THERMOREGULATION STRATEGIES OF DEEP DIVING ECTOTHERMIC SHARKS

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ABSTRACT

Temperature is one of the most important factors affecting the distribution, behavior, and physiological performance of animals. The rate of heat exchange between the body and surrounding environment determines the capacity for an individual to exploit various habitats. Some marine ectotherms move between drastically different thermal environments; probably to gain access to foraging opportunities or to find ambient temperatures that are optimal for bioenergetic efficiency. Here I used a novel package of telemetry devices to elucidate the thermoregulatory strategies of two large-bodied ectothermic sharks that routinely move between widely different thermal environments. My results revealed unexpected behavioral and physiological strategies of two relatively understudied species.

The bluntnose sixgill shark (*Hexanchus griseus*) is a primitive large-bodied vertically migrating shark that occupies deep waters of between 4-6˚C during the day and migrates to shallower depths with temperatures around 15˚C at night. In the first study to measure the body temperature of a deep-water shark *in situ*, I demonstrate that the large body mass of sixgill sharks provides sufficient thermal inertia to buffer against prolonged exposure to both low or high water temperatures and maintain what are assumed to be optimal body temperatures.

Scalloped hammerhead sharks (*Sphyrna lewini*) occupy the warm waters of the surface mixed layer during the day but exhibit an extraordinary ability to make repeated nighttime dives to depths exceeding 850m where water temperatures are as low as 4°C Presumably they are foraging on deep-sea squid. I show that scalloped hammerhead sharks maintain ‘warm’ core muscle temperatures throughout the deepest portion of each dive and that core muscle cooling only occurs during ascent to the surface. However, once initiated, this cooling is rapid, suggesting active physiological thermoregulation akin to a marine mammal-like dive strategy (i.e. breath holding). During deep dives, *S. lewini* exhibit intensive swimming activity which, coupled with reduced respiration, suggests a

high capacity for anaerobic metabolism in the white “burst swimming” muscle of this species. Analyses of white muscle poise suggests scalloped hammerhead sharks possess enzyme characteristics that facilitate anaerobic metabolism during deep dives and the necessary aerobic metabolism to allow for rapid recovery (through the breakdown of anaerobic end products) during inter-dive intervals.

These two species use highly contrasting approaches to achieve the same goal of broadening their thermal niche and presumably enabling them to exploit resources that would otherwise be out of reach.

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CHAPTER I
INTRODUCTION

Temperature is one of the most important abiotic factors affecting the distribution, behavior, and physiological performance of animals. Understanding the interplay between the physiology, behavior, and ecology of species in relation to environmental temperature is crucial for predicting changes in their movement, distribution, and ecological function in response to climate change (Hazen et al. 2013; Kleisner et al. 2017; Payne et al. 2016; Morley et al. 2018). Marine ectotherms, i.e. species whose body temperature is regulated by the ambient environment, must contend with changes in temperatures broadly (across latitudes) and locally (across depths). The rate of heat exchange between the body and surrounding environment determines the capacity of an ectothermic predator to exploit thermally heterogeneous habitats. Some marine predators such as sharks (superclass *Selachimorpha*) move between different thermal environments to take advantage of foraging habitats and select optimal ambient temperatures for bioenergetic efficiency (Sims 2006; DiSanto & Bennet 2011). Most fish, and sharks for that matter, are considered to be ectothermic with their body temperatures regulated by the ambient water temperature. The ability to access prey resources in thermally disparate habitats allows ectothermic predators to broaden their ecological niche and ultimately increase their fitness (Huey & Kingslover 1989; Block et al. 1993; Dickson & Graham 2004). To do this they must contend with the high thermal conductance of water and avoid changes in body temperature that are detrimental to physiological performance. Behavioral and physiological thermoregulations strategies can be used to passively and actively manage the exchange of body heat with the ambient environment (Holland et al. 1992; Hight & Lowe 2007; Pepino et al. 2015; Stoehr et al. 2018). Understanding the thermoregulatory strategies of marine predators is important for understanding the relationship of environmental temperature, behavior and physiological performance, and can ultimately reveal the limiting constraints to their distribution (Kearney & Porter 2009; Payne et al. 2018). Advances in biologging have allowed for fine-scale measurement of thermal physiology and behavior of free-swimming fish (Payne et al.

2014; Meyer 2017). Here I used a novel package of telemetry devices to elucidate the thermoregulatory strategies of two large-bodied ectothermic sharks that routinely move between widely different thermal environments.

Fish exchange heat between their body and the environment via convective and conductive heat transfer. Convective heat transfer occurs at the site of respiratory gas exchange (the gills) where heat is exchanged between the blood and the ambient water through the thin gill lamellae. It is assumed the majority of metabolic heat generated in muscle tissue is carried away by the blood and rapidly lost to the environment as blood flows through the gill lamellae due to the high thermal conductance of water (Stevens & Fry 1974; Stevens & Sutterlin 1976; Carey & Gibson 1987; Wegner et al. 2015). Conductive heat exchange occurs across the body wall between the body and the ambient water surrounding the fish (Neil et al. 1974; Fechhelm &Neill 1982; Brill et al. 1994; Dewar et al. 1994, Bernal et al. 2001a; Stoehr et al. 2018). Heat exchange models derived from Newton's law of cooling have been used to estimate the rate of heat exchange between the body of fish to their ambient environment (Stevens & Fry 1974; Stevens & Sutterlin 1976). The whole-body heat transfer coefficient (k) determines the rate of heat exchange between organism and environment, where a high coefficient indicates a higher rate of exchange between the body and the environment (Stevens & Sutterlin 1976). A thermal coefficient analysis can be used to discern passive (thermal inertia) versus active thermoregulation in fishes and determine whole-body heat transfer coefficients. Holland et al. (1992) used this approach to demonstrate the capacity for behavioral and physiological thermoregulation of bigeye tuna (*Thunnus obesus*).

Thermal inertia is the capacity of an animal to passively resist change in body temperature in the face of a changing external (ambient) environment. The mass of large body sizes provides an advantage in conserving heat and reducing the rate of heat exchange to the environment (Schmidt-Nielsen 1984). Large body sizes allow animals such as whale sharks, leatherback sea turtles (and possibly dinosaurs) to exploit a diverse range of thermal habitats due to their high thermal inertia (Paladino et al. 1990; Meekan

et al. 2015; Nakamura et al. 2020). The bluntnose sixgill shark (*Hexanchus griseus*) is an excellent candidate for studying the capacity of thermal inertia attributed to large body size. Bluntnose sixgill sharks, hereafter referred to as sixgill sharks, are members of the most primitive group of extant sharks (Hexanchiformes) and important large-bodied apex predators and scavengers of global deep-water ecosystems (Barnett et al. 2012; Churchill 2015; Bizzarro 2017; Drazen & Sutton 2017). Sixgill sharks in Hawaii move across steep thermal gradients during their routine diel vertical migrations, occupying depths of around 500-650m with temperatures around 4-6 ºC during the day and migrate to depths around 200-300m with temperatures around 15 ºC, sometimes reaching 17 ºC at night (Comfort & Weng 2015; Nakamura et al. 2015). It is possible that the large body sizes of adult sixgill sharks allows for sufficient thermal inertia to enable them to exploit different thermal habitats while maintaining their body temperatures within the thermal optimum or below critical temperatures (Sims et al. 2006; Papastamatiou et al. 2015). Here I examined how adult sixgill shark body temperature fluctuates between different thermal environments on a diel cycle through the use of multi-instrument packages capable of directly measuring depth, ambient water temperature, and core swimming muscle temperature on free swimming sharks. This is the first study to measure the body temperature of a deep-water shark *in situ.*

Scalloped hammerhead sharks (*Sphyrna lewini*) occupy warm surface waters in tropical environments but make repeated nocturnal dives to depths exceeding 800m where water temperatures are as low as 4°C (Jorgensen et al. 2009; Bessudo et al. 2011; Hoffmeyer et al. 2013; Spaet et al. 2017). The permanent inhabitants (e.g. fishes, cephalopods) of these deep, cold habitats move slowly compared to their surface counterparts, suggesting that ease of prey capture may motivate the deep-diving behavior seen in scalloped hammerhead sharks (Childress & Somero 1979; Childress et al. 1990; Somero et al. 1992; Childress et al. 1995, Seibel et al. 1997; Seibel & Drazen 2007). The high proportions of meso and bathypelagic species (fishes, cephalopods, crustaceans) in the stomachs of adult scalloped hammerhead sharks further support the notion that these deep dive events serve as foraging opportunities (Clarke 1971; Klimley & Nelson 1984;

Stevens 1989; Smale 1998; Vaske Júnior 2009; Galván-mahaña et al. 2013). However, deep-diving is also risky because body cooling could reduce visual acuity, cardiac function and muscle power potentially leading to death for obligate ram ventilators such as scalloped hammerhead sharks (Bennet 1984; Block & Finnerty 1994; Brill et al. 1998). So how do ectothermic species such as the scalloped hammerhead survive and function in frigid water temperatures experienced during deep dives? It is possible that they are actually regional endotherms with as yet undiscovered physiological or anatomical adaptations for heat retention, or they may rely exclusively on thermal inertia to carry them through their deep dives. In the latter case, the sheer physical size of the shark insulates the body core and provides a short window of opportunity for them to exploit deep, cold habitats before they must return to the surface waters to rewarm (Neil et al. 1974; Paladino et al. 1990; Kitagawa & Kimura 2006). Another possibility is that scalloped hammerhead sharks are able to modulate heat exchange between their bodies and the environment during vertical movements (Holland & Sibert 1994). This may allow them to have a faster rate of warming than cooling such as seen in the ectothermic blue shark (*Prionace glauca* (Carey & Gibson 1987; Carey & Sharrold 1990). This is achieved by modulating blood flow and hence convective heat transfer at the gills (Carey & Gibson 1987; Carey & Sharrold 1990; Kitagawa & Kimura 2006). To answer this question we equipped adult individuals with instrument packages capable of directly measuring depth, ambient water temperature, activity rates and swimming muscle temperature to determine how scalloped hammerhead sharks respond to cold ambient water temperatures experienced during deep dives. Our specific objectives were to: (1) characterize the swimming performance during deep, repetitive dives into cold water and (2) determine whether scalloped hammerhead sharks maintain core body temperature during deep dives via simple thermal inertia, or instead employ active (possibly physiological) thermoregulation.

I show that scalloped hammerhead sharks maintain ‘warm’ core muscle temperatures throughout the deepest portion of each dive and that core muscle cooling only occurs during ascent to the surface. However, once initiated, this cooling is rapid, suggesting

active physiological thermoregulation akin to a marine mammal-like dive strategy (i.e. breath holding). During deep dives, *S. lewini* exhibit intensive swimming activity which coupled with reduced respiration suggests a high capacity for anaerobic metabolism in the white "burst swimming" muscle of this species. Generally, elasmobranchs found in warm shallow water (above mesophotic depths) have higher metabolic rates and burst-locomotor capabilities in their white muscle than deep dwelling elasmobranchs (Treberg et al. 2003; Condon et al. 2012). Endothermic species (lamnids) have significantly higher capacities for both burst swimming (through glycolytic metabolism) and aerobic metabolism in their white muscle compared to ectotherms (Dickson et al. 1988; 1995; 1996; 2004; Bernal et al. 2003; Bernal 2005). The physiological advantages conferred by maintaining a warm body temperature at depth would enable scalloped hammerhead sharks to exploit warm tropical surface waters and cold mesopelagic depths (Bernal et al. 2001b). Maintaining a warm body while diving into cold depths should give scalloped hammerhead sharks performance advantages similar to those described in "high performance" endothermic fishes (Graham & Dickson 2001; Dickson & Graham 2004). These include faster swimming capability due to enhanced muscle power output and enzyme performance (Carey & Teal 1969, Carey et al. 1971; Brill 1996) and enhanced cardiac performance, visual acuity and neural processing (Carey & Teal 1966; Linthicum & Carey 1972; Block & Carey 1985). Tissue capacity for aerobic and anaerobic metabolism can be estimated by measuring the maximal activity levels of key enzymes involved in the production of ATP. Assays of the enzymes citrate synthase (CS) and malate dehydrogenase (MDH) have been widely used as indicators of aerobic capacity (Childress & Somero 1979; Bernal et al 2003; Chippari-Gomes 2005; Saavedra et al 2016; Condon et al. 2012). Assays of the enzymes pyruvate kinase (PK) and lactate dehydrogenase (LDH) have been used as indicators of anaerobic capacity (Childress and Somero 1979; Suarez et al 1986; Williams et al. 1997; Bernal et al 2003; Condon et al. 2012). Measurements of the maximal activity levels of all four of these enzymes have been widely used in previous studies to assess the aerobic and anaerobic capacity of muscle tissues (Childress & Somero 1979; Suarez et al. 1986; Dickson et al. 1988; Ballantyne 1997; Seibel et al. 1997; Panepucci et al. 2000; Bernal et al. 2003; Treberg et

al. 2003; Drazen and Seibel 2007; Seibel and Drazen 2007; Condon et al. 2012). These physiological adaptations should include enzyme characteristics that facilitate anaerobic metabolism during deep dives and the necessary aerobic metabolism to allow for rapid recovery (i.e. the breakdown of anaerobic end products) in well-oxygenated surface waters during intervals between dives. To test this hypothesis, the maximal activity rates of key enzymes citrate synthase (CS), malate dehydrogenase (MDH), pyruvate kinase (PK), and lactate dehydrogenase (LDH) were measured to examine the aerobic and anaerobic poise of the white muscle of adult scalloped hammerhead sharks. Activities levels of these enzymes were compared to those measured in other coastal tropical/temperate, deep water, and endothermic shark species.

Here I used a novel suite of tagging technology to overcome the logistical challenges of studying the thermal physiology and behavior of these two relatively understudied shark species that utilize deep-sea environments. The follow-up analysis of the aerobic and anaerobic poise of the swimming muscle of the scalloped hammerhead shark gains a further understanding of physiological requirements to conduct deep foraging dives. I hope that these findings can ultimately be used to reveal the proximate constraints that limit the distribution of these species in the face of a changing ocean environment.

CHAPTER II
THERMAL INERTIA OF A LARGE-BODIED DEEPWATER SHARK, THE BLUNTNOSE SIXGILL

ABSTRACT

The bluntnose sixgill shark (*Hexanchus griseus*), is a large bodied shark that inhabits cold waters around the world, and deep waters (> 200m) in tropical regions. Adult *H. griseus* in Hawaii conduct diel vertical migrations, swimming at depths around 500 - 650m with temperatures between 4-6˚C during the day and migrating to depths of 200-300m with temperatures around 15˚C at night. It is hypothesized that the large body mass of adult *H. griseus* may provide sufficient thermal inertia to maintain optimal body temperatures and buffer against prolonged exposure to elevated temperatures. There are no records of smaller juvenile or neonatal *H. griseus* around Hawaii, possibly because they are unable to migrate across steep thermal gradients on a diel basis. To examine how the body temperature of adult *H. griseus* fluctuates between different thermal environments on a diel cycle, we equipped individuals with instrument packages capable of directly measuring depth, ambient water temperature, and core swimming muscle temperature. Our specific objectives were to: (1) determine how core body temperature varies as sixgill sharks move across steep thermal gradients, (2) evaluate whether sixgill sharks employ simple thermal inertia or active physiological mechanisms to regulate core body temperature, (3) model the influence of body size on thermal inertia, and (4) identify ecological constraints of body size on sixgill shark diel vertical migrations.

We obtained 20 total days of data from 5 free-swimming *H. griseus*. Core muscle temperature warmed and cooled at a constant rate, was generally warmer than the ambient water during deep daytime phases, and cooler than ambient during shallower night phases, in a pattern consistent with simple thermal inertia. We modeled hypothetical body temperatures of each observed shark at three smaller body sizes with hypothetical precadual lengths of 100, 150 and 200cm. Our data indicates the core muscle of smaller juvenile sixgill sharks would experience greater temperature ranges

and more rapid approaches to ambient temperature after vertical movements compared with adult sharks.

INTRODUCTION

Some marine ectotherms move between thermal environments to take advantage of foraging habitats and select optimal ambient temperatures for bioenergetic efficiency (Sims et al. 2006; Di Santo & Bennet 2011). The rate of heat exchange between the body and surrounding environment determines the capacity for an individual to occupy habitats beyond their thermal optimum. Thermal conductance determines the rate of heat exchange between the body and the environment (Neil et al. 1974). Thermal inertia is the capacity of an animal to passively resist change in body temperature in the face of a changing external (ambient) environment. The mass of large body sizes provides an advantage in conserving heat and reducing the rate of heat exchange with the environment (Schmidt-Nielsen 1984). Large body sizes allow animals such as whale sharks, leatherback sea turtles (and possibly dinosaurs) to exploit a diverse range of thermal habitats (Paladino et al. 1990; Meekan et al. 2015). The whole-body heat transfer coefficient determines the rate of heat exchange between organism and environment, where a high coefficient indicates a higher rate of exchange between the body and the environment (Stevens & Sutterlin 1976). A thermal coefficient analysis can be used to discern passive (thermal inertia) from active thermoregulation in fishes and determine whole-body heat transfer coefficients (Holland et al. 1992; Holland & Sibert 1994).

The bluntnose sixgill shark (*Hexanchus griseus*) is a large-bodied predatory shark that moves between different thermal environemnts on a diel-cycle, and is therefore an excellent candidate for studying the capacity of thermal inertia attributed to large body size. Bluntnose sixgill sharks, hereafter referred to as sixgill sharks, are members of the most primitive group of extant sharks (Hexanchiformes) and important apex predators and scavengers of global deep-water ecosystems (Barnett et al. 2012; Churchill et al. 2015; Bizzarro et al. 2017; Drazen & Sutton 2017). Sixgill sharks in Hawaii move across

steep thermal gradients during their routine diel vertical migrations, occupying depths of around 500-650m with temperatures around 4-6 ºC during the day and migrate to depths around 200-300m with temperatures around 15 ºC, sometimes reaching 17 ºC at night (Comfort & Weng 2015; Nakamura et al. 2015). Fishing efforts from historic shark control programs, recent tagging studies, and deep baited remote video surveys have only recorded large sixgill sharks around Hawaii. There are no records of juveniles or neonates from Hawaii (Wetherbee et al. 1994; Yeh & Drazen 2009; Comfort & Weng 2015; Nakamura et al. 2015). It is hypothesized that the large body sizes of adult sixgill sharks allows for sufficient thermal inertia to enable them to exploit different thermal habitats while maintaining their body temperatures within the thermal optimum or below critical temperatures (Sims et al. 2006; Papastamatiou et al. 2015). Analysis of muscle temperature and activity patterns of sixgill sharks revealed that their optimum body temperatures were between 4.4 and 6 ºC and with sub-optimal high temperature > 13 ºC, and a lower boundary of < 4 ºC (Coffey 2019). These are the definitions of optimal and suboptimal temperatures used in the analyses of the current study.

In this study we examined the thermal inertia capacity of sixgill sharks. Our specific objectives were to: (1) determine how core body temperatures varies as sixgill sharks move across steep thermal gradients, (2) evaluate whether sixgill sharks employ active physiological mechanisms to regulate core body temperature, (3) model the influence of body size on thermal inertia in sixgill sharks, and (4) identify possible ecological constraints of body size on sixgill shark diel vertical migrations.

MATERIALS AND METHODS

Measuring Body Temperature During Diel Vertical Migration

To determine how core body temperatures varies as sixgill sharks move across steep thermal gradients, individual sharks were equipped with biologging packages capable of directly measuring depth, ambient water temperature and core muscle temperature.

Depth, water temperature and body temperature were sampled every 10 seconds using a TDR-Mk9 archival tag (71 × 17 × 17 mm, 120 mm stalk length, 29 g; Wildlife Computers, Redmond, WA, USA). Swimming behavior and environmental dissolved oxygen were also examined using these biologging packages using a TDR10-X accelerometer (57 × 38 × 24 mm, 69 g; Wildlife Computers, Redmond, WA, USA) and a prototype dissolved oxygen pop-up satellite archival tag (DO-PAT: 170 × 60 mm, 85 g; Wildlife Computers, Redmond, WA, USA) or a prototype TDR10-DO tag (109 × 53 × 21 mm, 118g; Wildlife Computers, Redmond, WA, USA). Analyses of data regarding oxygen-related phenomena are reported in Coffey et al. (2019). All of these components were housed in a syntactic foam float (2,000 m maximum depth rating) equipped with a timed release mechanism. Each package also contained either a miniPAT (115 × 40 mm, 53 g), SPOT6 (80 x 20 x 11mm, 30g) or SPOT-216E (80 × 20 × 11 mm, 30 g) to indicate the package surface position following release from the tagged animal.

