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The energetic, physiological, and behavioral response of lemon sharks (*Negaprion brevirostris*) to simulated longline capture

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A B S T R A C T

Commercial fisheries bycatch is a considerable threat to elasmobranch population recovery, and techniques to mitigate sub-lethal consequences can be improved with data on the energetic, physiological, and behavioral response of individuals to capture. This study sought to estimate the effects of simulated longline capture on the behavior, energy use, and physiological stress of juvenile lemon sharks (*Negaprion brevirostris*). Captive sharks equipped with acceleration biologgers were subjected to 1 h of simulated longline capture. Swimming behaviors were identified from acceleration data using a machine-learning algorithm, energetic costs were estimated using accelerometer-calibrated relationships and respirometry, and physiological stress was quantified with point-of-care blood analyzers. During capture, sharks exhibited nine-fold increases in the frequency of burst swimming, 98% reductions in resting, and swam as often as unrestrained sharks. Aerobic metabolic rates during capture were 8% higher than for unrestrained sharks, and accounted for a 57.7% increase in activity costs when excess post-exercise oxygen consumption was included. Lastly, sharks exhibited significant increases in blood lactate and glucose, but no change in blood pH after 1 h of capture. Therefore, these results provide preliminary insight into the behavioral and energetic responses of sharks to capture, and have implications for mitigating sub-lethal consequences of capture for sharks as commercial longline bycatch.

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1. Introduction

Unused or unmanaged capture (i.e., bycatch) is a considerable 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 when captured in different gear types (Brooks et al., 2012; Frick et al., 2012; Gallagher et al., 2014), but considerably less is known about post-release or chronic sub-lethal outcomes (Molina and Cooke, 2012; Skomal and Mandelman, 2012). While quantifying capture-induced mortality (both immediate and post-release) is important for estimating rates of population loss, defining sub-lethal consequences of bycatch may ultimately determine the extent to which individual fitness and population growth is affected (Wilson et al., 2014). For instance, changes in behavior and energy use can translate to organismal and population-level consequences if rates of energy

acquisition are altered (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 post-release fate of sharks, and for mitigating sub-lethal outcomes of capture for longline caught sharks.

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

swimming is generally linked to metabolic acidosis, an accumulation of lactate in the muscle and blood, and anaerobic metabolism (Cliff and Thurman, 1984), the effects of aerobically supported behaviors on whole-organism physiological responses to capture remain poorly understood. 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.

Modifying characteristics of fishing gear is one potential management action that could be employed to mitigate sub-lethal consequences or mortality, and facilitate behavioral and physiological recovery for sharks following capture (Dapp et al., 2016a, 2016b; Guida et al., 2016a). For instance, studies directed at longline capture have suggested that 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 should consider the problem of how an animal's ability to ventilate may be impaired, which is directly related to an individual's capacity for free-swimming behaviors (Dapp et al., 2016b). At present, little is known about how behavior influences energy expenditure for sharks during capture (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, strategies to mitigate sub-lethal outcomes for sharks are limited in effectiveness.

The primary goal of this study was to quantify the effect of simulated commercial longline fishing capture on the swimming behavior, energy use, and physiological stress response of a carcharhinid shark. To accomplish this, we conducted a series of laboratory experiments to allow us to measure behavior and aerobic metabolic rates from acceleration data, measure behavior, aerobic metabolic rates, and physiological stress in unrestrained and captured sharks, and measure the energetic costs of an exhaustive event, such as capture. Together, these three components allowed us to compare the proportion of time devoted to swimming behaviors, and changes in energy use and stress biomarkers of sharks during capture. Juvenile lemon sharks (*Negaprion brevirostris*) were selected as the study species because of their abundance at the study site and amenability to laboratory experimentation, and because calibrated relationships for acceleration data and aerobic metabolic rates have already been generated for this species (Bouyoucos et al., 2017). These data are necessary for providing new insight into the physiological and behavioral sub-lethal outcomes of longline capture, a potentially useful tool in gauging the suitability of strategies to mitigate sub-lethal outcomes for data deficient elasmobranch species.

2. Materials and methods

This study was conducted in accordance with University of Illinois Institutional Animal Care and Use Committee protocol #14163, and under permits MAF/FIS/17 and MAF/FIS/34 issued by the Bahamian Department of Marines Resources. 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

Juvenile lemon 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, and were transported by boat in coolers, in which water was constantly replenished, to a wet lab facility (Brooks et al., 2011). 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. Sharks were maintained in captivity for a minimum of three days prior to use in experiments to allow for behavioral recovery from the capture process

(Bullock et al., 2015). Each individual shark received a daily ration (6% body mass per day) of Spanish sardines (*Sardinella aurita*), with the exception of 48-hour fasts to ensure a post-absorptive state at experimental temperatures prior to all experiments (Cortés and Gruber, 1992). After experimentation, sharks were returned to their capture site using the same transportation methods detailed above.

