AEROBIC AND ANAEROBIC ACTIVTIY METABOLISM OF AN ELASMOBRANCH

BY

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THESIS

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ABSTRACT

Animals have finite energy stores and rates at which organisms acquire and use energy can have consequences for fitness. Furthermore, net rates of energy gain or loss can be affected in unknown or unpredictable ways in response to anthropogenic or environmental stressors. Elasmobranchs, in particular, represent some of the most exploited vertebrate taxa on the planet, and many species are data deficient with regard to a basic understanding of energetics or how stressors alter patterns of energy use and allocation. Therefore, the focus of this research was to define relationships between the behavior and energy use for a species of shark both undisturbed in the wild and subjected to the stress of fishing capture. To accomplish this goal, two complimentary series of experiments were conducted to define the frequency and energetic costs of different swimming behaviors in wild, free-swimming sharks (chapter one), and to define the effect of simulated commercial longline fishing capture on the swimming behavior and energy use of a shark (chapter two). The study presented in chapter one offered new insight into the energetics and behavioral partitioning of wild sharks, thereby providing a baseline from which to assess behavioral and, therefore, energetic responses of sharks to stressors. The study presented in chapter two offered an enhanced understanding of the energetics and behavioral responses of sharks to commercial longline capture, with implications for mitigating sub-lethal outcomes of fisheries capture for incidentally caught and released sharks. Together, these studies offer insight into why sharks behave the way they do, stressed or unstressed, and the energetic consequences of their actions, with application to elasmobranch conservation.

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CHAPTER 1: BEHAVIOR-SPECIFIC AEROBIC METABOLIC RATES OF WILD LEMON SHARKS

Abstract

Knowledge of how often animals engage in different behaviors with discrete energetic costs is important for understanding how activity-related energy expenditure is influenced by environmental or anthropogenic stressors. Fishes are subject to considerable exploitation and disturbance, yet studies on the energetics and behavior of wild fishes are lacking. This study sought to estimate the frequency of various swimming behaviors and their associated energetic costs for wild lemon sharks (*Negaprion brevirostris*). Behaviors were identified for captive animals and remotely observed for wild animals with accelerometry, and the energetic costs of behaviors were estimated for wild sharks using acceleration-calibrated relationships generated with respirometry. Sharks exhibited clear partitioning of swimming behaviors, favoring slow, aerobic swimming over resting and behaviors that characteristically recruit anaerobic metabolism. With the exception of resting, swimming behaviors with lower energetic costs occurred most frequently, and behaviors that approached the upper aerobic limit for activity were increasingly rare. Although behaviors that recruited anaerobic metabolism occurred 18 % of the time, the additional energetic costs incurred by anaerobic metabolism were predicted to be < 5 % of diel energy expenditure. Understanding behavioral partitioning and energy use in exploited taxa ultimately improves our ability to relate behavioral responses to stressors and changes in activity-related energy expenditure with survival and reproductive success.

Introduction

Wild fishes exhibit behaviors that involve both aerobic (*i.e.,* oxygen dependent) and anaerobic (*i.e.,* oxygen independent) metabolism and the contributions of anaerobic versus aerobic activities to diel energy expenditure are relatively unknown. Aerobic metabolism allows for slow, sustained and energetically efficient activity through oxidative phosphorylation, whereas anaerobic metabolism supports fast, short-lived, and energetically inefficient activity through glycolytic pathways that depletes stores of high-energy phosphates, accumulates metabolic waste (*e.g.,* lactate), and results in fatigue (Kieffer 2000). Fishes cannot optimize both aerobic and anaerobic activity metabolism; increased capacity for anaerobic activity metabolism comes at a cost to aerobic metabolism, and *vice versa* (Ejbye-Ernst *et al*. 2016). Generally, aerobic metabolism supports behaviors linked to routine swimming and resting activity (*e.g.,* Whitney *et al*. 2016) and social dominance (*e.g.,* aggressive behavior, Killen *et al*. 2014), while anaerobic metabolism supports behaviors linked to predator-prey interactions (*e.g.,* burst swimming, Killen *et al*. 2015) and reproduction (*e.g.,* upstream migrations, Burgetz *et al*. 1998; Lee *et al*. 2003a). Swimming activity can be further categorized into four discrete behaviors concerning aerobic and anaerobic contributions. First, non-swimming behaviors constitute zero swimming activity where fishes rest on the substrate or remain motionless in the water column, and can be defined by resting metabolic rates if the temperature is constant and digestion is not occurring (Chabot *et al*. 2016). Second, aerobic swimming constitutes slow, sustained swimming activity with a steady gait (Rummer *et al*. 2016), where all activity is fueled by aerobic red muscle (Shadwick and Goldbogen 2012) and can be defined by routine metabolic rates (*e.g.,* Whitney *et al*. 2016) or activity-specific calibrations with metabolic rate (*e.g.,* Lowe 2001). Third, anaerobic swimming constitutes swimming activity with an unsteady gait (Lee *et al*. 2003a), where fishes approach their upper aerobic oxygen consumption limit and begin to recruit anaerobic white muscle (Shadwick and Goldbogen 2012). Lastly, burst swimming consists of short-lived increases in velocity from one or more rapid tailbeats and relies entirely on oxidation of anaerobic substrates (Killen *et al*. In Press). Thus, different swimming

behaviors may be characterized by discrete energetic costs, and will, therefore, have different contributions to diel energy expenditure.

Understanding how energetically costly behaviors are is important for determining how wild fishes partition finite energy stores in response to environmental and anthropogenic stressors so as to maximize energy savings (Shepard *et al*. 2013) that may ultimately contribute toward an individual's fitness (Lemon 1991). However, fishes are inherently difficult to study in the wild (Cooke *et al*. In Press), and few studies have addressed the associated energetic costs of ecologically important behaviors (Treberg *et al*. In Press). For instance, provisioning whitetip reef sharks (*Triaenodon obsesus*) for ecotourism increased diel energy expenditure by stimulating sharks to swim at a time of day when sharks typically rest (Barnett *et al*. 2016), though studies of this nature are few in number. To fully account for energy expenditure of wild fishes, bioenergetics models need to include estimates for digestion (*e.g.,* specific dynamic action), somatic and gonadal tissue development, and heat lost through excretion, including a reliance on anaerobic pathways and variation with temperature and mass (Cooke *et al*. In Press). However, swimming behaviors comprise the largest and most variable component of a fish's energy budget (Boisclair and Sirois 1993; Lowe 2001), and variation in energy expenditure tied to swimming behaviors alone can considerably affect growth rates (Rennie *et al*. 2005) and ultimately fitness (Cooke *et al*. In Press). Therefore, understanding the energetic costs of swimming behaviors is important for quantifying contributions to diel energy expenditure and how stressors alter patterns of energy allocation.

Recent advances in biotelemetry and biologging technologies have made it possible to remotely observe behaviors and assign energetic costs to different activities for fishes and construct detailed bioenergetic models (Cooke *et al*. In Press). Because acceleration biologgers are capable of recording high-resolution behavioral data (*e.g.,* Brownscombe *et al*. 2014; Wilson *et al*. 2015), and acceleration-based behavioral metrics are reliable proxies of energy use (*e.g.,* Wilson *et al*. 2006; Halsey *et al*. 2009), acceleration biologgers can be used to remotely monitor behavior and estimate rates of energy expenditure for wild, free-swimming fish (Brown *et al*. 2013; Metcalfe *et al*. 2016). While these approaches have been

implemented for laboratory studies (*e.g.,* Whitney *et al*. 2007; Gleiss *et al*. 2010), few studies have applied these accelerometry techniques to wild fishes (*e.g.,* Murchie *et al*. 2011; Wilson *et al*. 2013), even though these techniques make it possible to associate ecologically meaningful energetic costs with behaviors observed in the wild.

The objective of this study was to estimate the frequency and energetic costs of different swimming behaviors in wild, free-swimming sharks. To accomplish this, we conducted a series of laboratory and field experiments to (1) generate predictive relationships between accelerometric activity levels and aerobic metabolic rates, (2) assign accelerometric characteristics to swimming behaviors for remote observation, and (3) generate acceleration data for wild sharks. Data on the energetic costs and occurrence of swimming behaviors provides a baseline for interpreting the effects of anthropogenic and environmental stressors, and insight into the magnitude of additional activity costs when fishes recruit anaerobic metabolism.

Materials and methods

Study site and species

All research was conducted at the Cape Eleuthera Institute (CEI), located at Cape Eleuthera, Eleuthera, The Bahamas (24˚49'46.43" N, 76˚19'41.49" W). Juvenile lemon sharks (*Negaprion brevirostris*) were selected as the subject for this study because their abundance at the study site (Harborne *et al*. 2016), strong site fidelity and no seasonal patterns in habitat use (Murchie *et al*. 2010) facilitate retrieval of biologging devices from free-swimming animals (Wilson *et al*. 2008). In addition, juveniles are small enough for use in modestly-sized respirometers (Nixon and Gruber 1988; Bushnell *et al*. 1989) and lemon shark energetics have been extensively studied relative to other elasmobranchs (*e.g.,* Bushnell *et al*. 1989; Sundström and Gruber 1998).

Animal collection and husbandry

Juvenile lemon sharks (n = 21, 71.6 ± 1.1 cm total length, 1.9 ± 0.1 kg, eight female and 13 male) were collected from tidal mangrove creeks using block seine netting between 2 June and 2 December 2015.

Sharks were transferred from the seine net to individual 200 L coolers with rubber dip nets for total length measurement and passive integrated transponder tagging (Manire and Gruber 1991; Feldheim *et al*. 2002). For field deployments, a subset of sharks was equipped with external acceleration biologgers and released to the site of capture. For laboratory experiments, a subset of sharks was transported by boat in 200 L coolers to CEI's wet lab facility. Of the 21 sharks captured throughout the entire study, seven were used across two components of the study; three sharks used to generate an ethogram were released to the wild and recaptured nine days later for field deployment of acceleration biologgers, one shark from the ethogram study was recaptured five months later for use in respirometry, and three sharks used for field deployments were recaptured four months later for use in respirometry. All other sharks were used only once. Transport lasted no longer than 60 minutes with half of the water replaced in coolers every five minutes of transit (Brooks *et al*. 2011). Upon arrival at the wet lab, sharks were moved to 13,000 L (3.7 m diameter by 1.25 m deep) flow-through holding tanks continuously supplied fresh seawater from an offshore pump. Captive sharks were maintained for a minimum of three days after initial capture on a daily ration of commercially available frozen Spanish sardines (*Sardinella aurita*) fed to satiation, with the exception of a 48-hour fast before swimming respirometry experiments. Sharks were maintained in captivity for no more than four weeks, and were released to their original site of capture using the same previously outlined transport methods.

Acceleration biologgers

Externally-attached tri-axial acceleration biologgers (hereafter, accelerometers) were used throughout each component of this study to generate an acceleration ethogram of swimming behaviors, remotely observe behaviors of free-ranging sharks, and estimate metabolic costs of discrete swimming behaviors. Accelerometers (X16-mini, Gulf Coast Data Concepts, Waveland, MS, USA; 5.1 cm × 2.5 cm × 1.3 cm, 17 g; 25 Hz recording frequency; ± 16 g acceleration range, where 1 g = 9.81 m s^{-2}) were prepared and attached to the right side of shark's first dorsal fin following standardized methods for X16-mini accelerometers from Wilson *et al*. (2015). Time to attach accelerometers to sharks lasted 120 ± 5 s (mean

± standard error) for laboratory experiments and 117 ± 9 s for field deployments. Accelerometers were uniformly oriented so that acceleration logged in the heave axis (ascents and descents) was positive on ascent, the surge axis (forward movement) was positive during forward motion, and the sway axis (lateral movement) was positive to the left. Prior to deployment, accelerometers were rotated 360 degrees through all three axes to calibrate device output against gravitational acceleration (Sakamoto *et al*. 2009; Gleiss *et al*. 2010).

Acceleration ethogram

Accelerometer-equipped sharks were observed swimming in an enclosed natural habitat over a period of three days to relate known behaviors with acceleration data and generate an acceleration ethogram. Five lemon sharks (70.8 ± 1.6 cm total length, 1.83 ± 0.1 kg, three females and two males) were outfitted with individually color-coded accelerometers and released into a 4000 m^2 saltwater pond (hereafter, mesocosm) immediately adjacent to the CEI wet lab facility during 19 – 23 June 2015. The mesocosm is characterized by silt substrate, red mangroves (*Rhizophora mangle*), along with water depths (0.5 – 1.0 m) and temperatures (32.1 ± 0.9 °C) typical of lemon shark nursery habitat around Cape Eleuthera (Murchie *et al*. 2010; Harborne *et al*. 2016). Sharks were given 24 hours to overcome tagging and handling stress (Sundström and Gruber 2002; Bullock *et al*. 2015) and to acclimate to the mesocosm before behavioral observation. Following acclimation, sharks were haphazardly selected for observation by an observer with a digital watch that was synchronized with the accelerometers. The start and stop times were recorded for four swimming behaviors: "resting" (remaining stationary on the substrate and buccal pumping), "swimming" (swimming supported by a continuous and consistent tailbeat), "fast swimming" (swimming supported by a fast, unsteady tailbeat), and "burst swimming" (one or more rapid tailbeats proceeded by a short-lived increase in speed) (Whitney *et al*. 2007; Gleiss *et al*. 2009; Whitney *et al*. 2010; Brownscombe *et al*. 2014). Sharks were observed between 700 – 800, 1100 – 1200, and 1500 – 1600 over three days, and water temperature was recorded from a fixed site at the mesocosm at each observation with a field dissolved oxygen and conductivity meter (YSI Pro2030, YSI Incorporated, Yellow Springs, OH, USA). After this

monitoring period, sharks were removed from the mesocosm, and accelerometers were removed so that data could be downloaded. The classification tree algorithm was generated for only four of the five sharks because one accelerometer flooded within the first day of mesocosm acclimation.

