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Integrating reflexes with physiological measures to evaluate coastal shark stress response to capture

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In both commercial and recreational fisheries, sharks are captured and released alive to comply with regulations or due to low economic value or voluntary conservation ethic. As a result, understanding the physiological and behavioural responses of sharks to capture stress is important for determining subsequent effects of fisheries interactions on a species-specific basis, as well as for identifying factors that influence mortality. Here, we employed a suite of conventional blood physiology endpoints (glucose, lactate, and haematocrit) integrated with assessments of reflex impairment on blacktip (*Carcharhinus limbatus*), great hammerhead (*Sphyrna mokarran*), nurse (*Ginglymostoma cirratum*) and sandbar sharks (*Carcharhinus plumbeus*) captured via experimental drumline gear. We documented a wide range of species-specific differences in all parameters assessed, with nurse sharks consistently having the lowest relative levels of physiological disturbance and reflex impairment; and with great hammerheads exhibiting the highest level of physiological disturbance and reflex impairment, suggesting higher vulnerability to fishing. In general, increases in lactate were positively associated with hook time and correlated with reflex impairment assessment. Moreover, reflex indices showed significant impairment with hook time, with the "jaw" reflex emerging as the most potential predictor of disturbance. Our study results connect previously reported species-specific at-vessel and post-release mortality rates to their physiological disturbance and reflex impairment.

Keywords: catch and release, conservation, fishing, physiology, reflex impairment, stress.

Introduction

Hundreds of millions of fishes are released in commercial and recreational fisheries every year (Arlinghaus *et al.*, 2007; Oliver *et al.*, 2015). Fishes are released for many reasons, whether it be to comply with fishery regulations, to mitigate incidental bycatch, due to low economic value, or as a result of voluntary conservation oriented behaviours from fishers (Stevens *et al.*, 2000). Released fishes may or may not survive these fishery interactions, but almost certainly incur some level of physiological disruption which can yield impairments in behaviour, growth, reproduction or disease resistance (Wilson *et al.*, 2014). Although the physiological disruptions that teleost fish experience from capture have been relatively well studied (Muoneke and Childress, 1994; Diodati and Richards, 1996; Tomasso *et al.*, 1996; Bartholomew and Bohnsack, 2005), there has been comparatively less research focussing on the physiological stress induced from capture and subsequent release in sharks (Moyes *et al.*, 2006; Mandelman and Skomal, 2009; Gallagher *et al.*, 2014a; Dapp *et al.*, 2016). Examining the effects of capture stress on shark survival is important given that these species are encountered in both commercial and recreational fisheries worldwide, and in many situations are released to an unknown fate (Arlinghaus *et al.*, 2007; Worm *et al.*, 2013; Oliver *et al.*, 2015). Furthermore, a significant portion of shark species are threatened with extinction due to their slow-growing nature and low rates of biological productivity (Gallagher *et al.*, 2012; Dulvy *et al.*, 2014).

Traditional methods for understanding capture stress profiles in fish species, including sharks, comprise the evaluation of blood physiological endpoints indicative of exercise, metabolism, and cellular activity (Skomal and Mandelman, 2012). In the last decade, the suite of tools available to researchers in this domain have expanded to include applications such as bio-telemetry and bio-logging devices (Hammerschlag *et al.*, 2011; Hussey *et al.*, 2015; Whitney *et al.*, 2016) as well as innovative methods for studying other aspects of fish behaviour. Reflex impairments integrate the effects of capture-related stressors by reflecting the status of physiological systems and predatory avoidance mechanisms (Davis, 2005; Davis and Ottmar, 2006) and have been used to indirectly measure delayed mortality in fishes (Chopin and Arimoto, 1995; Davis and Parker, 2004; Davis, 2005; Davis and Ottmar, 2006; Hueter *et al.*, 2006; Braccini *et al.*, 2012). In sharks, reflex impairments are an emerging tool to study the condition of sharks after capture, but their correlations with known blood parameters measured under stress have yet to be explored thoroughly. Although there has been some descriptive information provided on what happens to sharks after release from fisheries in terms of reflex impairment and shark condition (Hueter and Manire, 1994; Manire *et al.*, 2001; Hueter *et al.*, 2006; Benoit *et al.*, 2012; Braccini *et al.*, 2012; Afonso and Hazin, 2014; Danylchuk *et al.*, 2014; Gallagher *et al.*, 2014a; Hyatt *et al.*, 2016), there is still much more to understand about whether reflex impairments can be used as a relative proxy for understanding the condition and the physiological status in sharks exposed to fisheries interactions.