2.2. Experimental design

This study compared behavioral, energetic, and physiological responses of unrestrained and longline-captured juvenile lemon sharks. Behavioral responses were defined as changes in the proportion of time sharks exhibited three swimming behaviors, both free-swimming in tanks ("unrestrained") and on a longline gangion ("captured"), observed remotely with accelerometers. To accomplish this, acceleration criteria to define behaviors were first generated in an ethogram experiment, and acceleration data were gathered from unrestrained and longline captured sharks. Energetic responses to capture were defined as differences in aerobic metabolic rates between unrestrained and captured sharks, including the energetic costs of an exhaustive event. To accomplish this, acceleration-derived tailbeat frequency (TBF) was converted to rates of oxygen consumption using a calibration equation for this species, and the energetic costs of an exhaustive event were estimated using respirometry. Lastly, physiological responses to capture were defined as changes in the value of stress biomarkers between individuals before and after the longline capture event. Together, these allowed us to measure the behavioral, energetic, and physiological responses of juvenile lemon sharks to simulated capture.

2.3. Measuring behavior and aerobic metabolic rates from acceleration data

Sharks were equipped with accelerometers (X16-mini, Gulf Coast Data Concepts, Waveland, MS, USA) at their first dorsal fin in under 2 min following previously outlined methods for metabolic rate measurement and behavioral telemetry of lemon sharks (Wilson et al., 2015; Bouyoucos et al., 2017). A classification tree was generated to automatically distinguish behaviors based on acceleration data (De'ath and Fabricius, 2000; Nathan et al., 2012). Seven sharks (62.8 ± 1.9 cm total length, 1.2 ± 0.1 kg, mean ± S.D.) were individually observed swimming in holding tanks, and the occurrence of three swimming behaviors was recorded: resting, swimming, and burst swimming (Gleiss et al., 2009). Acceleration data during known instances of these behaviors was partitioned into dynamic (shark) and static (gravity) components with a two-second smoothing interval (Bouyoucos et al., 2017). Nine acceleration-based metrics were calculated using Igor Pro (6.3.3.5, WaveMetrics, Inc., Lake Oswego, OR, USA) and used to generate a classification tree using the "tree" package in R (R Core Team, 2015; Ripley, 2016): mean and standard deviation of acceleration in all three axes each second, overall dynamic body acceleration, TBF, and amplitude of the lateral acceleration signal (Brown et al., 2013; Kawabe et al., 2003; Wilson et al., 2006). Of these, standard deviations of acceleration in the anterior-posterior ($surge_{SD}$) and dorsal-ventral ($heave_{SD}$) axes together could successfully distinguish behaviors. It was, therefore, possible to use the classification tree to automatically distinguish swimming behaviors based on $surge_{SD}$ and $heave_{SD}$ values alone (Table 1).

To estimate aerobic metabolic rates, instantaneous TBF values for unrestrained and captured lemon sharks were converted to rates of oxygen consumption (MO_2) using the equation $MO_2 = 136.5 + 92.8 TBF$ generated for juvenile lemon sharks from Cape Eleuthera at 30 °C (Bouyoucos et al., 2017). Aerobic metabolic rates could not exceed the maximum aerobic limit for juvenile lemon sharks at Cape Eleuthera (398.0 mg O_2 kg^{-1} h^{-1}), and instantaneous values were averaged over an entire deployment (unrestrained and captured) to yield a single aerobic metabolic rate estimate per animal (Bouyoucos et al., 2017).

Table 1
Values for standard deviations of surging (anterior-posterior; "surge_{SD}") and heaving (dorsal-ventral; "heave_{SD}") 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 the mean and the respective range (standard error and the range).

Behavior (Observations)	Rest ($n = 4$)	Swim ($n = 7$)	Burst ($n = 7$)
Criteria	Surge$_{SD}$ < 0.01 g	Surge$_{SD}$ ≥ 0.01 g Heave$_{SD}$ < 0.09 g	Heave$_{SD}$ ≥ 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)

2.4. Measuring behavior, aerobic metabolic rates, and physiological stress in unrestrained and captured sharks

Sharks were subjected to simulated longline capture in experimental tanks to measure the behavior and aerobic metabolic rates of captured sharks, and to observe physiological responses to capture. Twenty-four hours prior to experiments, six sharks (74.8 ± 4.6 cm total length, 1.8 ± 0.2 kg, mean ± S.D.) were equipped with accelerometers and isolated in individual holding tanks. Acceleration data generated during the 24th hour of acclimation were used to measure behavior and aerobic metabolic rates for unrestrained sharks. After fasting, a monofilament gangion (custom-built to an individual's total length) with a 14/0 circle hook baited with little tunny (*Euthynnus alletteratus*) was suspended from a taught line at the water's surface in the center of the tank (Fig. 1). Water level was high enough to prevent sharks from resting on the tank bottom for the duration of capture (Frick et al., 2010). Acceleration data generated during capture were used to measure behavior and aerobic metabolic rates of captured sharks. Hooks were completely removed after 1 h of capture. Capture duration represented a "best-case" scenario where fishing gear could be checked periodically following deployment (Brooks et al., 2012). Mean water temperature during capture experiments was 30.0 ± 0.2 °C.