All sharks were caught using baited hooks on demersal longlines deployed in a submarine canyon outside of Kāneʻohe Bay (21.46°N, 157.80°W) on the island of Oahu (Hawaiʻi, USA). Longlines were set between 250m and 350m depth just before sunset and hauled after sunrise the next day. Captured sharks were brought along the side of a 21ft skiff and secured with a rope around the caudal peduncle. The stalk of the TDR-MK9 archival tag was inserted approximately 8cm into the dorsal musculature of the shark. The tag package was secured to the shark in parallel to the longitudinal axis by piercing a hole through the central dorsal musculature using a hollowed probe which allowed for a fusible stainless steel cable tie (360 mm, 8 g; Little Leonardo Co., Tokyo, Japan) to pass through the piercing and be secured around the syntactic foam float package. Each package contained a timed-release mechanism with a pre-programmed duration of 96 hours (RT-4, 16mm diameter x 19mm length 10g; Little Leonardo Co., Tokyo, Japan). When the countdown timer reached zero, a fusible capsule severed the stainless steel band allowing the package to detach from the shark and float to the surface. Package location and recovery was accomplished through initial position estimates from Argos satellite transmissions followed by use of hand held directional

radio receiver tuned to the Argos and VHF transmitter frequencies. These were used to guide a chase boat to the floating package. Contact information was also displayed on the packages in case they were found by members of the general public.

Tagging procedures were approved by the Institutional Animal Care and Use Committee of the University of Hawaii (Protocol #05-053).

Thermal Modeling

To evaluate whether sixgill sharks employ active physiological mechanisms or rely on simple thermal inertia to regulate core body temperature, whole-body thermal conductivity (k) was modelled to match the observed rates of body warming and cooling. The rate of temperature change is a function of heat exchange with the environment and internal (metabolic) heat production (Holland et al. 1992; Holland & Sibert 1994). Heat exchange is proportional to the difference between the intramuscular temperature and ambient water temperature:

Eq. 1

$$\frac{\mathrm{d}T_b(t)}{\mathrm{d}t} = k\big(T_a(t) - T_b(t)\big) + \dot{T}_o$$

where k is the whole-body heat transfer coefficient (ºC min^{-1} ºC^{-1}), $T_a(t)$ is the ambient water temperature (ºC) as a function of time t, $T_b(t)$ is the core muscle temperature (ºC) as a function of time t, and T_o rate of temperature change due to metabolic heat production (ºC min^{-1}) from the swimming muscles. The following two conditions of k were assumed:

(1) k = a constant value;

(2) $k = \begin{cases} k_1; Ta(t) < T_b(t) \\ k_2; Ta(t) \geq T_b(t) \end{cases}$,

where k_1 and k_2 are two values for the whole-body thermal conductivity coefficient. Model (1) assumes the rate of heat exchange between the shark and the environment is constant and not altered by the shark. Model (2) assumes the rate of heat exchange is different when the shark is cooling (k_1) compared to warming (k_2). The optimized parameters for each model were estimated based on minimization of the sum of squared errors (SSE).

Effect of Body Size on Thermal Inertia

To model the influence of body size on thermal inertia in sixgill sharks, a power regression was calculated using the optimized constant k values from model (1) from each shark in relation to their estimated body mass (Fig. 2.2). Body mass estimates were calculated using the relationship between body mass and total length of bluntnose sixgill sharks from Ebert (1986).

Constraints of Body Size on Thermal Inertia

To identify likely ecological constraints of body size on sixgill shark diel vertical migrations, the proportion of time spent at optimal and sub-optimal temperatures for the sharks observed in this study was used to predict the thermal and vertical preferenda for the smaller hypothetical sharks. The fitted power regression was used to calculate the whole-body thermal conductivity (k) for hypothetical sixgill sharks at body sizes with 100cm, 150cm, and 200cm precaudal lengths (body mass of 21.7kg, 56.5kg, 121.6kg respectively) (Table 4). These individual hypothetical sharks will be referred to as PCL100, PCL150, PCL200 respectively. A rate of temperature change due to metabolic heat production $T_o = 0.0023$ (°C min^{-1}) was assumed for each modeled shark, based on the average T_o from all five measured sixgill sharks (HG1 – HG5). A rearrangement of the heat exchange equation (Eq. 2.1) was used to calculate the body temperatures of the hypothetical sixgill sharks from the observed ambient water temperature of the five observed sharks:

Eq. 2.2

$$T_{b(t)} = k\left(T_o - T_a - \left(\frac{T_o}{k}\right)\right)e^{-kt} + T_a + \frac{T_o}{k}$$

The observed and modeled body temperatures were subsampled into 0.5 ºC bins and normalized to a probability density. Optimal (5.5 – 7 ºC) and suboptimal (>13 ºC) body temperatures are based on the thermal performance curve from Coffey (2019) that was generated using the body temperature and accelerometry-derived ODBA (overall dynamic body acceleration) telemetry data from the sharks in this study.

RESULTS

Diel Movements and Temperature

All five sixgill sharks undertook diel vertical migrations, occupying daytime depths between 500-650m (80%) at relatively stable temperatures between 5-7 °C (85%). At night they migrated to shallower depths between 200 – 350m (78%) and a broader temperature range 10-16 ºC (79%) (Table 2.1, Fig. 2.1). Results for the depth and ambient temperatures experienced by the sharks are reported in detail Coffey et al. (2020). Percentages (Table 2.5) refer to the duration of time spent within the temperature or depth ranges both during the day and the night (analyzed by Coffey 2019).

All five sixgill sharks experienced considerable changes in body temperature on a diel basis, with an average change of 7.82 °C. Core muscle temperature ranges (5.8-14.9 °C) were narrower than the range of ambient water temperatures (4.7-17.3 °C) (Table 2.1). The thermal inertia provided by the large body mass slowed the rates of heating and cooling across the shifts in thermal habitats to a rate where the core muscle temperature

was synchronized with the timing of the diel migrations between the cold deep and warm shallow habitats (Fig. 2.1).

Thermal Conductivity Analysis

The predicted intramuscular temperatures from the constant *k* model (1) and variable *k* model (2) were very similar to the observed intramuscular temperature for each shark (Fig. 2.1). The SSE for the variable *k* models (2) were lower than the constant *k* models (1) except for HG5. The large confidence intervals for the optimized values of k_1 in the variable *k* models (2) suggest these models overfit the data (Table 2.2). The predicted intramuscular temperatures from the two models (constant versus variable) were not significantly different (t-test; $P > 0.05$ for all individuals). Linear regressions of observed intramuscular temperatures versus predicted intramuscular temperatures from the constant *k* (1) models explained ≥99% of the variation for all individuals (slopes 0.99-1.03, y-intercepts -0.21 to 0.10). The constant *k* (1) model is selected as the most parsimonious explanation for whole-body thermal conductivity, indicating that sixgill sharks utilize simple passive thermal inertia.

Thermal Modeling of Body Sizes

The power regression calculated from the optimized constant thermal coefficient *k* values derived from model (1) in relation to estimated body mass (*m*) indicates a lower *k* value with increasing body mass (Fig. 2.3).

Eq. 2.3

$$k = 0.1512m^{-0.569}$$

The rate of heat exchange between the intramusculature and the ambient environment decreases at an exponential rate as body mass increases.

Size Constraints of Thermal Inertia

Hypothetical sixgill sharks with precaudal lengths of 100cm, 150cm, and 200cm would have *k* values of 0.0262, 0.0152, 0.0098 °C min^{-1} $°C^{-1}$ respectively (Table 2.4). The modeled body temperatures of the smaller hypothetical sharks had a larger range than the observed sharks, nearly equal to the range of ambient water temperature experienced (100PCL: 5.0 – 16.0 °C, 150PCL: 5.1 – 15.6 °C, 200PCL: 5.4 – 15.3 °C).

The hypothetical sixgill sharks came to equilibrium with the ambient water following crepuscular vertical migrations and experienced greater fluctuations in body temperature during the higher rates of nighttime vertical displacement of the type observed in the tracked sharks (Fig. 2.3). The mean time at temperature (TAT) for the PCL100 sharks was less than the mean TAT of the observed sharks for the optimal temperatures and greater for the critical temperatures (Fig. 2.3, 2.4). The mean TAT for the PCL150 sharks was equal to the mean TAT of the observed sharks for the optimal temperatures and greater for the critical temperatures. The TAT for the PCL200 sharks were greater than the mean TAT of the observed sharks for the optimal temperatures and greater for the critical temperatures (Table 2.5). The mean TAT for the optimal and critical temperatures of the observed sharks were not significantly different than the means from the hypothetical sharks (all $P > 0.05$).

DISCUSSION

The combination of biologging technologies used in this study has revealed fine-scaled behavior and physiology of this large bodied deepwater shark. The diel vertical migrations exhibited by the sharks in this study are consistent with the behavior observed in previous studies of sixgills around Hawaii (Comfort & Weng 2015; Nakamura et al. 2015). The sixgill sharks in this study conducted distinct diel vertical migrations, occupying depths of around 500-650m with temperatures around 4-6 ºC during the day and depths around 200-300m with temperatures around 15 ºC at night. The depth range

occupied by sixgill sharks coincides with a temperature profile that changes quite rapidly (Supp. 1). Sixgill shark core muscle temperature ranges (5.8-14.9 °C) were narrower than the range of ambient water temperatures that they experienced (4.7-17.3 °C). Heat-transfer coefficient analysis revealed a constant rate of heat exchange between the core muscle and ambient water temperature, indicating sixgill sharks rely on passive thermoregulation when moving between the two different thermal environments. The large body masses of adult sixgill sharks provide sufficient thermal inertia to slow the rate of heat exchange with the environment. Thermal inertia allows adult sixgill shark core muscle temperatures to remain warmer than the ambient deep water during daytime phases when the sharks are more active, and cooler than ambient water during night phases where shallower depths approach critical ambient temperatures.

Comfort & Weng (2015) inferred sixgill sharks in Hawaii are more active at night due to the higher rates of vertical displacement during their nighttime phase in shallower, warmer water. In contrast, the tri-axial accelerometer data from sharks in this study (reported in Coffey et al 2020) revealed greater activity during the deeper, colder daytime phases compared to the nighttime phases. These differences in activity rates were consistent with temperature differences between the core swimming muscles and ambient water. Similar to the sixgill sharks reported in Nakamura et al .(2015), the sharks in this study exhibited greater activity rates with relatively high tailbeat amplitudes during their dawn descents when their body temperatures were at their warmest, and passive gliding with little tailbeat amplitude during their nighttime ascents when their body temperatures were at their coolest. The simple thermal inertia provided by a large body size may provide an ecological advantage similar to that of active physiological thermoregulation strategies employed by some elasmobranchs and teleosts. These strategies allow species to make excursions from warm surface waters into deeper cooler waters to exploit food resources found at deeper depths. Such physiological mechanisms include whole-body endothermy (Holland et al. 1992; Weng et al. 2005; Bernal 2005; Madigan et al. 2015; Watanabe et al. 2015), regional endothermy such as eye or brain heaters (Weng & Block 2004; Thorrold et al. 2014) or changes in bloodflow rates (Carey & Gibson 1987;

Nakamura & Sato 2015; Stoehr et al. 2018). Active foraging with an elevated body temperature relative to the environment provides an advantage over smaller, non-migrating prey with body temperatures equivalent to the cooler ambient temperature (Madigan 2015). Most endothermic species such as lamnid sharks and tuna exhibit high metabolic rates that serve to generate enough heat to aid in temperature conservation (Watanabe 2019). Ectothermic species (such as blue sharks and *Mola mola*) must conduct frequent ascents to the surface to rewarm in order to forage in deep cold depths (Carey & Sharrold 1990; Nakamura & Sato 2015).

In contrast to the comparatively brief deep water excursions of these ectothermic species, sixgill sharks remained at depth during the daytime and exposed to cold temperatures (~5 ºC) while maintaining a constant rate of heat transfer. The comparatively low rates of body movement, slow swimming speed (Nakamura et al. 2015), low levels of aerobic and anaerobic enzyme activities in white swimming muscle (Garcia 2013), indicate that sixgill sharks have low metabolic rates compared to shallower species and low rates of metabolic heat production (Meekan et al. 2015). It is possible the thermal inertia provided by a large body size allows sixgill sharks to forage at colder depths for sparsely distributed prey while remaining relatively active and maintaining swimming speeds with low metabolic costs (Yeh & Drazen 2009; Drazen & Sutton 2017). Passive thermal inertia also dampened muscle warming when the sharks climbed into warmer water at night and this may be a mechanism for allowing muscle activity and other physiological functions to continue at optimal body temperature (Angilletta et al. 2002; Van den Burg et al. 2005).

Though the large bodies of sixgill sharks in this study allowed for sufficient thermal inertia to maintain elevated muscle temperatures, small-bodied sixgill sharks might not have adequate thermal inertia to function outside of optimal ambient temperatures. The core muscle temperatures of the smaller hypothetical sharks experienced greater ranges and rapidly approached equilibrium with the ambient temperature. Although muscle temperature change was more rapid and more variable, no statistically significant difference was observed in the time spent at optimal and sub-optimal muscle temperature

of the hypothetical smaller sharks. Generally, thermal performance declines rapidly as body temperature approaches the critical temperature. The slight increases in the mean proportion of time spent at higher, sub-optimal temperatures for the smaller sixgill sharks could have detrimental effects on their performance (Huey & Stevenson 1979; Huey & Kingsolver 1989; Angilletta et al. 2002).

No neonate or juvenile sixgill sharks have been observed in Hawaii. It is possible the thermal challenges of diel vertical foraging migrations prohibit smaller sixgills from occupying habitats around Hawaii. Water temperature data for juvenile sixgill sharks have been gathered in the eastern Atlantic off the continental shelf the Cantabrian Sea (Rodríguez-Cabello et al. 2018) and inside large estuaries (Puget Sound and Georgia Straight) of the eastern North Pacific (Andrews et al. 2009; King & Surry 2017). The single juvenile sixgill (121cm total length) in the Cantabrian Sea stayed within a narrow temperature range (8.0 – 10.8 ºC) for the entirety of its deployment (135 days) (Rodríguez-Cabello et al. 2018). The tagged juvenile sixgill sharks in Puget Sound exhibited diel vertical migrations but only experienced a 1 ºC change in water temperature, suggesting this behavior is driven by foraging behavior (prey availability) and not thermoregulation (Andrews 2009). All of the sharks observed by Andrews et al. (2009) are within the size ranges of the hypothetical sharks modeled in this study. It is hypothesized that sixgill sharks occupy deeper habitats as they mature (Ebert 2002; Andrews et al. 2010). This ontogenetic shift in habitat could be attributed to the increased capacity for thermal inertia in larger individuals, allowing them to exploit deeper habitats (Nakamura & Sato 2015; Nakamura et al. 2015). It is possible that the adult sixgill sharks in Hawaii originate from coastal estuaries and continental shelves where they occupy habitats within a narrow temperature range and later migrate to tropical oceanic islands when they are large enough to move between thermal habitats with sufficient thermal inertia as revealed by the current research.

CONCLUSIONS

This is the first study to measure the body temperature of a free-swimming deepwater shark *in situ*. The large body masses of adult bluntnose sixgill sharks allow for sufficient thermal inertia to allow them to exploit thermally disparate habitats. Juvenile sixgill sharks might not be able to exploit the same habitats as adults due to their reduced capacity for thermal inertia. Further studies on assessing the Q_{10} values of juvenile sixgill sharks may reveal their physiological sensitivity to temperature shifts. Long-term tracking of habitat use of juvenile sixgill sharks through ontogeny might validate shifts in thermal habitat use, and potential movements to regions with thermal profiles that are only suitable for mature individuals.

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TABLES

Table 2.1. Deployment summary of the tagged bluntnose sixgill shark (*Hexanchus griseus*). Depths and ambient water temperature recordings during crepuscular migration phases were omitted. Body temperature range refers to the maximum and minimum body temperatures experienced.

Shark ID	Sex	Total Length (cm)	Deployment Duration (h)	Depth (m)			Ambient Water Temperature (°C)			Body Temperature (°C)
				Day Median (IQR)	Night Median (IQR)	Range	Day Median (IQR)	Night Median (IQR)	Range	Range
HG1	M	352	112	588 (528-616)	267 (249-284)	180-684	6.2 (5.9-6.6)	13.4 (12.5-14.2)	4.9-16.0	6.4 – 13.8
HG2	F	412	110	580 (561-604)	350 (296-456)	228-667	6.2 (6.0-6.4)	10.4 (7.6-12.7)	5.3-15.6	6.3 – 12.1
HG3	M	326	151	614 (593-634)	277 (253-313)	182-693	5.7 (5.5-6.0)	13.6 (12.2-14.5)	5.1-17.3	5.8 – 14.9
HG4	F	309	112	566 (535-619)	284 (241-314)	185-699	5.8 (5.5-6.1)	11.9 (10.5-14.2)	4.9-17.7	5.9 – 13.9
HG5	F	346	96	545 (508-588)	296 (274-382)	231-816	6.8 (6.3-7.3)	13.4 (9.5-14.5)	4.7-16.0	5.8 – 14.6

Table 2.2. Results from whole-body heat transfer coefficient models for bluntnose sixgill shark (*Hexanchus griseus*). Parameter estimates include ± 95% confidence intervals. T_b, intramuscular temperature; k, whole-body heat transfer coefficient; T_o, rate of temperature change due to internal (metabolic) heat production; SSE, sum of squared errors

Shark ID	T_b Range (°C)	Model	k (°C min^{-1} °C^{-1})	k_1 (°C min^{-1} °C^{-1})	k_2 (°C min^{-1} °C^{-1})	T_o (°C min^{-1})	SSE
HG1	6.4-13.9	(1)	0.00546 ± 0.00009			0.00270 ± 0.00003	6025.6
		(2)		0.07264 ± 0.01085	0.00791 ± 0.00006	0.00288 ± 0.00002	5513
HG2	6.3-12.1	(1)	0.00531 ± 0.00004			0.00133 ± 0.00001	409.3
		(2)		0.11076 ± 0.04178	0.00694 ± 0.00007	0.00152 ± 0.00002	271.8
HG3	5.8-14.9	(1)	0.00759 ± 0.00006			0.00274 ± 0.00002	1679.7
		(2)		0.014133 ± 0.00054	0.00451 ± 0.00003	0.00133 ± 0.00008	1472.5
HG4	5.9-13.9	(1)	0.00896 ± 0.00008			0.00245 ± 0.00003	844.9
		(2)		0.04497 ± 0.00655	0.00881 ± 0.00007	0.00243 ± 0.00002	696.5
HG5	5.8-14.6	(1)	0.00691 ± 0.00007			0.00189 ± 0.00002	1006.7
		(2)		0.03591 ± 0.01146	0.00541 ± 0.00009	0.00271 ± 0.00003	1142.9

Table 2.3 Summary of sharks used to generate bodymass vs. thermal coefficient regressions. *k*, whole-body heat transfer coefficient; T_o, rate of temperature change due to internal (metabolic) heat production.

Shark ID	Species	PCL (cm)	TL (cm)	Body mass (kg)	T_o (°C min^{-1})	k (°C min^{-1} °C^{-1})
HG1	*H. griseus*	255	352	247	0.0027	0.0055
HG2	*H. griseus*	295	412	408	0.0013	0.0053
HG3	*H. griseus*	235	326	195	0.0027	0.0076
HG4	*H. griseus*	220	309	166	0.0024	0.0090
HG5	*H. griseus*	251	346	234	0.0019	0.0069

Table 2.4 Summary of the body mass and whole-body heat transfer coefficient of smaller hypothetical bluntnose sixgill shark (*Hexanchus griseus*). Model *k* assumes T_o = 0.0026. PCL, precaudal length; TL, total length; *k*, whole-body heat transfer coefficient

PCL (cm)	TL (cm)	Body mass (kg)	k (°C min^{-1} °C^{-1})
100	139.4	21.7	0.0262
150	208.7	56.5	0.0152
200	278.0	121.6	0.0098

Table 2.5. Summary of proportion of time spent at optimal and near-critical temperatures for the observed bluntnose sixgill sharks (*Hexanchus griseus*) and modeled temperatures at smaller sizes. TAT, time at temperature; Opt., optimal temperature; Sub., sub-optimal temperature.

