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The stress physiology of extended duration tonic immobility in the juvenile lemon shark, *Negaprion brevirostris* (Poey 1868)

Edward J. Brooks [a,b,*], Katherine A. Sloman [c], Stephanie Liss [a,d], Laila Hassan-Hassanein [a,b], Andy J. Danylchuk [e], Steven J. Cooke [f], John W. Mandelman [g], Gregory B. Skomal [h], David W. Sims [b,i], Cory D. Suski [d]

[a] Shark Research and Conservation Program, Cape Eleuthera Institute, Eleuthera, The Bahamas
[b] School of Marine Science and Engineering, Marine Institute, University of Plymouth, Plymouth, United Kingdom
[c] School of Science, University of the West of Scotland, Paisley, Scotland, United Kingdom
[d] Department of Natural Resources and Environmental Science, University of Illinois, Urbana, IL, USA
[e] Department of Environmental Conservation, University of Massachusetts, Amherst, Massachusetts, USA
[f] Fish Ecology and Conservation Physiology Laboratory, Department of Biology, Carleton University, Ottawa, ON, Canada
[g] Research Department, New England Aquarium, Central Wharf, Boston, MA, 02110-3399, USA
[h] Massachusetts Division of Marine Fisheries, 838 South Rodney French Blvd., New Bedford, MA 02744, USA
[i] Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill, Plymouth, United Kingdom

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ABSTRACT

Tonic immobility (TI) is a reversible coma-like stasis inherent to a variety of terrestrial and aquatic taxa, including elasmobranchs, yet virtually nothing is known about its underlying neurological and physiological processes in any taxa. The purpose of this research was to investigate the physiological effects of TI on the juvenile lemon shark (*Negaprion brevirostris*). Eight juvenile lemon sharks were subjected to four, three-hour treatments during which blood was sampled at 0, 30, 90 and 180 min, over a 6 week period. Treatments were differentiated by the method of maintaining the shark, either in TI, or allowed to swim freely between blood samples and the presence or absence of a pre-treatment exercise period designed to simulate the capture induced exhaustion that usually precedes the use TI in the field. The results suggest that TI is an inherently stressful experience, which magnifies the degree of perturbation observed in a number of blood chemistry parameters. It is thought that TI induced a short term reduction in ventilatory efficiency, which appeared to be countered by a series of compensatory mechanisms that include increased ventilation rates, and maintenance of the primary stress response. TI remains one of the most enigmatic areas of biology for all taxa and further research into its underlying psychological, physiological and neurological processes is recommended.

1. Introduction

Tonic immobility (TI) is an unlearned, reversible, coma-like stasis displayed by a large number of taxa (Gallup, 1974). In general, TI is thought to be the final stage of a ‘defensive cascade’ of behaviours initiated in response to the presence of a predator (Ratner, 1967). This cascade, which begins with a period of voluntary immobility intended to decrease the probability of detection and heighten responsiveness, then transitions through the ‘flight or fight’ response, and if escape is unsuccessful resulting in capture and restraint (i.e. by a predator), terminates with the onset of TI (Marx et al., 2008). TI is characterised by a catatonic motionless posture and a profound but reversible physical immobility which, in terrestrial vertebrates, is caused by muscle rigidity and unresponsiveness to painful stimulation (Marx et al., 2008; Ratner, 1967).

Nearly all research into TI has focused on terrestrial vertebrates such as lizards (e.g. Edson and Gallup, 1972), chickens (e.g. Gallup et al., 1976), guinea pigs (e.g. Bis Vieira et al., 2011) and humans (e.g. Marx et al., 2008). However, this phenomenon is also exhibited by a large number of elasmobranchs (Henningsen, 1994; Watsky and Gruber, 1990; Whitman et al., 1986). Like many terrestrial vertebrates, TI in sharks is characterised by a state of immobility (Henningsen, 1994; Watsky and Gruber, 1990), yet in contrast to their terrestrial counterparts, sharks exhibit relaxed muscle tone (the “limp” response; Whitman et al., 1986). In addition, for species with the ability to self ventilate via buccal pumping, individuals in TI exhibit deep rhythmical ventilations (Watsky and Gruber, 1990). In sharks, TI is typically induced by rapid dorsoventral inversion (Watsky and Gruber, 1990; Whitman et al., 1986) and its onset is relatively rapid (<1 min—Henningsen, 1994; Whitman et al., 1986), lasting for less than a minute to several hours in unrestrained individuals (Henningsen, 1994; Watsky and Gruber, 1990).

* Corresponding author at: Shark Research and Conservation Program, Cape Eleuthera Institute, PO Box EL-26029, Eleuthera, Bahamas. Tel.: +1 242 334 8552.
E-mail address: eddbrooks@ceibahamas.org (E.J. Brooks).

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TI is commonly used to safely restrain and handle sharks following capture for both scientific research (e.g. Brooks et al., 2011b; Holland et al., 1999; Murchie et al., 2009) and aquarium husbandry (e.g. Gruber, 1980; Henningsen, 1994). However, at present, the effects of TI on the physiological homeostasis of elasmobranchs is unknown, especially when coupled with the exhaustive anaerobic exercise and acute physiological disruption associated with most elasmobranch capture events (e.g. Mandelman and Skomal, 2009; Skomal, 2007). The little research that has been conducted to date suggests that TI is relatively benign, given the limp muscle tone and deep rhythmical ventilations exhibited (Watsky and Gruber, 1990). In addition, heart rate and blood pressure have been found to remain stable in blacktip reef sharks (*Carcharhinus melanopterus*) maintained in TI and provided with branchial irrigation (Davie et al., 1993).

