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Behavioral modification of visually deprived lemon sharks (*Negaprion brevirostris*) towards magnetic fields

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ABSTRACT

The ability of elasmobranchs to orient to weak electromagnetic fields is well documented. Recently, scientists have employed the use of strong electrosensory stimuli, such as permanent magnets, as a means to evaluate the repellent responses of elasmobranchs and assess the utility of these materials for bycatch repellent technologies. However, several studies have produced contrasting results both between and within species. To explain these results, we hypothesized that conditions leading to vision loss (i.e. turbid water) may be a factor affecting electrosensory repellent success. To simulate a visually deprived environment, the nictitating membranes of juvenile lemon sharks (*Negaprion brevirostris*) were temporarily sutured closed and the behavioral responses of sharks towards a magnetic apparatus were observed in a pen within the shallows of Bimini, Bahamas. Results demonstrate that the magnet-associated behavior of visually deprived sharks significantly differed from control sharks in regard to: (1) avoidance distance, (2) visit quantity prior to first entrance through the magnet zone, and (3) total entrances/total visits. These findings suggest context-dependent switching, where elasmobranchs may exhibit a heightened reliance on their electrosensory system when the extent of their visual range is reduced. These findings also provide insight into favorable environments (e.g. estuary or other coastal ecosystems) and applications (e.g. inshore fisheries and beach nets) that may yield more consistent and successful future implementations of electrosensory repellents for sharks.

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1. Introduction

Context-dependent switching – the capacity to flexibly tailor behavior based on the current ecological and biological state – has been extensively demonstrated in a wide variety of both marine and terrestrial organisms (Hoare et al., 2004; Leahy et al., 2011; McIntyre and McCollum, 2000; Ranåker et al., 2012; Smith and Belk, 2001; Zuberbühler, 2001). For example, in teleosts, ecological factors, such as competition or presence of predators, can impact shoal size (Hoare et al., 2004), whereas environmental factors, such as turbidity, can impact chemo-sensory system reliance (Leahy et al., 2011; Ranåker et al., 2012).

Studies related to context-dependent switching have been conducted on diverse taxa, including teleosts (Hoare et al., 2004; Krause, 1993), amphibians (McIntyre and McCollum, 2000) and mammals (Zuberbühler, 2001); however, there is little information pertaining to elasmobranchs (i.e. sharks, skates, and rays). Elasmobranchs have highly developed vision and electroreception which they use for, *inter alia*, prey detection (Cohen, 1991; Gruber and Cohen, 1978, 1985; Kalmijn, 1974). For example, sharks are equipped with an intraocular reflecting structure known as the tapetum lucidum (Bernstein, 1961; Best and Nicol, 1967; Braekevelt, 1994), a feature that enhances visual sensitivity in low light levels, therefore giving sharks advanced nocturnal vision (Arnott et al., 1970; Ollivier et al., 2004).

An elasmobranch's unique electrosensory system, known as the ampullae of Lorenzini (Kalmijn, 1966, 1971; Murray, 1960), serves a variety of functions, including the detection of bioelectric fields produced by prey (Kalmijn, 1974; Kajiura and Holland, 2002), conspecifics (Bratton and Ayers, 1987; Tricas et al., 1995) and predators (Peters and Evers, 1985; Sisneros et al., 1998). This system is also suspected to enable the detection of magnetic fields that have been hypothesized to provide geolocation information and navigational cues (Kalmijn, 1982; Klimley, 1993; Klimley et al., 2002). Recently a number of studies have exploited this acute sensitivity to weak electric and magnetic fields. These studies investigate the applicability and efficacy of much stronger electrosensory stimuli, such as magnets and electropositive metals, to overstimulate the ampullary systems of elasmobranchs, produce repellent responses and minimize elasmobranch bycatch in fisheries and beach nets (e.g. Rigg et al., 2009; Stoner and Kaimmer, 2008). Laboratory and field analyses have produced varying results, finding that repellent efficacy can be affected by a variety of factors including organismal satiation (O'Connell

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et al., 2012; Stoner and Kaimmer, 2008; Tallack and Mandelman, 2009), habituation (Brill et al., 2009; O'Connell et al., 2011), and conspecific density (Brill et al., 2009; Jordan et al., 2011; Robbins et al., 2011). However, none of these studies have revealed if the visual environment plays a role in repellent effectiveness.

