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Circadian clock and sun compass orientation in hatchlings of the turtle *Eretmochelys imbricata* at Sisal, Yucatán, México

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The sun compass orientation implies a circadian clock and the sun's azimuth in order to establish a direction. This behavior has been observed in a diversity of invertebrate and vertebrate species including reptiles. Shifting an organism's internal clock by a given period of time (via alterations in photoperiod) helps to demonstrate the presence of a sun compass and circadian clock mechanisms. This study aims to test whether a sun compass orientation mechanism exists in hatchlings of the hawksbill turtle *Eretmochelys imbricata* at the shore of Sisal, Yucatán, México. In order to mask the magnetic sensitivity, turtles were observed with and without a neodymium magnet attached to their carapaces. One group of animals was held in regular LD, while another group was maintained for three days in a 6-h-delayed LD. Observations were made by photographs in an open circular arena near the shore. Results obtained from these observations indicate that hatchlings exposed to a 6-h-delayed LD show a difference in angular direction of near 60° and that magnets do not change such difference. Swimming activity recorded during the first four days after hatching indicates an organized crepuscular rhythm. The implications of multiple orientation mechanisms in these species and conservation efforts are discussed.

Keywords: hatchlings; hawksbill turtle; sun compass; orientation; circadian; magnetic field

Introduction

Orientation is the behavior in an organism defining a direction. The elements used by the orientating organisms may depend on diverse environmental cues according to the richness of the landscape (Alerstam 2006). An inherent clock-and-compass program may be part of the diverse variety of cues that a migratory species requires to maintain orientation during migration and homing. Sea turtles provide a particularly interesting model, since they hatch in places that eventually they will find again to lay eggs when adults (Bjorndal & Zug 1995; Sale & Luschi 2009). Animals migrating through the sea have a cues environment different from that of the terrestrial habitats, because visual landmarks are poor or absent and celestial cues, such as the position of the sun and stars, cannot be clearly perceived underwater (Lohman et al. 2008). However, marine air breathers may be frequently exposed to this cue environment when swimming at the surface (Narazaki et al. 2009). Hatchling sea turtles migrating from their nest on the beach to deep water may just need a way to maintain a consistent offshore heading, while mapping a

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migration route may require more diverse information from the environment (Lohmann & Lohmann 1996; Lohmann et al. 2001, 2004). The Earth's magnetic field is an environmental feature present throughout the day/night cycle and unaffected by weather and season; hatchling loggerhead turtles detect both magnetic inclination angle and field intensity (Lohmann & Lohmann 1994, 1996) suggesting that young turtles have a "magnetic map" where magnetic field functions as navigational marker (Lohmann et al. 2008).

Sun compass orientation has been proposed as a mechanism that may also contribute to migrating processes, and it has been studied in orientation and navigation in terrestrial turtles (DeRosa & Taylor 1978; Southwood & Avens 2011), but few studies have investigated these mechanisms in marine species (Alerstam 2006). Of particular interest in marine animals lacking landmark cues, skylight polarization patterns (Mäthger et al. 2012), and sun compass (Avens & Lohmann 2003; Mott & Salmon 2011), odors (Grassman et al. 1984) or phototropotactic response (Mrosovsky et al. 1979) have been studied among others. From the investigation, the following question arises: what is the relative importance of the individuals' multiple compass mechanisms and how are the different compasses calibrated in relation to each other? (Alerstam 2006). Magnetic orientation behavior of hatchling sea turtles is disrupted by attaching a small magnet to the carapace (Irwin & Lohman 2003), which offers an excellent possibility to mask the effect of natural magnetic fields, allowing to test other different mechanisms for orientation, such as the clock-based sun compass orientation.

The hypothesis of sun compass orientation has been useful to test whether a circadian clock regarding the sun azimuth is useful for establishing a direction in migrating birds (Hoffman 1960). Since then, the use of a circadian time-compensated sun compass for orientation has been observed in a diversity of both invertebrate and vertebrate species including reptiles (Ugolini 2003; Reppert et al. 2010; Southwood & Avens 2011). The location of the sun's azimuth can be used as information for animal's orientation, but a circadian clock is needed to compensate the direction for the changes in the sun's position throughout the day. Due to the temporal component of this directional sense, shifting an organism's internal clock by a given period of time (via alterations in photoperiod) helps to demonstrate the presence of suncompass and circadian clock mechanism (Schmidt-Koenig 1990; Southwood & Avens 2011).

