Final Report

Brilliant Marine Research Idea 2024

This report should be submitted no later than 28 February 2025 via filantropie@vliz.be and consists of the following documents:

- A final report listing the work done and the problems encountered. This report will be made available online. If any of the tasks has not been completely finished, the report should clearly mention this, including a short explanation. max. 5 pages
- An overview of all expenditures including invoices.
- A set of five pictures (low resolution in this document). The five high resolution pictures should be delivered to VLIZ by email to karen.rappe@vliz.be. Pictures should be free from use - to upload on the VLIZ website and to use in VLIZ communications.

Keep in mind that VLIZ should be mentioned in the acknowledgements of publications following the results of this Brilliant Marine Research Idea.

1. General information

Title of the idea	Measuring stress in a heartbeat in spiny dogfish
Name PhD student	Shamil Debaere
Name supervisor	Gudrun De Boeck
Flemish University or University College	UAntwerpen

2. Brilliant Marine Research Idea – Report about the activities

Abstract

Given the global threats facing shark populations, a comprehensive understanding of their stress physiology and reliable indicators of chronic stress are urgently needed to inform and refine conservation efforts. In this study, we tested the overarching hypothesis that chronic stress alters energy mobilization in a representative shark species, the spiny dogfish (*Squalus suckleyi*). We applied a stress paradigm based on commonly encountered and easily reproducible stressors to experimentally induce the chronic phase of the stress response in adult male spiny dogfish over the course of a 3-week experiment. We subsequently aimed to validate heamatological metrics by monitoring the heart rate of the sharks during acute and chronic stress. Our data demonstrated that conventional vertebrate stress biomarkers may not reliably reflect chronic stress in sharks, and we introduced β-hydroxybutyrate as a potential novel biomarker for chronic stress in this and likely other species of sharks. These insights will be instrumental in devising management strategies that are vital for the conservation of shark populations both today and into the future.

Intro

Since 1970, the global abundance of oceanic sharks and rays has declined by 71% and 59% of the reef-associated shark and ray species are now threatened with extinction. Because sharks encounter so many human and environmental stressors throughout their lives, a detailed investigation into their physiological response to stress is crucial, especially considering that many of these stressors will increase both in frequency and severity over the coming decades. However,

directed studies on the physiological stress response in sharks are rare, even though a profound knowledge of the stress response in sharks is pivotal to inform and refine future conservation efforts. Although the hypothalamus-pituitary-interrenal (HPI) axis, the pathway that mediates the chronic phase of the stress response across the fishes in general, appears to be an evolutionary conserved neuroendocrine system, key physiological differences between sharks and the well-studied teleost fishes preclude directly applying teleost stress theory to sharks. Indeed, sharks are characterized by a unique dominant stress hormone 1α-hydroxycorticosterone or 1α-OHB (as opposed to cortisol and corticosterone widely understood across vertebrates) and a high reliance on ketone bodies and amino acids as oxidative fuels in cardiac and skeletal muscle tissues. A clear understanding of the primary (endocrine) response to stress is currently lacking due to the difficulty to synthesize their unique dominant corticosteroid 1α-OHB for use as standard in measurements. Therefore, finding alternative indicators of stress are of utmost importance to underpin management decisions and conservation strategies.

Their unique physiological characteristics necessitate a tailored approach to understanding the full spectrum of the stress response in sharks and necessitates a re-evaluation of conventional stress biomarkers used in vertebrates. While glucocorticoids (i.e., cortisol and corticosterone), along with plasma glucose and lactate, lipid and fatty acid concentrations, and haematocrit and haemoglobin concentrations, serve as established indicators of chronic stress in tetrapods and teleost fishes, their applicability to sharks is not as straightforward. Evidence for a glucocorticoid role of 1α-OHB in sharks remains equivocal, and, as previously mentioned, sharks predominantly utilise ketone bodies and amino acids as oxidative fuel sources, rather than carbohydrates and fatty acids. These distinctions in their energy metabolism therefore warrant a cautious approach in determining the utility of such biomarkers for assessing chronic stress in sharks.

