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Swimming speeds and metabolic rates of semi-captive juvenile lemon sharks (*Negaprion brevirostris*, Poey) estimated with acceleration biologgers

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ABSTRACT

There is much interest in being able to quantify the swimming speeds and metabolic rates of wild aquatic animals such as sharks to develop bioenergetics models and evaluate the metabolic consequences of different stressors. This study sought to calibrate tri-axial acceleration biologgers (accelerometers) such that it would be possible to estimate swimming speeds and metabolic rates of semi-captive sharks in an enclosed natural mesocosm. Juvenile lemon sharks (*Negaprion brevirostris*, Poey; 60–75 cm total length, 1–2 kg) were equipped with accelerometers and swum at stepwise velocity increments in a Blazka-style swim tunnel respirometer using a critical swimming speed protocol. Metabolic rates and acceleration-derived metrics (overall dynamic body acceleration and tailbeat frequency) were measured concurrently during forced swimming, and accelerometer-equipped sharks were released to an enclosed mesocosm habitat to estimate average daily metabolic rate (ADMR) and swimming velocities across diel and tidal cycles. Acceleration-derived tailbeat frequency was a stronger predictor of metabolic rate than overall dynamic body action, and predicted an active metabolic rate of 249.7 ± 1.9 mg O_2 kg^{-1} h^{-1} and an ADMR of 88.7 ± 0.7 kJ kg^{-1} d^{-1} at 30 °C. Following exhaustive exercise a maximum metabolic rate of 398.0 ± 19.6 mg O_2 kg^{-1} h^{-1} was achieved and over the subsequent 55-minute recovery period excess post-exercise oxygen consumption was 31.2 mg O_2 kg^{-1}. The critical swimming speed of the sharks was 0.71 ± 0.03 body lengths per second (BL s^{-1}), and swimming speed in the mesocosm was 0.19 ± 0.01 BL s^{-1}. Locomotor activity levels of semi-captive sharks in the mesocosm were influenced by tide state and diel period, with sharks having highest swimming velocities during diurnal periods and flooding tides. Overall, accelerometry is a suitable means for estimating swimming speed and metabolic rate in this species, and additional research to address anaerobic energy expenditure of wild sharks is warranted.

1. Introduction

Understanding rates of energy expenditure in wild fishes (i.e., swimming speeds and metabolic rates) is important for estimating how energy use changes when fishes are exposed to environmental or anthropogenic stressors (Treberg et al., in press). Elasmobranchs were initially thought to have the lowest metabolic rates and activity levels of all fishes (Brett and Blackburn, 1978), though evidence suggests that elasmobranchs have comparable metabolic capacities as ecologically similar teleosts (e.g., Bushnell et al., 1989; Dickson et al., 1993; Lowe, 2001). Several studies have addressed the effects of mass and temperature on aerobic metabolic rates (e.g., Dowd et al., 2006; Whitney et al., 2016), fewer address conservation issues (e.g., Barnett et al., 2016), and only one has addressed anaerobic energy expenditure (Brett and Blackburn, 1978). Elasmobranch studies, however, are advancing to the point where they have begun to elucidate patterns in metabolism across species with different “pace-of-life” traits (e.g., Whitney et al., 2016). For instance, inactive

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Abbreviations: ADMR, average daily metabolic rate; EPOC, excess post-exercise oxygen consumption; MMR, maximum metabolic rate; ODBA, overall dynamic body acceleration; RMR, resting metabolic rate; SMR, standard metabolic rate; TBF, tailbeat frequency.

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species typically have very low metabolic rates (e.g., Whitney et al., 2007, 2016), whereas obligate ram-ventilating species have considerably higher metabolic rates attributed to the increased costs of maintaining larger gill surface area (Bernal et al., 2012). Thus, while studies are beginning to define trends in aerobic metabolic performance, the understanding of the anaerobic performance of sharks is limited (Bernal et al., 2012). Studies have estimated metabolic rates of wild sharks using laboratory defined relationships between metrics of energy expenditure and activity, but a comprehensive picture of the activity metabolism of wild sharks is incomplete without understanding an animal's full aerobic and anaerobic capacities (Whitney et al., 2016). Therefore, quantifying metabolic rates and activity levels in wild sharks will improve estimates of changes in energy use in response to environmental or anthropogenic stressors.

Comprehensive laboratory and field metabolism studies would ultimately allow researchers to provide ecologically meaningful estimates of metabolic rates that could be used to address energy requirements of populations and address conservation issues (Treberg et al., in press). Studies that incorporate a field component to “bridge the gap” between the laboratory and field are lacking for sharks, partially owing to logistically difficulties associated with shark respirometry and that a single method to remotely measure metabolic rates and activity levels has not been universally adopted (Lowe and Goldman, 2001). Metabolic rates are typically measured indirectly as rates of oxygen consumption using respirometry, though the logistics of respirometry can be prohibitive for many species (Bernal et al., 2012). In general, shark respirometry studies have been limited by the size of the animal that commercially available respirometers can accommodate (e.g., Graham et al., 1990) and whether the animal is an obligate ram-ventilator, because measurement of standard metabolic rate (SMR, the metabolic rate of a fasted shark at rest and stable temperature; Chabot et al., 2016) and excess post-exercise oxygen consumption (EPOC, the mass of oxygen consumed above SMR to restore anaerobic substrates and resolve physiological disturbance after exhaustive activity; Gaesser and Brooks, 1984) require that sharks are motionless (e.g., Dowd et al., 2006). Furthermore, swimming sharks at a range of activity levels necessary for sensor tag calibration can be further complicated by confinement stress from respirometers (e.g., Lowe, 1996, 2001) or uncooperative behavior of the study species (e.g., Whitney et al., 2007, 2016). Where researchers have been successful, however, studies have provided key insights into basic animal ecology (e.g., Lowe, 2002; Sundström and Gruber, 1998), and have addressed changes in diel energy expenditure owing to anthropogenic stressors (e.g., Barnett et al., 2016). While metabolic rates measured for captive animals can be extrapolated to estimate energy requirements of wild animals, combined laboratory and field studies ultimately provide ecologically meaningful estimates of energy requirements.

Acceleration biologgers (accelerometers) are a valuable tool that may help to bridge the gap between laboratory and field studies of elasmobranch metabolism (Whitney et al., 2012; Cooke et al., in press). Specifically, acceleration-based metrics provide high resolution and fine-scale behavioral measurement (Brown et al., 2013), and are activity-specific proxies for expenditure in free-ranging animals (e.g., Halsey et al., 2009b). The metric overall dynamic body acceleration (ODBA; Wilson et al., 2006), which assumes energy expenditure scales linearly with acceleration generated from muscle contraction (Gleiss et al., 2011), correlates metabolic rates with activity (Wilson et al., 2006) and has been validated for scalloped hammerhead sharks (*Sphyrna lewini*; Gleiss et al., 2010). While ODBA is a promising proxy of energy expenditure, studies have questioned whether logging acceleration at high frequencies in three axes is any better than logging in fewer axes because of the associated memory and battery requirements (Halsey et al., 2009a). In addition to ODBA, tailbeat frequency (TBF) can be derived from acceleration in the lateral axis of movement (Kawabe et al., 2003), and has previously been used to quantify metabolic rates and swimming speeds in sharks using a tailbeat-sensing acoustic transmitter (Lowe, 2002; Lowe et al., 1998). Studies have recently begun to apply accelerometry for shark research to determine its suitability for remote behavioral (Whitney et al., 2007; Wilson et al., 2015) and physiological (Gleiss et al., 2010) assessments, yet few have applied accelerometry to define activity-specific metabolic rates for elasmobranchs (Barnett et al., 2016). Given the capacity for accelerometers to measure behavior and estimate rates of energy expenditure, accelerometry should be capable of bridging the gap between field and laboratory studies of elasmobranch metabolism.

The primary objective of this study was to telemeter activity levels in semi-captive lemon sharks (*Negaprion brevirostris*, Poey) to estimate metabolic rates, swimming velocities, and diel energy expenditure, using laboratory calibrations of metabolic rates with acceleration-derived activity metrics using swim tunnel respirometry. A secondary objective was to quantify recovery of sharks from the exhaustive exercise protocol employed for calibration in an attempt to incorporate anaerobic metabolic costs into metabolic rate estimations by measuring EPOC. Lemon sharks were selected as the study species because lemon shark metabolism has been well documented relative to other elasmobranchs, including several studies that sought to calibrate metabolic rate with various sensor tags (e.g., heart rate: Scharold and Gruber, 1991; speed-sensing tag: Sundström and Gruber, 1998). Furthermore, studies have begun to validate the use of accelerometry for remote behavioral measurement, and have suggested that accelerometry is a promising method for metabolic rate measurement in lemon sharks (Gleiss et al., 2009). Therefore, the use of this species allows for comparison with previous metabolism and telemetry studies.