Swimming respirometry

Accelerometer-equipped sharks were swum in two swimming respirometers to calibrate acceleration-derived metrics of activity level with rates of aerobic oxygen consumption, thereby making it possible to estimate the energetic costs of resting, swimming, fast swimming, and burst swimming behaviors observed during field deployments. Specifically, mass-specific rates of oxygen consumption (MO_2, in units of mg O_2 kg^{-1} h^{-1}) were quantified *via* intermittent-flow respirometry (Steffensen 1989) using custom-built annular and Blazka swim tunnel respirometers. Both volitional and forced swimming were employed to cover the range of activity levels expected for swimming, fast-swimming, and burst-swimming sharks because the range of activity levels and, therefore, acceleration values generated by forced swimming are not entirely representative of values derived for free-swimming sharks (Lowe 1996, Gleiss *et al*. 2010).

Volitional swimming respirometry

Five sharks (75.6 ± 4.4 cm total length, 2.3 ± 0.4 kg, two females and three males) were swum in a custom-built annular respirometer (Fig. 1.1a) between 17 November and 9 December 2015 to produce activity-specific calibration of MO_2 and acceleration values at volitional swimming velocities. The 197.7 L respirometer (86:1 respirometer-to-fish volume ratio) was composed of 6.1 m of 20.3 cm diameter corrugated clear PVC tubing with flanges at either end. To close the respirometer, both flanged ends were brought together and bolted shut, forming an oblong circle with a water-tight and an air-tight seal provided by a rubber gasket. Inflowing water was controlled by four aquarium flush pumps with a combined flush rate of 300 L h^{-1} fed to four t-shaped inlets that were positioned to divert flow perpendicular to the swimming section of the tunnel to avoid generating a directional current. Sharks, therefore, had to swim to sufficiently mix water in the respirometer for MO_2 measurements. Two additional inlets at opposite ends

of the respirometer were connected to a continuous recirculating pump line that housed a fiber optic oxygen probe and temperature probe externally (Pyroscience, Aachen, Germany). Three outlets opposite the flush pumps allowed for water outflow between measurement cycles and were buoyed at the surface to create a water barrier to oxygen diffusion from the air during measurement cycles (Svendsen *et al*. 2016a). The entire respirometer was submerged in a 3.7 m diameter by 0.5 m deep flow-through holding tank constantly supplied fresh seawater and aerated with air stones.

Sharks were fasted for a minimum of 48 hours before swimming respirometry to ensure a post-absorptive state at experimental temperatures (26.0 ± 0.3 °C; Cortés and Gruber 1992). Following fasting, a shark was weighed, outfitted with an accelerometer as described above, and placed in the respirometer for a minimum 12-hour overnight acclimation. Measurements were made the following day between 700 – 2100 only when sharks were continuously swimming for at least five minutes and when water in the respirometer was sufficiently mixed. Start and stop times of measurements were recorded for reference with acceleration data. Depending on how long a shark would swim, water inflow was shut off to measure the rate of decline in dissolved oxygen until a drop of 5 – 10 % was observed, after which water inflow was resumed for at least 40 minutes (*i.e.,* the time necessary for flush pumps to flush the entire volume of the respirometer) or until the shark transitioned from resting to continuous swimming. A typical trial consisted of four to ten iterations of this process, after which the trial was concluded, the shark was removed from the respirometer, and its accelerometer was removed for data download. Following each trial, the respirometer was drained, rinsed with fresh water, and allowed to dry completely between trials. Oxygen probes were calibrated before each trial.

Forced swimming respirometry

Eight sharks (71.2 ± 1.9 cm total length, 1.9 ± 0.1 kg, two females and six males) were swum in a custom-built Blazka swim tunnel respirometer (Fig. 1.1b) during 3 – 11 November 2015 to produce activity-specific calibration of MO_2 and acceleration values at swimming velocities characterized by anaerobic and burst swimming. The 108.7 L respirometer (57:1 respirometer-to-fish volume ratio) was composed of two

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concentric clear acrylic tubes: sharks swam in the 25.4 cm diameter inner tube, or swimming section, and inlets mounted in the outer tube allowed for water inflow and outflow and housed oxygen and temperature probes. Flow was generated by a Leeson Washguard 3-Phase AC Motor (Leeson Electric Corp., Grafton, WI, USA), which was controlled by a Leeson Speedmaster Adjustable Speed Motor Controller (Leeson Electric Corp, Grafton, WI, USA). Motor controller readings (in Hz) were calibrated against known flow velocities with a mechanical flow meter (General Oceanics, Miami, FL, USA). The front portion of the swim tunnel was covered to promote swimming at that end.

Following a 48-hour fast to ensure a post-absorptive state at experimental temperatures (29.1 ± 0.1 °C), sharks were weighed, measured, equipped with accelerometers as described above, and loaded into the swim tunnel. Six hours after loading a shark, trials began by increasing water velocity to the slowest speed at which a shark would swim steadily at the front of the tunnel. Once sharks began swimming steadily, water flow was shut off for five minutes to observe at least a 10% decline in dissolved oxygen concentration. Water flow was subsequently turned back on for an additional ten minutes to replenish dissolved oxygen levels and before increasing water velocity. Water velocity was increased by 5 cm s^{-1} and the next measurement began once the shark began swimming steadily at the new velocity. This process was repeated until sharks could no longer swim at the front end of the tunnel without the assistance of burst swimming, and trials were ended when the shark could not swim off of the background after 20 s of contact (Lee *et al*. 2003b). Times were recorded during measurement periods at each water velocity when sharks exhibited steady swimming for reference with acceleration data. Three determinations of background respiration were made before and after each trial (for a total of six) by recording the decline in dissolved oxygen concentration in the respirometer without a shark over consecutive 15-minute measurement cycles (*i.e.,* a five-minute measurement and ten-minute flush). Oxygen probes were recalibrated, and the swim tunnel was bleached before each series of consecutive trials.

Field deployments

Accelerometers were deployed on juvenile lemon sharks captured from and released to tidal mangrove creeks to generate acceleration data to estimate the frequency of swimming behaviors and energy expenditure of wild sharks. Ten sharks (70.2 ± 1.2 cm total length, four females and six males) were collected from Kemps Creek, which is a high-use tidal mangrove creek for lemon sharks (Murchie *et al.* 2010) and movement in and out of the creek is restricted to a single opening that can be blocked off with a seine net. All sharks were recaptured using block seining after 39.9 – 130.4 hours at liberty (mean = 79.2 ± 7.6 hours). Accelerometers were quickly removed by cutting the line at the backing plate opposite the accelerometer, and sharks were released back to their capture site. One accelerometer stopped logging immediately after deployment, yielding nine deployments and 897.6 hours of acceleration data collected from nine of ten individuals between 1 June and 31 July 2015. Water temperatures during deployments were 29.6 ± 0.2 °C.

Data analysis

Acceleration ethogram

Acceleration data generated during mesocosm observations were categorized into one of four swimming behaviors (resting, swimming, fast swimming, and burst swimming) to generate a classification tree (Breiman *et al.* 1984) that was used to assign swimming behaviors to acceleration data generated by field deployed sharks. Classification trees are a type of machine-learning algorithm that predict categorical outputs (*i.e.,* swimming behaviors) from continuous data (*i.e.,* acceleration data) using hierarchical decision rules generated from a training dataset (*i.e.,* the acceleration ethogram), and typically predict behaviors of free-ranging animals with high accuracy (80-90 %; Nathan *et al.* 2012; Brownscombe *et al.* 2014).

Eleven acceleration metrics (described below) were derived from raw acceleration data and input into the training dataset. Means and standard deviations were calculated from raw acceleration data (in units of standard gravity, where 1 g = 9.81 m s^{-2}) in each axis over one-second intervals (*i.e.,* 25 values per second; Brown *et al.* 2013; Graf *et al.* 2015). Overall dynamic body acceleration (ODBA; Wilson *et al.*

2006), a strong proxy across taxa that relates acceleration generated by muscular contraction to oxygen consumption (Gleiss *et al*. 2011), was derived by separating raw acceleration into static (gravity) and dynamic (shark movement) acceleration with a 2 s smoothing interval (Shepard *et al*. 2008a; Wilson *et al*. 2015; Metcalfe *et al*. 2016), subtracting static acceleration from raw acceleration in each axis, and summing the absolute value of dynamic acceleration in each axis to produce a single metric (*g*). Pitch and roll (°) were calculated according to

$$Pitch = (Surge_{static} - \mu) * \frac{180}{\pi}$$

$$Roll = (Sway_{static} - \mu) * \frac{180}{\pi}$$

where surge$_{static}$ and sway$_{static}$ refer to values of static acceleration in the sway and surge axes, and μ refers to the mean surge$_{static}$ or sway$_{static}$ value of a shark at rest for one minute (Shepard *et al*. 2008b; Brownscombe *et al*. 2014). Lastly, tailbeat frequency (TBF; Hz) and tailbeat acceleration amplitude (TBAA; *g*) were calculated through spectral analysis of dynamic swaying acceleration (Sakamoto *et al*. 2009). All 11 metrics (mean and standard deviation of surging, swaying, and heaving acceleration, ODBA, roll, pitch, TBF, and TBAA) were calculated over one-second intervals (*i.e.,* every 25 values). One instance of each behavior was analyzed for each shark for each time of day and included in the training dataset. Acceleration metrics were derived from raw acceleration data using Igor Pro (Version 6.3.3.5, WaveMetrics Inc., Lake Oswego, OR, USA) and Ethographer (Sakamoto *et al*. 2009), and the classification tree was generated using the tree package (Ripley 2016) in R (R Core Team 2015). Tree size (*i.e.,* the number of behavior categories) was selected using V-fold cross-validation (De'ath and Fabricius 2000). This made it possible to determine whether observed behavior categories had different acceleration criteria (*e.g.,* an optimal tree with three terminal nodes would indicate that two behavior categories were indistinguishable from acceleration characteristics), and whether an observed behavior category would be better off split into more than one behavior category.

Swimming respirometry

Mass-specific oxygen consumption rates were correlated with acceleration metrics to provide an equation to predict activity-specific metabolic rates for field deployed sharks. Mass-specific rate of oxygen consumption was calculated according to

$$MO_2 = \Delta O_2 \frac{v}{mt}$$

where ΔO_2 is the change in dissolved oxygen concentration (mg O_2 L^{-1}) every second, v is the volume of water in the respirometer (*i.e.,* the respirometer's volume minus the shark's volume assuming 1 kg of shark equals 1 L of seawater; Lee *et al*. 2003b), m is the mass of the animal, and t is the time interval over which ΔO_2 is measured (h). Overall dynamic body acceleration and TBF were derived from raw acceleration during measurement periods and averaged over each measurement. Acceleration (ODBA and TBF) and MO_2 data were analyzed for each respirometer separately and combined for the two respirometers to generate six predictive relationships for MO_2 with simple linear regressions. Of these, a single model was selected to predict field metabolic rates on the basis of model fit (adjusted R^2; Zuur *et al*. 2007).

For the selected model, the influence of temperature and individual variation on MO_2 was assessed with a linear mixed effects model, where MO_2 was the response variable, the acceleration metric and temperature were continuous fixed effects and shark ID was a random effect. The importance of fixed effects was estimated by running 1000 posterior simulations of each fixed effect. Significance was determined if the 95% credible interval (CI) for the distribution of fixed effect estimates did not overlap zero (*e.g.,* Guida *et al*. 2016). Linear model outputs are therefore presented as the values of the upper (97.5% CI) and lower bounds (2.5% CI) of the CI.

Field deployments

Acceleration data generated during field deployments were analyzed to determine the frequency of occurrence of swimming behaviors and their associated aerobic metabolic costs. The beginning of each deployment was analyzed to determine the period of time to exclude post-release until consistent swimming

behavior resumed. Specifically, ODBA was averaged every minute and binned into ten-minute bins, the means of which were compared in chronological order with Student's *t*-tests until the value of one bin was not significantly different from the chronological next bin (Bullock *et al*. 2015). Elevated activity levels post-deployment may be indicative of tagging and handling stress (Sundström and Gruber 2002; Bullock *et al*. 2015), and data characteristic of elevated activity levels immediately post-release were excluded from further analysis (Lowe 2002). The same 11 metrics used to generate the classification tree were calculated for every second of deployment until the time seine nets were set to recapture to exclude any effects of researcher presence on animal behavior. In one instance where a shark was not recaptured during an initial attempt, 12 hours were excluded from the start of that recapture attempt. Data for each shark were subsequently run through the classification tree model to estimate the frequency of each behavior, and instantaneous metabolic rates were estimated using linear equations generated from swimming respirometry studies.

Instantaneous metabolic rates were used to determine field metabolic rate (FMR; the average metabolic rate of the entire deployment) and the average metabolic rate of each behavior. Specifically, instantaneous metabolic rates for active swimming behaviors (swimming, fast swimming, and burst swimming) were estimated using linear model equations. Maximum metabolic rate (MMR; the highest metabolic rate achieved during aerobic swimming; Norin and Clark 2016) calculated as the mean of the highest MO_2 measured from Blazka swim tunnel experiments (Sepulveda *et al*. 2007) was used as an upper limit for estimation, *i.e.,* it was assumed that, for wild sharks, metabolic rates could not exceed lab-derived MMR, and all field estimates exceeding MMR were set equal MMR. Standard metabolic rate (SMR; the metabolic rate of a postprandial shark at rest; Chabot *et al*. 2016) was estimated by extrapolating linear models to zero activity (Bushnell *et al*. 1989; Roche *et al*. 2013), and the metabolic rate estimated during each instance of resting was set to SMR.

Results

Acceleration ethogram

The optimal classification tree with four behavior categories (*i.e.,* resting, swimming, fast swimming, and burst swimming) predicted behaviors in the training dataset with 95.4 % accuracy (*i.e.,* the algorithm correctly classified 95.4 % of the data used to construct it) using only ODBA and TBF as criteria for decision rules. The number of observations and criteria for each behavior, as well as descriptive statistics for only ODBA and TBF from the training dataset, are presented in Table 1.1.

