ESTIMATING METABOLIC RATE OF ELASMOBRANCHS BY MEASURING OXYGEN EXTRACTION AT THE GILLS

by

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A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Bachelor of Science in Environmental Science with Distinction

Spring 2020

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ACKNOWLEDGMENTS

First, I would like to thank my entire lab. I cannot express how much I appreciate Dr. Carlisle for taking me under his wing and helping me grow and teaching me so many vital skills for the marine science world. Without Kathryn and Devon's constant help for shark husbandry and support, my life would have been significantly harder. I also need to give a huge thank you to Haley Oleynik for providing edits for this thesis, for her constant support, and for being an exceptional female role model in marine science. And most importantly, I would like to thank Rachel Roday for being an incredible partner during this project. She is a strong, hard-working, bright young woman and I am very excited to see where life takes her. I would like to thank Dr. Jonathan Cohen for being an intimidating yet kind guider and pushing me to think outside the box. Thank you to the entire University of Delaware College of Earth, Ocean, and Environment staff and faculty for helping with every technical and physical issue that was encountered. Thank you to Dr. Art Trembanis and Dr. Mark Parcells for taking the time to review my work and for joining my committee. The support from both of you is extremely important to the success of this project. I would like to thank the Undergraduate Research Program for allowing this project to move forward and succeed, and for providing me this opportunity. Lastly, I would like to thank my family and friends for being an unwavering support system through this almost year long journey. I truly appreciate every single person that has been involved in this process. Thank you.

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ABSTRACT

Metabolism is the series of chemical reactions that convert food into energy, influencing all aspects of an organism's biology. Oxygen is a key substrate used in metabolism and therefore, rates of oxygen consumption are used as a proxy for metabolic rates. We placed fiber optic oxygen sensors at the gills of two shark species: *Carcharhinus plumbeus* (sandbar shark) and *Mustelus canis* (smooth dogfish shark) to measure oxygen content in the gills. Elasmobranchs were anesthetized using MS-222, and an oxygen probe was placed at specific locations along all five gills to measure the spatial variability in oxygen content of gill exhalant. The differential values in oxygen content between ambient water and the gill exhalant were used to estimate the degree of oxygen extraction at the gills. We found significant differences (Two factor ANOVA, $P < 0.05$) in the oxygen content of gill exhalant between gills and the second and third gills were the most efficient for oxygen extraction. We then tested for the effect that water flow rate (mL/s) had on the oxygen content of gill exhalant and used these flow rates to calculate the metabolic rate of a *C. plumbeus* individual. The efficiency of oxygen extraction varied by the activity level of the species, with more active species such as *C. plumbeus* being able to extract oxygen more efficiently than sluggish, demersal species like *M. canis*. This experiment was the first step to calculating the metabolic rates of freely swimming elasmobranchs and fishes in the wild.

Chapter 1

INTRODUCTION

1.1 Metabolism Introduction

An organism's metabolism uses the energy it acquires and stores to support all aspects of its life. Understanding the factors that influence metabolic rates in elasmobranchs is important to understanding their biology and ecology. Historically, it has been difficult to accurately measure an organism's metabolic rate in the wild, particularly for gill breathing animals like fishes. As a result, metabolic rate experiments on fishes have all been conducted within a laboratory setting and to date, no direct measurements of metabolic rate have been collected for free-swimming, gill-breathing animals.

The goal of this study was to measure the amount of oxygen extracted by the gills of two elasmobranch species and develop an approach for extrapolating these point measurements of oxygen consumption to a whole animal metabolic rate. The amount of oxygen extracted by the gills can be used as a proxy to determine the metabolic rates of these three species, since oxygen plays a fundamental role in aerobic metabolism. As Chabot et al. (2016) explain, "Because MR (metabolic rate) has almost always been measured as O_2 removal from the water by a fish, MR is actually oxygen uptake ($\dot{M}O_2$) rather than oxygen consumption by tissues.". In this experiment, we measured the Standard Metabolic Rate (SMR), which is measured when an animal is at complete rest, and only using energy to fulfill its most basic biological requirements.

1.2 Gill Morphology

Most elasmobranch species, with a few exceptions, have five gills on each side of their body. To obtain oxygen, water needs to pass over the gills. Elasmobranchs use two different mechanisms to pass water over the gills, ram ventilation and buccal pumping (see next section). As water moves through the gills, small filaments called lamellae promote gas exchange from the saltwater to the blood stream. After, the oxygen is dispersed throughout the elasmobranch's body where it is used for all biological processes.

