

POPULATION LEVEL AND BEHAVIORAL INVESTIGATIONS OF GEOMAGNETIC  
IMPRINTING AND NATAL HOMING IN SEA TURTLES

John Roger Brothers

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Approved by:

Kenneth J. Lohmann

John F. Bruno

Sönke Johnsen

Keith W. Sockman

R. Haven Wiley

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## **ABSTRACT**

John Roger Brothers: Population Level and Behavioral Investigations of Geomagnetic Imprinting and Natal Homing in Sea Turtles  
(Under the direction of Kenneth J. Lohmann)

Diverse animals migrate long distances before returning as adults to reproduce in the same location where they began life. This phenomenon, called natal homing, is exemplified by sea turtles. Turtles travel immense distances through seemingly featureless open ocean and sometimes cross entire ocean basins before laying eggs on the same stretch of coastline where they themselves hatched. Although natal homing is widespread among sea turtles, how it is accomplished has remained a long-standing mystery of animal behavior.

One idea, called the geomagnetic imprinting hypothesis notes that Earth's magnetic field varies across the globe; as a result different geographic areas are characterized by different magnetic fields. Therefore, animals that derive navigational information from Earth's field might learn the magnetic signature that marks their natal area when they are young and use this information to return as adults. This hypothesis carries with it two central tenets: Firstly, it proposes that adult animals use magnetic navigation to guide reproductive migrations to the natal area. Secondly, it suggests that young animals learn the local magnetic field of the natal location prior to leaving.

The research presented hereafter provides evidence that sea turtles use Earth's magnetic field to accomplish natal homing. Results from a behavioral experiment indicate that adults use magnetic navigation to guide their nesting migrations and return to the natal

beach. Additional findings examine the ecological implications of geomagnetic imprinting; as predicted by the hypothesis, population level analyses revealed that natural changes in Earth's field result in detectable shifts in where sea turtles choose to nest. Moreover, spatial variation in Earth's field is strongly related to genetic differentiation between nesting beaches suggesting that magnetic navigation can play a role in shaping population genetic structure. Finally, evidence indicates that sea turtle embryos orient non-randomly inside the egg and might use Earth's magnetic field to do so.

Taken together these findings represent four independent lines of evidence that are consistent with geomagnetic imprinting and suggest that turtles use Earth's magnetic field to accomplish natal homing. The results provide insight into an enigmatic phenomenon in animal behavior and are likely applicable to diverse migratory animals.

To my grandfather Don Dee Brothers who introduced me to sea turtles. He was a scientist himself, and always knew that I would be as well. He died in 2017 when I was in the field.  
Grandpa — go like a turtle, and find your way home.

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## **CHAPTER 1: INTRODUCTION**

Natal homing refers to a pattern of behavior during which animals migrate away from their geographic area of origin when they are young before returning as adults to reproduce in the same location where they began life [1–4]. Although first observed more than a century ago in birds [5], there is now widespread evidence for natal homing in diverse animals, including certain fishes [3, 6, 7], birds [8, 9], reptiles [1, 4, 10], amphibians [11, 12], and mammals [13].

In some instances natal homing has clear benefits, especially in cases where birth (or oviposition and hatching) take place at the same location as mating. For example, the migratory range of elephant seals spans thousands of kilometers in the Pacific Ocean, but reproduction is terrestrial and limited to specific stretches of beach within a considerably smaller range [14]. Each year males and females return to their natal beach where females give birth to young from the previous season before mating again. During the non-breeding season, however, males and females favor different foraging grounds; males feed on benthic organisms along the continental shelf and females feed on pelagic prey in the open ocean [15]. Although sexual segregation is common during foraging, both sexes can ensure reliable access to potential mates by returning to their natal beach. As a result, for elephant seals natal homing facilitates mate finding and large mating aggregations form on natal beaches during the breeding season.

Salmon provide another extreme example of natal homing [16]. Young fish hatch out of eggs in freshwater streams and migrate downriver to the sea before foraging in the open

ocean as juveniles and adults. Only at the very end of their lives do adult salmon return to spawn in their natal stream before dying. This impressive migration consists of two stages; the first involves open-sea homing to the same river mouth where the fish first entered the ocean, and the second involves swimming upriver, against flow, until the fish reaches its natal stream and spawns. Similar to the case of elephant seals, natal homing in salmon facilitates access to mates but for salmon it also ensures an appropriate environment for successful spawning and incubation. For example, developing embryos require a freshwater stream with an appropriate gravel substrate and flow, both of which are difficult to assess from downstream [2]. In addition, both egg size and male body size tend to be locally adapted to a population's natal stream [6, 17]; females tend to produce eggs that are sized appropriately for the grain size of the gravel substrate in their natal stream and larger males are less likely to spawn successfully in shallow streams due to higher rates of predation [6, 17]. Therefore, returning to the natal stream increases the likelihood that an adult will find a location that is favorable for both successful spawning and incubation [2].

In sea turtles, however, the benefits of natal homing might be less evident in that mating is thought to often occur away from the nesting beach and there is little evidence for local adaptation to the natal beach. Yet, sea turtles provide an iconic example of natal homing and females return as adults to nest on the same stretch of beach where they themselves hatched [4, 18, 19]. In fact, during nesting migrations turtles regularly bypass countless suitable nesting beaches, on which other individuals of the same species nest successfully [1].

Although this may seem counterintuitive, it is important to consider that successful nesting and embryo development each require a certain set of environmental conditions that

are difficult to assess from afar and are not geographically widespread [1, 20]. Specifically, nesting females require a sandy beach that is favorable for nest construction and has a relatively shallow slope so that they can emerge from the sea. Moreover, the beach must be free of egg predators, wide enough that embryos are not drowned by high tides, and have sand characteristics (e.g. temperature, grain size, etc.) that are conducive to successful incubation [1, 2]. Finally, even if conditions on the beach are appropriate for nesting and incubation, certain coastal areas are more favorable than others for hatchling survival because of their proximity to major ocean currents, which can facilitate transport of young turtles to developmental habitats [21, 22].

All of the characteristics described above are likely to be difficult to assess from the sea and impossible from the open ocean. Therefore, because suitable reproduction requires a coastal area with an uncommon suite of environmental characteristics that is difficult to evaluate, it seems reasonable that natural selection would favor turtles that return to nest on their natal beach, a location that is almost certain to be appropriate [1]. In other words, the simple fact that a nesting female is alive provides evidence that her natal beach has conditions that are favorable for reproduction.

Although all species of sea turtle accomplish natal homing, this dissertation will focus on two extreme cases: 1) Loggerhead sea turtles (*Caretta caretta*), which traverse entire ocean basins before returning to nest [19, 23, 24]. 2) Olive ridley sea turtles (*Lepidochelys olivacea*), which sometimes nest in groups of hundreds of thousands on remarkably small lengths of coastline [25].

The southeastern USA supports one of the largest loggerhead sea turtle nesting aggregations in world. The young turtles that leave these nesting beaches as hatchlings

undertake an immense trans-oceanic migration and can circumnavigate the entire north Atlantic ocean basin before taking up residency in foraging areas in the western Atlantic [10, 26]. As adults, females return to nest on the same stretch of coastline where they themselves hatched more than a decade earlier [27, 28].

Although most sea turtles nest on their own, as do the loggerheads described above, olive ridley turtles (*Lepidochelys olivacea*) sometimes nest *en masse*. During mass nesting events, tens and sometimes hundreds of thousands of turtles emerge to nest in synchrony [25, 29, 30]. The nesting females travel thousands of kilometers through the open ocean and bypass countless suitable nesting beaches in search of specific stretches of coastline that in some places span no more than 5 kilometers. The density of turtles on the beach is so great that nesting females regularly dig up each others eggs and crawl over one another when heading back to sea.

