE METERS FOR USE IN *C. TARUS*

Elasmobranchs (sharks and rays) are regularly captured by commercial and recreational anglers, as well as captured for scientific research. This capture, which involves exhaustive fighting and air exposure, can induce a measurable stress response. One way to measure stress is by changes in blood chemistry that indicate increased energy production (glucose) and metabolic state (lactate), and energy store use (ketones). Most elasmobranch fieldwork is remote and keeping most species in the lab is not feasible, so researchers need to be able to analyze blood in the field. Small, battery powered, point of care (POC) blood meters make this possible. However, these devices were developed for mammals and have not thoroughly been validated for use in elasmobranchs (Stoot et al., 2014). To validate POC meters for use in sand tiger sharks, whole blood samples were tested on meters in the field, and plasma samples will be tested again on these meters in the lab. Biochemical assays were then ran to elucidate the plasma concentrations of glucose, lactate, and BHB. The following point of care meters were be used: the iStat (Abbot Point of Care Inc., Princeton, NJ, USA), The iPet Pro (Ultimed Inc. Excelsior, MN, USA) glucose meter, Contour next (Senseonics, Inc. Parsippany, NJ, USA) glucose meter, Ketosense (i-Sens Inc, Torrance, CA, USA) ketone meter, and the Lactate Plus (Nova Biomedical Inc. Waltham, MA, USA). To assess the hypothesis that the POC meters will produce representative values like the biochemical assays, Spearmans rank correlations were ran to compare the meter and biochemical assay data for glucose, lactate, and ketones. The iPet Pro glucose meter when used to run whole blood or plasma, does not produce representative glucose values that correlate with biochemical laboratory assays (whole blood vs assay: Spearmans rank $\rho = -0.18$, $p = 0.3632$, $N = 29$; plasma vs assay: Spearmans rank $\rho = 0.22$, $p = 0.1692$, $N = 39$). The lactate plus meter produced representative values to the laboratory assays when used with whole blood (Spearmans rank $\rho = 0.52$, $p = 0.0043$, $N = 28$) and plasma (Spearmans rank $\rho = 0.80$, $p < 0.0001$). More investigation is needed on the Ketosense meter and ketones in elasmobranchs in general to establish whether the Ketosense is a useful tool for analyzing circulating ketone bodies in

elasmobranchs. Values produced by whole blood and plasma on the Ketosense both correlated with the values produced by laboratory assays (whole blood vs assay: Spearmans rank $\rho = 0.51$, $p = 0.004$, $N = 30$; plasma vs assay: Spearmans rank $\rho = 0.48$, $p = 0.0021$, $N = 39$). This validation has an implication for development of more accurate and affordable field and laboratory blood sampling methods in elasmobranchs.

APPENDIX II

TABLES

Table 1: Mean, standard deviation, range, and sample size of plasma metabolite levels combined between sexes, females, and males, from the initial blood sample. Data are pooled from all three sampling years (2021, 2022, and 2023).

Metabolite initial value (mM)	Mean	Standard Deviation	Min - Max	N
Glucose	2.98	0.76	1.37 - 5.47	50
Females	3.03	0.79	1.37 – 5.47	24
Males	2.94	0.75	1.79 – 5.09	26
Lactate	3.12	2.31	0 – 12	48
Females	2.98	1.66	0.65 – 7.5	24
Males	3.28	2.84	0 – 12	24
AcAc	0.74	0.74	0 - 2.9	35
Females	0.81	0.68	0 – 2.18	16
Males	0.70	0.81	0 – 2. 9	19
BHB	0.22	0.17	0 - 0.59	28
Females	0.69	0.07	0.57 – 0.84	14
Males	0.77	0.09	0.64 – 0.93	8

Table 2: Mean, standard deviation, range, and sample size of environmental factor and longline soak time.

Variable	Mean	Standard Deviation	Min - Max	N
Fork Length	2094 mm	177 mm	1720mm - 2460mm	48
Water Temperature	17.92 °C	0.95 °C	16.6 °C - 19.6 °C	47
Dissolved Oxygen	8.09 mg/L	0.36 mg/L	7.5 mg/L - 8.8 mg/L	47
Longline Soak Time	131 minutes	47 minutes	54 minutes - 313 minutes	51

*Table 3: Results of multiple regression comparing water temperature, dissolved oxygen, and long line soak time against initial plasma glucose. * indicates variables that are significant, standard betas only provided for significant variables. DF = 3, 43*

Predictor Variable	t-value	p-value	Standard Beta
Water Temperature	-2.64	0.0116*	-0.54
Dissolved Oxygen	-0.98	0.3348	
Longline Soak Time	0.12	0.9073	

*Table 4: Results of multiple regression comparing water temperature, dissolved oxygen, and long line soak time against initial plasma lactate. * indicates variables that are significant, standard betas only provided for significant variables. DF = 3, 41*

Predictor Variable	t-value	p-value	standard beta
Water Temperature	-2.1	0.0423*	-0.37
Dissolved Oxygen	0.7	0.4882	
Longline Soak Time	3.41	0.0015*	0.43

*Table 5: Results of multiple regression comparing water temperature, dissolved oxygen, and long line soak time against initial plasma AcAc. * indicates variables that are significant. DF = 3, 28*

Predictor Variable	t-value	p-value
Water Temperature	-1.2	0.2399
Dissolved Oxygen	-1.39	0.1741
Longline Soak Time	-0.62	0.5393

*Table 6: Results of multiple regression comparing water temperature, dissolved oxygen, and long line soak time against initial plasma BHB. * indicates variables that are significant. DF = 3, 21*

Predictor Variable	t-value	p-value
Water Temperature	-1.37	0.1841
Dissolved Oxygen	-0.71	0.4826
Longline Soak Time	1.03	0.317

Table 7: Table of previously published plasma glucose values of sand tiger sharks.

