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Comparison of Temperature Preference and Metabolic Thermal Sensitivity Between Two Juvenile  
Coastal Shark Species.

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Marine Biology

by

Zachary Robert Skelton

Committee in charge:

Philip A. Hastings, Chair  
Octavio Aburto-Oropeza  
Brice X. Semmens  
Nicholas C. Wegner

2020

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Chair

University of California San Diego

2020

DEDICATION

For Mom and Rachel.

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This thesis, in part, is currently being prepared for submission for publication of the material. The thesis author was the primary investigator and author of this material. The thesis is coauthored with Tanya Prinzing, Nicholas Wegner, and committee chair Philip Hastings.

## ABSTRACT OF THE THESIS

Comparison of Temperature Preference and Metabolic Thermal Sensitivity Between Two Juvenile Coastal Shark Species.

by

Zachary Robert Skelton

Master of Science in Marine Biology

University of California San Diego, 2020

Professor Philip Hastings, Chair

Ectotherms, including sharks, behaviorally thermoregulate in order to optimize physiological processes. Juvenile sharks often utilize estuaries, which can provide warm water temperatures that increase metabolism and facilitate growth. Both the California Horn Shark (*Heterodontus francisci*) and the Leopard Shark (*Triakis semifasciata*) occupy estuaries of southern California as juveniles and represent contrasting activity levels; horn sharks are relatively sedentary while leopard sharks are more active and mobile. The primary aim of this study was to better understand the relationship of behavioral thermoregulation and metabolism in response to temperature in these two species. The objectives were

threefold: 1) identify the temperatures juveniles prefer, 2) assess the sensitivity of metabolism ( $Q_{10}$ ) to temperature via measurements of oxygen consumption, and 3) compare these results between species, sex, and seasonal acclimation treatments. Using a shuttlebox, this study showed juvenile horn sharks and leopard sharks have comparable thermal preferences and temperature ranges. Respirometry trials at different temperatures showed both species exhibited increasing metabolic rates with increasing temperature, and leopard sharks exhibited higher metabolic rates than horn sharks across all temperatures. However, horn sharks exhibited a higher overall  $Q_{10}$  suggesting their metabolism has greater thermal dependence. Underlying acclimation to seasonal temperatures did not appear to have an effect on any of these parameters. Horn sharks likely target environments (i.e., day-time resting places) closer to their preferred temperature and more stable in temperature fluctuation than leopard sharks. Lab-based determination of physiological and behavioral responses to temperature are important in understanding habitat usage during vulnerable life stages.

## INTRODUCTION

Temperature is an important driver of habitat use and, although organisms may make exploratory movements across a broad range of temperatures within the environment, selection exists for organisms to target preferred temperatures that are optimal to their metabolism through physiological or behavioral controls (Fry, 1947; Jobling, 1981; Schulte, 2015). Sharks, for instance, are known to behaviorally thermoregulate by physically moving across temperature regimes in order to target bioenergetically favorable thermal conditions (Carey et al., 1990; Klimley, 1993; Stokesbury et al., 2005; Grubbs et al., 2007; Heupel and Simpendorfer, 2008; Kessel et al., 2014), often associated with processes such as foraging, digestion, and parturition (Wallman and Bennett, 2006; Sims et al., 2006; Hight and Lowe, 2007). The degree to which an organism may target their preferred temperature is dependent on the sensitivity of the organism's underpinning physiology to changes in temperature. Interspecific variability in the thermal sensitivity ( $Q_{10}$ ) – a measure of how a physiological system changes across a range of temperature – of metabolism suggests certain species may be more susceptible to changes in environmental temperature than others (Schmidt-Nielsen, 1997). Metabolic rate can vary based on lifestyle with more active species usually having higher proportional metabolic rates than less active benthic species (Kleiber, 1961; Schulte, 2015) and, due to the direct effect of temperature on metabolic rate, higher temperatures can also lead to an increase in activity levels (Grady et al., 2019). Understanding the behavioral and physiological responses of organisms to temperature can allow for more refined conservation and management decisions in the face of habitat alteration such as human encroachment or anthropogenic-induced climate change.

Currently, there exists a gap in the literature on behavioral thermoregulation in elasmobranchs, as only a handful of studies have isolated the preferred temperature of species via experimental means (Casterlin and Reynolds, 1979; Wallman and Bennett, 2006; Dabruzzi et al., 2013; Gervais et al., 2018). Most behavioral thermoregulation studies focus on *in situ* movement data (Matern and Hopkins, 2000; Campana et al., 2011; Speed et al., 2012; Thums et al., 2013; Vaudo and Heithaus, 2013; Andrzejaczek et

al., 2018), and are constrained by the fact that there are various drivers of habitat utilization that can act simultaneously. Therefore, the goal of this study was to isolate temperature as a single variable and study the convergence of behavioral thermoregulation and metabolism in a controlled setting. Considering differences in activity likely contribute to differences in metabolism and underlying thermal optimum, this study also compared two co-occurring species of juvenile sharks with contrasting activity levels.

Both the California Horn Shark (*Heterodontus francisci*) and the Leopard Shark (*Triakis semifasciata*)—two common mesopredators of southern California—occupy warmer shallow embayments during early life stages (Talent, 1985; Ebert, 2003). Juvenile leopard sharks have been found within estuaries throughout all seasons, suggesting this size class are year-round residents of estuaries (Hopkins and Cech, 2003; Carlisle and Starr, 2009), and observational data suggests this is also true for horn sharks. Many shark species utilize embayments and estuaries for nursery grounds (Castro, 1993; Merson and Pratt, 2001; Espinoza et al., 2011) as the dynamic chemical and physical parameters of coastal environments can provide certain biological advantages. In addition to providing abundant prey and a refuge from predation, the shallow waters may offer bioenergetically favorable thermal conditions for growing juveniles due to the shallow and enclosed nature of the habitat (Springer, 1967; Bass, 1978; Branstetter, 1990; Heupel et al., 2007). Horn sharks are benthic species that are generally inactive during the day often residing within crevices of rocky reefs. The geographic range of horn sharks extends from central California, USA to southern Mexico (including the Gulf of California) and they typically occupy depths between 2-11 meters (Ebert, 2003). Horn sharks will actively forage over mud, sand flats, or eelgrass bed at night and exhibit site fidelity in foraging area and shelter sites (Nelson and Johnson, 1970; Talent, 1985; Ebert, 2003; Meese, 2019). Contrarily, leopard sharks are a more mobile, demersal species, commonly found up to 20 meters deep and occur from Oregon, USA to the Gulf of California, Mexico (Ebert, 2003). Like horn sharks, they generally forage at night over mud, sand flats, or eelgrass beds of the surf and subtidal zone (Talent, 1985; Ebert, 2003). As readily accessible ectotherms in critically important environments, these two species serve as unique study organisms to understand how vital abiotic factors such as temperature drive habitat usage.

Evidence for behavioral thermoregulation has been reported in previous studies for both species (Crawshaw and Hammel, 1973; Hight and Lowe, 2007), and the present study builds upon these by further investigating the relationship between the behavioral response to temperature and underpinning metabolism. In order to better understand behavioral thermoregulation, this study employed a shuttlebox system (Loligo Systems Inc., Tjele, Denmark) to determine the temperature preference of juvenile horn sharks and leopard sharks. To assess the effects of temperature on metabolism, this study examined resting oxygen consumption (a proxy for metabolic rate) during daylight hours when both species naturally exhibit lower levels of activity. To simulate the natural heterogeneous environments in which these species are found, metabolic rate was measured with acute changes in temperature (<24 hours) indicative of daily thermal regimes experienced by both study species within the estuaries and embayments of Southern California. Metabolic thermal sensitivity ( $Q_{10}$ ) was then calculated across experimental temperatures. Both metabolic rate and temperature preference are known to change with season (Carlson and Parsons, 1999; Mortensen et al., 2007), therefore sharks were subjected to long-term acclimations between 6-11 weeks at two different temperatures prior to the experiments. Although previous studies reported the thermal sensitivity in both horn sharks (Luongo and Lowe, 2018) and leopard sharks (Miklos et al., 2003), the present study built upon these previous studies by synthesizing and adding to the different experimental approaches.

This study aimed to elucidate the adaptive thermal biology of juvenile horn sharks and leopard sharks through lab-based examination of behavioral thermoregulation and metabolism. Baseline assessments of thermal biology are lacking for sharks, and this was one of the first studies to pair lab-based preferenda and metabolic rate in elasmobranchs, alongside the study on juvenile ribbontail rays (*Taeniura lymma*) by Dabruzzi et al. (2013). Due to species-specific differences in thermal sensitivity, the movements of sharks in the field may differ from lab-determined thermal optima. Therefore, lab-based approaches are important complements to field-based studies when assessing habitat usage.