Swimming respirometry

While regressions of ODBA (Fig. 1.2a) and TBF (Fig. 1.2b) against MO_2 yielded significant linear regressions within each respirometer (except for ODBA against MO_2 in the annular respirometer; Fig. 1.2a), models that combined respirometers were more optimal from an objective model selection perspective. Of these combined models for ODBA (Fig. 1.3a) and TBF (Fig. 1.3b), TBF (adjusted $R^2 = 0.67$) was objectively the better predictive model by explaining more variation in metabolic rate than ODBA (adjusted $R^2 = 0.35$). Therefore, the linear model for TBF was used to estimate field metabolic rates. Despite the variation in temperature experienced between measurements made for each respirometer (24.1 – 28.6 °C annular, 28.4 – 29.1 °C Blazka), temperature did not influence variation in metabolic rates (2.5% CI = 30.7, 97.5% CI = -0.7); only TBF influenced variation in metabolic rate (2.5% CI = 130.4, 97.5% CI = 29.8). The TBF model predicted a SMR of 160.8 mg O_2 kg^{-1} h^{-1}, and MMR was 462.6 mg O_2 kg^{-1} h^{-1} estimated from Blazka trials.

Field deployments

Juvenile lemon sharks spent the majority of each day swimming (76.7 ± 1.2 %), followed by fast swimming (17.9 ± 0.9 %), resting (4.9 ± 0.6 %), and burst swimming (0.4± 0.1 %; Fig. 1.4). Activity levels (*i.e.,* ODBA) were elevated post-release for most sharks (range = 0 – 70 minutes), but sharks resumed consistent activity levels 25.6 ± 6.6 minutes post-release. Sharks had a mean FMR of 255.5 ± 1.0 mg O_2

kg^{-1} h^{-1}, mean swimming metabolic rate was 249.1 ± 0.7 mg O_2 kg^{-1} h^{-1}, mean fast swimming metabolic rate was 304.9 ± 1.3 mg O_2 kg^{-1} h^{-1}, mean burst swimming metabolic rate was 357.9 ± 2.0 mg O_2 kg^{-1} h^{-1}, and resting was assumed to have a metabolic rate equivalent to SMR.

Discussion

Wild sharks exhibited clear partitioning of swimming behaviors, favoring slow and consistent activity levels and minimizing time at rest. Specifically, lemon sharks engaged in slow, aerobic swimming 77 % of the time, anaerobic and burst swimming 18 % of the time, and resting only 5 % of the time. Despite possessing a unique adaptation among Carcharhiniformes to buccal pump (Dapp *et al*. 2016) and the energetic savings afforded by resting relative swimming (*e.g.,* Whitney *et al*. 2016), lemon sharks were active 95 % of the time. Bonefish (*Albula vulpes*), a tropical teleost with a detailed behavioral time budget, exhibited burst swimming behaviors with identical frequencies as wild lemon sharks (0.4 – 0.5 %; Murchie *et al*. 2011), but bonefish swam considerably less (26 – 51.7 %; Murchie *et al*. 2011, Brownscombe *et al*. 2014) despite having higher metabolic rates at comparable temperatures (Nowell *et al*. 2015). Generally, routine swimming velocities across shark and teleost species scale positively with metabolic rate (Watanabe *et al*. 2012; Jacoby *et al*. 2015), though these species have disparate feeding and spatial ecologies. Specifically, a preference for continuous swimming activity over periods of rest may be driven by this species' feeding ecology, whereby lemon sharks are opportunistic piscivores (Cortés and Gruber 1990) and bonefish forage in the benthos. Furthermore, predator-prey interactions may drive activity levels in juvenile lemon sharks, given that lemon sharks must avoid inter- and intra-specific predation within a limited daily home range (Murchie *et al*. 2010; Guttridge *et al*. 2012). Lastly, ventilation efficiency may drive a propensity toward continuous activity, given that ram ventilation is generally a more efficient method of gas exchange than active ventilation methods (Steffensen 1985), and lemon sharks are characterized by an internal hypoxic state (Bushnell *et al*. 1982). Therefore, although lemon sharks exhibited a range of swimming behaviors, animals spent the majority of daily activity maintaining slow, aerobic swimming.

Swimming behaviors had discrete energetic costs, and more costly behaviors occurred less frequently during daily activity. Slow, aerobic swimming had an estimated energetic cost of 249.1 ± 0.7 mg O_2 kg^{-1} h^{-1} and occurred most frequently, whereas fast, anaerobic and burst swimming behaviors had estimated costs of 304.9 ± 1.3 mg O_2 kg^{-1} h^{-1} and 357.9 ± 2.0 mg O_2 kg^{-1} h^{-1}, and occurred 17.9 % and 0.4 % of the time, respectively. Activity levels with high associated metabolic rates are less frequently observed in free-swimming obligate ram-ventilating sharks (Lowe 1996; 2001; Gleiss *et al*. 2010), and this study suggests that an active buccal pumping species also frequently exhibits swimming velocities that minimize transportation costs in the wild (Scharold and Gruber 1991; Lowe 2001). Lemon sharks were predicted to have higher maintenance costs (SMR) and rest less often than a relatively inactive sympatric species (*Ginglymostoma cirratum*) with lower maintenance costs (Whitney *et al*. 2016), though bonefish have even higher SMR than lemon sharks and rest considerably more often (Murchie *et al*. 2011; Brownscombe *et al*. 2014; Nowell *et al*. 2015), and SMR might not be a good indicator of behavior in the wild (Laskowski *et al*. 2016). Similar to bonefish, however, slow, aerobic swimming behaviors predominated, and resting and bursting behaviors were observed less frequently (Murchie *et al*. 2011). Based on these data, a shark captured in fishing gear might be expected to exhibit more frequent anaerobically-fueled behaviors to support high-energy escape responses or to maintain sufficient swimming velocities to improve gas exchange necessary to resolve physiological disturbance, thereby increasing diel consumption requirements. Furthermore, sharks in coastal habitat subjected to pollution or degradation (*e.g.,* dredging effects on nursery habitat; Jennings *et al*. 2008) might be expected to increase activity costs searching for diminished prey populations, or reduce energy allocation to tissue production if activity-influenced consumption requirements cannot be met. Though sharks may adjust behaviors accordingly in response to stressors, increases in diel energy consumption requirements that exceed actual energy intake may have long-term consequences by reducing the energy available for somatic or gonadal tissue production. Therefore, these data provide a baseline for determining the energetic consequences of an individual's behavioral response to a stressor.

Anaerobically-fueled swimming behaviors were prevalent in daily activity and represent a source of additional energetic costs. Fast and burst swimming behaviors that recruit anaerobic metabolism represented 18 % of daily swimming activity, though the additional anaerobic costs of these swimming behaviors could not be directly estimated. Burst swimming is powered almost exclusively by white, glycolytic muscle and anaerobic metabolism (Shadwick and Goldbogen 2012) and prolonged reliance on burst swimming at the final velocity increment in Blazka trials resulted in exhaustion (Norin and Clark 2016). Furthermore, fishes can recruit anaerobic metabolism to support swimming at high-end aerobically-supported velocities between 59 – 70 % of a fish's maximum aerobically-sustained swimming velocity, and anaerobic costs can account for an additional 21.4 – 50.5 % of energy expended (Burgetz *et al*. 1998; Lee *et al*. 2003a; Svendsen *et al*. 2010). While this study could not determine when lemon sharks recruit anaerobic metabolism, previous studies have accounted for anaerobic costs of swimming by applying an anaerobic tax to swimming data exceeding a fixed percentage of a fish's maximum aerobically-sustained speed (*e.g.,* Rand and Hinch 1998). Using 70 % (Burgetz *et al*. 1998) of a lemon shark's maximum aerobically-sustained TBF as a conservative estimate of the extent to which sharks may have recruited anaerobic metabolism, and applying a 20 % (Lee *et al*. 2003a) increase in instantaneous energy expenditure as a conservative estimate of anaerobic costs increased overall activity energy expenditure by 4.6 ± 0.2 %. While instantaneous energetic costs may be 21.4-50.5 % (Burgetz *et al*. 1998; Lee *et al*. 2003a; Svendsen *et al*. 2010) higher when anaerobic metabolism is recruited to support swimming, this may translate to a much lower increase in daily energy expenditure. Minimal recruitment of anaerobic metabolism to support routine activity may, therefore, serve to minimize daily energy expenditure or to maintain sufficient anaerobic energy stores to fuel escape from predators. Thus, while it was not possible to measure anaerobic metabolic costs of swimming to calculate field costs, studies in teleosts suggest that swimming behaviors exhibited by wild sharks incurred minimal costs from anaerobic metabolism.

Combining swimming respirometer techniques was a suitable means for obtaining a broad range of ecologically relevant metabolic rates for juvenile lemon sharks. Specifically, combining data collected

from two different swimming respirometers made it possible to generate a predictive relationship between TBF and metabolic rate that predicted a biologically sound SMR. An annular respirometer appeared to compliment metabolic rates and activity levels measured in a Blazka swim tunnel by allowing sharks to swim at velocities more typical of slow swimming behavior (see Lowe 1996; Gleiss *et al*. 2010), and allowed us to predict metabolic rates across a broad range of activity levels. With regard to SMR, our estimate is lower than temperature-corrected estimates for lemon sharks based on SMR extrapolated from power-performance curves derived at lower temperatures (*e.g.,* 189 – 236 mg O_2 kg^{-1} h^{-1} corrected to 30 °C with a Q_{10} of 2.3 from 22 °C and 25 °C, respectively; Bushnell *et al*. 1989; Scharold and Gruber 1991); however, direct measurement of SMR for juvenile lemon sharks at Cape Eleuthera for a concurrent study indicates that SMR at 30 °C is approximately 155 mg O_2 kg^{-1} h^{-1} (I.A. Bouyoucos unpublished data). While using two respirometers expanded the range of activity levels and metabolic rates that could be measured, the authors acknowledge potential concern for combining data generated by subjecting animals to two different experimental devices. While both respirometers had similar internal conditions, *i.e.,* similar internal diameters (annular = 20.3 cm, Blazka = 25.4 cm) and the same water source, acclimation times and length time spent in respirometers was different, and it was not possible to quantify background respiration for the annular respirometer. In addition, the relationship between activity level and metabolic rate did vary between respirometers (*i.e.,* different slopes), though this may have resulted from individual variation in metabolic rates because there were no apparent gait transitions in either respirometer, sharks did not transition respiratory modes while swimming (Steffensen 1985), and respirometers had reasonable respirometer-to-fish volume ratios (Svendsen *et al*. 2016b). Furthermore, differing sources of stress experienced in either respirometer, *e.g.,* noise in the Blazka respirometer (Lowe 1996) or lack of current in the annular respirometer (Farrell *et al*. 2003; Chabot *et al*. 2016) and circular swimming pattern (Weihs 1981) may have influenced measurements. Therefore, while combining data from different swimming respirometers produced a reliable predictive relationship (R^2 = 0.67), these results should be interpreted with caution given differential sources of stress and measurement error associated with each respirometer type.

In conclusion, our study provides a preliminary view of the energetic costs of discrete swimming behaviors for a wild fish. These data improve our ability to address responses to stressors in this species, whereby behavioral modifications have associated energetic costs that can be linked to changes in diel energy expenditure. By linking energetic costs to behaviors, behavioral responses to a stressor can be scaled to address potential changes in energy allocation to production of somatic and gonadal tissues, which can ultimately address changes survival or reproductive success for exploited populations. Therefore, this study offers an enhanced understanding of the basic ecology of the wild juvenile lemon shark, and it is our intention that these findings further support the importance of including behavior-specific metabolic rates when constructing energetics models for wild fishes (Cooke *et al*. In Press; Treberg *et al*. In Press).

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Tables

Table 1.1. Values for overall dynamic body acceleration (ODBA) and tailbeat frequency (TBF) used to generate a classification tree machine learning algorithm to distinguish four swimming behaviors (resting, swimming, fast swimming, and burst swimming) from acceleration data generated by juvenile lemon sharks in the wild. For instance, data with an overall dynamic body acceleration (ODBA) value greater than 0.03 *g* but a tailbeat frequency (TBF) less than 1.26 Hz would be classified as swimming. Values for each metric are presented as mean ± standard error and the range (*i.e.,* maximum minus minimum). Tailbeat frequency values for resting sharks were estimated as greater than zero because spectral analysis detected periodicity in dynamic swaying acceleration signals, though TBF was coincidentally not used to distinguish resting from other behaviors.