This study utilized assessments of both physiological measures (blood-based endpoints) and reflex impairments to investigate the sub lethal consequences associated with capture stress in sharks. We focused primarily on four large coastal shark species while also incorporating additional data collected for other species encountered at lower replication. The objectives of this study were: (i) to test for relationships between physiological variables (blood glucose, lactate, and haematocrit) and hooking duration, shark species, and body size; (ii) to evaluate for relationships between reflex impairments and hooking duration, species, body size; and (iii) to determine if any patterns existed amongst physiological blood indicators (glucose, lactate, haematocrit) and reflex impairments.

Material and methods

Study site and species

Research was conducted within Florida state waters between latitudes N 25.791 and N 25.597. Sampling was conducted from June 2015 to May 2016, across the wet and dry seasons (wet = June to November; dry = December to May). Species encountered include: blacknose (*Carcharhinus acronotus*), blacktip (*Carcharhinus limbatus*), bull (*Carcharhinus leucas*), great hammerhead (*Sphyrna mokarran*), lemon (*Negaprion brevirostris*), nurse (*Ginglymostoma cirratum*), sandbar (*Carcharhinus plumbeus*), scalloped hammerhead (*Sphyrna lewini*), and tiger (*Galeocerdo cuvier*).

Fishing and capture methods

Sharks were caught using a standardized circle-hook drumline system as described by Gallagher *et al.* (2014a). In short, gear consisted of a submerged weight with two attachment points: (i) a line running to the surface with buoy floats and (ii) a swivel connecting a 23-m monofilament gangion line (~400 kg test) that terminated with a baited 16/0 5°-offset circle hook. The proximal end of the monofilament line was connected to the weight via a hook timer (Lindgren Pitman HT600) that consisted of a magnetic release timer to display the time each animal has been on the line. This permitted us to measure hooking duration (i.e. hook time). Due to gear malfunction and damage, hooking duration was not guaranteed for all individuals. Gear was deployed between 9 and 23 m depth depending on location and allowed to soak for up to 1 h until retrieval began. Upon capture of a shark, the animal was secured either alongside the boat or on a semi-submerged platform, depending on size and species. A water pump was placed in the mouth of each individual to allow for continued respiration. All data collected for this project, including reflex evaluation (described below), occurred within 1 min of capture and securing (up to 1 min) of individuals.

Experimental procedures and animal husbandry were approved by the University of Miami Institutional Animal Care and Use Committee (Protocol 15-238) and research permits from Florida Fish and Wildlife Conservation Commission, Biscayne National Park and National Marine Fisheries Service.

Reflexes

To assess potential reflex impairments of sharks from capture, we used an approach following Davis and Ottmar (2006), whereby each reflex was graded with either a 0 (zero, non-impaired) or a 1 (one, impaired). All reflexes were evaluated immediately once the shark was landed within a 30-s time frame to standardize the procedure, entirely by the first author. We evaluated a total of five reflexes described in chronological detail below.

Retrieval reflex

The first reflex evaluated was the individual's "vigour" when the fishing line was initially retrieved (hereafter termed "retrieval"). For this test, the individual's relative resistance to being reeled in on the fishing line was determined. A grade of 0 was characterized by individuals actively resisting or swimming against the force of being reeled into the boat. Individuals graded with a 1 did not show any swimming activity upon retrieval.

Nictitating reflex

Once individuals were brought to the boat, a nictitating membrane test was conducted (hereafter termed "nictitating") following Gallagher *et al.* (2014a). The nictitating test consisted of a small burst of seawater at a distance of 4 cm away from the eye of the shark, from a 10 mL plastic, needleless syringe. A grade of 0 was characterized by the obvious firing of the nictitating membrane within 2 s of contact. A grade of 1 was given to individuals whose nictitating membrane did not appear or was delayed past 2 s.

Jaw reflex

Next, a PVC pipe attached to a submerged pump was placed in each individual's mouth to allow water to continuously flow over their gills and to evaluate their biting reflex (hereafter termed "jaw"). A grade of 0 was assigned if individuals maintained downward pressure during an attempt to open their mouth with upward pressure on the snout. Individuals whose mouths were opened easily without the presence of downward pressure were given a grade of 1.