1.3 Study Species

Two different elasmobranch species were used for this study: *Carcharhinus plumbeus* (sandbar shark) and *Mustelus canis* (smooth dogfish). Both species differ in the physiology and activity levels. One factor that may influence metabolic rate is how an organism respires. Ram ventilating species such as *C. plumbeus*, need constant motion to push oxygen through the gills. They swim with their mouths open to allow water to move through their gills. Species that can use buccal pumping such as *M. canis*, pull water into their mouths using suction and expel it through their gills. Buccal pumping does not require the animals to be in constant motion and is generally found in sedentary, less active species, and therefore we can predict that the metabolic rate of these organisms will be lower, since they are generally less active. Also, we note that *M. canis* can switch between ram ventilation and buccal pumping. These species were picked because they are good model organisms to represent the wide range of elasmobranchs in the wild, since they encompass a range lifestyles and modes of respiration commonly found in elasmobranch species, and because they are locally available in the Delaware Bay. Furthermore, metabolic rate is influenced by a range of

factors, including temperature and mass (Dowd et al. 2006). In this study, we held temperature constant at ~22°C but used animals of differing weight (Table 1), with *C. plumbeus* individuals being large than *M. canis*. We would predict that the larger size of *C. plumbeus* will result in a higher whole-animal metabolic rate when compared to *M. canis*.

Table 1. Metadata for elasmobranchs used in this study. Size measurements include total length (TL), fork length (FL), precaudal length (PCL), and for the skates, disc width (DW). Girth measurements were taken, by wrapping a tape measure around the circumference of the sharks right below the pectoral fin.

Date of Measurement	Shark	Mass(kg)	TL (cm)	FL (cm)	PCL (cm)	DW (cm)	Girth (cm)	Sex	Species
6/14/19	1	1.3	62	52	46		27	F	Sandbar
6/17/19	2	1.4	63	52	47		27	M	Sandbar
6/17/19	3	1.0	60	50	45			M	Sandbar
7/30/19	4	0.3	47	41	37			F	Dogfish
7/30/19	5	0.3	46	40	38			M	Dogfish
8/06/19	6	0.2	41	36	53			F	Dogfish
8/12/19	7	1.4	65					M	Sandbar
8/12/19	8	1.0	59					F	Sandbar

Chapter 2

MATERIALS AND METHODS

2.1 Collection of Specimens and Husbandry

All elasmobranchs were caught in the Delaware Bay using several methods. The first three *C. plumbeus* specimens were caught in June using hook and line. Three *M. canis* were caught in the end of July and the beginning of August using a longline. The final two *C. plumbeus* individuals were caught by NOAA as their team was sampling in the Delaware Bay in the beginning of August. All specimens were stored in a 2,000-gallon saltwater tank and the University of Delaware Sharp Campus. Water quality in the tank was monitored daily, and water changes were performed as necessary in the summer months, and then switched to weekly in the fall months.

Animals were fed ~1-2% of their body mass each day following the Elasmobranch Husbandry Manual II (Smith, 2017). Elasmobranch specific vitamins (Mazuri Exotic Animal Nutrition) were added to the food, which included a mix of shrimp, mantis shrimp, menhaden, and squid.

2.2 Data Collection

Water oxygen saturation data were collected using PyroScience optical oxygen sensors and logged using Logger O_2 software. One oxygen sensor in the water of the experimental tank collected data on the oxygen content of ambient water, and another sensor placed at the gills of the elasmobranch was used to measure oxygen content of gill exhalant (water exiting the gills). We determined how much oxygen the animal was extracting by subtracting the percent oxygen saturation of the water in the tank by the water flowing out of the gills of the animal.

To assess how oxygen extraction may vary across gills and at different locations in the gills, we measured oxygen at six different locations at each gill (Fig. 1). Then, we tested whether there was any difference in oxygen extraction between the right and left gills. We did this for each species and then compared differences in oxygen extraction across species.

All data analyses were done using RStudio and SigmaPlot software.