Reference	Mean Glucose (mM)	Glucose Range (mM)	N	Capture Method	Location	Notes
Hoopes et al., 2022	3.55 ± 0.66	2 – 5.38	153	Longline	Delaware Bay, Delaware, USA	
Otway 2015	2.7 ± 0.2	2.5 – 2.9	30	Lassoed by divers, put in tonic immobility, and brought to surface	East Australia	
Anderson et al., 2012	1.88 ± 0.08	Unpublished	23	Varied	Captive from 18 separate institutions	Healthy sharks unaffected by spinal deformities.
Anderson et al., 2012	1.81 ± 0.15	Unpublished	10	Varied	Captive from 18 separate institutions	Sharks affected by spinal deformities.
This study	2.88 ± 0.76	1.3 – 5.47	50	Longline	St. Helena Sound, South Carolina, USA	Values from post-capture but pre-handling blood sample

Table 8: Table of previously published values of plasma lactate in sand tiger sharks.

Reference	Mean Lactate (mM)	Lactate Range (mM)	N	Capture Method	Location	Notes
Hoopes et al., 2022	8.8 ± 3.3	2.8 – 18.1	153	Longline	Delaware Bay, Delaware, USA	
This study	3.12 ± 2.3	0.0 – 12.02	48	Longline	St. Helena Sound, South Carolina, USA	Values from pre-handling blood sample

Table 9: Table of previously published plasma BHB values in various shark species. Table modified from Moorhead 2019. Only wild captured animals not under experimental conditions included.

Reference	Species	BOH Mean mM	BOH Range mM	N	Location
Zammit and Newsholme 1979	Spotted catshark *(Scyliorhinus canicula)*	0.06 ± 0.04	0.013 – 0.140	8	Plymouth, UK
Zammit and Newsholme 1979	Spiny dogfish *(Squalus acanthias)*	0.20	0.173 – 0.287	4	Plymouth, UK
Moorhead, 2019	Nurse shark *(Ginglymostoma cirratum)*	1.36	0.00 – 6.69	107	Miami, FL, USA
Watson and Dickson 2001	Shortfin Mako Shark	0.978	0.08 – 2.89	9	Southern CA, USA
Watson and Dickson 2001	Blue shark	0.26	0.08 – 0.45	6	Southern CA, USA
Current study	Sand tiger shark	0.22 ± 0.14	0.00 – 0.59	28	St. Helena Sound, South Carolina, USA

Table 10: Table of previously published plasma AcAc values in various shark species. Table modified from Moorhead 2019. Only wild captured animals not under experimental conditions included.

Reference	Species	AcAc Mean (mM)	AcAc Range (mM)	N	Location
Zammit and Newsholme 1979	Spotted catshark *(Scyliorhinus canicula)*	0.07±0.034	0.045 – 0.109	8	Plymouth, UK
Zammit and Newsholme 1979	Spiny dogfish *(Squalus acanthias)*	0.13	0.078 – 0.156	4	Plymouth, UK
Moorhead, 2019	Nurse shark *(Ginglymostoma cirratum)*	1.46	0.00 – 7.05	107	Miami, FL, USA
Current Study	Sand tiger shark	0.75 ± 0.744	0.00 – 29	35	St. Helena Sound, South Carolina, USA

APPENDIX III

FIGURES

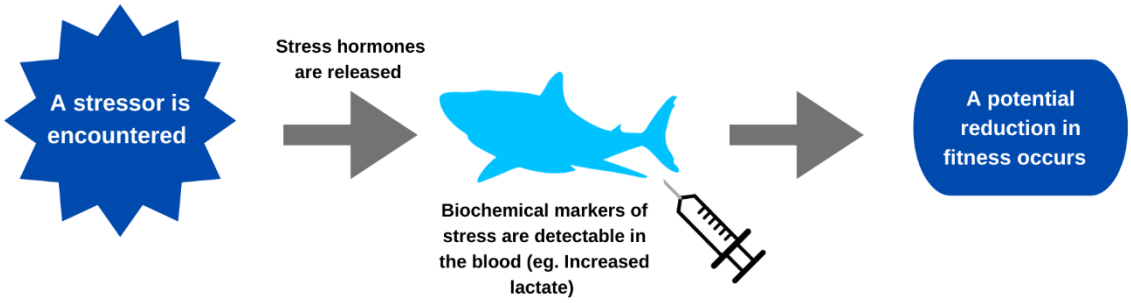

Figure 1: General flowchart of the vertebrate stress response.