Although it is clear that natal homing exists in diverse animals, including all species of sea turtle, we still know relatively little about how it is accomplished. A large body of evidence indicates that salmon complete the final freshwater stages of their spawning migration through olfactory imprinting and returning to tributaries that have the same chemical composition as their natal stream [31]. How long-distance marine migrants accomplish natal homing in the open sea, however, remains less clear.

Over the past decade, evidence has accumulated that is consistent with the hypothesis that sea turtles and salmon, use Earth's magnetic field to return to their natal areas [1, 2, 32–35]; some of this evidence is presented within the following chapters of this dissertation. Specifically, a recent idea known as the geomagnetic imprinting hypothesis for natal homing,

notes that disparate geographic areas are often characterized by different magnetic fields [2]. Therefore, animals that detect magnetic fields, such as sea turtles [36, 37], might learn the magnetic signature of their natal area before leaving and use this information to return years later as adults.

The main dipole magnetic field of the Earth approximates that of a large bar magnet, with field lines leaving the southern hemisphere of the planet, wrapping around the globe and re-entering the planet in the northern hemisphere (Figure 1.1A). At any given location Earth's magnetic field can be described by two main parameters, the geomagnetic inclination angle (angle at which magnetic field lines intersect the surface of the planet) and the total geomagnetic intensity or strength (Figure 1.1B). Both of these parameters vary across the globe; there are relatively shallow inclination angles and weak intensities near the equator and relatively steep inclination angles and strong intensities near the poles. Because Earth's magnetic field varies predictably with latitude, magnetic isolines (i.e. lines along which either inclination or intensity are constant) typically trend from east to west. In addition, most major sea turtle rookeries worldwide typically occur on continental coastlines that trend from north to south [1]. As a result, most coastal areas along continental coastlines, and therefore most sea turtle nesting beaches, are marked by different inclination angles and different intensities [1, 2, 32].

Additionally, sea turtles derive positional information from Earth's magnetic field by detecting subtle variations in both inclination and intensity [38–40]. Therefore, for a turtle that imprinted on the magnetic signature of her natal beach as a hatchling, returning might in principle be relatively simple. The turtle could locate the appropriate magnetic isoline in the open ocean and then swim along it until she intersects the coastline at the natal beach.

Alternatively, the turtle could locate the coastline and then swim north or south along it until she encounters the correct magnetic signature [1, 2, 32]. Evidence from a behavioral experiment indicates that juvenile sea turtles use Earth's magnetic field in a similar way to determine their position relative to a goal along a coastline [39]. Turtles were captured in coastal feeding grounds and subjected to magnetic fields that exist at far away locations. Turtles that were exposed to a magnetic field that exists north of the foraging area responded by swimming overwhelmingly to the south; by contrast, turtles exposed to a field that exists in the south responded by swimming to the north.

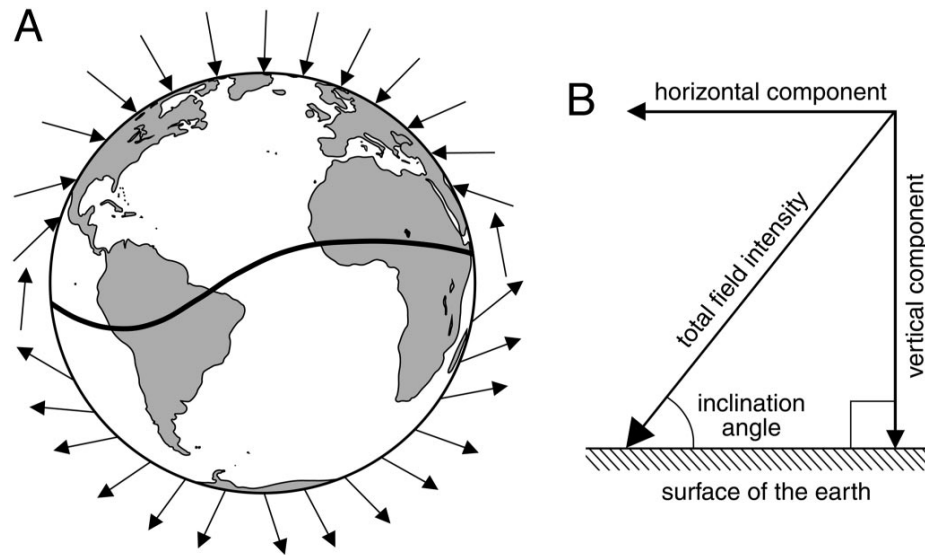
In recent years, findings consistent with geomagnetic imprinting have accumulated [32–35, 41]. Several studies have investigated natural changes in Earth's magnetic field, called secular variation, and shown that typical rates of field change are compatible with geomagnetic imprinting and natal homing [2, 32, 35]. In addition, within the context of secular variation both sea turtles and salmon appear to alter their reproductive migrations in ways that the geomagnetic imprinting hypothesis predicts [32, 33]. Subtle but natural changes in Earth's field seem to influence both where sea turtles return to nest [32], and the route that salmon choose to take during the final stage of their open sea migration to the spawning river [33, 41].

In my dissertation I have expanded on these results and the findings presented hereafter provide strong evidence that sea turtles use Earth's magnetic field to accomplish natal homing. I have used an interdisciplinary approach to investigate both whether sea turtles use Earth's magnetic field to guide their nesting migrations, and the ecological implications of geomagnetic imprinting. Chapter Two presents some of the first experimental evidence that adult sea turtles use earth's magnetic field to locate the nesting beach. At the

time of its publication, Chapter Three represented the first evidence for geomagnetic imprinting in sea turtles and the results indicate that geomagnetic imprinting combined with subtle changes in earth's magnetic field can elicit detectable population level shifts in sea turtle nesting distributions. The findings presented in Chapter Four suggest that geomagnetic imprinting can have a profound effect on the genetic structure of a population; spatial variation in Earth's magnetic field is strongly related to genetic differentiation between sea turtle nesting beaches even after accounting for geographic distance and environmental similarities. Finally, Chapter Five begins to examine whether sea turtle embryos detect Earth's magnetic field, as is required for turtles to learn the magnetic signature of their natal beach prior to hatching. The results provide evidence from two species of sea turtle that embryos orient non-randomly during the later stages of development and that they might use Earth's magnetic field to do so.

Taken together the findings presented in my dissertation represent four independent lines of evidence for geomagnetic imprinting in sea turtles and suggest that turtles use Earth's magnetic field to accomplish natal homing. The results provide insight into an enigmatic phenomenon in animal behavior and are likely applicable to diverse migratory animals.

## Figures



**Figure 1.1: Earth's magnetic field [2]**

A diagram of the Earth (A) showing how magnetic field lines (represented by arrows) leave the southern hemisphere of the planet, wrap around the globe and enter into the northern hemisphere of the planet. The inclination angle (the angle at which magnetic field lines intersect the surface of the planet) varies predictably with latitude. At the magnetic equator, represented by the curved line across the Earth, field lines are parallel to the planet and the inclination angle is  $0^\circ$ . As latitude increases the magnetic field lines become progressively steeper until they are perpendicular to the planet at the poles where inclination angle is  $90^\circ$ . Although it is not shown, geomagnetic intensity varies in a similar way, with weaker magnetic fields near the equator and stronger magnetic fields near the poles. (B) At any given location the geomagnetic field can be described as a vector and has both an inclination angle and total field intensity (magnitude of the vector). Additionally, the total field intensity can be resolved into two vector components, the horizontal intensity and the vertical intensity.