## METHODS

### *Collection and Holding*

Horn sharks (37-49 cm total length, TL, n=5 males and n=5 females) and leopard sharks (72-90 cm TL, n=5 males and n=3 females) were collected from Mission Bay (32°46'30.8"N 117°13'47.5"W) and San Diego Bay (32°39'40.2"N 117°08'13.4"W) CA, respectively, as bycatch in Hubbs SeaWorld's White Seabass Gillnet Survey in June, 2018 (California Fish and Wildlife Scientific Collecting Permits SC-2481 and SC-13908). Captured individuals were size-selected to only retain immature individuals, with maturity being assessed by total length (Kusher et al., 1992; Ebert, 2003) and confirmed via absence of clasper calcification for males. Immediately following capture, sharks were temporarily held at Scripps Institution of Oceanography in tanks containing flow-through ambient seawater until they began eating, and were subsequently moved to the Southwest Fisheries Science Center (SWFSC) Experimental Aquarium for experimentation. At SWFSC, horn sharks were held in a 300 × 150 × 90 cm ( $l \times w \times h$ ) oval tank (approximately 3200 l), while leopard sharks were held in a 300 × 90 cm ( $d \times h$ ) round tank (approximately 4500 l), both with flow-through filtered seawater. Sharks were fed a diet of squid and mackerel until satiation every 72 hours and fasted for 72 hours prior to any experimentation to avoid metabolic effects associated with specific dynamic action. All animal care and experimentation was conducted in accordance with IACUC protocols S00080 (University of California San Diego) and SW1801 (SWFSC).

In order to assess the effect of temperature on shark behavior and physiology, sharks were acclimated to two seasonal temperatures. These seasonal acclimation temperatures were informed through mean sea surface temperature (SST) data from NOAA Station SDBC1 (32°42'51" N 117°10'26" W) in San Diego Bay (NOAA National Data Buoy Center). In the first series of experiments, each species was subjected to a long-term "warm acclimation" for a minimum of 8-11 weeks at a target temperature of 21°C representing a typical summer temperature (due to water flow rates and tank dynamics actual temperatures were 20.2 ± 0.1°C for leopard sharks and 20.7 ± 0.5°C for horn sharks). All ten horn sharks

and all eight leopard sharks were used in these "warm acclimation" experiments. In the second series of experiments, individuals were subjected to a "cold acclimation" of 6-9 weeks at a target temperature of 15.0 °C representing a typical winter temperature (actual temp: 14.9 ± 0.5°C for leopard sharks and 15.3 ± 0.4°C for horn sharks). Only five of the horn sharks and seven leopard sharks were used for the "cold acclimation" experiments, as five horn sharks were used in a non-related study and one leopard shark died while in captivity. From the start of the "warm acclimation" to the end of the "cold acclimation" (32 weeks) horn sharks increased in mass by 22.9 ± 3.74% (mean percent mass increase ± SE) and leopard sharks gained 29.2 ± 5.37% in mass (Table 1).

#### *Shuttlebox System and Temperature Preference*

Temperature preference was assessed via a behavioral choice experiment using an automated shuttlebox (Loligo Systems Inc., Tjele, Denmark) (Figure 1) consisting of two 130 x 60 cm (*d x h*) circular chambers connected by a 35 x 30 x 60 cm (*l x w x h*) shuttling channel. One of the circular chambers contained water that was always kept 1.5°C warmer (warm side) than the other (cool side). Each chamber was connected to an external buffer tank for temperature control. Water temperature in each buffer tank was maintained by pumping water through recirculating loops with stainless steel heating / cooling coils immersed in water baths of either 30 or 6°C water. Pumps to each heating / cooling bath were modulated by a temperature sensor situated on the return from the shuttlebox to the buffer tank. The in-flow and out-flow into each chamber of the shuttlebox (~1.8 gpm) created a slight circular current within each chamber to promote mixing and temperature uniformity within each chamber, while mixing between the warm and cold chambers was minimized by the shuttle channel. The total volume of water in the system including chambers, hoses, and buffer tanks was approximately 600 liters. An experimental fish was able to behaviorally thermoregulate by "shuttling" back and forth between the warm and cool sides. When the fish swam to the warm side it triggered a system-wide temperature increase of 2°C per hour, while maintaining the 1.5°C difference between the warm and cool tanks. When the fish switched to the cool side, system-wide cooling was triggered at the same rate. The position of the fish was monitored



by an overhead video camera (uEye USB camera, Imaging Development Systems, Dimbacher, Germany) connected to a computer with accompanying animal tracking software (Shuttlesoft, Loligo Systems Inc., Tjele, Denmark) that in turn automatically controlled the pumps and water temperatures via a Daq-M. Dissolved oxygen concentration was maintained throughout the system at saturation by air stones located in the buffer tanks.

To reduce the potential of experimental results being affected by an individual fish's side preference, the warm and cool sides of the shuttlebox system were switched for half of the experiments and sharks were randomly assigned a starting side when first introduced into the system. Each shark was placed in the shuttlebox system with the system presenting a static difference of 1.5°C around the acclimation temperature (20.75°C cool side and 22.25°C warm side during 21°C warm acclimation experiments, 14.75°C cool side and 16.25°C for the cold acclimation experiments). Once in the tank, the experiment began and the shark was continuously tracked for a target of 72 hours (range of 68-96 hours). Due to the small heat transfer coefficient of the sharks obtained from previous studies (Stevens and Fry 1974; Hight and Lowe 2007), the difference between internal body temperature and the occupied water temperature were assumed to be negligible. As in other studies, the final temperature preferendum was therefore reported as median occupied water temperature (Stol et al., 2013; Macnaughton et al., 2018). To account for individual variability in learning to navigate the shuttlebox, a broken stick model was run using the R "Segmented" package to determine the time when the shark began targeting its preferred temperature (Figure 2; Muggeo, 2017). Median occupied temperature was then determined from this break point (subsequently assigned as time zero) to the end of the trial (typically resulting in 12-48 h of shuttling behavior). Temperature preference between species, warm and cold acclimation, and sex was then compared using an analysis of variance (ANOVA) followed by post hoc Tukey's honest significance test ( $\alpha = 0.05$ ).

### *Respirometry and Metabolic Rate*

In order to examine the effect of short-term temperature changes on resting metabolism at different seasonal acclimation temperatures, resting oxygen consumption rate was measured for both species via closed respirometry. Warm-acclimated (21°C) individuals were subjected to respirometry trials at 16°C, 20°C and 24°C, while cold-acclimated (15°C) individuals were subjected to trials at 12°C in addition to 16°C, 20°C, and 24°C. Each shark was placed in a respirometry system comprised of a cylindrical acrylic holding chamber proportional to the size of the individual (5.7-16.4 L for horn sharks and 40.0-52.5 L for leopard sharks; Loligo Systems, Inc.) with a recirculating loop containing a fiber optic oxygen sensor and temperature sensor connected to a Fibox 4 fiber optic oxygen transmitter (PreSens Precision Sensing GmbH, Regensburg, Germany). The respirometer was placed within a buffer tank that was used to maintain the desired temperature and provide aerated seawater for flushing between trials. Each shark was placed into the respirometer at its acclimation temperature which was then slowly brought down to the lowest experimental temperature (0.5°C h<sup>-1</sup>) controlled by an automated temperature mixing station. Each individual was held at the starting temperature for 12 hours prior to measuring oxygen consumption to account for acute temperature stress (Miklos et al., 2003; Luongo and Lowe, 2018). To begin a trial, the inflow and outflow of the respirometer were manually closed to seal it off from the surrounding buffer tank, and the oxygen concentration was measured within the recirculating loop every five seconds until oxygen concentration was brought down to between 70-80% air saturation. Following each trial, the respirometer was flushed with saturated seawater from the buffer tank, and the process was repeated. A minimum of two trials were conducted at each temperature following which the system temperature was slowly raised (0.5°C h<sup>-1</sup>) and trials were repeated at the next temperature following another 12 h acclimation period. Once respirometry measurements were collected at all temperatures, the shark was removed and oxygen levels were measured for an additional 1.5 hours at the warmest experimental temperature (24°C) to account for background microbial respiration (change in background respiration with change in temperature was negligible). During each trial, shark movement was visually observed to omit (and, if possible, repeat) any trials with spontaneous activity resulting in

elevated oxygen consumption rates (Ferry-Graham and Gibb, 2001). During the warm acclimation trials, metabolic rates of five of the ten horn shark individuals at 24°C were not included in the final analyses due to their excessive movement throughout measurement periods. Likewise, during the cold acclimation, respirometry trials from one leopard shark individual at 16°C and three at 24°C were excluded from further analysis. Ultimately, the two trials with the lowest mass adjusted oxygen consumption rates were then combined to find a single mean metabolic rate of the individual at that temperature (Clark et al., 2012; Wegner et al., 2018).