2.3 First Coordinate System

The experimental tank included a pump system that recirculated the saltwater continuously through the tank, with two air stones to ensure proper aeration of the saltwater. A PVC pipe was attached to the recirculating tubing as a mouthpiece to provide a constant and continuous flow of water across the animal's gills. Prior to each experiment, the animal was placed in a sedation tank containing a dosage of approximately 150mg/L of the sedative MS-222 and monitored until it was unresponsive, yet still breathing. The individual was then moved to the experimental tank which contained a lower dosage of approximately 122mg/L of MS-222 so that the animal would remain sedated and calm until the experiment was complete. After oxygen extraction data were obtained, the animal was revived in fresh seawater and released back into the holding tank.

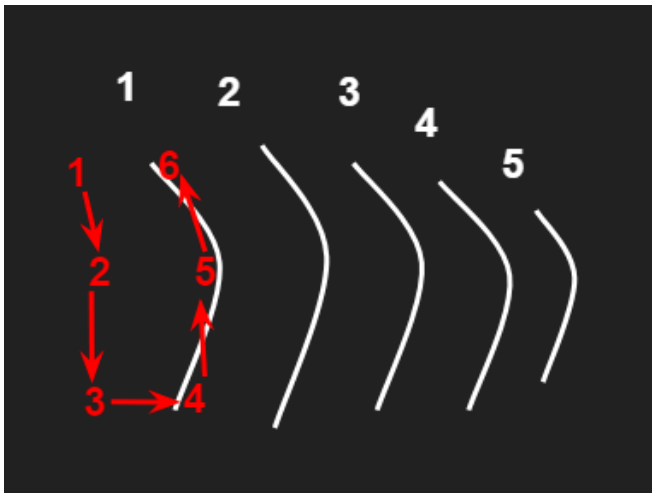

Figure 1. Gill coordinate system used to describe the oxygen extraction trends (see Figure 4). The coordinate system can be read as "1.1" corresponding to the first position in the first gill, "2.1" being the first position in the second gill and so on.

Figure 2. *C. plumbeus* with a Pyroscience optical oxygen sensor 2mm inside the second gill. This sensor position corresponds to coordinate position 2.1.

2.4 Modified Coordinate System

Based on our preliminary results, which showed no significant difference in the horizontal and vertical placement of the oxygen sensor, which is explained further in the results section, we modified our experimental design to increase efficiency in data collection. Figure 3 shows the modified gill coordinate system used for oxygen extraction measurements. The second coordinate system involved a single point measurement approximately two millimeters inside the center of the gill. We then took measurements on both sides of the shark to determine if there was any significant difference between the two sets of gills. The same experimental apparatus is used throughout this experiment, with some adjustments for plumbing issues.

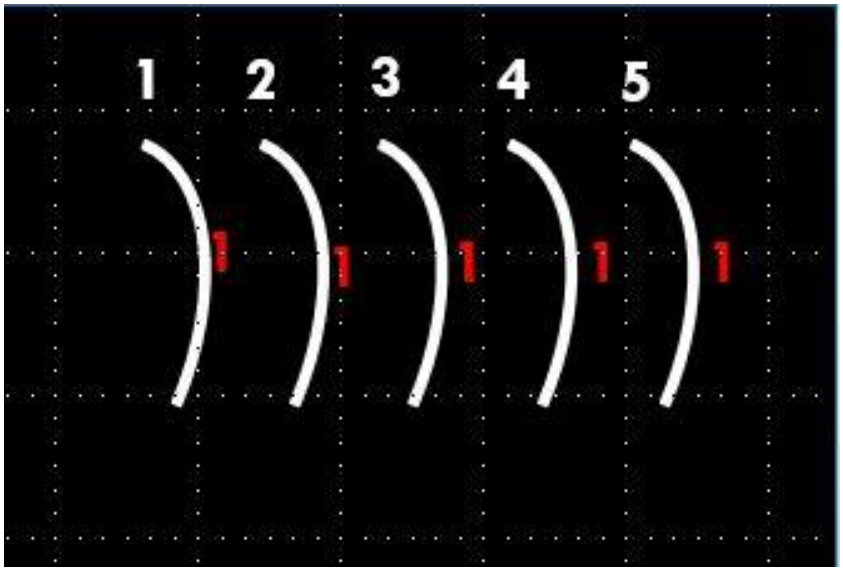

Figure 3. Modified gill coordinate system used for the oxygen extraction tests following the initial experiments. Statistical tests (explained further in chapter 3), demonstrated that there were no significant differences between the six vertical and horizontal positions used in the initial experiments. Thus, a single position that was easiest to place the sensor was used for the remainder of the experiments.