	Observed body temp		**at 100cm PCL**		**at 150cm PCL**		**at 200cm PCL**	
Shark ID	**TAT Opt.**	**TAT Sub.**	**TAT Opt.**	**TAT Sub.**	**TAT Opt.**	**TAT Sub.**	**TAT Opt.**	**TAT Sub.**
HG1	0.20	0.07	0.29	0.13	0.31	0.12	0.31	0.09
HG2	0.53	0.00	0.46	0.01	0.50	0.00	0.51	0.00
HG3	0.24	0.17	0.12	0.23	0.14	0.22	0.26	0.21
HG4	0.26	0.05	0.21	0.13	0.23	0.08	0.33	0.05
HG5	0.17	0.26	0.27	0.25	0.20	0.26	0.14	0.26
Means ± SD	0.28 ± 0.14	0.11 ± 0.10	0.27 ± 0.13	0.15 ± 0.10	0.28 ± 0.14	0.14 ± 0.10	0.31 ± 0.13	0.12 ± 0.11

FIGURES

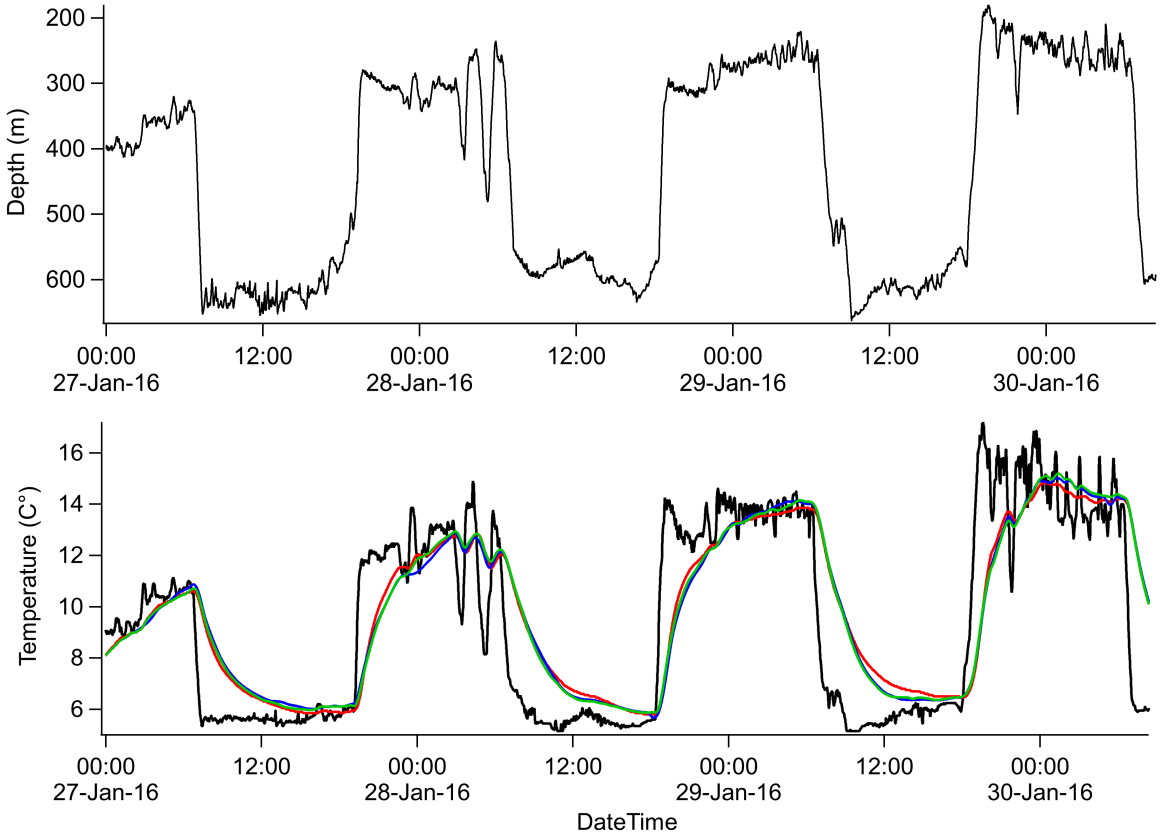


Figure 2.1. Time series of depth (top), ambient water temperature (below), and intramuscular temperature (colored lines) for bluntnose sixgill shark (*Hexanchus griseus*) individual HG3. Intramuscular temperature colors indicate observed temperatures (red line) and estimated temperatures derived from models using a constant (blue) and variable (green) whole-body heat-transfer coefficient.

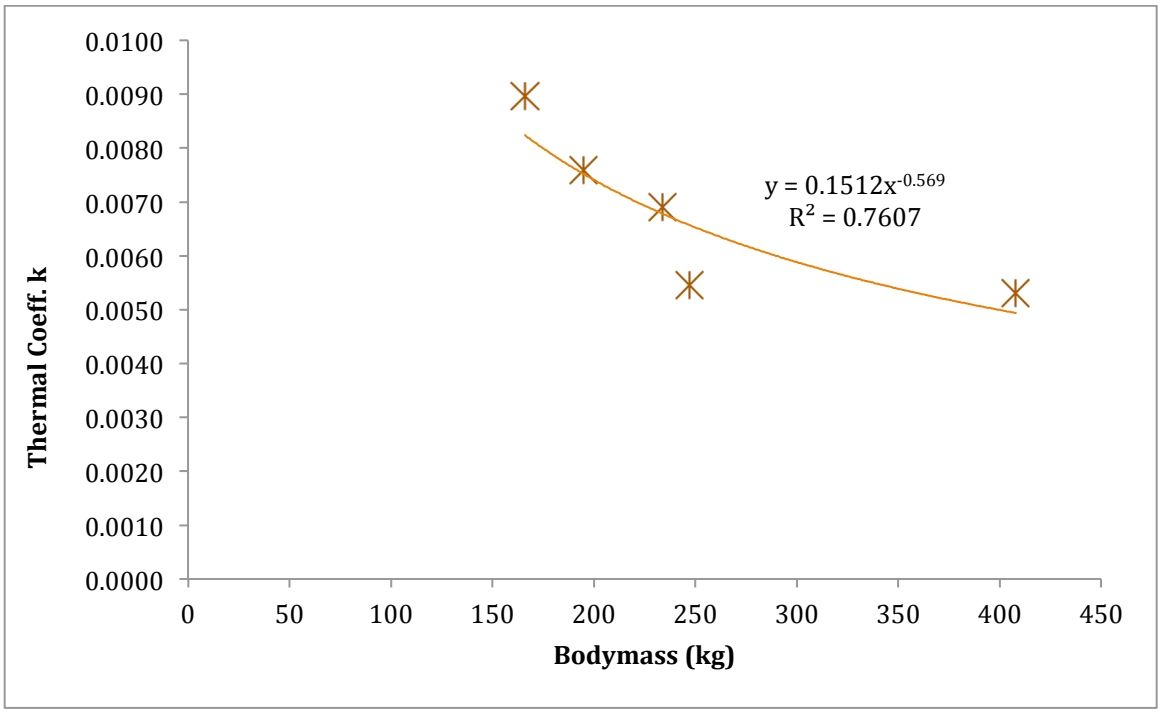
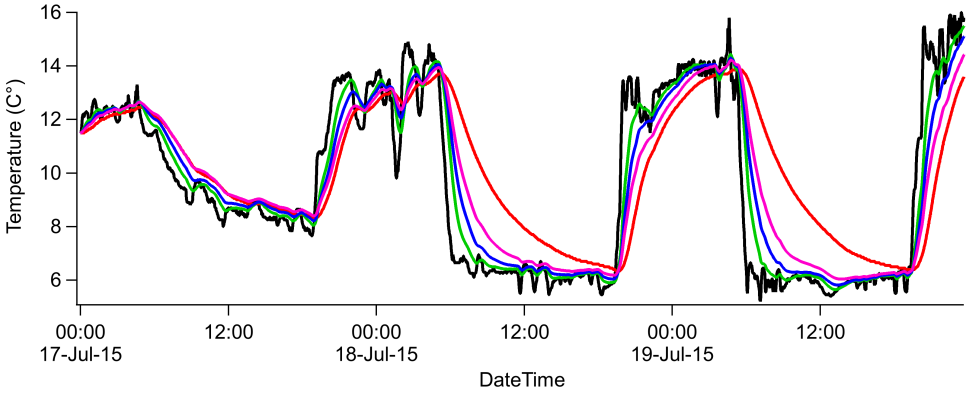
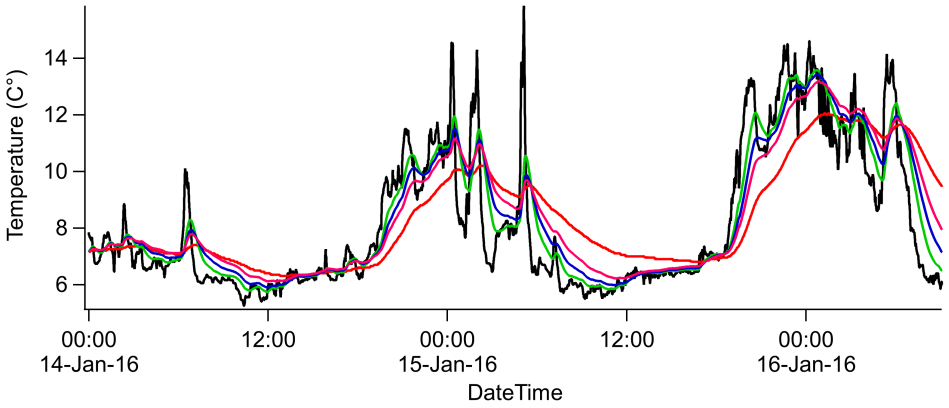


Figure 2.2. Regression for bodymass and thermal coefficient from the sixgill sharks.

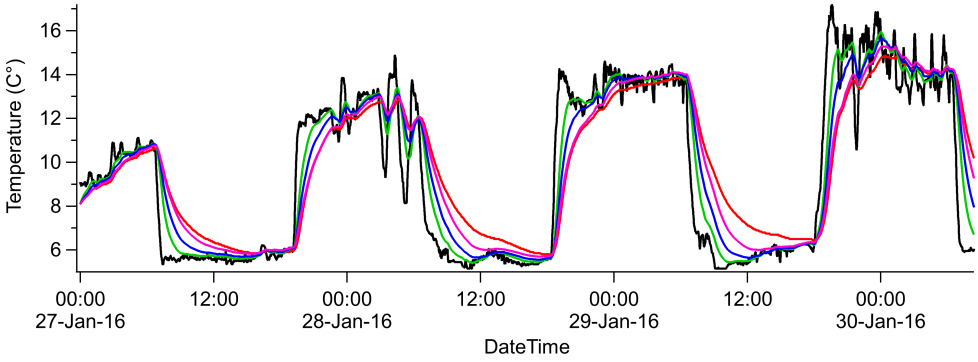
HG1


Temperature (C°)
16
14
12
10
8
6
00:00
17-Jul-15
12:00
00:00
18-Jul-15
12:00
00:00
19-Jul-15
12:00
DateTime

HG2


Temperature (C°)
14
12
10
8
6
00:00
14-Jan-16
12:00
00:00
15-Jan-16
12:00
00:00
16-Jan-16
DateTime

HG3


Temperature (C°)
16
14
12
10
8
6
00:00
27-Jan-16
12:00
00:00
28-Jan-16
12:00
00:00
29-Jan-16
12:00
00:00
30-Jan-16
DateTime

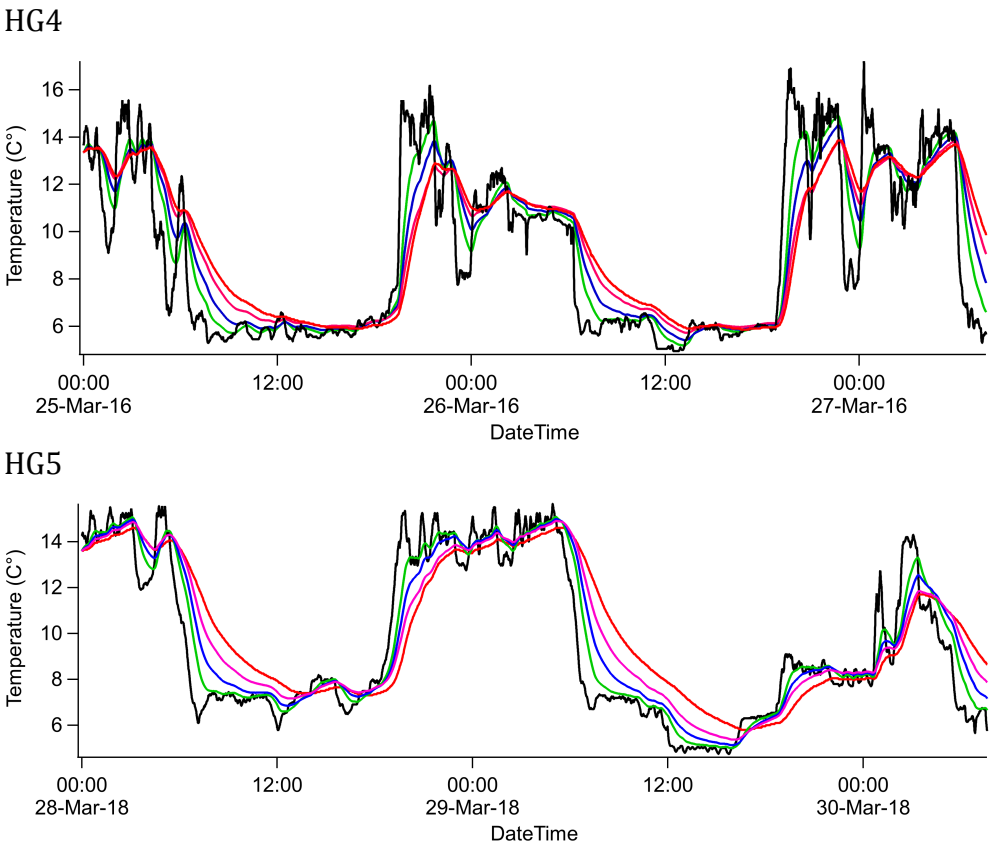


Figure 2.3. Time series of ambient water temperature (black), observed intramuscular temperature (red), and modeled intramuscular temperatures of hypothetical sharks at 100, 150 and 200cm PCL lengths (green, blue, pink respectively). HG1 (top) to HG5 (bottom).

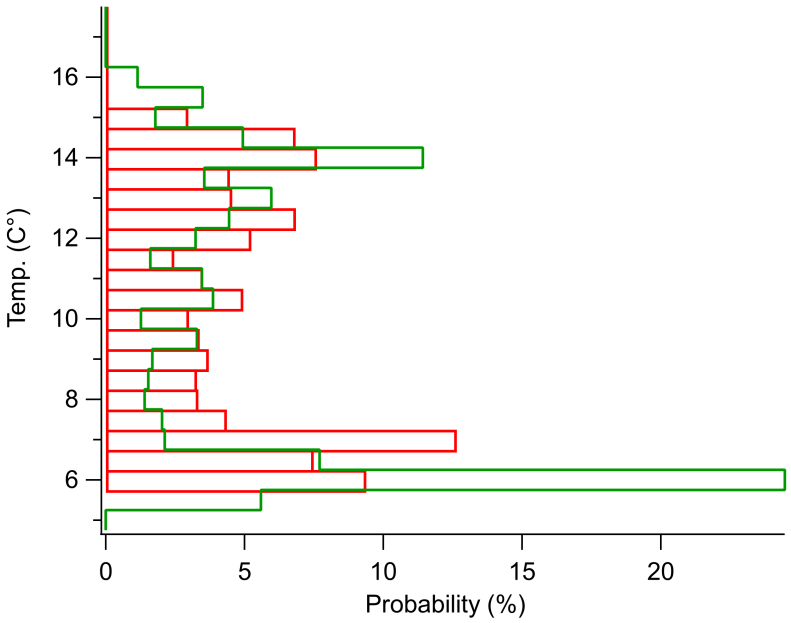


Figure 2.4. Histogram of proportion of time spent at body temperatures for bluntnose sixgill shark (*Hexanchus griseus*) individual HG3. Observed data from the actual shark is in red, the modeled body temperature at body size 100cm PCL in green.

CHAPTER III
"BREATH HOLDING" AS A THERMOREGULATION STRATEGY IN A DEEP-DIVING TROPICAL SHARK, THE SCALLOPED HAMMERHEAD

ABSTRACT

Adult scalloped hammerhead sharks (*Sphryna lewini*) utilize oceanic habitats around the Hawaiian Islands where at night they dive repeatedly to depths exceeding 800m and water temperatures as low as 4°C, presumably to forage on deep-dwelling prey. We hypothesized that *S. lewini* dive duration is limited by ambient water temperature at depth because body cooling associated with excursions into cold water can reduce muscle power output, cardiac function and visual acuity. To determine how *S. lewini* respond to cold ambient water temperatures experienced during deep dives, we equipped adult individuals with instrument packages capable of directly measuring depth, ambient water temperature, activity rates, orientation and intramuscular temperature. Our specific objectives were to determine (1) whether *S. lewini* maintain core body temperature during deep dives via simple thermal inertia or instead employ active, physiological thermoregulation and (2) whether swimming performance changes during deep, repetitive dives into cold water. We obtained 180 total days of data from 9 free-swimming adult *S. lewini* with individual deployment durations ranging from 7 to 23 days. Our data show 'warm' core muscle temperatures are maintained throughout the deepest portion of each dive and core muscle cooling only occurs during ascent to the surface. However, once initiated, this cooling is rapid. After sharks return to the warm surface layer, it takes 45 to 75 minutes for swimming muscles to fully equilibrate to ambient mixed layer water temperatures, but the shark will begin the next dive while the core temperature is still rising. The delayed onset and subsequent rapid rate of cooling followed by the slower rate of rewarming indicate evidence of physiological thermoregulation akin to a marine mammal-like dive reflex (i.e. breath holding and/or modulation of blood flow). Understanding how *S. lewini*, a warm-water species, are physiologically able to exploit

resources in deep, cold habitats provides important insights into the broader ecology of this regionally endangered shark.

INTRODUCTION

Scalloped hammerhead sharks (*Sphyrna lewini*) occupy warm surface waters in tropical environments but make repeated nocturnal dives to depths exceeding 800m where water temperatures are as low as 4°C (Jorgensen et al. 2009; Bessudo et al. 2011; Hoffmeyer et al. 2013; Spaet et al. 2017). The permanent inhabitants (e.g. fishes, cephalopods) of these deep, cold habitats move slowly compared to their surface counterparts, indicating that ease of prey capture may motivate the deep-diving behavior observed in scalloped hammerhead sharks (Childress et al. 1990; Somero 1992; Childress 1995; Seibel et al. 1997; Drazen and Seibel 2007). The high proportions of meso and bathypelagic species (fishes, cephalopods, crustaceans) in the stomachs of adult scalloped hammerhead sharks further support the notion that these deep dive events serve as foraging opportunities (Clarke 1971; Klimley & Nelson 1984; Stevens 1989, Smale & Cliff 1998; Vaske Júnior et al. 2009). However, deep-diving is also challenging because body cooling could reduce visual acuity, cardiac function and muscle power potentially leading to vulnerability for obligate ram ventilators such as scalloped hammerhead sharks (Bennet 1984; Block & Finnerty 1994; Brill 1998).

Fish exchange heat between their body and the environment via convective and conductive heat transfer. Convective heat transfer occurs at the site of respiratory gas exchange (the gills) where heat is exchanged between the blood and the ambient water through the thin gill lamellae. Conductive heat exchange occurs between the body wall and the ambient water (Neil & Stevens 1974; Fechhelm and Neill 1982; Brill et al. 1994; Dewar et al. 1994; Bernal et al. 2001; Wegner et al. 2015; Stoehr et al. 2018).

Lamnid sharks such as the great white (*Carcharodon carcharias*), shortfin mako (*Isurus oxyrynchus*), salmon (*Lamna ditropis*), and common thresher (family *Alopidae*, *Alopias*

vulpinus) and certain species of devil rays (family *mobulidae*) are regional endotherms with anatomical adaptions to retain metabolic heat produced by swimming muscles. These adaptations reduce heat loss and in some cases maintain steady-state elevated temperatures of swimming muscles and critical tissues (e.g., brain) as their bodies and blood at the gills are exposed to rapid drops in ambient temperature during repetitive deep dives below the thermocline (Carey and Teal 1969; Carey et al. 1985; Schweitzer & Notarbatolo-di-Sciara 1986; Brill & Bushnell 1991; Block & Finnerty 1994; Sepulveda et al. 2004; Weng et al. 2005; Patterson et al. 2011; Cartamil et al. 2011; Thorrold et al. 2014; Coffey et al. 2017; Skomal et al. 2017; Bernal et al. 2018). Most other sharks, including the scalloped hammerhead, are ectotherms that lack morphological and vascular adaptations to conserve body temperature as they move into cold environments.

