

## Magnet-induced disorientation in hatchling loggerhead sea turtles

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### Summary

Laboratory experiments have indicated that hatchling loggerhead sea turtles (*Caretta caretta*) are able to establish and maintain courses using information from the Earth's magnetic field. In previous experiments, turtles were tested in relatively uniform magnetic fields generated by large coil systems surrounding an orientation arena. In this study, we investigated the orientation behavior of hatchlings with either magnets or magnetically inert brass bars attached to their carapaces. Control turtles (with brass bars) oriented significantly towards the east whereas

turtles bearing magnets were not significantly oriented as a group. The two distributions were statistically different. These results indicate that magnetic orientation behavior of hatchling sea turtles can be disrupted by attaching a small magnet to the carapace. This finding may prove useful both in field experiments and in efforts to localize magnetoreceptors.

Key words: orientation, navigation, magnetoreception, sea turtle, *Caretta caretta*.

### Introduction

Sea turtle hatchlings on the east coast of Florida, USA emerge from nests on sandy beaches, crawl to the surf, enter the water and swim offshore to the Gulf Stream. The ability to sense the Earth's magnetic field probably assists turtles in maintaining an appropriate offshore direction after they have migrated beyond shallow coastal waters (Lohmann and Lohmann, 1994a, 1998; Goff et al., 1998). Additionally, the ability to garner positional information from the geomagnetic field may aid young turtles during their transatlantic migration (Lohmann et al., 2001).

Several approaches have been used to investigate magnetic orientation behavior in animals. One technique is to use large magnetic coil systems to generate relatively uniform fields over a considerable area (Merritt et al., 1983; Kirschvink, 1992). When the magnetic field is changed artificially, researchers can determine if the orientation of animals inside the coil shows a corresponding shift (Wiltschko and Wiltschko, 1995). An advantage of this method is that the field experienced by the animal can be carefully controlled and the response to specific components of the field can thus be determined (Semm and Beason, 1990; Lohmann and Lohmann, 1994b, 1996a; Fischer et al., 2001). However, large coils are unsuitable for manipulating fields around animals moving freely through their natural environment and they cannot be used to localize magnetoreceptors within an animal by selectively altering the field around small, restricted parts of the animal's body.

A second method of investigation is to attach a small magnet or electromagnetic coil directly to an animal in an attempt to disrupt or alter magnetic orientation behavior. If the imposed field produced is strong enough to interfere with the Earth's field and redundant cues are not available, changes in

orientation behavior (Keeton, 1971; Walcott and Green, 1974; Mathis and Moore, 1988) or the performance of a conditioned response (Walker and Bitterman, 1989; Haugh et al., 2001) can sometimes be observed. This approach has been used in field studies for the purpose of blocking access to normal magnetic information (Keeton, 1971; Sinsch, 1987; Mathis and Moore, 1988) and also in attempts to localize magnetoreceptors by disrupting the field around a specific part of an animal's body (Haugh et al., 2001). A disadvantage of this technique is that fields produced by magnets and small coils typically change rapidly with distance from the source, so that quantifying and describing the field presented to an animal can be challenging.

To date, all laboratory experiments investigating magnetic orientation behavior in sea turtles have involved large magnetic coil systems (e.g. Lohmann, 1991; Light et al., 1993; Lohmann and Lohmann, 1994b, 1996a; Goff et al., 1998). To investigate whether small magnets can be used to disrupt orientation behavior of sea turtles, we monitored two groups of hatchlings under laboratory conditions in which they are known to orient magnetically. The results demonstrate that magnetic orientation behavior in hatchling turtles can be disrupted by attaching a small magnet to the carapace.

### Materials and methods

#### Animals

Hatchling loggerhead sea turtles, *Caretta caretta* L., were obtained from nests at a beach hatchery in Hillsboro, FL, USA. Nests at this hatchery had been relocated from the Fort Lauderdale and Pompano Beach areas within 24 h after being

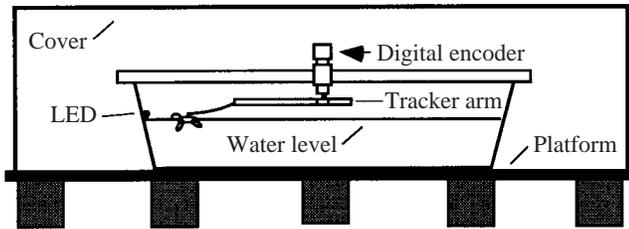


Fig. 1. Experimental arena. Hatchlings were harnessed to a rotatable tracker arm that was coupled to a digital encoder, so that the heading of the turtle was relayed continuously to a data-acquisition computer (not shown). A green light-emitting diode (LED) was affixed to the eastern wall of the arena at the water line. The arena in which the turtle swam was enclosed by a lightproof cover so that the hatchling swam in darkness when the LED was not on. See text for additional details.

deposited by adult turtles. Each nest was marked with a stake that indicated the number of eggs and date of deposition.