Physiological responses to capture were quantified by measuring blood glucose, pH, and lactate. Sharks were phlebotomized twice: 24 h into fasting to provide unstressed reference values and after capture to provide stressed values (Brooks et al., 2012; Hoffmayer et al., 2015). Sharks were manually restrained in supine position to draw 1 mL of blood from the caudal vein with 21 gauge 3.8 cm needles and sodium heparin coated syringes (Skomal and Mandelman, 2012). Blood was analyzed within 3 min of phlebotomy with an Accu-chek portable blood glucose meter and an i-STAT clinical analyzer for pH and lactate (Brooks et al., 2011; Harter et al., 2015). Blood pH values from the i-STAT were temperature-corrected to blood temperature using the i-STAT's internal temperature correction function (Harter et al., 2015).

2.5. Measuring the energetic costs of an exhaustive event

Resting intermittent-flow respirometry was employed to estimate the energetic cost of an exhaustive event (Svendsen et al., 2016). Rates of oxygen consumption were measured in 38.6 L PVC respirometry chambers. Chambers were submerged in a common tank that was mostly covered that allowed for observation of the condition or activity of sharks during measurement. Sharks were, therefore, exposed to a natural photoperiod during measurements. Dissolved oxygen was measured each second using a fiber optic oxygen probe (calibrated before each use) and temperature probe externally (Loligo Systems, Copenhagen, Denmark). Measurements were automated using AutoResp software (Loligo Systems, Copenhagen, Denmark), where MO$_2$ was measured over 3 min every 15 min. Background respiration was accounted for by measuring MO$_2$ in respirometry chambers before and after each respirometry trial (Rodgers et al., 2016).

Energetic costs were quantified by measuring excess post-exercise oxygen consumption (EPOC), which is an indirect measure of the energy used to resolve physiological disturbance and replenish anaerobic substrates used during exhaustive activity (Gaesser and Brooks, 1984). To measure EPOC, MO$_2$ of seven minimally-stressed sharks (the same individuals used to generate the acceleration ethogram) was first measured over 24 h to estimate resting metabolic rate (RMR), which is the metabolic rate of a resting, fasted shark at stable temperature, and is a necessary baseline value for the calculation of recovery time and EPOC (Bouyoucos et al., 2017). For RMR measurement, minimally-stressed sharks were transferred directly from holding tanks to respirometry chambers for 24 h of measurements without acclimation. Four days after measuring RMR, the same individuals were equipped with accelerometers and chased to exhaustion following the methods outlined in Brooks et al. (2011), because chasing as an exhaustion technique simulates the stress of fishing capture (Clark et al., 2012). Exhaustion occurred in under 2 min (60.0 s ± 12.3 s S.D.), and sharks were subsequently transferred to respirometry chambers, where the first MO$_2$ measurements were made within 6 min of exhaustion. Rates of
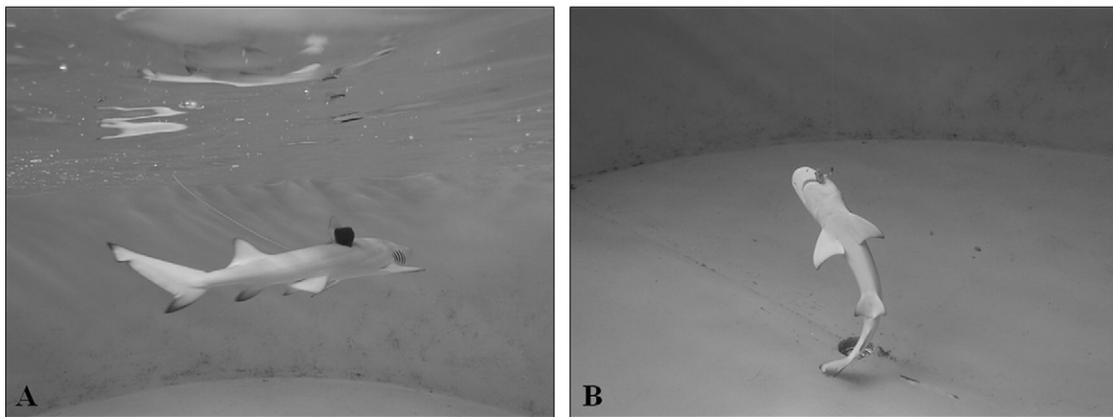

Fig. 1. Simulated longline capture of juvenile lemon sharks. Swimming and non-swimming behaviors, and aerobic metabolic rates, were estimated with acceleration biologgers mounted at the first dorsal fin (A). Gangion length and water level were manipulated so that sharks could not rest on the tank bottom, in order to better simulate capture on a mid-water or pelagic longline (B). Resting sharks could hang from gangions and were fully capable of buccal pumping.

oxygen consumption were measured over 12 h post-exhaustion, after which sharks were returned to holding tanks prior to release. All respirometry experiments were conducted at 30.7 ± 0.1 °C.