Behavior (Observations)	Rest (n = 9)	Swim (n = 12)	Fast swim (n = 8)	Burst (n = 8)
Criteria	**ODBA < 0.03 *g***	**TBF < 1.26 Hz** **ODBA ≥ 0.03 *g***	**ODBA < 0.31 *g*** **TBF ≥ 1.26**	**ODBA ≥ 0.32 *g***
ODBA (g)	0.01 ± 0.00 (0.02 – 0.06)	0.08 ± 0.00 (0.04 – 0.28)	0.15 ± 0.00 (0.05 – 0.50)	1.28 ± 0.18 (0.13 – 6.77)
TBF (Hz)	0.57 ± 0.00 (0.35 – 2.03)	1.09 ± 0.00 (0.51 – 5.28)	1.48 ± 0.02 (0.46 – 3.84)	4.16 ± 0.29 (0.63 – 8.08)

Figures

A.
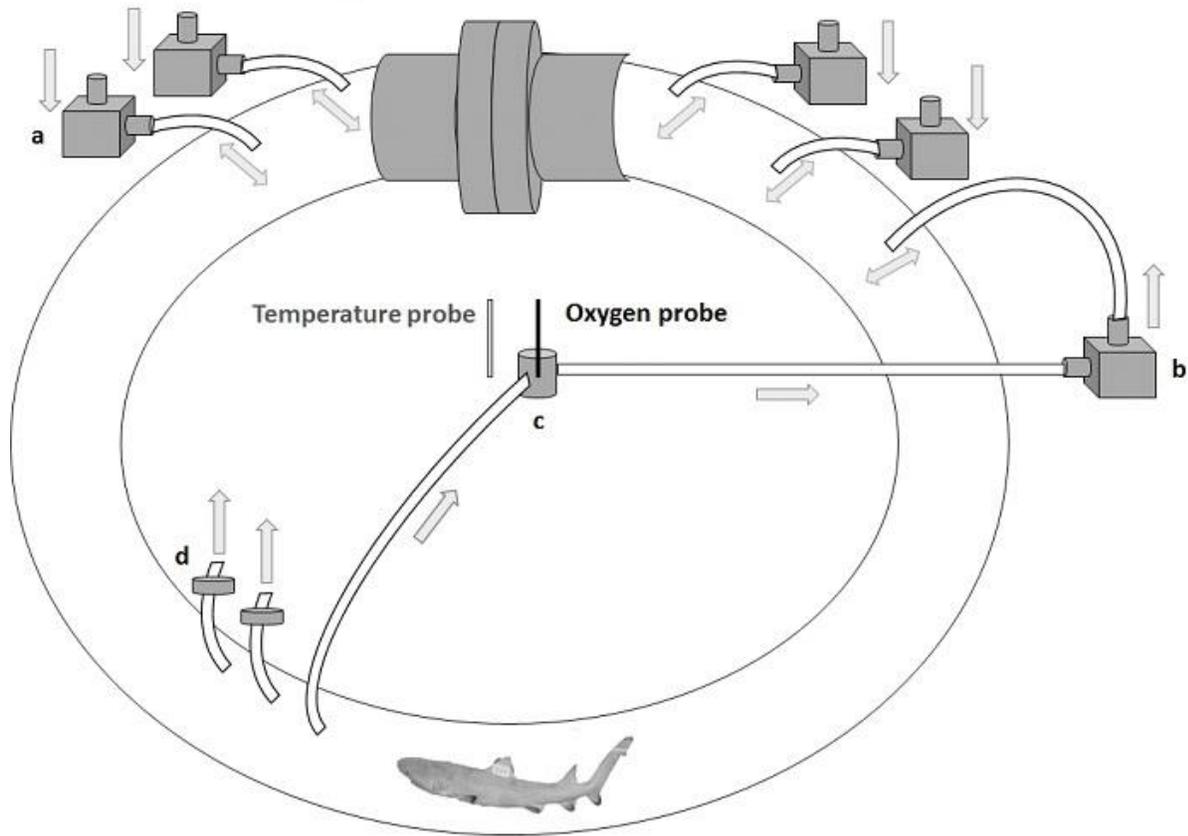
B.
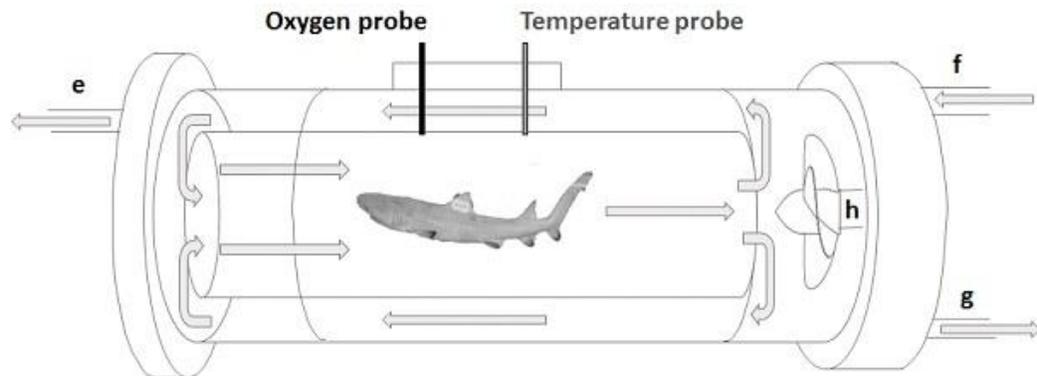
Figure 1.1. Annular (A) and Blazka (B) swim tunnel respirometer diagrams. Arrows represent the direction of water flow into, out of, and through respirometers. Lowercase letters denote specific components of each respirometer. Annular: (a) flush pumps (×4), (b) recirculating pump and closed recirculating loop, (c) internally-housed fiber optic oxygen probe and externally-housed temperature probe, and (d) water outflow (×2) buoyed to the surface of a common tank. Blazka: (e) water outflow, (f) water inflow, (g) drain/outflow, and (h) impellor.
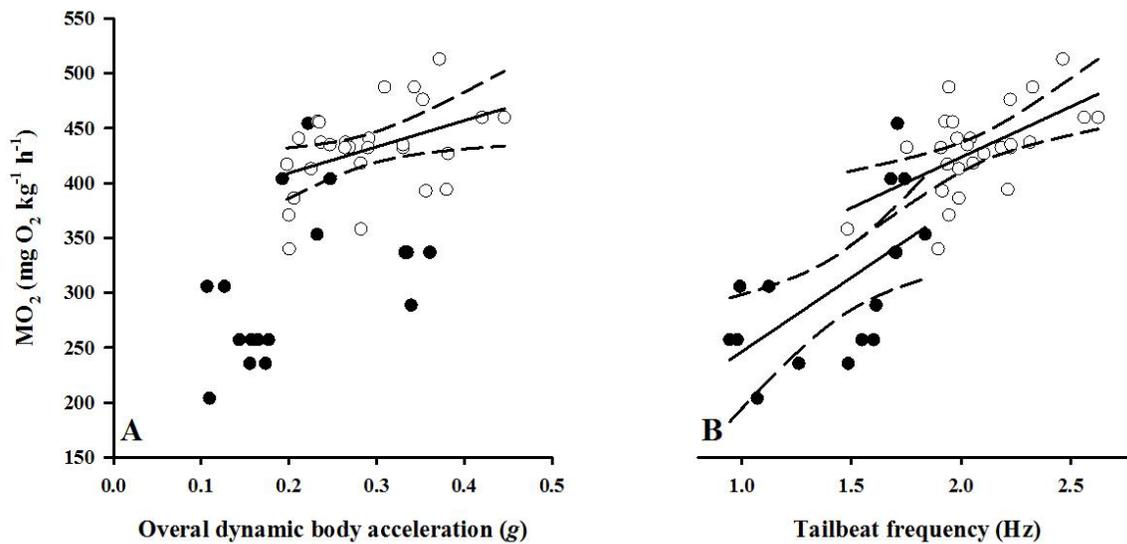
Figure 1.2. Linear regressions of overall dynamic body acceleration (ODBA; A) and tailbeat frequency (TBF; B) on metabolic rate (MO_2) generated with two different swimming respirometers. Filled and empty circles represent measurements taken in an annular and Blazka swim tunnel respirometers, respectively. Significant predictive relationships were generated with an annular respirometer for TBF (Linear regression, $R^2 = 0.35$, $F_{1, 15} = 9.2$, p = 0.008) but not ODBA (Linear regression, $R^2 = 0.18$, $F_{1, 15} = 3.6$, p = 0.078), and with a Blazka swim tunnel respirometer for TBF (Linear regression, $R^2 = 0.30$, $F_{1, 26} = 12.5$, p = 0.002) and ODBA (Linear regression, $R^2 = 0.16$, $F_{1, 26} = 5.9$, p = 0.022). Regression lines are presented with 95 % confidence intervals.
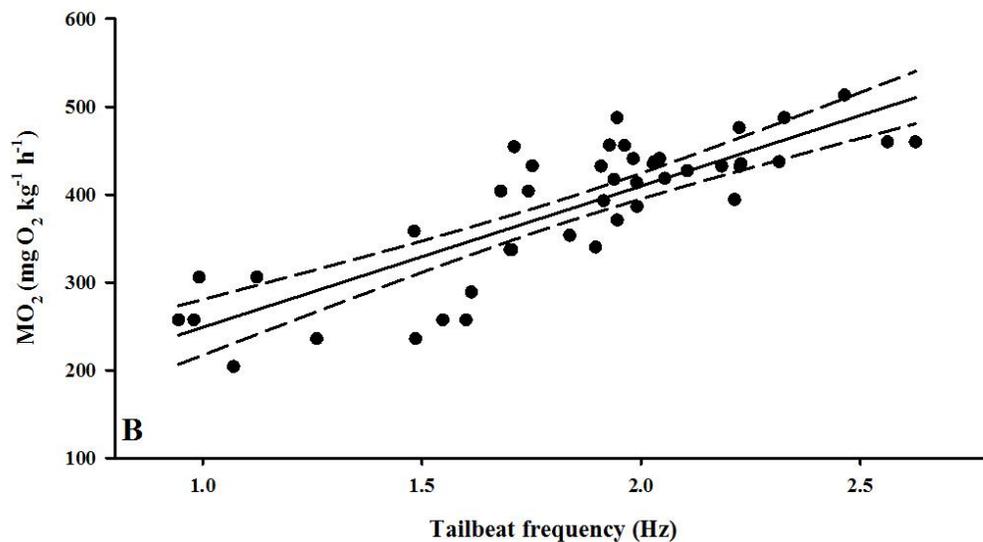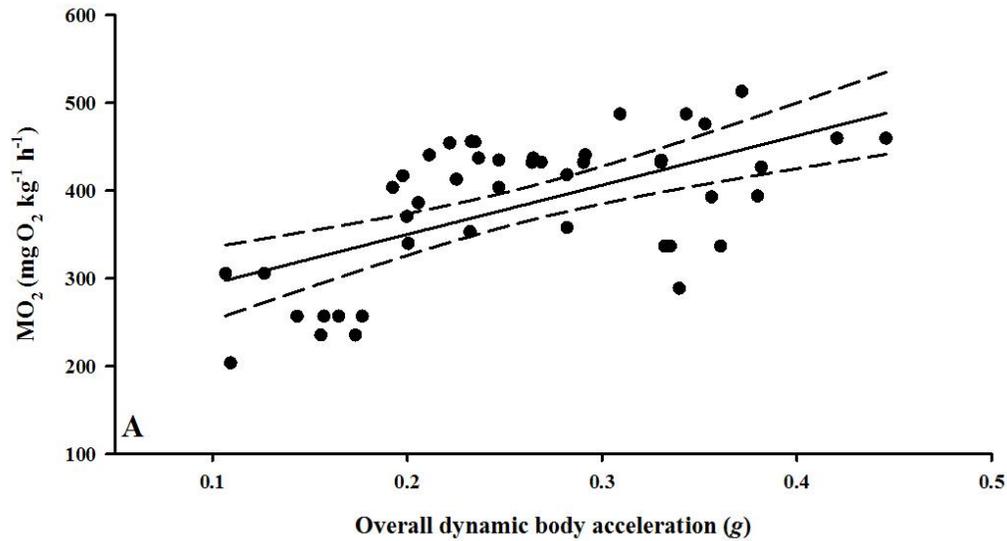
Figure 1.3. Linear regressions of overall dynamic body acceleration (ODBA; A) and tailbeat frequency (TBF; B) on metabolic rate (MO_2) for combined data generated by two swimming respirometers. Significant predictive relationships were generated when combining data from both respirometers for ODBA (Linear regression, $R^2 = 0.35$, $F_{1, 43} = 24.3$, $p < 0.0001$) and TBF (Linear regression, $R^2 = 0.67$, $F_{1, 43} = 91.7$, $p < 0.0001$). Regression lines are presented with 95 % confidence intervals.
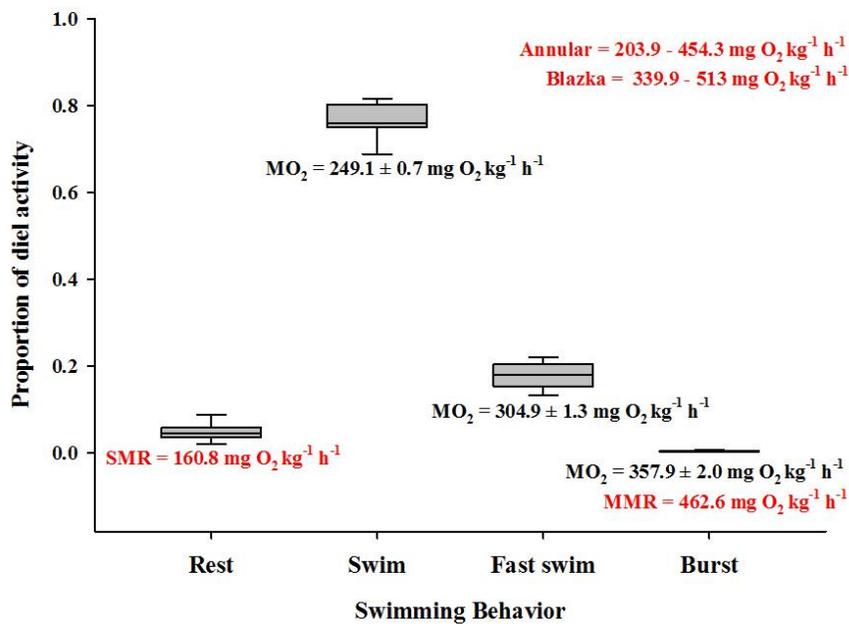
Figure 1.4. Boxplot of the frequencies of four swimming behaviors (resting, swimming, fast swimming, and burst swimming) estimated from acceleration data generated by sharks in the wild. Metabolic rates in black represent the mean (± standard error) metabolic rate incurred by a given behavior estimated from a predictive relationship established between tailbeat frequency and metabolic rate from respirometry experiments. Metabolic rates in red represent values estimated directly from respirometry experiments. Specifically, standard metabolic rate (SMR) was estimated from the predictive relationship mentioned above as the metabolic rate of a shark at rest, and therefore the field and laboratory estimates are identical. The maximum metabolic rate is the maximum aerobic metabolic rate a shark can sustain, and it was assumed that metabolic rate data extrapolated by the predictive relationship could not exceed this value. Lastly, ranges of metabolic rates measured during swimming respirometry in an annular respirometer and Blazka swim tunnel respirometer are presented.