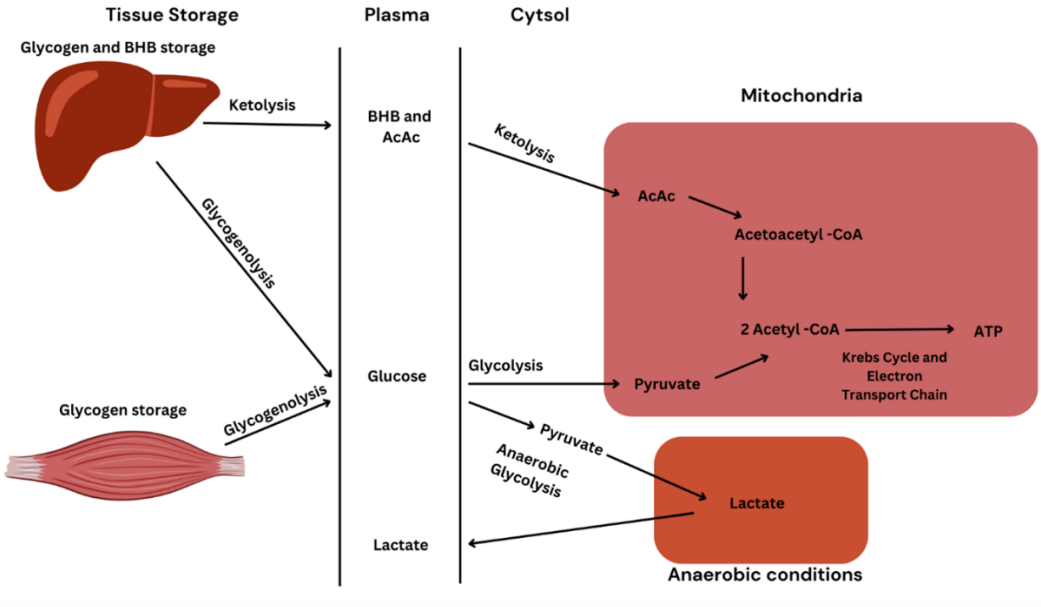

Figure 2: Pathways and fate of mobilized energy stores (BHB and Glycogen).

Figure 3: Scientific illustration of a sand tiger shark (Florida Museum of Natural History, University of Florida).

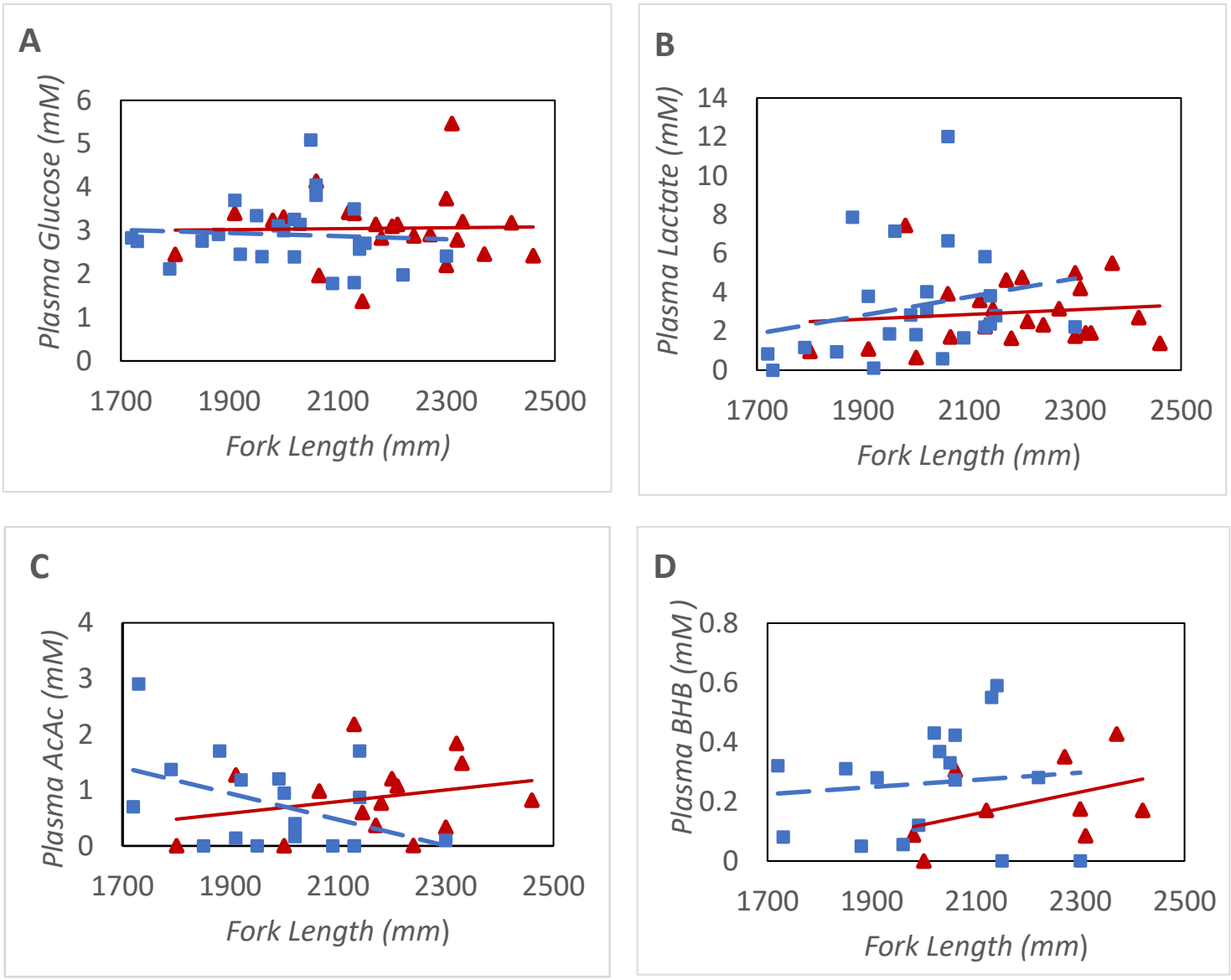

Figure 4: Line graphs of initial plasma metabolites (mM) plotted against fork length (mm). Triangles (▲) and solid trend line represent female sharks, squares (■) and dashed trend line represent male sharks. A) Initial circulating glucose verses fork length by sex. N =48, p = 0.75 B) Initial circulating lactate verses fork length by sex. N = 46, p = 0.45 C) Initial circulating AcAc verses fork length by sex N = 33, p = 0.040. D) Initial circulating BHB verses fork length by sex. N = 26, p = 0.60. * indicates a significant interaction.*