Due to the variability in body size of the sampled individuals and because metabolic rate is known to change with size, the oxygen consumption rate of each individual was mass adjusted to the mean mass of each species at each seasonal acclimation treatment using the scaling exponent of 0.80 (Brett and Groves 1979; Luongo and Lowe, 2018). This was done by first calculating the scaling intercept ( $a$ ) for each individual ( $n$ ). The intercept was determined using the following equation:

$$a_n = \frac{R \times 3600 \text{ s} \times (V - D)}{M^{0.80}} \quad (1)$$

where  $R$  is the rate of oxygen draw-down during the trial minus the oxygen draw-down during the background run (in mg O<sub>2</sub> L<sup>-1</sup> s<sup>-1</sup>),  $V$  is volume of the respirometer (in liters),  $D$  is the volume of water displaced by the shark (in liters),  $M$  is the mass of the individual (in kilograms), and  $b$  is the scaling exponent. The displacement volume of the shark was determined using estimates from Luongo and Lowe (2018) where 1 mg = 0.957 L. Mass adjusted oxygen consumption ( $M_{O_2}$ ) in mg O<sub>2</sub> hr<sup>-1</sup>kg<sup>-1</sup> was calculated as:

$$M_{O_2} = a_n \bar{M}^{0.80} \quad (2)$$

where  $\bar{M}$  is the mean mass of all individuals during that acclimation.

The metabolic thermal sensitivity quotient ( $Q_{10}$ ) was determined by the Schmidt-Nielsen (1997) equation:

$$Q_{10} = \left( \frac{MO_2(2)}{MO_2(1)} \right)^{\frac{10}{T_2 - T_1}} \quad (3)$$

where  $MO_{2(1)}$  is the metabolic rate at temperature  $T_1$  and  $MO_{2(2)}$  is the metabolic rate at temperature  $T_2$ . Due to constraints on only utilizing two points to calculate the quotient,  $Q_{10}$  for this study was also calculated through non-linear regression using the R “Respirometry” package (Birk 2018; R package version 1.0.0).

#### *Statistical Analysis*

To compare  $MO_2$  across experimental temperature and across acclimation treatments analysis of variance (ANOVA) was used followed by post hoc Tukey’s honest significance test. Shapiro-Wilk normality tests were run prior to ensure a normal distribution at each temperature. To compare  $MO_2$  between species an analysis of covariance (ANCOVA) was used followed by post hoc Tukey’s honest significance test ( $\alpha = 0.05$ ).

## RESULTS

The mean preferred temperatures and temperature ranges determined through shuttlebox experimentation were compared between acclimation treatments and species as reported in Table 2. Mean preferred temperature  $\pm$  SE did not differ between acclimation treatments within either species or when compared between the two species. Leopard sharks preferred  $18.3 \pm 1.44^{\circ}\text{C}$  and  $17.7 \pm 1.15^{\circ}\text{C}$  after warm and cold acclimations, respectively (ANOVA:  $F_{(1, 13)} = 0.28$ ,  $P > 0.05$ ). Horn sharks preferred  $20.6^{\circ}\text{C} \pm 0.81$  and  $18.0^{\circ}\text{C} \pm 1.92$  after warm and cold acclimations, respectively (ANOVA:  $F_{(1, 13)} = 2.0$ ,  $P > 0.05$ ). When looking at the temperature ranges, the lower bound temperatures did not differ between acclimation treatments within either species or when compared between species (ANCOVA:  $P > 0.05$ ). For leopard sharks, the upper bound of the temperature ranges did not differ between acclimation treatments. However, horn sharks had significantly higher upper bound temperatures ( $24.2 \pm 0.84^{\circ}\text{C}$ ) during warm acclimation than cold acclimation ( $20.6 \pm 2.26^{\circ}\text{C}$ ; ANOVA:  $P < 0.05$ ). When compared between species, the upper bound temperature of horn sharks was also significantly higher than that of leopard sharks during warm acclimation only (ANOVA:  $F_{(1, 17)} = 4.8$ ,  $P < 0.05$ ). Sex-specific comparisons of preferred temperatures and temperature ranges are reported in Table 3. The mean lower bound temperature was significantly higher in warm acclimated female leopard sharks than in males (ANOVA:  $F_{(3,7)} = 6.2$ ,  $P < 0.05$ ). Despite a lack of statistical significance, there was a general trend in which females preferred warmer temperatures and had a higher shifted thermal range than males in both species and with both acclimation temperatures.

The resting oxygen consumption rates ( $M_{\text{O}_2}$ ) of both shark species under both acclimation treatments and each experimental temperature are reported in Table 4 and Figures 3a-c. Leopard sharks had higher resting oxygen consumption rates than horn sharks at all temperatures regardless of the acclimation treatment (ANCOVA:  $p < 0.05$ ). Post hoc Tukey test found the mean  $M_{\text{O}_2}$  of horn sharks significantly increased with experimental temperature in both acclimation temperatures. The mean  $M_{\text{O}_2}$  of

leopard sharks significantly increased across all experimental temperatures during the warm acclimation, however, leopard shark mean  $M_{O_2}$  was only significantly different between 12-20°C and 12-24°C during cold acclimation ( $\alpha = 0.05$ ). Resting oxygen consumption at a given temperature did not differ between warm and cold acclimation in either species (ANCOVA:  $p > 0.05$ ).

The metabolic thermal sensitivities of both shark species as measured through estimates of  $Q_{10}$  are shown in Table 5. Across all experimental temperatures, the  $Q_{10}$  for horn sharks was higher than that of leopard sharks. From 12-24°C, horn sharks had a  $Q_{10}$  of 2.89 while leopard sharks had a  $Q_{10}$  of 1.84.

## DISCUSSION

Experimental approaches to understand behavioral thermoregulation and physiological responses to temperature allow for insight into the habitat selection of ectotherms. In combination with previous field studies, the lab-based thermal preferences and metabolic sensitivity to temperature determined in this study, provide a clearer picture of how temperature may influence habitat selection of juvenile horn sharks and leopard sharks. This study reported that juvenile horn and leopard sharks both prefer similar temperatures regardless of season suggesting that both species actively seek to remain within their thermal optimum. The metabolic thermal sensitivity of neither species changed with seasonal acclimation, and horn sharks showed greater metabolic changes (higher  $Q_{10}$ ) with acute changes in water temperature than leopard sharks. In order to reduce physiological costs associated with these greater changes in metabolic rate across the same change in temperature, the proportion of time in which horn sharks spend near their preferred temperature is predicted to be higher than that of leopard sharks. Despite the examination of metabolic sensitivity by other studies on horn and leopard sharks, the present study was the first to report the temperature preferences of juvenile horn sharks and leopard sharks as well as the first to run temperature preference and metabolic rate using the same experimental sharks. When interpreted together, these findings illustrate that differences in lab-based thermal preferences and *in situ* movements can be species-specific, and more holistic habitat preference studies are needed to elucidate true drivers of movement.

### *Juvenile California Horn Sharks*

In the context of habitat utilization, the lab-based thermal preference of juvenile horn sharks corresponds with previous field studies and therefore the horn sharks likely target physiologically optimal temperatures. In this study, juvenile horn sharks preferred temperatures between 18.0-20.6°C and spent the majority of time in waters above ~16°C. These parameters were consistent with underlying seasonal acclimation, although warm acclimated horn sharks explored warmer waters (mean  $\pm$  SD: 24.2  $\pm$  0.84°C)

than cold acclimated horn sharks (mean  $\pm$  SD:  $20.6 \pm 2.26^\circ\text{C}$ ). The preferred temperatures (and ranges) of horn sharks falls within normal temperatures experienced across their geographic distribution (Central California, USA to Gulf of California, Mexico). Sea surface temperature in southern California—where the individuals from this study were captured—typically has an annual range between  $14\text{-}22^\circ\text{C}$ , but sea surface temperatures can even dip below  $12^\circ\text{C}$  and exceed  $30^\circ\text{C}$  across the entire geographic expanse of this species (NOAA National Data Buoy Center; Walker et al., 2020). Advection of deeper, colder water into the shallow coastal environment (7-17 m depth) along the coast of California can also drop bottom temperatures to between  $10\text{-}12^\circ\text{C}$  over periods of hours to weeks (Booth et al., 2012; Frieder et al., 2012).