Here, we tested the overarching hypothesis that chronic stress alters energy metabolism in the spiny dogfish (*Squalus suckleyi*). We hypothesised that chronic stress (1) enhances blood oxygen transport to match metabolic demands and assist in oxygen debt recovery, reflecting their need for efficient aerobic energy utilisation, and (2) leads to dose-dependent shifts in energy mobilisation to fuel recovery from a stressor. We applied a stress paradigm based on commonly encountered and easily reproducible stressors to experimentally induce the chronic phase of the stress response in spiny dogfish. Additionally, we aim to validate these heamatological metrics using heart rate measurements via small, implanted heart rate loggers, providing a unique insight in the cardio-respiratory stress response of shark, which we found is only possible through measurements in undisturbed free ranging shark. Through this approach, we aimed to illuminate how chronic stress might reconfigure energy strategies in a representative temperate shark species, providing insights that could have profound implications for their conservation.

Material & Methods

Adult male spiny dogfish (*Squalus suckleyi*, n=25) were collected by hook-and-line in Barkley Sound and transported to the Bamfield Marine Sciences Centre (BC, Canada) for experiments. We surgically implanted a heart rate logger in 20 dogfish to track heart rates during acute and prolonged stress (loggers were programmed to make ECGs every hour). The dogfish were divided over 4 experimental treatments: a small-tank group (n=6; sharks were maintained in two 2000-l tanks), a large-tank group (n=7; sharks were maintained in a 150.000-l tank), a confined group (n=7, sharks were maintained in the 150.000-l tank but periodically confined in 50-l blacked-out boxes for 48h), and a non-surgery control (n=5; maintained in the 150.000-l tank). Sharks were allowed to recover from surgery for 5 days prior to the start of experimentation, after which a daily stressor (chase protocol) was applied to all treatments to elicit a chronic stress response over a 3-week period. Once per week, all sharks were hand-caught from their tank to collect a blood sample. Whole blood was used to measure hematocrit, glucose, lactate, β-hydroxybutyrate with validated point-of-care devices. Additionally, plasma was used to determine osmolality and urea concentration. After blood sampling, all sharks were subjected to a 20-minute exhaustive exercise. During this exercise, heart rate loggers were programmed to collect ECGs every 5 minutes.

Afterwards, the small-tank, large-tank, and non-surgical control animals were allowed to recover from the exercise in their holding tanks; the confined treatment was transferred to 50-l blacked-out isolation boxes for 48h to assess the effect of confinement on recovery rates. During confinement in the boxes, respiration rates were recorded via intermittent-flow respirometry to investigate cardiorespiratory effects of stress. At the end of the experiment, sharks were terminally sampled and organs were harvested for gene expression at the University of Antwerp. Interrenal tissues were also collected to try an purify 1α-OHB to allow for measurements of this corticosteroid in plasma samples in the future.

Results/Conclusions

This was the first study to successfully implant heart rate loggers in an experimental setting with a shark species. Sharks have a thick pectoral girdle making automatic heart rate calculations difficult and inaccurate. Heart rates therefore have to be calculated manually from the collected ECGs. We are currently still in the process of analyzing these ECGs, but preliminary data suggest that heart rates do not appear to come back to baseline levels during sustained stress (i.e., the sharks cannot fully recover from an exhaustive exercise when chronically stressed). Eventually, these heart rate data will be matched with hematological data collected throughout the experiment to validate blood biomarkers of chronic stress. From the whole blood analyses, we have observed elevated levels of β-hydroxybutyrate with chronic stress, whereas blood glucose, lactate, and osmolality remain stable throughout the experiment. Interestingly, urea levels also appear to drop with chronic stress, which may indicate osmoregulatory disturbances. A next step will be to quantify amino acids concentrations to shed light on their mobilization during stress. Indeed, glutamine is readily used as an oxidative fuel during stress but also plays an important role in maintaining osmotic balance (urea synthesis) in sharks, so a trade-off may exist between serving as an energy source versus an essential compound in the synthesis of urea. Although part of the data still have to be analyzed, our data demonstrate that conventional vertebrate stress biomarkers (e.g., glucose, lactate) may not reliably reflect chronic stress in sharks, and we introduce β-hydroxybutyrate as a potential novel biomarker for chronic stress in this and likely other species of sharks. These insights will be instrumental in devising management strategies that are vital for the conservation of shark populations both today and into the future.

3. Overview of the expenditures

Describe in detail how the requested fund was spent within the implementation period (1 March 2024 and 28 February 2025). Be as specific as possible.

We used the BMRI funding to purchase 8 Star-Oddi DSL-milli HRT ACT heartrate loggers (€700 each, totaling €5600).

4. Pictures

A set of five pictures (low resolution in this document). The five high resolution pictures should be delivered to VLIZ by email to karen.rappe@vliz.be.