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## **CHAPTER 2: EVIDENCE FOR MAGNETIC NAVIGATION IN THE NATAL HOMING OF MASS NESTING SEA TURTLES**

### **Summary**

Natal homing refers to a pattern of behavior in which animals migrate away from their area of origin when young, before returning to reproduce in the same location where they began life [1–3]. An extreme example exists in sea turtles, which travel immense distances through open ocean to nest on the same stretch of coastline where they themselves hatched [4–7]. Although natal homing is widespread among diverse taxa [2, 3, 8–10], little is known about how it is accomplished [7]. One idea is that animals return as adults by seeking out the unique magnetic signature that marks the natal area [1]. Although recent evidence consistent with this hypothesis has been acquired [11–13], experimental corroboration has remained elusive [7]. Here we report direct behavioral evidence that adult sea turtles use earth's magnetic field to guide their nesting migrations and return to the natal beach. When exposed to the uniform magnetic field that exists 500km northwest of their nesting beach olive ridley sea turtles (*Lepidochelys olivacea*) responded by swimming southeast, a direction that would take them back to the nesting beach had they actually been displaced geographically. By contrast, when strong magnets were placed around the tank, eliminating any reliable geomagnetic information, turtles swam in random directions. These findings suggest that sea turtles can use magnetic information alone to determine their position relative to the nesting beach. In addition, the results provide experimental evidence that

magnetic navigation underlies natal homing and are likely applicable to diverse long-distance migrants [2, 3, 8–10].

## **Introduction**

At a few specific locations around the world olive ridley sea turtles (*Lepidochelys olivacea*) participate in mass nesting events, during which hundreds of thousands of turtles nest in synchrony on remarkably small lengths of coastline [14–16]. During these events, which can last up to 10 days, the density of nesting females is so great that turtles regularly dig up each other's eggs and crawl over one another while heading back to sea. Before converging on the nesting beach, which in some cases spans no more than five kilometers of coastline, turtles travel through thousands of kilometers of open ocean and bypass countless other suitable nesting beaches. Although this impressive homing represents one of the greatest displays of animal navigation, how turtles arrive at such precise locations has remained enigmatic.

Recent evidence suggests that sea turtles and other animals accomplish natal homing through magnetic navigation [11–13, 17, 18]. Earth's magnetic field varies predictably across the globe [1], and as a result, each area of coastline is typically marked by a unique magnetic signature (Figure 2.1) [1, 7, 11]. In addition, sea turtles and other animals detect two of the geomagnetic parameters that vary, the total field intensity and the inclination angle (the angle at which magnetic field lines intersect Earth's surface) [19, 20]. Therefore, for a turtle, identifying a specific stretch of coastline and returning to the natal beach might be relatively simple: a turtle might need only locate the coast and swim along it until she encounters the correct magnetic signature [7, 11]. Although initial reports suggest that salmon and sea

turtles accomplish natal homing through magnetic navigation [11–13], evidence from behavioral experiments is lacking.

To directly test if adult turtles use Earth's magnetic field to return to the natal beach we conducted a behavioral experiment with nesting females. We captured turtles as they crawled out of the water but before they began to nest, placed them in a water-filled arena and observed their swimming direction in response to a simulated magnetic displacement. Some of the turtles swam in the magnetic field that exists 500km northwest of the nesting beach (Figure 2.1), a location within their normal migratory range [21, 22], and other turtles swam in an irregular artificial magnetic field produced by a series of magnets placed outside the tank.

## **Methods**

### *Animals*

We conducted our experiment in Costa Rica at the Ostional National Wildlife Refuge (latitude 9.99° N, longitude 85.70° W). This area has regular mass nesting events during which hundreds of thousands of olive ridley sea turtles (*Lepidochelys olivacea*) emerge from the Pacific Ocean to nest in synchrony [14]. Each night during three mass-nesting events we captured female turtles (n=65) after they crawled out of the water but before they began to nest. Although the age of the animals cannot be determined all were mature, adult females and ranged in curved carapace length from 57.4 cm to 72.5 cm. Each turtle was carried by hand from the nesting beach to the test site (less than 300m away) and some were temporarily held in a non-magnetic wooden pen prior to testing (never more than one hour). Immediately before an orientation trial the turtle was fitted with a custom harness; each front flipper was placed through a loop of lycra fabric and a nylon rope was tied around the

carapace immediately anterior to the rear flippers. The harness was fastened with non-magnetic carabiners and care was taken so that flipper movement was not obstructed.

### *Orientation Trials*

Each turtle was tethered to a non-magnetic swivel at the center of a circular water-filled arena (1.5m diameter) so that she could swim comfortably in all directions but not make forward progress towards the side of the tank. An observer who was unaware of the treatment group used an infrared camera to remotely monitor the turtle for a five-minute acclimation period. If the turtle was swimming consistently and naturally it was allowed to swim for an additional ten-minute trial period. At the end of the trial each turtle was marked with zinc oxide and released back onto the nesting beach in the general vicinity of where it was captured. We did not collect any data from those few turtles that did not swim naturally during the acclimation period.

### *Magnetic Manipulation*

The arena was positioned at the center of a magnetic coil system that measured 3.42 meters on each side and was used to precisely control the magnetic field inside the arena [23]. The coil was calibrated to produce the exact magnetic field that exists 500 km northwest of the nesting beach at a location near El Salvador (latitude 13.17° N, longitude 88.96° W), a location within the normal migratory range of turtles that nest at Ostional [21, 22]. The magnetic field used to simulate the conditions at this location had an inclination angle of 40.3° and an intensity of 36.9 $\mu$ T calculated as the mean of four independent measurements with a MEDA FVM-400 three-axis fluxgate magnetometer. The deviation from perfect uniformity of the magnetic field inside the arena was less than 0.5% (both calculated and measured). Because of the nature of the geomagnetic field in the region we

were able to produce the magnetic field necessary for our experiment by modifying only the vertical component of the local magnetic field.

Although some turtles swam in the uniform magnetic field that exists at a faraway location (as described above), other turtles swam in a highly irregular magnetic field produced by eight strong neodymium magnets that were evenly spaced around the outside of the tank. To ensure that the uniformity of the magnetic field inside the arena was sufficiently disrupted the north pole of every other magnet was directed towards the center of the arena. This resulted both in strong gradients for the inclination and intensity inside the tank, as well as drastic variations in the direction of magnetic north throughout the arena.

Rather than replicating a magnetic field that exists southeast of the nesting beach for a second treatment we used magnets to disrupt the field uniformity for two reasons. Firstly, because of the nature of the geomagnetic field in this part of the world there is no relevant magnetic signature that exists unmistakably south of Ostional. Instead, the magnetic isolines in the region curve slightly north and west in the Pacific Ocean (Figure 2.1). As a result, the magnetic signatures that mark coastal locations south of Ostional are ambiguous and also exist to the west or even slightly north of the nesting beach. Secondly, although tracking studies have revealed that the migratory range of turtles that nest at Ostional spans well north of the nesting beach, it remains unclear whether turtles migrate south of Ostional, and if so, how far they might go [21, 22].

The trials took place during three separate mass-nesting events between November 2016 and August 2017. The majority of trials took place in a single mass-nesting event and the two treatments were alternated ( $n=21$  per treatment). To compensate for the fact that a portion of the El Salvador data came from previous mass-nesting events ( $n=17$ ), however, we

used a duplicate setup to increase our sample size in the magnet treatment group by conducting additional trials (n=6) at the same time that El Salvador trials were being conducted in the primary arena. We found no evidence of a difference across mass nesting events or the two setups so the data were combined.