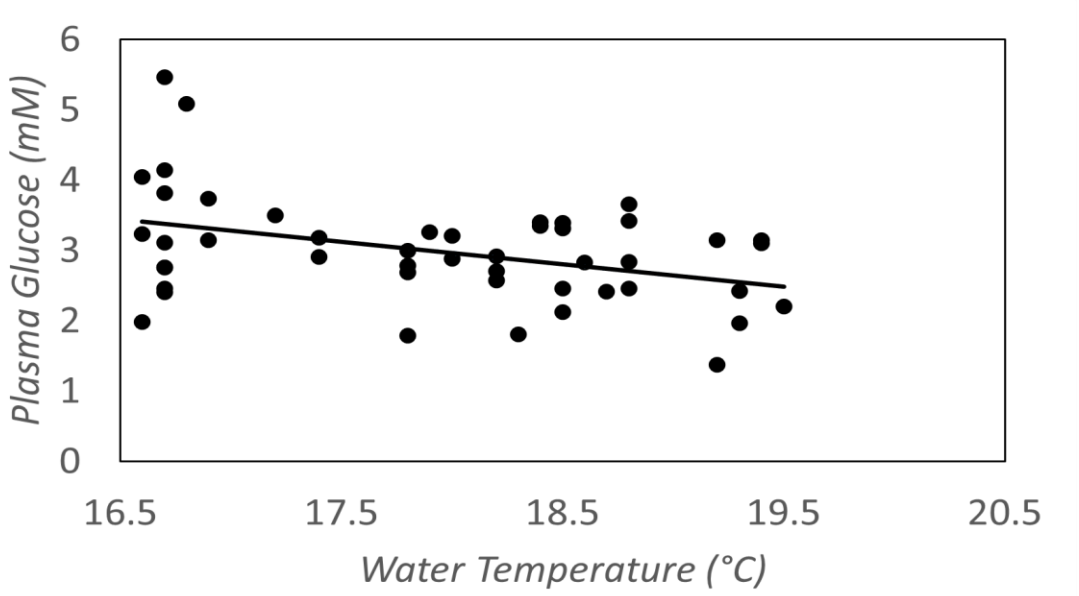

Figure 5: The relationship between water temperature and initial plasma glucose. Points represent individual sharks. N = 48. p = 0.0116.

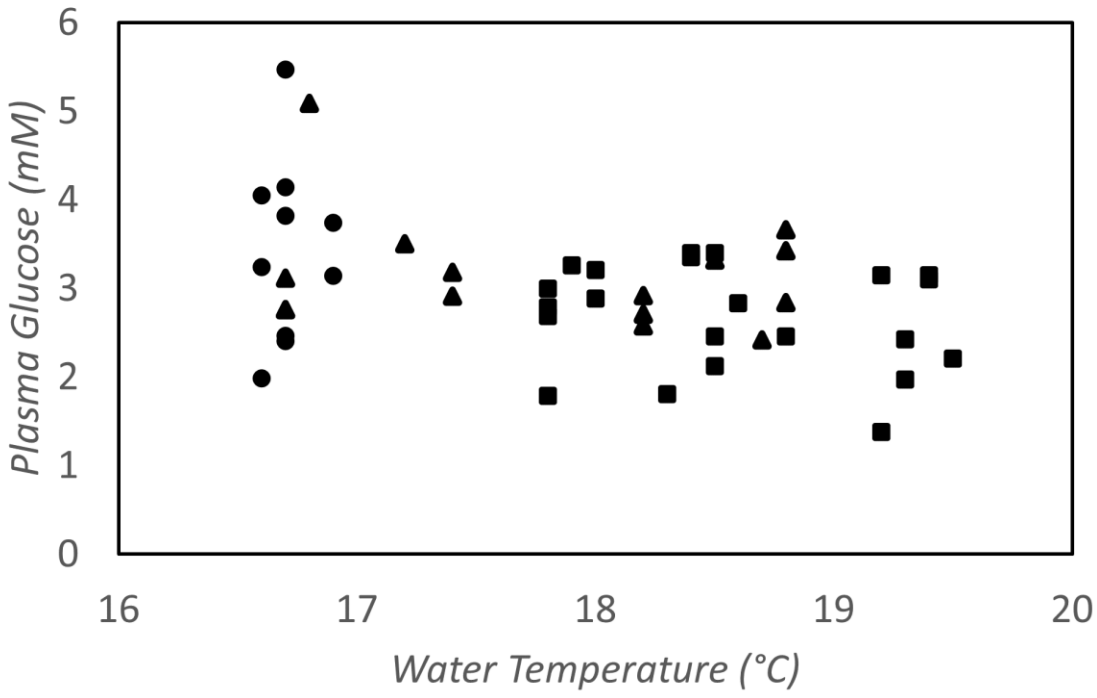

Figure 6: Plasma glucose concentrations across water temperatures, differing symbols represent sharks sampled in 2021 (●) 2022 (▲), and 2023 (■).