2. Materials and methods

All research was conducted under research permits MAF/FIS/17 and MAF/FIS/34 issued by the Bahamian Department of Marine Resources and followed the Cape Eleuthera Institute (CEI) animal care protocols based on guidelines set forth by the Association for the Study of Animal Behavior and the Animal Behavior Society (Rollin and Kessel, 1998). Permission to capture sharks within the Bahamian Shark Sanctuary was established in accordance with Bahamian Department of Marine Resources Form 20A, Regulation 36D (3), permitting fishing, possession, and exportation of sharks or shark parts.

2.1. Animal collection and husbandry

Sharks were collected from tidal mangrove creeks around Cape Eleuthera, Eleuthera, The Bahamas (24°49′46.43″N, 76°19′41.49″W) between 31 March and 8 August 2014 for laboratory calibration, and between 31 August and 22 September 2014 and 2–4 June 2015 for field deployments. All animals were captured using seine netting and brought back to CEI's wet lab within 45 minutes post-capture. During transit, half of the water in holding totes was replaced every 5 min to provide adequate aeration and quality (Brooks et al., 2011). Sharks were maintained in a 13,000 L (3.7 m diameter by 1.25 m depth) open-circulating tank continuously aerated and supplied with fresh seawater. The wet lab is an open-sided, covered, outdoor facility exposing animals to ambient seasonal water temperatures and a natural photoperiod. Feeding occurred daily and consisted of one thawed commercially available Spanish sardine (*Sardinella aurita*) per shark. All sharks were acclimatized to wet lab conditions for at least three days prior to use in experiments.

2.2. Acceleration measurement

To quantify activity, lemon sharks were fitted with an accelerometer (X8M-3, Gulf Coast Data Concepts, Waveland, MS, USA; 5.1 cm × 2.5 cm × 1.3 cm, 17 g; 25 Hz recording frequency; ±8g acceleration range), secured to plastic frontal and backing plates through the base of the dorsal fin using 36 kg break strength braided Dacron line (Wilson et al., 2015). Accelerometers were mounted on the right side of the fin (Gleiss et al., 2010) so that heave (dorsal-ventral movement), surge (anterior-posterior movement), and sway (lateral movement) axes were uniformly oriented.

Prior to deployment accelerometers were individually calibrated to gravitational acceleration at known angles by slowly rotating tags 360° through each axis (Gleiss et al., 2010). Raw acceleration from accelerometers was used to derive overall dynamic body acceleration (ODBA) and tailbeat frequency (TBF) with Igor Pro (Igor Pro 6.3.3.5, WaveMetrics, Inc., Lake Oswego, OR, USA). To derive dynamic acceleration, a smoothing interval of 2 s was applied to total acceleration (Wilson et al., 2015), yielding static acceleration (Shepard et al., 2008). Static acceleration was then subtracted from total acceleration yielding dynamic acceleration. The absolute values of dynamic acceleration in all three axes were then summed, yielding ODBA (Wilson et al., 2006). Tailbeat frequency was derived from spectral analysis of dynamic swaying acceleration (Kawabe et al., 2003) using the Igor Pro compatible freeware Ethographer (Sakamoto et al., 2009).

2.3. Swim tunnel respirometry

Sharks ($n = 25$, 64.69 ± 0.72 cm total length, 1.38 ± 0.05 kg) were swum at known velocities in a custom-built 108.7 L Blazka-style swim tunnel respirometer. The swim tunnel was composed of two concentric transparent acrylic tubes with inner diameters of 24.0 cm and 34.4 cm, with plastic grates at either end of the 100.4 cm long swimming section of the tunnel. Flow was generated by a Leeson Washguard 3-Phase AC Motor (Leeson Electric Corp., Grafton, WI, USA) 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 tunnel was covered to promote swimming at the front end.

2.3.1. Swimming protocol

Prior to experimentation sharks were fasted for 48 h in their holding tanks, allowing sufficient time for gastric evacuation at experimental temperatures (Cortés and Gruber, 1992). After fasting, sharks were mounted with accelerometers following the methods detailed above, placed in the respirometer and held for a six-hour acclimation period (0700–1300) at a flow velocity of <1.0 cm s^{-1}. After the acclimation period, sharks were swum at 10 cm s^{-1} until they oriented to the direction of flow and swam steadily. Once sharks could maintain a constant speed and position at the front half of the tunnel, flow velocity was increased in 10 cm s^{-1} increments (Lowe, 1996) every 15 min until sharks exhausted, which was defined as when a shark began burst swimming and could not swim off of the rear grate for >20 s (Lee et al., 2003a). Upon exhaustion, testing immediately ceased and flow velocity was decreased to 1.0 cm s^{-1}. Critical swimming speed (i.e., the maximum aerobically-sustained swimming speed) was quantified according to Brett (1964) as

$$U_{crit} = U_f + \frac{T_f}{T_i} U_i$$

where U_f is the last full velocity increment at which the shark swam steadily, T_f is the time the shark swam at the final velocity before becoming exhausted, T_i is the time interval over which velocity is increased (15 min), and U_i is the velocity increment at each time interval (10 cm s^{-1}). Times were recorded at each velocity increment when lemon sharks swam steadily at the front of the tunnel (i.e., no change in forward or lateral position) so mean acceleration data existed for at least 1 min at each velocity.

At the conclusion of each trial, body width and height (excluding fins), and curvilinear total length (TL) were measured for solid blocking corrections given animals represented a significant portion of the total cross-sectional area of the swim tunnel (8.0 ± 0.4%). Solid blocking was calculated as

$$U_{corrected} = U\left(1 + 0.5\tau \frac{l}{t}\left[\frac{A_o}{A_t}\right]^{\frac{3}{2}}\right)$$

where U is swimming speed measured in cm s^{-1}, τ is a dimensionless factor for any tunnel sectional shape ($\tau = 0.8$), l is the curvilinear total length of the shark, t is its thickness (width plus height), A_o is the cross-sectional area of the shark, and A_t is the cross-sectional area of the swim tunnel (Bell and Terhune, 1970).

2.3.2. Metabolic rate measurements

Oxygen consumption during U_{crit} tests was quantified using intermittent-flow respirometry (Svendsen et al., 2016). Rates of oxygen consumption were quantified to define (1) resting metabolic rates (RMR; the metabolic rate of a postprandial shark at rest and stable temperature; Chabot et al., 2016), (2) metabolic rates at each velocity step during the U_{crit} test, (3) maximum metabolic rate (MMR; the highest metabolic rate during the entire trial; Norin and Clark, 2016), and (4) recovery from exhaustion (EPOC). Metabolic rates were measured for 10 min every 20 min during the six-hour morning acclimatization period to estimate RMR. Following exhaustion, metabolic rates were measured for 10 min every 15 min for up to 70 min to estimate EPOC. After removing animals from the swim tunnel, the tunnel was refilled and run for an additional 10 min at 20 cm s^{-1} to measure any background respiration. The swim tunnel loaded from the front portion, requiring that the tunnel was partially drained to add and remove animals. Therefore, background respiration could not be quantified with the same water sharks swam in.

Water from the swim tunnel was tested for dissolved oxygen concentration (mg L^{-1}) every second to observe the rate of mass-specific oxygen consumption (MO_2) with a FireSting one-channel fiber optic oxygen sensor and accessory temperature probe (Pyroscience, Aachen, Germany). Oxygen sensors were calibrated prior to each trial, and salinity for calibration was measured using a YSI Pro2030 field dissolved oxygen and conductivity meter (YSI Incorporated, Yellow Springs, OH, USA).

Rates of oxygen consumption were measured as

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

where $\Delta[O_2]$ is the change in dissolved oxygen concentration during the ten-minute closed period, v is the volume of the swim tunnel minus the animal's volume assuming 1 kg of sea water is equivalent to 1 kg of animal, m is the mass of the animal, and t is the time interval over which $\Delta[O_2]$ is assessed (Lee et al., 2003b). During acclimation, RMR was calculated as the mean of the lowest six measurements with coefficients of determination >0.95 (Shultz et al., 2011). During forced swimming, MO_2 was measured for the first 10 min of each 15-minute measurement at each speed. Excess post-exercise oxygen consumption was calculated by fitting a five-parameter bi-exponential decay curve (Scarabello et al., 1991; Svendsen et al., 2010) to mean post-exercise MO_2 data at each measurement following 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 (Scarabello et al., 1991; Svendsen et al., 2010). All constants except the intercept were derived using non-linear regression; the intercept was replaced with RMR (Scarabello et al., 1991; Svendsen et al., 2010). Time of recovery was assessed by determining when the recovery curve intersected the upper 95% confidence interval limit for RMR (Bushnell et al., 1994; Svendsen et al., 2010). Excess post-exercise oxygen consumption was calculated by integrating the area beneath the recovery curve bound by the initial measurement, recovery time, and the upper 95% confidence interval limit RMR (Lee et al., 2003a; Svendsen et al., 2010).

