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The physiological response of the Caribbean reef shark (*Carcharhinus perezi*) to longline capture☆

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ABSTRACT

Longline fishing is the most common elasmobranch capture method around the world, yet the physiological consequences of this technique are poorly understood. To quantify the sub-lethal effects of longline capture in the commonly exploited Caribbean reef shark (*Carcharhinus perezi*), 37 individuals were captured using standard, mid-water longlines. Hook timers provided hooking duration to the nearest minute. Once sharks were landed, blood samples were taken and used to measure a suite of physiological parameters. Control data were obtained by sampling an additional three unrestrained Caribbean reef sharks underwater at an established shark feeding site. The greatest level of physiological disruption occurred after 120–180 min of hooking, whereas sharks exposed to minimal and maximal hook durations exhibited the least disturbed blood chemistry. Significant relationships were established between hooking duration and blood pH, pCO_2, lactate, glucose, plasma calcium and plasma potassium. Longline capture appears more benign than other methods assessed to date, causing a shift in the stress response from acute at the onset of capture to a sub-acute regime as the capture event progresses, apparently facilitating a degree of physiological recovery. Continued investigation into the physiological response of elasmobranchs to longline capture is vital for the effective management of such fisheries.

1. Introduction

Longline fishing is thought to be the predominant method of commercial capture for sharks (Beerkircher et al., 2002; Lewison et al., 2004; Gilman et al., 2008). In the majority of longline fisheries all or part of the shark is harvested, however, in some areas large numbers of sharks are released alive, due to low species-specific commercial value (Beerkircher et al., 2002) and/or to comply with fisheries regulations (Morgan et al., 2009). The emerging discipline of conservation physiology is focused on using physiological tools and knowledge to understand and address conservation problems (Wikelski and Cooke, 2006; Cooke and O'Connor, 2010). Documenting the physiological consequences of longline capture in sharks is a vital undertaking for effective fisheries management because it can provide insights into the underlying causes of at-vessel mortality and into the viability of animals post release (Skomal, 2007).

The capture and release of a shark induces various degrees of physical trauma and physiological stress, the magnitude of which is thought to be dependent on the capture method, capture duration, and the specific metabolic capacity of the species (Skomal, 2006; Skomal, 2007; Mandelman and Skomal, 2009). If physiological stress, physical trauma, or a combination of the two is excessive, then immediate or delayed (post-release) mortality is possible (Skomal, 2007). In cases where sharks survive the capture event, a suite of homeostatic disruptions can potentially impact growth, feeding, swimming behaviour, and the immune system, leading to population-level consequences (Cooke et al., 2002; Skomal, 2007).

Although the study of elasmobranch stress physiology is very much in its infancy, the physiological effects of capture have been quantified

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for a number of different species and gear types. The majority of research to date has focused on the physiological effects of recreational rod and reel capture (e.g. Heberer et al., 2010), however, the effects of both gillnet (e.g. Frick et al., 2010) and trawl capture (Mandelman and Farrington, 2007) have also been quantified. In those studies that quantified capture duration, the magnitude of the stress response was found to be proportional to the magnitude of the stressor, which in the case of most capture events is determined by capture duration and gear type (Skomal and Bernal, 2010). Despite being the most common capture method for sharks, the physiological consequences of longline capture have received very little attention, with only four published studies to date (Moyes et al., 2006; Hight et al., 2007; Mandelman and Skomal, 2009; Frick et al., 2010); none of these studies quantified the magnitude of the stress response relative to capture duration.

It has been suggested that there is considerable inter-specific variation in the stress response in sharks, likely associated with differences in metabolic scope (Mandelman and Skomal, 2009). However, the response to capture has yet to be assessed for the majority of species commonly captured on longlines. This information is vital to understanding the causative factors of at-vessel-mortality and the viability of sharks post release, both of which have implications for elasmobranch fisheries management (Moyes et al., 2006).