2.5 Water Velocity Analysis Through the Gills

Because the rate of water flow over the gills will influence efficiency of oxygen extraction, we evaluated how flow rate of impacts oxygen extraction efficiency across the different species. Food dye was injected into the tubing leading directly into the elasmobranch's mouth. A GoPro (GoPro Inc.), positioned above the experimental tank using a ring stand and clamp, was used to record the dye traveling through the mouth and out through the gills. Tracker software (Open Source Physics) was used to measure the velocity at which the water was leaving the gills by tracking the rate of movement of the dye leaving the gills. Six different flow rates of 10, 15, 20,

25, 30, and 40 mL/s, were recorded to determine differences in water velocity traveling through the gills. From there, we took the water flow rate, velocity of water leaving the gills, oxygen extraction data, and physical parameters such as temperature, volume of the experimental tank, and pressure to calculate a metabolic rate of the species.

2.6 Comparison Across Species

We compared oxygen extraction rates across both species to determine how oxygen extraction differs between a more active species, *C. plumbeus* and more sluggish, demersal species, *M. canis*. We measured oxygen extraction rates at the unaltered water flow rate of the system (40 mL/s), and the six altered flow rates previously listed and compared oxygen extraction from the gills for each species.

2.7 Statistical Analyses

Statistical analyses were performed to determine whether there were significant differences between treatments within each experiment. For the first experiment and second experiments, two-factor ANOVA tests were performed to determine any variability within the data.

Chapter 3

RESULTS

3.1 First Coordinate System

We found that there was high oxygen extraction at gills one, two, and three, and lower extraction at gills four and five (Figure 4). Oxygen extraction at gills two and three was relatively consistent across trials and individuals. The highest oxygen extraction levels with the lowest variability was observed at gill two, while the lowest levels were observed at gill five (Figure 5).

We found that there was no significant difference in oxygen extraction between the inside and outside of the gill (Two-factor ANOVA, $P > 0.05$). We also found that there was no significant difference in the vertical placement of the oxygen sensor inside the gill (Two-factor ANOVA, $P > 0.05$). However, we found that there was a significant difference in oxygen extraction across gills (Two-factor ANOVA, $P > 0.05$).

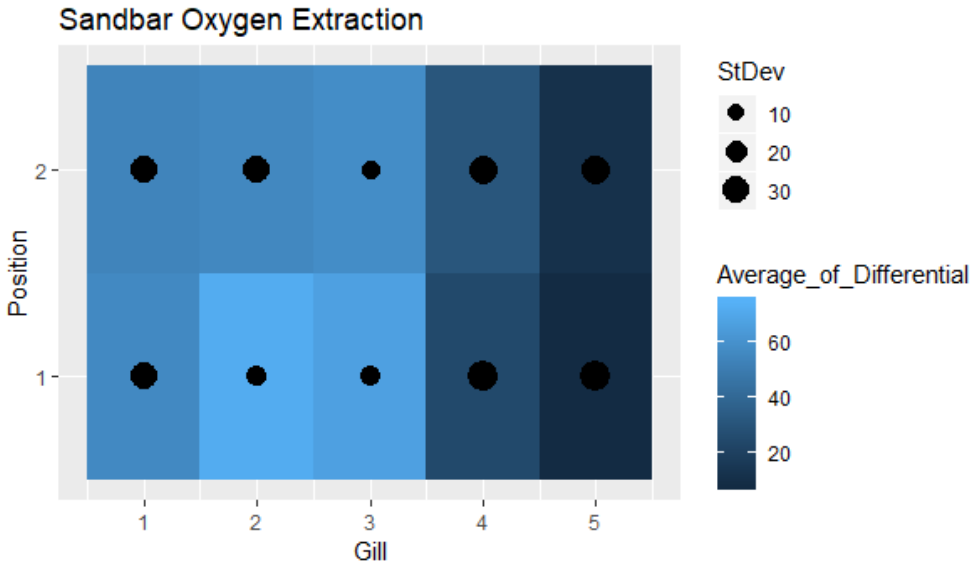

Figure 4. Heatmap depicting the differences in oxygen extraction in three sandbar sharks, showing that the highest oxygen extraction measurements with the lowest variability were observed in gills two and three. Gill number is shown on the x-axis with sensor position (1: inside the gill, 2: outside the gill) on the y-axis.