So how do ectothermic species such as the scalloped hammerhead function in frigid water temperatures experienced during deep dives? It is possible that they are actually regional endotherms with as yet undiscovered physiological or anatomical adaptations for heat retention, or they may rely exclusively on thermal inertia to carry them through deep dives. In the latter case, the sheer physical size of the shark insulates the body core and provides a short window of opportunity for them to exploit deep, cold habitats before they must return to warm surface waters (Neil & Stevens 1974; Paladino et al. 1992; Kitagawa & Kimura 2006; Coffey et al. 2020). Another possibility is that scalloped hammerhead sharks are able to modulate heat exchange between their bodies and the environment during vertical movements (Holland and Sibert 1994). This may allow a faster rate of warming than cooling as observed in the ectothermic blue shark (*Prionace glauca* (Carey & Gibson 1987; Carey & Sharrold 1990)). This is achieved by modulating blood flow and convective heat transfer at the gills (Carey & Gibson 1987; Carey & Sharrold 1990; Kitagawa & Kimura 2006).

To address this question we equipped adult individuals with instrument packages capable of directly measuring depth, ambient water temperature, activity rates and swimming muscle temperature, to determine how scalloped hammerhead sharks respond to cold

ambient water temperatures during deep dives. Our specific objectives were to: (1) characterize the swimming performance during deep, repetitive dives into cold water and (2) determine whether scalloped hammerhead sharks maintain core body temperature during deep dives via simple thermal inertia, or instead employ active (possibly physiological) thermoregulation. Here we define physiological thermoregulation as being distinct from the animal simply employing habitat selection (preference) behaviors to achieve optimal body temperature (Neil 1979; Matern et al. 2000; Sims et al. 2006)

MATERIALS AND METHODS

Measuring swimming performance, environment, and body temperature

To measure scalloped hammerhead shark swimming performance, depth, ambient and muscle temperatures we used an instrument package consisting of a tri-axial accelerometer tag and a depth and temperature archival tag housed in a syntactic foam float (2,000 m depth rating) equipped with a timed release mechanism and telemetry tags to facilitate recovery. The tri-axial accelerometer tag was either a TDR10-XB-340 (56 x 38 x 24mm 69g; Wildlife Computers., Redmond, WA) or a TDR10-Daily Diary-278 (74 x 57 x 36mm, 117g; Wildlife Computers., Redmond, WA). Tri-axial acceleration was sampled at either 16Hz or 32Hz. Depth, water temperature and body temperature were sampled every 5 or 10 seconds using a MK9 archival tag with a 8cm thermistor stalk (Wildlife Computers, Redmond, WA). Each package also contained a SPOT5 or SPOT6 satellite-linked transmitter (80 x 20 x 11mm, 30g; Wildlife Computers., Redmond, WA) to indicate the packages position when it floated to the surface following release from the tagged animal. Packages that contained a SPOT5 tag were also equipped with a VHF transmitter (MM130B; 16mm diameter, 60mm length, 20g; ATS, USA). Two packages deployed on two separate sharks were also equipped with a Little Leonardo video logger (20 x 11 x 52mm, 16g; Little Leonardo Co., Tokyo, Japan).

Shark capture and handling

All sharks were caught using baited hooks on demersal longlines inside Kāneʻohe Bay (N 21.45°, W 157.80°), Oahu (Hawaiʻi, USA). To ensure captured sharks were in good condition, longlines were checked every 30 minutes. Captured sharks were brought along the side of a 17ft skiff, secured with a rope around the caudal peduncle and provided with constant gill ventilation during measurement and instrumentation via a hose inserted into the mouth and connected to an in-water bilge pump. The tag package was attached to each shark via a fusible stainless steel cable tie (360 mm, 8 g; Little Leonardo Co., Tokyo, Japan) passed through two holes drilled through the base of the dorsal fin and secured around the syntactic foam float. The sensor stalk of the MK9 was inserted approximately 8 cm below the skin into the dorsal musculature while the package was being secured to the dorsal fin. Each package contained a timed-release mechanism (RT-4, 16mm diameter x 19mm length 10g; RT-5, 20mm diameter x 38mm length, 20g; Little Leonardo Co., Tokyo, Japan) set to release after 7 (n=2), 21 (n=1), or 23 (n=8) days. At release time, an integrated capsule severed the stainless steel band, releasing the package to float to the surface. We used a small vessel and hand held receivers equipped with directional antennas to home in on and recover the floating package locator beacons. Contact information was written on the packages in case they were found by members of the general public. All shark capturing and handling procedures were approved by the ethics committee at the University of Hawaiʻi (Institutional Animal Care and Use Committee Protocol #05-053).

Accelerometer Data Processing

Archived data were downloaded from ten recovered tag packages and all 32Hz tri-axial acceleration data were resampled at 16Hz to facilitate analyses. Acceleration and depth data were analyzed using the 'Ethographer' package (Sakamoto et al. 2009) in Igor Pro 8 (WaveMetrics Inc., Portland, OR, USA). A low pass filter of 0.3 Hz was used to estimate the static (gravitational) and dynamic (tail stroking) components of the acceleration signal for each axis. The static acceleration components from the x,y,z axes were used to calculate the roll and pitch angles of the shark, where x is the surge axis, y is sway axis, and z is the heave axis (Collins et al. 2015):

Eq. 1

$$\text{Roll} = \arctan(y/x^2+z^2)^{1/2})(180/\pi)$$

Eq. 2

$$\text{Pitch} = \arctan(x/y^2+z^2)^{1/2})(180/\pi)$$

The tag attachment angle was corrected to zero degrees centered for roll and to horizontal for pitch by quantifying tag pitch during upright swimming at constant depth and subtracting this value from the archived data (Andrzejaczek et al. 2019; Kawatsu 2010). The Ethographer 'mask' function was used to separate daytime and nighttime values for each shark based on local sunrise and sunset times obtained from the Astronomical Applications Department of the U.S. Naval Observatory (http://aa.usno.navy.mil/). We calculated overall dynamic body acceleration (ODBA), tailbeat frequency, and tailbeat acceleration amplitude as proxies of energy expenditure. ODBA was calculated by summing the absolute values of the dynamic acceleration from all three orthogonal axes (Gleiss et al. 2011; Quasam et al. 2012). Tailbeat acceleration amplitude, hereafter referred as tailbeat amplitude, was the amplitude of the dynamic acceleration of the sway (y) axis measured by the 3D accelerometer on board the tag. We used continuous wavelet transformation to generate a spectrogram of the dynamic component of swaying acceleration, classified the dominant peak as tail beat cycles, and calculated tail beat frequency and amplitude of acceleration at every second.

Thermal conductivity coefficient analysis

To evaluate whether scalloped hammerhead sharks actively retain body heat or rely on simple thermal inertia during deep dives into cold water, predicted values of the whole-body heat transfer coefficient (k) and the rate of temperature change due to metabolic heat production (T_o) were modeled to match the observed rates of body warming and

cooling (*sensu* Holland et al. 1992; Holland & Sibert 1994). The whole-body heat transfer coefficient (k) accounts for both convective heat transfer (i.e., heat exchange between blood flowing through the gills and ambient seawater) and conductive heat exchange across the body wall (Neil et al. 1974; Fechhelm and Neill 1982; Brill et al. 1994; Dewar et al. 1994; Bernal et al. 2001; Pepino et al. 2015; Stoehr et al. 2018). Heat exchange is proportional to the difference between the intramuscular temperature and ambient water temperature:

Eq. 3

$$\frac{\mathrm{d}T_b(t)}{\mathrm{d}t} = k\big(T_a(t) - T_b(t)\big) + \dot{T}_o$$

where k is the whole-body thermal rate coefficient (ºC min^{-1} $ºC^{-1}$), $T_a(t)$ is the ambient water temperature (ºC) as a function of time t, $T_b(t)$ is the core muscle temperature (ºC) as a function of time t, and T_o is the rate of temperature change due to metabolic heat production (ºC min^{-1}) from the swimming muscles (Holland et al. 1992; Holland & Sibert 1994). The modeling used in Holland et al. 1992, Holland & Sibert 1994, Nakamura et al. 2015, and the previous chapter (Chapter 2) assumed the following conditions for k;

(a) k = a constant value;

(b) $k = \begin{cases} k_1; Ta(t) < T_b(t) \\ k_2; Ta(t) \geq T_b(t) \end{cases}$,

where k_1 and k_2 are two values for the whole-body heat transfer coefficient. Model (a) assumes the rate of heat exchange between the shark and the environment is constant and not altered by the shark. Model (b) assumes the rate of heat exchange is different when the shark is cooling (k_1) compared to warming (k_2). These conditions were not able to adequately model the body temperature of either of the sharks (all R^2 fits >0.60). A post-hoc analysis of swimming activity (ODBA, tailbeat frequency and amplitude) revealed high activity levels during each deep dive. Body temperature was observed to increase throughout the deepest portion of each dive and then followed by a sharp cooling rate

during ascent to the surface and subsequent decrease in swimming activity. These high activity rates will affect metabolic heat generation (T_o) and possibly rates of blood flow through the core muscles or through the gills. These phenomena would ultimately affect the rate of change of body temperature ($dT_b(t)/dt$) and whole-body thermal rate coefficient (k) (equation 3) (Brill et al. 1994; Stoehr et al. 2018). We therefore modeled predicted values of the whole-body heat transfer coefficient (k) and the rate of temperature change due to metabolic heat production (T_o) based on different conditions associated with the periods intense swimming activity during each dive. The following four conditions of k and T_o were assumed:

(1) k = *a constant value*, T_o = *a constant value;*

(2) k = *a constant value,* $T_o = \begin{cases} T_{o1}; normal\ swimming \\ T_{o2}; deep\ diving \end{cases}$;

(3) $k = \begin{cases} k_1; normal\ swimming \\ k_2; deep\ diving \end{cases}$, T_o = *a constant value;*

(4) $k = \begin{cases} k_1; normal\ swimming \\ k_2; deep\ diving \end{cases}$, $T_o = \begin{cases} T_{o1}; normal\ swimming \\ T_{o2}; deep\ diving \end{cases}$;

where k_1 and k_2 are two values for the whole-body heat transfer coefficient. Model (1) assumes the rate of heat exchange between the shark and the environment (k) and rate of temperature change due to metabolic heat production (T_o) is constant and not altered by the shark. Model (2) assumes rate of heat exchange between the shark and the environment (k) is constant while the rate of temperature change due to metabolic heat production (T_o) alters between (T_{o1}) during normal swimming and (T_{o2}) during the high activity phases of a deep dive. Model (3) assumes (k_1) when the shark is swimming normally and (k_2) during the high activity phases of a deep dive while the rate of temperature change due to metabolic heat production (T_o) is constant. Model (4) assumes (k_1) and (T_{o1}) when the shark is swimming normally and (k_2) and (T_{o2}) during the high

activity phases of a deep dive. The determination of switching (models 3 & 4) between (k_1) (normal swimming) and (k_2) (deep diving) was based on established thresholds of vertical velocity between the descent phase and ascent phase of each dive (Supp. 2). The same thresholds were used for the determination of switching (models 2 & 4) between (T_{o1}) (normal swimming) and (T_{o2}) (deep diving). The optimized parameters for each model were estimated based on coefficient of determination (R-squared) between the observed and predicted body temperature. Models were run for each night that consisted of more than one deep dive for each shark. Start and stop times for each model duration were arterially set to include all deep dives within a night duration and to include the durations where body-temperature reaches the ambient temperature after the conclusion of a series of deep dives.

Thermal conductivity of dead sharks

To account for the rate of temperature change in the absence of convective heat transfer (blood flow across the gills), the body temperatures of two dead adult male scalloped hammerhead sharks with total lengths 240cm and 273cm were recorded by individually placing the sharks in an insulated tub and inducing abrupt alternations of water temperature from 23 ºC to 6 ºC and vice versa (Carey & Gibson 1987; Nakamura et al. 2015). Core muscle and ambient water temperatures were measured using the same TDR-Mk9 archival tag used in field experiments. Constant heat-transfer coefficients *k* were calculated using the heat budget model with metabolic heat production (T_o) = 0 (Table 3.3).

RESULTS

Biologger deployments

We deployed biologging packages on 11 adult male scalloped hammerhead sharks ranging in size from 204cm to 270cm Total Length (TL, Table 3.1) of which 10 were

successfully recovered. Of the recovered tags; 8 recorded the full suite of tri-axial acceleration, body temperature, depth and water temperature profile data. One deployment recorded all parameters except body temperature (HH11), and 1 recorded all parameters except acceleration (HH2). In total, we obtained 196.7 total days (4720 hours) of combined accelerometer, depth, ambient temperature, and body temperature data with individual deployment durations ranging from 7 to 27 days (3.1). For recovered tag packages, horizontal distance from tagging location to pop-up point ranged from 8.1 to 51.1 kilometers, and all packages surfaced within 5 kilometers of the coast of Oahu. The package timer for HH11 was set for 7 days but it stayed on the animal for 29 days, most likely due to damage sustained to the wires connecting the fusible capsule to the timer. The package for HH3 was programmed for 21 days but was knocked off prematurely 14 days into the deployment. The package from HH11 failed to transmit any satellite or VHF positions due to damaged sustained during deployment but was discovered 248 kilometers away from the tagging location on the shore of the island of Ni‘ihau approximately 320 days after the expected pop-up time.

Deep diving behavior

Deep dives were defined as dives exceeding 400m with starting and ending depths <50m. A total of 103 deep dives were recorded from 7 out of the 10 scalloped hammerheads in this study. Average maximum depths of deep dives were 635.5m (± 91.8m) with a maximum depth of 825m (HH10). All deep dives were conducted at night. Four sharks (HH7, HH8, HH9, HH10) conducted repetitive deeps dives (more than one dive per night). For sharks HH7, HH8, and HH10, the number of deep dives per night increased through the deployments, with each shark conducting single dives each night for 3 to 6 nights, followed by 2 to 3 nights with 2 to 3 deep dives per evening, and eventually followed by nights with 5 to 8 consecutive deep dives each (see example of HH7, Fig. 3.1). This pattern of gradually increasing the number of deep dives per a night is similar to behavior previously observed from pop-of archival tag data of this species (Jorgensen et al. 2009; Bessudo et al. 2011; Hoffmeyer et al. 201; Spaet et al. 2017).

All deep dives conducted by sharks in this study consisted of 5 visually distinctive phases (Figure 3.5):

1) *Slow descent to pause at 100m*: Starting between the surface to 60m depth, the sharks would swim to 100m with a gradually decreasing pitch angle from 0 to -15 degrees with an average ODBA of 0.3 m/s^2, average tailbeat frequency of 3.2 seconds per cycle, and an average tailbeat amplitude of 0.08 m/s^2. This phase lasted 7 to 10 minutes. This is considered a reduced intensity of swimming activity in comparison to the normal (average) nighttime swimming behavior. Before transitioning to the next phase of the dive there was a brief 20 – 50 second "pause" period where the shark would orient to a level pitch angle and further reduce their swimming activity with ODBA decreasing to 0.2 m/s^2, tailbeat frequency slowing to 3.9 seconds per cycle and tailbeat amplitude reducing to 0.2 m/s^2.

2) *Fast descent to bottom*: After the pause period at around 100m depth the sharks would then abruptly orient at a steep pitch angle between 70 to 80 degrees and increase their swimming intensity with ODBA averaging at 1.14 m/s^2, tailbeat amplitudes increasing to 0.42 m/s^2 and an average tailbeat frequency of 3.43 seconds per cycle. At depths between 150m to 500m and while the sharks were at a steep dive angle, they would conduct 3 to 5 bursts of intense swimming activity that would last between 5 to 10 seconds, with ODBA and tailbeat amplitude reaching more than a whole orders of magnitude greater (at 19.6 m/s^2 and 4.75 m/s^2) respectively and with tailbeat frequencies at 0.6 seconds per cycle. As the sharks approached the last 100m to 150m of their dive (between 650m to 800m depth) they would increase the frequency of these bursts with an average of 4 bursts per minute.

3) *Bottom time*: The bottoms of the deep dives exhibited "V", "U", and "Uv" shaped depth profiles as seen in deep diving marine mammals such as seals (Le Boeuf 1992). Sharks would either hold a steady depth within a 20m range (V and U shaped dives) or

move between depths ranging greater than 100m (Uv shaped dives). Sharks exhibited intense swimming activity either in frequent bursts or sustained periods lasting during the entire duration of the bottom time with average ODBA values of 0.8 m/s^2 with bursts to 15 m/s^2 and increased tailbeat amplitudes of 0.4 m/s^2 with bursts up to 4 m/s^2. Tailbeat frequency was difficult to distinguish due to the erratic nature of the movement at the bottom as indicated by rapid fluctuations in pitch and roll angles. Bottom times ranged from 2 to 7 minutes from the moment a shark would level out from the dive to the start of the ascent.

4) *Fast ascent to inflection point*: Ascents began with a steep, abrupt change in pitch angle to 70 – 80 degrees and sudden increase in swimming intensity with tail beat amplitudes up to 3 m/s^2 at a frequency of 1.4 seconds per cycle and ODBA averaging 2.6 m/s^2 with peaks at 7.08 m/s^2. Sharks sustained this intense swimming effort and steep pitch angle for 7-8 minutes during their ascent until they reached depths of between 200 and 300m. At this point their dive profiles show an inflection point characterized by an abrupt change in ascent rate associated with a sudden change to a shallow pitch angle and reduction in swimming intensity (indicated by drops in ODBA, tailbeat frequency and amplitude).

5) *Slow ascent to surface*: After the inflection point at 250 – 300m depth in the ascent profile, the sharks would continue their ascent at a slower rate to a depth <50m over a period of about 16 minutes. Swimming activity was greatly reduced in comparison to the fast ascent phase, with ODBA averaging 1.2 m/s^2, tailbeat frequency at 2.21 seconds per cycle and an amplitude of 0.26 m/s^2.

Surface intervals were defined as the time between deep dives when sharks would level out their pitch angle within the top 50m of the water column after completing a deep dive and the time to the initiation of the slow descent phase of the next dive. During surface intervals sharks would stay within the top 50m of the water column and resume their routine nighttime rolling behavior that they normally conduct during steady swimming

(Fig. 3.3) (Royer et al. 2020). Surface intervals lasted an average of 40 minutes (Fig. 3.2, 3.3, 3.4, 3.5).

Temperature profiles

Of the 7 sharks that conducted deep dives, tags from 4 sharks contained useful body temperature data. HH11 did not have a thermal probe inserted into the dorsal musculature and sharks HH2 and HH10 had erroneous body temperature readings, possibly due to the thermal probe slipping out of the dorsal musculature. Outside of deep dive events, sharks spent the majority of the time (98%) above the thermocline in the top 170m of the water column with ambient water temperatures averaging 26.1 °C (±1.6°C). Scalloped hammerhead intramuscular temperatures (measured approximately 8 cm below the skin) averaged 26.3 °C and had a narrow range of variation (minimum 25.3 °C, maximum 27.1 °C).

At the bottom of deep dives, sharks experienced ambient temperatures of 5 °C to 6.6 °C (mean 5.7 °C (±1.2°C), more than 20° below surface temperature. Thus during the fast descent phase of a dive, sharks experienced a 20 °C drop in ambient water temperature over a period of 10 minutes. However, the body temperature of the scalloped hammerhead sharks did not cool during their fast descents into cold water. Although shark body temperatures initially cooled slightly (0.1 °C) between 100m and 280m depth, this was immediately followed by an 0.3 °C increase in body temperature that was sustained to the bottom of the dive resulting in sharks body temperatures of between 25 to 26 °C through at least half of their bottom time. After 2 to 3 minutes of bottom time and during the initial fast ascent, shark body temperatures began to drop slowly (0.02 degrees a minute). At the "inflection point" of the ascent (250-300m depth), the rate of heat loss increased by an order of magnitude to 0.4 degrees a minute. Shark body temperatures continued to drop at this faster rate until the final phase of the dive when sharks leveled off in the mixed surface layer (top 50m) (Fig. 3.6, 3.7). Thus scalloped hammerhead sharks experienced an average drop in body temperature of 2.5 °C from the

bottom of their dive to the start of their surface interval, with the greatest loss of heat occurring after inflection point of the ascent when swimming intensity abruptly declines. Sharks rewarmed during the surface interval but surprisingly began their next dive deep before their body temperatures reached full equilibrium with the surface waters (Fig. 3.6, 3.7).

Whole-body heat transfer coefficient modeling

Of the four sharks that contained useful body temperature data (HH6, HH7, HH8, HH9), three were used in the heat transfer coefficient analysis (HH7, HH8, HH9) because HH6 only conducted single deep dives for each evening that consisted of diving. Whole-body heat transfer coefficient modeling revealed that more than one k value is needed to accurately describe the rate of heat transfer for scalloped hammerhead sharks during deep dives. The single k model (1) greatly overestimated shark body temperature at the start of a deep dive and underestimated body temperature for any consecutive dives. Modeling also revealed that changes in the rate of temperature change due to metabolic heat production (T_o) alone (Model (2)) do not accurately predict rate of heat transfer for scalloped hammerhead sharks during deep dives (3.3). For the single night consisting of deep dives analyzed for HH9, in order for Model (2) to be a good fit ($R^2 = 0.98$), the rate of metabolic heat production (T_{o2}) during a deep dive was not a biologically realistic value (Table 3.2). Models (3) and (4), which employed two k values based on the phases of the deep dives, were the most accurate at predicting the core body temperature of each shark, with model (4) (which employed two k and two T_o values based on the phases of the deep dives) being the more accurate of the two (Table 3.2, Fig. 3.8).