The purpose of this project was to investigate the physiological and behavioural effects of extended duration tonic immobility in juvenile lemon sharks. Lemon sharks are members of the largest of the shark families, the carcharhinids, and are widely distributed throughout the tropical and sub-tropical western Atlantic and Caribbean (Compagno, 1984). Juvenile lemon sharks are easily captured and maintained in captivity (Dallas et al., 2010; Gruber, 1980), and are commonly found in the mangrove creeks surrounding Cape Eleuthera, making it an ideal subject animal for this study. To the authors' knowledge, this study represents the first investigation into the physiological effects of TI in any species to date.

2. Methods

This study was conducted between June 9th and October 1st 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, transport and husbandry

Juvenile lemon sharks were collected from local mangrove creeks using conventional hook-and-line angling gear that consisted of a standard spinning rod, a steel leader and a 9/0 circle hook. Upon capture, lemon sharks were transferred to a 200 l cooler of seawater, the hook was removed, the total length (cm) measured and the sex identified. The cooler was transferred to a boat for the journey back to the CEI laboratory, typically taking between 8 and 16 min. To ensure adequate oxygenation during transport, ~50% of the water in the cooler was exchanged with fresh seawater every 5 min during the journey. Upon arrival at the laboratory, sharks were housed individually in 13,000 l (3.7 m diameter × 1.25 m depth) circular tanks continuously supplied with fresh seawater at a rate of approximately 120 l h^{-1} (Dallas et al., 2010). Individual sharks remained in captivity for 4–6 weeks during the experimental period prior to being released back into the creek from which they were caught. Sharks were offered food daily in the form of chunks of bonito tuna (*Euthynnus alletteratus*) or Spanish sardines (*Sardinella aurita*) (Gruber, 1980). Dissolved oxygen, temperature and salinity were measured twice daily with a YSI 85 oxygen, salinity and temperature probe (YSI Inc, Yellow Springs, Ohio, USA). Between June and October 2009, four male and four female juvenile lemon sharks (x Total Length = 679 mm, ±21.8 S.E.) were captured and maintained according to these protocols. During the course of the experiments the ambient water temperature ranged from 19.2 to 37.1 °C (x = 25.8, ±0.15 SE), dissolved oxygen ranged from 5.12 to 9.46 mg l^{-1} (x = 6.58, ±0.02 SE) and salinity 34.1–39.8 ppt (x = 36.6, ±0.19 SE).

2.2. Experimental design

Juvenile lemon sharks were subjected to a series of four treatments with a minimum rest period of four days between trials. In all cases the subject animal resumed feeding the same day, or the day following a trial. Prior to a trial the water level in the tank was lowered to a depth of approximately 60 cm to facilitate the capture and handling of the animals. During previous laboratory experiments it was observed that shark swimming speed was temporarily elevated immediately following a change in water level; as such, a 24 h acclimation period was established between the lowering of the water level and the trial. All eight sharks were subjected to four treatments in a random order and each shark was subjected to an individual treatment only once.

During all treatments, blood samples were taken 0, 30, 90 and 180 min from the commencement of the trial. Treatments were differentiated by the method of maintaining the subject animal during the course of the three hours, either held in TI by a field assistant, or allowed to swim freely between blood samples, both coupled with the presence or absence of an initial exercise period. Exhaustive exercise, in the form of three minutes of chasing and tail grabbing, was incorporated into the study design to simulate the physiological stress that typically precedes the use of TI. Chasing and tail grabbing have been shown to produce physiological responses similar to those imposed by angling (Kieffer, 2000; Suski et al., 2006, 2007; Wood, 1991). The four treatments consisted of all possible interactions of these two variables, consisting of two treatments whereby a subject animal was maintained in TI for three hours, one of which had an initial exercise period and one which did not, and two treatments where the subject animal was allowed to swim freely between blood samples, one of which had an initial exercise period and one which did not. Across all treatments, sharks were netted in under 25 s (x = 11.6, ± 2.3 SE), inverted until the onset of tonic immobility, which took less than 100 s (x = 60.2, ±4.8 SE), and immediately blood sampled. The time elapsed from netting to blood draw was typically less than 120 s (x = 104.7, ±7.4 SE).

2.3. Behavioural observations

Lemon sharks supplement ram ventilation by buccal pumping, which allows them to remain stationary on the sea bed for long periods of time (Kessel et al., 2009). Depending on the treatment, one of two behaviours important for respiratory regulation was quantified. For treatments that required the maintenance of animals in TI for an extended period, preventing the use of ram ventilation, ventilation rates were determined by counting the number of contractions of the buccal chamber in one minute (v m^{-1}; Barreto and Volpato, 2004; Chapman et al., 2010; Shultz et al., 2011). Ventilation rates were measured six times over the course of a 15 min observation period immediately after blood sampling at 0, 30, 90 and 180 min. Swimming speed, which is an important behaviour for respiratory regulation in ram ventilating sharks (Parsons and Carlson, 1998), was quantified for treatments where the subject animal was allowed to swim freely between blood samples. Relative swimming speed was determined by counting the number of tail beat cycles per minute (tbc m^{-1}), defined by the complete movement of the tail through the cycle returning to the start position (Graham et al., 1990). This measure was not designed to give absolute swimming speeds, but rather to identify relative changes in swimming speed both within and between treatments. Relative swimming speed was quantified on a similar schedule to ventilation rates, measured six times over the course of a 15 min observation period immediately after blood sampling at 0, 30, 90 and 180 min. Control values for ventilation rates and tail beat frequencies were taken during the 4–6 day rest periods between treatments, but not within 24 h of the completion of a treatment. Sharks were observed for a minimum of five, 15 min observation periods during which either ventilation rates, or tail beat frequencies