The Caribbean reef shark (*C. perezi*) is an abundant, large bodied, reef-associated, apex predator distributed throughout the tropical and sub-tropical western Atlantic and greater Caribbean (Compagno, 1984). Although fisheries data pertaining to the Caribbean reef shark are sparse, there are indications that it is commonly captured by longline fisheries throughout its range (Amorim et al., 1998; Arocha et al., 2002; Rosa et al., 2006). In the United States, it is a prohibited species, which is commonly captured and subsequently released in the bottom set longline fishery off the Florida Keys (Morgan et al., 2009). Despite its abundance and common interactions with fisheries, there has been no investigation into the capture driven stress physiology of this species to date. Thus, the aim of this project was to quantify and characterise physiological disturbances induced by varying duration of longline capture in the Caribbean reef shark.

2. Methods

This study was conducted between 9th June and 1st October 2009, at the Cape Eleuthera Institute (CEI), Eleuthera, The Bahamas (24.54° N 76.12° W). All research was carried out under research permits MAF/FIS/17 and MAF/FIS/34 issued by the Bahamian Department of Marine Resources and in accordance with CEI animal care protocols developed within the guidelines of the Association for the Study of Animal Behaviour and the Animal Behaviour Society (Rollin and Kessel, 1998).

2.1. Animal collection and sampling structure

Stationary, mid-water longlines, 500 m in length with 25 baited gangions, were set in 15–30 m of water for up to 8 h. Gangions were 2.5 m in length and spaced ~15 m apart along the mainline with a support buoy every five hooks. Each gangion was connected to the mainline via a tuna clip and hook timer rigged with monofilament line (Lindgren Pitman HT600 Hook Timer, Pompano Beach, FL, USA). The gangion itself consisted of 2 m of braided polyester cord, 0.5 m of monofilament line, 0.5 m of steel leader, and a 16/0 circle hook. Gangion materials were connected with 8/0 swivels and each hook was baited with a 100 g chunk of bonito tuna (*Euthynnus alletteratus*). Line checks were performed every hour to identify candidate sharks and to release non-candidate sharks and by-catch. In order to minimise additional stress, line checks were conducted visually when water conditions permitted (i.e. flat water and good visibility). When this was not possible, the section of line already containing a hooked shark was avoided.

Once a candidate shark was identified, its position on the line was marked with a numbered and coloured marker and the time of hooking back-calculated from the elapsed time on the hook timer. Only animals cleanly hooked in the corner of the jaw were selected for sampling. To quantify the physiological stress response over a broad time range, the hooking duration of each candidate animal was manipulated to encompass up to four hours on the line. The shark was then retrieved from the line, secured alongside the boat, and placed in tonic immobility (Henningsen, 1994) for blood sampling by caudal venipuncture. Hook duration was defined as the time between hooking and phlebotomy. Following blood sampling, the hook was removed and the animal released.

2.2. Blood collection and analyses

Blood (~3 mL) was drawn by caudal venipuncture using a 38 mm, 20 gauge needle and a 3 mL syringe (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). All syringes were washed with the anticoagulant sodium heparin prior to drawing blood. Approximately 95 μl of whole blood was immediately transferred from the syringe into an iStat CG4+ cartridge, which was in turn inserted into an iStat point of care device (Heska Corporation, Fort Collins, CO, USA) for immediate analysis of pCO_2, pH, and lactate levels (Mandelman and Farrington, 2007; Mandelman and Skomal, 2009; Gallagher et al., 2010). The time from blood draw to the insertion of the cartridge into the iStat was <30 s. Glucose was measured by adding 10 μL of whole blood directly from the syringe to an Accu-Chek glucose metre (Roche Diagnostics, Basel, Switzerland), which had previously been validated for use on fish (Cooke et al., 2008). To calculate haematocrit, a small volume of whole blood was transferred to a 75 mm micro-haematocrit tube (Drummond Scientific, Broomall, PA, USA), the base of which was sealed with Critoseal putty (McCormick Scientific, St Louis, MO, USA). The sample was spun in a micro-haematocrit centrifuge (LW Scientific Zippocrit, Atlanta, GA, USA) at 4400 *g* for 5 min. The balance of the sample was transferred to a 3 mL vacutainer containing lithium heparin (Becton, Dickinson and Co.) and spun in a centrifuge (Clay Adams Compact II Centrifuge) for 5 min at 10,000 *g* to separate plasma from red blood cells. Plasma was then transferred to three microcentrifuge tubes using a pipette and stored in crushed ice until it could be frozen at −20 °C upon returning to the laboratory.