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CHAPTER TWO: ENERGETICS AND BEHAVIORAL RESPONSES OF LEMON SHARKS TO SIMULATED LONGLINE CAPTURE

Abstract

Commercial fisheries bycatch is the greatest perceived threat to elasmobranch population recovery, and techniques to mitigate sub-lethal outcomes can be improved with data on the behavior of captured animals. This study sought to estimate the effects of simulated longline capture on the behavior and energy use of juvenile lemon sharks (*Negaprion brevirostris*). Captive sharks equipped with acceleration biologgers were subjected to one hour of simulated longline capture and 15 hours of post-capture observation. Swimming behaviors were identified from acceleration data using a machine-learning algorithm, and energetic costs were estimated using accelerometer-calibrated relationships generated with respirometry. Captured sharks exhibited nine-fold increases in the frequency of burst swimming events, 97% reductions in resting, and maintained swimming behaviors similar to free-swimming sharks. Furthermore, sharks were estimated to resume routine bursting, resting, and swimming behaviors 4.0, 9.8, and 7.9 hours after capture, and physiological recovery was estimated to occur within 5.4 hours. Ram ventilation may be a preferred strategy over stationary ventilation for lemon sharks to resolve physiological stress from capture, and these data support the notion that longline capture is a benign capture method because hooked sharks can approximate the aerobic swimming behavior and energy expenditure of free-swimming animals. Therefore, these results have implications for mitigating sub-lethal outcomes of capture for sharks as commercial longline bycatch by supporting the development of minimally-restrictive fishing gears, increasing gangion length, and periodic gear checks.

Introduction

Unused or unmanaged capture (*i.e.,* bycatch) is the greatest perceived threat to global shark populations (Stevens *et al*. 2000; Oliver *et al*. 2015). At present, the majority of our understanding of how sharks respond to the stress of fishing capture focuses on the secondary stress response of various species in different gear types (*e.g.,* longline: Brooks *et al*. 2012; trawl: Heard *et al*. 2014; gill-net: Frick *et al*. 2012; drum-line: Gallagher *et al*. 2014a), but considerably less is known about post-release or chronic sub-lethal outcomes (Molina and Cooke 2012; Skomal and Mandelman 2012). While mortality (both immediate and post-release) is an important outcome of capture to quantify for managing shark populations, defining sub-lethal consequences of bycatch may ultimately determine the extent to which individual fitness is affected (Wilson *et al*. 2014). For instance, changes in behavior (*e.g.,* Frick *et al*. 2012) and energy use (*e.g.,* Guida *et al*. 2016b) can translate to organismal and population-level consequences if rates of energy acquisition are altered (*e.g.,* Lemon 1991). Specifically, energy allocated to recovery may reduce energy investment in growth or reproduction (Romero *et al*. 2009), though this link needs to be established for elasmobranchs (Skomal and Mandelman 2012). Therefore, quantifying sub-lethal consequences of capture, including behaviors and energy use, is necessary to improve our understanding of the fate of shark bycatch post-release, and for mitigating sub-lethal outcomes of capture for sharks as commercial longline bycatch.

Behavioral responses of sharks to capture include brief bouts of high-intensity thrashing or burst swimming (*e.g.,* Frick *et al*. 2009; 2010), resting on the bottom or suspended in fishing gear (*e.g.,* Frick *et al*. 2009; 2010; Guida *et al*. 2016a), and steady swimming (*e.g.,* Frick *et al*. 2010). The magnitude and duration of these behaviors are thought reflect pace-of-life traits (*e.g.,* stamina; Gallagher *et al*. 2014a) and have the potential to mitigate the severity of stress responses (Guida *et al*. 2016a) or facilitate recovery (Brooks *et al*. 2012). However, our understanding of sharks' behavioral responses to capture is mainly from laboratory experiments with a limited number of species (*e.g.,* Frick *et al*. 2010). Of these studies, behavioral responses are defined coarsely as struggling bouts inferred from pressure-sensing (*e.g.,* Guida *et al*. 2016a) or load-bearing data loggers (*e.g.,* Frick *et al*. 2009), and almost no work has sought to directly

define relationships between physiological outcomes and behavioral correlates (*e.g.,* Frick *et al*. 2009). Although burst swimming is generally linked to metabolic acidosis through an accumulation of lactate resulting from a reliance on anaerobic metabolism (Cliff and Thurman 1984), the effects of aerobically supported behaviors on whole-organism physiological responses to capture remain unknown. Understanding behavioral responses in relation to energy use and stress physiology would provide fisheries management with information to gauge the suitability of mitigation measures for sharks caught as bycatch in commercial fisheries.

Because it is impossible to eliminate shark bycatch all together, modifying characteristics of fishing gear is one potential management action that could be employed to mitigate sub-lethal outcomes or mortality, and facilitate behavioral and physiological recovery for sharks following capture (Guida *et al*. 2016a; Dapp *et al*. 2016a; 2016b). For instance, studies directed at longline capture have suggested lengthening gangions could improve an animal's ability to swim while hooked, which is thought to mitigate physiological disturbance (Dapp *et al*. 2016b; Guida *et al*. 2016a). Discussions of the potential benefits of gear modification consider how an animal's ability to ventilate may be impaired (*e.g.,* Dapp *et al*. 2016b), which is directly related to an individual's capacity for swimming and non-swimming behaviors. At present, little is known about how behavior influences energy expenditure for sharks during capture (but see Frick et al. 2009; Guida et al. 2016a), making it challenging to confidently define gear modifications that can facilitate recovery and/or minimize disturbance. Therefore, without understanding behavior as a factor influencing sub-lethal outcomes of capture, bycatch mitigation strategies for sharks are limited in effectiveness.

Our study objective was to quantify the effect of simulated commercial longline fishing capture on the swimming behavior and energy use (aerobic and anaerobic) of a carcharhinid shark. To accomplish this, we conducted a series of laboratory experiments to (1) generate predictive relationships between accelerometric activity levels and aerobic metabolic rates, (2) estimate anaerobic costs of exhaustive exercise, (3) assign accelerometric characteristics to swimming behaviors for remote observation, and (4)

generate acceleration data for sharks before, during, and after a simulated capture event. Together, these four components allowed us to compare the proportion of time devoted to swimming behaviors, as well as energy use, of sharks before, during, and after a capture event. These data are necessary for providing new insight into the physiological and behavioral sub-lethal outcomes of longline capture, which can be used to gauge the suitability of mitigation strategies for many data deficient species.

Methods

Animal collection and husbandry

Juvenile lemon sharks (*Negaprion brevirostris*) were selected as a model species for simulated longline capture experiments because juvenile lemon sharks (1) exhibit comparable aerobic metabolic capacities relative to carcharhinid sharks (Bernal *et al*. 2012), (2) can serve as surrogates for similar sized, small-bodied sharks that interact with commercial longline gear (*e.g.,* Morgan *et al*. 2010), and (3) lemon shark energetics (*e.g.,* Nixon and Gruber 1988; Bushnell *et al*. 1989; Graham *et al*. 1990; Scharold and Gruber 1991) and physiological responses to capture stress (*e.g.,* Brooks *et al*. 2011; Hyatt *et al*. 2012; Danlychuk *et al*. 2014; Gallagher *et al*. 2014a) have been well documented. However, the authors acknowledge that (1) energy use will differ between adult and juvenile sharks, and at different temperatures (Bernal *et al*. 2012), (2) lemon sharks are generally regarded as a physiologically resilient to capture stress relative to obligate ram ventilating species (Hyatt *et al*. 2012; Gallagher *et al*. 2014a; Dapp *et al*. 2016b), and (3) the magnitude of the stress response experienced by sharks caught on commercial versus simulated longline gear may differ (Frick *et al*. 2010). Therefore, comparisons between simulated capture of juvenile lemon sharks in a laboratory and capture of larger species on commercial gear in the wild should be made with caution.

Sharks were collected from tidal mangrove creeks around Cape Eleuthera (Eleuthera, The Bahamas, 24˚49'46.43" N, 76˚19'41.49" W) using block seining between 2 June and 2 December 2015. Captured sharks were transported from seine nets to 200 L coolers with rubber dip-nets for total length

measurement and passive integrated transponder tagging. Water in coolers was replenished every five minutes (Brooks *et al*. 2011), and sharks were subsequently transported by boat to a wet lab facility. At the facility, sharks were held in 13,000 L (3.7 m diameter by 1.25 m depth) flow-through holding tanks, continuously supplied with fresh seawater from an offshore pump, and exposed to natural photoperiod. Sharks were fed a daily ration of commercially available frozen Spanish sardines (*Sardinella aurita*), with the exception of a 48-hour fast to ensure a post-absorptive state at experimental temperatures (Cortés and Gruber 1992) prior to all experiments. After experimentation, sharks were returned to their capture site using the same transportation methods detailed above.

Acceleration biologgers

Externally-attached tri-axial acceleration biologgers (hereafter, accelerometers) were used to quantify activity levels of sharks and to estimate the occurrence of different swimming behaviors before, during, and after simulated capture. Accelerometers (X16-mini, Gulf Coast Data Concepts, Waveland, MS, USA; 5.1 cm × 2.5 cm × 1.3 cm, 17 g; 25 Hz recording frequency; ± 16 *g* acceleration range, where 1 *g* = 9.81 m s^{-2}) were prepared and uniformly mounted on the right side of the dorsal fin following standardized protocols for the X16-mini used by Wilson *et al*. (2015). Time to attach accelerometers to sharks lasted 125 ± 4 s (standard error) for all laboratory experiments. Prior to deployment, accelerometers were rotated 360 degrees through all three axes (*i.e.,* anterior-posterior, dorsal-ventral, and lateral) to calibrate device output against gravitational acceleration (Sakamoto *et al*. 2009; Gleiss *et al*. 2010).

Pre-exhaustion respirometry

Aerobic metabolic rates were measured for resting sharks (n = 12, 67.5 ± 1.8 cm total length, 1.5 ± 0.1 kg, five female and seven male, caught 16 – 28 July 2015) prior to chasing to define resting metabolic rate (RMR; the metabolic rate of a resting, fasted shark at a stable temperature; Chabot *et al*. 2016), which was also used to define when animals had fully recovered from exercise associated with a capture event. Resting metabolic rate was measured using static, intermittent-flow respirometry (Svendsen *et al*. 2016). Twenty-four hours into fasting, sharks were isolated and equipped with accelerometers to standardize

handling and tagging procedures with subsequent chasing experiments. After 48 hours of fasting had elapsed, sharks were loaded into static respirometers. Accelerometers were removed before loading sharks to avoid the possibility of sharks damaging accelerometers by attempting to turn around in the respirometers. Prior to beginning measurements, respirometers were covered to minimize stress from light and traffic through the wet lab facility. Measurements were recorded over 24 hours before sharks were returned to holding tanks for at least four days prior to use in chasing experiments. Trials were conducted between 24 June and 21 August 2015 when water temperatures averaged 30.2 ± 0.2 °C.

Static respirometry chambers (38.6 L; 76.2 cm length by 25.4 cm diameter) were composed of a clear acrylic tube with a PVC cap at one end, and a PVC threaded plug at the other. Because lemon sharks were flexible enough to fully turn around in the 25.4 cm diameter chambers, sharks were placed in plastic 2.5 cm mesh inserts (76.2 cm length by 15.2 cm diameter) within chambers to minimize spontaneous movement. Two inlets at either end of a chamber were attached to a 600 L h^{-1} flush pump, an outflow tube buoyed to the surface (Svendsen *et al*. 2016), and a 300 L h^{-1} pump connected to a closed recirculating loop that housed a fiber optic oxygen probe (calibrated before each use) and temperature probe externally (Loligo Systems, Copenhagen, Denmark). Four chambers were submerged in two flow-through tanks ($3.1 \times 0.7 \times 0.2$ m) supplied UV- and mesh- (300 μm, 50 μm, and 25 μm) filtered seawater. The automated respirometry system (AutoResp software, Loligo Systems, Copenhagen, Denmark) was set to run 15-minute measurement cycles, where flush pumps were shut-off for three minutes to measure a 10-15% decline in dissolved oxygen concentration, and turned on for the remaining 12 minutes to oxygenate chambers and remove metabolic wastes (Svendsen *et al*. 2016). Background respiration was accounted for by running blank chambers before and after respirometry trials, and where possible, blank chambers were run concurrently with animals to account for bacterial respiration for the entire respirometry system (Rodgers *et al*. 2016).

Exhaustive exercise

Approximately four days after RMR measurements, the same sharks used in the RMR study were chased to exhaustion to generate acceleration data characteristic of swimming behaviors and to induce physiological exhaustion, thereby making it possible to quantify maximum metabolic rate (MMR; the highest aerobic metabolic rate measured after the cessation of exhaustive activity; Norin and Clark 2016), anaerobic costs and recovery from exhaustive exercise. Prior to each day of chasing, two to four sharks were equipped with accelerometers and moved to individual holding tanks with reduced water level (3.7 m diameter by 0.6 m depth) for a minimum of 24 hours to acclimate to the reduced water level (Brooks *et al*. 2011) and overcome handling and tagging stress (Sundström and Gruber 2002). In addition, sharks were observed resting (n = 9; three sharks never rested) and swimming (n = 12) to generate acceleration criteria characteristic of those behaviors. The following day, two personnel with rubber dip-nets entered the tank at opposite ends and immediately chased sharks by splashing behind the caudal fin until sharks exhausted, which was defined as slow, sustained swimming, and an inability to respond to splashes with bursting (e.g. McKenzie *et al*. 1996). Sharks were additionally observed during chasing trials to generate acceleration criteria characteristic of burst swimming (n = 12). Once exhausted, sharks were immediately netted and transferred to 100 L coolers filled with seawater that were then brought to the facility's respirometry equipment to measure post-exhaustion metabolic rates.

Post-exhaustion respirometry

Aerobic metabolic rates were measured for exhausted sharks after chasing to define MMR, and the anaerobic costs and recovery time for an exhaustive event. Maximum metabolic rate and excess post-exercise oxygen consumption (EPOC; a measure of energy used to resolve physiological disturbance and replenish anaerobic substrates used during exhaustive activity; Gaesser and Brooks 1984) were measured using intermittent-flow respirometry and the same static respirometers described above. Sharks were chased one at a time, with chases staggered by 15 minutes to coincide with the beginning of new measurement cycles in the respirometry system. Accelerometers were removed prior to loading, and load times were 68

± 5 s. From the end of chasing to the beginning of the first metabolic rate measurement, total handling time across all individuals was 231 ± 33 s, of which 6 ± 1 s encompassed two brief periods of air exposure (*i.e.,* moving sharks from the chasing tank to the cooler and from the cooler into the respirometer). Measurements were recorded over 12 hours before sharks were removed and returned to holding tanks prior to release. Trials were conducted between 30 July and 24 August 2015 when water temperatures averaged 30.7 ± 0.1 °C.