Surveys indicate horn sharks spend the majority of the day (~90%) resting on the bottom, and half of the night resting between bouts of activity (Strong, 1990; Meese, 2019; Meese and Lowe, 2020a). Horn sharks also exhibit site fidelity and homing behavior often returning to the same rest sites (Ebert, 2003; Meese, 2019). Active tracking by Meese and Lowe (2020a) determined that adult horn sharks rested in shallow waters (0.2-35 m) with mean temperatures of  $18.1 \pm 0.31^\circ\text{C}$  that fluctuated  $3.3 \pm 0.3^\circ\text{C}$  (range:  $11.0\text{-}23.0^\circ\text{C}$ ) during the day. At night (a time of greater activity), the same adult horn sharks exhibited directed movements to specific foraging areas away from areas of shelter. While sometimes moving to deeper or shallower depths experiencing larger temperature fluctuations ( $7.4 \pm 0.4^\circ\text{C}$ , range:  $10\text{-}23.8^\circ\text{C}$ ; Meese and Lowe 2020a), these horn sharks appeared to continue to track the same mean temperatures as when resting ( $18.0 \pm 0.5^\circ\text{C}$ ).

Horn sharks may seek habitat with more stable temperature fluctuations due to the high thermal sensitivity of their metabolism to temperature. It appears short-duration changes in water temperature have a larger effect on the change in metabolic rates (present study:  $Q_{10} = 2.89$  from  $12\text{-}24^\circ\text{C}$ ) as opposed to longer term acclimation (Luongo and Lowe, 2018:  $Q_{10}=2.01$  from  $14\text{-}22^\circ\text{C}$ ). Horn sharks in areas that experience broad thermal changes may require more energy to keep up with the metabolic requirements and therefore seek a stable thermal environment to reduce metabolic costs. While some elasmobranchs alternate between environments with different mean temperatures often related to resting and foraging (Hopkins and Cech, 1994; Matern et al., 2000; Sims et al., 2006; Wallman and Bennett, 2006;



DiGirolamo et al., 2012), horn sharks likely do not exhibit this pattern (Meese, 2019; Meese and Lowe, 2020a). Due to the high thermal sensitivity of their metabolism and the low thermal inertia due to their small body size, juvenile horn sharks are likely to limit movements across broad thermal regimes during periods of activity (i.e., foraging), and will remain in areas with more stable temperatures close to their thermal preference during periods of inactivity (i.e., resting).

Although tracked adult horn sharks selected for habitat that corresponded with the preferred temperature of the juveniles utilized in this study, population surveys by Meese and Lowe (2020b) found immature individuals rested in warmer (mean  $\pm$  SD:  $20.3 \pm 1.6$  °C, range: 16.3–23.2 °C), shallower (mean  $\pm$  SD:  $8.5 \pm 3.4$  m, range: 2.7– 15.5 m) waters than mature individuals during the day. While it is possible that temperature preference changes with ontogeny, it is more likely that adult sharks exhibit a lower thermal sensitivity associated with inherent changes due to increased body size (Grubbs, 2010), and therefore adults can occupy shelters away from their temperature preference and with higher thermal variance. Thermal habitat may be more important for shelter selection in juvenile horn sharks due to inherent decrease in thermal inertia associated with increase in body size (bigger sharks have a smaller surface area to volume ratio and therefore gain/lose heat at a lower rate), and adults may take other factors into consideration such as shelter quality and proximity to foraging sites (Meese and Lowe, 2020b). Future studies should assess if changes in temperature preference or metabolic sensitivity are potential drivers of segregation between adults and juveniles. Insight into seasonal movement patterns and how these parameters may change with geographic distribution are also needed.

### *Juvenile Leopard Sharks*

The findings of this study indicate that juvenile leopard shark habitat utilization may be different than horn sharks, despite overlap in their thermal preferences. Juvenile leopard sharks preferred waters between 17.7–18.3°C and spent 95% of their time between ~16–21°C regardless of acclimation to seasonal temperatures. These parameters correspond with temperatures typically experienced across the geographic range of leopard sharks (Oregon, USA to the Gulf of California, Mexico) where surface

temperatures can range from below 8°C to above 30°C (NOAA National Data Buoy Center). Unlike the sedentary horn shark, leopard sharks actively patrol coastal habitats throughout the diel cycle and tracked adult leopard sharks were shown to explore a mean range of 17.0-21.6°C and experienced waters as cool as 12.7°C and as warm as 23.9°C (Nosal et al., 2013).

Juvenile leopard sharks exhibit lower thermal sensitivities ( $Q_{10} = 1.84$  from 12-24°C) than horn sharks ( $Q_{10} = 2.89$  from 12-24°C), and thus likely do not need to continuously target their preferred temperature to keep up with energetic demands. As more mobile mesopredators, the higher movement rate of leopard sharks means that they likely spend more time moving across these thermal gradients and are consequently able to effectively deal with sudden temperature changes. In addition to low thermal sensitivity, leopard sharks also have low thermal inertia (the body temperature of adult leopard sharks changes 3°C/hour when in warmer or colder environments), which allows individuals to move through heterogeneous thermal environments over short periods of time without much change to the internal body temperature (Hight and Lowe, 2007). With less reliance on surrounding environmental temperature, leopard sharks can thus allocate more time towards other purposes such as foraging and maintain activity across varied thermal environments. Previous studies have found fine-scale *in situ* movement of leopard sharks has been correlated with a variety of factors besides temperature including tide, photoperiod, salinity, and dissolved oxygen (Manley, 1995; Ackerman et al., 2000; Carlisle and Starr 2009). Although temperature may not be the major driver of fine-scale movement, the seasonal abundance of leopard sharks in shallow embayments or calm coastal regions is largely determined by temperature (Hopkins and Cech 2003; Carlisle and Starr 2009; Nosal et al., 2013).

The thermal sensitivity of leopard sharks may also vary across the species' geographic range. Leopard sharks exhibit population differences with latitudinal distribution along the coast of California (Lewallen et al., 2007; Barker et al., 2015), which could possibly be driven by local adaptations to differences in selective pressures including temperature. The present study reports a lower thermal sensitivity (present study:  $Q_{10} = 1.84$ ) than previously reported (Miklos et al., 2003:  $Q_{10} = 2.51$ ) in juvenile leopard sharks across the same experimental temperature change (12-24°C). Individuals used in

the study by Miklos et al. (2003) were captured from Elkhorn Slough, CA, a tidal estuary in the northern geographic extent of leopard shark range, whereas individuals from the current study were captured within the southern distribution. Latitudinal differences in metabolic rate have been shown in bony fishes acclimated to extreme high temperatures (Gardiner et al., 2010) and thus it is possible that leopard sharks acclimated to extreme low temperatures may also exhibit metabolic differences across their latitudinal distribution. In southern California, sea surface temperature typically ranges from 14-22°C and can reach extremes below 12°C and above 26°C, while in the northern California sea surface temperatures typically range from 10-16°C and can drop below 8°C or rise above 24°C (NOAA National Data Buoy Center). In addition to a shift in thermal sensitivity, the thermal tolerances of leopard sharks may also be lower or broader along the northern extent of the geographic range. The findings of the present study indicate that temperatures below 16°C are outside the preferred thermal range of juvenile leopard sharks caught from the southern distribution, whereas the lower thermal threshold of tracked leopard sharks within the northern distribution appears to be closer to 10°C (Hopkins and Cech, 2003). Exposure to colder environments outside of their preferred temperature ranges likely leads to an increase in behavioral thermoregulation to keep temperatures close to the metabolic optimum. When temperatures decrease below this threshold, leopard sharks may explore outside these semi-enclosed habitats in search for more suitable environments such as open coastal waters. While the reported thermal sensitivity suggests that leopard sharks can move across variable thermal regimes within these habitats with reduced metabolic costs and minimized physiological debt accrual, future studies need to look into possible difference across their geographic distribution and at more extreme acclimation temperatures. Possible disparities in metabolic thermal sensitivity, temperature preference, and thermal tolerance may mean differences in both fine-scale movement and/or seasonal abundance within these nearshore environments, which can ultimately lead to region-specific management and conservation concerns.

### *Sex Comparisons*

An emergent trend, while not statistically significant, was that females preferred warmer temperatures than males in both species and in both seasonal acclimation treatments. The present study was constrained by a low number of individuals from each sex and a greater sample size is needed to make concrete conclusions. However, sexual segregation is observed in many shark species and is believed to be influenced by high energetic expenditure associated with aggressive mating, or sex-specific energetic demands such as reproductive expenditures (e.g., gamete production, gestation of embryos), or somatic growth (Klimley, 1987; Sims, 2005, Wearmouth and Sims, 2008; Mucientes et al., 2009; Jacoby et al., 2011). Klimley (1987) suggests in shark species where females grow larger than males, sexes will segregate by thermal habitat both prior to maturity and post-maturation in order to increase food consumption and maximize growth rate, consequently increasing fecundity. Females of both horn sharks and leopard sharks grow larger than males and reach sexual maturity at a larger size than males (Ebert, 2003), and therefore immature females may target warmer waters to increase body temperature and growth rate in order to reach maturity faster (Economakis and Lobel, 1998). Studies on the sexual segregation in immature elasmobranchs are scarce, but immature females have also been shown to prefer warmer waters than immature males in bony fishes, and this difference in thermal preference was linked to higher testosterone levels in males (Johansen and Cross, 1980). It is possible these apparent differences of preferred temperatures between sexes were a result of physiological modification induced by higher testosterone levels in developing males, although further investigation is needed. Interestingly, immature female blue sharks (*Prionace glauca*) were found to occupy colder water than immature males during summer months (Maxwell et al., 2017; Vandeperre et al., 2014; Howey et al., 2017), which is a different pattern than found in the present study. Maxwell et al. (2017) suggest that this sexual segregation may be a result of the thicker skin of females in this species, which in addition to a protection against mating males, may act as thermal insulation and consequently opens up access to resources in colder waters. Movement studies of juveniles in both leopard and horn sharks are extremely limited, and so whether there is a difference of thermal habitat utilization between sexes at this life stage is yet to be determined.