The values for k_2, (applied during the intense swimming phases of deep dives) were an order of magnitude less than k_1 values (Table 3.2, Table 3.4). This indicates a substantially slower rate of heat transfer during the intense swimming phases of deep dives and suggests the existence of a physiological mechanism enabling scalloped hammerheads to reduce heat loss during deep dives and conserve body temperature until

they are approaching the surface during ascent. Additionally the values for T_{o2} (applied during the intense swimming phases of deep dives) were an order of magnitude greater than T_{o1} values (Table 3.2, Table 3.4), indicating a substantially greater rate of heat metabolic generation during the intense swimming phases of deep dives.

Additionally the whole-body heat transfer coefficient was modeled using the single *k* model (1) for the two dead scalloped hammerheads. Rates of heating and cooling for the dead sharks were assumed to be equal in the absence of any physiological processes. The *k* values from the dead sharks can only be attributed to conductive heat transfer across the body wall as convective heat-transfer across the gills is not possible due to a lack of blood flow. The whole-body heat transfer coefficients (*k*) from the dead sharks (3.3) were similar (within 0.0007 °C min^{-1} $°C^{-1}$) to the k_2 values estimated for the tagged scalloped hammerheads during their deep dives using Model (4) (Table 3.2, Table 3.4). The similarities in the heat transfer coefficients (*k*) from the dead sharks, which are not affected by convective heat transfer at the gills, and the k_2 coefficients from the live sharks, indicates that live scalloped hammerheads do not lose body heat through convective heat transfer at the gills during the intense swimming phases of their deep dives.

DISCUSSION

Our data provide detailed insight into the swimming behavior and thermoregulatory capabilities of scalloped hammerhead sharks during deep dives. Scalloped hammerhead sharks maintain an elevated (up to 20 °C warmer than ambient) body temperature during high-activity deep dives into cold water followed by a rapid drop in body temperature and swimming intensity during the final ascent phase of a dive. This behavioral and physiological pattern has not been observed in any other ectothermic shark and implies the existence of a physiological mechanism to retain heat.

The body temperature of strict ectotherms should equilibrate with their environment once

thermal inertia has been overcome but some deep diving species have evolved mechanisms to limit heat transfer. For example, ectothermic blue sharks (*Prionace glauca*) alter their rate of heat exchange with the environment above and below the thermocline, presumably by modulating blood flow to the gills (Carey & Gibson 1987; Carey & Sharrold 1990). However, blue shark heat exchange patterns differ from those observed in scalloped hammerhead sharks. Although blue shark heat exchange rates are slower during cooling than warming, they nevertheless begin to cool on encountering colder water. By contrast, the scalloped hammerhead sharks in this study showed a slight body temperature *increase* during their descent and then maintained a steady-state temperature during their bottom time despite being immersed in water 20 °C colder than their bodies, a temperature difference five times greater than those experienced by blue sharks (Carey & Gibson 1987; Carey & Sharrold 1990). This behavior is also in stark contrast to endothermic tuna which only initiate the upward (warming) phase of their dives once their body temperatures have declined to a threshold minimum level (Holland et al.1992; Holland and Sibert 1994). During ascents from deep dives, scalloped hammerhead sharks displayed an inflection in the ascent profile at a depth of around 300m which corresponded with an abrupt increase in heat exchange resulting in more rapid cooling while still below the thermocline, and equally rapid warming on reaching the surface mixed layer (Fig. 3.6, 3.7). The rapid change in heat exchange and body temperature occurring around the inflection point of the upward phase of each dive indicates that a profound physiological change has occurred.

For fish moving into colder water, the greatest and most rapid loss of body heat to the environment is through the gills. Metabolic heat generated in muscle tissue is carried away by the blood and rapidly lost to the environment as blood flows through the gill lamellae (Stevens & Sutterlin 1976; Carey & Gibson 1987). Thus with water flowing over the gills, body cooling in scalloped hammerhead sharks should occur during the descent phase of deep dives, yet this is not the case. Instead scalloped hammerhead sharks maintained an elevated body temperature for the majority of each dive. Our observations of delayed body cooling (and evidence of a lack of convective heat transfer)

suggest that scalloped hammerheads possess a mechanism to prevent heat loss from their gills during excursions into cold water. Possible mechanisms include 1) shunting blood away from the gills or 2) reducing the flow of water across the gills by reducing ram-ventilation by closing the mouth and/or gill slits to reduce convective heat loss. Either mechanism will temporarily inhibit the shark's ability to absorb oxygen from the environment, resulting in the fish equivalent of "breath-holding". The rapid change in heat exchange at or near the inflection point of the ascent, causing a more rapid decline in body temperature, likely reflects the resumption of convection heat transfer as blood flow comes into contact with ambient water flow at the gills. Further anatomical studies could reveal whether scalloped hammerhead sharks can modulate blood supply to the gills. ROV footage of an adult scalloped hammerhead shark swimming along the seafloor at 1008 meters depth off Tanzania appears to have its gill slits closed (Moore & Gates 2015). Camera loggers attached to free swimming sharks that can view the gill slits and mouth during deep dives could help confirm if the sharks rely on closing either of them to restrict the flow of cold water during deep dives.

The physiological advantages conferred by maintaining a warm body temperature at depth enable scalloped hammerhead sharks to exploit warm tropical surface waters and adjacent cold mesopelagic depths (Graham & Dickson 2000). Maintaining a warm body while diving into cold depths should give scalloped hammerhead sharks advantages similar to those described in "high performance" endothermic fishes (Dickson & Graham 2004). These include faster swimming capability due to enhanced muscle power output and enzyme performance (Carey & Teal 1969; Carey et al. 1971; Brill 1978; Wardle et al. 1989) and enhanced cardiac performance, visual acuity and neural processing (Carey & Teal 1966, Linthicum & Carey 1972; Block & Carey 1985). These anticipated benefits are consistent with our empirical observations of scalloped hammerhead behavior during deep dives characterized by burst swimming events and high activity rates indicating that they are actively pursuing prey at depth.

Maintaining warm blood flow to the brain and eyes should allow for the neural

processing required for highly active visual-based hunting behavior. Scalloped hammerhead sharks are known to have the largest brain encephalization quotient among sharks with relatively large and heavily foliated cerebellums (Yopak et al. 2007). They also have enhanced binocular vision and high visual acuity in dark environments (McComb el al. 2009).

By reducing convective heat loss at the gills, scalloped hammerhead sharks are able to prevent drops in their heart temperature allowing them to preserve cardiac function during deep dives. Vertical excursions into cold water by "high performance" endotherms such as tunas, billfish, and lamnid sharks are limited by reductions in heart function due to cold temperature (Brill 1978; Korsmeyer et al. 1997; Brill 1998; Brill et al. 1999; Brill and Bushnell 2001; Blank et al. 2004, Weng et al. 2005; Shiels et al. 2014; Wegner et al. 2015). Despite their (regionally) elevated body temperatures, their hearts operate at ambient temperatures due to the convective heat loss at the site of gills (Brill & Bushnell 2001; Wegner et al. 2015). Even species such as salmon sharks (*Lamna ditropis*) that have enhanced expression of excitation-contraction coupling proteins still experience reduce cardiac output at lower temperatures (Brill 1998; Weng et al. 2005).

Limited available diet data suggest scalloped hammerheads exploit deep-dwelling cephalopods (Clarke 1971; Klimley & Nelson 1984; Stevens 1989; Smale 1998; Vaske Júnior et al. 2009) as do a variety of cetaceans such as sperm whales (*Physeter macrocephalus*) (Clarke et al. 1998), pygmy sperm whales (*Kogia breviceps*) (West et al. 2009), Blainsville beaked whales (*Mesoplodon densirostris*), and shortfinned pilot whales (*Globicephala macrorhynchus*) (Abecassis et al. 2015). Scalloped hammerhead shark deep diving behavior is strikingly similar to that of shortfin pilot whales (Soto et al. 2008). Both species conduct vertical gliding descents at steep pitch angles (70 to 80 degrees), increase burst swimming approaching depths of 600-800 meters, and have short bottom times (less than 5 minutes) with consistently high swimming activity followed by a steep-pitched ascent with strong swimming activity.

"Breath holding" scalloped hammerhead sharks and shortfinned pilot whales face similar physiological challenges during deep dives. These include oxygen depletion during the dive and resultant reliance an anaerobic metabolism (and the concomitant buildup of lactate). The aerobic dive limit, defined as the dive duration that can be performed aerobically (Kooyman et al 1980; Williams et al. 2011), should be assessed for scalloped hammerhead sharks. Aerobic dive limit can be calculated by either measuring the blood lactate levels after a dive, or by dividing the total body oxygen stores of the sharks tissues by the diving metabolic rate (Kooyman et al. 1980; Meir et al. 2013). Physiological factors such as myoglobin concentration, a hallmark of deep diving birds and mammals (Butler & Jones 1997; Kooyman & Ponganis 1998), mitochondrial density, hematocrit level, and hemoglobin O2-binding affinity should be examined to assess the oxygen storage capability of scalloped hammerhead swimming tissues. (Kooyman et al. 1980; Butler & Jones 1997; Kooyman & Ponganis 1998). Diving metabolic rate can be estimated from high resolution body activity telemetry using kinematic modeling (Iosilevkii et al. 2012; Iosilevskii & Papastamatiou 2016). Scalloped hammerhead sharks and shortfinned pilot whales may possess similar high oxidative and glycolytic capabilities in their locomotor muscles (Velten et al. 2013). Examining the activity levels of aerobic and anaerobic enzymes in the fast-glycolytic swimming muscle tissue will help shed light on the aerobic and anaerobic metabolic capability of the scalloped hammerhead shark and reveal whether they can utilize aerobic or anaerobic metabolism (or both) during deep dives (Condon et al. 2012).

CONCLUSIONS

Scalloped hammerhead sharks employ a "breath holding" technique when they conduct deep dives into cold water. This technique is evident by the suspension of convective heat transfer at the site of the gills. This behavioral-physiology innovation allows scalloped hammerhead sharks to maintain a warm body as they actively pursue prey in cold deep depths. The discovery of this unique behavioral-physiological innovation in an

ectothermic raises interesting questions to hypothesis regarding thermal niche expansion and the physiology of "breath-hold" deep diving in a gill-breathing vertebrate.

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TABLES

Table 3.1. Deployment summary of scalloped hammerhead sharks that conducted deep dives.

Shark	Tagging Date	PCL (cm)	TL (cm)	Tagging lat (DD)	Tagging long (DD)	Pop-off lat (DD)	Pop-off long (DD)	Total # of nights with deep dives	Total # of deep dives	Body Temp. Data from Deep Dives?	Accel. Data from Deep Dives?
HH6	6/5/17	164	240	21.480	-157.830	21.489	-157.705	3	3	Yes	Yes
HH7	6/26/17	187	270	21.484	-157.827	21.352	-158.151	11	43	Yes	Yes
HH8	6/27/17	136	204	21.481	-157.829	21.588	-158.302	6	14	Yes	Yes
HH9	7/18/17	165	245	21.483	-157.828	21.492	-157.728	3	4	Yes	No
HH10	7/26/17	181	263	21.483	-157.828	21.572	-158.313	15	40	No	Yes
HH11	5/17/18	179	256	21.482	-157.828	-	-	2	2	No	Yes

Table 3.2. Results from representative whole-body heat coefficient models for scalloped hammerheads HH7, HH8, and HH9. PCL, pre-caudal length; T_a, ambient temperature during deep dives, T_b, intramuscular temperature during deep dives; k, constant whole-body heat transfer coefficient; k_1, whole-body heat transfer coefficient during normal swimming; k_2, whole-body heat transfer coefficient during the high activity phase of a deep dive; T_o, constant rate of temperature change due to internal (metabolic) heat production; T_{o1}, rate of temperature change due to internal (metabolic) heat production during normal swimming; T_{o2}, rate of temperature change due to internal (metabolic) heat production during the high activity phase of a deep dive; R^2, coefficient of determination of modeled body temperature with measured body temperature.

Shark ID PCL (cm)	T_a Range	T_b Range	Model	k (°C min^{-1} °C^{-1})	k_1 (°C min^{-1} °C^{-1})	k_2 (°C min^{-1} °C^{-1})	T_o (°C min^{-1})	T_{o1} (°C min^{-1})	T_{o2} (°C min^{-1})	R^2 fit
HH7	**5.9 – 27.1**	**23.3 – 26.5**	**(1)**	**0.0058**	**-**	**-**	**0.0145**	**-**	**-**	**0.60**
187			**(2)**	**0.0069**	**-**	**-**	**-**	**0.0445**	**0.0109**	**0.61**
			(3)	**-**	**0.204**	**0.0018**	**0.0057**	**-**	**-**	**0.81**
			(4)	**-**	**0.0176**	**0.0034**	**-**	**0.0030**	**0.0399**	**0.84**
HH8	**6.5 – 27**	**21.4 – 26.8**	**(1)**	**0.0326**	**-**	**-**	**0.0663**	**-**	**-**	**0.34**
136			**(2)**	**0.1297**	**-**	**-**	**-**	**0.0246**	**1.6719**	**0.67**
			(3)	**-**	**0.1362**	**0.0019**	**0.0105**	**-**	**-**	**0.81**
			(4)	**-**	**0.1297**	**0.0047**	**-**	**0.0184**	**0.0701**	**0.85**
HH9	**6.5 – 26.9**	**22.3 – 26.1**	**(1)**	**0.0096**	**-**	**-**	**0.0150**	**-**	**-**	**0.86**
165			**(2)**	**0.0185**	**-**	**-**	**-**	**0.0067**	**0.1850**	**0.97**
			(3)	**-**	**0.0317**	**0.0018**	**0.0049**	**-**	**-**	**0.98**
			(4)	**-**	**0.0273**	**0.0032**	**-**	**0.0056**	**0.0368**	**0.98**

Table 3.3. Sizes (PCL, pre-caudal length; TL, total length) and whole-body heat coefficient (k) estimates for two dead adult scalloped hammerheads.

Shark	PCL (cm)	TL (cm)	k (°C min^{-1} °C^{-1})
SL1	182	258	0.0039
SL2	194	273	0.0038

Table 3.4. Results from representative whole-body heat coefficient model (4) for scalloped hammerheads HH7, HH8, and HH9. Dive Nights refers to the number of total number of evenings of deep diving that had more than one deep dive that were incorporated into the modeling analysis. Total Dives refer to the total number of deep dives that were incorporated into the modeling analysis. Parameter estimates include ± 95% confidence intervals. k_1, whole-body heat transfer coefficient during normal swimming; k_2, whole-body heat transfer coefficient during the high activity phase of a deep dive; T_{o1}, rate of temperature change due to internal (metabolic) heat production during normal swimming; T_{o2}, rate of temperature change due to internal (metabolic) heat production during the high activity phase of a deep dive.

Shark	Dive Nights	Total Dives	$k_{1\ normal}$	$k_{2\ diving}$	$T_{o1\ normal}$	$T_{o2\ diving}$
HH7	8	38	0.0154 ± 0.0018	0.0031 ± 0.0010	0.0064 ± 0.0048	0.0215 ± 0.0190
HH8	3	11	0.1059 ± 0.0264	0.0037 ± 0.0009	0.0186 ± 0.0056	0.0338 ± 0.0368
HH9	1	2	0.2730	0.0032	0.0056	0.0368

FIGURES

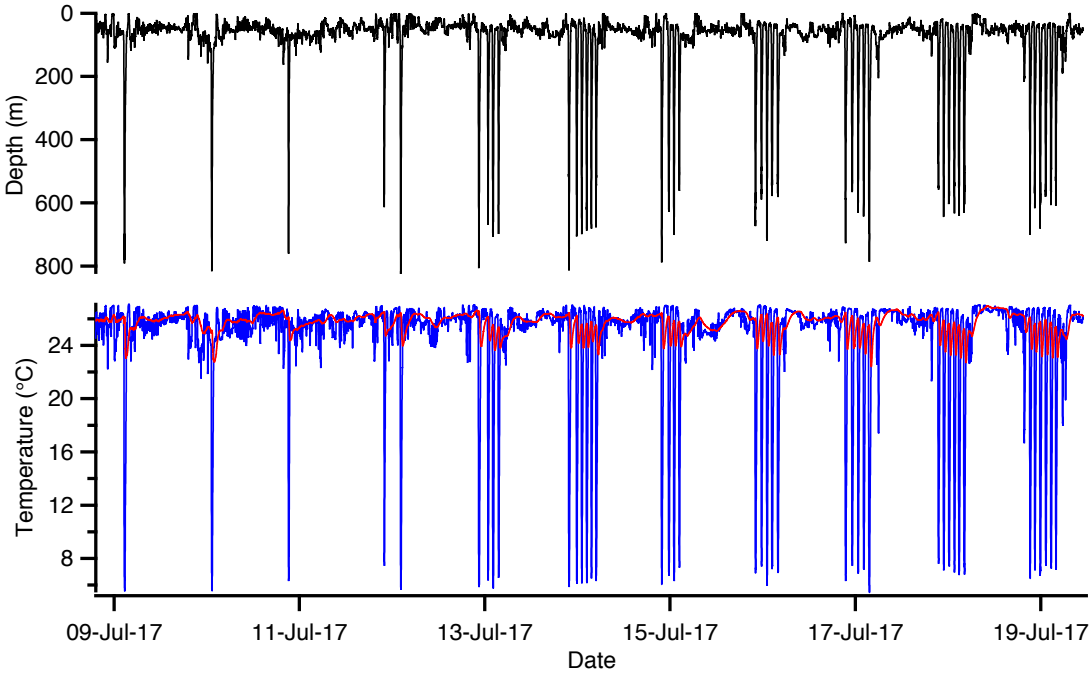

Figure 3.1. Eleven days of depth (top), ambient (blue) and intramuscular (red) temperature profiles from scalloped hammerhead shark HH7. All deep dives were conducted at night.

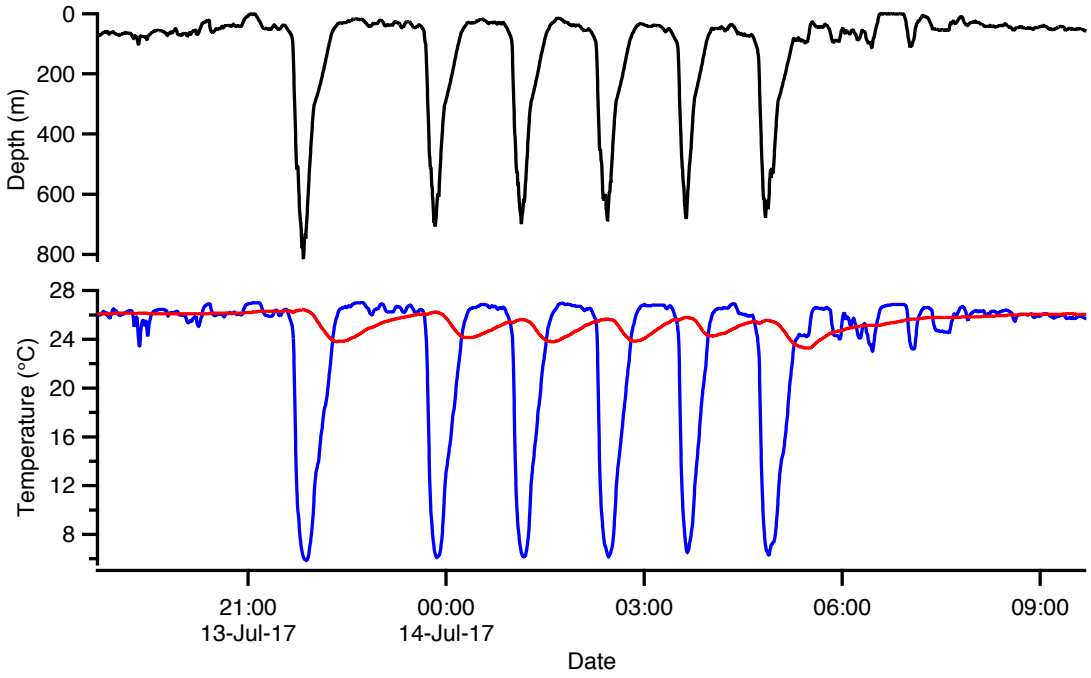

Figure 3.2. Depth (top), ambient (blue) and intramuscular (red) temperature profiles from scalloped hammerhead shark HH7 during six deep dives conducted in a single evening.