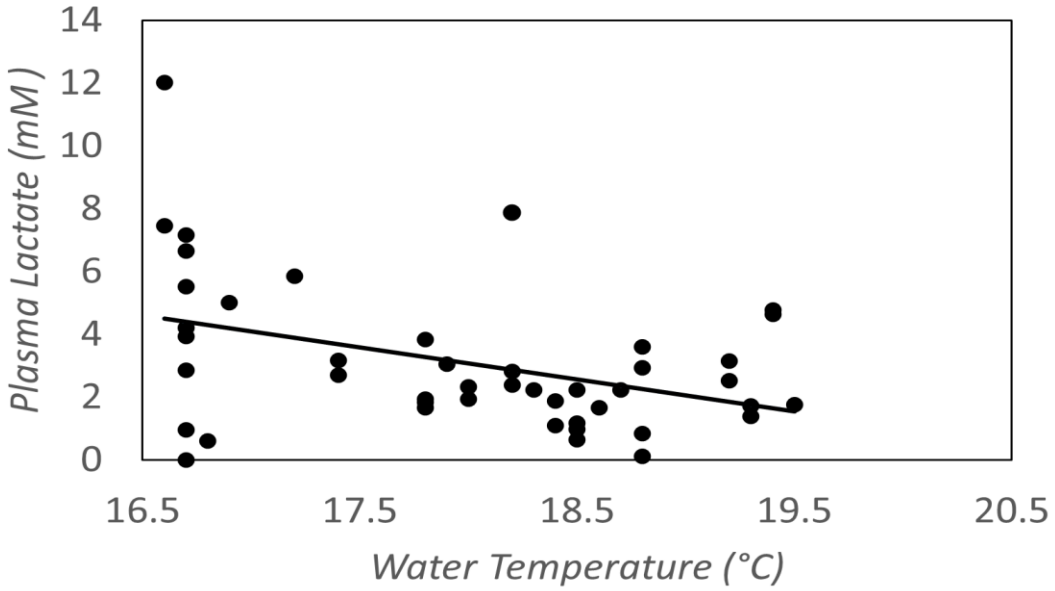

Figure 7: The relationship between water temperature and initial plasma lactate. Points represent individual sharks. N = 45, p = 0.043.

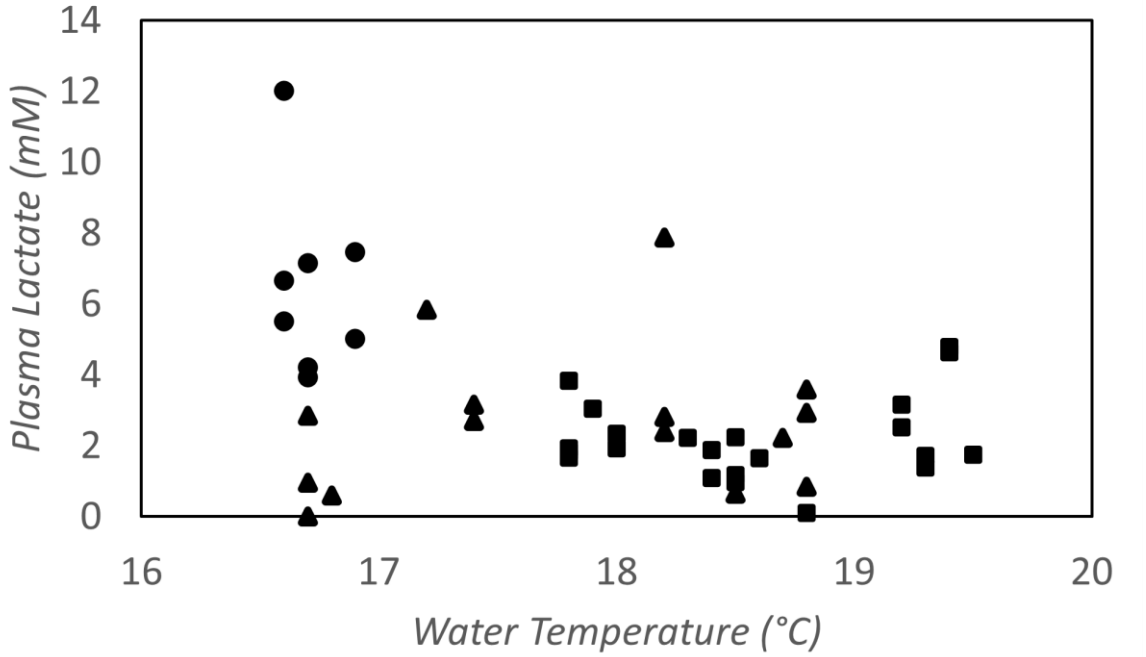

Figure 8: Plasma lactate concentrations across water temperatures, differing symbols represent sharks sampled in 2021 (●) 2022 (▲), and 2023 (■).