### *Quantification and Statistical Analysis*

We used infrared illuminators placed in each of the four cardinal directions in combination with a custom infrared GoPro to monitor the swimming direction of each turtle. Because infrared does not penetrate water very effectively we measured the turtle's heading each time she came to the surface to breathe. An observer who was unaware of each turtle's treatment group watched each video to evaluate swimming behavior and used ImageJ to measure the turtle's direction relative to north at each breath. If a turtle did not swim comfortably or steadily during the ten-minute trial period she was excluded from analyses; all decisions were made by an observer who was unaware of the turtles treatment group. Some examples of unacceptable swimming behavior include extended periods of inactivity, frantic struggling with the harness, or excessive interaction with the side of the tank.

All statistical analyses were done using R Version 3.3.2 [24]. We used the Rayleigh test to determine if the turtles in each treatment showed significant orientation as a group. The Watson two-sample test was used to determine if the two groups were significantly different from each other. For the El Salvador treatment group that showed significant orientation we constructed bootstrap confidence intervals for the mean angle of orientation (1,000 iterations).

## Results

We found a significant difference in the orientation of turtles exposed to a uniform magnetic field and the orientation of turtles exposed to a highly irregular magnetic field produced by magnets ( $p < 0.05$ , Watson test). Turtles subjected to the magnetic field that exists 500km northwest of the nesting beach responded by swimming significantly to the southeast ( $145^\circ$ ,  $r = 0.365$ ,  $p = 0.006$ ,  $n = 38$ ; Rayleigh test, Figure 2.2A). By contrast, turtles that experienced a distorted magnetic field produced by magnets placed outside of the tank swam in directions that were statistically indistinguishable from random ( $r = 0.17$ ,  $p = 0.462$ ,  $n = 27$ ; Rayleigh test, Figure 2.2B).

## Discussion

These results provide strong evidence that sea turtles use geomagnetic cues to guide their nesting migrations and accomplish natal homing. Additionally, the findings suggest that adult turtles can determine their position relative to the nesting beach using the geomagnetic field alone. Turtles swimming in a distorted magnetic field did not orient significantly in any particular direction, but turtles subjected to a simulated magnetic displacement responded by swimming in an appropriate direction that would bring them back to the nesting beach had they actually been geographically displaced.

Our results do not, however, indicate that turtles rely exclusively on Earth's magnetic field during natal homing. Rather, in certain situations turtles, like salmon, might find the nesting beach through multimodal navigation using geomagnetic cues to travel long-distances and more local secondary cues during the final stage of natal homing [7, 13, 25, 26]. This is particularly likely in instances of extremely precise homing such as island

finding and mass-nesting events during which nesting is consistently limited to only a few kilometers.

Similarly, multimodal navigation that combines geomagnetic cues with local ones (e.g. chemical, visual, etc.) might mitigate potential navigational errors due to natural changes in Earth's magnetic field [26]. Although these changes, known as secular variation, can cause the magnetic signatures that mark natal locations to drift along the coast, numerous studies have indicated that the typical rates of field change are compatible with magnetic navigation and natal homing [1, 11, 17].

In addition, although our findings provide strong evidence that sea turtles use geomagnetic cues to guide their nesting migrations, they do not reveal the resolution of the magnetic map. Because of the extensive evidence for magnetic maps in hatchling sea turtles [27], and because juveniles have been shown to use Earth's magnetic field to determine their position along a coastline [28], we favor the hypothesis that adult turtles use geomagnetic positional information throughout the nesting migration to determine their precise location relative to the natal beach. In the specific case of mass nesting olive ridley turtles in Costa Rica, however, turtles might be able to achieve precise homing through a relatively simple navigational mechanism. Southeastern swimming at any latitude higher than the nesting beach might be sufficient to return if turtles subsequently encounter a local cue that differentiates the nesting beach from other locations. Therefore, our data alone cannot dismiss the hypothetical possibility that turtles might return to Ostional using a combination of a magnetic compass and local cues at the nesting beach. With the context of previous work, however, and the wealth of existing evidence for magnetic maps in sea turtles [27, 28],

our results suggest that adult turtles also derive positional information from Earth's magnetic field.

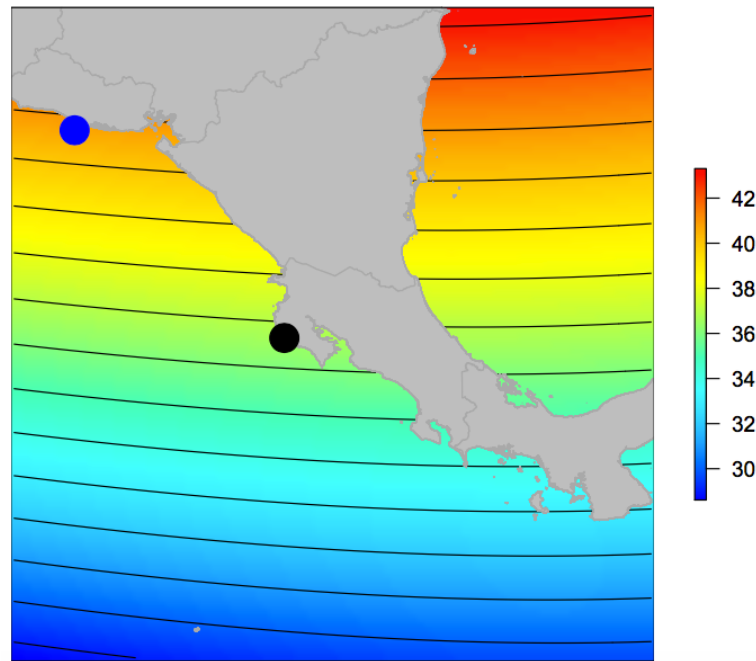
It is also not yet possible to determine which geomagnetic parameter(s) turtles use during natal homing. The most likely candidates appear to be intensity, inclination, or both together. Because we manipulated the two simultaneously, however, our results cannot identify which magnetic parameter, if either, is of primary importance.

Regardless of these considerations, our results provide strong evidence that sea turtles can use Earth's magnetic field alone to determine their position relative to the nesting beach. In addition, the findings provide behavioral and experimental corroboration that adult sea turtles use magnetic navigation to accomplish natal homing and are likely applicable to diverse long distance migrants including certain fishes [9, 12], reptiles [2, 7], birds [10], and mammals [8].

### **Acknowledgements**

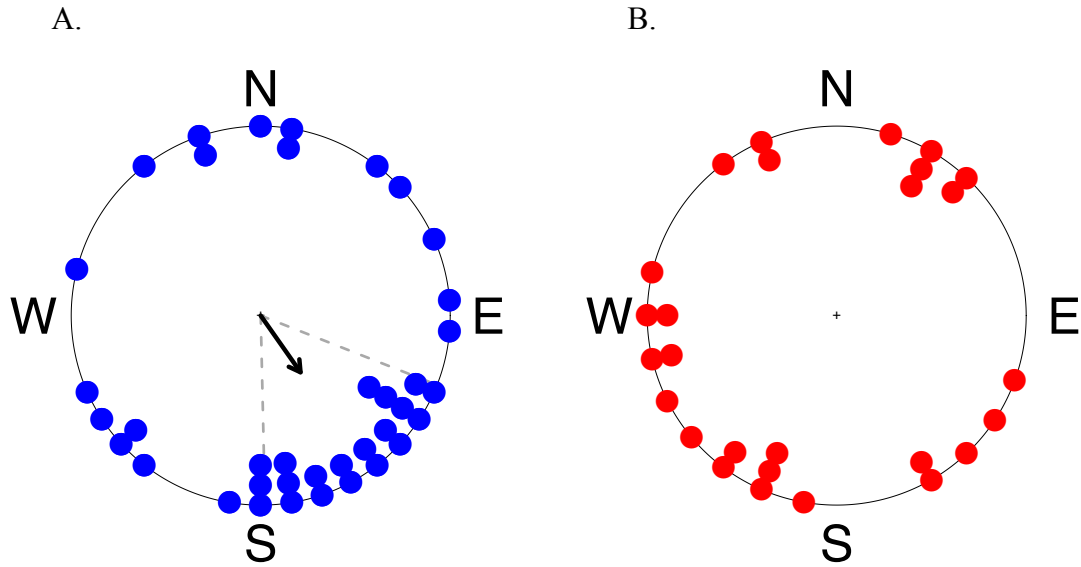
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## Figures