Rates of oxygen consumption during RMR and EPOC respirometry trials were calculated as.

$$MO_2 = \beta O_2 V_{RE} W_o^{-1} \frac{\delta O_2}{\delta t}$$

where βO_2 is the solubility of oxygen in water at experimental temperature, salinity, and atmospheric pressure, V_{RE} is the volume of the respirometer minus the volume of the fish, W_o is the mass of the fish, and $\frac{\delta O_2}{\delta t}$ is the slope of the linear decline in dissolved oxygen during each measurement cycle (Svendsen et al., 2016). Resting metabolic rate was calculated for each shark as the mean of the six lowest MO_2 values with coefficients of determination >0.95 after removing the first 6 h of data to account for artificially elevated MO_2 from the initial stress of handling (Shultz et al., 2011; Chabot et al., 2016). Lastly, EPOC was calculated for each shark by fitting hourly mean MO_2 values during the 12-hour recovery period with a five parameter bi-exponential decay curve, using an individual shark's RMR as the curve's intercept (Scarabello et al., 1991; Svendsen et al., 2010). Integrating the area under the curve that was bound by the first MO_2 measurement, the time when the curve intersected the upper 95% confidence interval limit for RMR, and the upper 95% confidence interval limit yielded EPOC (Bushnell et al., 1994; Scarabello et al., 1991; Lee et al., 2003; Svendsen et al., 2010).

Lastly, to provide a conservative estimate of the total energetic cost of a capture event, values for aerobic energy expenditure, measured during capture, and anaerobic energy expenditure, measured from exhaustive chasing, were combined. The total energetic cost of capture (in mg O_2 kg^{-1}) was calculated as the sum of oxygen consumed during 1 h of capture added to EPOC, which was converted to kJ kg^{-1} using an oxygen equivalent of 14.14 J mg O_2^{-1} (Elliott and Davison, 1975).

2.6. Statistical analysis

To determine the effects of capture on behavior, aerobic metabolic rates, and physiological stress, comparisons between unrestrained/unstressed and captured/stressed sharks were made with paired t-tests. Because raw proportion data are subject to unit-sum constraint and are, therefore, not independent, behavior proportion data were log-ratio transformed (Aebischer et al., 1993).

3. Results

3.1. Measuring behavior, aerobic metabolic rates, and physiological stress in unrestrained and captured sharks

Sharks captured in simulated longline experiments exhibited statistically significant differences in swimming, resting, and bursting behaviors relative to unrestrained animals. Captured sharks swam as often as unrestrained sharks (paired t-test, $t = 2.678$, df = 5, $P = 0.267$), which was 64.9% ± 9.1% standard error of the mean when data were pooled across treatment (Fig. 2). Captured sharks rested significantly less than unrestrained sharks (paired t-test, $t = 1.248$, df = 5, $P = 0.038$), representing a 98% decrease in occurrence (Fig. 2). Unrestrained sharks rested 48.7 ± 15.7% of the time, and captured sharks rested 0.9 ± 0.6% of the time. Lastly, captured sharks burst significantly more often than unrestrained sharks (paired t-test, $t = 3.919$, df = 5, $P = 0.011$), representing a nine-fold increase in bursting activity (Fig. 2). Unrestrained sharks burst 1.9 ± 0.5% of the time, while captured sharks burst 18.6 ± 5.9% of the time.

In response to capture, sharks exhibited increases in activity levels and aerobic energy expenditure. Captured sharks exhibited a marginally statistically significant increase in TBF of 20% from 1.06 ± 0.07 to 1.28 ± 0.02 tailbeats s^{-1} relative to unrestrained sharks (Fig. 3a; paired
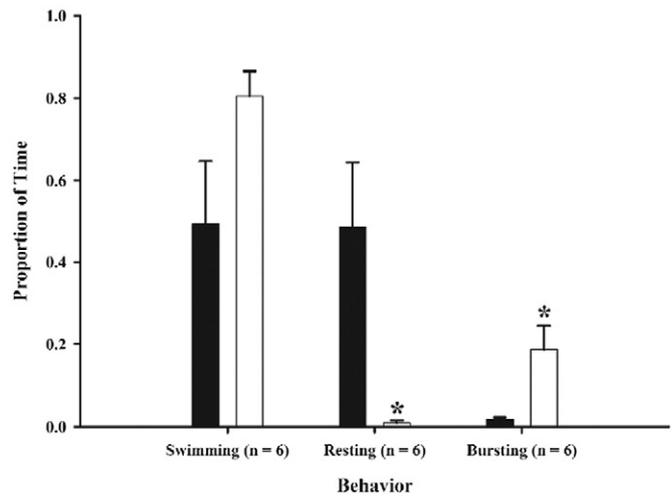
Fig. 2. Mean and standard error of proportions of swimming (A), resting (B), and burst swimming (C) behaviors of juvenile lemon sharks unrestrained and captured in simulated longline fishing gear. Six individuals were used to generate data characteristic of both unrestrained and captured sharks. Data are presented as raw proportions, but statistical analyses were performed on log-ratio transformed data to account for unit-sum constraint of proportion data. Asterisks indicate statistically significant differences between values for unrestrained and captured sharks within behaviors at α = 0.05, and sample sizes are denoted beside behavior categories.