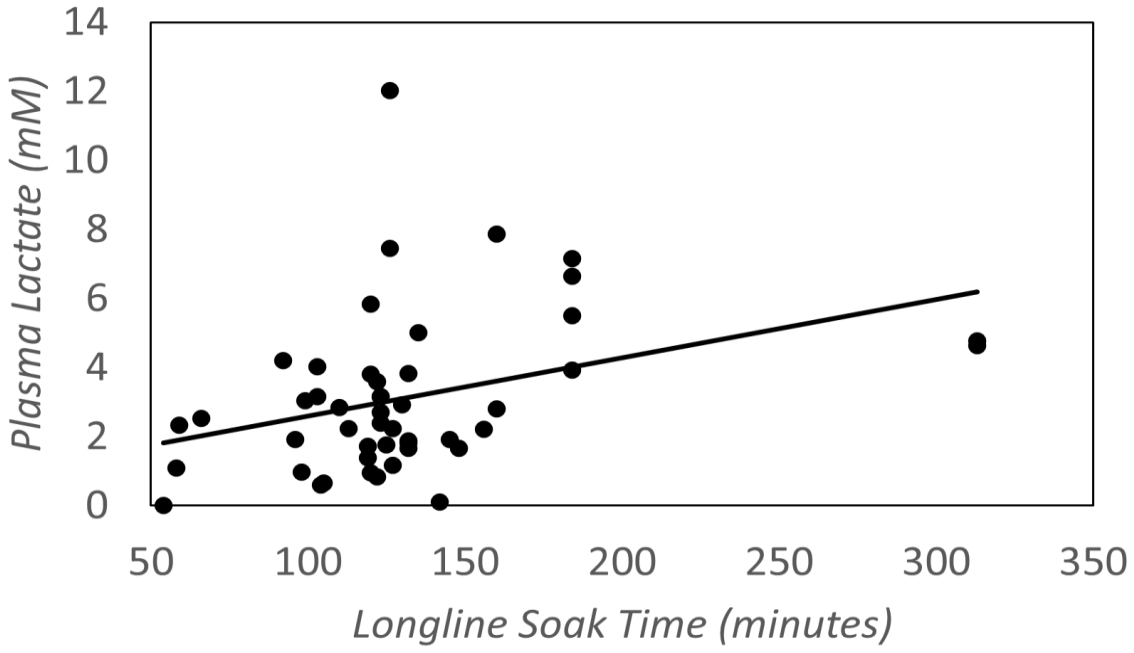

Figure 9: The relationship between long line soak time and initial plasma lactate. Points represent individual sharks. N = 45, p = 0.0015.

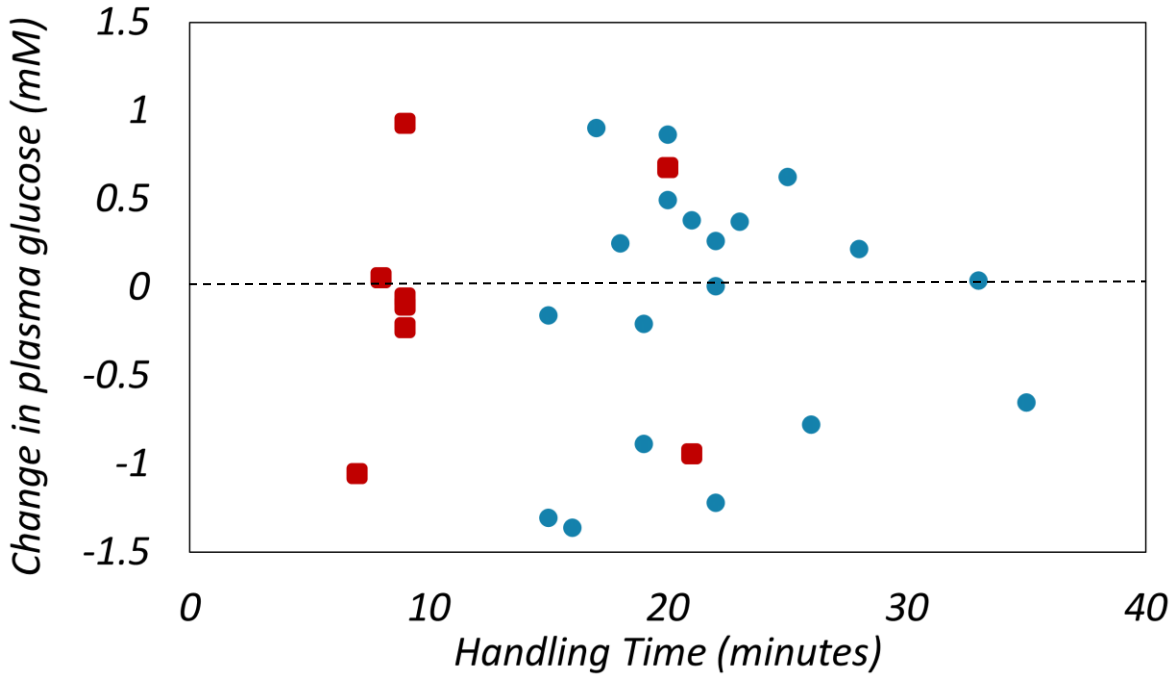

*Figure 10: Change in plasma glucose across handling time by surgery treatment (surgery or no surgery). Each point represents an individual shark. The ● indicates sharks that received surgery and the ■ indicates sharks that did not receive surgery. N = 27, 8 sharks had no surgery, 19 sharks had surgery. Surgery * handling time, F = 0.025, p = 0.875; surgery, F = 0.053, p = 0.81; Handling time, F = 0.06, p = 0.79. All points above the dotted line at zero indicate sharks that had an increase in glucose concentration across handling, points below the dotted line indicate sharks that had a decrease in glucose concentration across handling.*