Gill reflex

The fourth reflex was monitored visually by closely examining the gills of the animal and watching for the contraction and relaxation of the gill musculature (herein termed "gill"). A grade of 0 was characterized by movement of the hyoid constrictor (i.e. gill) muscles, an attempt by the animal to pump water for respiration. A grade of 1 was given to individuals who did not contract or relax their gill musculature.

Flex reflex

During the reflex workup, each individual was monitored closely for any type of body flexion or purposeful body movement (hereafter termed "flex"). To account for individual differences in whole body movement, if no obvious movements were detected during our monitoring period, we grabbed the tail and pulled laterally to induce a response. A grade of 0 resulted from any purposeful body movement or a response to the tail grab. A grade of 1 was given to individuals who did not show any purposeful body movement nor a response to the tail grab.

From the reflex results from each individual, a probability of impairment score was calculated as a simple proportion of the measured reflexes that were impaired in an individual shark (probability of impairment score of 0 = no reflexes impaired; 1 = all reflexes impaired) (Davis, 2007; Raby *et al.*, 2012).

Blood physiology

To assess potential physiological indicators of capture stress, whole blood (5–7 ml) was drawn from each individual via caudal venepuncture using 18-gauge needles and plastic non-heparinized syringes and subsequently processed for glucose, lactate, and haematocrit levels. Haematocrit was determined through centrifugation (13, 000*g* × 3 min) of micro haematocrit tubes on board the vessel and measuring the packed red blood volume (% Hct) with a crito-caps card reader in duplicates. Glucose was measured on whole blood by adding 10 µl of blood to a glucose metre (ACCU-CHEK glucose metre; Roche Diagnostics, Basel, Switzerland; see Cooke *et al.*, 2008 for validation study with fish). Four millilitre of whole blood was separated into two vials and spun via centrifugation (13 000*g* for 5 min) for plasma extraction. Vials containing plasma were stored on ice until frozen at −20° C. Lactate values were measured on plasma by adding 10 µl of plasma to a lactate metre (Lactate Pro LT-1710 portable lactate analyser; Arkray Inc., Kyoto, Japan; see Cooke *et al.*, 2008 for validation study with fish). Though the lactate metre is designed for use with whole blood, plasma samples were used to obtain lactate values. This was accomplished through fitting raw values to an equation ($y = 0.8202x + 0.1292$, $R^2 = 0.987$) that was obtained through regression analysis of whole blood and plasma samples from the same individuals.

Statistical analysis

Glucose values were ranked then arc-sin square root transformed while lactate values were log transformed to obtain normality and homogeneity of variances. Generalized linear models (GLMs) were used to explore potential relationships among blood (glucose, lactate, and haematocrit), biological (shark species, length) and operational (hook time) parameters. A stepwise approach was taken starting with fully saturated models and then removing factors with *P*-values > 0.05. Final models included all significant factors and second order interactions. *Post hoc* pairwise comparisons generated mean ranks to examine for inter-specific differences after controlling for hook time and/or size effects. Ordinal logistic regression was used to explore potential relationships between the probability of impairment scores and biological (shark species, length) and operational (hook time) variables. Parameter estimates were produced from dummy variables and log-odds were examined for differences among categorical variables. Each reflex (retrieval, nictitating, jaw, gill, flex) was evaluated against blood variables and hook time using binomial logistic regression. For a one versus one binary model, variables were evaluated independently against each reflex to examine the relative predictability of each variable without the influence of others in the model. Kruskal-Wallis H tests were used to explore differences among species type for glucose, lactate, haematocrit, and probability of impairment scores. Pairwise comparisons were performed using Dunn's (1964) procedure with Bonferroni correction for multiple comparisons. First and second order regression analyses were employed to test for possible effects of hook time on the four parameters, on a species-specific basis. Only species with $n \geq 5$ individuals were included for each statistical test. All statistical analyses were performed using SPSS (Ver. 24, 2016) and statistical significance was declared at $p < 0.05$.

Results

Hook times ranged from 1 to 128 min (mean ± *SD* = 45.4 ± 29.1) for 116 individual sharks of 9 total species. An additional 107 individuals that lacked hooking duration values were sampled for a total of 223 blood and reflex-sampled sharks (Table 1). Sizes of sharks sampled were as follows: blacknose (121.0 ± 4.0; mean total length, cm ± SD), blacktip (157.1 ± 16.2), sandbar (202.2 ± 10), nurse (220.8 ± 38.2), tiger (225.3 ± 53.6), lemon (228.4 ± 33.2), bull (240.8 ± 25.6), scalloped hammerhead (267.6 ± 28.8), and great hammerhead (269.2 ± 44.7). Based on published length at maturity information (Compagno *et al.*, 2005), all sampled sharks in the study were either sub-adults or adults, with the exception of six individuals that were juveniles [lemon (two), nurse (three), tiger (one)].