Plasma samples were subsequently transported in liquid nitrogen to a laboratory at the University of Illinois where they were stored in an ultra-cold freezer (−80 °C) prior to analysis of ions and urea concentrations. Plasma sodium and potassium levels were quantified using a flame photometer (Cole-Parmer Single-Channel Digital Flame Photometer, model WU-02655-00, Vernon Hills, IL, USA). Plasma urea, chloride, magnesium, and calcium levels were quantified using commercially available kits (BioAssay Systems, Hayward, CA, USA – Urea DIUR-500, Chloride DICL-250, Magnesium DIMG-250; Calcium DICA-500).

2.3. Conversions

The iStat point of care device is designed for use on homeothermic animals, and, as such, is thermostatted to 37 °C. To accurately quantify *in vivo* blood gas and pH values for ectothermic species, it is necessary to correct for temperature (Reeves, 1977). Each pH and pCO_2 value generated was, therefore, adjusted using sea surface water temperature taken at the time of capture using Eqs. (1) and (2) respectively, based on research conducted on the larger spotted dogfish (*Scyliorhinus stellaris*), where pH_M and pCO_{2M} represent the values derived directly from the iStat, and where pH_{TC} and pCO_{2TC} represent the temperature corrected values (Mandelman and Skomal, 2009).

$$pH_{TC} = pH_M - 0.011(T - 37) \quad (1)$$

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$$pCO_{2TC} = pCO_{2M}\left(10^{-0.019\Delta T}\right) \quad (2)$$

More recent research has suggested that species-specific conversions are required to generate absolute blood gas and pH values from raw iStat data (Gallagher et al., 2010). Given the present lack of specific conversion values for the Caribbean reef shark, established protocols described by Mandelman and Skomal (2009) were used, which provide relative differences in pH and pCO_2 whilst taking into account variation in water temperature over the course of the study.

Bicarbonate (HCO_3^-) level was calculated via the Henderson–Hasselbalch equation using temperature corrected pCO_2 and pH values (Mandelman and Skomal, 2009). Values for pK were derived from Eq. (3) and the value of αCO_2 was set at 0.414 following investigations into the ventilatory responses to hypercapnia in the lesser spotted dogfish (*Scyliorhinus canicula*) (Randall et al., 1976).

$$pK' \ (at\ 20\ ^\circ C) = -0.1003(pH_{TC}) + 6.67 \quad (3)$$

2.4. Baseline blood chemistry estimates

The present study took a novel approach to generating baseline estimates of blood chemistry by sampling minimally stressed, free-swimming Caribbean reef sharks underwater at an established shark diving operation on New Providence, The Bahamas. Experienced shark handlers are able to induce a mild form of tonic immobility in free-swimming individuals as they come to feed, allowing an experienced phlebotomist equipped with SCUBA to draw a blood sample underwater. Samples were obtained from three Caribbean reef sharks using identical needles and syringes to those described previously. The time from initial contact by the shark handler to blood draw was estimated to be less than three minutes, and, as such, represents an approximation of a stress-free (control) sample. Underwater blood sampling has seen *a priori* use for both elasmobranchs (Skomal, 2006) and teleosts (Pankhurst, 1990; Pankhurst, 2011). Although blood processing followed identical protocols to the field-based sampling described above, ~1 mL of blood was discarded prior to processing so as to avoid any potential contamination of the sample by seawater (Pankhurst, 1990).