For the present study, we aim to examine how visual deprivation, simulating a turbid environment, may influence the repellent success of a grade C8 barium-ferrite ($BaFe_{12}O_{19}$) magnetic barrier on the lemon shark (*Negaprion brevirostris*). Using a similar experimental design, identical species, and magnet-type as in O'Connell et al. (2011), we aim to evaluate how turbidity, which will be simulated by nictitating membrane closure, may affect elasmobranch electroreception/magnetoreception capabilities. Similar to the studies pertaining to context-dependent switching, we hypothesize that the magnet-associated behavior of visually deprived sharks will significantly differ from control and procedural control sharks. More specifically, we predict: (1) the avoidance ratios (total avoidances/total visits) will be significantly greater and the entrance ratios (total entrances/total visits) will be significantly reduced towards the magnet zone in comparison to the control zone, (2) the avoidance ratios and avoidance distance with respect to the magnet zone will be significantly greater in visually deprived sharks in comparison to all other shark types, (3) the entrance ratio with respect to the magnet zone will be significantly lower in visually deprived sharks in comparison to all other shark types, and (4) the quantity of visits prior to first entrance through the magnet zone will be significantly greater in visually deprived sharks in comparison to all other shark types.

2. Material and methods

The study was conducted at Bimini, Bahamas (25°44′N, 79°16′W), a small series of islands approximately 85 km east of Miami, Florida, USA. A total of 24 juvenile lemon sharks (mean ± standard deviation, precaudal length (PCL) = 58.6 ± 8.24 cm) were used in the experiments, with 14 being male and 10 being female. Sharks were captured using 180 m long × 2 m deep gillnets and promptly transported to a 4m diameter holding pen. Upon arrival, each shark was restrained in a trough (10 × 100 cm), sexed, measured for PCL (tip of snout to precaudal pit, see DiBattista et al., 2008), tagged intramuscularly with a passive integrated transponder (PIT) tag (see DiBattista et al., 2008), and fitted with a color code tag to permit visual identification of individual animals (see Guttridge et al., 2011). All sharks were held in semi-captive pens that exposed them to ambient environmental conditions (i.e. changes in tides, salinity, temperature, and light) (see Guttridge et al., 2009) and given one week acclimation period. All sharks were fed to satiation during non-experimental periods on a mixed diet of fresh and frozen great barracuda (*Sphyraena barracuda*). No sharks died during these experiments and all were released at the site of their capture. A permit (MAF/LIA/22) to conduct scientific marine animal research was supplied by the Department of Marine Resources, Bahamas.

2.1. Experimental setup

A pen consisting of three compartments was constructed, including: 1) recovery/acclimation pen (5 m diameter), 2) corridor (3 × 1 m), and 3) experimental arena (4 × 4 m) (Fig. 1A). Each compartment was built with diamond-shaped construction mesh (5 cm × 5 cm) with evenly spaced steel reinforcing bar. Sliding mesh doors were constructed between compartments allowing researchers to usher sharks without the stress associated with handling. The experimental arena consisted of four zones: the separation, observation, control, and magnet zones. The separation zone was a 2 m section of construction mesh placed perpendicular to the substrate that was used to separate the control and magnet zones. The control zone consisted of three 1.75 m (height) polyvinyl chloride (PVC) columns spaced by 0.5 m and placed perpendicular to the substrate. At 0.5 m intervals on each column a slot was cut and a