2.4. Mesocosm deployments

Lemon sharks ($n = 13$) were equipped with accelerometers and released to a 4000 m^2 semi-enclosed natural habitat (hereafter, mesocosm) to measure activity levels in semi-captive sharks in 2014 ($n = 8$, total length = 70.59 ± 2.49 cm total length, 1.24 ± 0.11 kg, five female and three male) and 2015 ($n = 5$, total length = 70.84 ± 1.56 cm total length, 1.78 ± 0.10 kg, three female and two male). The mesocosm exposes fishes to natural photoperiod and seasonal temperatures, is 0.5–1.0 m deep, and is continuously supplied seawater as runoff from the adjacent wet lab facility. While the mesocom is natural in origin and situated 10.0 m from the ocean, it only receives tidal water flux during extreme high tides (Murchie et al., 2011; Brownscombe et al., 2014; Wilson et al., 2015). In addition, the mesocosm is fringed with red (*Rhizophora mangle*) and black (*Avicennia germanius*) mangroves, and inhabited by bonefish (*Albula vulpes*), great barracuda (*Sphyraena barracuda*), mangrove (*Lutjanus griseus*) and schoolmaster (*L. apodus*) snapper, and yellowfin mojarra (*Gerres cinereus*). Sharks were released to the mesocosm for three to five days, and were fed cut sardines (*S. aurita*) daily to supplement foraging of aforementioned prey and insure animal health (Wilson et al., 2015). Water quality (temperature, dissolved oxygen, and salinity) was monitored daily.

Acceleration data were analyzed for an entire deployment (i.e., from immediate release to the mesocosm to recapture) by calculating instantaneous metabolic rates and swimming speeds from calibration equations generated for ODBA and TBF (Lowe, 2002), and categorized by tide state (flooding and ebbing) and diel period (day and night) using times from an on-island tide recording station. It was assumed that aerobic metabolic rates could not exceed MMR, and estimated values were therefore capped at MMR. Metabolic rates were averaged to define active metabolic rate, and the mass of oxygen consumed by each shark during a deployment was converted to energy consumption using an oxycalorific coefficient of 14.8 J mg O_2^{-1} (Elliott and Davison, 1975). Energy expenditure was subsequently converted to average daily metabolic rate (ADMR, presented in kJ kg^{-1} d^{-1}) for an entire deployment.

2.5. Statistical analysis

Predictive relationships of MO_2 against activity metrics (ODBA, TBF, and U_{rel}), and U_{rel} against ODBA and TBF were generated with simple linear regressions. Statistically significant linear predictors of MO_2 and U_{rel} were fit with linear mixed effects models with the activity metric and temperature as fixed effects, and shark ID as a random effect. Lastly, MO_2 and U_{rel} were fit with generalized least squares mixed effects models with tide state, diel period, and their interaction as fixed effects, and shark ID as a random effect. Generalized least squares was employed because it allowed for heteroscedasticity among the nominal variables tide state, diel period, and the interaction of the two (Zuur et al., 2009). For linear mixed effects models, importance of fixed effects was determined 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 (Hasler 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. All statistical analyses were run in R (R Core Team, 2015) using the nlme package (Pinheiro et al., 2016).

3. Results

3.1. Swim tunnel respirometry

Between 7 April and 14 August 2014, forced swimming trials were run with 25 lemon sharks. Of these, ten sharks swam to exhaustion (61–69.8 cm total length, 1.09–1.70 kg, four females and six males) while the others failed to swim despite repeated attempts to encourage steady swimming behavior. Resting metabolic rate was measured from eight individuals between 3 July and 14 August 2014 (59.4–69.8 cm total length, 1.03–1.72 kg, five females and three males). Mean RMR was 155.19 ± 3.56 mg O_2 kg^{-1} h^{-1} measured at a mean temperature of 30.27 ± 0.11 °C. Maximum metabolic rate was measured from ten individuals across the entire experimental period as 398.02 ± 19.60 mg O_2 kg^{-1} h^{-1} at a mean temperature of 29.13 ± 0.64 °C. Critical swimming speed for these same sharks was 0.71 ± 0.03 BL s^{-1}. Recovery from exhaustive exercise (Fig. 1), was modeled by the equation $MO_2 = 155.19 + 132.24e^{-322.48\text{time}} + 92.49e^{-2.77\text{time}}$. Recovery was estimated to occur within 55 min. Integrating the area bound by this curve and RMR over the 55-minute recovery period yielded an EPOC of 31.2 mg O_2 kg^{-1}.

Relative swimming speed had a positive linear relationships with MO_2 measured at each velocity step during swim tunnel respirometry (Fig. 2A), but this relationship was influenced by water temperature (Table 1). Overall dynamic body acceleration did not have a clear predictive relationship with MO_2 (Fig. 2B). Conversely, TBF displayed a positive linear relationship with MO_2 (Fig. 2C), and temperature did not influence relationships between MO_2 and TBF (Table 1). Overall dynamic body acceleration also did not exhibit a linear relationship with U_{rel} (Fig. 3A). Specifically, high ODBA values were associated with both low and high values for U_{crit}. Tailbeat frequency did exhibit a strong positive linear relationship with U_{rel} (Fig. 3B), and this relationship was not affected by temperature (Table 1).

3.2. Mesocosm deployments

Sharks were deployed in the mesocosm between 25 September and 10 October 2014, and 19–23 June 2015. Of 13 deployments, 12 sharks (eight in 2014 and four from 2015) had functioning accelerometers. Deployments ranged from 29.4 to 95.0 h (mean = 50.2 ± 6.9 h), at 30.8 ± 0.3 °C (2014) and 32.1 ± 0.9 °C (2015). From the equation predicting MO_2 from TBF, sharks had an active metabolic rate of 249.7 ± 1.9 mg O_2 kg^{-1} h^{-1}, a mean U_{rel} of 0.19 ± 0.01 BL s^{-1}, and an ADMR of 88.7 ± 0.7 kJ kg^{-1} d^{-1}. There was a significant interaction effect

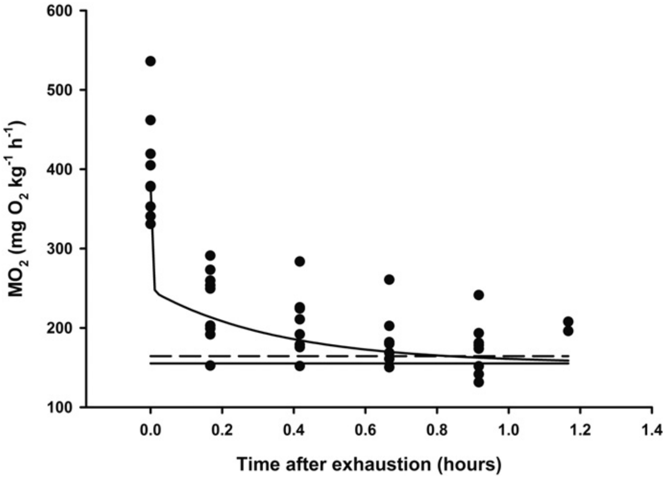

Fig. 1. Recovery from exhaustive exercise. The recovery curve was modeled by a five-parameter bi-exponential decay curve ($R^2 = 0.80$), following the equation $MO_2 = 155.19 + 132.24e^{-322.48 * \text{time}} + 92.49e^{-2.77 * \text{time}}$. Time at recovery was approximately 0.92 h (55 min). Excess post-exercise oxygen consumption (EPOC) was calculated by integrating the area under the recovery curve that was bound by $t = 0$ h, time of recovery ($t = 0.92$ h), and upper 95% confidence interval limit of mean resting metabolic rate (dashed line; 162.34 mg O_2 kg^{-1} h^{-1}), yielding an EPOC of 31.2 mg O_2 kg^{-1}. The solid line represents mean resting metabolic rate (155.19 mg O_2 kg^{-1} h^{-1}).