The aim of this study is to test whether hatchlings of the Hawksbill turtle exposed to natural day light, with or without a magnet in their carapace, present different orientation regarding controls, and if a 6-h delay, with an artificial LD photoperiod, may be evidence of sun compass orientation, based on a circadian clock.

Methods

Animals

Hatchlings of hawksbill turtle (*Eretmochelys imbricata*) were obtained the day of hatch at The Conservation Turtle Camp, Sisal, Yucatán, México, in accordance with the federal authorization SEMARNAT SGPA/DGVS/02507/10. After completion of all the observations, turtles were released in to the sea. No physical harm was caused to any organisms tested.

After hatch ($n=115$), turtles were separated into two groups: one group was maintained in artificial 12:12 LD conditions in regular schedule ($n=85$), according to the geographical time (06:00–18:00), and a second group ($n=30$) in delayed LD (12:00–24:00) in controlled conditions at the facilities of Facultad de Ciencias (Unidad Multidisciplinaria de Investigación y Docencia, Sisal, Yucatán). Turtles were held in

water-circulating tanks (40 cm × 60 cm × 30 cm) with about 12 animals each. Water quality was constantly filtered and checked for dissolved oxygen (25.86 mg/L) temperature (27.9 °C) and salinity (35 ppt). Animals fed raw fish stripes *ad libitum* until released.

Experimental protocol

To see whether a sun's position at morning or dawn might influence the observations, two schedules for observation were chosen under natural light: morning (7:00–10:00 h) and evening (16:00–19:00 h).

Observations were performed on alternating hatchlings, with or without a neodymium magnet (Mega Brands) attached to their carapace with a small piece of wax. Orientation was observed in animals held in regular LD during the first three days after hatching and by the fourth day, animals held in delayed LD were tested. Magnet's deformation of the magnetic field was tested at the Institute of Geophysics, UNAM, with the help of Dr Jaime Urrutia (Fuentes-Farias et al. 2008). An area of 40 cm × 60 cm in the laboratory's floor was tested for magnetic complete deformation before and after placing a neodymium magnet (Figure 1(A)). The symbol inside graphs indicates the place of magnet position before (left panel) and after being placed (right panel). Observations were tested in a circular plastic pool (2 m diameter and 45 cm high), with