Differences among species were reported for glucose, lactate, haematocrit and reflexes (Table 2). *Post hoc* examination of mean ranks for each of the blood parameters and reflex indices revealed significant differences. Great hammerhead sharks showed significantly higher glucose values than sandbar and blacktip sharks, while nurse sharks had significantly lower glucose and lactate values than all species (Figure 1). Haematocrit values were significantly lower in nurse sharks compared with blacktip and great

Table 1. Summary of replication for physiological samples and reflex assessments, and hook time (min) and total length (cm) mean ± *SD* and range for all species included in the present study.

Species (n)	Glucose	Lactate	Haematocrit	Reflexes	Hook time	Total length
Blacknose (5)	5	0	2	4	35 ± 26.5 6–58	121 ± 4 115 – 125
Blacktip (74)	71	18	41	31	46.5 ± 24.6 8–106	160 ± 13.3 118–192
Bull (17)	17	1	8	8	56 ± 43.4 3–128	234.3 ± 24.1 189–270
Great hammerhead (17)	16	5	10	12	49.3 ± 27.4 4–100	262.1 ± 50.2 200–385
Lemon (10)	10	1	3	2	50 ± 32.9 4–100	235 ± 33.9 179–270
Nurse (67)	66	25	34	28	40.7 ± 28.2 1–100	215.2 ± 38.5 104–267
Sandbar (22)	17	11	12	14	57.9 ± 23.5 34–106	204.6 ± 9.7 182–222
Scalloped hammerhead (5)	5	4	4	4	38.3 ± 9.2 29–51	247.7 ± 9.7 237–256
Tiger (6)	6	1	2	3	52.5 ± 43.1 22–83	225.3 ± 53.6 120–269

Table 2. Mean values ± *SD* examined for glucose, lactate, haematocrit, and reflexes for the primary four species assessed in this study: great hammerhead, blacktip, sandbar, and nurse sharks.

Species	Glucose (mmol/l)	Lactate (mmol/l)	Haematocrit (%)	Reflexes (proportion)
Blacktip	(71) 5.2 ± 1.0	(18) 6.3 ± 3.4	(41) 25.5 ± 4.1	(31) 0.43 ± 0.26
Great hammerhead	(16) 10.2 ± 1.4	(5) 5.6 ± 2.8	(10) 26.8 ± 5.2	(12) 0.45 ± 0.17
Nurse	(66) 1.9 ± 0.4	(25) 1.2 ± 0.5	(34) 20.0 ± 3.9	(28) 0.06 ± 0.13
Sandbar	(17) 4.6 ± 0.9	(11) 3.5 ± 2.2	(12) 24.3 ± 5.4	(14) 0.17 ± 0.22

Sample sizes in parenthesis.

hammerhead. Nurse and sandbar sharks had the lowest probability of impairment compared with blacktip and great hammerhead which had the highest (Figure 1).

Species effects were detected in the final models for all three blood parameters, whereas size was not (Table 3). Glucose significantly increased with hook time, but no such relationship was detected for haematocrit (Table 3). The interaction between hook time and species had a significant effect on the final model for lactate (i.e. an increase in lactate as a result of increasing hook time was dependant on species type). This interaction was non-significant for glucose and haematocrit (Table 3).

The effects of hook time, species, size and their interactions on the probability of impairment were assessed through two model variations to alleviate the discrepancies between the probability of impairment values for nurse sharks and the remaining species. Nurse sharks lack a nictitating membrane and therefore formed dissimilar impairment probabilities than other species. For nurse sharks, hook time and size had no significant effect on the probability of impairment (Wald $X^2(1) = .198$, $p > 0.05$ and Wald $X^2(1) = .235$, $p > 0.05$, respectively). For blacktip, bull, great hammerhead, and sandbar sharks (combined as a unit), the final model could significantly predict the probability of impairment ($X^2(5) = 19.144$, $p < 0.005$), indicating that at least one of the independent variables was significant. An increase in hook time (expressed in minutes) was associated with an increase in the odds of a higher probability of impairment, with an odds ratio of 1.018 (95% CI, 1.000 to 1.036), Wald X^2 (1) 4.028, $p < 0.05$ (i.e. with every minute on the line, the probability of impairment increased 1.018 times). Species effects significantly influenced the probability of impairment with log-ratios indicating sandbar sharks have a significantly lower probability of impairment when compared with blacktip, bull, and great hammerhead sharks (Figure 2). As a result, sandbar sharks were used as the reference category in the model. Model log-ratios revealed that bull, blacktip, and great hammerhead sharks are more likely to have a higher probability of impairment (19.71, 26.51, and 48.02 times; respectively) when compared with sandbar sharks (Figure 2). Shark size and interaction terms were non-significant ($p > 0.05$).