Although sex-specific differences in depth distribution or temperature were not observed in a recent field survey (Meese and Lowe, 2020b), the authors do not rule out sexual segregation due to a insufficient sample size of immature males, mature females, and adolescent individuals (45-55 cm TL), and/or could be also be an artefact of timing as the authors only sampled during the warmer season. A closely related species to horn sharks, the Port Jackson Shark (*Heterodontus portjacksonii*), does indeed exhibit sexual and ontogenetic habitat segregation (McLaughlin and O’Gower, 1971).

Evidence suggests that adult female leopard sharks behaviorally thermoregulate in concordance with reproductive periods (Hight and Lowe, 2007; Nosal et al., 2013), and thus it is possible that females may be seeking warmer waters prior to maturity as well. Leopard sharks exhibit sexual segregation at specific aggregation sites during warmer months such as in La Jolla, CA, where 97% of sampled sharks were found to be pregnant females (Nosal et al., 2013). Seven adult females from this aggregation were tracked and occupied waters with a mean temperature of 20.6°C (Nosal et al., 2013), and tagged adult females from Santa Catalina Island, CA, were found to be in areas where mean water temperature was  $21.8 \pm 1.2^\circ\text{C}$  (Hight and Lowe, 2007). In both cases, it is hypothesized that females use the warm, shallow waters to facilitate growth of the embryos and to avoid unwarranted mating with males (Hight and Lowe, 2007; Nosal et al., 2013). Although both of these past studies were on adult females, the occupied temperatures are remarkably close to that found in the present study in which (warm-acclimated) immature female leopard sharks preferred  $21.1 \pm 1.3^\circ\text{C}$  waters, and therefore it is possible this thermal preference extends across ontogeny. Further, males at a nearby mixed-sex aggregation site in Del Mar, CA began to congregate at about 16.3°C whereas the seasonal day-time aggregation of females in La Jolla began when mean water temperature were above 17.6°C (Nosal et al., 2014). Even small differences in temperature can have large physiological consequences, and a study on pregnant Atlantic Stingrays showed a 1°C increase in temperature can reduce gestation time by almost two weeks (Wallman and Bennett, 2007). Therefore, it is vital for sex-specific differences in temperature preference and movement to be investigated further.

## *Implications*

The present study highlights a gap in current research on the ecophysiology and behavioral ecology of cartilaginous fishes. Few studies focus on the drivers of habitat use for early life stages of elasmobranchs (Kraska and Gaskins, 2015), and especially those in southern California. Various shark species, including both horn sharks and leopard sharks, utilize warm, shallow coastal waters such as estuaries and bays during early life stages in order to optimize growth and fitness (Castro, 1993; Merson and Pratt, 2001; Espinoza et al., 2011). Almost 60% of the global human population lives along the coast leading to an increased pressure on estuarine habitats (Gleason et al., 2011). This is certainly the case in southern California (Gillander et al., 2003) where the unique geomorphology and dynamic tectonics have led to shallower and smaller estuaries (14 of 16 estuaries in San Diego County are <10 km<sup>2</sup>) with low freshwater input (Emmett et al., 2000; California Department of Fish and Game Marine Resources Region, 2014). Changes in estuarine ecosystems (e.g., human encroachment and climate change) coupled with the small size of the estuaries in southern California can reduce the availability of suitable habitat to estuarine-dependent fishes such as leopard sharks and horn sharks. Such changes can have unfavorable effects and may even threaten these potential nursery areas and the species dependent on them. Thus, it is imperative to further understand how juvenile sharks utilize these coastal environments and assess the relative importance of local estuaries and bays as viable nursery grounds.

In particular, warming ocean temperatures may affect the activity and distribution of fishes utilizing these habitats (Sunday et al., 2012; Grady et al., 2019). Increased mean water temperatures can increase energetic demands which may result in increased activity such as foraging or movement of sharks within the environment (Grady et al., 2019). An energetic model by Luongo and Lowe (2018) suggests that horn sharks experience annual variability of standard metabolic rate due to annual fluctuations in temperature and that this species has already seen an increase in mean standard metabolic rate from 2005-2017 due to rise in mean sea surface temperature across this time period. Daily variation in temperature is also expected to change within the environment (Easterling et al., 2000), and it is possible that this shift could alter viable habitat important for growth. Change in daily temperature

variation can potentially lead to a shift in optimal temperatures (Paaijmans et al., 2013), ultimately affecting the daily habitat usage of these sharks. This change in habitat utilization could also differ across the geographic distribution of these species. Sharks within the northern extent of the range are expected to experience a decrease in the occurrence of lower temperatures and leopard sharks that typically leave shallow waters when temperatures drop too low may be able to stay within embayments at a higher proportion as mean water temperature rises during cooler months. Across their range there could also exist areas in which temperatures may become too high, ultimately restricting the habitat usage of sharks within these shallow environments. For example, leopard sharks are known to actively avoid water temperatures warmer than 26 °C (Carlisle and Starr, 2009; Hight and Lowe, 2007) with temperatures of 27 °C beginning to have deleterious effects on heart function (Cox and Wegner, unpublished). Even at 24°C, several leopard sharks used in the current study were visibly restless within the respirometer and measurements could not be taken as a result. Current temperatures can exceed 30°C in the Gulf of California, and 26°C within waters off of southern California (NOAA National Data Buoy Center; Walker et al., 2020), and it is likely sharks would avoid these pockets of the environment due to the associated physiological costs. As global water temperatures rise, uninhabitable thermal pockets will likely increase both within estuaries and along the coast which can decrease suitable habitat within the shallows for juvenile sharks particularly along their southern geographic range. However, while warmer temperatures may lead to lower nursery ground capacities within the shallows, ectothermic organisms like sharks may be able to move to deeper depths (e.g., to forage, for refuge) along the coast as the thermocline broadens. Ultimately, warm climatic shifts in temperature can lead to northward expansion of marine organisms (Cheung et al., 2009), and geographic expansion to higher latitudes has already been observed in certain species (Nicolas et al., 2011; Hobbs, 2011; Walker et al., 2020). Changes in species composition in the California Current is strongly correlated with climate change (Doney et al., 2011), and therefore it is likely horn shark and leopard shark populations will be affected. In fact, the rise in temperature over the last few decades has been linked to the population increase of horn sharks and decrease of swell sharks (*Cephaloscyllium ventriosum*) along Catalina Island (Grover, 1972; Ebert, 2003).

Despite this, global warming could be potentially detrimental for future populations due to the already limited number of suitable nursery grounds and the fact that many of the potential nurseries along the north coast of the western United States are more riverine-based and not suitable environments for many species including horn and leopard sharks (Hughes et al., 2014).



## CONCLUSIONS

The relationship between physiology and behavior is critically important for making educated judgements related to the effects of climate change on habitat utilization. Baseline assessments, such as the one presented here, are necessary for species in habitats that are particularly vulnerable to change. The data suggests that juvenile horn sharks and leopard sharks may be able to acclimatize to temporal changes in temperature on the scale of weeks to months. As observed in other species (Lowe, 2001; Neer et al., 2006; Di Santo and Bennett, 2011; Whitney et al., 2016; Luongo and Lowe, 2018), both horn sharks and leopard sharks displayed large intraspecific variability in oxygen consumption rate which may be attributed to individual differences in cellular physiology (Norin and Malte, 2012; Salin et al., 2016). This intraspecific variability suggests there may be a proportion of the population of each species that is better suited to shifting climatic conditions, but the degree to which these species may be resilient needs further investigation. The present study indicates that thermal dependence is largely contingent on the baseline activity levels and ecology of the shark species in question. Co-occurring species, like horn sharks and leopard sharks, may overlap in time and space yet their habitat utilization may be different—particularly during periods of low activity—due to the disparity in their metabolic dependence on the thermal environment. These parameters may also change with ontogeny and geographic distribution and it is crucial that future studies focus on collecting baseline physiological, bioenergetic, and behavioral data to help refine approaches for conservation and fisheries management. It is also imperative that future studies use a synergistic approach in pairing lab-based experimentation with *in situ* tracking, particularly during early life stages, to more fully resolve spatiotemporal models and discern true drivers of habitat use.