t-test, $t = 2.678$, df = 5, $P = 0.044$). In addition, aerobic metabolic rates of captured sharks significantly increased by 8% from 235.76 ± 7.02 mg O_2 kg^{-1} h^{-1} for unrestrained sharks to 255.38 ± 1.53 mg O_2 kg^{-1} h^{-1} (Fig. 3b; paired t-test, $t = 2.678$, df = 5, $P = 0.044$).

Following simulated longline capture, sharks experienced changes in the values for several stress biomarkers. Captured sharks exhibited a statistically significant increase in blood glucose relative to unrestrained sharks (paired t-test, $t = 5.040$, df = 5, $P = 0.004$). Blood glucose increased by 41% from 112.50 ± 4.71 mg dL^{-1} to 158.83 ± 9.39 mg dL^{-1} (Fig. 4a). Captured sharks exhibited over an eight-fold increase in blood lactate relative to unrestrained sharks (paired t-test, $t = 3.979$, df = 5, $P = 0.011$), increasing from 0.61 ± 0.15 mmol L^{-1} to 5.66 ± 1.29 mmol L^{-1} (Fig. 4b). Lastly, pH was not significantly different between captured and un-restrained sharks (paired t-test, $t = -1.598$, df = 5, $P = 0.171$), and was 7.45 ± 0.02 across both treatments (Fig. 4c).

3.2. Measuring the energetic costs of an exhaustive event

Metabolic rates of all sharks returned to pre-exercise (RMR) levels within 12 h post-exhaustion, which allowed for calculation of EPOC and recovery time (Fig. 5). Chasing sharks to exhaustion resulted in an EPOC of 116.4 ± 34.4 mg O_2 kg^{-1} from which sharks took 5.4 ± 2.5 h to recover. Resting metabolic rate was 154.1 ± 10.6 mg O_2 kg^{-1} h^{-1}. The total energetic cost of a one-hour simulated longline capture event for juvenile lemon sharks was 5.3 kJ kg^{-1}, in which energy consumption requirements for captured sharks represents a 57.7% increase in energy demand above that estimated for unrestrained sharks.

4. Discussion

Relative to unrestrained sharks, captured sharks burst more frequently, rested less often, and did not change the amount of time devoted to swimming. Captured sharks exhibited a nine-fold increase in the proportion of burst activity and a 98% reduction in the proportion of resting activity relative to unrestrained sharks. Burst swimming is a typical response to capture (Gallagher et al., 2016; Guida et al., 2016b), whereby a shark utilizes anaerobic metabolism to support a high-energy escape response (Skomal and Bernal, 2010). Because prolonged
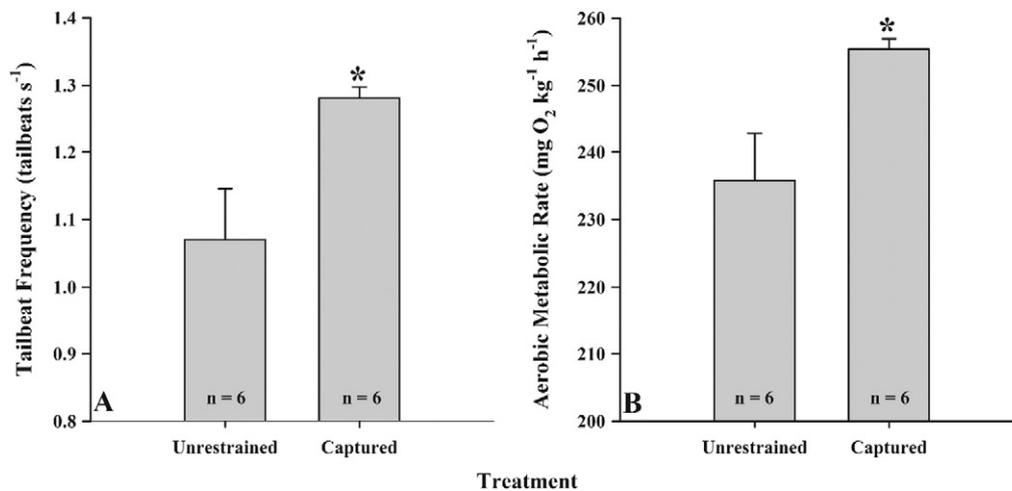
Fig. 3. Mean and standard error of tailbeat frequencies (A) and aerobic metabolic rates (B) of unrestrained and captured juvenile lemon sharks. Six individuals were used to generate data characteristic of un-restrained and longline captured sharks in a paired design. Tailbeat frequencies were measured from acceleration data generated by sharks, and aerobic metabolic rates were estimated using a calibration equation that relates tailbeat frequency and aerobic metabolic rates for juvenile lemon sharks from Cape Eleuthera at 30 °C (Bouyoucos et al., 2017). Asterisks indicate statistically significant differences between values for unrestrained and captured sharks at $\alpha = 0.05$ and sample sizes are denoted within bars for each treatment.