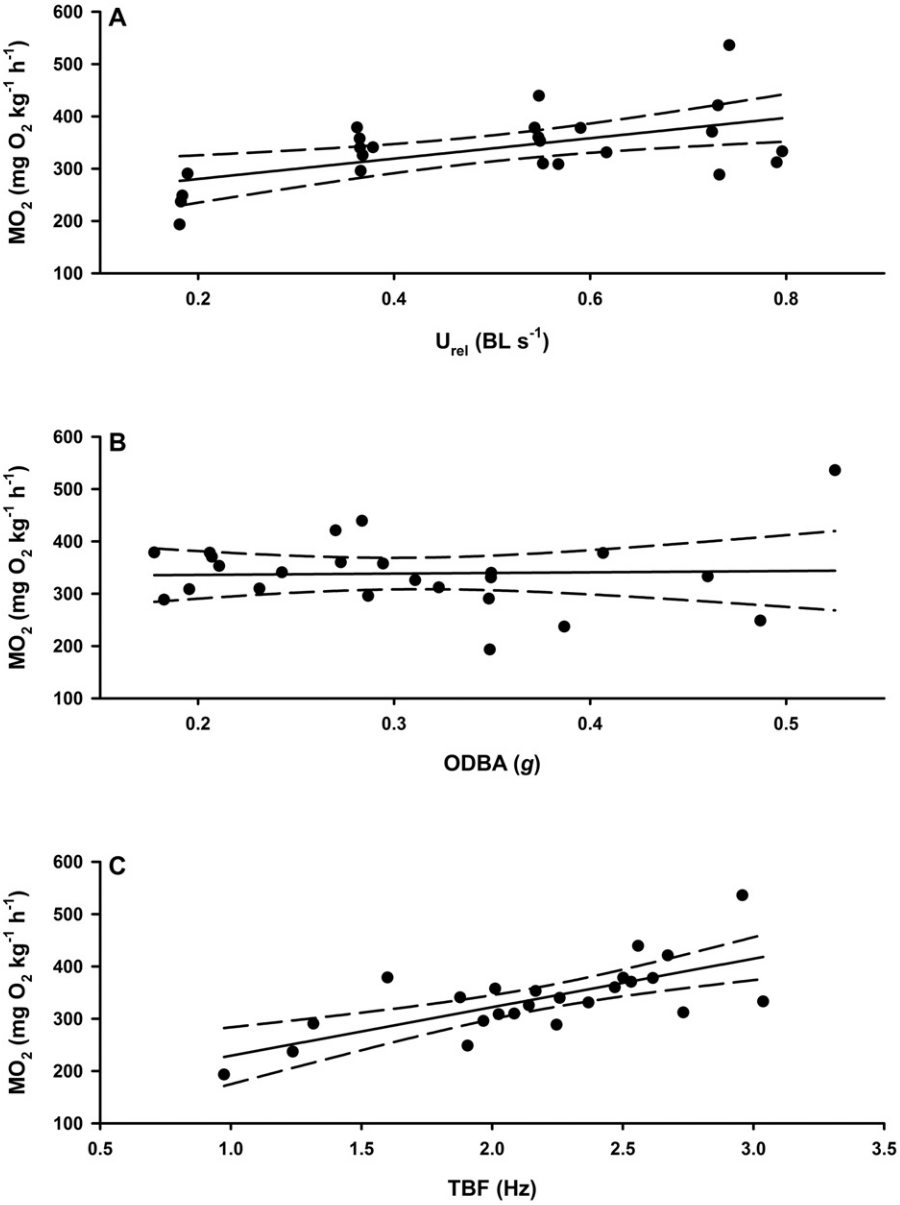

Fig. 2. Predictive relationships for metabolic rate (MO_2) against relative swimming speed (U_{rel}) (A), overall dynamic body acceleration (ODBA) (B), and tailbeat frequency (TBF) (C) from swim tunnel respirometry with 95% confidence intervals. Metabolic rate was related to U_{rel} by the equation $MO_2 = 241.3 + 194.8U_{rel}$ (linear regression, $R^2 = 0.32$, $F_{1, 22} = 10.19$, $p < 0.01$), ODBA by the equation $MO_2 = 330.9 + 24.7ODBA$ (linear regression, $R^2 = 0.001$, $F_{1, 22} = 0.03$, $p = 0.87$), and TBF by the equation $MO_2 = 136.5 + 92.8TBF$ (linear regression, $R^2 = 0.48$, $F_{1, 22} = 20.64$, $p < 0.001$).

Table 1

Linear mixed effects model output for laboratory calibrations. Mixed effects models for metabolic rate (MO_2) was fit with significant linear predictors, relative swimming speed (U_{rel}) and tailbeat frequency (TBF) as fixed effects, with temperature as a fixed effect and shark ID as a random effect. In addition, a model for U_{rel} was fit with TBF and Temperature as fixed effects, and shark ID as a random effect. Significance of fixed effects was determined by running 1000 posterior simulations of each fixed effect and observing whether the 95% credible interval (CI) for the distribution of fixed effect estimates did not overlap zero. Bolded terms indicate significant fixed effects whose CI does not overlap zero.

Response variable	Fixed effect	97.5% CI	2.5% CI
MO_2	**U_{rel}**	**262.66**	**45.36**
	Temperature	**38.11**	**1.29**
MO_2	**TBF**	**119.64**	**32.13**
	Temperature	33.19	−3.12
U_{rel}	**TBF**	**0.45**	**0.26**
	Temperature	0.03	−0.05

between diel period and tide state on both MO_2 and U_{rel} (Table 2). Sharks had the highest metabolic rates during the day at flooding tides (253.0 ± 0.1 mg O_2 kg^{-1} h^{-1}), followed by flooding tides at night (249.1 ± 0.1 mg O_2 kg^{-1} h^{-1}), ebbing tides during the day (245.1 ± 0.0 mg O_2 kg^{-1} h^{-1}), and ebbing tides at night (243.9 ± 0.0 mg O_2 kg^{-1} h^{-1}) (Fig. 4A). In addition, sharks swam fastest during the day at flooding tides (0.27 BL s^{-1}), followed by flooding tides at night (0.22 BL s^{-1}), ebbing tides during the day (0.18 BL s^{-1}), and sharks swam slowest during ebbing tides at night (0.17 BL s^{-1}) (Fig. 4B).

4. Discussion

Swimming velocities and metabolic rates of juvenile lemon sharks varied with diel period. Sharks in this study were more active during the day than night across tide states. These findings contradict previous studies on diel activity patterns of lemon sharks in The Caribbean, with

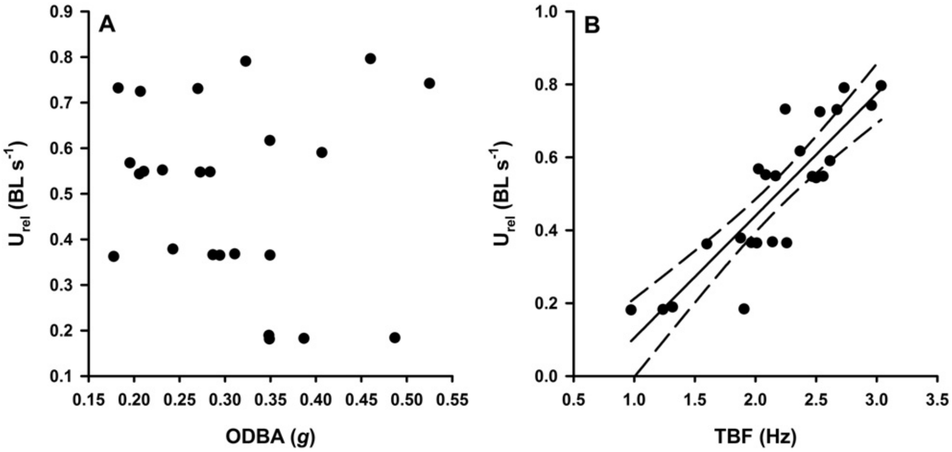

Fig. 3. Relationships (with 95% confidence intervals) for relative swimming speed (U_{rel}) against the acceleration metrics overall dynamic body acceleration (ODBA) (A) and tailbeat frequency (TBF) (B). Overall dynamic body acceleration did not have a linear relationship with U_{rel} (linear regression, $R^2 = 0.01$, $F_{1,\ 22} = 0.29$, $p = 0.59$). Tailbeat frequency correlated strongly with U_{rel}, and was modeled by the equation $U_{rel} = 0.33\text{TBF} - 0.23$ (linear regression, $R^2 = 0.76$, $F_{1,\ 22} = 68.53$, $p < 0.0001$).

studies claiming that lemon sharks are either nocturnal (e.g., Cortés and Gruber, 1990; Gruber et al., 1988; Nixon and Gruber, 1988; Wilson et al., 2015) or that lemon sharks do not exhibit diel patterns in activity levels (e.g., Legare et al., 2015; Murchie et al., 2010; Wetherbee et al., 2007). In this study's mesocosm, however, diel variation in swimming activity has been observed for juvenile lemon sharks and bonefish observed over 5–8 days, as compared to this study's 1–4 days of observation (Brownscombe et al., 2014; Wilson et al., 2015). Diel patterns in activity can be highly plastic in fishes (Fraser et al., 1993; Reebs, 2002), and diel variation in activity levels may have been more pronounced if this study could have incorporated variation in water temperatures (Brownscombe et al., 2014). Thus, these data support the notion that lemon sharks may possess a circadian component to their activity cycle, though differences with previous reports of lemon shark activity cycles may suggest differences in local biotic and abiotic factors that influence when sharks are active. It should be noted, however, that transplanting sharks and the conditions of the mesocosm likely had an influence on shark activity levels and, therefore, metabolic rates (Wilson et al., 2015). Specifically, the mesocosm was considerably smaller than the home range of juvenile lemon sharks around Cape Eleuthera, and sharks were not exposed to predation risk or the full range of environmental conditions (e.g., changing depth with tide, dissolved oxygen, salinity) that could be experienced in the wild (Murchie et al., 2010). Furthermore, the influence of feeding sharks during the day on hunting motivation likely affected diel activity levels, though lemon sharks are not known to feed at any particular time of day (Cortés and Gruber, 1990). Thus, activity levels of sharks in the mesocosm should not be considered natural, and comparison with activity of unrestricted, wild sharks should be made with caution. Regardless, fishes observed in this study's mesocosm exhibited variation in diel activity levels, and this study's lemon sharks exhibited higher activity levels during the day across tidal states.