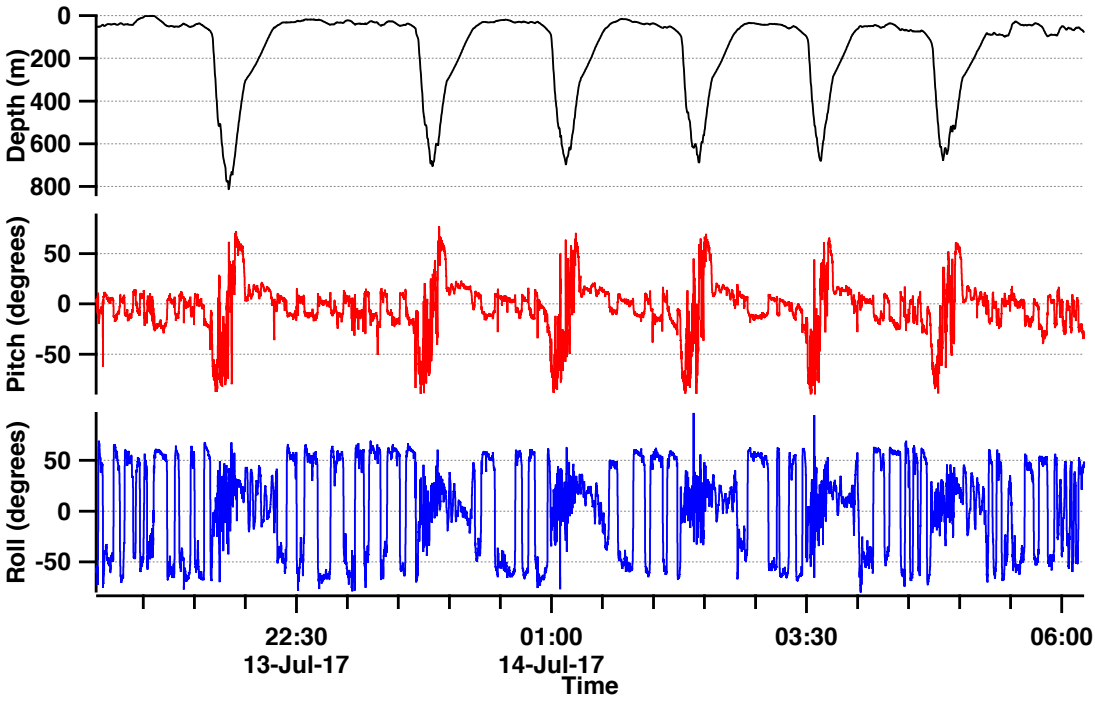

Figure 3.3. Depth profiles and body orientation (pitch and roll) during six deep dives in one evening from scalloped hammerhead shark HH7.

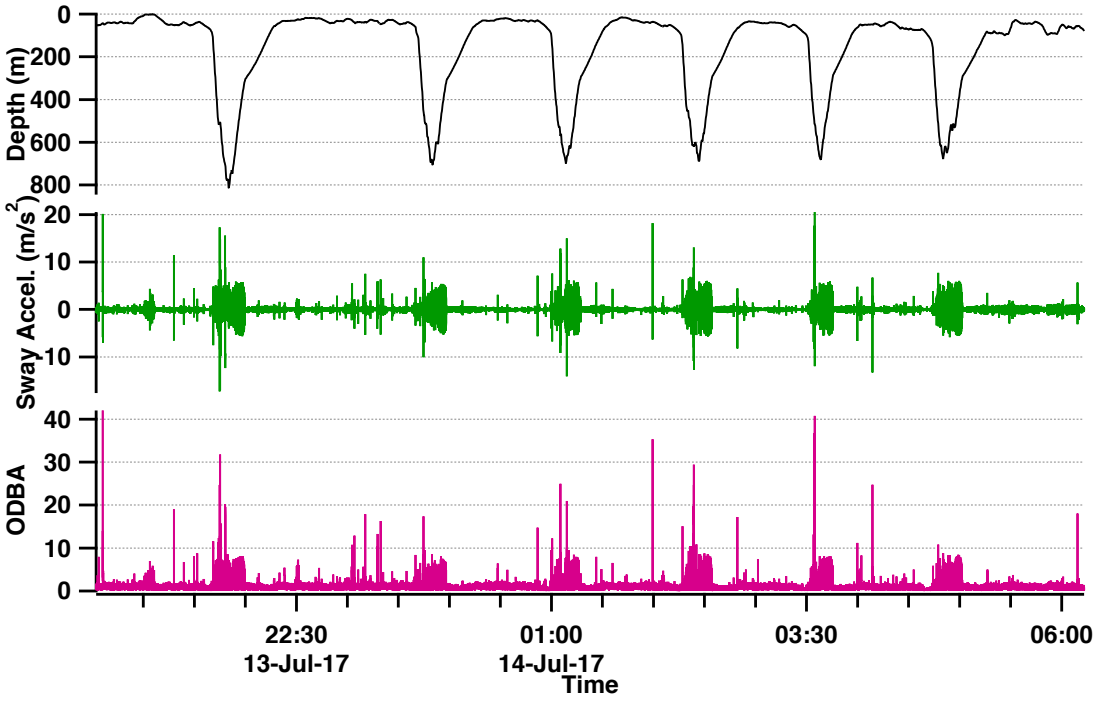

Figure 3.4. Depth profiles (top) and swimming activity (tailbeat sway acceleration (middle) and ODBA (bottom)) during six deep dives in one evening from scalloped hammerhead shark HH7.

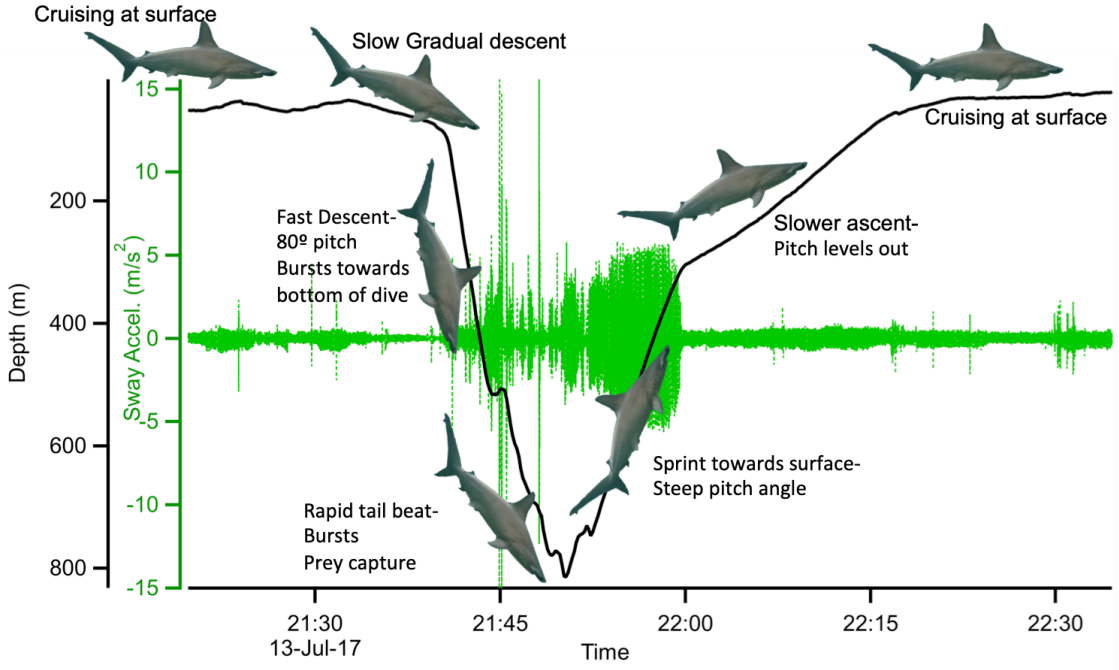

Figure 3.5. Distinct phases of a deep dive using a representative dive from scalloped hammerhead shark HH7. Depth (black), intramuscular temperature (red) and tailbeat sway acceleration (green).

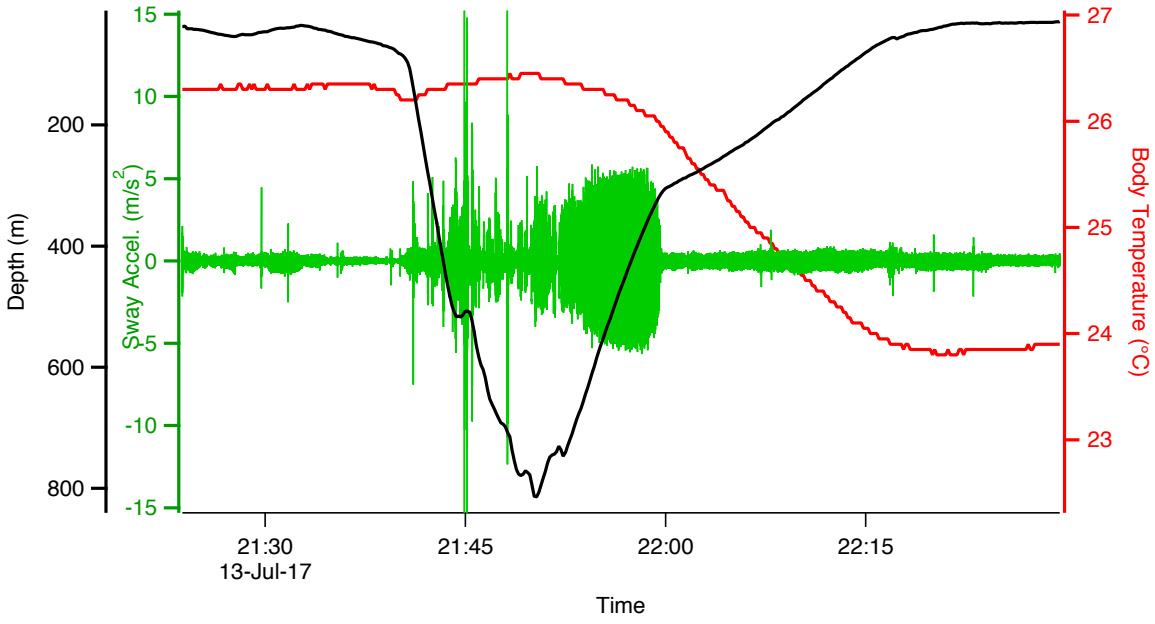

Figure 3.6. Depth (black), intramuscular temperature (red) and tailbeat sway acceleration (green) during a single dive from scalloped hammerhead shark HH7.

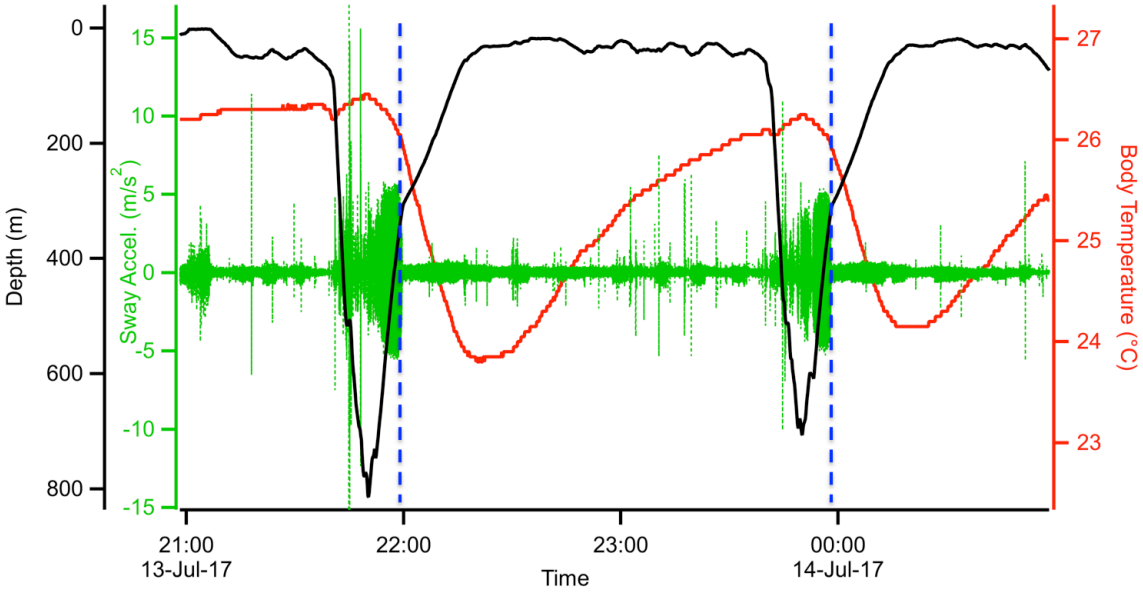

Figure 3.7. Depth profiles (top), body temperature (middle) and swimming activity (tailbeat sway acceleration (middle) and ODBA (bottom)) during two successive deep dives from scalloped hammerhead shark HH7. Dotted lines indicate the inflection point of the ascents when the swimming activity, pitch, and body temperature abruptly decrease.

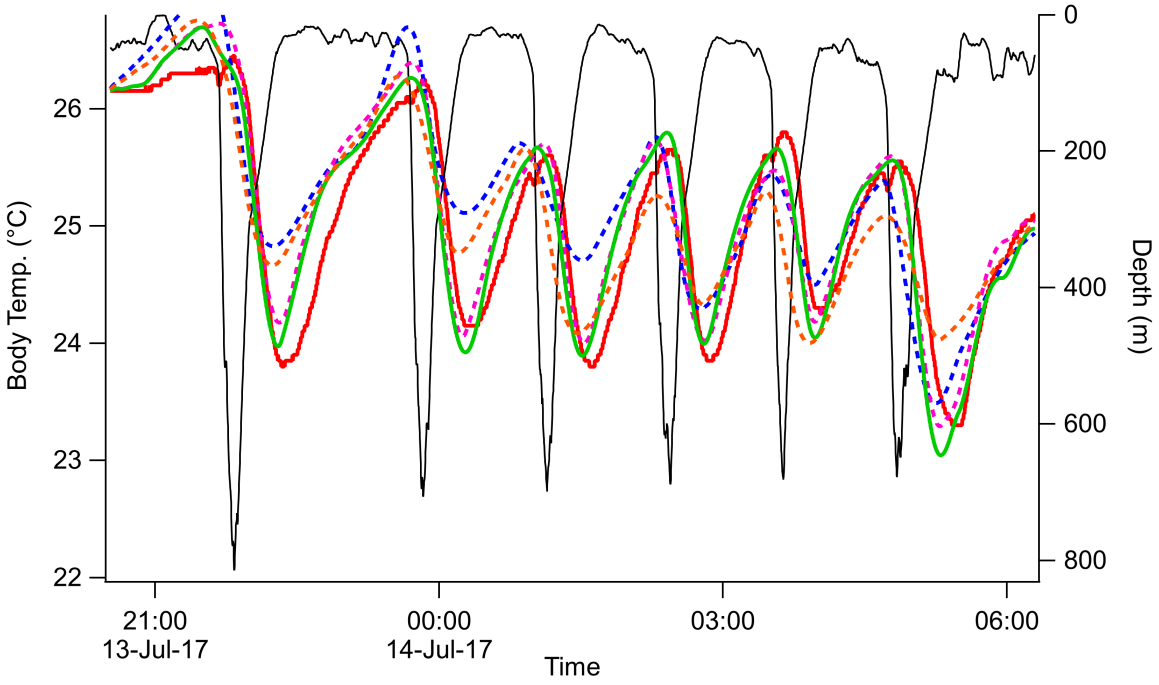

Figure 3.8. Observed (red) and modeled intramuscular temperature during six deep dives from scalloped hammerhead shark HH7 with depth (black). Model (1) (blue dashed) assumes the rate of heat exchange between the shark and the environment (k) and rate of temperature change due to metabolic heat production (T_o) is constant and not altered by the shark. Model (2) (orange dashed) assumes rate of heat exchange between the shark and the environment (k) is constant while the rate of temperature change due to metabolic heat production (T_o) alters between (T_{o1}) during normal swimming and (T_{o2}) during the high activity phases of a deep dive. Model (3) (magenta dashed) assumes (k_1) when the shark is swimming normally and (k_2) during the high activity phases of a deep dive while the rate of temperature change due to metabolic heat production (T_o) is constant. Model (4) (solid green) assumes (k_1) and (T_{o1}) when the shark is swimming normally and (k_2) and (T_{o2}) during the high activity phases of a deep dive.

CHAPTER IV
AEROBIC AND ANAEROBIC POISE OF BURST-SWIMMING MUSCLES OF A DEEP-DIVING SHARK

ABSTRACT

Scalloped hammerhead sharks (*Sphryna lewini*) routinely perform rapid deep dives to forage on meso and bathypelagic prey. These deep dives consist of intensive burst swimming followed by resting periods in the surface mixed layer. Swimming muscle temperature profiles suggest *S. lewini* suppress their gill function as a means to reduce convective heat loss. Such intensive swimming behavior coupled with supposedly reduced respiration raises questions concerning the aerobic and anaerobic metabolic capacity of the white swimming muscle tissue of this species. Measuring the activity rates of key enzymes used in aerobic and anaerobic metabolism provides an indirect indicator of the metabolic potential ("poise") of a tissue. Here we measured the maximal activities (units $gram^{-1}$ of wet tissue) of aerobic enzymes citrate synthase (CS), malate dehydrogenase (MDH) and anaerobic enzymes pyruvate kinase (PK) and lactate dehydrogenase (LDH) from white swimming muscle of *S. lewini*. Enzyme activity rates and ratios that indicate glycolytic poise (the relative reliance on anaerobic versus aerobic metabolism) were compared to those measured from other coastal, deep-dwelling, and highly active endothermic shark species. *Sphyrna lewini* had high activity levels of LDH, an anaerobic enzyme, and MDH, an aerobic enzyme. The high MDH levels are surprising in that they are greater than observed in other endothermic species. However, the anaerobic (PK) and aerobic (CS) enzyme activity levels were not higher than those found in other species. Glycolytic poise of *S. lewini* white muscle, as assessed by enzyme activity ratios, showed a strong reliance on glycolytic capability. These results indicate the white muscle tissue of *S. lewini* is highly anaerobic dependent for burst swimming but has a high potential for lactate buildup. High activity rates of MDH indicate the potential for lactate to be rapidly reduced under aerobic conditions such as those experienced in the

surface mixed layer between dives. These physiological adaptations likely enable *S. lewini* to remain highly active while suppressing gill function during deep dives and thereby exploit a very different ecological niche from sympatric shark species (e.g. coastal carcharhinids).

INTRODUCTION

Scalloped hammerhead sharks, *Sphryna lewini*, conduct repetitive nocturnal deep (over 600m) dives into cold (as low as 5 ºC) water presumably to forage on meso and bathypelagic cephalopods in the oxygen minimum zone. These deep dives are characterized by steep descents with swimming bursts when approaching maximum depth, intense activity throughout the deepest phase and a consistent high-powered tailbeat during ascent (see Chapter 3 for details). At an ascent depth of ~200m, swimming intensity decreases abruptly, sharks level out and ascend more slowly. On reaching the surface mixed layer, sharks resume "normal" tailbeats until starting the next deep dive. *Shyrna lewini* temperature profiles show these sharks maintain warm swimming muscles during deep dives, possibly by suppressing normal gill function to prevent convective heat loss (Chapter 3). Powering the swimming muscles during these repetitive, intense, deep dives necessitates a high energetic output. It is possible that *S. lewini* exceed their aerobic capacity during these deep dives due to the combination of intense burst swimming activity and suppression of normal respiration. This highly active swimming combined with apparent "breath holding" should be reflected in the activity levels of key muscle enzymes involved with locomotion and energy mobilization.

Anaerobic metabolism becomes an essential process when aerobic potential cannot sustain cellular energetic demands (Pörtner 2002). Burst swimming typically requires anaerobic metabolism and a greater reliance on muscle energy stores (Williams et al. 1997). In fishes, fast-twitch glycolytic white (type II) muscle is specialized for anaerobic burst swimming (Bone 1988; Bernal et al. 2003) and is the largest tissue mass (Bone 1988). Burst swimming events that rely on anaerobic metabolism deplete muscle

creatine-phosphate and glycogen stores and there is a concomitant buildup of lactate and H^+ byproducts (Guppy & Hochachka 1978; Bernal et al. 2003; Kane 2014).