2.5. Data analysis

All analyses were performed using JMP 7.0.1 (SAS Institute, Cary, NC, USA) and the level of significance (α) for all tests was 0.05. The Box–Cox procedure was used to select the optimum data transformation to improve normality and homogeneity of variances (Box and Cox, 1964), as part of a standard data cleaning process (Osborne, 2010). Regression analysis was used to estimate relationships between hook duration and blood parameters (Zar, 1984). Because it was not assumed that the relationship between variables would be linear, both linear and second order polynomial equations were applied as lines of best fit. In all cases, the best fit line, defined by the highest r^2 value, is reported. Following transformation, only two parameters deviated significantly from a normal distribution: urea ($p = 0.009$ Shapiro-Wilk W Test) and potassium ($p = 0.028$ Shapiro-Wilk W Test). For these parameters, hook duration was assigned to 60 min bins and analysed with a non-parametric Kruskal–Wallis tests. Where significant differences were found, post-hoc Mann–Whitney U tests were used to explore the data further (Zar, 1984). The use of Bonferroni corrections when performing multiple comparisons has been strongly contested because it reduces the probability of Type I error at the cost of inflating the probability of the equally deleterious Type II error (Rothman, 1990; Perneger, 1998; Nakagawa, 2004). As such, there was no adjustment to the threshold of significance (α) for the post-hoc Mann–Whitney U tests. To promote access to physiologically meaningful data, all graphical plots are based on untransformed data, not the Box–Cox transformed data used for statistical analysis.

3. Results

Between June and October 2009, a total of 40 Caribbean reef sharks were sampled: 37 by longline with hook durations ranging from 14 to 244 min ($\bar{x} = 115$ min, ± 11.5 S.E.), and three underwater-sampled control animals. Sea surface temperatures for both longline-sampled and control animals ranged from 26.3 °C–29.3 °C ($\bar{x} = 28.2\ ^\circ C \pm 0.2$ S.E.).

Significant second-order quadratic relationships were estimated between hooking duration and blood pH_{TC} ($r^2 = 0.221$, $p = 0.016$), carbon dioxide ($r^2 = 0.203$, $p = 0.024$), lactate ($r^2 = 0.622$, $p = <0.001$), and glucose ($r^2 = 0.362$, $p = <0.001$) (Fig. 1 A–D) with disturbances reaching their highest level after 120–180 min of hook duration. There was no significant relationship between hook duration and haematocrit or bicarbonate (Fig. 1 E–F). A significant relationship was also established between hook duration and plasma calcium ($r^2 = 0.332$, $p = 0.002$) and plasma potassium ($\chi^2 = 9.969$, $p = 0.041$) (Fig. 2A and E). Maximum disturbance for both plasma calcium and potassium occurred at approximately 120–180 minutes of hooking. No significant relationships were detected between hook duration and plasma sodium, chloride, magnesium, or urea (Fig. 2C–F).

4. Discussion

The goals of this study were to quantify and characterise the physiological perturbation induced by longline capture in the Caribbean reef shark, the results of which suggest a non-linear (parabolic) response of a number of blood chemistry parameters to hook duration. However, despite statistically significant results, and homeostatic perturbations of a magnitude capable of inducing mortality in other carcharhinid species (Moyes et al., 2006; Mandelman and Skomal, 2009; Frick et al., 2010), some caution should be employed when interpreting them given unavoidable limitations in the study design. The sample size of 37 experimental animals is relatively small and only six of those sampled were subjected to hook durations in excess of 200 min. Furthermore, r^2 values, though comparable to a large number of published field physiology studies (e.g., Skomal, 2006; Cooke et al., 2008; Butcher et al., 2011), are fairly low and there is a great deal of individual variation within the data.

The majority of research published on capture events in sharks suggests that the magnitude of homeostatic disruption initiated by a capture event is proportional to the duration of capture, the type of capture gear, and the metabolic scope of the species in question (Skomal, 2007; Mandelman and Skomal, 2009). However, the results of the current study indicate that this might not be the case for longline-captured Caribbean reef sharks as sharks subjected to maximal and minimal hook durations exhibited the least disturbed blood chemistry across all parameters with statistically significant trends. This result can potentially be attributed to the characteristics of the longline capture experience, which, when compared to other elasmobranch capture techniques, are relatively benign. Longlines lack the continual active retrieval of rod-and-reel capture, which induces continuous muscular exertion (Skomal, 2006) or the forward momentum and physical compaction of a trawl net (Mandelman and Farrington, 2007). Moreover, unlike gillnets, longlines do not usually completely entangle the captured animal, which can impede ventilation and consequently gas exchange (Manire et al., 2001; Frick et al., 2010). It is likely that the negative stimuli persisting through the duration of these other capture experiences trigger an acute "fight-or-flight" stress response for the duration of the capture event. In contrast, longline-captured animals are not actively fought following hooking and, in the present study, had relatively