**Figure 2.1: A map showing isolines of magnetic inclination angle near Central America.**

In this map each black line represents an isoline of inclination angle (i.e. a line along which inclination angle is constant). Adjacent isolines represent increments of  $1^\circ$ . Because the coastline trends north-south and the magnetic isolines trend east-west, each area on the Pacific coast has a different inclination angle and thus a different magnetic signature. Intensity isolines are not shown, but the pattern is similar to that of inclination isolines with a different intensity marking each location along the Pacific coast. The black dot indicates the location of the nesting beach in Ostional, Costa Rica where the experiment was conducted. The blue dot near El Salvador indicates the location of the simulated magnetic field that exists 500km northwest of the nesting beach. The magnetic field used to simulate the conditions at this location had an inclination of  $40.3^\circ$  and an intensity of  $36.9\mu\text{T}$ ; the magnetic field at the nesting beach had an inclination of  $36.7^\circ$  and an intensity of  $34.7\mu\text{T}$ . We did not use an additional treatment that replicates a magnetic field southeast of the nesting beach for two reasons. Firstly, because the magnetic isolines in this region curve slightly north and west in the Pacific Ocean, the magnetic signatures that mark coastal locations south of Ostional also exist to the west and, even north, of the nesting beach. Therefore, it is impossible to provide a magnetic inclination angle or field intensity that a turtle would only encounter southeast of Ostional. Secondly, tracking studies indicate that turtles nesting at Ostional regularly migrate to the north, but it remains unclear whether turtles consistently travel to the south [21, 22].



**Figure 2.2: Orientation of mass-nesting adult turtles in response to a uniform magnetic field near El Salvador (A) and a non-uniform artificial magnetic field (B).**

Each dot represents the average swimming direction of one turtle during a ten-minute orientation trial. The arrow depicts the mean angle of the group and the length of the arrow is proportional to the magnitude of the mean vector ( $r$ ) with the radius of the circle corresponding to  $r=1$ . The dotted lines indicate bootstrap 95% confidence intervals for the mean angle. Turtles tested in a uniform magnetic field that exists 500km northwest of the nesting beach (A) were significantly oriented southeast ( $145^\circ$ ,  $r=0.365$ ,  $p=0.006$ ,  $n=38$ ; Rayleigh test). By contrast, turtles tested in a non-uniform magnetic field that had been disrupted by magnets placed around the tank (B) swam in directions that were statistically indistinguishable from random ( $r=0.17$ ,  $p=0.462$ ,  $n=27$ ; Rayleigh test). The two groups were significantly different from each other ( $p<0.05$ , Watson test).

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### CHAPTER 3: EVIDENCE FOR GEOMAGNETIC IMPRINTING AND MAGNETIC NAVIGATION IN THE NATAL HOMING OF SEA TURTLES<sup>1</sup>

#### Summary

Natal homing is a pattern of behavior in which animals migrate away from their geographic area of origin and then return to reproduce in the same location where they began life [1-3]. Although diverse long-distance migrants accomplish natal homing [1-8], little is known about how they do so. The enigma is epitomized by loggerhead sea turtles (*Caretta caretta*), which leave their home beaches as hatchlings and migrate across entire ocean basins before returning to nest in the same coastal area where they originated [9,10]. One hypothesis is that turtles imprint on the unique geomagnetic signature of their natal area and use this information to return [1]. Because Earth's field changes over time, geomagnetic imprinting should cause turtles to change their nesting locations as magnetic signatures drift slightly along coastlines. To investigate, we analyzed a 19-year database of loggerhead nesting in the largest sea turtle rookery in North America. Here we report a strong association between the spatial distribution of turtle nests and subtle changes in Earth's magnetic field. Nesting density increased significantly in coastal areas where magnetic signatures of adjacent beach locations converged over time, whereas nesting density decreased in places where magnetic signatures diverged. These findings confirm central predictions of the geomagnetic imprinting hypothesis and provide strong evidence that such imprinting plays an important

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role in the natal homing in sea turtles. The results give credence to initial reports of geomagnetic imprinting in salmon [11,12] and suggest that similar mechanisms might underlie long-distance natal homing in diverse animals.

## **Results and Discussion**

Ever since John James Audubon tied silver threads to the legs of young songbirds and observed their return the following year [13], evidence has accumulated that many animals return to their natal areas after migrating to distant locations [1-8]. An extreme example exists in loggerhead sea turtles, which leave their natal beaches as hatchlings and traverse entire ocean basins before returning to nest, at regular intervals, on the same stretch of coastline where they themselves hatched [9, 10, 14]. How sea turtles accomplish natal homing has remained an enduring mystery of animal behavior [1, 14-16].

Turtles derive long-distance navigational information from the geomagnetic field by detecting the intensity and inclination angle (the angle at which field lines intersect Earth's surface) [17-20]. These parameters vary predictably across the globe [21-22]. As a result, each area of coastline is typically marked by a different isoline of inclination and a different isoline of intensity (Figure 3.1A), and thus has a unique magnetic signature [1]. In principle, if turtles were to imprint on the inclination angle and/or intensity of their natal beach, then returning might be relatively simple; a turtle might need only to locate the coast and then swim north or south until it encounters the correct magnetic signature (Figure 3.1A). No evidence presently exists, however, to support or refute this hypothesis.

An important consideration for the geomagnetic imprinting hypothesis is that Earth's magnetic field changes slowly over time. Because of this field change, known as secular variation [23], the magnetic signatures that mark natal sites often drift slightly along the coast

while turtles are gone [1, 24]. Thus, if an adult female selects her nesting sites by seeking out the magnetic signature on which she imprinted as a hatchling, then she will inevitably change her nesting location in accordance with secular variation [25, 26]. Such individual changes might result in detectable population-level shifts in nesting distributions, providing a unique opportunity to test the geomagnetic imprinting hypothesis.

Specifically, the hypothesis predicts that when isolines of inclination or isolines of intensity converge along the coast, the magnetic signatures marking natal locations between those isolines will also converge (Figure 3.1). Thus, returning turtles will nest on a shorter length of coastline, and the number of nests per kilometer should increase (Figures 3.1B and 3.1C). By contrast, when isolines diverge, magnetic signatures also diverge, so returning turtles will nest over a longer length of coastline and the concentration of nests should decrease (Figures 3.1B and 3.1C). Until now, this possibility has never been investigated.

We analyzed a 19-year (1993-2011) database of loggerhead nesting for each of the 12 counties on the east coast of Florida, USA [27], an area comprising the largest sea turtle rookery in North America. To evaluate secular variation, we developed an objective metric (convergence index) that quantifies the degree of isoline movement along the coast within each county during 17 two-year time steps (see methods). A positive convergence index indicates that isolines within a particular coastal area moved closer together, with higher values indicating greater convergence. A negative convergence index indicates that isolines moved apart, with more negative values indicating greater divergence. For each county and time step combination, we calculated two different convergence indices, one based on the movement of inclination isolines and the other based on the movement of intensity isolines.