Pooled species analysis revealed that lactate proved to be the most informative blood parameter as it related to reflex performance, evidenced by the finding that an increase in lactate levels were significantly associated with impairment for 4 of the 5 reflexes (all except for nictitating membrane; $p < 0.05$; Table 4; Figure 3). Hook time, glucose, and haematocrit were all non-significant for impairment for the entire set of assessed reflexes (Table 4). Hook time was significantly associated with the impairment of jaw and gill reflexes, while haematocrit was associated with only the jaw reflex. Glucose was significantly associated with the impairment of jaw and flex reflexes. Finally, lactate was significantly associated with the impairment of the retrieval, jaw, gill, and flex reflexes (Table 5).

Discussion

Fisheries interactions elicit a complex set of responses that can alter both fish physiology and behaviour. Although these responses

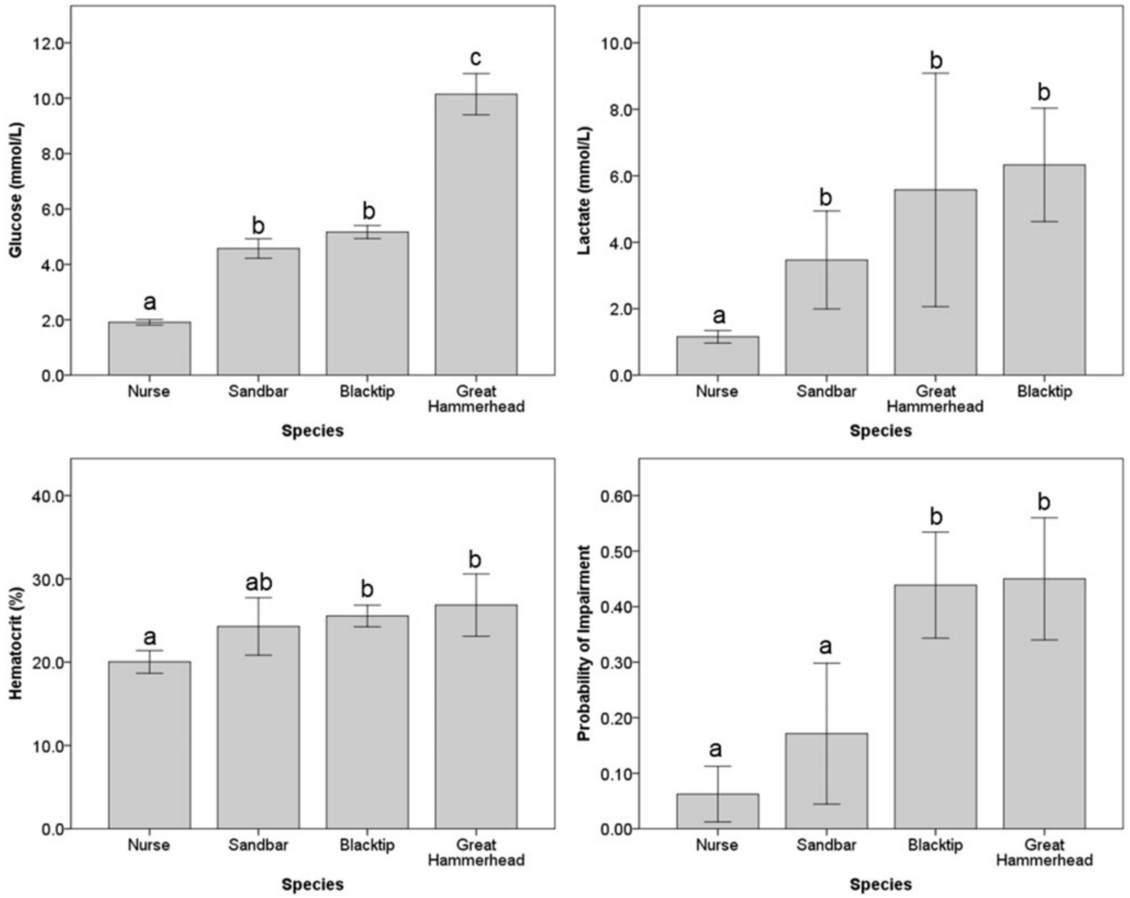

Figure 1. Mean values for blood parameters and reflexes for the primary four species in the present study. Bars with different lower case letters indicate significant differences. Error bars represent 95% *CI*.

Table 3. Results from stepwise GLMs evaluating lactate, glucose, and haematocrit.

	Lactate	Glucose	Haematocrit
Hook time	*	*	ns
Species	*	*	*
Size	ns	ns	ns
Hook time x Species	*	ns	ns

Significance (*) declared at $p < 0.05$. Non-significant terms are indicated by "ns".

allow animals to survive day-to-day activities, their persistence can impede normal function and have the potential to lead to mortality. The responses involved span several systems (circulatory, respiratory, locomotory, and neuro-endocrine) and their severity seems to be linked with the duration and magnitude of a capture event (Cliff and Thurman, 1984; Wilson *et al.*, 2014; Dapp *et al.*, 2016). By examining both the physiological and reflex responses to a standardized experimental fishing technique, we documented differences in the stress response to capture among multiple large coastal shark species, as well as the influence of various biological and operational factors inherent to the stress event.