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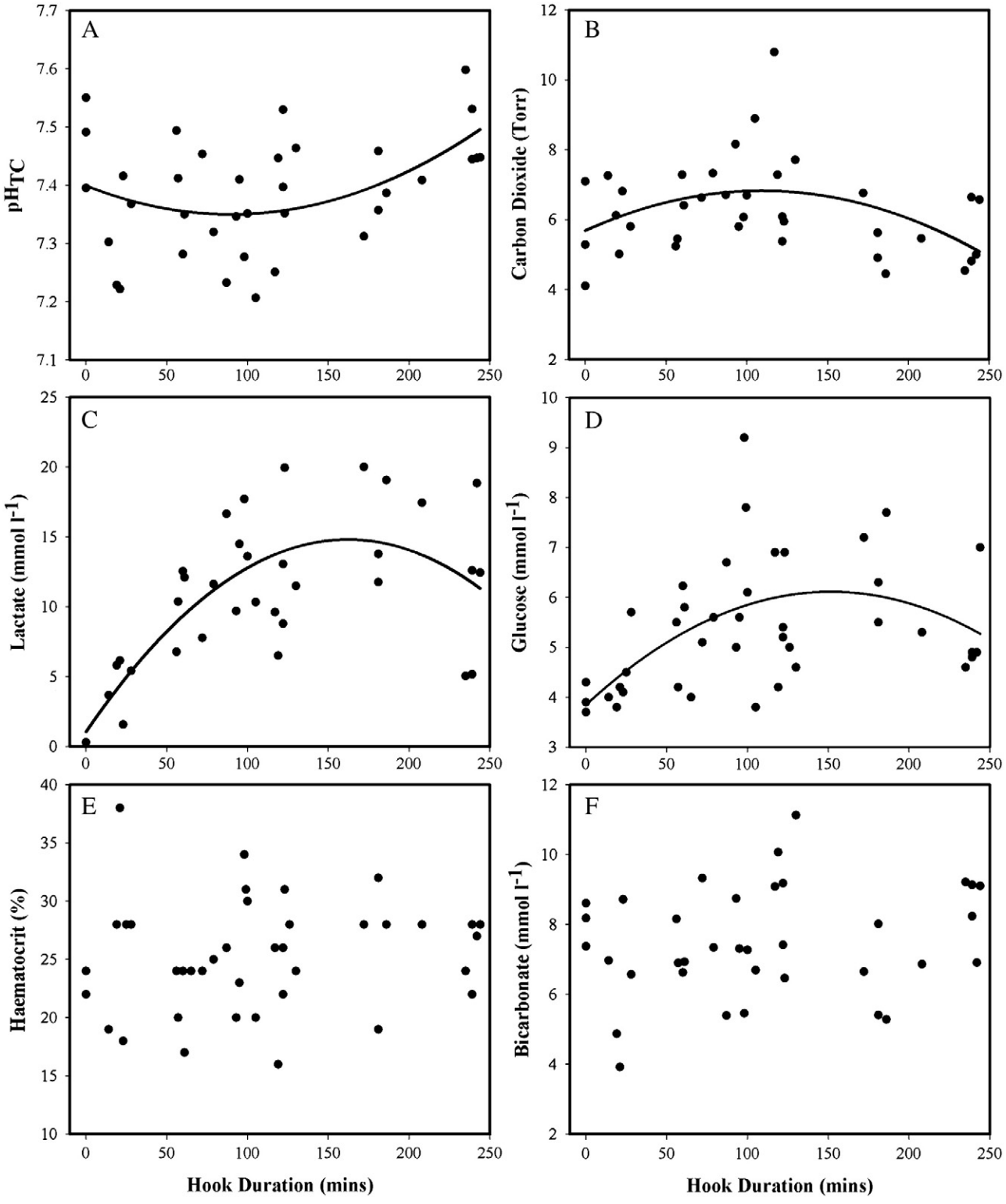