*This thesis, in part, is currently being prepared for submission for publication of the material.*

*The thesis author was the primary investigator and author of this material. The thesis is coauthored with Tanya Prinzing, Nicholas Wegner, and committee chair Philip Hastings.*

**Table 1.** Horn and leopard shark size data immediately preceding shuttlebox and respirometry trials following both the warm and cold acclimation periods. Total length (TL) and mass are reported for the same individuals.

Species	Sex	Individual	TL (cm)	Mass Warm	TL (cm)	Mass Cold	% Mass Gained
			Warm Acclimation	(kg)	Cold Acclimation	Acclimation (kg)	
Horn Shark ( <i>Heterodontus francisci</i> )	F	1	45.6	0.738			
	F	2	48.2	0.852			
	F	3	41.1	0.496	41.9	0.669	34.8
	F	4	48.7	0.755			
	F	5	37.6	0.393	38.7	0.439	11.7
	M	6	41.1	0.515	42.6	0.642	24.9
	M	7	43.7	0.746	45.2	0.860	15.3
	M	8	38.8	0.443	39.3	0.565	27.7
	M	9	38.2	0.408			
	M	10	37.1	0.433			
		<b>Mean ± SE</b>	<b>42.0 ± 1.3</b>	<b>0.578 ± 0.052</b>	<b>41.5 ± 1.1</b>	<b>0.635 ± 0.062</b>	<b>22.9 ± 3.7</b>
Leopard Shark ( <i>Triakis semifasciata</i> )	F	1	88	2.72	96.0	3.62	33.1
	M	2	75.6	1.68			
	F	3	89.9	2.70	95.0	3.30	22.2
	F	4	70.2	1.42	79.3	2.07	45.8
	F	5	84.5	2.32	91.5	2.81	21.1
	M	6	77.8	2.10	84.2	2.20	4.8
	M	7	72.1	1.44	77.5	1.84	27.8
	F	8	76.2	1.86	82.0	2.78	49.5
		<b>Mean ± SE</b>	<b>79.3 ± 2.4</b>	<b>2.03 ± 0.16</b>	<b>86.5 ± 2.7</b>	<b>2.66 ± 0.23</b>	<b>29.2 ± 5.4</b>

**Table 2.** Preferred temperatures and temperature ranges (means ± SE) of horn and leopard sharks compared between two seasonal acclimation treatments. Temperature ranges were determined as the bounds between which sharks spent 95% of their time.

Species	Acclimation	n	Preferred Temperature (°C)	Range of Temperature (°C)	
				Lower Bound	Upper Bound
Horn Shark ( <i>Heterodontus francisci</i> )	Warm (21°C)	10	20.6 ± 0.81	16.6 ± 0.92	24.2 ± 0.84
	Cold (15°C)	5	18.0 ± 1.92	16.1 ± 0.92	20.6 ± 2.26
Leopard Shark ( <i>Triakis semifasciata</i> )	Warm (21°C)	8	18.3 ± 1.44	16.3 ± 1.03	20.7 ± 1.41
	Cold (15°C)	7	17.7 ± 1.15	15.9 ± 1.08	19.8 ± 1.41

**Table 3.** Preferred temperatures and temperature ranges (means  $\pm$  SE) of horn and leopard sharks compared between two seasonal acclimation treatments and sex. Temperature ranges were determined as the temperature bounds between which sharks spent 95% of their time.

Species	Acclimation	Sex	Preferred Temperature ( $^{\circ}$ C)	Range of Temperatures ( $^{\circ}$ C)	
				Lower Bound	Upper Bound
Horn Shark ( <i>Heterodontus francisci</i> )	Warm (20.5 $^{\circ}$ C)	Male (n=5)	19.5 $\pm$ 1.1	15.0 $\pm$ 0.8	23.4 $\pm$ 1.2
	Warm (20.5 $^{\circ}$ C)	Female (n=5)	21.7 $\pm$ 0.8	18.1 $\pm$ 1.2	24.9 $\pm$ 1.0
	Cold (15 $^{\circ}$ C)	Male (n=2)	17.7 $\pm$ 2.4	16.7 $\pm$ 2.1	18.6 $\pm$ 2.5
	Cold (15 $^{\circ}$ C)	Female (n=3)	18.9 $\pm$ 2.0	15.2 $\pm$ 1.7	23.5 $\pm$ 2.1
Leopard Shark ( <i>Triakis semifasciata</i> )	Warm (20.5 $^{\circ}$ C)	Male (n=3)	15.9 $\pm$ 1.7	13.8 $\pm$ 1.1	18.9 $\pm$ 2.5
	Warm (20.5 $^{\circ}$ C)	Female (n=5)	21.1 $\pm$ 1.3	17.8 $\pm$ 0.8	21.9 $\pm$ 1.3
	Cold (15 $^{\circ}$ C)	Male (n=2)	17.1 $\pm$ 0.8	15.3 $\pm$ 0.8	19.7 $\pm$ 0.8
	Cold (15 $^{\circ}$ C)	Female (n=5)	18.2 $\pm$ 1.2	16.1 $\pm$ 1.4	19.9 $\pm$ 1.7

**Table 4.** Mean resting oxygen consumption rate for horn and leopard sharks at each experimental temperature and seasonal acclimation treatment in comparison to oxygen consumption rates from the current study compared with available values reported from previous studies. Sharks from Miklos et al. (2003) were captured when ambient temperatures were between 12-14 $^{\circ}$ C and held for 5-7 days in flow through seawater at these temperatures prior to the experiment. For more direct comparison, oxygen consumption rates from previous studies were converted to the mean mass of each species at each acclimation from the present study (see Table 1) and scaled as in Equations 1 and 2 using a scaling exponent of 0.8. During cold acclimation, the mean mass was 0.58 kg for horn sharks and 2.03 kg for leopard sharks. During warm acclimation, the mean mass was 0.63 kg for horn sharks and 2.66 kg for leopard sharks.

Species	Study	Acclimation	Mean $M_{O_2}$ (mgO <sub>2</sub> h <sup>-1</sup> ) $\pm$ SE			
			12 $^{\circ}$ C	16 $^{\circ}$ C	20 $^{\circ}$ C	24 $^{\circ}$ C
Horn Shark ( <i>Heterodontus francisci</i> )	<b>Present study</b>	<b>Cold</b> (15 $^{\circ}$ C)	<b>16.7 <math>\pm</math> 4.1</b>	<b>23.3 <math>\pm</math> 4.3</b>	<b>40.7 <math>\pm</math> 8.6</b>	<b>57.7 <math>\pm</math> 6.7</b>
	Luongo & Lowe (2018)	Cold (15 $^{\circ}$ C)		35.2 $\pm$ 2.4	46.7 $\pm$ 2.5	
	<b>Present</b>	<b>Warm</b> (20.5 $^{\circ}$ C)		<b>22.5 <math>\pm</math> 7.1</b>	<b>30.0 <math>\pm</math> 5.6</b>	<b>46.2 <math>\pm</math> 8.5</b>
	Luongo & Lowe (2018)	Warm (20.5 $^{\circ}$ C)		33.0 $\pm$ 2.2	43.7 $\pm$ 2.3	
Leopard Shark ( <i>Triakis semifasciata</i> )	<b>Present study</b>	<b>Cold</b> (15 $^{\circ}$ C)	<b>160 <math>\pm</math> 44.7</b>	<b>219 <math>\pm</math> 64.4</b>	<b>275 <math>\pm</math> 43.2</b>	<b>342 <math>\pm</math> 86.9</b>
	Miklos et al. (2003)	Cold (15 $^{\circ}$ C)	109	184	259	333
	Scharold et al. (1989)	Cold (15 $^{\circ}$ C)		200.6 $\pm$ 30		
					242 $\pm$	
	<b>Present study</b>	<b>Warm</b> (20.5 $^{\circ}$ C)		<b>189 <math>\pm</math> 32.4</b>	<b>59.8</b>	<b>283 <math>\pm</math> 52.2</b>
	Miklos et al. (2003)	Warm (20.5 $^{\circ}$ C)		148	209	269
Scharold et al. (1989)	Warm (20.5 $^{\circ}$ C)		161 $\pm$ 24.5			

**Table 5.** Thermal sensitivity of oxygen consumption ( $Q_{10}$ ) in horn sharks and leopard sharks across temperature and seasonal acclimation treatments by (A) using mean metabolic rate of all individual sharks to find species-specific  $Q_{10}$  via the Schmidt-Nielsen equation, (B) using the Schmidt-Nielsen equation to find  $Q_{10}$  for each individual and then taking mean  $Q_{10}$  of the individuals to find species-specific  $Q_{10}$ , and (C) non-linear regression in R “respirometry” package to find  $Q_{10}$ .