Simulated capture

A new group of accelerometer-equipped sharks (n = 5, 72.5 ± 1.5 cm total length, 1.8 ± 0.06 kg, two female and three male, caught 9 September – 13 October 2015) was subjected to simulated longline capture in experimental tanks to generate acceleration data for animals before, during, and after capture. Twenty-four hours into a 48-hour fast, sharks were equipped with accelerometers and isolated in holding tanks with elevated water levels (3.7 m diameter by 1.5 m depth) for an additional 24 hours to overcome handling and tagging stress. Water level was set high enough to prevent sharks from being able to rest on the tank bottom for the duration of capture (*e.g.,* Frick *et al*. 2010) and to simulate capture conditions of mid-water or pelagic longline sets that contribute to the majority of incidences of shark bycatch (Oliver *et al*. 2015). After 48 hours of fasting, a monofilament gangion with a 14-gauge non-offset circle hook with a depressed barb made to the total length of the shark was baited with little tunny (*Euthynnus alletteratus*) and suspended from a taught line across the water's surface in the middle of the tank. Gangion length relative to shark total length was typical of scientific longline gear used to catch juvenile sharks (*e.g.,* Hyatt *et al*. 2012) and prevented sharks from resting on the tank bottom, though the size of gangions and sharks in commercial scenarios are quite variable (*e.g.,* Broadhurst *et al*. 2014). Once sharks became hooked, they were monitored for one hour until they were removed from gangions by clipping the exposed barb or hook shank with bolt cutters and passing the free end of the hook through the wound. Capture duration represented a "best-case" scenario where fishing gear could be checked periodically following deployment (*e.g.,* Brooks *et al*. 2012), though typical commercial longline sets can last at least seven hours (*e.g.,*

Gallagher *et al*. 2014b; Butcher *et al*. 2016) without being checked. Sharks were subsequently returned to the experimental tank for an additional 15 hours to generate acceleration data characteristic of post-capture behavior, after which accelerometers were removed to download data. Because capture was voluntary to approximate natural behavior (*i.e.,* sharks were not forcibly hooked; Frick *et al*. 2010), only five of 18 individuals were caught. Water temperatures during this series of experiments averaged 30.0 ± 0.2 °C.

Data analysis

Acceleration biologgers

To process raw acceleration data for sharks chased to exhaustion, a smoothing interval of 2 s (Bullock *et al*. 2015; Wilson *et al*. 2015; Metcalfe *et al*. 2016) was applied to raw acceleration data individually for all three axes to separate raw acceleration into static (gravity) and dynamic (shark movement) components (Kawabe *et al*. 2003; Shepard *et al*. 2008). To generate an acceleration-based metric that could be used to predict aerobic metabolic costs, sway frequency (signal frequency in the lateral axis that is analogous to tailbeat frequency; Kawabe *et al*. 2003) was estimated *via* spectral analysis of dynamic acceleration in the lateral axis using Igor Pro (Version 6.3.3.5, WaveMetrics Inc., Lake Oswego, OR, USA) compatible freeware, Ethographer (Sakamoto *et al*. 2009).

To estimate the occurrence of swimming behaviors (*i.e.,* proportion of time devoted to a given behavior), it was necessary to construct a classification tree machine-learning algorithm (Breiman *et al*. 1984). Classification trees predict categorical outputs (swimming behaviors) for free-ranging animals from continuous data (acceleration data) using hierarchical decision rules generated from a training dataset with high accuracy (80-90%; Nathan *et al*. 2012). The training dataset was generated from one instance of three swimming behaviors (resting, swimming, and burst swimming) for each shark observed during exhaustive exercise. Nine acceleration-based metrics were calculated and input into the training dataset: mean and standard deviation of acceleration in all three axes calculated over 1 s intervals (*i.e.,* 25 data points; Brown *et al*. 2013; Graf *et al*. 2015), overall dynamic body acceleration (ODBA; Wilson *et al*. 2006), sway frequency, and sway amplitude (derived from spectral analysis of dynamic swaying acceleration). A

classification tree was generated using the R package "tree" (Ripley 2016). Optimal tree size (*i.e.,* the number of behaviors represented by the classification tree) was determined with V-fold cross validation (De'ath and Fabricius 2000). The classification tree automatically selected standard deviations of acceleration in the anterior-posterior and dorsal-ventral axes as criteria to distinguish behaviors (Table 2.1).

Pre-exhaustion respirometry

Aerobic metabolic rates (MO_2) were calculated according to

$$MO_2 = \Delta O_2 \frac{v}{mt}$$

where ΔO_2 is the change in dissolved oxygen concentration (mg O_2 L^{-1}) every second, v is the volume of water in the respirometer (*i.e.,* the respirometer's volume minus the shark's volume assuming 1 kg of shark equals 1 L of seawater; Lee *et al.* 2003b), m is the mass of the animal, and t is the time interval over which ΔO_2 is measured (h). Specifically, linear regression was used to define the change in dissolved oxygen concentration per unit time (slope), and each MO_2 value, therefore, had a coefficient of determination (R^2). Resting metabolic rate (RMR) was quantified by removing the first six hours of data to give sharks sufficient time to overcome handling stress, and calculating the mean of the six lowest MO_2 values with coefficients of determination greater than 0.95 (Shultz *et al.* 2011; Chabot *et al.* 2016).

Post-exhaustion respirometry

The maximum metabolic rate was defined as the highest MO_2 recorded during the 12-hour recovery period (Murchie *et al.* 2011). Excess post-exercise oxygen consumption was calculated by fitting hourly mean MO_2 values during the 12-hour recovery period with the equation

$$MO_2 = ae^{k_1 time} + be^{k_2 time} + c$$

where a, b, c, k_1, and k_2 are constants, and e is Euler's number. All constants were estimated by non-linear regression using a five parameter bi-exponential decay curve, except for c, which was set as an individual shark's RMR (Scarabello *et al.* 1991; Svendsen *et al.* 2010). Excess post-exercise oxygen

consumption was calculated by integrating the area under the curve that was bound by the initial MO_2 measurement time, recovery time, and the upper 95% confidence interval limit (Scarabello *et al*. 1991; Lee *et al*. 2003a; Svendsen *et al*. 2010). Sharks were considered to have recovered from the chasing bout when metabolic rate following chasing declined to the point that it intersected the upper 95% confidence interval limit for RMR (Bushnell *et al*. 1994; Svendsen *et al*. 2010).

Simulated capture

Acceleration data generated by sharks after capture were analyzed to determine whether hourly proportions of resting, swimming, and burst swimming behaviors were consistent (*i.e.,* recovered) following simulated longline capture. In so doing, it was possible to generate values for recovered behaviors that could be compared to pre-capture and capture values. Following capture and release to experimental tanks, 15 hours of acceleration data post-capture were run through the classification tree algorithm, and proportions of each behavior were estimated in one-hour bins (Whitney *et al*. 2013). To determine if a behavior recovered, the hourly proportion of a given behavior was fit with a non-linear mixed effects model (Whitney *et al*. 2013) with hour post-capture as a continuous fixed effect, and individual shark as a random effect using the "lme4" package (Bates *et al*. 2015) in R (R Core Team 2015). Given that a curve can never reach its asymptote, behavioral recovery was defined as the time (in hours) when the curve intersected 80% of the asymptote (Whitney *et al*. 2013).

Acceleration data generated by sharks before, during, and after capture were analyzed to quantify the effects of capture on resting, swimming, and burst swimming behaviors. One hour of acceleration data for each capture period for each shark was run through the classification tree algorithm, and proportions of time sharks spent performing each behavior were estimated. Specifically, pre-capture acceleration data were analyzed for the hour immediately before capture, *i.e.,* the 24th hour of acclimation to experimental tanks. Capture data were generated over the hour-long capture event. Acceleration data for recovered behaviors were analyzed for the hour when behavioral recovery was estimated to occur. Given that sharks cannot exhibit more than one behavior at any given time, raw proportion data were subject to unit-sum

constraint (Aebischer *et al*. 1993). To account for this, proportion data were log-ratio transformed using the equation

$$y_i = ln((x_i + 0.05)(x_j + 0.05)^{-1})$$

where x_i is the proportional value for a given behavior, x_j is the sum of the proportional values of the remaining two behaviors, and 0.05 is added to account for zeros in the dataset (Aebischer *et al*. 1993). Means of transformed proportions for each behavior before, during, and after capture were compared with a generalized least squares model with capture status (*i.e.,* pre-capture, capture, and recovered) as a fixed effect, and shark identification, nested within capture status, included and as a random effect. Random effects were used because the same sharks were used across each capture status such that data might not be independent, and behaviors and metabolic rates might be correlated within an animal (Laird and Ware 1982; Lindstrom and Bates, 1990). Generalized least squares were used to account for heteroscedasticity among levels of capture status (Zuur *et al*. 2009). Models were generated using the "nlme" package in R (Pinheiro *et al*. 2016), and *post hoc* multiple comparisons were conducted by rerunning models using different levels of capture status as a baseline.

Additionally, acceleration data generated by sharks before, during, and after capture were analyzed to quantify the effect of capture on sway frequencies and aerobic metabolic rates. A predictive equation was generated with linear regression pairing MMR and RMR with sway frequency data for each shark chased to exhaustion and observed at rest in holding tanks, respectively (Murchie *et al*. 2011). Aerobic metabolic rates were estimated by converting acceleration data to sway frequencies every second and applying these data to the predictive relationship generated for sway frequency and MO_2. It was assumed that aerobic metabolic rates could not exceed MMR, and predicted metabolic rates that exceeded that value were capped at MMR. Sway frequencies and aerobic metabolic rates were generated from the same acceleration data as behavioral metrics for pre-capture and capture periods, and acceleration data for recovered sharks were analyzed for the hour post-capture when sharks were estimated to recover from

exhaustive exercise. Physiological recovery was therefore defined as EPOC recovery time because fishing capture and exhaustive exercise elicit similar physiological responses (Kieffer 2000; Brooks *et al*. 2011). Means of sway frequencies and aerobic metabolic rates were compared with generalized least squares, and multiple comparisons were conducted by rerunning models using different levels of capture status as a baseline.

Results

Acceleration biologgers

The classification tree algorithm to distinguish resting, swimming, and burst swimming behaviors correctly predicted behaviors with 98.3% accuracy (*i.e.,* the model categorized 98.3% of behaviors used to construct it correctly). Acceleration data with standard deviations of acceleration in the anterior-posterior axis (surge S.D.) less than 0.01 *g* were defined as resting. Data with surge S.D. values greater than or equal to 0.01 *g* and standard deviations of acceleration in the dorsal-ventral axis (heave S.D.) less than 0.09 *g* were defined as swimming. Lastly, data with heave S.D. values greater than 0.09 *g* were defined as burst swimming (Table 2.1).

Pre- and post-exhaustion respirometry

Resting metabolic rate was 154.3 ± 5.6 mg O_2 kg^{-1} h^{-1}, and MMR was 260.9 ± 6.8 mg O_2 kg^{-1} h^{-1} (n = 12). Excess post-exercise oxygen consumption could only be estimated for seven sharks (62.8 ± 0.7 cm total length, 1.2 ± 0.03 kg, one female and six male) and was 116.4 ± 34.4 mg O_2 kg^{-1}. The other sharks exhibited considerable spontaneous activity in respirometers during recovery that resulted in elevated metabolic rates well above the upper 95% confidence interval limit of RMR for the entire 12-hour measurement period. The time required for metabolic rate elevated by chasing to return to RMR (physiological recovery time) was 5.4 ± 0.9 hours.

Simulated capture

The non-linear model for burst swimming estimated that recovered sharks burst approximately 1% of the time by 4.03 hours after capture (Fig. 2.1a). Resting behaviors were estimated to recover when sharks rested 32% of the time, and behavioral recovery for resting behaviors occurred 9.75 hours after capture (Fig. 2.1b). Lastly, recovered sharks were estimated to swim 66% of the time, which occurred 7.92 hours after capture (Fig. 2.1c).

Captured sharks burst nine times more frequently than pre-capture and recovered sharks, and burst swimming occurred equally as often pre-capture as for recovered sharks (Fig. 2.2a). Conversely, captured and recovered sharks rested as often as pre-capture sharks, but recovered sharks rested more often than captured sharks (Fig. 2.2b). Lastly, sharks did not exhibit changes in the proportion of swimming behavior pre-capture, during capture, and when recovered (Fig. 2.2c). Model outputs for burst swimming, resting, and swimming behaviors are summarized in Table 2.2.

Aerobic MO_2 and sway frequency had a positive relationship (Linear regression, $R^2 = 0.75$, $F_{1,\,19} = 56.34$, $p < 0.001$) defined by the equation

$$MO_2 = 141.3 + 37.6 \times sway\ frequency$$

(Fig. 2.3). Capture status had no effect on sway frequencies (Fig. 2.4a) or aerobic metabolic rates (Fig. 2.4b). Model outputs for sway frequency and metabolic rate are summarized in Table 2.2.

Discussion

Relative to pre-capture status, sharks responded to simulated longline capture by bursting more frequently, resting less often, and without changing the amount of time devoted to swimming. Captured sharks exhibited a nine-fold increase in the proportion of burst activity and a 97% reduction in the proportion of resting activity relative to free-swimming sharks. Burst swimming is a typical response to capture (Guida *et al*. 2016b), whereby a shark recruits anaerobic metabolism to support a high-energy

escape response (Skomal & Bernal 2010), and, because prolonged reliance on anaerobic metabolism results in exhaustion (*e.g.,* Frick *et al*. 2012), frequent burst activity ceases early in the capture event (Brooks *et al*. 2012). Resting in response to capture has been observed for buccal pumping species, like lemon sharks, with the ability to ventilate while stationary (Frick *et al*. 2010; Guida *et al*. 2016a), and it has been suggested that resting serves to mitigate the severity of the stress response (Guida *et al*. 2016a). Conversely, ram ventilation, relative to buccal pumping, is a more efficient method of mitigating the severity of a stress response for lemon sharks (Brooks *et al*. 2011), and studies in teleosts have documented considerable movement-related energy savings when fishes transition from buccal pumping to ram ventilation (Clark & Seymour 2006). For wild fishes, relying on behaviors than maximize ventilation efficiency should increase metabolic scope by maximizing oxygen uptake and minimizing movement-related costs, thereby improving the capacity to resolve physiological perturbations that characteristically invoke EPOC (*e.g.,* ion imbalance, lactate accumulation, and acid-base derangements; Wood 1991), and promoting recovery during potentially long capture events (*e.g.,* more than four hours; Brooks *et al*. 2012). However, behavioral responses to capture are likely much different for obligate ram ventilating species that cannot ventilate while stationary and must maintain sufficient swimming velocities to support oxygen demand that is typically higher than buccal pumping species (Lowe 2002), which could be a potential factor contributing to higher mortality rates of ram ventilating sharks relative to buccal pumping sharks (Dapp *et al*. 2016b). Therefore, in response to longline capture, lemon sharks increase the frequency of burst events but rest less often than free-swimming sharks.