We then analyzed the relationship between each convergence index and changes in nesting density.

Analyses confirmed the predictions of the geomagnetic imprinting hypothesis. For inclination, regardless of year or location, isoline convergence was associated with increased nesting density, whereas isoline divergence was associated with decreased nesting density ( $p=5.34 \times 10^{-4}$ ) (Figure 3.2). Moreover, a linear mixed-effects model revealed a highly significant relationship between the magnitude of isoline movements and the magnitude of changes in nesting density ( $p=3.67 \times 10^{-4}$ ) (Figure 3.3, Table 1); the highest convergence indices were associated with the greatest increases in nesting density, and the lowest convergence indices were associated with the greatest decreases in nesting density. This trend persisted within each of the 12 counties on Florida's Atlantic coast (Figure 3.4, Table 2).

For intensity, there were no areas along the coast where isolines converged; thus, all convergence indices were negative. In all other regards, however, the results of the analysis were qualitatively identical to those of the inclination analysis. A linear mixed-effects model revealed a strong positive relationship between convergence index and changes in nesting density ( $p=8.2 \times 10^{-4}$ ) (Figure 3.3, Table 1); as convergence index increased, so did the percent change in nesting density. This trend persisted within all 12 counties on Florida's Atlantic coast (Figure 3.4, Table 2).

These results provide strong evidence that nesting sea turtles use Earth's magnetic field to locate their natal beaches. Although the exact geomagnetic component(s) exploited by turtles cannot be determined from the analyses, the findings are consistent with the

hypothesis that nest site selection depends at least partly on magnetic signatures comprised of inclination angle, field intensity, or a combination of the two.

In a previous study, the migratory route of salmon approaching their natal river was shown to vary with subtle changes in the Earth's field [11]. Whereas the endpoint of the salmon spawning migration was presumably the same regardless of route, our findings demonstrate for the first time a relationship between changes in Earth's magnetic field and the locations where long-distance migrants return to reproduce.

Sea turtles are long-lived and females undertake reproductive migrations periodically throughout their adult lives [28]. Thus, the population of turtles that migrates to a given beach to nest each year is comprised of two subsets: a group of first-time nesters, and another, typically larger group of older 're-migrants' that have nested in the area during previous years. Genetic analyses indicate that both groups display natal homing [3, 5, 9, 14]. An unresolved question, however, is whether both reach the natal region by using the same navigational strategy and sensory cues [25].

At least two possibilities are compatible with the data. One is that hatchling turtles imprint on the magnetic signature of the natal beach and retain this information into adulthood [1]. Alternatively, nesting females might somehow reach the natal beach the first time without relying on magnetic information (e.g., by following an experienced nester or by using non-magnetic cues), then learn the magnetic signature of the beach and use it to return during subsequent nesting migrations. Although neither possibility can be excluded, we presently favor the first because 'socially facilitated' migration has never been observed in sea turtles [3, 30] and because no non-magnetic cue has been identified that can provide the necessary positional information for long-distance navigation [16]. Regardless of how the

first return to the natal region is accomplished, turtles might periodically update their knowledge of the magnetic field at the nesting area each time they visit so as to minimize navigational errors that might otherwise accrue due to secular variation [24, 25].

Given the strong relationship between subtle changes in Earth's magnetic field and sea turtle nesting density, one possibility is that turtles are highly sensitive to small differences in magnetic fields. Alternatively, however, the same relationship can be explained if, in a typical nesting area, numerous error-prone individuals seek out a magnetic signature but miss the target by varying distances. Such imperfect navigation might, through a process resembling a 'wisdom of the crowd' phenomenon [31, 32], give rise to a nesting distribution centered on the magnetic signature and, in effect, coupled to it. Thus, when Earth's field changes slightly and magnetic signatures move, the population-level nesting distribution might change even if individual turtles have relatively low magnetic sensitivity and make considerable navigational errors.

Our findings do not imply that turtles reflexively nest at a particular magnetic signature regardless of other environmental conditions, or that nesting distributions will track the steady movement of isolines along a coast no matter what. Successful nesting requires deposition of eggs in a location suitable for incubation. Factors such as beach erosion, sand quality, visual cues, and predation are known to influence where turtles nest on a local scale [1, 25]. Because these and other environmental conditions also affect the likelihood that a nest will yield viable hatchlings [25, 33], natural selection is likely to act against turtles that choose nesting locations by relying on magnetic cues to the exclusion of all else. Moreover, sensory cues other than geomagnetism are likely to help guide natal homing, especially once turtles have arrived in the vicinity of the nesting area [24, 25].

Given that geomagnetic cues appear to play an important role in natal homing, an intriguing speculation is that, over evolutionary time, turtles might have had difficulty locating their natal beaches during brief periods of rapid field change, as are thought to have occurred during some magnetic polarity reversals [34]. During these intervals, turtles might have had a tendency to stray into new nesting areas, which subsequent generations could then locate reliably as the field stabilized and geomagnetic imprinting once more became an effective strategy for natal homing [1].

Because sea turtles nest in different environmental settings worldwide, it is possible that different nesting populations exploit geomagnetic cues in different ways during natal homing [1, 16, 35]. Our analysis suggests that turtles use geomagnetic cues to locate natal areas along continental coastlines, the most common setting for large sea turtle rookeries worldwide [16]. In other settings, such as on small islands, turtles must nest in specific, restricted areas because no alternative exists. In such situations, a clear relationship between field changes and nesting sites is unlikely because, over time, magnetic signatures that once marked the natal site drift offshore where nesting is impossible [1, 25]. In these cases turtles might use magnetic cues to navigate into the vicinity of the island and then use odorants or other supplemental, local cues to locate the nesting beach [16, 35, 36].

Regardless of these considerations, our results provide the strongest evidence to date that sea turtles find their nesting areas, at least in part, by navigating to unique magnetic signatures along the coast. In addition, the results are consistent with the hypothesis that turtles accomplish natal homing largely on the basis of magnetic navigation and geomagnetic imprinting. These findings, in combination with recent studies on Pacific salmon [11, 12],

suggest that similar mechanisms might underlie natal homing in diverse long-distance migrants such as fishes [2, 4], birds [33, 34], and mammals [6].

### **Methods Summary**

Using data from Florida's Statewide Nesting Beach Survey [27], which reports the number of kilometers surveyed within each county and the corresponding number of sea turtle nests counted, we calculated the loggerhead nesting density in Florida's 12 Atlantic coast counties for each of 19 years (1993-2011). We then calculated each county's percent change in nesting density for 17 two-year time steps (e.g., from 1993 to 1995, 1994 to 1996, and so on). Because the total number of loggerhead nests on Florida's east coast varied from year to year [39], we estimated the change in nesting density attributable to population fluctuations by calculating the average change in nesting for all counties and time steps. We then calculated the difference between this average and each data point and used the resulting value in our analyses.

Two-year time steps were used because adult female loggerheads typically return to nest on their natal beach every two or three years [28]; thus, this time step reflects isoline movement that turtles realistically encounter during successive reproductive migrations. Ideally, an analysis of nesting data designed to test geomagnetic imprinting would be limited to first-time migrants and would also use a longer time step that coincides with the interval between hatching and first migration, but this is impractical because no existing data set spans a sufficient time period or distinguishes between first-time and experienced migrants.