Table 2
Generalized least squares mixed effects model output for field deployment data. Models for metabolic rate (MO_2) and relative swimming speed (U_{rel}) collected from mesocosm deployed sharks were fit with tide state (flooding or ebbing), diel period (day or night), and their interaction (denoted by ":") as fixed effects, with shark ID as a random effect. Models incorporated variance structures that allowed for heteroscedasticity among levels of the fixed effects. Significance of fixed effects was determined by running 1000 posterior simulations of each fixed effect and observing whether the 95% credible interval (CI) for the distribution of fixed effect estimates did not overlap zero. Bolded terms indicate significant fixed effects whose CI does not overlap zero.

Response variable	Fixed effect	97.5% CI	2.5% CI
MO_2	Diel period	−4.89	−5.15
	Tide state	−8.44	−8.22
	Diel:tide	3.40	3.06
U_{rel}	Diel period	−0.06	−0.06
	Tide state	−0.09	−0.09
	Diel:tide	0.05	0.05

Lemon sharks also exhibited variation in swimming speeds and metabolic rates across tidal states. Specifically, sharks were more active during flooding tides than ebbing tides within each time of day. Sharks at the study site experienced semi-diurnal tides and were exposed to flooding and ebbing tides during the day and night, therefore, making it unlikely that differential responses to tide state or diel period were confounded. Changes in swimming velocities and metabolic rates were less pronounced across tide state than time of day, though changes in activity with the tide have been demonstrated in lemon sharks (e.g., Guttridge et al., 2012; Harborne et al., 2016) and teleosts inhabiting tidal mangrove creeks, including bonefish tested in this study's mesocosm (e.g., Brownscombe et al., 2014; Krumme, 2009; Murchie et al., 2011). In particular, bonefish observed over five days in the mesocosm exhibited significant variation in activity levels with tides despite water levels not being affected by tides, which suggests a mechanism (e.g., entrained circadian rhythms) by which this coastal species can detect changes in tide (Brownscombe et al., 2014). In the wild, small changes in activity levels with tide state may reflect predator avoidance strategies, given that sharks were typically more active on flooding tides when water depths would be too shallow for sharks to seek refuge in mangrove creeks (Wetherbee et al., 2007). While this study did not attempt to understand foraging activity, it has been suggested that lemon sharks feed intermittently and asynchronously (Cortés and Gruber, 1990), and predation from teleosts, raptors, and conspecifics is likely a predominant factor influencing activity across tidal and diel cycles. In the mesocosm, however, changes in activity levels across tidal states tied to predator-prey interactions would be entirely in response to the motivation to hunt, with no apparent threat of predation. Thus, the influence of foraging over predator avoidance on tidal activity of juvenile lemon sharks around Cape Eleuthera remains unclear, but feeding sharks in the absence of predation risk likely biased tide-based activity. Therefore, while sharks in the mesocosm did not experience predation risk or significant changes in water level associated with tides, sharks in this study exhibited higher activity levels at flooding tides over ebbing tides across time of day.

Metabolic rates estimated from swim tunnel respirometry for juvenile lemon sharks relative to sympatric elasmobranch species are higher

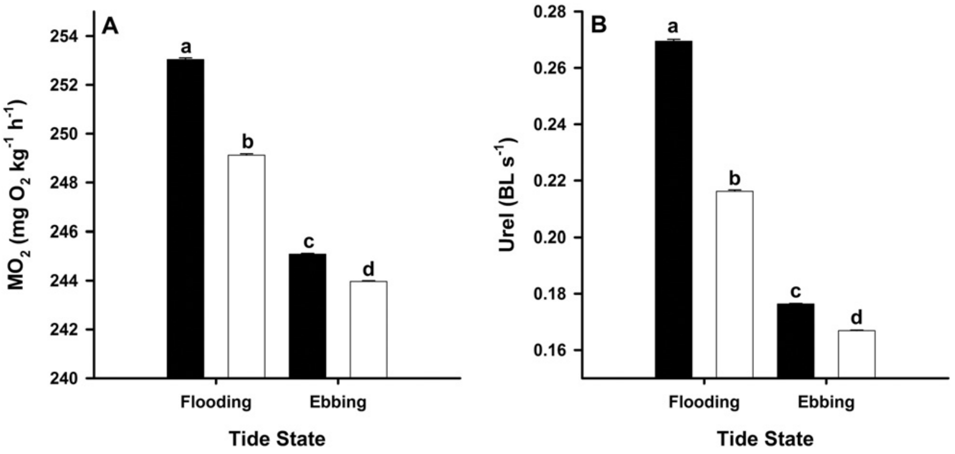

Fig. 4. Metabolic rates (MO$_2$) (A) and relative swimming speed (U$_{rel}$) (B) for mesocosm deployed sharks by diel period (time of day) and tide state (flooding or ebbing). Sharks had the highest metabolic rates and relative swimming velocities at flooding tides during the day (253.0 mg O$_2$ kg^{-1} h^{-1}, 0.27 BL s^{-1}), followed by flooding tides at night (249.1 mg O$_2$ kg^{-1} h^{-1}, 0.22 BL s^{-1}), ebbing tides during the day (245.1 mg O$_2$ kg^{-1} h^{-1}, 0.18 BL s^{-1}), and ebbing tides at night (243.9 mg O$_2$ kg^{-1} h^{-1}, 0.17 BL s^{-1}). Black and white bars represent daytime and nighttime values, respectively. Differing lowercase letters indicate statistically significant differences ($\alpha < 0.05$).

than sedentary species, lower than obligate ram-ventilating species, and differ from previous estimates for this species. Juvenile lemon sharks had a RMR of 155.19 ± 3.56 mg O$_2$ kg^{-1} h^{-1}, and a MMR of 398.02 ± 19.60 mg O$_2$ kg^{-1} h^{-1} measured at approximately 30 °C. Relative to sympatric sub-tropical elasmobranchs, lemon sharks had considerably higher resting metabolic rates than sedentary nurse sharks (*Ginglymostoma cirratum*; 60.0 ± 17.0 mg O$_2$ kg^{-1} h^{-1} at 28–30 °C; Whitney et al., 2016), and a lower metabolic rate than continuously active obligate ram-ventilating blacknose sharks (*Carcharhinus acronotus*; 239.8 mg O$_2$ kg^{-1} h^{-1} at 27–29 °C; Carlson et al., 1999). Resting metabolic rate for sharks in this study was equal to that measured at 25 °C (152.6 ± 7.3 mg O$_2$ kg^{-1} h^{-1}; Scharold and Gruber, 1991) and higher than measured at 22 °C (94.6 ± 4.3 mg O$_2$ kg^{-1} h^{-1}; Bushnell et al., 1989) suggesting population-specific metabolic rates with lemon sharks (e.g., Lee et al., 2003a) or that lemon sharks exhibit metabolic compensation, i.e., the ability to maintain constant metabolic rates despite temperature variation (e.g., Dickson et al., 2002; Sandblom et al., 2014). Maximum metabolic rate has been measured for few elasmobranchs, but MMR of sharks in this study was considerably lower than measured for a lemon shark (MMR = 627 mg O$_2$ hg^{-1} h^{-1} at 22–25 °C; Graham et al., 1990) and scalloped hammerhead sharks (501–701 mg O$_2$ kg^{-1} h^{-1} at 26 °C; Lowe, 2001; Gleiss et al., 2010). While it is a reasonable supposition that lemon sharks exhibit lower MMR than ram-ventilating hammerheads by maintaining less gill surface area (Carlson et al., 1999; Lowe, 2001), lower MMR relative to conspecifics at lower temperatures may indicate an undefined thermal optimum for lemon shark MMR (Lee et al., 2003b). Therefore, lemon sharks have intermediate metabolic rates relative to sedentary and continuously active tropical species, though metabolic rates appear to be variable across populations of lemon sharks.

Experimental conditions of the mesocosm may have additionally influenced metabolic rates and swimming speeds of lemon sharks. Mesocosm deployed sharks exhibited an ADMR of 88.7 ± 0.7 kJ kg^{-1} d^{-1}, an active metabolic rate of 249.7 ± 1.9 mg O$_2$ kg^{-1} h^{-1}, and a U$_{rel}$ of 0.19 ± 0.01 BL s^{-1}. Compared to other studies of lemon sharks, ADMR and U$_{rel}$ was 69 kJ kg^{-1} d^{-1} and 0.25 BL s^{-1} at 23 °C for sharks in an annular respirometer (Nixon and Gruber, 1988), and 34–37 kJ kg^{-1} d^{-1} and 0.37 BL s^{-1} at 18–24 °C for wild lemon sharks (Sundström and Gruber, 1998). Thus, sharks in this study consumed more energy swimming at relatively slower U$_{rel}$. Though differences in estimates resulting from water temperature and animal mass likely influenced these ADMR and U$_{rel}$ estimates (Bernal et al., 2012), the influence of differences in experimental design across these experiments is unclear. Lemon sharks in this study were provisioned to insure animal health, though this feeding may have had unintended consequences for activity level and therefore, active metabolic rate, because provisioning has been demonstrated to increase activity levels and energy expenditure of whitetip reef sharks (*Triaenodon obesus*; Barnett et al., 2016). Furthermore, it is unclear how enclosure in this study's mesocosm may have affected activity levels or metabolic rates, given that mean volitional swimming speeds of respirometer-confined and free-swimming sharks were both higher than in this study (Nixon and Gruber, 1988; Sundström and Gruber, 1998). Overall, there is a need for standardized approaches to estimation of metabolic rates of sharks, as comparison across elasmobranch metabolism studies here was impractical even within the same species (Lowe and Goldman, 2001). Thus, conditions of the mesocosm may have influenced metabolic rates and activity levels of lemon sharks in this study, though variability in experimental design across elasmobranch metabolism studies may preclude meaningful comparisons.