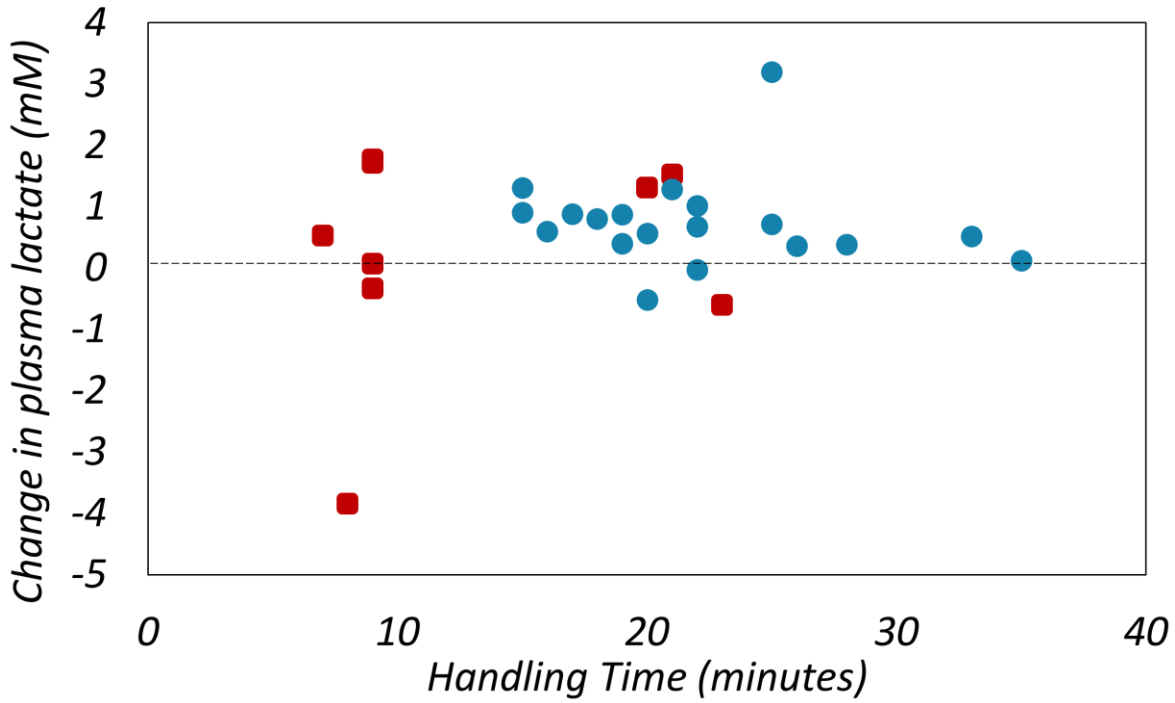

*Figure 11: Change in plasma lactate across handling time by surgery treatment (surgery or no surgery). Each point represents an individual shark. The ● indicates sharks that received surgery and the ■ indicates sharks that did not receive surgery. N = 28, 9 sharks had no surgery, 19 sharks had surgery. Surgery * handling time, F = 0.79, p = 0.382; surgery, F = 0.44, p = 0.51; Handling time, F = 0.07, p = 0.78. All points above the dotted line at zero indicate sharks that had an increase in lactate concentration across handling, points below the dotted line indicate sharks that had a decrease in lactate concentration across handling.*

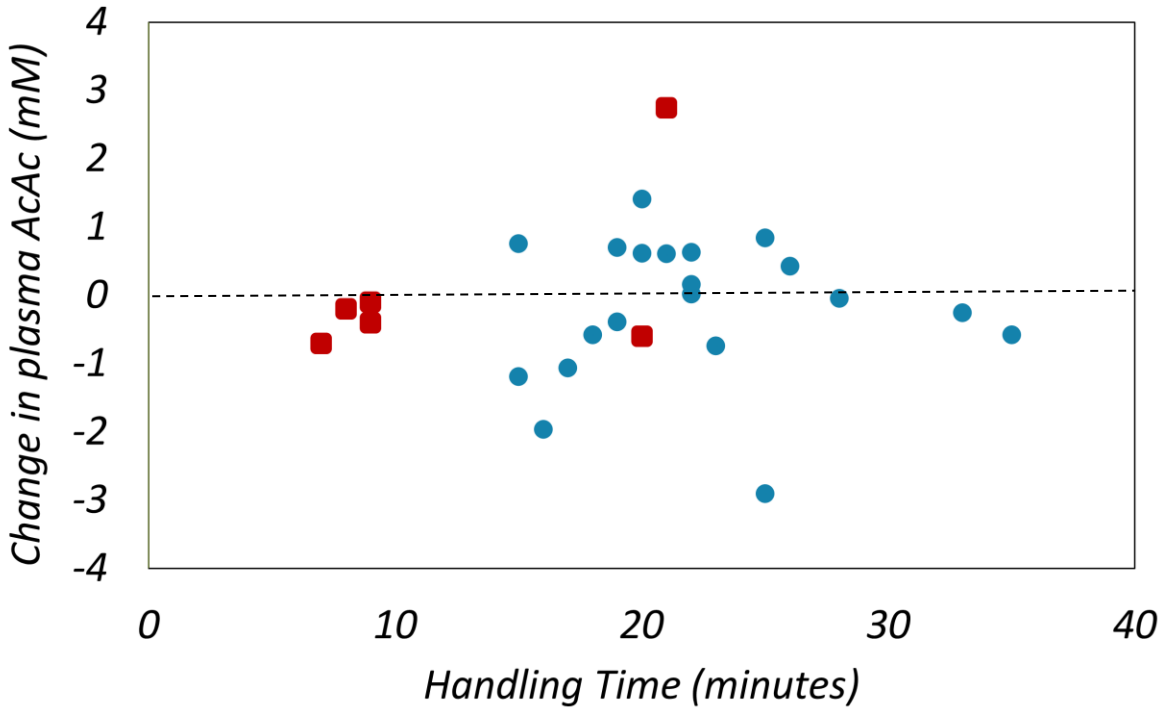

*Figure 12: Change in plasma AcAc across handling time by surgery treatment (surgery or no surgery).. Each point represents an individual shark. The ● indicates sharks that received surgery and the ■ indicates sharks that did not receive surgery. N = 26, 6 sharks had no surgery, 20 sharks had surgery. Surgery * handling time, F = 1.9, p = 0.18; surgery, F = 0.97, p = 0.33; Handling time, F = 0.75, p = 0.39. All points above the dotted line at zero indicate sharks that had an increase in AcAc concentration across handling, points below the dotted line indicate sharks that had a decrease in AcAc concentration across handling.*