reliance on anaerobic metabolism results in exhaustion, lemon sharks were likely unable to sustain burst activity as an escape response for the duration of the capture event (Brooks et al., 2012; Frick et al., 2012; Gallagher et al., 2016). Resting in response to capture has been observed for buccal pumping species like lemon sharks (Port Jackson sharks *Heterodontus portusjacksoni* and gummy sharks *Mustelus antarcticus*), and it has been suggested that resting can mitigate the severity of the stress response for these species (Frick et al., 2010; Guida et al., 2016a). Gummy sharks, for instance, rested 89.6–96.0% of the time during demersal longline capture (Guida et al., 2016a). Though sharks in this study could not rest on the tank bottom, animals observed hanging from gangions were fully capable of buccal pumping (Fig. 1). The reduction in time spent resting for captured sharks likely reflects how ram ventilation, relative to buccal pumping, can enhance gas exchange at the gills and reduce energy expenditure while swimming (Clark and Seymour, 2006). In addition, ram ventilation is a more efficient method of mitigating the severity of the secondary stress response for lemon sharks, and increases in swimming speed and mouth gape can improve oxygen uptake in ram ventilating sharks (Parsons and Carlson, 1998; Brooks et al., 2011). Furthermore, lemon sharks typically have low internal oxygen tensions and no oxygen reserves, which supports ventilation strategies that improve oxygen uptake following exercise, such as ram ventilation (Bushnell et al., 1982). Relying on behaviors that 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, and promoting recovery during potentially long capture events. However, behavioral responses to capture and mortality rates are quite different for obligate ram ventilating species (Dapp et al., 2016b; Gallagher et al., 2016). Therefore, captured lemon sharks increased the frequency of burst events and rested less often than unrestrained sharks.

Captured sharks exhibited higher TBF and aerobic metabolic rates than unrestrained sharks. Tailbeat frequencies significantly increased by 20% between unrestrained and captured sharks, and aerobic metabolic rates significantly increased by 8%. Blacktip sharks (*Carcharhinus limbatus*) and nurse sharks (*Ginglymostoma cirratum*) have been documented to increase dynamic body acceleration during capture, which has been shown to correlate with metabolic rates for these species, and suggests increased energy expenditure above routine levels for captured sharks (Gallagher et al., 2016; Lear et al., 2016). The aerobic metabolic rate of captured sharks in this study was 8% higher than for unrestrained sharks, but only 3% higher than for semi-captive juvenile

lemon sharks swimming in an enclosed natural habitat (Bouyoucos et al., 2017). Unfortunately, there are very few instances of correlative relationships between activity levels and metabolic rates of sharks being applied (or even existing) to address the responses of sharks to anthropogenic stressors, such as the responses of whitetip reef sharks, *Triaenodon obesus*, (Barnett et al., 2016) to ecotourism, or the responses of lemon sharks to capture (this study). In this study, lemon sharks may have increased TBF to increase swimming speed to improve oxygen uptake and carbon dioxide offloading at the gills in response to exercise-induced metabolic or respiratory acidosis. Increased activity and energy expenditure have been observed in obligate ram ventilating bonnethead (*Sphyrna tiburo*) and blacknose sharks (*C. acronotus*) that increase swimming speeds and mouth gape to regulate oxygen delivery in response to hypoxia (Parsons and Carlson, 1998; Carlson and Parsons, 2001). Tailbeat frequency has a linear relationship with swimming speed for juvenile lemon sharks, so observed changes in TBF likely coincided with increases in swimming speed (Graham et al., 1990; Bouyoucos et al., 2017). Alternatively, increases in activity levels could have been a behavioral response to capture not linked to resolving physiological stress. This is unlikely, however, given that this study's assessment of physiological stress and EPOC suggests that lemon sharks have elevated metabolic rates above routine or resting levels following exhaustive exercise. Thus, captured juvenile lemon sharks exhibited a change in tailbeat frequencies and aerobic metabolic rates relative to unrestrained sharks.