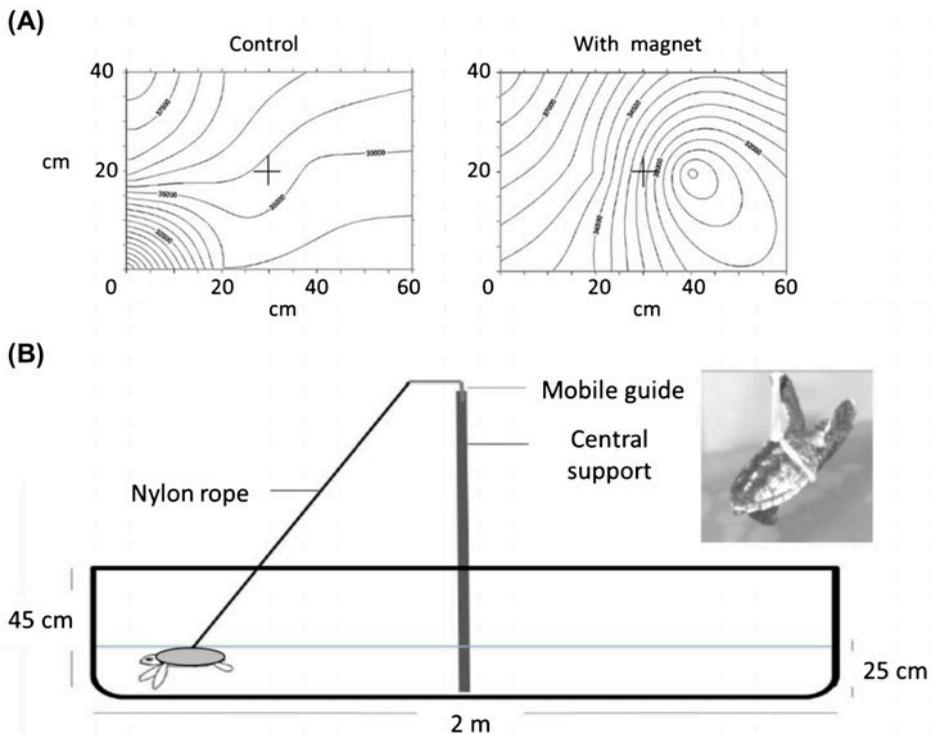


Figure 1. In A, is shown the way in which the magnetic profile is deformed after locating a neodymium magnet used in this study, at laboratory environment. The deformation seen in the left is due to the activity of electric equipment. The cross in the middle indicates the site where the magnet was placed. Units inside the graphs are expressed in nanoTeslas (nT). In B, a diagram of the observation arena is shown. Hatchlings were held with a rubber band (see photo) and tied to a nylon rope. Observations were taken with an automated camera above the pool.

the floor covered with a homogenous layer of sand (Figure 1(B)). The pool located at least 10 m of the shore, did not have any image inside, and its internal walls were white. It was filled with seawater to a depth of 25 cm and uncovered, allowing the turtles to view the sky, but blocking natural horizon and surroundings.

During observations, each turtle was partially held with a rubber band encircling the carapace and tethered to a rotatable aluminum arm mounted at the center of the pool (Figure 1(B)). As the turtle swam, the tether pulled the arm indicating swimming direction continuously. After 10 min of swimming, a series of six photographs, every 20 s, were taken with an automated digital camera in a fixed holder located at the edge south of the pool.

Locomotor activity recordings

Locomotor activity was recorded from individual turtles, by means of infrared light beams coupled into an aquarium (18 cm × 40 cm × 20 cm); swimming activity was recorded only with an infrared crossing at surface, and therefore, any other locomotor activity other than swimming on the surface was not recorded. Data were obtained by acquisition data equipment (Fac. Psicología, URIDES, UNAM, México) in a PC by means of the software ACTIBIO and then plotted with the ACTIVIEW software. Data were summarized every ten minutes (10 min bin cell) and stored in a PC until further analysis. Eight aquaria were placed in a humid room near a window, in such way that natural illumination was possible, but avoiding direct sunlight. Ventilation and temperature inside the room were kept constant using a circulation air system, and circulating sea water was maintained at 25 ± 3 °C. Activity was evaluated during four consecutive days after hatch, and then, hatchlings were released.

Data analysis

Each series of six photographs taken from the same organism was used to calculate an average vector by means of the Rayleigh's test (Batschelet 1981). If the direction was significant ($p < 0.05$), the resultant orientation was then used as one case, representing the resultant direction that the turtle swam. The significant main vectors were compared with the Mardi-Watson test (Batschelet 1981) by means of the software Oriana 2.0 (Kovach Computing Services, UK).

Results

Thirty out of 44 hatchlings, maintained in regular LD and with no magnet, did show significant orientation (68%), while only 25 out of 44 turtles tested with magnet (57%) showed significant orientation ($p < 0.05$, Rayleigh test), but 93% of turtles after three days in delayed LD photoperiod, either with or without magnet, did show significant orientation.

Table 1 presents the average orientation angle (Considering the South as 0 degrees) among different groups tested. Comparisons between morning and evening data of the same group did not show statistical differences. Neither significant difference in direction was observed when comparing turtles with or without magnets. The differences indicated with a star ($p < 0.05$, Marty-Watson test) were observed between groups of animals held in regular with its correspondent group at delayed LD.

Figure 2 shows the summarized orientation for observations performed with no difference in time of day. Each point corresponds to an individual statistically significant direction. In A and B, groups of turtles in regular LD without (Ctl) and with a magnet

Table 1. Average (\pm SE) orientation observed in all groups tested. All groups showed a significant vector (Rayleigh's test). Main differences among groups (*) occur between LD regular and LD advanced cases (Marty-Watson test, $p < 0.05$).

	Regular LD		Delayed LD	
	Morning	Evening	Morning	Evening
Control	*151.8 \pm 6.41°	*142.63 \pm 8.48°	207.1 \pm 4.05°	212.29 \pm 8.9°
Magnet	*157.7 \pm 5.89°	*163.35 \pm 11.7°	214.4 \pm 4.6°	209.46 \pm 4.6°

in their carapace (Mgt), respectively. No significant differences were found between them. In Figure 2(C) and (D), circular plots of turtles orientation after three days in LD were delayed. A change on the main vector of near 60° may be observed in turtles without (C) and with a magnet (D). Significant differences between groups are indicated with a double-headed arrow ($p < 0.05$). The inner circle in each graph indicates statistical threshold ($p < 0.05$) for the resultant vector plotted.