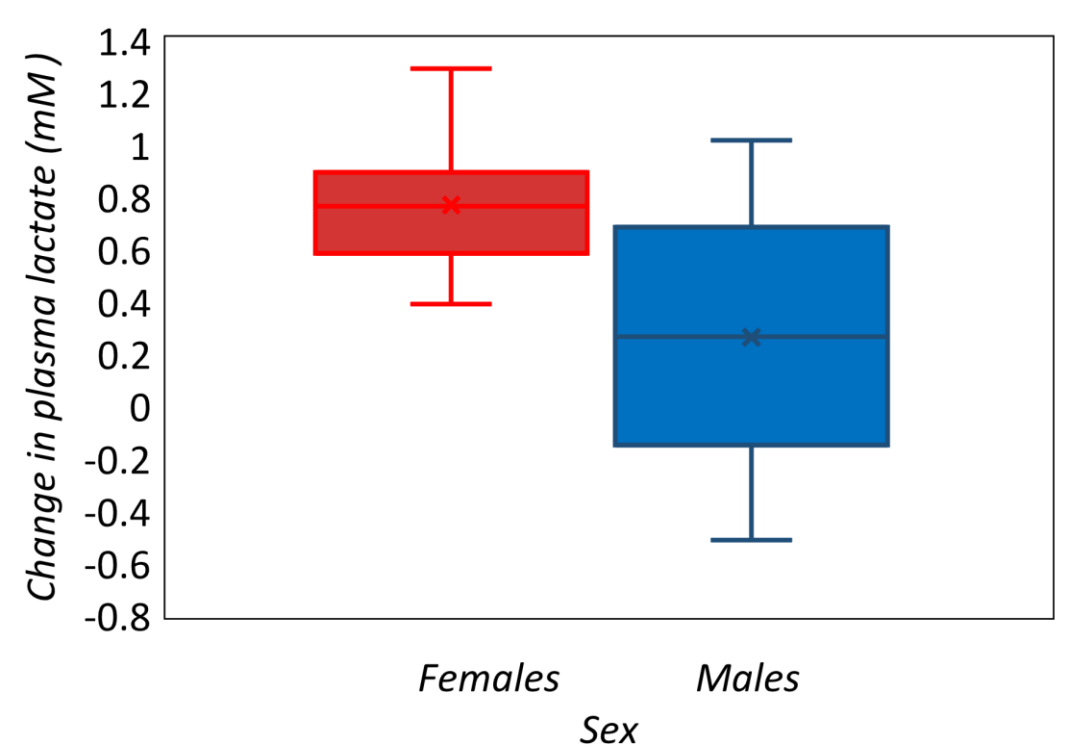

Figure 13: Box and whisker plot of lactate change across sexes. The outer whiskers represent the range of the data, the box represents the interquartile range of the data, the line is representative of the median, and the X is the location of the mean of the data. Female N = 10, Male N = 6, p = 0.0202

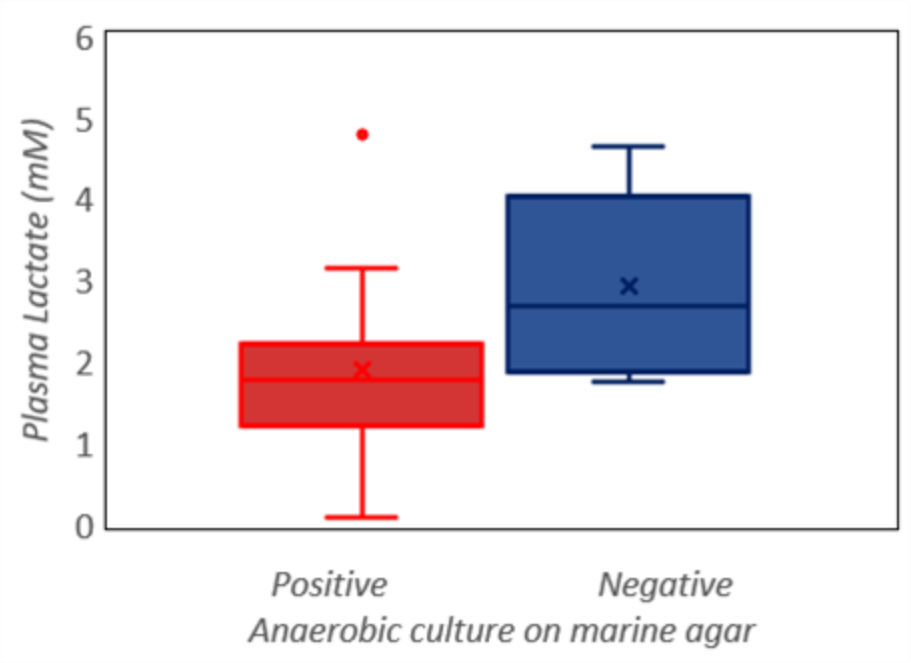

Figure 14: Box and whisker plot of initial plasma lactate in sand tiger sharks by positive or negative anaerobic marine agar culture. The whiskers represent the maximum and minimum range of the data, the box represents the interquartile range, the line within the box represents the median of the data, the X represents the mean, and any dots outside the whisker range represent outliers of the data. N = 22, p =0.057.