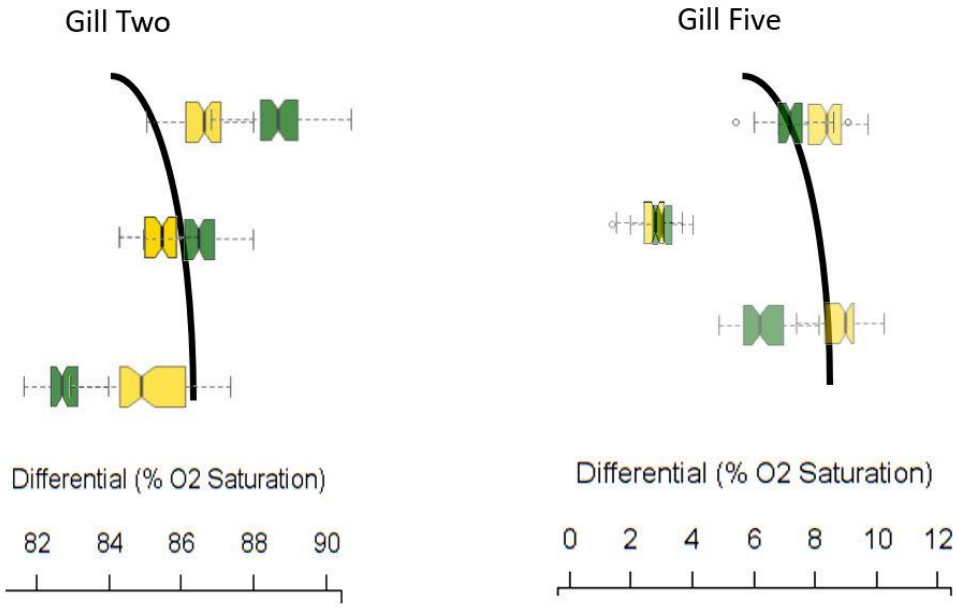

Figure 5. Boxplots depicting differences in oxygen extraction between gills two and five for three trials for three sandbars. The x-axis shows the differential and the y-axis is considered as the coordinate system for the gill (Figure 1).The green color represents when the sensor is placed 2mm inside the gill and the yellow color represents the sensor being placed outside the gill.

3.2 Modified Coordinate System

Since there were no significant differences between vertical and lateral sensor placements in the gills, we modified the experimental design to simplify testing (Figure 3). We found that overall oxygen extraction at the gills of *C. plumbeus* was higher than *M. canis* individuals (Figure 6).