Our study documented significant species-specific differences in shark physiological and reflex parameters in response to capture. Inter-specific patterns in the physiological response of sharks are well documented (Marshall *et al.*, 2012; Skomal and Mandelman, 2012; French *et al.*, 2015; Guida *et al.*, 2016), however most of the species which have been assessed are those commonly captured as target or bycatch in offshore commercial fisheries. Thus, our work on coastal species expands this growing body of work. Nurse sharks consistently revealed significantly low values, suggestive of a dampened behavioural and physiological stress response to capture. Even with hook times of up to 70 min, blood and reflex parameters did not increase significantly for nurse sharks, and remained the lowest of any species reported in this study. This is consistent with recent data using accelerometers on fishing gear to measure the behavioural responses of sharks to capture (Gallagher *et al.*, 2017). When captured, nurse sharks exerted minimal force and often settled on the bottom when hooked (Gallagher *et al.*, 2017), likely due to their ventilation strategy and low metabolic rate (Dapp *et al.*, 2016; Whitney *et al.*, 2016).

In comparison to nurse sharks, the opposite capture response was found for more active species. Great hammerhead sharks were the only species in the current study where hook time significantly affected their probability of reflex impairment and showed the highest values for glucose and lactate, findings that are corroborated by other work (Gallagher *et al.*, 2014a, this study). The increased probability of impairment in this species may be a result of a heightened capture stress response, whereby this species is exhausted and in an anaerobic state when landed. Indeed, this

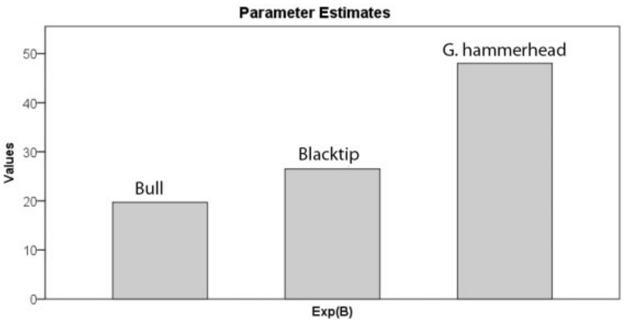

Figure 2. Log ratio estimates of the probability of impairment with sandbar sharks as the reference category.

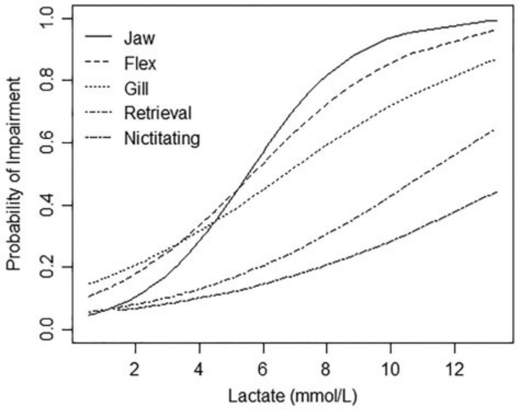

Figure 3. Probability of impairment for sharks compared with predicted lactate values.