To assign coastal position, we used Google Earth to calculate distance along a line parallel to the east coast of Florida (Figure 3.5). We then used data from the International Geomagnetic Reference Field model (IGRF-11) [40] to calculate the distance isolines

traveled along the coast over the same two-year time steps for which we evaluated changes in nesting density. We described isoline movement as a function of coastal position (Figure 3.6A). The derivative of this function, with respect to position, quantifies isoline convergence or divergence (Figure 3.6B). This metric, referred to as the convergence index, was calculated at the midpoint of each county for each time step. A convergence index was calculated for both inclination and intensity isolines.

Over the past two decades, isolines near Florida have moved northward, but at variable rates. At some times and places, isolines to the south moved less than those to the north, resulting in the divergence of isolines. In such cases the derivative (convergence index) is negative (Figure 3.6). At other times and places, isolines to the south traveled farther than those to the north, resulting in the convergence of isolines. In these places the derivative (convergence index) is positive (Figure 3.6). In addition, the degree of isoline convergence or divergence is proportional to the magnitude of the derivative; a more positive derivative indicates high rates of convergence while a more negative derivative indicates high rates of divergence.

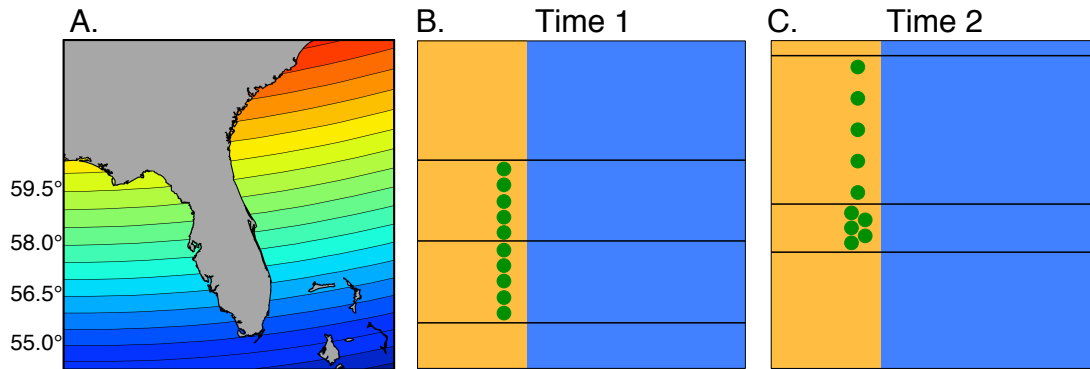
To characterize the relationship between convergence index and change in nesting density, we evaluated several linear models, including ordinary least-squares regression, mixed-effects regressions with random effects for time step, and mixed-effects regressions with random effects for county. The random effects included in the models take into account the year-to-year variations in nesting density along the Florida coast, as well as the county-to-county variations. While all models revealed equivalent results, the best-fit models for both the inclination analysis and for the intensity analysis include convergence index as a fixed effect and a random intercept and slope for time step (Table 1). We evaluated the difference

between nesting changes for areas with converging or diverging inclination isolines using a mixed-effects model with convergence or divergence as a fixed effect and time-step as a random effect. This last analysis was not done for intensity isolines because there were no coastal areas with converging intensity isolines.

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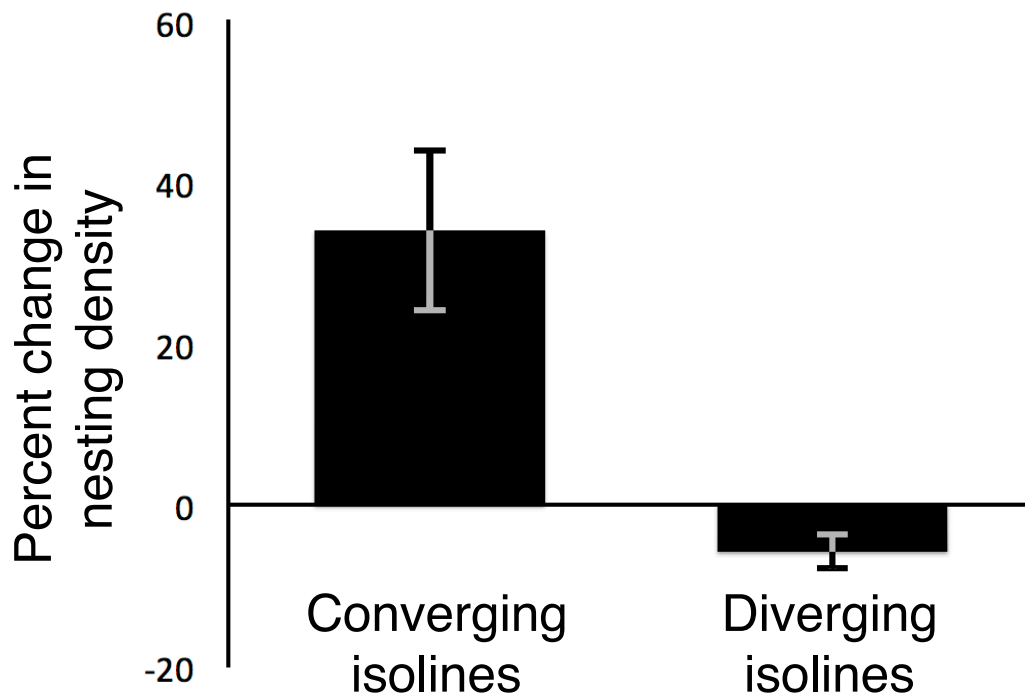
## Figures



**Figure 3.1: Map showing inclination isolines near Florida and diagrams showing predicted effects of isoline movement on nesting density.**

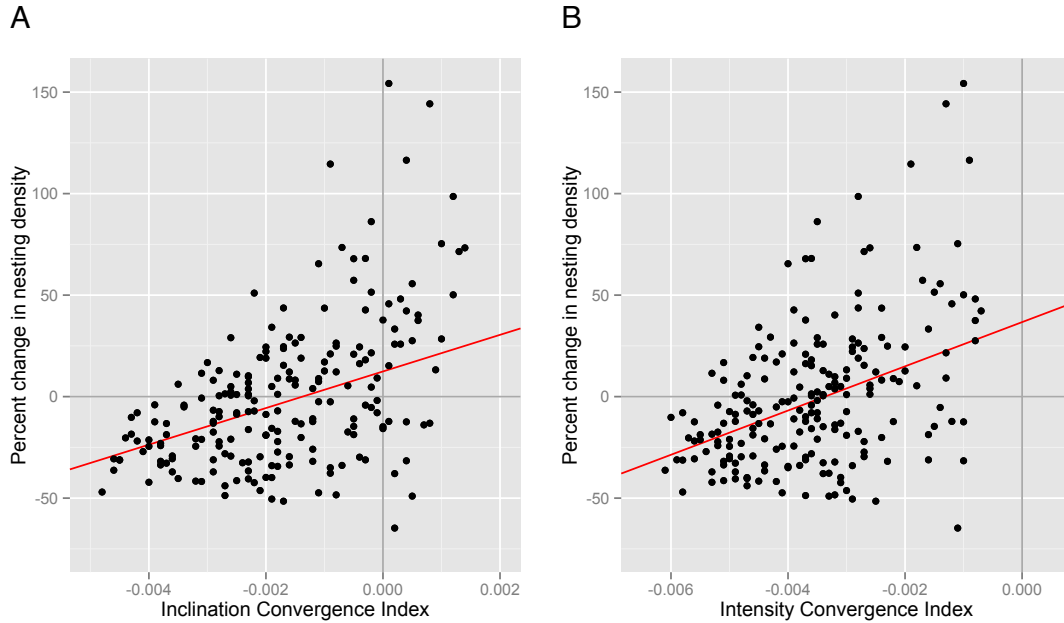
(A) Because these isolines trend east-west while the coastline trends north-south, a unique inclination angle marks each area along Florida's east coast. Thus, turtles might locate natal beaches by returning to the appropriate isolines; locations to the north of the target area have steeper inclination angles while locations to the south have shallower inclination angles. Black isolines bordering each color indicate increments of  $0.5^\circ$  and were derived from the IGRF model 11 [40] for the year 2012. The map for intensity isolines is not shown but is qualitatively similar, with different isolines of intensity existing at each area along Florida's east coast [16].