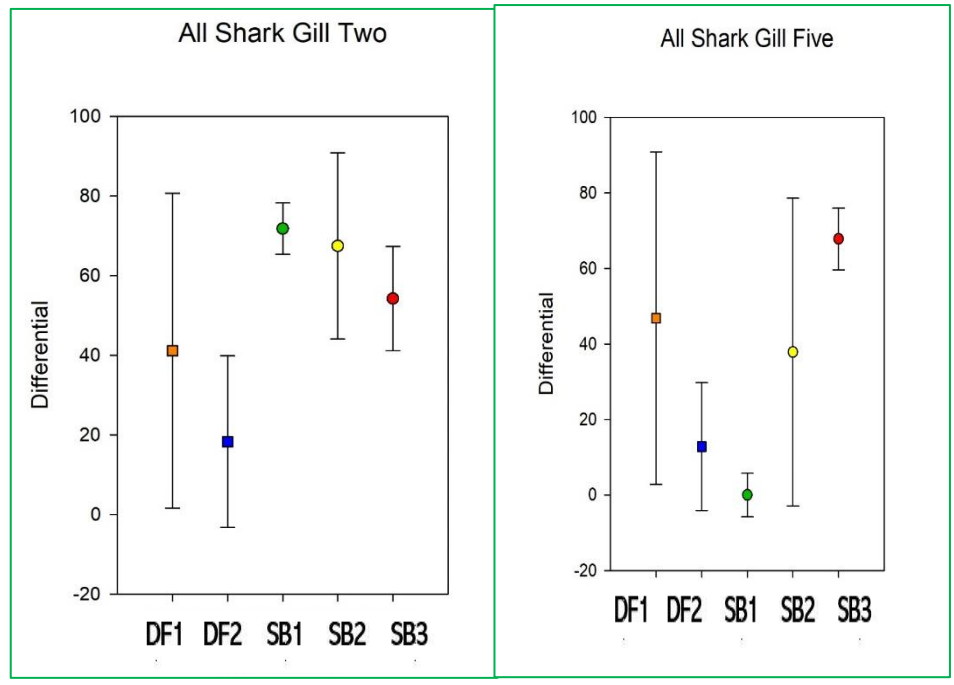

Figure 6. This graph shows the difference in oxygen extraction between three *C. plumbeus* individuals and two *M. canis* individuals. The x-axis represents the five individuals and the y-axis represents the amount of oxygen extracted from the water

3.3 Dye Experiments

Dye experiments were performed to calculate the velocity of water flowing from the gills. Velocity (m/s^2) was measured at seven different water flow rates (mL/s): 10, 15, 20, 25, 30, 35, and 40. A table of the average velocities that were gathered for one skate individual are placed below. Each gill at each velocity was

tracked four times to receive four velocity values. From this, we took the average of the four velocity values to have one average value for each gill at each velocity. From this we can see that the fastest velocity is typically around gill four. The slowest velocities are seen in gills one and two. A further explanation of these trends is described in the discussion chapter.

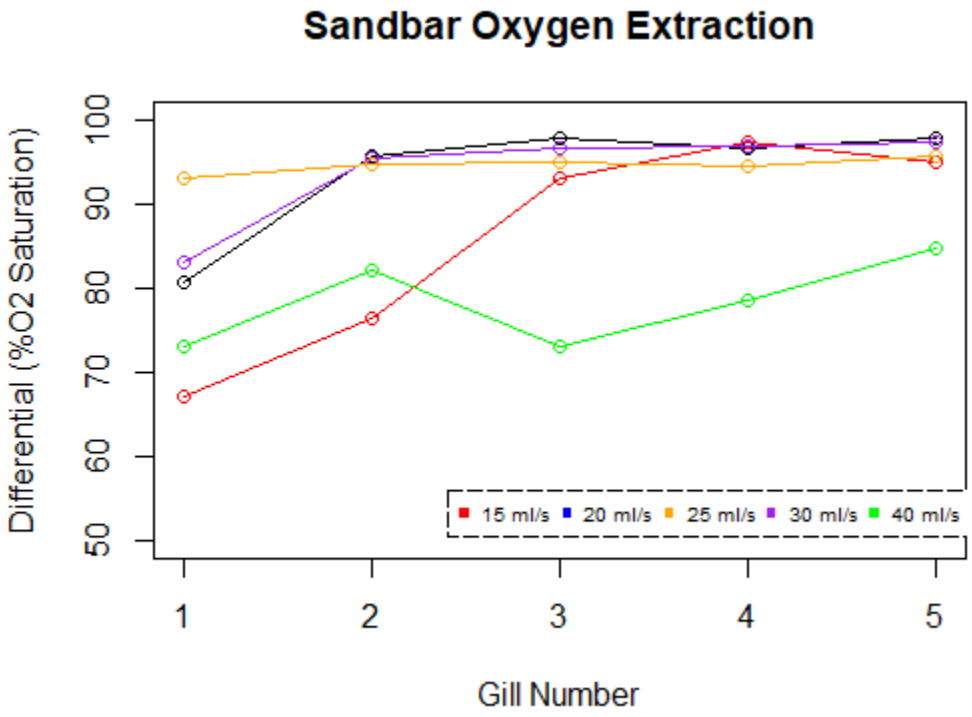

Figure 7. Effect of flow rate on oxygen extraction measurements (%O_2 saturation) in *C. plumbeus* (n = 1) at five different testing flows. The flows are measured in mL/s.

3.4 Metabolic Rates

Standard metabolic rates were calculated at all six water velocity flows using RespR in RStudio. The data from flows 10 mL/s and 40 mL/s were anomalously high and were omitted. For flow rates 15, 20, 25, and 30 mL/s we estimated standard metabolic rates of 100-250 mg/O_2/h/g for sharks of 1-2kg mass. These SMR estimates were similar to those reported for the same species by Dowd et al. (2006) who reported SMR measurements of 100-200 mgO_2/hour for individuals of the same mass range as sharks in our study (Table 1).