Tailbeat frequency was a better acceleration-derived predictor of metabolic rates than ODBA for juvenile lemon sharks. Acceleration-derived estimates of TBF were the best predictor of MO$_2$ for juvenile lemon sharks, albeit with lower predictive power than ODBA generated for other aquatic species (i.e., this study's R^2 of 0.48 compared to R^2 values > 0.71; Gleiss et al., 2010; Wright et al., 2014). Tailbeat frequency has been used to estimate swimming speeds and metabolic rates for a shark (Lowe, 2002; Lowe et al., 1998), though studies in elasmobranchs are seeking to implement ODBA as a metric to telemeter activity-specific metabolic rates (Gleiss et al., 2010; Whitney et al., 2012). While ODBA has shown promise as a proxy for energy expenditure in juvenile lemon sharks (Gleiss et al., 2009), unsuccessful calibration of ODBA with MO$_2$ in this study was likely due to sharks exhibiting considerable dorso-ventral and lateral movement at slower speeds to remain stable within the water column (Lowe, 1996), while maintaining a stable tailbeat (Fig. 3A). Acceleration-derived estimates of tailbeat frequency may not be a sufficient metric for estimating energy expenditure tied to non-steady or complex swimming behaviors occurring in three planes (Gleiss et al., 2010), though the majority of swimming behaviors for sharks are generated from the tailbeat and recent work for a teleost suggests TBF is a better proxy than ODBA (Mori et al., 2015). Forced swimming may not be the best method to calibrate ODBA and metabolic rates in active buccal-pumping elasmobranchs (e.g., Brett and Blackburn, 1978; Whitney et al., 2007), given that lemon sharks inhabit environments with minimal flow and either voluntarily swim or rest (Wilson et al., 2015), unlike salmonids that inhabit environments characterized by high flow and are comparatively well-suited to forced swimming protocols (e.g., Lee et al., 2003a, 2003b). Indeed, more than half of the sharks introduced into the swim tunnel failed to swim when exposed to increases in velocity that suggests a lack of motivation, which is an increasingly common finding in forced swim trials (Nelson et al., 2002). Furthermore, sharks that swam exhibited relatively low values for U_{crit} (see Graham et al., 1990) suggesting that confines of the swim tunnel prompted fatigue as a behavioral response (Peake and Farrell, 2006). While calibration of ODBA was unsuccessful likely by virtue of spontaneous activity at low swimming velocities, TBF is generally a strong proxy for other acceleration-based metrics that correlate well with energy expenditure (e.g., Clark et al., 2010; Gleiss et al., 2009; Wilson et al., 2013). Furthermore, for telemetry devices with greater demands on battery life (e.g., tags that log at very high frequencies or have multiple sensors), logging acceleration in fewer axes (e.g., the sway axis to estimate TBF; Kawabe et al., 2003) reduces power demand and little predictive power is gained by logging in more than one axis (Halsey et al., 2009a). Therefore, TBF is still a suitable metric for active metabolic rate estimation, despite it being inadequate for characterizing complex behaviors.

This study was the second to estimate EPOC for an elasmobranch, and highlights logistical constraints for measuring EPOC in sharks. Juvenile lemon sharks subjected to a U_{crit} protocol experienced an EPOC of 31.2 mg O_2 kg^{-1} at 29 °C that resolved within 55 min. In the only other account of shark EPOC, spiny dogfish (*Squalus acanthias*) subjected to manual chasing experienced an EPOC of 105 mg O_2 kg^{-1} at 10 °C that resolved within 180 min (Brett and Blackburn, 1978), though interspecific comparison of EPOC is limited because of the considerable temperature difference and different exhaustive exercise technique employed (Roche et al., 2013). Compared with high-performance salmonids (*Onchorhyncus* spp.) exercised with a U_{crit} protocol at cooler water temperatures (7.9–17.5 °C), salmonids experienced an EPOC at least twice that of juvenile lemon sharks (61.6–254.2 mg O_2 kg^{-1}), and recovered over comparable durations (42–78 min; Lee et al., 2003a), suggesting that lemon sharks may have limited anaerobic capacity relative to high-performance teleosts. Bonefish (*A. vulpes*), a prey item for juvenile lemon sharks experienced a lower recovery scope (MMR-RMR = 154 mg O_2 kg^{-1} h^{-1}) following 4 min of exhaustive chasing at 23.5 °C (Shultz et al., 2011) than lemon sharks in this study (recovery scope = 242.8 mg O_2 kg^{-1} h^{-1}), yet bonefish exhibited faster blood lactate clearance at comparable temperatures and exercise protocols (Brooks et al., 2011; Suski et al., 2007). Though exercise physiology data are typically lacking for tropical marine fishes at high temperatures (Kieffer, 2010) and inter-specific comparisons with this study are limited, juvenile lemon sharks do not appear well-suited to extended durations of high-end aerobic swimming at high water temperatures. Lemon sharks likely recruit anaerobic metabolism at aerobically-supported swimming velocities below their U_{crit}, but the velocity at which sharks recruit anaerobic metabolism could not be determined (Burgetz et al., 1998; Lee et al., 2003a; Svendsen et al., 2010). Excess post-exercise oxygen consumption is typically measured in fishes at rest with minimal or absent current (e.g., Farrell et al., 2003), making lemon sharks one of the few tropical shark species that are appropriate for EPOC measurement given that they are small enough for use in modestly-size respirometers (e.g., Bushnell et al., 1989), they are active enough to swim against controlled currents for extended durations (e.g., Graham et al., 1990), and most importantly they can buccal pump while sedentary, which allows for direct measurement of SMR (see Lowe, 2001). Therefore, additional research is required on the exercise physiology of elasmobranchs especially given its relevance to fisheries research as a method of defining energetic costs of exhaustive events, such as fisheries capture.

In conclusion, acceleration derived-estimates of TBF were a better predictor of metabolic rates for wild lemon sharks than ODBA, which appears to be a better proxy for obligate ram-ventilating species (e.g., Gleiss et al., 2010). While TBF may not be a sufficient metric for identifying complex behaviors occurring in three-dimensional space, this study demonstrates that acceleration data analyzed in a single acceleration axis can adequately telemeter active metabolic rates for wild sharks. Furthermore, TBF was sensitive enough to observe variation in activity levels correlated with variation in environmental variables (i.e., tide state and diel period), though these may not represent natural activity levels given the semi-captive and predator-free conditions of the mesocosm. In comparison with other studies, this study suggests that lemon sharks have intermediate aerobic metabolic capacities relative to inactive sedentary species and obligate ram ventilators, yet it is unclear how mesocosm conditions and daily feeding influenced active metabolic rates relative to natural field metabolic rates. While these results were in discordance with previously published reports of lemon shark metabolic rates, variation in temperature, body mass, and perhaps respirometer design may have ultimately made comparison between these studies difficult. The latter point highlights the need for comprehensive studies of elasmobranch metabolism that account for scaling of metabolic rate with mass and temperature (e.g., Whitney et al., 2016). Lastly, this study provided the second account of EPOC for a shark species, though the significance of this finding is limited given the paucity of exercise physiology research on elasmobranchs and the inherent difficulty of elasmobranch respirometry in static systems. Lemon sharks appear to have limited capacity for anaerobic metabolism relative to high-performance teleosts, yet how other species characterized by different “pace-of-life” traits differ in their anaerobic capacities. Thus, in the context of lemon shark metabolism and telemetry studies, accelerometry is a suitable means for active metabolic rate and swimming speed measurement, and this species is in need of comprehensive metabolism studies given the opportunity to measure both aerobic and anaerobic activity metabolism for this species.

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References

Barnett, A., Payne, N.L., Semmens, J.M., Fitzpatrick, R., 2016. Ecotourism increases the field metabolic rate of whitetip reef sharks. Biol. Conserv. 199, 132–136.

Bell, W.M., Terhune, L.D.B., 1970. Water tunnel design for fisheries research. Fish. Res. Board Canada Tech. Rep. 195, 1–69.

Bernal, D., Carlson, J.K., Goldman, K.J., Lowe, C.G., 2012. Energetics, metabolism, and endothermy in sharks and rays. In: Carrier, J.C., Musick, J.A., Heithaus, M.C. (Eds.), Biology of Sharks and Their Relatives, second ed. Taylor and Francis Group, LLC, Boca Raton, FL, pp. 211–237.

Brett, J.R., 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Board Can. 21, 1183–1226.