Table 4. Results from binomial logistic regressions including blacktip, bull, great hammerhead, lemon, nurse, sandbar, scalloped hammerhead, and tiger sharks where variables were pooled for each reflex.

Variable	Retrieval	Nictitating	Jaw	Gill	Flex
Hook time	ns	ns	ns	ns	ns
Glucose	ns	ns	ns	ns	ns
Lactate	*	ns	*	*	*
Haematocrit	ns	ns	ns	ns	ns

Significance (*) declared at $p < 0.05$. Non-significant terms are indicated by "ns".

Table 5. Results from binomial logistic regressions including blacktip, bull, great hammerhead, lemon, nurse, sandbar, scalloped hammerhead, and tiger sharks where regressions were run for each variable independently.

	Retrieval	Nictitating	Jaw	Gill	Flex
Hook time	ns	ns	*	*	ns
Glucose	ns	ns	*	ns	*
Lactate	*	ns	*	*	*
Haematocrit	ns	ns	*	ns	ns

Significance (*) declared at $p < 0.05$. Non-significant terms are indicated by "ns".

species has been shown to exhibit pronounced fighting behaviours when captured on a fishing line, characterized by repeated burst accelerations (Gallagher *et al.*, 2017). This data along with their high metabolite values agrees with the available data on at-vessel mortality rates for great hammerheads, which can be upwards of 90% (Morgan and Burgess, 2007).

Interestingly, blacktip sharks had a significantly higher probability of reflex impairment compared with sandbar sharks despite no significant differences in their blood parameters. We interpret this finding as indicative of a pronounced behavioural stress response in blacktips, an inference that is consistent with the force they exert when captured on fishing lines (Gallagher *et al.*, 2017). Although there are no current published studies on sandbar behaviour during capture, Brill *et al.* (2008) saw that oxygen delivery following exercise was likely not compromised, suggesting that hook and line capture and release may not increase mortality rates. This is consistent with data from the U.S. pelagic longline fisheries, in which a relatively high proportion of sandbar sharks (75%) are alive upon gear retrieval (Gallagher *et al.*, 2014b). Similarly, Morgan and Burgess (2007) reported a large difference in at-vessel mortality for these two species with blacktips showing 88% mortality compared with 36.1% for sandbars. The combination of these data suggests that reflex impairments for these species may be a better predictor of mortality than the physiological blood parameters used.

Lactate consistently emerges as the most informative and predictive physiological parameter in shark stress literature (Brill *et al.*, 2008; Skomal and Mandelman, 2012; Bouyoucos *et al.*, 2017), a finding that is supported here. Gallagher *et al.* (2017) found a positive and significant relationship between maximum acceleration of sharks captured on fishing lines (i.e. maximum acceleration) and their corresponding plasma lactate values when landed, a result that demonstrates that intense swimming behaviours during capture can result in anaerobic processes and metabolic acidosis. This behavioural "fight" response of sharks when captured may also be responsible for observed reflex impairment. Previous work on capture stressed blacktips have revealed linear trends in the relationship between hook time and lactate as well as significantly higher mean values when compared with the second order quadratic relationship seen here. Manire *et al.* (2001) saw a steady increase in lactate for blacktip sharks subjected to gill net capture, however this capture technique fully restrains animal movement which prevents respiration in the obligate ram ventilating species. Additionally, mean lactate values for blacktip sharks captured on commercial longline gear was 36.8 mmol/l compared with 6.3 mmol/l found in this study (Marshall *et al.*, 2012). These results, however, reflect soak times ranging from 4 to 12 h without delineation. Similar to this study, Brooks *et al.* (2011) saw a parabolic trend with lactate values in longline captured Caribbean reef sharks (*Carcharhinus perezi*) with peak values attained at 120–180 min of hooking. In the present study, mean hook times for blacktips were 46.5 ± 24.6 min and the max hook time for any individual where lactate was collected was 96 min. The parabolic trend and overall lower lactate values seen in this study may be attributed to the experimental drumline system used which was designed to permit ram ventilation and enable movements of sharks, thereby reducing capture stress compared with other traditional fishing methods such as gill nets and longlines. Additionally, soak times (average 1 h) were relatively short compared with other fishing methods. Further, a delayed accumulation of lactate in the blood, as seen in previous studies (Frick *et al.*, 2012), may explain the parabolic trend. Thus, the sharks in this study may have had a chance to recover after bouts of burst swimming that initiated the increase in lactate levels and subsequently the following decline.