Fig. 1. The stress response of the Caribbean reef shark in response to longline capture. Data represent the relationships between hook duration and the physiological response variables (A) pH, (B) carbon dioxide, (C) lactate, (D) glucose, (E) haematocrit and (F) bicarbonate. All parameters were derived from whole blood with field-based portable blood analysers. Curves represent significant second order polynomials and were fitted by $y = 5.248\ (1 - e^{-0.0118x})$. To promote ease of access and comparability with other studies, the data plotted represents untransformed values in contrast to the transformed values on which the statistical analysis was conducted.

unrestricted movement within a 5 m diameter sphere (twice the length of the gangion).

Based on the apparently parabolic response of many blood parameters to hook duration, one hypothesis is that the Caribbean reef shark responds to longline capture with an initial energetic escape response (high anaerobic muscular activity) to the acute stress of hooking, followed by a period of reduced activity associated with a concomitant attenuation of the secondary stress response. The allostatic overload associated with the initial stress of longline hooking is designed to increase fitness and the capacity for escape

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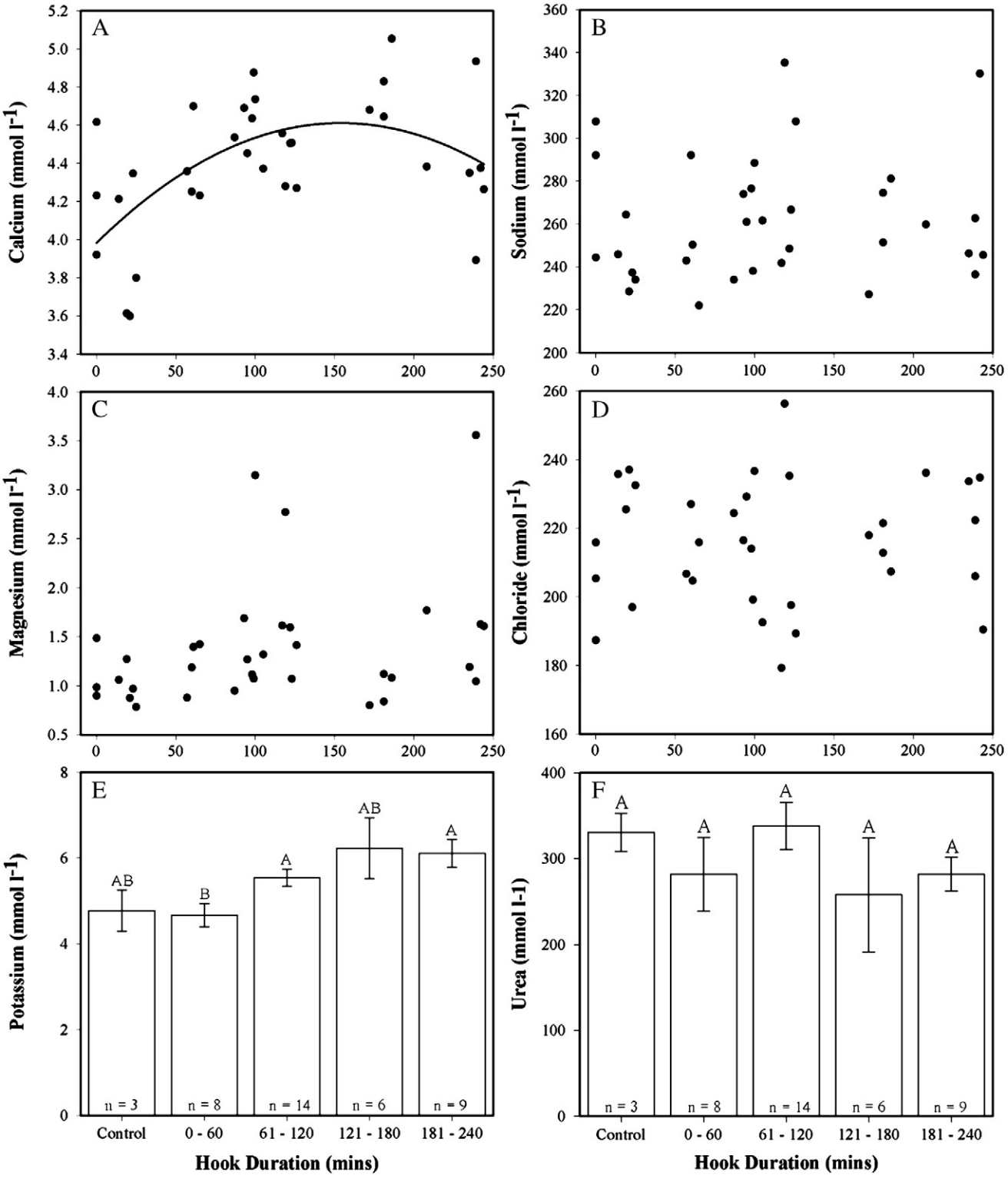