Each day, one or two nests were selected based on the average time to emergence of other nearby hatchery nests of similar size. Turtles from these nests were collected several hours before they would otherwise have emerged naturally and were immediately placed into lightproof coolers. They were then transported to a laboratory at Florida Atlantic University in Boca Raton, FL, where they were maintained in darkness. Each hatchling was tested only once and released on the beach following experiments each night.

#### Experimental arena

Experiments were conducted in a black, plastic, circular pool 1.7 m in diameter and 0.5 m high. The pool was filled with water to a depth of 0.3 m and was enclosed by a removable lightproof cover (Fig. 1). Observers dark-adapted for 30 min or longer were unable to perceive any light through the cover when stationed beneath it. A light-emitting diode (LED; peak output 565 nm) was attached to the inside wall of the pool directly east of the center of the arena. The LED was controlled by a 3 V power supply (two 1.5 V alkaline batteries) located outside the arena and could be turned on and off as needed (see below).

To minimize field distortions from buildings or electrical wiring, the arena and its cover were placed outdoors on a plywood and cinderblock platform approximately 30 m from the room in which the data-acquisition computer was housed (see below). Measurements with a Schonstedt model DM2220 magnetometer indicated that the magnetic field was uniform in the area where the turtles swam. The inclination angle of the field was  $57.0^\circ$  and the intensity was  $46 \mu\text{T}$ . These magnetic field conditions were nearly identical to those measured at the beach hatchery where the eggs developed ( $56.8^\circ$ ,  $46 \mu\text{T}$ ).

#### Procedure

For each trial, a hatchling was placed in a Lycra harness that encircled the carapace but did not inhibit swimming movements (Salmon and Wyneken, 1987). Turtles were



Fig. 2. Loggerhead hatchling in Lycra harness with monofilament line attached. The magnet was attached to the harness above the carapace using Velcro.

assigned to one of two groups. One set of turtles ( $N=13$ ) had a 24 g, 14 mm SpinBar<sup>®</sup> magnet attached by Velcro to the harness on the dorsal side of the turtle approximately 1 cm posterior to the nuchal scute (Fig. 2). The magnet was positioned with its poles perpendicular to the long axis of the turtle and the north-seeking end of the magnet towards the turtle's left. The magnet produced a field of  $7400 \mu\text{T}$  near its poles (measured with a Model 912 digital gaussmeter, Magnetic Instrumentation, Inc. Indianapolis, IN, USA); this field diminished with distance but was always greater than Earth-strength over the entire body of the turtle (Fig. 3). The other set of turtles ( $N=15$ ) had a magnetically inert brass bar of equivalent size and mass attached in the same manner and location as the magnet.

The harness was attached by monofilament line to a wooden tracker arm (0.6 m length, 6.4 mm diameter) that was affixed to a rotary digital encoder mounted above the center of the pool (Fig. 1). The tracker arm could rotate freely within the horizontal plane and thus tracked the movement of the turtle as it swam. Information was relayed, *via* the digital encoder, to a data-acquisition computer that continuously monitored the heading of the turtle throughout each trial.

All trials were conducted between 20:00 h and 01:00 h, the period when most loggerhead hatchlings emerge from their nests (Witherington et al., 1990). At the beginning of each trial, the LED in the east side of the tank was turned on. Harnessed hatchlings were then released in the south quadrant of the arena and observed for 3–5 min. Healthy hatchlings are known to swim vigorously towards a light source under such conditions (Salmon and Wyneken, 1987; Lohmann, 1991); thus, the response of each turtle to the light served to verify that the hatchling was developmentally and behaviorally competent to maintain an oriented course. Those few hatchlings that failed to orient towards the LED were replaced with other turtles. Once a turtle began swimming steadily towards the light, the arena cover was lowered over the tank and the data-acquisition

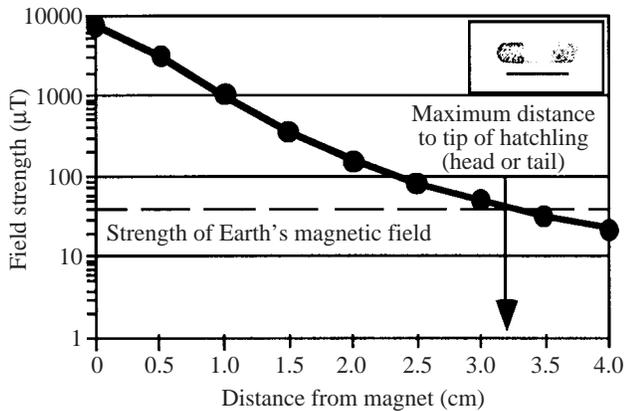


Fig. 3. Strength of magnetic field as a function of distance from the magnet. The strength of the Earth's field at the test site ( $46\mu\text{T}$ ) is represented by the broken line. The strength of the field produced by the magnet matched or exceeded the Earth's field over the entire body of each hatchling. Inset shows a photograph of the magnet (scale bar, 10 mm).

computer was started. The computer recorded the magnetic heading of the turtle every 10 s throughout the trial. The turtle was allowed to swim towards the light for 60 min. The light was then turned off. After a 3 min adjustment period, the orientation of the turtle was monitored as it swam in darkness during the next 60 min.