Brett, J.R., Blackburn, J.M., 1978. Metabolic rate and energy expenditure of the spiny dogfish, *Squalus acanthias*. J. Fish. Res. Board Can. 35, 816–821.

Brooks, E.J., Sloman, K.A., Liss, S., Hassan-Hassanein, L., Danylchuk, A.J., Cooke, S.J., Mandelman, J.W., Skomal, G.B., Sims, D.W., Suski, C.D., 2011. The stress physiology of extended duration tonic immobility in the juvenile lemon shark, *Negaprion brevirostris* (Poey 1868). J. Exp. Mar. Biol. Ecol. 409, 351–360.

Brown, D.D., Kays, R., Wikelski, M., Wilson, R., Klimley, A., 2013. Observing the unwatchable through acceleration logging of animal behavior. Anim. Biotelem. 1, 1–16.

Brownscombe, J.W., Gutowsky, L.F.G., Danylchuk, A.J., Cooke, S.J., 2014. Foraging behaviour and activity of a marine benthivorous fish estimated using tri-axial accelerometer biologgers. Mar. Ecol. Prog. Ser. 505, 241–251.

Burgetz, I.J., Rojas-Vargas, A., Hinch, S.G., Randall, D.J., 1998. Initial recruitment of anaerobic metabolism during sub-maximal swimming in rainbow trout (*Oncorhynchus mykiss*). J. Exp. Biol. 201, 2711–2721.

Bushnell, P., Lutz, P., Gruber, S., 1989. The metabolic rate of an active, tropical elasmobranch, the lemon shark (*Negaprion brevirostris*). Exp. Biol. 48, 279–283.

Bushnell, P.G., Steffensen, J.F., Schurmann, H., Jones, D.R., 1994. Exercise metabolism in two species of cod in arctic waters. Polar Biol. 14, 43–48.

Carlson, J., Palmer, C., Parsons, G., 1999. Oxygen consumption rate and swimming efficiency of the blacknose shark, *Carcharhinus acronotus*. Copeia 1999, 34–39.

Chabot, D., Steffensen, J.F., Farrell, A.P., 2016. The determination of standard metabolic rate in fishes. J. Fish Biol. 88, 81–121.

Clark, T.D., Sandblom, E., Hinch, S.G., Patterson, D.A., Frappell, P.B., Farrell, A.P., 2010. Simultaneous biologging of heart rate and acceleration, and their relationships with energy expenditure in free-swimming sockeye salmon (*Oncorhynchus nerka*). J. Comp. Physiol. B. 180, 673–684.

Cooke, S.J., Brownscombe, J.W., Raby, G.D., Broell, F., Hinch, S.G., Clark, T.D., Semmens, J.M., 2016. Remote bioenergetics measurements in wild fish: opportunities and challenges. Comp. Biochem. Physiol. A Mol. Integr. Physiol. http://dx.doi.org/10.1016/j.cbpa.2016.03.022 (in press).

Core Team, R., 2015. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria (URL). https://www.R-project.org/.

Cortés, E., Gruber, S.H., 1990. Diet, feeding habits and estimates of daily ration of young lemon sharks, *Negaprion brevirostris* (Poey). Copeia 1990, 204–218.

Cortés, E., Gruber, S.H., 1992. Gastric evacuation in the young lemon shark, *Negaprion brevirostris*, under field conditions. Environ. Biol. Fish 35, 205–212.

Dickson, K.A., Gregorio, M.O., Gruber, S.J., Loefler, K.L., Tran, M., Terrell, C., 1993. Biochemical indices of aerobic and anaerobic capacity in muscle tissues of California elasmobranch fishes differing in typical activity level. Mar. Biol. 193, 185–193.

Dickson, K.A., Donley, J.M., Sepulveda, C., Bhoopat, L., 2002. Effects of temperature on sustained swimming performance and swimming kinematics of the chub mackerel *Scomber japonicus*. J. Exp. Biol. 205, 969–980.

Dowd, W.W., Brill, R.W., Bushnell, P.G., Musick, J.A., 2006. Standard and routine metabolic rates of juvenile sandbar sharks (*Carcharhinus plumbeus*), including the effects of body mass and acute temperature change. Fish. Bull. 104, 323–331.

Elliott, J.M., Davison, W., 1975. Energy equivalents of oxygen-consumption in animal energetics. Oecologia 19, 195–201.

Farrell, A.P., Lee, C.G., Tierney, K., Hodaly, A., Clutterham, S., Healey, M., Hinch, S., Lotto, A., 2003. Field-based measurements of oxygen uptake and swimming performance with adult Pacific salmon using a mobile respirometer swim tunnel. J. Fish Biol. 62, 64–84.

Fraser, N.H., Metcalfe, N.B., Thorpe, J.E., 1993. Temperature-dependent switch between diurnal and nocturnal foraging in salmon. Proc. R. Soc. Lond. B 252, 135–139.

Gaesser, G.A., Brooks, G.A., 1984. Metabolic bases of excess post-exercise oxygen consumption: a review. Med. Sci. Sports Exerc. 16, 29–43.

Gleiss, A., Gruber, S., Wilson, R., 2009. Multi-channel data-logging: towards determination of behaviour and metabolic rate in free-swimming sharks. In: Nielsen, J.L., Arrizabalaga, H., Fragoso, N., Hobday, A., Lutcavage, M., Sibert, J. (Eds.), Tagging and Tracking of Marine Animals With Electronic Devices. Springer, Dordrecht, Netherlands, pp. 211–228.

Gleiss, A.C., Dale, J.J., Holland, K.N., Wilson, R.P., 2010. Accelerating estimates of activity-specific metabolic rates in fishes: testing the applicability of acceleration data-loggers. J. Exp. Mar. Biol. Ecol. 385, 85–91.

Gleiss, A.C., Norman, B., Wilson, R.P., 2011. Moved by that sinking feeling: variable diving geometry underlies movement strategies in whale sharks. Funct. Ecol. 25, 595–607.

Graham, J.B., Dewar, H., Lai, N.C., Lowell, W.R., Arce, S.M., 1990. Aspects of shark swimming performance determined using a large water tunnel. J. Exp. Biol. 151, 175–192.

Gruber, S.H., Nelson, D.R., Morrissey, J.F., 1988. Patterns of activity and space utilization of lemon sharks, *Negaprion brevirostris*, in a shallow Bahamian lagoon. Bull. Mar. Sci. 43, 61–76.

Guttridge, T.L., Gruber, S.H., Franks, B.R., Kessel, S.T., Gledhill, K.S., Uphill, J., Krause, J., Sims, D.W., 2012. Deep danger: intra-specific predation risk influences habitat use and aggregation formation of juvenile lemon sharks *Negaprion brevirostris*. Mar. Ecol. Prog. Ser. 445, 279–291.

Halsey, L.G., Green, J.A., Wilson, R.P., Frappell, P.B., 2009a. Accelerometry to estimate energy expenditure during activity: best practice with data loggers. Physiol. Biochem. Zool. 82, 396–404.

Halsey, L.G., Shepard, E.L.C., Quintana, F., Gomez Laich, A., Green, J.A., Wilson, R.P., 2009b. The relationship between oxygen consumption and body acceleration in a range of species. Comp. Biochem. Physiol. A. 152, 197–202.

Harborne, A.R., Talwar, B., Brooks, E.J., 2016. The conservation implications of spatial and temporal variability in the diurnal use of Bahamian tidal mangrove creeks by transient predatory fishes. Aquat. Conserv. Mar. Freshw. Ecosyst. 26, 202–211.

Hasler, C.T., Midway, S.R., Jeffrey, J.D., Tix, J.A., Sullivan, C., Suski, C.D., 2016. Exposure to elevated pCO2 alters post-treatment diel movement patterns of largemouth bass over short time scales. Freshw. Biol. 61, 1590–1600.

Kawabe, R., Kawano, T., Nakano, N., Yamashita, N., Hiraishi, T., Naito, Y., 2003. Simultaneous measurement of swimming speed and tail beat activity of free-swimming rainbow trout *Oncorhynchus mykiss* using an acceleration data-logger. Fish. Sci. 69, 959–965.

Kieffer, J.D., 2010. Perspective – exercise in fish: 50+ years and going strong. Comp. Biochem. Physiol. A. 156, 163–168.

Krumme, U., 2009. Diel and tidal movements by fish and decapods linking tropical costal ecosystems. In: Nagelkerken, I. (Ed.), Ecological Connectivity Among Tropical Coastal Ecosystems. Springer, Dordrecht, Netherlands, pp. 271–324.

Lee, C.G., Farrell, A.P., Lotto, A., Hinch, S.G., Healey, M.C., 2003a. Excess post-exercise oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon following critical speed swimming. J. Exp. Biol. 206, 3253–3260.

Lee, C.G., Farrell, A.P., Lotto, A., MacNutt, M.J., Hinch, S.G., Healey, M.C., 2003b. The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon stocks. J. Exp. Biol. 206, 3239–3251.