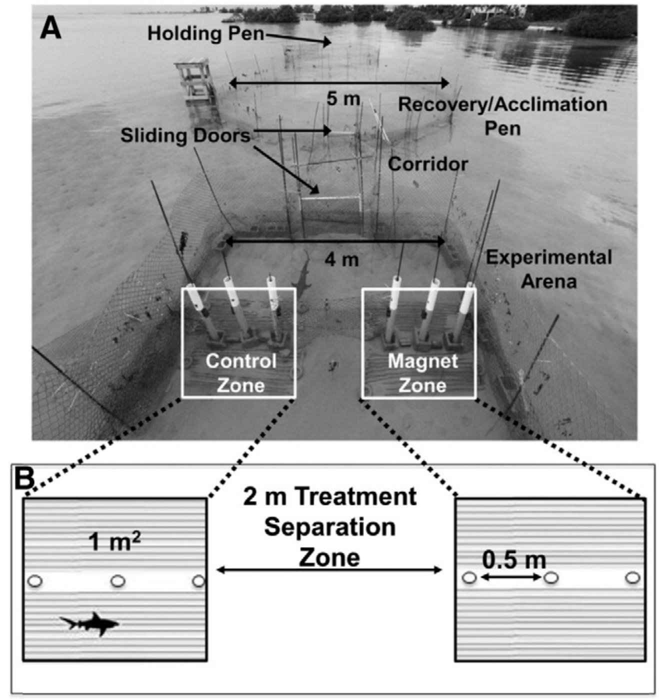

Fig. 1. Experimental setup. A) Perspective view, from near to far: experimental arena (4 m × 4 m) with PVC pipes (0.5 m apart) containing either barium-ferrite permanent magnets (magnet zone) or clay bricks (control zone); the transfer corridor, the recover/acclimation pen (5 m diameter) and the holding pen (3 m diameter). B) Surrounding the PVC columns/treatment zones and placed flush against the substrate was an observation zone containing flex pipe that were spaced at 5 cm increments from 0 to 50 cm (represented by the gray parallel and horizontal lines), as a means to determine the distance of avoidance.

0.15 m (length) × 0.10 m (width) × 0.05 m (height) clay brick, or sham magnet, was inserted. The magnet zone was identical in structure and dimension to the control zone; however, sham magnets were replaced with 0.15 m (length) ×0.10 m (width) × 0.05 m, grade C8 barium-ferrite ($BaFe_{12}O_{19}$) magnets (Fig. 1A). Throughout experimentation, control and magnet zone locations were randomized to avoid any side preference-based behaviors. Furthermore, to standardize the location of observable behaviors around each treatment zone and to determine the distance of avoidance in reference to each zone, an observation zone (1 × 0.5 m) was placed flush against the substrate surrounding the control and magnet zones. Within each observation zone, PVC piping (1.3 cm diameter) was placed parallel at 5 cm increments from 0 to 50 cm as a means to determine avoidance distance in reference to the treatment zones (Fig. 1B). In addition, in the center of the treatment separation region HD Go Pro 1080p cameras were positioned to permit a post-hoc identification and measurement of avoidance distance.

2.2. Surgery and shark type

All sharks (*N* = 24) were randomly assigned to one of four types/treatments (*N* = 6 per treatment): 1) 'control' — no manipulation, 2) procedural control 'eyebrow' — one suture above each eye, 3) procedural control 'one eye' — one suture used to temporarily close the nictitating membrane (note: for this treatment, three sharks had the left eye closed and three sharks had the right eye closed, and 4) 'visually deprived' — one suture for each eye to close the nictitating membrane and to severely compromise its visual acuity (Fig. 2). For treatments, sharks were lightly anesthetized in a 1:20,000 solution of tricane methanesulfonate (MS-222) in seawater to facilitate safe handling (see Newman et al., 2010). Once anesthetized, one 3–0 silk suture was used per eye (Fig. 2). After surgery, sharks were transferred to the recovery/acclimation pen, monitored until typical captive behaviors

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