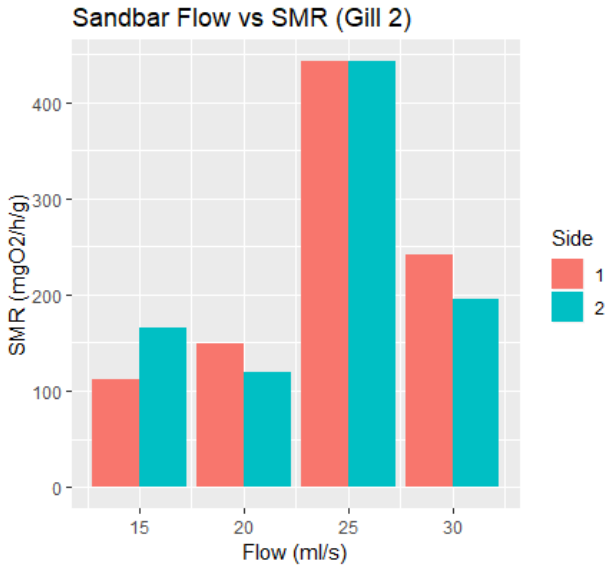

Figure 8. Effect of flow rate (mL/s) on standard metabolic rate (SMR) in one *C. plumbeus*.

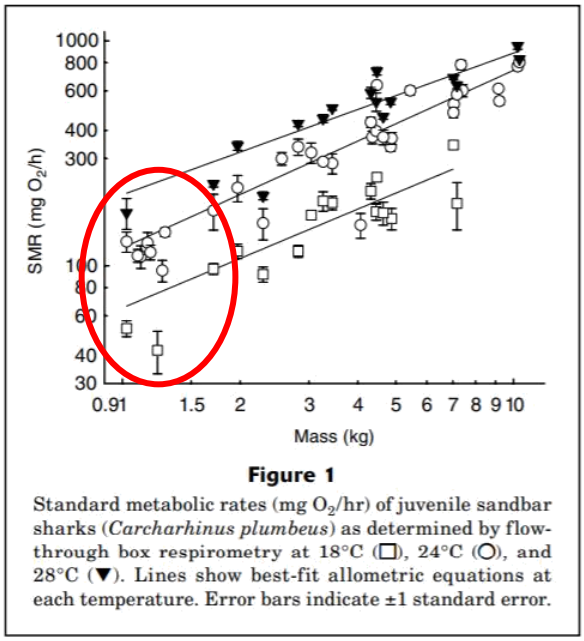

Figure 1

Standard metabolic rates (mg O_2/hr) of juvenile sandbar sharks (*Carcharhinus plumbeus*) as determined by flow-through box respirometry at 18°C (□), 24°C (○), and 28°C (▼). Lines show best-fit allometric equations at each temperature. Error bars indicate ±1 standard error.

Figure 9. Data from Dowd et al. 2006 showing the SMR relative to mass. The red circle indicates range of masses for the individuals used in our research.

Chapter 4

DISCUSSION

Our results suggest that differences in activity level and physiology affect oxygen extraction and metabolic rates in both study species. We were able to determine the optimal placement for an oxygen sensor to measure the most consistent oxygen extraction and use that point measurement to extrapolate a metabolic rate for an entire individual. *Carcharhinus plumbeus*, a more active species, has a higher oxygen extraction than *M. canis*, which is a less active, demersal species. The increased activity level and need for constant motion in *C. plumbeus* is a likely explanation for the difference in oxygen extraction as opposed to the demersal species, which does not require constant motion for survival. The two different modes of breathing, ram ventilation and buccal pumping, could also be a viable explanation for the difference in oxygen extraction and resulting estimates of metabolic rate. The increased amount of oxygen extracted in *C. plumbeus* compared to the two species is best explained by the ram ventilating characteristics of *C. plumbeus*. For *M. canis* lower oxygen extraction rates compared to *C. plumbeus* are due to the decrease in activity level. The increased activity levels of *C. plumbeus* require a significant amount of energy, with sharks using between 34-100% of their metabolic scope to sustain routine continuous activity (Dowd et al. 2006).