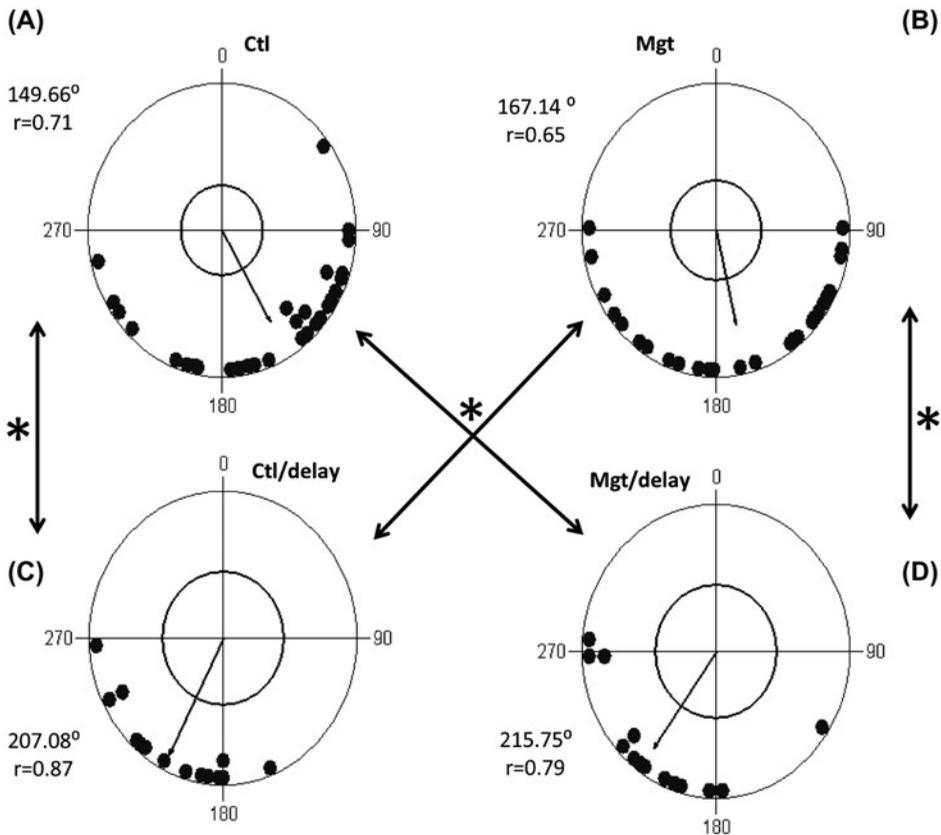


Figure 2. Orientation of hatchlings in regular LD, without (Ctl, A) and with a neodymium magnet (Mgt, B). A change in the main vector was observed in animals exposed three days to delayed LD, without (Ctl/delay, C) and with a magnet (Mgt/delay, D). Double-headed arrows indicate significant differences between groups ($p < 0.05$). Angle 0, is located in landside, south, while 180 is located at open sea, north.

Swimming activity recordings

Figure 3 shows four single-plotted actograms of swimming activity from independent turtles. Below each graph, the correspondent average waveform is plotted. In all the eight organisms tested since the first day of hatch, a diurnal-bimodal pattern of swimming activity was clearly observed.

Discussion

In this work, we show that turtles exposed to shadowed daylight do show a bimodal daily rhythm in activity, entrained to dusk and dawn (Figure 3). Tested hatchlings were more swimming-active early in the morning and in late evening since the first day after hatching. Our observations at two different times of day showed not difference between groups (Table 1). In fact, when observations were performed near the sunset or at midday, a particular rest position of flippers was frequently observed in many animals.