(A)

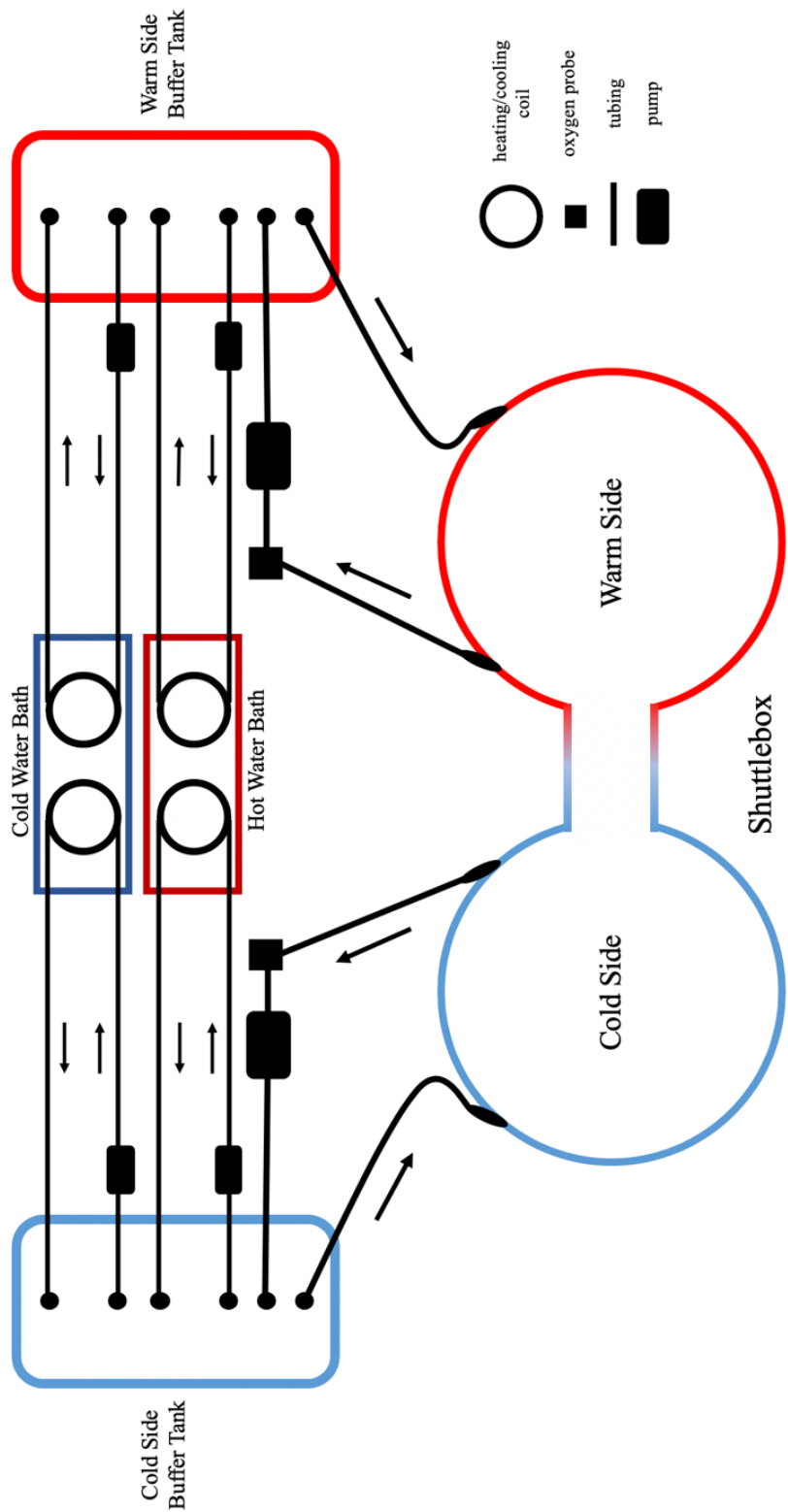
Species	Acclimation	Metabolic Thermal Sensitivity ( $Q_{10}$ )				
		12-16°C	16-20°C	20-24°C	16-24°C	12-24°C
Horn Shark ( <i>Heterodontus francisci</i> )	Warm (20.5°C)	-	2.31	3.75	2.93	-
	Cold (15°C)	2.39	4.01	2.43	3.14	2.87
Leopard Shark ( <i>Triakis semifasciata</i> )	Warm (20.5°C)	-	1.78	1.50	1.65	-
	Cold (15°C)	2.20	1.73	1.73	1.73	1.87

(B)

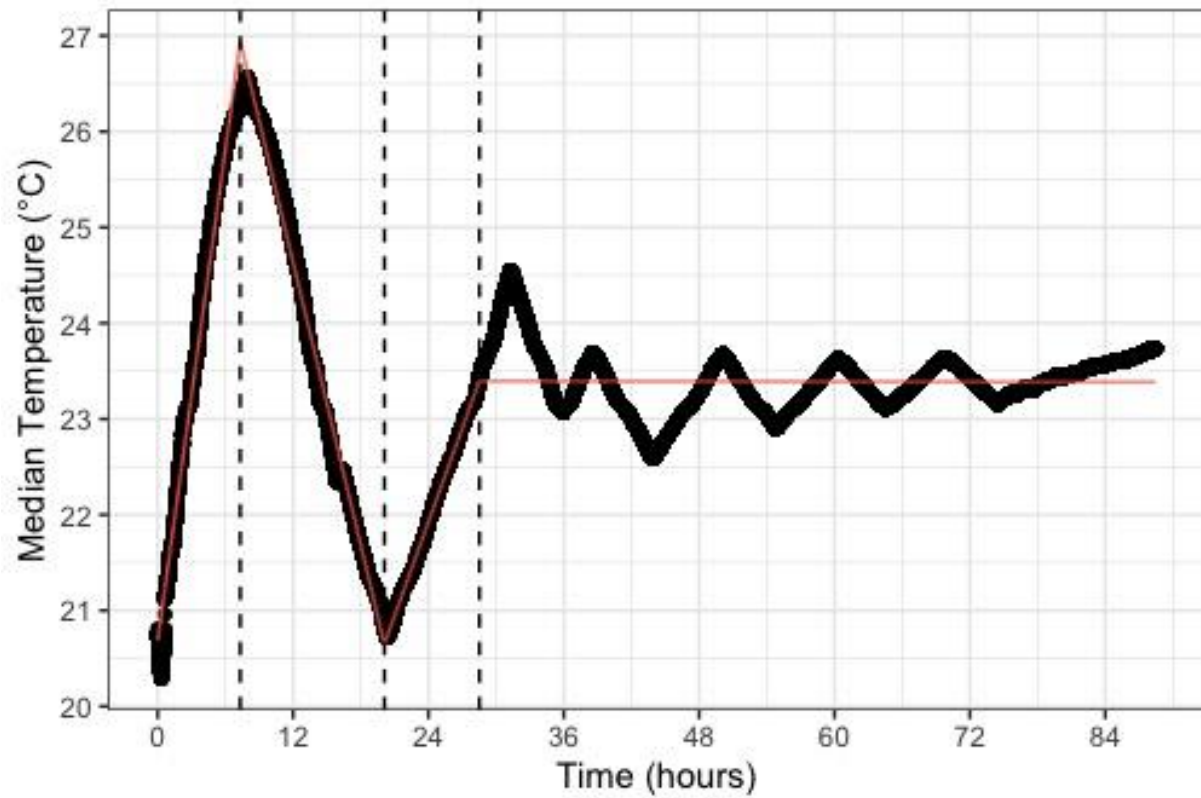
Species	Acclimation	Metabolic Thermal Sensitivity ( $Q_{10}$ )				
		12-16°C	16-20°C	20-24°C	16-24°C	12-24°C
Horn Shark ( <i>Heterodontus francisci</i> )	Warm (20.5°C)	-	3.53	3.20	2.28	-
	Cold (15°C)	2.50	4.26	2.60	3.27	2.98
Leopard Shark ( <i>Triakis semifasciata</i> )	Warm (20.5°C)	-	1.82	1.73	1.68	-
	Cold (15°C)	2.76	2.94	2.45	1.82	1.78

(C)

Species	Acclimation	Metabolic Thermal Sensitivity ( $Q_{10}$ )				
		12-16°C	16-20°C	20-24°C	16-24°C	12-24°C
Horn Shark ( <i>Heterodontus francisci</i> )	Warm (20.5°C)	-	2.32	3.69	3.02	-
	Cold (15°C)	2.37	3.94	2.39	2.89	2.89
Leopard Shark ( <i>Triakis semifasciata</i> )	Warm (20.5°C)	-	1.81	1.53	1.64	-
	Cold (15°C)	2.26	1.8	1.73	1.74	1.84