Glucose levels here peaked between 50 and 80 min of hook time, before decreasing for blacktip and great hammerhead sharks. The initial increase may be attributed to the hormonal component of the stress response wherein glucocorticoid stress hormones are released that activate glycogen stores to be dispersed from the liver to satisfy the increased energetic demand that a stressor causes (Cliff and Thurman, 1984; Hoffmayer and Parsons, 2001; Frick *et al.*, 2010). The following period of hypoglycaemia is consistent with other findings (Manire *et al.*, 2001; Frick *et al.*, 2010; Brooks *et al.*, 2011) and may suggest a complete utilization of the glucose mobilized through the stress response or a total depletion of hepatic glycogen stores, or a combination of the two.

Body size can impact physiological responses in terms of available energy such as glycogen stores. In both teleosts and elasmobranchs, size has been significantly correlated with glucose levels and suggests that larger fish are at an advantage due to higher volumes of energy available in the form of glycogen which could be used during a "fight" response to capture (Ferguson *et al.*, 1993; Meka and McCormick, 2005; Gingerich and Suski, 2012; Barkley *et al.*, 2017). However, shark size did not alter glucose, lactate, haematocrit or the probability of impairment in this study. This may be attributed to the fact that only sub-adults and adult sharks were sampled and that the effect of shark size on these parameters may be more present in other species.

The duration of capture continues to emerge as an important factor in dictating the severity of key physiological stress response indicators in sharks and the current study further demonstrates that hook time can also predict probability of impairment in sharks. However, a relatively small sample size, which required pooling of all species in analysis, may have hindered this relationship. Blacktip, great hammerhead, and sandbar sharks retrieval reflexes were rarely impaired in this study. In fact, the retrieval reflex was the second least impaired reflex (14.1% overall impairment), just behind nictitating (11.5%). When probing into the reflex impairments further, we revealed that increases in lactate were associated with an increased likelihood of impaired retrieval, jaw, gill, and flex reflexes. Although Manire *et al.* (2001) was able to demonstrate that lactate values increased as release condition decreased (i.e. sharks became less robust) for blacktip and bonnethead sharks, and Hyatt *et al.* (2016) saw a coarse relationship between behavioural release condition scores and acid-base status in bull and bonnethead sharks, this study successfully linked increases in physiological stress parameters with increased reflex impairment. In this study, jaw reflex impairment yielded the greatest number of significant relationships with all blood parameters and hook time. As stated prior, Danylchuk *et al.* (2014) found reflex indices to be positively correlated with post-release mortality, with the congruent "BITE" reflex impaired in three of their four post-release mortality occurrences. Together, these results may indicate that reflexes involving the mouth of sharks may be most suitable at reflecting physiological disruptions associated with the stress response and the potential for delayed mortality.

A limitation of our study is an absence of control data to represent a state of resting or minimally stressed animals, making it difficult to infer the extent to which disturbances were affected by the capture event. We were not able to assess whether reflex impairments recover or not overtime; therefore, it remains unknown if reflexes are reflective of costs to the maintenance of homeostasis, metabolic processes, or survivability. Last, the capture method used for this study (experimental drumline) does not fully reflect the capture methods used in recreational and commercial fisheries (rod and reel, gill net, longline, trawl, etc.) and therefore likely produces a more benign capture stress response. Therefore, our results should be considered conservative and capture stress responses observed less than that off traditional fishing gears. In addition, this gear was allowed to soak for a maximum of 1.5 h, a considerably less amount of time compared with other fishing techniques that can often range from 1 to 12 h depending on the fishery.

In conclusion, this study links capture stress physiology and reflex impairment for the species examined while generating results that agree with previously reported species-specific at-vessel and post-release mortality rates. Collectively, our results indicate that lactate should continue to be used as a primary measure of large shark capture stress, and jaw reflex impairments should be further explored as a useful reflex in future studies. However, there is need for additional research on understanding the relationship between mortality and the factors evaluated here, especially for threatened or understudied species. Future investigations involving expanded hook times and simulated recovery periods may point to stress thresholds, as well as increased susceptibility to disease, impacts on movements post-release, and other sub lethal and lethal impacts.

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