This information suggests that a circadian clock, even though none tested in free running, may be functional after hatching. If a sun compass clock compensated mechanism exists in hatchlings, it should be functional after hatch. We observed that most of the organisms tested displayed a significant direction (about 80% of the total used). However, the proportion of animals with more dispersion was found in those with magnets in their carapace indicating the possible contribution of magnetic field orientation. The magnets used were extracted from a toy brand (Magnetix, 5 mm diameter, 4 mm wide) and they may modify clearly the magnetic profile as shown in Figure 1; therefore, a kind of magnetic masking may occur; however, no differences found in average orientation

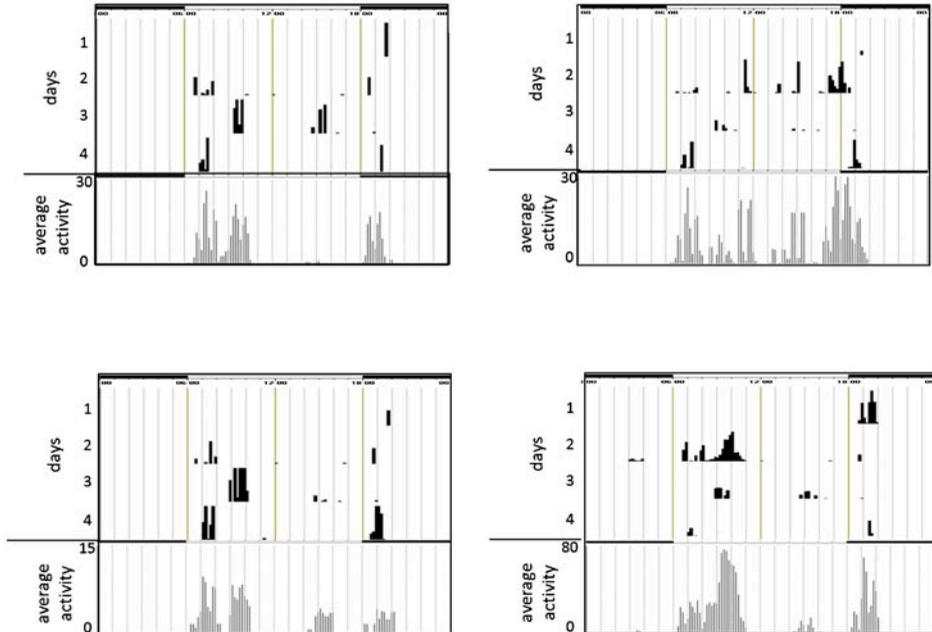


Figure 3. (Colour online) Swimming activity of four different turtles held under shadowed natural illumination. Below each actogram, an average waveform of the four consecutive days is shown. Main activity is present early in the morning and around late evening. LD bars are an arbitrary representation of daylight.

between turtles with or without magnets were found indicating that a stronger mechanism allows them to keep direction. By the fourth day on advanced LD, a clear change in the average direction occurs (Figure 2), with a difference of about 60°, and therefore, it is possible that an angular correction of 15° may occur daily (Schmidt-Koenig 1990).

Orientation in a migrating species may require multiple cues and a combination of diverse mechanisms that may assure keeping direction. Magnetic fields are one of the most stable signals but require highly sensitive physiological systems (Lohmann & Johnsen 2000). Therefore, a combination with other cues such as sun compass orientation may reinforce migrating behavior.

Sun compass orientation has proved to be present in a diversity of animals, (Hoffman 1960; Southwood & Avens 2011) including marine turtles (Mott & Salmon 2011). It would not be surprising that sea turtles, when swimming on the surface, calibrate other compasses according to sun's position, or other cues reliable at the surface (Narazaki et al. 2009). Once at sea, currents may play a fundamental passively transportation over long distances. Combined reliance on mechanisms of map-based navigation and local orientation cues close to the target may allow turtles to accomplish the difficult task of returning to specific sites after years spent wandering in a moving medium (Sale & Luschi 2009).

At the present work, we show that the main orientation in hatchlings of the hawksbill turtle *E. imbricata* can be shifted after being exposed to artificial delayed LD photoperiod for at least three full days. This response was not changed in turtles with a possible magnetic disorientation, which means that, at least during the day, sun compass may be strong enough for orientation. The calibration of each system as well as the dominance may change according to the progressive learning of the habitat as well as the age. Sun compass orientation seems to be an alternative way to guide the initial migration offshore.

Nevertheless, we present data of animals of only one nest; more studies will be required with different nests with different positions on the shore. Sisal beach in Yucatan is a place where hawksbill turtles lay their eggs; after hatching, turtles migrate in diverse routes in the Caribbean Sea (Abreu-Grobois et al. 2005).

Conservational effort to protect endangered species needs a combination of studies that may help to better understand animal's biology and their habitat. The hawksbill turtle is a protected species in the Caribbean Sea, and multiple efforts have contributed to increase their population (Garduño-Andrade et al. 1999).

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