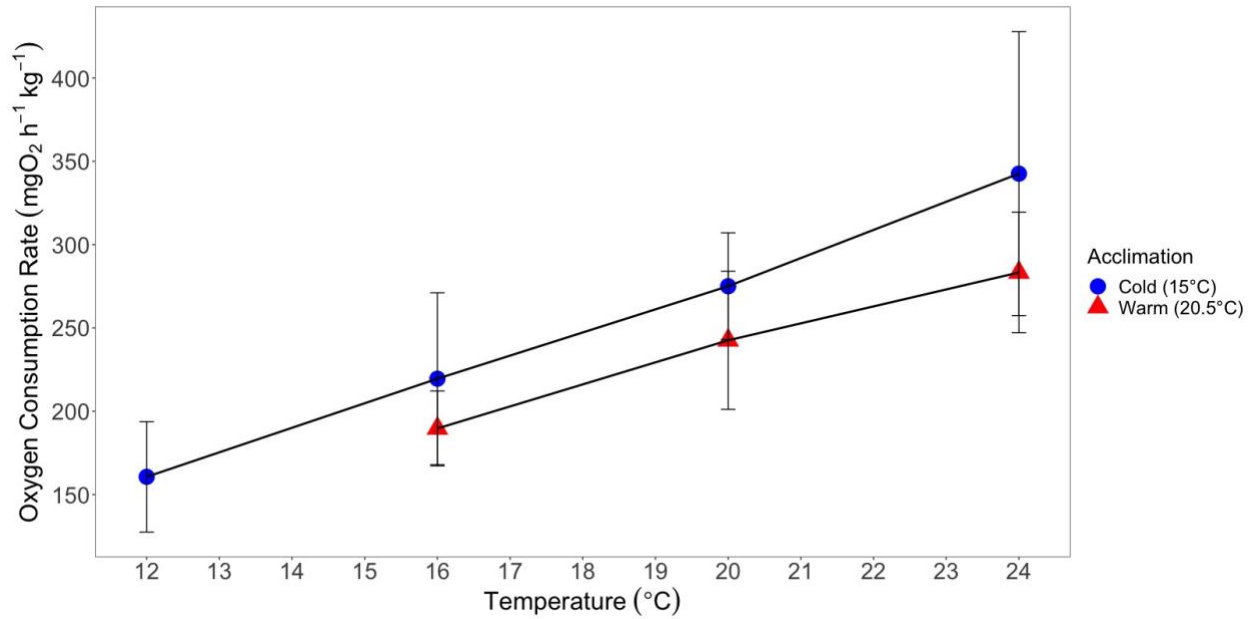


**Figure 1.** Schematic of closed loop shuttlebox system adapted from Loligo, Inc. Red circle indicates chamber in which water is 1.5°C warmer than the water in the cold chamber (blue circle). Shuttlebox is monitored from above by a camera and Shuttlesoft software that provides feedback to the corresponding pumps.

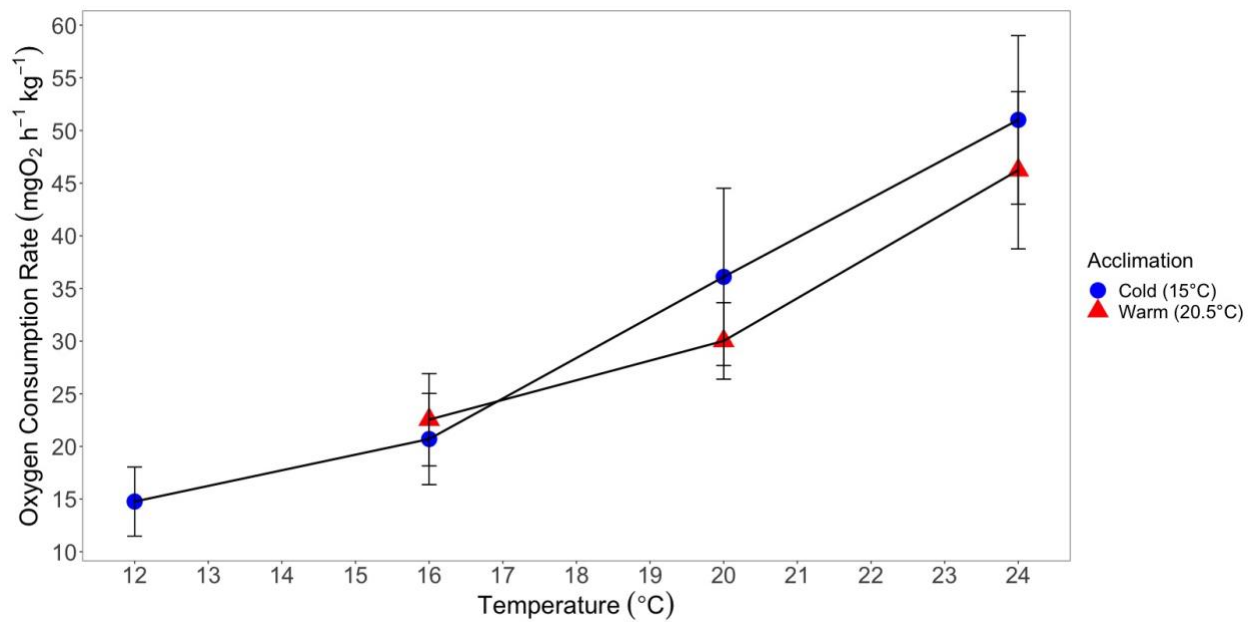


**Figure 2.** Example of the water temperature experienced by a 0.4 kg horn shark over an 88 h period in the shuttlebox. The broken stick model used to find the breakpoint (dashed lines) in slopes is overlaid on the graph. The final breakpoint (dashed line farthest to right) indicates the point at which the individual started to target its preferred temperature. All points after the dashed line were then used to determine the final preferred temperature and temperature ranges.

(A)



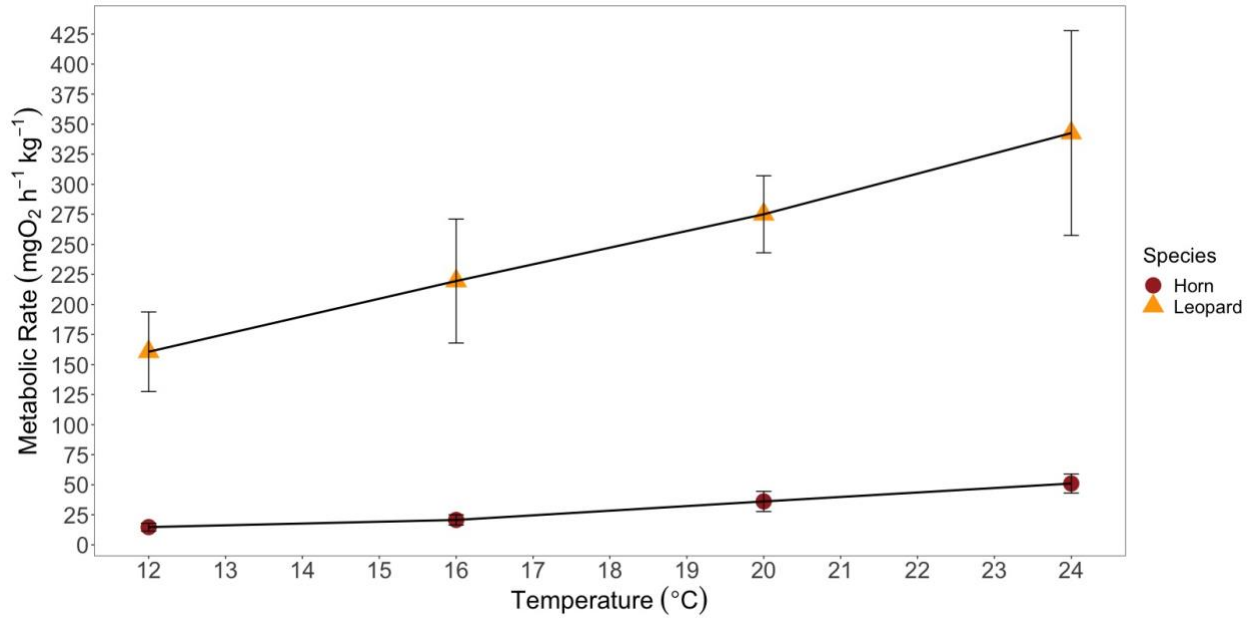
(B)



**Figure 3.** Mean resting oxygen consumption rates compared across experimental temperatures and between acclimation treatments for (A) leopard sharks, and (B) horn sharks. (C) Mean resting oxygen consumption rates were also compared between species during the cold acclimation (15 °C).

Figure 3. Continued

(C)





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