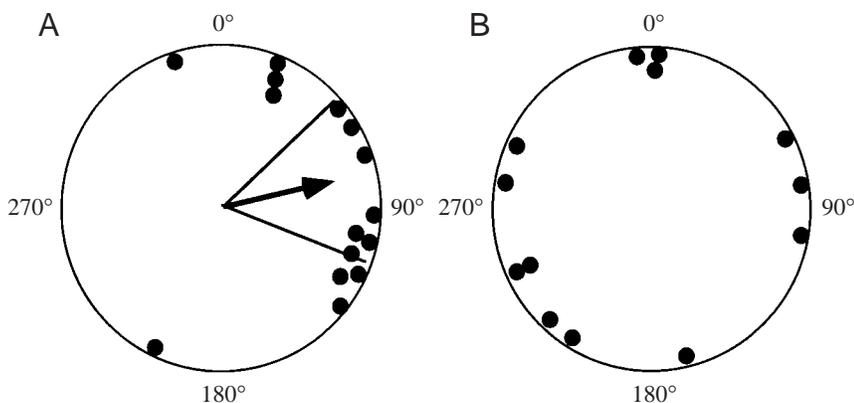
Using these procedures, two or three turtles were tested each evening. No two turtles from the same nest were tested in the same condition (i.e. with a magnet or brass bar).

#### Data analysis and statistics

The data-acquisition computer calculated the mean heading for each turtle based on all data collected during the final 60 min of the trial (i.e. the period beginning 3 min after the light was turned off). The orientation of each group of turtles was analyzed using a Rayleigh test and the distributions of the two groups were compared using Watson's  $U^2$  test (Batschelet, 1981).

### Results

Turtles with a brass bar attached were significantly oriented



as a group with a mean heading of  $77.5^\circ$  ( $r=0.64$ ,  $N=15$ ,  $P<0.001$ , Rayleigh test; Fig. 4A). The 95% confidence interval of the mean angle overlapped the direction of the initial light stimulus ( $90^\circ$ ). Turtles with the magnet attached were not significantly oriented as a group ( $r=0.0026$ ,  $N=13$ ,  $P>0.9$ , Rayleigh test; Fig. 4B). The two distributions were significantly different ( $U^2=0.196$ ,  $P<0.05$ , Watson's  $U^2$  test).

### Discussion

Two groups of hatchlings were tested under laboratory conditions in which turtles are known to orient magnetically (Lohmann, 1991; Light et al., 1993; Lohmann and Lohmann, 1993, 1994a). Turtles with brass bars attached to their carapace were significantly oriented as a group with a mean heading of  $77.5^\circ$  (Fig. 4A). This eastward orientation closely resembles that observed in several previous studies in which similar procedures were used (Lohmann, 1991; Light et al., 1993; Lohmann and Lohmann, 1994a) and probably represents a magnetic directional preference acquired on the basis of light cues provided at the start of each trial (Lohmann and Lohmann, 1994a). Under natural conditions, when such cues are provided by the ocean horizon, this response may function in guiding hatchlings eastward from the coast of Florida to the Gulf Stream current (Salmon and Wyneken, 1994; Lohmann et al., 1997).

Whereas turtles with brass bars attached to their carapaces oriented eastward, those with magnets attached were not significantly oriented as a group (Fig. 4B). The orientation of the two groups was statistically different. Because the size and mass of the brass bars and magnets were nearly identical, the results imply that the magnetic field produced by the bar magnets disrupted the ability of the turtles to maintain consistent orientation under the test conditions.

#### Magnetic compasses and magnetic positional information

Hatchling sea turtles have been shown to derive both directional information (Lohmann, 1991) and positional information (Lohmann and Lohmann, 1994b, 1996b; Lohmann et al., 2001) from the Earth's magnetic field. In principle, the magnets attached to the turtles might have interfered with either or both of these abilities. One possibility is that the field disrupted the turtles' magnetic compass sense, so that they could not hold a reliable course. Alternatively or additionally, however, the field from the magnet might have distorted the field line inclination and intensity around the turtles, so that an accurate assessment of positional information was not possible. The

Fig. 4. (A) Control turtles with a brass bar attached to the harness [mean heading (arrow) =  $77.5^\circ$ ,  $r=0.64$ ,  $N=15$ ,  $P<0.001$ , Rayleigh test]. Lines indicate 95% confidence interval. (B) Experimental turtles with a magnet attached to the harness ( $r=0.0026$ ,  $N=13$ ,  $P>0.9$ , Rayleigh test).

present data are insufficient to distinguish among these different possibilities.