Generally, elasmobranchs found in warm shallow water (above mesophotic depths) have higher metabolic rates and stronger burst-locomotor capabilities in their white muscle relative to deep dwelling elasmobranchs (Treberg 2003; Condon et al. 2012). Endothermic species (lamnids) have significantly higher capacities for both burst swimming (through glycolytic metabolism) and aerobic metabolism in their white muscle compared to ectotherms (Dickson et al. 1988; Dickson 1995; Bernal et al. 2003). Elevated muscle temperatures and higher energetic mobilization rates from aerobic metabolism allow endothermic species to maintain high levels of burst activity and more rapid rates of end-product breakdown (Dickson et al. 1988; Dickson 1995; 1996; Bernal et al. 2003).

Tissue capacity for aerobic and anaerobic metabolism can be estimated by measuring the maximal activity levels of key enzymes involved in the production of ATP. Assays of the enzymes citrate synthase (CS) and malate dehydrogenase (MDH) have been widely used as indicators of aerobic capacity (Moyes et al. 1992, Duong et al. 2006, Saavedra et al 2016). CS is the first catalyst in the citric acid cycle (Childress & Somero 1979) and is a good indicator of tissue mitochondrial density (Dickson et al. 1988; Dickson 1995; Moyes et al. 1992; Duong et al. 2006). MDH is used in the citric acid cycle in the shuttling of reducing agents between the mitochondria and cytosol. MDH is also involved in gluconeogenesis, the synthesis of glucose from smaller molecules such as pyruvate (Kane 2014; Rogatzki et al. 2015). Assays of the enzymes pyruvate kinase (PK) and lactate dehydrogenase (LDH) have been used as indicators of anaerobic capacity (Childress & Somero 1979; Dickson 1988; Somero 1992;). PK is a good indicator of the capacity for glycolysis, as it is used in the final stage of pyruvate formation during glycolysis (Somero & Childress 1980). LDH is involved in the terminal step of glycolysis, catalyzing the reduction of pyruvate to lactate and acts as a reducing agent for NADH in the reversible reaction Pyruvate + NADH <=> Lactate + NAD^+. By

maintaining the redox balance between NADH and NAD^+, LDH allows anaerobic production of ATP to continue in the cytosol and is therefore considered a strong indicator of anaerobic capacity (Hochachka et al. 1983). Measurements of the maximal activity levels of all four of these enzymes have been widely used in previous studies to assess the aerobic and anaerobic capacity of muscle tissues (Sullivan & Somero 1980; Dickson et al 1988; Dickson et al 1993; Vetter & Lynn 1997; Seibel et al. 2000; Bernal et al 2003; Treberg et al 2003; Drazen & Seibel 2007; Seibel & Drazen 2007; Ombres et al 2011; Condon et al 2012).

The combination of intensive swimming activity and likely suppressed gill function suggests *S. lewini* rely heavily on anaerobic metabolism during deep dives. These physiological adaptations should include enzyme characteristics that facilitate anaerobic metabolism during deep dives, and the necessary aerobic metabolism to allow for rapid recovery (i.e. the breakdown of anaerobic end products) in well-oxygenated surface waters during intervals between dives. To test this hypothesis, the maximal activity rates of key enzymes citrate synthase (CS), malate dehydrogenase (MDH), pyruvate kinase (PK), and lactate dehydrogenase (LDH) were measured to examine the aerobic and anaerobic poise of the white muscle of adult *S. lewini*. Activities levels of these enzymes were compared to those measured in other coastal tropical/temperate shark species; ectothermic sandbar shark (*Carcharhinus plumbeus*), blacktip shark (*Carcharhinus limbatis*), tiger shark (*Galeocerdo cuvier*), deep water sharks; bluntnose sixgill (*Hexanchus griseus*), prickly shark (*Echinorhinus cookie*), and endothermic species; common thresher shark (*Alopias vulpinus*), and shortfin mako shark (*Isurus oxyrynchus*).

MATERIALS AND METHODS

Tissue Sampling

White muscle tissue samples were collected from mature sharks caught on demersal long-lines set in waters off Kāneʻohe Bay, Hawaiʻi (see previous two chapters for further

details on shark capture and handling). Muscle samples were collected from a 2cm incision below the base of the first dorsal fin using 5mm or 8mm biopsy punch. Samples were placed in a cryovial and immediately dropped into a Dewar containing liquid nitrogen. Samples were later transferred and stored in an -80 ºC freezer for 3-24 months. This storage time falls within the allowable timeframe between tissue sampling and assay running without compromising (decreasing) tissue enzyme activity. (Dickson et al. 1993; Condon et al 2012).

Enzyme Assays

Assays were performed to measure the maximal activities of the enzymes CS, PK, LDH, and MDH for each white muscle tissue sample. Enzyme assay protocols were based on those established by previous studies (Childress & Somero 1979; Treburg et al 2003; Drazen & Seibel 2007; Condon et al 2012; Friedman et al 2012). Frozen samples were weighed and homogenized in a Kontes Duall ground glass tissue grinder with ice-cold 10 mM Tris-HCL buffer (titrated to pH = 7.55 at 10 ºC) at a ratio of 0.1g of tissue to 1 ml of buffer. Duplicate homogenates were prepared for each sample unless the total sample was less than 0.05g, in which case a single homogenate was prepared. CS assays were performed before centrifugation for the other enzyme assays. Homogenates were then centrifuged at 5000 min^{-1} for 5 minutes with the supernatants used for PK, LDH, and MDH assays.

All assays were run at volumes of 2 mL of substrate at 10 ºC in a Shimadzu UV 1601 spectrophotometer with a water-jacketed 12-cell cuvette holder attached to a water chiller. A temperature of 10 ºC was chosen to facilitate comparison to other studies that using this standardized temperature (Condon et al. 2012; Garcia 2013). Activity levels were measured as a function of change in absorbance over time and reported in international units (U; µmol substrate converted to product per min) per gram of wet tissue mass. PK, LDH, and MDH were run at 340 nm for 40 seconds and CS was run at 412 nm for 3 minutes. Enzyme assays were run under the following substrate saturating

conditions: citrate synthase: 10 mM T50 mM Imidazol (titrated to pH 8.0 at 10 ºC), 2 mM MgCl, 0.1 mM acetyl-CoA, 0.1 mM 5,5-dithiobis-nitrobenzoic acid (DTNB). A control assay of the substrate conditions were run to adjust for any changes in the absorbance from the presence of the enzyme. A 0.5 mM Oxaloacetate solution was then added to initiate the CS reaction. Pyruvate kinase: 80 mM tris, 100 mM KCl, 10 mM $MgSO_4$, titrated to pH = 7.8 at 10 ºC, 10 U/ml lacate dehydrogenase, 0.1 mM fructose 1-6 bisphosphate, 5.0 mM adenosine diphosphate, 150 µM NADH. PK reaction was initiated by the addition of 1.0 mM phosphoenol pyruvate. Lactate dehydrogenase: 80 mM imidazole (titrated to Ph = 7.8 at 10 ºC), 100 mM KCl, 150 µM NADH, 2 mM sodium pyruvate. Malate dehydrogenase: 100 mM tris-HCl (titrated to pH = 8.1 at 10ºC), 20 mM $MgCl_2$, 150 µM NADH, 0.5 mM oxaloacetatic acid. LDH and MDH reactions were initiated with the addition of the supernatant.

Data Analysis

Enzyme activity data collected during this study (*S. lewini* , *Galeocerdo cuvier, Carcharhinus plumbeus, Carcharhinus limbatis*) were combined with previous measurements collected and analyzed using the same sampling protocol by Garcia (2013) (*Galeocerdo cuvier, Carcharhinus plumbeus, Hexanchus griseus, Ecinorhinus cookie, Alopias vulinus, Isurus oxyrynchus*). Combining data sets provided an overall sample size of 99 individuals from among 8 shark species and enabled us to compare muscle enzyme activity in shallow coastal, deep diving species, including deep water ectotherms and deep diving endotherms (*Isurus oxyrinchus* and *Alopias vulpinus*).

We used regression analyses to evaluate whether muscle enzyme activity scaled with shark body size for each species. We explored data characteristics to identify the most appropriate tests for comparing muscle enzyme activity among species sampled. We assessed normality of enzyme activity level data by examining distribution histograms for each species and enzyme, and used Levene's test to assess homogeneity of variance. Due to the non-normal (based on histograms and NQQ plots) and heteroscedastic (Levene's test, all $P < 0.05$) nature of the data and the unequal sample sizes among species, non-

parametric Welch's ANOVAs with post-hoc Games-Howell tests were used to assess interspecific differences in muscle enzyme activity.

To compare the glycolytic poise (the relative reliance on anaerobic versus aerobic metabolism) in the white swimming muscle between species, the following ratios of enzymatic activity were calculated: MDH/LDH, CS/PK, PK/LDH and CS/LDH (Hochachka et al 1983; Suarez et al 1986; Duncan 2009; Friedman et al 2012). Ratios were compared between species for each enzyme using Welch's ANOVA with a post-hoc Games-Howell text.

RESULTS

Body size scaling effects on enzyme activity

All *S. lewini* sampled were mature adults (PCL: 161 – 202) and individuals of other species sampled were mostly adults and large sub-adults. Regression analyses showed no significant scaling of enzyme activity with body size for each species (all *P* values >0.05), therefore we did not scale enzyme activity rates with body size.

White muscle enzyme activity levels

Sphyrna lewini white muscle exhibited high activity levels of LDH, an anaerobic enzyme, and MDH, an aerobic enzyme. *S. lewini* had the highest levels of MDH and the second highest activity levels of LDH of all species analyzed (*I. oxyrynchus*, an endotherm, had the highest LDH activity) (Table 4.1).

All *P*-values reported below are results of Welsh's ANOVA post-hoc Games-Howell tests for each enzyme and enzyme ratio.

MDH activity in *S. lewini* was very high compared to all of the coastal and deep water

species (all $P \geq 0.007$) but was not significantly different than the endothermic species (*A. vulpenus*, $P = 0.38$; *I. oxyrynchus,* $P = 0.99$).

LDH activity in *S. lewini* was significantly greater than in the coastal sharks *C. limbatis* ($P = 0.02$) and *C. plumbeus* ($P = 0.003$) and the deep water shark *H. griseus* ($P = 0.006$). LDH activity in *S. lewini* was not significantly different from the endothermic *A. vulpenus* ($P = 0.90$) but was significantly lower than *I. oxyrynchus* ($P < 0.001$).

CS activity in *S. lewini* was not significantly different from that measured in coastal and deep water species (all $P > 0.05$) (Fig. 4.1), but was significantly less than that measured in the endothermic species *A. vulpinus* ($P = 0.002$) and *I. oxyrynchus* ($P = 0.008$).

PK activity in *S. lewini* was not significantly different from that measured in the coastal sharks *C. limbatis* and *C. plumbeus,* both deep water sharks, or the endothermic *A. vulpinus* (all $P > 0.05$), but was significantly below that of *G. cuvier* ($P < 0.001$) and the endothermic *I. oxyrynchus* ($P < 0.001$).

Enzyme Ratios

S. lewini white muscle, as assessed by enzyme activity ratios, showed a strong reliance on glycolytic (oxygen-independent) capability. There was little difference in the glycolytic poise of *S. lewini* in comparison to the other species. None of the species examined showed a greater reliance on aerobic over anaerobic capability. Low levels of MDH/LDH, PK/CS, and CS/LDH indicate down-regulation of oxidative capabilities (Duncan & Macron). Low levels of PK/LDH further clarify the reliance of anaerobic metabolism for energy production due to the increased potential of available pyruvate (from PK) to be converted to lactate (from LDH).

Sphyrna lewini had a significantly lower CS/LDH ratio compared to *C. plumbeus* ($P = 0.046$) and similar ratios to all other species. There was no significant difference between

the MDH/LDH ratios of *S. lewini* to any other species, with all averages below 1 (Table 4.2). *S. lewini* had a significantly lower PK/LDH ratio to *G. cuvier* ($P = 0.002$) and *C. plumbeus*. ($P < 0.001$), and the lowest overall ratio compared to all other species (Table 4.2). *S. lewini had* significantly lower PK/CS ratio to *G. cuvier* ($P = 0.006$) and *I. oxyrynchus*.($P = 0.052$) .

DISCUSSION

Analyses of white muscle poise suggests scalloped hammerhead sharks possess enzyme characteristics that facilitate anaerobic metabolism during deep dives and the necessary aerobic metabolism to allow for rapid recovery (through the breakdown of anaerobic end products) during inter-dive intervals. Based on the comparatively high activity levels of LDH and MDH, *S. lewini* have higher anaerobic and aerobic capacity than other coastal carcharhinids and bathyal shark species which do not exhibit the repetitive nocturnal deep dives observed in *S. lewini* (Fig. 4.2). This is supported to a lesser extent by the activity levels of CS in *S. lewini*. These physiological adaptations likely enable *S. lewini* to remain highly active while suppressing gill function during deep dives into cold habitats and then to recover quickly in well-oxygenated surface waters through efficient metabolism of lactate built up during anaerobic activity**.** These behavioral and physiological adaptations enable *S lewini* to exploit a very different ecological niche than other sympatric shark species (eg. coastal carcharhinids whose daytime distributions overlap with *S. lewini*). Important caveats that must be taken into consideration of the results of this study. Enzymatic activity can change based on a number of circumstances such as sampling period, time of day, recent feeding or mating events, and activity level (Yang & Somero 1993; Dalhoff 2004; Li et al. 2012); none of which are known from any of the sharks that were captured for sampling.

The overall higher levels of PK and LDH in comparison to CS and MDH across all species examined are consistent with previous findings on the anaerobic poise of fish white muscle (Dickson et al. 1988, 1993; Condon et al. 2012). Based on PK and CS

activity levels, *S. lewini* has less anaerobic and aerobic capacity than the endothermic species *A. vulpinus* and *I. oxyrynchus*. However *S. lewini* had higher activity levels of LDH and MDH than *A. vulpinus* and the highest overall rate of MDH.

High activity levels of LDH and the low ratios of LDH/CS, LDH/MDH, and PK/LDH, indicate that *S. lewini* white muscle is anaerobically poised with high capacity for ATP production regardless of oxygen availability (Panepucci et al. 2000; Saavedra et al 2016). Higher levels of LDH allow for higher rates of glycolysis due to the high rates of reduction of NADH to NAD^+, preventing the inhibitory impact of NADH buildup but also causes a high rate of lactate buildup in the cytosol. The amount of accumulated lactate can be altered by factors such as O2 tension, metabolic rate, and available mitochondrial activity (Rotgatzki et al 2015). Under ample O2 conditions, lactate can be converted to pyruvate during gluconeogenesis. MDH plays a key role in the malate-asparate cycle, a key step in gluconeogenesis, where NADH is regenerated from the reversal of the LDH reaction and its pair of electrons are shuttled across the inner mitochondrial membrane. The high activity levels of MDH observed in *S. lewini* white tissue, in comparison to all other species examined, indicates a high potential for the conversion of accumulated lactate into glycogen through the process of gluconeogenesis under aerobic conditions. This process may be initiated when *S. lewini* ascend to approximately 200 m and slow their rate of ascent and start rapid cooling. Available evidence indicates that this is the moment when the sharks open their gills and start to quite rapidly repay the anaerobic debt accumulated during the dive.

Previous studies indicate that teleosts retain lactate produced from strenuous exercise in their white muscle (Milligan & Wood 1986; Girard & Milligan 1992;). Similar studies on elasmobranchs are conflicting. Results from Richards et al (2003) indicates that *Squalus acanthias* retains lactate in white muscle which is then used in gluconeogenesis and glycogen synthesis rather than oxidation. However, Backey (2007) did not find evidence for white muscle as the site of glycogen synthesis in three shark species (blue shark *Prionace glauca*, leopard shark *Triakis semifasciata*, and *I. oxyrynchus*). Future studies

should investigate the activity levels of other key enzymes in the gluconeogenesis pathway in the white muscle of *S. lewini*.

S. lewini high capacity for anaerobic metabolism and hence high activity during deep "breath hold" dives likely confers an advantage over deep-dwelling prey which may be relatively sluggish due to low body temperatures and slow metabolisms (Childress 1995; Seibel et al 2000; Seibel & Drazen 2007). Previous studies revealed a significant correlation between decreased burst swimming capacities of the white muscle of deep-dwelling teleost and cephalopods and minimum depths of occurrence (Childress & Somero 1979; Sullivan & Somero 1980; Drazen & Seibel 2007). By maintaining a high capacity for burst swimming, *S. lewini* have an energetic advantage in capturing deep-dwelling prey during their deep foraging dives. Their enzyme profile indicates this deep water hunting capacity is facilitated by rapid recovery in surface waters between dives.

CONCLUSIONS

Analyses of white muscle poise suggests scalloped hammerhead sharks possess enzyme characteristics that facilitate anaerobic metabolism during deep dives and the necessary aerobic metabolism to allow for rapid recovery (through the breakdown of anaerobic end products) during inter-dive intervals. Recovery closer to the surface in oxygenated water is likely important for lactate clearance and glycogen restoration, accomplished with high levels of MDH. This study provides insight on the aerobic and anaerobic metabolic capabilities of this species and how it allows them to conduct intensive deep dives. This study raises further questions on the metabolic pathways of aerobic and anaerobic metabolism in relation to muscle power output and restoration of energy stores. This adaptation to deep foraging and rapid recover is likely a key to the success of the scalloped hammerhead, which has historically been abundant in coastal and off shore waters on a global scale.

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TABLES

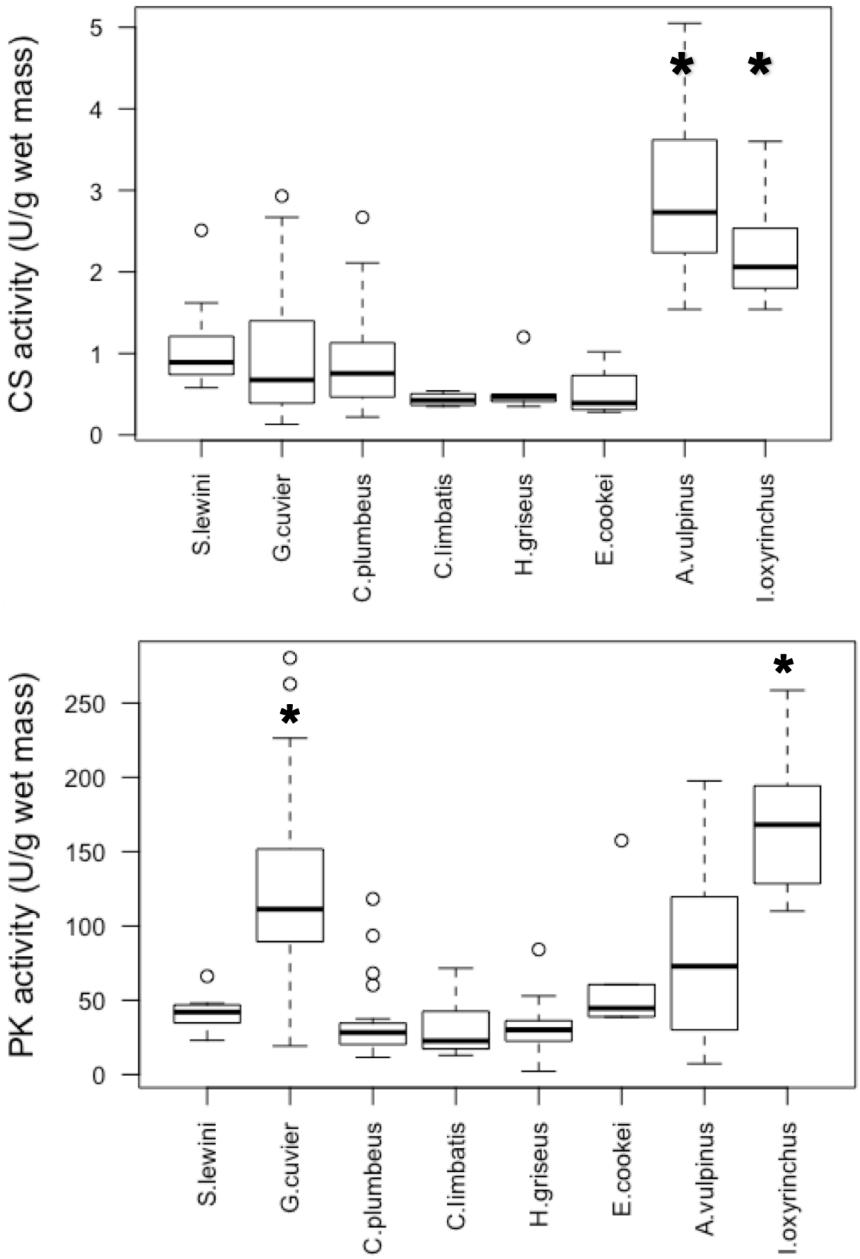
Table 4.1. White muscle enzyme activity averages (± standard deviations) reported in (U/g wet mass) for each species.