Fig. 2. The stress response of the Caribbean reef shark in response to longline capture. Data represent the relationships between hook duration and the physiological response variables (A) calcium, (B) sodium, (C) magnesium, (D) chloride, (E) potassium and (F) urea. The curve represents significant second order polynomials and were fitted by $y = 5.248(1 - e^{-0.0118x})$. Potassium and urea (Panels E and F) deviated significantly from a normal distribution, and as such, hook duration was binned at 60 min intervals and the data analysed with a non-parametric Kruskal–Wallis test. Data are means, error bars indicate ± 1 standard error and sample sizes are indicated at the base of the bars. Dissimilar letters above the error bars indicate statistically significant differences. To promote ease of access and comparability with other studies, the data plotted represents untransformed values in contrast to the transformed values on which the statistical analysis was conducted.

in the short term, however, as seen in other aquatic and terrestrial vertebrates, the high metabolic costs of maintaining the stress response can reduce fitness in the long term (Romero, 2004; Busch and Hayward, 2009; Romero and Wikelski, 2010; Schreck, 2010). We postulate that Caribbean reef sharks experience a shift in their stress response from acute at the onset of hooking to a sub-acute physiological regime, which promotes physiological recovery and preserves fitness over prolonged capture events.

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Blood acid–base properties of Caribbean reef sharks exhibited similar stress responses to those of other carcharhinids (e.g. Cliff and Thurman, 1984; Hoffmayer and Parsons, 2001; Spargo, 2001; Mandelman and Skomal, 2009). In this study, blood pH was significantly depressed following moderate hook durations. Pronounced blood acidosis has been reported in many other elasmobranchs subjected to hook and line (Cliff and Thurman, 1984; Hoffmayer and Parsons, 2001) and longline (Mandelman and Skomal, 2009) capture. This acidosis is thought to be driven by elevated levels of pCO_2, a common stress response in elasmobranchs (e.g. Cliff and Thurman, 1984; Hoffmayer and Parsons, 2001; Mandelman and Skomal, 2009), and the metabolic production of H^+ ions in response to capture stress, although the specific metabolic source of the latter is at present uncertain (Lindinger, 2004; Robergs et al., 2004). Lactate anion concentrations increased significantly for animals subjected to 0–150 min of hook duration, however, they declined in animals exposed to extended hook durations (>150 min). Due to the lag between intramuscular lactic acid production and the leaching of lactate loads into the blood, the plateau and subsequent decline of lactate concentrations in sharks exposed to longer hook durations suggests that burst exercise might have ceased early in the capture event.

The Caribbean reef shark ranks as a species more resilient to longline capture than several others assessed to date. Mandelman and Skomal (2009) ranked the relative resilience of five species of carcharhinid sharks according to the level of blood acid–base perturbation at the time of longline capture, the characteristics of which are comparable to the current study in terms of gangion length, hook type, and blood analysis protocols. Individual hooking durations were not obtained by Mandelman and Skomal (2009), but were estimated to range between 3 and 330 min compared to the present study which ranged between 14 and 244 min. Using the near maximally depressed 10th percentile blood pH values reported by Mandelman and Skomal (2009) and corresponding values from the present study, the Caribbean reef shark ranks among the more resilient species assessed to date, second only to the tiger (*Galeocerdo cuvier*) and sandbar (*C. plumbeus*) sharks. This resilience is supported by the lack of at-vessel-mortality encountered over the course of the present study and the proportionally lower at-vessel-mortality rates for both tiger and sandbar sharks reported by Mandelman and Skomal (2009).