#### *Use of magnets in field studies*

Hatchlings migrating offshore initially use directional information from the orbital motion of oceanic waves (Salmon and Lohmann, 1989; Lohmann and Lohmann, 1992; Lohmann et al., 1995) but later proceed on a course that diverges from wave direction (Witherington, 1995). Turtles have been hypothesized to maintain headings in deep water by relying on their magnetic compass sense (Lohmann et al., 1997; Goff et al., 1998; Lohmann and Lohmann, 1998). It has been difficult to test this hypothesis under natural conditions due to the logistical difficulties of using large electromagnetic coil systems in the open ocean.

In several studies, magnets have been attached to animals that have then been released into their natural habitats. The effect of the magnets on orientation behavior has then been assessed as the animal moves through its environment. Although results have been highly variable (Wiltschko and Wiltschko, 1995), the orientation of homing pigeons (*Columba livia*; Keeton, 1971), toads (*Bufo bufo*; Sinsch, 1987) and box turtles (*Terrapene carolina*; Mathis and Moore, 1988) has reportedly been altered by magnets under at least some conditions. With sea turtles, the few attempts that have been undertaken all relied on small sample sizes and have yielded results that are difficult to interpret. In a pioneering study on remote tracking, a few adult green turtles (*Chelonia mydas*) were outfitted with magnets and their paths were compared with control turtles carrying brass bars (Baldwin, 1972). The author concluded that the magnets affected orientation ability while acknowledging that the sample sizes were too small to support a statistical analysis. In a field study of leatherback (*Dermochelus coriacea*) hatchlings migrating out to sea, dispersion of the turtles reportedly increased with the strength of the magnets attached to them (Kloc et al., 1996); sample sizes were again small, however, and no statistical analysis was provided in support of the conclusion. Finally, adult green turtles were tracked by satellite telemetry during post-nesting migration from Ascension Island to the coast of South America. Magnets were attached to some of the turtles while brass bars were attached to others. The authors reported a statistically significant difference between the two groups in the latitude at which the turtles reached Brazil, yet concluded that the magnets did not have an effect because the paths of all turtles were generally similar (Papi et al., 2000). Given the ambiguous nature of these initial results, additional work is needed to determine whether magnets alter or disrupt the orientation of sea turtles under some or all field conditions.

Our results demonstrate that, at least under laboratory conditions, magnets can be used to disrupt the magnetic orientation behavior of hatchling turtles. This information may prove useful in designing field experiments to assess the importance of magnetic information in the migrations of hatchlings. An important caveat, however, is that many animals use multiple, redundant cues for orientation and

navigation. Thus, eliminating a single cue such as magnetism may cause no obvious change in behavior if alternative sources of directional information are present (Able, 1993).

#### *Localizing magnetoreceptors*

Although behavioral experiments have demonstrated that diverse animals can sense the Earth's magnetic field, relatively little is known about the physiological mechanisms that underlie this sensory ability (reviewed by Wiltschko and Wiltschko, 1995; Lohmann and Johnsen, 2000). Despite significant progress (Beason and Semm, 1987; Walker et al., 1997; Deutschlander et al., 1999a; Diebel et al., 2000), primary magnetoreceptors have not yet been identified conclusively in any animal.

Several factors have made locating magnetoreceptors difficult. One is that magnetic fields pass freely through biological tissue. Thus, magnetoreceptors need not contact the external environment and might plausibly be located anywhere within the body of an animal (Lohmann and Johnsen, 2000). In addition, magnetoreceptors might be tiny and dispersed throughout a large volume of tissue (Kirschvink et al., 2001), or the transduction process might occur as a set of chemical reactions, so that no obvious organ or structure devoted to magnetoreception necessarily exists (Deutschlander et al., 1999b; Ritz et al., 2000).

Given these circumstances, a helpful first step towards identifying magnetoreceptors is to determine the approximate anatomical location where the search should be focused. One promising method is to attach small magnets to specific parts of an animal's body, thus producing localized field distortions over limited anatomical areas (Walker and Bitterman, 1989; Haugh et al., 2001). By varying the location of the magnet and monitoring behavior that is dependent on magnetic field detection, researchers can infer the approximate anatomical location where magnetoreceptors are likely to exist.

In our study, magnets capable of distorting the field over a hatchling's entire body were found to disrupt magnetic orientation behavior. This finding sets the stage for attempts to localize magnetoreceptors by using smaller, weaker magnets that alter the field only around specific anatomical target sites.

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