Juvenile lemon sharks exhibited signs of physiological stress following simulated longline capture. Specifically, captured sharks exhibited significant increases in blood lactate and glucose, but no change in blood pH relative to unrestrained sharks. Blood lactate values for longline-captured lemon sharks after 1 h were comparable to values for lemon sharks 30 min after exhaustive exercise and 1–8 min of rod-and-reel angling (Brooks et al., 2011; Danylchuk et al., 2014). Blood glucose levels of juvenile lemon sharks exercised to exhaustion did not exceed glucose levels of unrestrained sharks after 3 h of serial sampling, and values for this study's captured sharks are comparable to the upper limit observed for rod-and-reel angled juvenile lemon sharks (Brooks et al., 2011; Danylchuk et al., 2014). Blood pH did not differ between captured and unrestrained sharks, though lemon sharks exercised to exhaustion did not demonstrate recovery in blood pH until 3 h, and EPOC data from this study suggest that physiological recovery should take at least 5 h (Brooks et al., 2011). Comparatively, the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*), which are similar-sized (1.4–5.5 kg) and have been studied at a comparable
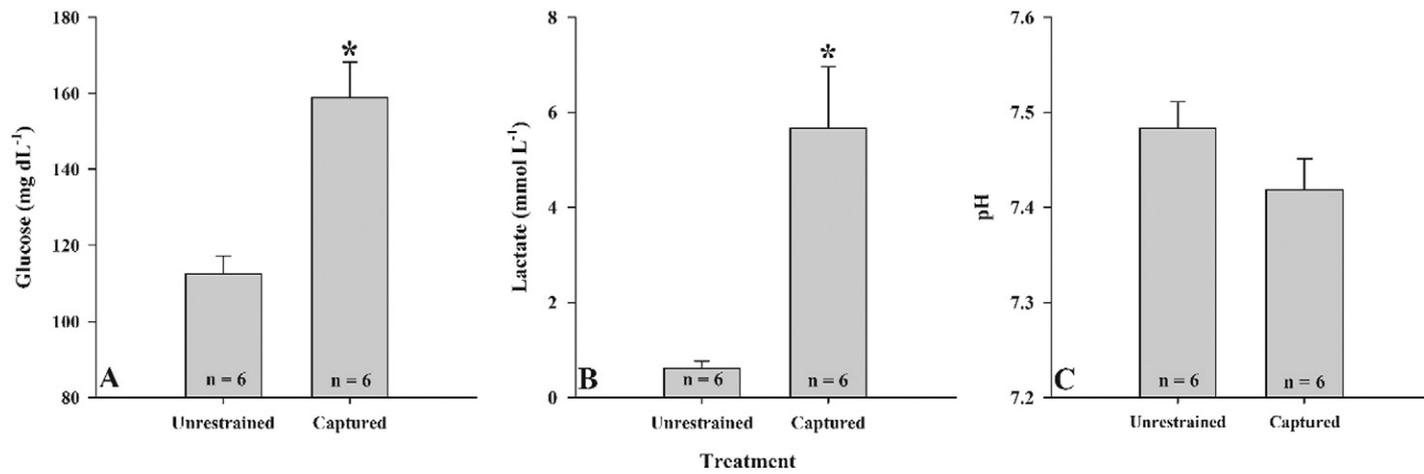
Fig. 4. Mean and standard error of blood glucose (A), blood lactate (B), and blood pH (C) values for unrestrained and longline captured juvenile lemon sharks in a laboratory environment. Data were generated for six individuals that were phlebotomized 24 h prior to capture (unrestrained) and after 1 h of simulated longline capture (captured). Asterisks indicate statistically significant differences between values for unrestrained and captured sharks at $\alpha = 0.05$ and sample sizes are denoted within bars for each treatment.
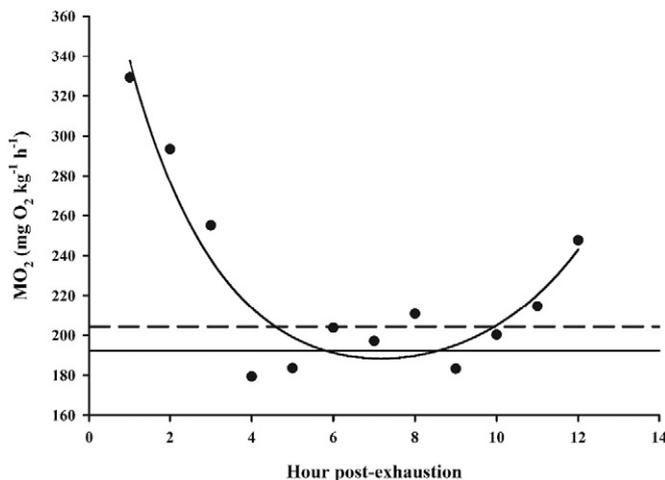
Fig. 5. Exemplary metabolic rate (MO$_2$) data for a juvenile lemon shark measured over 12 h post-exhaustion. Excess post-exercise oxygen consumption (EPOC) was measured by calculating the area under an exponential decay curve fitted to MO$_2$ data that is bound by the time of the first MO$_2$ measurement, the time when the MO$_2$ curve intersects the upper 95% confidence interval limit (dashed horizontal line) for the sharks' resting metabolic rate (RMR; solid horizontal line), and the value of the upper 95% confidence interval limit. For this shark, RMR was 192.2 mg O$_2$ kg^{-1} h^{-1}, the upper 95% confidence interval limit was 204.2 mg O$_2$ kg^{-1} h^{-1}, EPOC was 175.5 mg O$_2$ kg^{-1}, and the time for MO$_2$ to return to pre-exercise levels was 4.5 h. Increasing MO$_2$ at 12 h indicates spontaneous activity of the shark within the respirometry chamber.