In addition to mild acid–base disruption, Caribbean reef sharks exhibited elevations in whole blood glucose levels in response to capture. Glucocorticoid stress hormones mobilise hepatic glycogen stores leading to hyperglycaemia, which has been identified as a response to stress in a number of elasmobranchs (e.g., Cliff and Thurman, 1984; Hoffmayer and Parsons, 2001; Skomal, 2006; Frick et al., 2010). It addition, it has also been suggested that the stress response in some species of sharks is characterised by a hypoglycaemic period immediately following the initial hyperglycaemia (Manire et al., 2001; Frick et al., 2010). This could account for the parabolic trend in blood glucose identified in the present study. However, given the consistency of the trends across multiple parameters, it is not thought to be the case.

Only two ions (Ca^{2+} and K^+) showed significant variation with hooking duration in the present study. Elevated levels of plasma potassium (hyperkalemia) have been previously attributed to intracellular acidosis, which causes a net efflux of potassium from the tissue into the blood stream (Cliff and Thurman, 1984; Moyes et al., 2006; Mandelman and Farrington, 2007; Frick et al., 2010). Hyperkalemia is thought to alter the electrochemical gradients that control the function of excitable tissues such as muscle and, as a result, induce cardiac arrhythmia and muscle fatigue (Martini, 1974; Moyes et al., 2006). In mammals, the reported threshold for the onset of cardiac disruption is approximately 7 mmol L^{-1} (Cliff and Thurman, 1984), a threshold which appears to be similar in elasmobranchs (see Martini, 1974; Moyes et al., 2006; Frick et al., 2010). In the present study, five Caribbean reef sharks had potassium values in excess of 7 mmol L^{-1}, however, the fate of these five animals post-release cannot be ascertained, even though none of the sharks required any form of resuscitation and all swam away strongly upon release. This is in contrast to the muscle tetany observed in moribund gummy sharks (*Mustelus antarcticus*) which was attributed to hyperkalemia by Frick et al. (2010). Elevated plasma calcium is thought to be a natural consequence of acidosis that can impair muscular contraction and neuromuscular nerve transmission (Cliff and Thurman, 1984; Moyes et al., 2006). In contrast, elevated levels of calcium might also act as a compensation mechanism to help offset acidosis induced cardiac damage (Wells et al., 1986; Mandelman and Farrington, 2007). Although the mean plasma calcium levels in Caribbean reef sharks were higher than those reported in other studies (e.g., Cliff and Thurman, 1984; Moyes et al., 2006), it is unclear what the physiological implications of this might be.

In conclusion, the Caribbean reef shark responds to capture stress in a manner similar to other carcharhinids and, according to the magnitude of acid–base disruption, is ranked as one of the more resilient species assessed to date. The apparently non-linear relationship between physiological stress and hook duration is likely unique to longline capture due to its relatively benign characteristics when compared to other common capture techniques. It is possible that these characteristics cause a shift from a metabolically costly, escape-driven stress response immediately following hooking, to a sub-acute regime over longer hook durations, which facilitates the recovery of physiological homeostasis. The veracity of the parabolic responses identified in the present study require further testing, potentially via the quantification of the strength of the escape response (e.g., degree of struggling) over the course of a longline capture event, and additionally by the serial sampling of Caribbean reef sharks in a more experimental setting, even though it has been suggested that the repeated restraint and sampling of a single animal can itself exacerbate and, thus, potentially mask the effects of the capture event (Frick et al., 2009; Frick et al., 2010). Further investigation of the physiological effects of longline capture in elasmobranchs can help develop protocols to reduce at-vessel-mortality and promote post release survivorship, contributing to the effective management of elasmobranch longline fisheries on a global scale.

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