Legare, B., Kneebone, J., DeAngelis, B., Skomal, G., 2015. The spatiotemporal dynamics of habitat use by blacktip (*Carcharhinus limbatus*) and lemon (*Negaprion brevirostris*) sharks in nurseries of St. John, United States Virgin Islands. Mar. Biol. 162, 699–716.

Lowe, C.G., 1996. Kinematics and critical swimming speed of juvenile scalloped hammerhead sharks. J. Exp. Biol. 199, 2605–2610.

Lowe, C.G., 2001. Metabolic rates of juvenile scalloped hammerhead sharks (*Sphyrna lewini*). Mar. Biol. 139, 447–453.

Lowe, C.G., 2002. Bioenergetics of free-ranging juvenile scalloped hammerhead sharks (*Sphyrna lewini*) in Kāne'ohe Bay, Ō'ahu, HI. J. Exp. Mar. Biol. Ecol. 278, 141–156.

Lowe, C.G., Goldman, K.J., 2001. Thermal and bioenergetics of elasmobranchs: bridging the gap. Environ. Biol. Fish 60, 251–266.

Lowe, C.G., Holland, K.N., Wolcott, T.G., 1998. A new acoustic tailbeat transmitter for fishes. Fish. Res. 36, 275–283.

Mori, T., Miyata, N., Aoyama, J., Niizuma, Y., Sato, K., 2015. Estimation of metabolic rate from activity measured by recorders deployed on Japanese sea bass *Lateolabrax japonicus*. Fish. Sci. 81, 871–882.

Murchie, K.J., Schwager, E., Cooke, S.J., Danylchuk, A.J., Danylchuk, S.E., Goldberg, T.L., Suski, C.D., Philipp, D.P., 2010. Spatial ecology of juvenile lemon sharks (*Negaprion brevirostris*) in tidal creeks and coastal waters of Eleuthera, The Bahamas. Environ. Biol. Fish 89, 95–104.

Murchie, K.J., Cooke, S.J., Danylchuk, A.J., Suski, C.D., 2011. Estimates of field activity and metabolic rates of bonefish (*Albula vulpes*) in coastal marine habitats using acoustic tri-axial accelerometer transmitters and intermittent-flow respirometry. J. Exp. Mar. Biol. Ecol. 396, 147–155.

Nelson, J.A., Gotwalt, P.S., Reidy, S.P., Webber, D.M., 2002. Beyond Ucrit: matching swimming performance tests to the physiological ecology of the animal, including a new fish "drag strip". Comp. Biochem. Physiol. A. 133, 289–302.

Nixon, A., Gruber, S., 1988. Diel metabolic and activity patterns of the lemon shark (*Negaprion brevirostris*). J. Exp. Zool. 248, 1–6.

Norin, T., Clark, T.D., 2016. Measurement and relevance of maximum metabolic rate in fishes. J. Fish Biol. 88, 122–151.

Peake, S.J., Farrell, A.P., 2006. Fatigue is a behavioural response in respirometer-confined smallmouth bass. J. Fish Biol. 68, 1742–1755.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2016. _nlme: Linear and Nonlinear Mixed Effects Models_ (R package version 3.1-124, <URL: http://CRAN.R-project.org/package=nlme>).

Reebs, S.G., 2002. Plasticity of diel and circadian activity rhythms in fishes. Rev. Fish Biol. Fish. 12, 349–371.

Roche, D.G., Binning, S.A., Bosiger, Y., Johansen, J.L., Rummer, J.L., 2013. Finding the best estimates of metabolic rates in a coral reef fish. J. Exp. Biol. 216, 2103–2110.

Rollin, B.E., Kessel, M.L., 1998. Guidelines for the treatment of animals in behavioural research and teaching. Anim. Behav. 55, 251–257.

Sakamoto, K.Q., Sato, K., Ishizuka, M., Watanuki, Y., Takahashi, A., Daunt, F., Wanless, S., 2009. Can ethograms be automatically generated using body acceleration data from free-ranging birds? PloS One 4, e5379.

Sandblom, E., Gräns, A., Axelsson, M., Seth, H., 2014. Temperature acclimation rate of aerobic scope and feeding metabolism in fishes: implications in a thermally extreme future. Proc. R. Soc. B 281, 2141490.

Scarabello, M., Heigenhauser, G.J.F., Wood, C.M., 1991. The oxygen debt hypothesis in juvenile rainbow trout after exhaustive exercise. Respir. Physiol. 84, 245–259.

Scharold, J., Gruber, S.H., 1991. Telemetered heart rate as a measure of metabolic rate in the lemon shark, *Negaprion brevirostris*. Copeia 1991, 942–953.

Shepard, E.L.C., Wilson, R.P., Halsey, L.G., Quintana, F., Laich, A.G., Gleiss, A.C., Liebsch, N., Myers, A.E., Norman, B., 2008. Derivation of body motion via appropriate smoothing of acceleration data. Aquat. Biol. 4, 235–241.

Shultz, A.D., Murchie, K.J., Griffith, C., Cooke, S.J., Danylchuk, A.J., Goldberg, T.L., Suski, C.D., 2011. Impacts of dissolved oxygen on the behavior and physiology of bonefish: implications for live-release angling tournaments. J. Exp. Mar. Biol. Ecol. 402, 19–26.

Sundström, L., Gruber, S., 1998. Using speed-sensing transmitters to construct a bioenergetics model for subadult lemon sharks, *Negaprion brevirostris* (Poey), in the field. Hydrobiologia 241–247 (371/372).

Suski, C.D., Cooke, S.J., Danylchuk, A.J., O'Connor, C.M., Gravel, M., Redpath, T., Hanson, K.C., Gingerich, A.J., Murchie, K.J., Danylchuk, S.E., Koppelman, J.B., Goldberg, T.L., 2007. Physiological disturbance and recovery dynamics of bonefish (*Albula vulpes*), a tropical marine fish, in response to variable exercise and exposure to air. Comp. Biochem. Physiol. A. 148, 664–673.

Svendsen, J.C., Tudorache, C., Jordan, A.D., Steffensen, J.F., Aarestrup, K., Domenici, P., 2010. Partition of aerobic and anaerobic swimming costs related to gait transitions in a labriform swimmer. J. Exp. Biol. 213, 2177–2183.

Svendsen, M.B.S., Bushnell, P.G., Steffensen, J.F., 2016. Design and setup of an intermittent-flow respirometry system for aquatic organisms. J. Fish Biol. 88, 26–50.

Treberg, J.R., Killen, S.S., MacCormack, T.J., Lamarre, S.G., Enders, E.C., 2016. Estimates of metabolic rate and major constituents of metabolic demand in fishes under field conditions: methods, proxies, and new perspectives. Comp. Bioch. Physiol. A. http://dx.doi.org/10.1016/j.cbpa.2016.04.022 in press.

Wetherbee, B.M., Gruber, S.H., Rosa, R.S., 2007. Movement patterns of juvenile lemon sharks *Negaprion brevirostris* within Atol das Rocas, Brazil: a nursery characterized by tidal extremes. Mar. Ecol. Prog. Ser. 343, 283–293.

Whitney, N.M., Papastamatiou, Y.P., Holland, K.N., Lowe, C.G., 2007. Use of an acceleration data logger to measure diel activity patterns in captive whitetip reef sharks, *Triaenodon obesus*. Aquat. Living Resour. 20, 299–305.

Whitney, N.M., Papastamatiou, Y.P., Gleiss, A.C., 2012. Integrative multisensor tagging: emerging techniques to link elasmobranch behavior, physiology, and ecology. In: Carrier, J.C., Musick, J.A., Heithaus, M.C. (Eds.), Biology of Sharks and Their Relatives, second ed. Taylor and Francis Group, LLC, Boca Raton, FL, pp. 265–290.

Whitney, N.M., Lear, K.O., Gaskins, L.C., Gleiss, A.C., 2016. The effects of temperature and swimming speed on the metabolic rate of the nurse shark (*Ginglymostoma cirratum*, Bonaterre). J. Exp. Mar. Biol. Ecol. 477, 40–46.

Wilson, R.P., White, C.R., Quintana, F., Halsey, L.G., Liebsch, N., Martin, G.R., Butler, P.J., 2006. Moving towards acceleration for estimates of activity-specific metabolic rate in free-living animals: the case of the cormorant. J. Anim. Ecol. 75, 1081–1090.

Wilson, S.M., Hinch, S.G., Eliason, E.J., Farrell, A.P., Cooke, S.J., 2013. Calibrating acoustic acceleration transmitters for estimating energy use by wild adult Pacific salmon. Comp. Biochem. Physiol. A. 164, 491–498.

Wilson, A.D.M., Brownscombe, J.W., Krause, J., Krause, S., Gutowsky, L.F.G., Brooks, E.J., Cooke, S.J., 2015. Integrating network analysis, sensor tags, and observation to understand shark ecology and behavior. Behav. Ecol. 26, 1577–1586.

Wright, S., Metcalfe, J., Hetherington, S., Wilson, R., 2014. Estimating activity-specific energy expenditure in a teleost fish, using accelerometer loggers. Mar. Ecol. Prog. Ser. 496, 19–32.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology With R. Springer, New York.