range of temperatures (23.5–31.0 °C), exhibited much higher values for blood lactate and glucose, and lower values for pH when subjected to 1 h of rod-and-reel angling than longline-caught lemon sharks (Hoffmayer and Parsons, 2001; Hoffmayer et al., 2012, 2015). However, minimally stressed sharpnose sharks exhibited higher blood glucose values and lower blood pH than lemon sharks (Hoffmayer and Parsons, 2001; Hoffmayer et al., 2012, 2015), and these species-specific differences in the secondary stress response may be attributable to respiratory mode (sharpnose sharks are obligate ram ventilators) and/or metabolic scope, though the latter hasn't been measured for sharpnose sharks (Dapp et al., 2016b). Furthermore, lemon sharks are generally regarded as physiologically resilient to capture stress relative to obligate ram ventilating species, and the magnitude of the stress response experienced by sharks caught on commercial versus simulated longline gear may differ, which may ultimately make inter- or intraspecific comparisons of lemon sharks captured in the laboratory or field difficult (Frick et al., 2010; Danylchuk et al., 2014; Gallagher et al., 2014; Dapp et al., 2016b). Therefore, juvenile lemon sharks exhibited significant changes in stress biomarkers in response to simulated longline capture.

Juvenile lemon sharks exhibited increased energy expenditure associated with simulated longline capture. Captured sharks were estimated to expend 5.3 kJ kg^{-1} of energy during a one-hour simulated longline capture event, representing a 57.7% increase in energy expenditure relative to unrestrained sharks. Bouyoucos et al. (2017) measured EPOC for juvenile lemon sharks exercised to exhaustion in a swim tunnel at 30 °C to be 0.5 kJ kg^{-1}, but different exhaustion techniques resulted in disparate values. However, chasing to exhaustion, rather than exercise in a swim tunnel, is a widely used exercise technique to illicit post-exercise metabolic responses in fishes similar to what would be experienced during fishing capture (Clark et al., 2012; Norin and Clark, 2016). Spiny dogfish (*Squalus acanthias*) chased to exhaustion expended 1.5 kJ kg^{-1} of energy at 10 °C, which would increase the average daily metabolic rate (ADMR) of spiny dogfish of 15.9 kJ kg^{-1} d^{-1} by 9.3% (Brett and Blackburn, 1978). Compared to juvenile lemon sharks, 1 h of simulated longline capture represents an increase in ADMR of 88.7 kJ kg^{-1} d^{-1} by 2.2% (Bouyoucos et al., 2017). Barnett et al. (2016) observed a 6.4% increase in daily aerobic energy expenditure for whitetip reef sharks provisioned at ecotourism sites. Together, these suggest that chronic stressors affecting energy use incur a higher

energetic cost on diel scales than acute events like capture, where temperature and metabolic rates are similar (Barnett et al., 2016; Bouyoucos et al., 2017). Though energy expenditure of captured sharks was higher than for unrestrained sharks, it is unclear whether this acute increase in energy use translates to chronic sub-lethal outcomes, such as reduced growth rates or reproductive impairments. Indeed, brief bouts of exhaustive activity (bursting) can substantially increase energy expenditure (Baker and Gleeson, 1998), but the significance of increased energy expenditure associated with capture needs to be further examined for sharks vulnerable to fisheries bycatch. Therefore, longline captured lemon sharks exhibited increases in aerobic energy expenditure and, likely, anaerobic energy use.

5. Conclusions

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 increases our understanding of the condition of animals during a capture event, which can help management gauge the suitability of mitigation measures for many data deficient species (Dapp et al., 2016b). Longline capture of lemon sharks resulted in significant increases in energy use, induced physiological stress, and influenced the behavior of captured sharks, though the long-term consequences of these effects and potential mitigation measures require further research. For instance, understanding the effect of gangion length on responses to capture may shed light on the efficacy of regulating gangion lengths as a management strategy (Dapp et al., 2016b; Guida et al., 2016a). Furthermore, shortened soak times, or periodic gear checks may serve to reduce post-release mortality (Broadhurst et al., 2014; Marshall et al., 2015), though additional research is necessary to observe the effect of longer capture duration on behavior and energy use (Brooks et al., 2012). Lastly, techniques employed to minimize the intensity of a shark's initial escape response when caught, such as hooks with bending strength that can be exceeded by sharks versus the target catch, may reduce EPOC-related costs incurred by capture (Watson and Kerstetter, 2006). Therefore, these data offer new insight into the behavior and energy use of longline captured sharks, and have implication for mitigating sub-lethal consequences of sharks as commercial longline bycatch.

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