Species	N	CS avg	PK avg	LDH avg	MDH avg
S. lewini	10	1.08 ± 0.59	41.03 ± 11.92	203.19 ± 96.84	55.15 ± 19.09
G. cuvier	22	0.99 ± 0.82	126.61 ± 71.40	144.53 ± 95.37	25.19 ± 9.51
C. plumbeus	24	0.92 ± 0.66	35.05 ± 26.35	31.81 ± 24.09	16.75 ± 6.37
C. limbatis	5	0.44 ± 0.09	33.39 ± 24.16	76.56 ± 25.3	19.02 ± 9.78
H. griseus	9	0.57 ± 0.32	32.95 ± 24	50.38 ± 44.62	10.49 ± 6.98
E. cookei	5	0.52 ± 0.34	68.05 ± 50.79	141.55 ± 44.86	14.49 ± 5.88
A. vulpinus	11	3.01 ± 1.10	83.79 ± 63.95	141.97 ± 123.93	39.66 ± 10.94
I. oxyrinchus	12	2.29 ± 0.70	168.47 ± 44.76	539.40 ± 79.89	49.84 ± 7.97

Table 4.2. Ratios of white muscle enzyme activity averages (± standard deviations) for each species.

Species	CS / LDH	MDH / LDH	PK / LDH	PK / CS
S. lewini	0.008 ± 0.008	0.363 ± 0.257	0.301 ± 0.263	44.11 ± 14.83
G. cuvier	0.008 ± 0.006	0.257 ± 0.207	1.19 ± 0.791	210.09 ± 169.02
C. plumbeus	0.057 ± 0.061	0.945 ± 0.840	1.389 ± 0.768	59.74 ± 60.62
C. limbatis	0.005 ± 0.001	0.230 ± 0.115	0.550 ± 0.611	56.90 ± 36.80
H. griseus	0.016 ± 0.010	0.369 ± 0.289	0.848 ± 0.521	56.98 ± 27.08
E. cookei	0.004 ± 0.001	0.106 ± 0.045	0.449 ± 0.179	144.96 ± 39.37
A. vulpinus	0.036 ± 0.027	0.433 ± 0.259	0.722 ± 0.644	28.65 ± 20.11
I. oxyrinchus	0.004 ± 0.002	0.095 ± 0.025	0.306 ± 0.045	82.73 ± 33.16

FIGURES


CS activity (U/g wet mass)
0
1
2
3
4
5
S.lewini
G.cuvier
C.plumbeus
C.limbatis
H.griseus
E.cookei
A.vulpinus
I.oxyrinchus
PK activity (U/g wet mass)
0
50
100
150
200
250
S.lewini
G.cuvier
C.plumbeus
C.limbatis
H.griseus
E.cookei
A.vulpinus
I.oxyrinchus

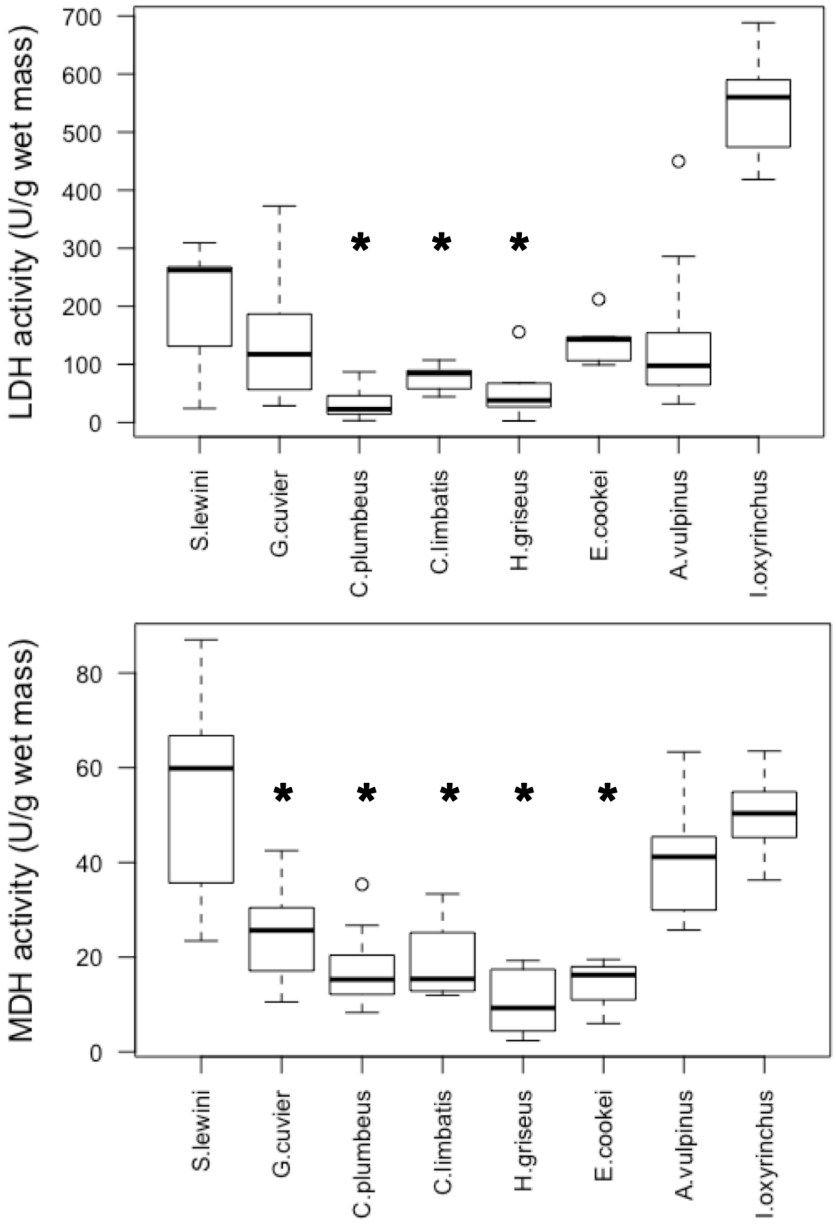


Figure 4.1. Boxplots of white muscle CS, PK, LDH, and MDH activities (U g^{-1} wet mass at 10º C) from (left to right) *Syphrna lewini,* coastal species *(Galeocerdo cuvier*, *Carcharhinus plumbeus*, *Carcharhinus limabtis*), deep water species (*Hexanchus griseus*, *Ecinorhinus cookie*), and endothermic species (*Alopias vulpinus*, *Isurus oxyrynchus*). Thick line is mean, box is standard error, error bars are standard deviations, circles are outliers. Endothermic species are omitted to make it easier to compare the levels of *S. lewini* to the other ectothermic coastal and deep water sharks. Asterisk (*) indicates significant difference (*P*-values < 0.5, Welsh's ANOVA post-hoc Games-Howell test) in comparison to *S. lewini*.

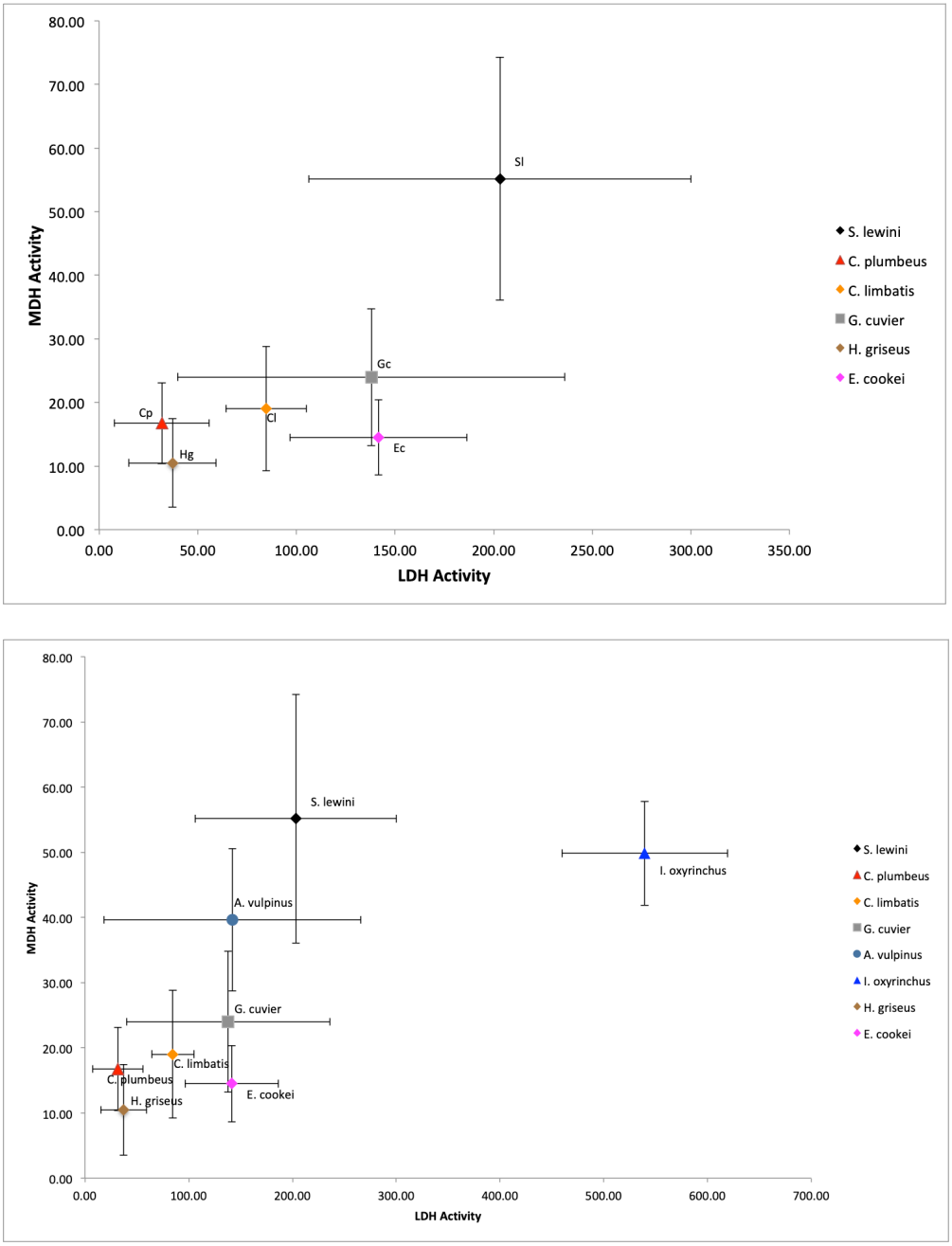


Figure 4.2. Scatter plots of average LDH activity and MDH activity of *Syphrna lewini* with coastal and deep waters species (top) and with the endothermic species included (bottom). Bars indicate 95% standard deviations. The activities of LDH and MDH of *S. lewini* stand out at much higher levels in comparison to the coastal and deepwater species (Top). *S. lewini* MDH activities are greater than the endothermic *Alopias vulpinus* and *Isurus oxyrynchus* (bottom).

CHAPTER V
CONCLUSION

The overarching goal of my dissertation was to elucidate the thermoregulation strategies of two ectothermic sharks, the bluntnose sixgill and the scalloped hammerhead shark, as they move vertically between drastically different thermal environments. I used a novel suite of tagging technology to overcome the logistical challenges of studying the thermal physiology and behavior of these two relatively understudied shark species that utilize deep-sea environments. My dissertation reached new milestones in the field of biologging and contributed novel insight to the understanding of the thermal physiology of marine ectotherms. I gathered unprecedented simultaneous high-resolution body activity and body temperature datasets from these sharks. My second chapter is the first study to measure the body temperature of a free-swimming deep-water shark *in situ*. The third chapter describes a physiological and behavioral deep-diving strategy that has never been observed in a non-air breathing deep diving vertebrate. The metabolic capacity required for this behavioral/physiological innovation is supported by the findings of my fourth chapter which reveals an enzyme profile in the white swimming muscle of scalloped hammerhead sharks that distinguishes them from other sympatric species.

Bluntnose sixgill and scalloped hammerhead sharks use different thermoregulation strategies to achieve the same goal of broadening their thermal niche - presumably enabling them to exploit resources that would otherwise be beyond reach. Large body masses allow adult bluntnose sixgill sharks to exploit thermally disparate habitats through simple thermal inertia. In contrast, scalloped hammerhead sharks employ a complex 'breath holding' technique to reduce heat loss when they conduct rapid deep dives into cold water. This technique involves the suspension of convective heat transfer at the site of the gills. This behavioral-physiological innovation allows scalloped hammerhead sharks to maintain a warm body as they actively peruse prey in deep cold habitats. Enzyme activity results support the hypothesis that *S. lewini* are physiologically adapted for highly active 'breath hold' dives. These physiological adaptations likely enable

scalloped hammerhead sharks to remain highly active while suppressing gill function during deep dives and thereby exploit a very different ecological niche from sympatric shark species (e.g. coastal carcharhinids).

The discovery of this unique behavioral-physiological innovation in an ectothermic shark raises interesting questions regarding thermal niche expansion and the physiology of 'breath-hold' deep diving of a non-air breathing vertebrate. Several studies have been conducted on the ecology, behavior, and physiology of mammals, birds, and reptiles that conduct deep foraging dives (reviewed by Kooyman & Ponganis 1998; Tyack et al. 2006). Key physiological adaptations of these air-breathing deep diving vertebrates include increased oxygen storage capacity via high concentrations of hemoglobin and hematocrit in blood, high concentrations of myoglobin in locomotor tissue, high blood volume, decreased heart rates during dives, and shunting of blood away from peripheral tissue (Kooyman & Ponganis 1998).

Future studies should examine the oxygen storage capacity of scalloped hammerheads by examining the concentrations of hemoglobin and hematocrit in their blood and myoglobin concentrations in their swimming muscles. Blood oxygen binding curves should also be examined to understand the relation of O2 binding affinity in relation to the temperatures scalloped hammerheads experience during deep dives. By maintaining a high capacity for burst swimming, scalloped hammerhead sharks have an energetic advantage in capturing deep-dwelling prey during their deep foraging dives. Their enzyme profile indicates this deep water hunting capacity is facilitated by rapid recovery in near-surface waters between dives. According to optimal foraging theory, the energy gained from foraging during a deep dive must be greater than the energy spent to execute such behavior, including the recovery period where energy stores are replenished (Thomas & Fedak 2001). Diving metabolic rate and the energy required for scalloped hammerheads to conduct deep dives can be estimated using kinematic modeling from the high-resolution body activity data gathered from this study (Iosilevkii et al. 2012; Iosilevskii & Papastamatiou 2016). A greater understanding of the physiological

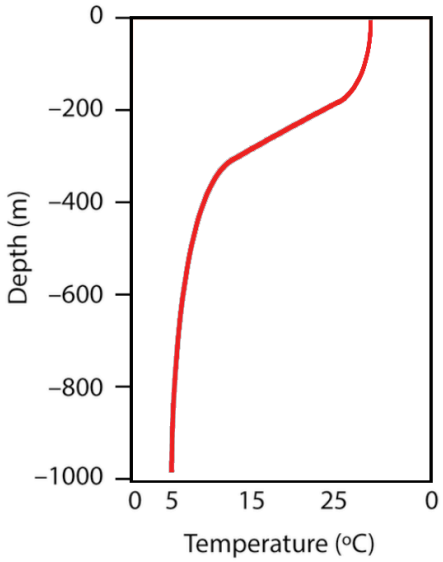
requirements for scalloped hammerheads to conduct deep dives can help us understand the biotic and abiotic requirements of essential foraging habitats for this regionally endangered species. Such factors include prey density for optimal forging, water temperature for physiological performance, and oxygen saturation required during recovery at the surface.

The vertical movements of sixgill and scalloped hammerhead sharks between thermally disparate habitats in the water column provide energetic linkages between these habitats. The specific ecological context of the observed behavioral-physiological strategies of these species can be addressed using new advances in tagging technology such as animal-borne cameras and feeding tags (Meyer & Holland 2012; Hussey et al. 2015; Meyer 2017). Future studies utilizing these technological innovations can address ecological questions such as 1) what are the specific prey species, 2) what is the rate of foraging success, 3) at what specific habitats does foraging occur, 4) what is the quantity of prey consumed and subsequent rate of digestion, 5) what is the relation between foraging and digestion to vertical movement and body temperature? Camera tag deployments on the pectoral fins of scalloped hammerhead sharks can validate whether the sharks reduce ram-ventilation by closing their mouths and/or gill slits when they conduct rapid deep dives.

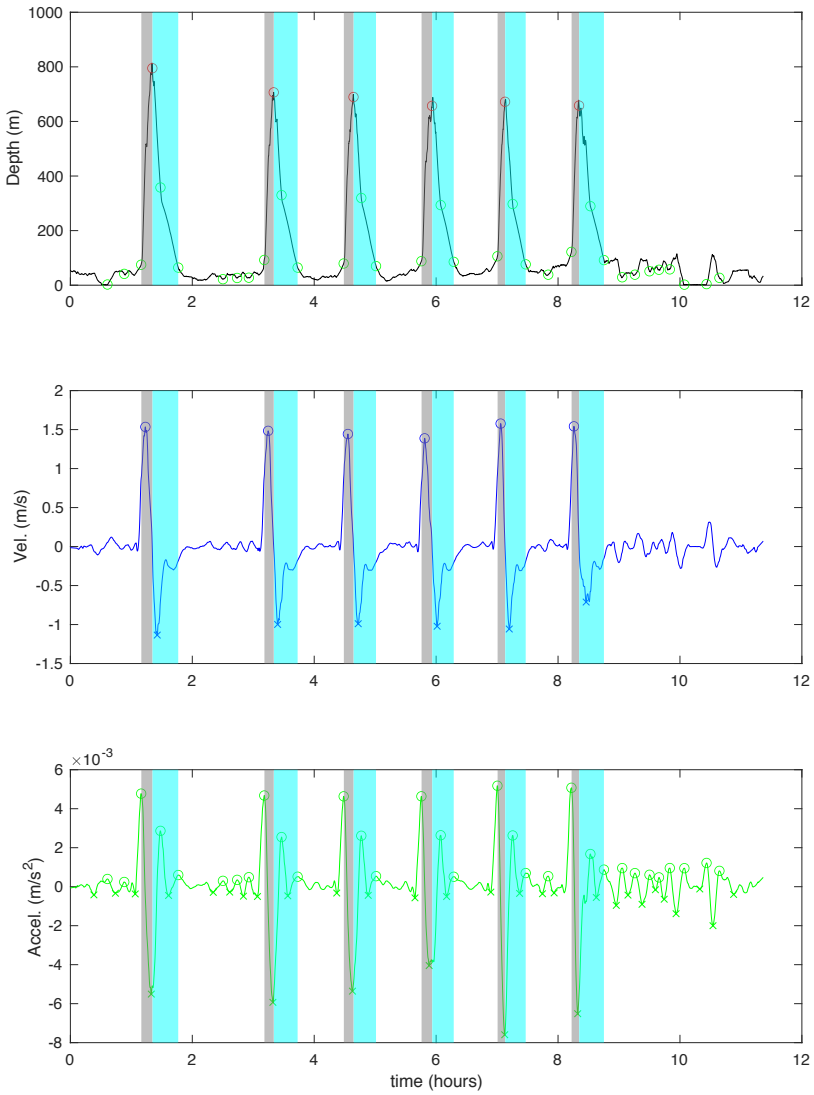
The behavioral-physiological strategies I observed from the bluntnose sixgill and the scalloped hammerhead sharks, one of the oldest extant and one of the most derived species of elasmobranchs respectively, represent highly contrasting evolutionary strategies for thermal niche expansion of large marine ectotherms. It is my hope that the findings from this dissertation can be used to apply an eco-physiological understanding of functional role of these species to the environment and what effect projected changes in ocean temperatures will have on them.

APPENDIX

SUPPLEMENTARY FIGURES



Supp 1. Typical temperature-depth profile around Hawaii. Figure modified from https://manoa.hawaii.edu/exploringourfluidearth/



Supp. 2: Identifying diving duration for thermal coefficient modeling (Chapter III), using vertical velocity and acceleration for determining the switching from k_1 (normal) to k_2 (diving) (models 3 & 4) and/or T_{o1} (normal) to T_{o2} (diving) (models 2 & 4) during descent and ascent for 6 dives from scalloped hammerhead shark HH7. The start of the gray area indicates an immediate switch from k_1 (normal) to k_2 (diving) (models 3 & 4) and/or T_{o1} (normal) T_{o2} (diving) (models 2 & 4) during descent. The extent of the blue area indicates a linear return from k_2 (diving) to k_1 (normal) (models 3 & 4) and/or T_{o2} (diving) to T_{o1} (normal) (models 2 & 4).

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