Despite changes in the proportion of time devoted to a given behavior, sharks did not modulate sway frequencies relative to pre-capture status, and therefore aerobic metabolic rates tied to swimming activity were not affected during capture. Sway frequencies (*i.e.,* acceleration signal frequency in the lateral axis that is analogous to tailbeat frequency and an accelerometric proxy of metabolic rate; Kawabe *et al*. 2003) and aerobic metabolic rates were 1.06 ± 0.09 Hz and 180.58 ± 3.51 mg O_2 kg^{-1} h^{-1} before capture, respectively, and 1.27 ± 0.01 Hz and 187.91 ± 0.73 mg O_2 kg^{-1} h^{-1} during capture, respectively. Longlining

is a relatively benign capture method compared to trawl and gill-net capture (Brooks *et al*. 2012; Dapp *et al*. 2016b) in that captured animals can freely swim in a radius restricted by the length of the gangion (Dapp *et al*. 2016b) or rest on the substrate for demersal sets (*e.g.,* Guida *et al*. 2016a). Given that struggling effort may be inversely related to gangion length (Guida *et al*. 2016a), captured animals should have the capacity to behave as free-swimming animals provided that gangions are sufficiently long relative to the length of the shark, and there is no risk of entanglement or depredation (O'Shea *et al*. 2015). However, captured sharks in this study were expected to exhibit higher sway frequencies associated with escape responses, though preliminary data providing behavioral profiles of capture events suggest that escape attempts occur briefly at the beginning of capture, and infrequently throughout (Frick *et al*. 2009; Guida *et al*. 2016a). If capture limits behavioral responses either by physically restricting animals or inducing a mild form of tonic immobility (a natural state of paralysis brought on by various forms of restraint; Brooks *et al*. 2011), sharks might appear to behave as free-swimming animals, by adopting different strategies to improve oxygen uptake without increasing swimming activity, such transitioning from buccal pumping to ram ventilation (Clark and Seymour 2006), or increasing mouth gape (Carlson and Parsons 2001). Conversely, a reliance on anaerobic metabolism to support an initial burst response could have exhausted sharks, and the slow aerobic swimming characteristic of free swimming sharks could be a behavioral response to the onset of anaerobic metabolism (Peake and Farrell 2006; Norin and Clark 2016). However, lemon sharks should have had elevated metabolic rates during capture because exhaustive exercise resulted in lemon sharks having EPOC and chasing and capture illicit similar physiological responses (Kieffer 2000), though our inability to detect changes in activity levels may have been the case that the calibration technique (*e.g.,* Murchie *et al*. 2011) was inappropriate, or because sway frequency doesn't account for animal movement in three planes as does dynamic body acceleration (*e.g.,* Gleiss *et al*. 2010). Regardless, this study did not document a change in aerobic metabolic rates tied to swimming for capture sharks relative to pre-capture sharks.

This study used two different metrics to define behavioral (*i.e.,* bursting, resting, and swimming) and physiological (*i.e.,* metabolic rate) recovery, and both methods successfully estimated recovery times.

Proportions of time spent bursting, resting, and swimming behaviors estimated at hours four, nine, and seven post-capture were identical to pre-capture levels, respectively, and sway frequencies and aerobic metabolic rates five hours post-capture were identical to pre-capture levels. Blacktip sharks (*Carcharhinus limbatus*) subjected to angling in the field and 72 hours of post-capture remote observation with accelerometers had behavioral recovery times ranging from 7.4 to 14.4 hours with a mean of 9.9 hours (Whitney *et al*. 2013), compared to this study's range of 4.0 to 9.8 hours with a mean of 7.2 hours. Though capture techniques (longline versus rod-and-reel; Whitney *et al*. 2013) and fight times (one hour versus 2-16 minutes; Whitney *et al*. 2013) varied between these studies, longer recovery times for blacktip sharks might be explained by underlying physiological (*e.g.,* higher metabolic rates) or behavioral (*e.g.,* inability to rest) qualities of obligate ram ventilating species, such as blacktips, relative to buccal pumping species, such as lemon sharks (Dapp *et al*. 2016b). Increasing swimming speed (and tailbeat frequency; Lowe 1996; but see Webb and Keyes 1982) relative to routine swimming, is a potential behavioral response to increase oxygen supply (Carlson and Parsons 2001), and a lack of this response by five hours post-capture may indicate that lemon sharks no longer had EPOC and had recovered from capture stress. It was surprising, however, that lemon sharks resumed bursting behavior before sharks were estimated to recover physiologically from capture, though anaerobic substrates (*e.g.,* creatine phosphate) in white muscle can be replenished within several hours of exhaustive activity (*e.g.,* Scarabello *et al*. 1991; Richards *et al*. 2003). It was also surprising that predominantly aerobic behaviors (resting and bursting) did not recover until two to four hours after predicted physiological recovery, though EPOC incurred by capture may have been larger than measured for chasing, necessitating longer recovery. In situations where capture duration is short relative to the length of typical longline sets, routine swimming behaviors of sharks may recover over hours as opposed to days post-capture for lengthy sets (*e.g.,* Campana *et al*. 2009). However, while a resilient species like a lemon shark may have the potential to recover physiologically over the course of a long capture duration (Brooks *et al*. 2011; 2012), and given that physiological responses to capture are repeatable (*e.g.,* Frick *et al*. 2009), the present study cannot infer the post-release state of fine-scale behavioral responses. Given that behavioral responses may serve to mitigate the severity of physiological

responses to capture (Gallagher 2015; Guida *et al*. 2016a), behavioral responses may persist as long as physiological perturbations and *vice versa*, though additional research is required to establish that relationship. Therefore, sharks were estimated to recovery physiologically within five hours, and sharks were estimated to recovery behaviorally within nine hours.

When taken together, our results provide new insight into the physiological and behavioral sub-lethal outcomes of longline capture for a carcharhinid shark. Specifically, our research allows us to fill gaps in our understanding of the condition of animals during a capture event, as well as their fate post-release, which can help management gauge the suitability of mitigation measures for many data deficient species (Dapp *et al*. 2016b). For instance, longline capture was a relatively benign method because it allowed captured sharks to exhibit identical sway frequencies, aerobic metabolic rates, and proportion of time swimming as free-swimming animals, given that conditions of the fishing gear (*i.e.,* gangion length) were not overly restrictive. Additional research into the effect of gangion length on responses to capture may shed light on the efficacy of regulating gangion lengths as a management strategy (*e.g.,* Dapp *et al*. 2016b; Guida *et al*. 2016a). Furthermore, when capture durations are short or sharks are released from fishing gear within several hours of being caught, behavioral recovery of swimming and non-swimming behaviors occurs only on the scale of hours (mean recovery time = 7.2 hours). Shortened soak times, or periodic gear checks may serve to reduce post-release mortality (*e.g.,* Broadhurst *et al*. 2014; Marshall *et al*. 2015), though additional research is necessary to observe the effect of capture duration on sub-lethal outcomes post-release. Lastly, these data suggest that excess post-exercise oxygen consumption, not elevated aerobic metabolic rates, is the only source of additional energy expenditure tied to brief durations of longline capture, and that techniques employed to minimize the intensity of a shark's initial escape response when caught (*e.g.,* hooks with bending strength that can be exceeded by sharks versus the target catch; Watson and Kerstetter 2006) may serve to reduce the additional energetic costs incurred by capture. Therefore, these data clearly have implication for mitigating sub-lethal outcomes of capture for sharks as commercial

longline bycatch by supporting increases in gangion length and the implementation of periodic gear checks, which can be used to mitigate the negative consequences of unintended bycatch for shark populations.

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Tables

Table 2.1. Values for standard deviations of surging (anterior-posterior; "surge S.D.") and heaving (dorsal-ventral; "heave S.D.") acceleration used to generate a classification tree machine learning algorithm to distinguish three swimming behaviors (resting, swimming, and burst swimming) from acceleration data generated by captive sharks. Values for each metric are presented as mean ± standard error and the range (*i.e.,* maximum minus minimum). The model had 98.3% accuracy (*i.e.,* the model categorized 98.3% of behaviors used to construct it correctly).

Behavior (Observations)	Rest (n = 4)	Swim (n = 7)	Burst swim (n = 7)
Criteria	**Surge S.D. < 0.01 *g***	**Surge S.D. ≥ 0.01 *g*** **Heave S.D. < 0.09 *g***	**Heave S.D. ≥ 0.09 *g***
Surge S.D. (*g*)	0.004 ± 0.001 (0.002 – 0.022)	0.07 ± 0.05 (0.01 – 0.25)	0.38 ± 0.29 (0.02 – 1.34)
Heave S.D. (*g*)	0.005 ± 0.001 (0.002 – 0.019)	0.03 ± 0.02 (0.00 – 0.24)	0.29 ± 0.27 0.01 – 2.04)

Table 2.2. Statistical output for generalized least squares models for proportion of time devoted to a given behavior (burst swimming, resting, or swimming), sway frequency (signal frequency in the lateral acceleration axis that is analogous to tailbeat frequency), and metabolic rate (aerobic MO_2) for different capture status (pre-capture, capture, and recovered) for sharks caught on simulated longlines. For all models, individual shark identification was nested within capture status (*i.e.,* the same sharks were used across all capture status treatments) and included in the model as a random effect. Proportion data (burst swimming, resting, and swimming) were log-ratio transformed to address the unit-sum constraint because raw proportion data are not independent (*i.e.,* sharks can only exhibit one behavior at a time). Significant treatment effects are indicated with bold text.

| Response variable	Fixed effect	F	d.f.	p
Burst swimming	**Capture status**	**6.78**	**2, 8**	**0.0189**
Resting	**Capture status**	**7.94**	**2, 8**	**0.0126**
Swimming	Capture status	0.76	2, 8	0.5724
Sway frequency	Capture status	3.12	2, 8	0.0997
Aerobic MO_2	Capture status	2.59	2, 8	0.1359

Figures
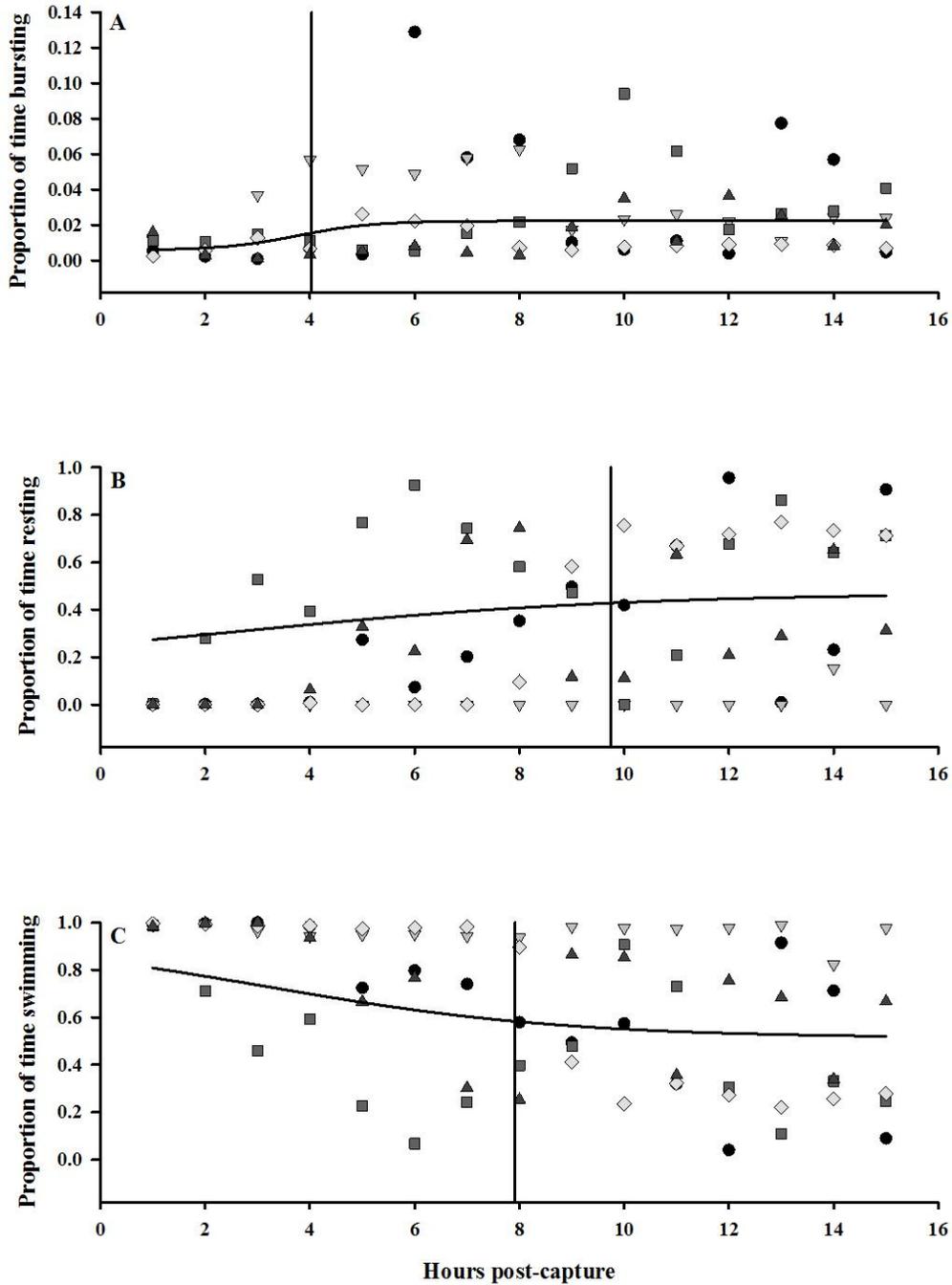
Figure 2.1. Non-linear regressions of the hourly proportion of burst swimming (A), resting (B), and swimming (C) behaviors against hours after simulated longline capture. Proportion data were fit with four-parameter logistic curves. Solid vertical lines denote the time (in hours) until sharks reached 80% of curve asymptotes, indicating behavioral recovery. Individual sharks (n = 5) are represented by different shapes, and the same shark is represented by the same shape across graphs.