4.1 First Coordinate System

We completed the first step of the study by determining a pinpoint location from all five gills where oxygen is extracted most consistently. This location is at in the middle of the second gill at the gill opening. This position was chosen because gill two had the highest amount of oxygen extracted and the smallest standard deviation.

We want to find the location that has the most oxygen extracted because this provides us with the most accurate data for a metabolic rate.

As described in the results, there were issues encountered with the water circulation through the mouth that could have influenced our data. We experienced some issues with plumbing and creating a stable system for water recirculation, which is reflected in some of the differences in oxygen extraction between the individuals.

4.2 Modified Coordinate System

Using the modified experimental design, we found that there was a difference in oxygen extraction between *C. plumbeus* and *M. canis*. These differences could very well be linked to differences in their physiology. As discussed in Dowd et al. (2006), there is a correlation between body mass in *C. plumbeus* and metabolic rate, with larger animals having increased whole-animal metabolic rates. Since oxygen can be used as a proxy for metabolic rate, we can conclude that the increase in oxygen extraction also correlates with the increase in body mass. As seen in Table 1. there is a large difference in mass between the *C. plumbeus* and *M. canis*. This reasoning justifies the trend seen from these tests showing that *C. plumbeus* extracts more oxygen than *M. canis*. The reasoning behind the variability observed in some of the sharks could be linked to the previous technical issues in the filtration system in the experimental tank, specifically at the tube distributing water into the elasmobranch's mouth.

4.3 Dye Experiments

From the dye experiments, we found a general trend of increasing water velocity flowing through the gills as we measured oxygen extraction from gill one to

gill five. Although we encountered some issues in video clarity, it was clear that water flows faster out of gill four compared to gill one. If water is flowing faster out of these gills, there is less of an opportunity for oxygen to be extracted from the water. This matches the trends that we observed from the previous experiments that show that gill four did not extract as much oxygen as gill two. So, the faster the water flowing through the gills, the less oxygen that is being extracted. The video quality was not always clear enough to see the dye flowing across the frames, and there were multiple instances where the dye velocity was unable to be analyzed. Along with this, there was an issue of the concentration of the dye in the water increasing over time, making it extremely difficult to see the dye coming out of the gills. In future experiments we would suggest testing each flow rate as a separate trial. Increased camera quality would also help see the dye traveling through the frames.

4.4 Metabolic Rate

The difference in metabolic rate at water flow 25 ml/s is best explained by the variability of the placement of the breathing tube in the elasmobranch's mouth. There is also the possibility that the elasmobranch began to recover from the anesthetic, which could cause an increase in stress and therefore a jump in metabolic rate. As the sharks are anesthetized, oxygen consumption should not vary across flow rates. Therefore, the anomalously high metabolic rates at the water flow rate of 40 mL/s are likely due to the same issues which is why this data was omitted.

4.5 Future Experiments

For future experiments, we will look at the routine metabolic rate, which is the metabolic rate of an animal in motion, using a swim tunnel respirometer. Dowd et al.

(2006) used a swim tunnel respirometer in their study, and so we hope to compare our data to theirs to further confirm that oxygen pinpoint measurements can be used and quantified to a whole animal metabolic rate.

The last step of this project will be to create a new biologging tag package that will be able to measure the amount of oxygen flowing out of the gills of a freely swimming elasmobranch, and convert this number to a metabolic rate. This way, we will be able to measure the metabolic rate of an elasmobranch in the open ocean, which has never been accomplished before.

Chapter 5

CONCLUSION

We found that oxygen extraction measurements were not influenced by the location of the sensor within the gill (vertical and horizontal location); thus, the placement of the optical oxygen sensor can be dictated by experimental requirements and not physiological ones. However, there was a significant difference in oxygen extraction across the gills, and we suggest that sensors should be placed at the opening of gill two. We also found that more active, ram ventilating species will have a higher metabolic rate than buccal pumping, benthic species. Thus, *C. plumbeus* has higher oxygen extraction capabilities than *M. canis*. In terms of flow rate, we see a trend in consistent oxygen extraction across all different flows measured. There are some exceptions for technical issues. For metabolic rate, we can conclude that the method used for this experiment is a viable option for calculating metabolic rate since the values obtained match that of Dowd et al. 2006.

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