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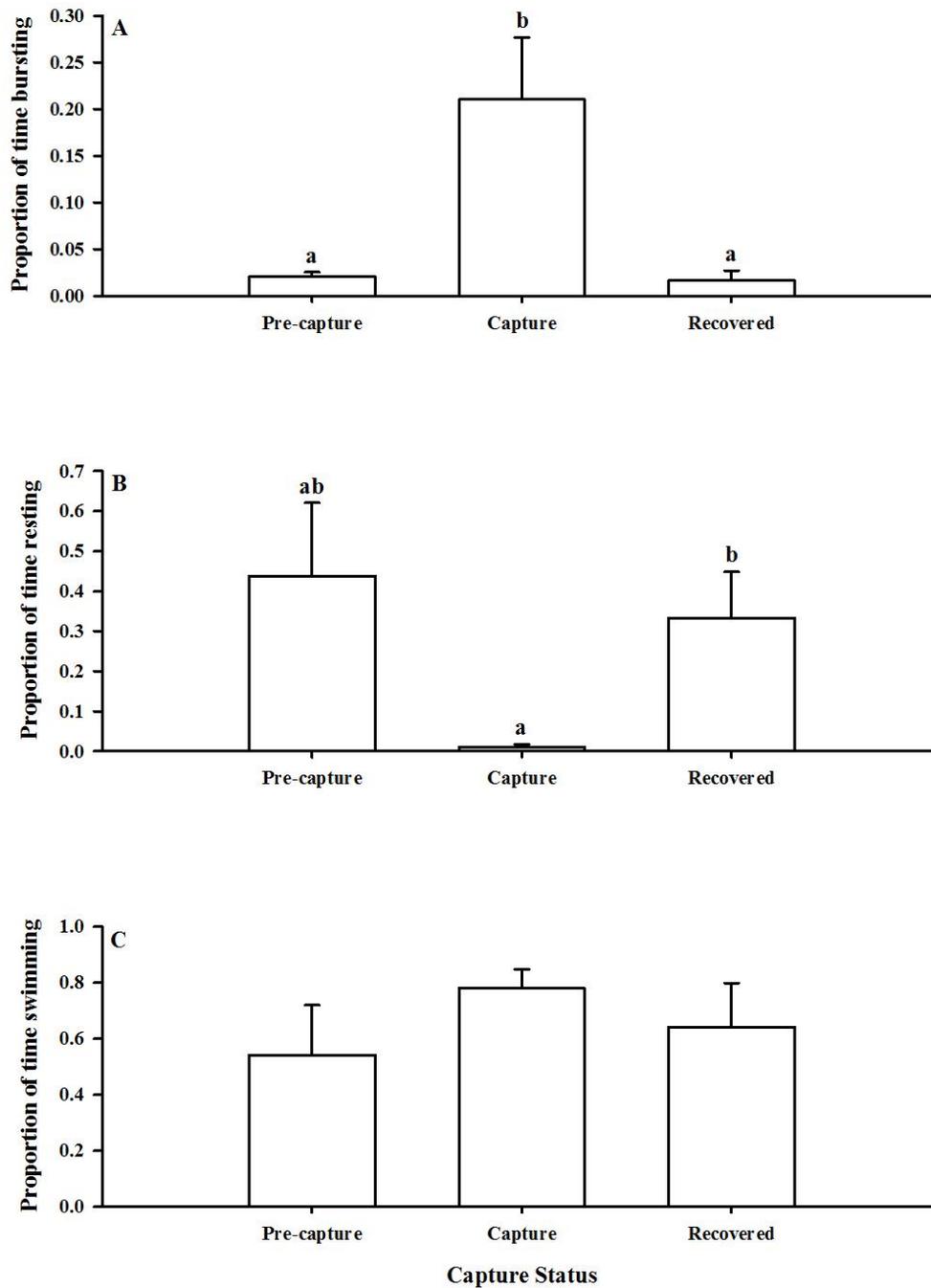
Figure 2.2. Mean and standard error of proportions of burst swimming (A), resting (B), and swimming (C) behaviors one hour before, during, and after simulated longline capture. Data for behaviors observed after capture were generated during the hour that a given behavior was predicted to recover (Fig. 2.1) Differing lowercase letters indicate statistically significant differences between means within a behavior. There was no effect of capture status (*i.e.,* pre-capture, capture, recovered) on swimming.
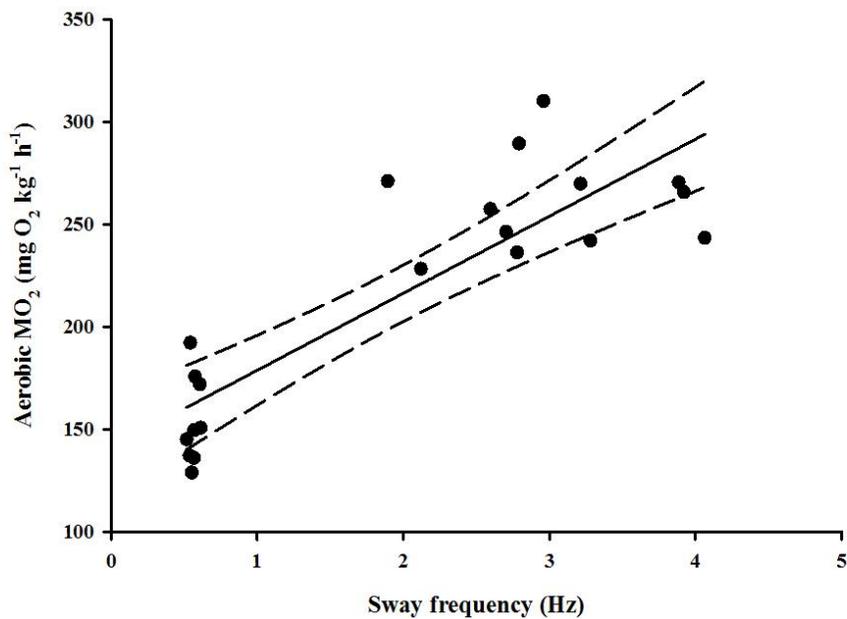
Figure 2.3. Linear model of aerobic metabolic rate (MO_2) against acceleration-derived sway frequency. A predictive equation was generated by pairing maximum post-exercise metabolic rate and resting metabolic rate (the metabolic rate of a fasted shark at rest and stable temperature) with sway frequency data from sharks chased to exhaustion and observed at rest in holding tanks, respectively. Each data point was a paired metabolic rate and sway frequency from an individual shark. Dashed lines represent 95% confidence interval lines for the regression.
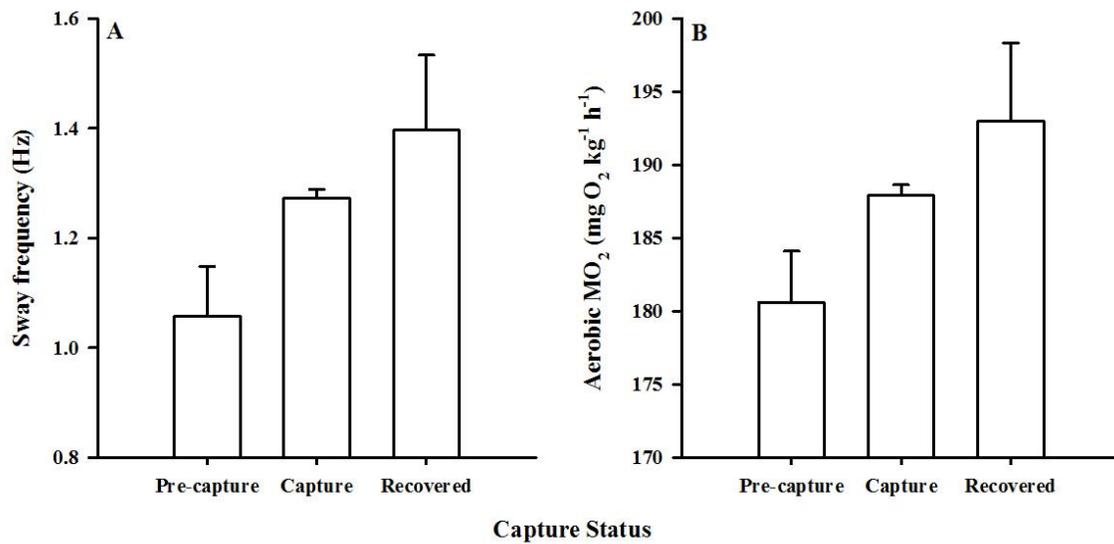
Figure 2.4. Mean and standard error of sway frequencies (Hz; A) and aerobic metabolic rates (MO$_2$, mg O$_2$ kg^{-1} s^{-1}; B) one hour before, during, and after simulated longline capture. Data for sharks after capture were generated during the hour that sharks were predicted to recover physiologically from exhaustive exercise. There was no effect of capture status on sway frequencies or aerobic metabolic rates.

CHAPTER THREE: CONCLUSION

The purpose of this research was to define relationships between the behavior and energy use for a species of shark both undisturbed in the wild and subjected to the stress of fishing capture. To accomplish this goal, I conducted two complimentary series of experiments. For my first study, I remotely observed four swimming behaviors that differentially rely on aerobic and anaerobic metabolism, and predicted the energetic costs of these behaviors. Therefore, it possible for me to observe how wild sharks partition behaviors and manage energy expenditure over diel scales. For my second study, I subjected sharks to simulated commercial fisheries capture and defined recovery endpoints for swimming behaviors and physiological disturbance, thereby making it possible to observe changes in behavior and energy use associated with capture and recovery. Together, these studies offer insight into why sharks behave the way they do, stressed or unstressed, and the energetic consequences of their actions. Therefore, this research has defined preliminary relationships between the behavior and energy use for a species of shark, with implications for addressing organismal responses to stressors.

Chapter one concluded that more energetically costly swimming behaviors occur less frequently for wild lemon sharks (*Negaprion brevirostris*) and that non-swimming behaviors did not follow this trend. From a basic perspective, these data advance our current understanding of this species' biology and ecology by providing a detailed energy-based behavioral time budget. From an applied perspective, these data offer a baseline for evaluating how stressors alter the behavior, and therefore energy expenditure of wild sharks. For instance, coastal shark species (*e.g.,* lemon sharks) are particularly vulnerable to habitat degradation (*e.g.,* Jennings *et al.* 2008), fishing pressure (*e.g.,* Worm *et al.* 2013), and environmental stressors stemming from climatic change throughout their ontogeny (Crain *et al.* 2009) with unknown sub-lethal consequences. Knowledge of the energetic costs of behaviors for coastal elasmobranchs could improve the potential for ecological risk assessments to be effectively employed in management decisions (Gallagher *et al.* 2012). It is my intention that these data, and studies similar to this one, can be applied to develop an understanding of fitness consequences resulting from increasing anthropogenic pressure and that studies of this nature can

extend to species on the forefront of exploitation (*e.g.,* deep-sea Chondrichthyes; Simpfendorfer and Kyne 2009). Before these goals can be addressed, however, future studies are needed to expand upon the work presented here to offer a more robust and comprehensive understanding of the relationship between behavior on energetics.

Chapter two concluded that captured lemon sharks behave differently than free-swimming animals, yet they minimize activity-based energy expenditure to facilitate physiological recovery. These data are directly applicable to measures aimed at mitigating sub-lethal outcomes of fisheries capture for incidentally caught and released sharks. For instance, these data suggest the allowing for captured animals to approximate behavior of free-swimming animals can reduce energy use, and that overly restrictive gear types may prolong recovery. The majority of global shark production constitutes longline fisheries bycatch (Oliver *et al*. 2015), and these data could be applied to fisheries guidelines mandating minimum lengths for gangions, thereby increasing a hooked shark's capacity to swim (Dapp *et al*. 2016). In addition to suggesting gear modification to mitigate sub-lethal outcomes, it is the intention of this work to be applied and expanded upon to understand how energy expenditure tied to capture influences long-term processes like growth and reproduction. Knowledge of how energy expenditure ultimately affects energy investment in pups, and reproductive success would provide some of the strongest evidence to date of the fitness consequences of fisheries bycatch (Skomal and Mandelman 2012) for a taxonomic class (*i.e.,* Chondrichthyes) already characterized by low population regrowth rates (Stevens *et al*. 2000). Thus, future studies are needed to determine how energy expenditure tied to processes other than swimming and recovery are affected by capture.

In conclusion, this research provides new insight into the relationship between behavior and energetics of a shark with implication for addressing whole-organism responses to stressors. Elasmobranch metabolism is severely understudied relative to what is known about teleosts (Carlson *et al*. 2004), and advances in this field are important, first and foremost, to improve our understanding of these species' biology and ecology. Furthermore, energetics and behavior represent novel techniques for assessing stress

in elasmobranchs (Wilson *et al*. 2014) despite elasmobranchs being an increasingly exploited and threatened taxa (Dulvy *et al*. 2008; 2014). For instance, measures of performance (*e.g.,* aerobic scope) have become increasingly popular among teleost studies and have been employed extensively (Clark *et al*. 2013), from predicting an individual's vulnerability to fisheries capture (*e.g.,* Killen *et al*. 2015), to observing reductions in performance at near-future climate change scenarios (*e.g.,* Rummer *et al*. 2013), yet elasmobranch energetics studies are decades behind teleost research. Therefore, there is an implicit need to advance elasmobranch energetics research so that studies, like this one, can begin effectively addressing relevant issues at the forefront of elasmobranch conservation.

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