(B and C) Brown indicates land and blue indicates sea. (B) Horizontal lines indicate 3 hypothetical isolines and green dots represent nesting turtles, each of which has imprinted on the magnetic signature that marked her natal site as a hatchling. Over the past 2 decades, isolines near Florida have moved northward, but at variable rates. Sometimes, isolines to the south moved less than those to the north resulting in divergence (C; upper 2 isolines). In these situations, the geomagnetic imprinting hypothesis predicts a decrease in nesting density because turtles imprinted on the fields between the isolines should return to nest over a larger area. In places where isolines converged (because those to the south moved more than those to the north), the hypothesis predicts that nesting density should increase (C; lower 2 isolines).



**Figure 3.2: Changes in nesting density for coastal areas with converging and diverging inclination isolines.**

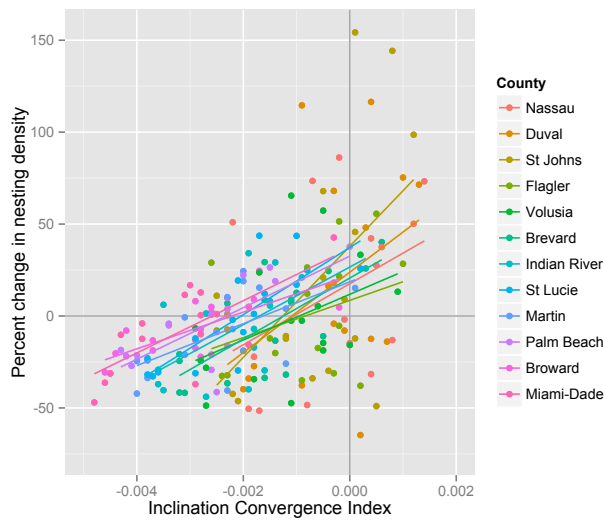
At times and places in which isolines converged ( $n=29$ ), nesting density increased by an average of 35%. At times and places in which isolines diverged ( $n=172$ ), nesting density decreased by an average of 6%. The mean changes of the two groups were significantly different ( $p=5.34 \times 10^{-4}$ ). Error bars represent standard error of the mean.



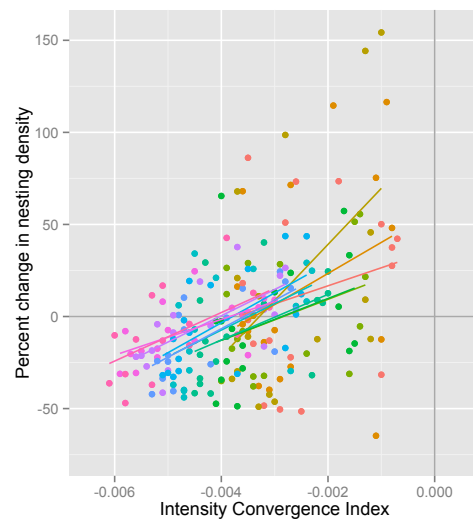
**Figure 3.3: Relationship between isoline movement and change in nesting density.**

Each data point represents values for one county in one time step. (A) For inclination, a significant, positive relationship exists between convergence index and change in nesting density ( $p=3.67 \times 10^{-4}$ ,  $n=204$ ) (Table S1). As the degree of isoline convergence increased so did the change in nesting density; the greatest increases in nesting were associated with the highest rates of convergence and the greatest decreases in nesting were associated with the highest rates of divergence. (B) For intensity, a significant positive relationship also exists between convergence index and change in nesting density ( $p=8.2 \times 10^{-4}$ ,  $n=204$ ) (Table S1). The slope and intercept for each red line were estimated with mixed-effects models including convergence index as a fixed effect and a random slope and intercept for time step.

A

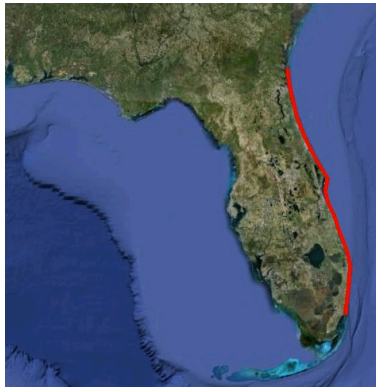


B



**Figure 3.4: Relationship between isoline movement and change in nesting density for individual counties.**

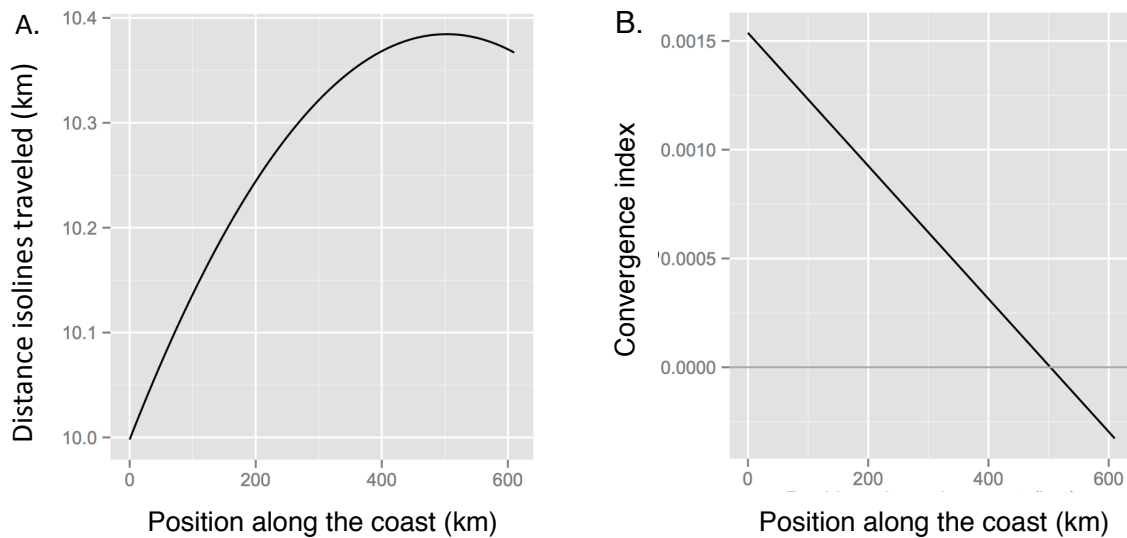
Each data point represents values for one county in one time step; each county is represented by a different color. In the color key, counties are arranged from north (top) to south (bottom). For both the inclination analysis (A) and the intensity analysis (B) all counties show a positive relationship between convergence index and change in nesting density ( $n=17$  for each county) (Table 3.2). The greatest increases in nesting were associated with the highest rates of convergence and the greatest decreases in nesting were associated with the highest rates of divergence. For inclination, this relationship is significant in 8 individual counties ( $p<0.05$ ) and the trend is present in all. For intensity, the relationship is significant in 7 individual counties ( $p<0.05$ ) and the trend is present in all.



County	County Midpoint (km)	Northern Boundary (km)	Southern Boundary (km)
Nassau	42	28 (30.71°N, 81.45°W)	56 (30.51°N, 81.43°W)
Duval	70	56 (30.51°N, 81.43°W)	84 (30.25°N, 81.43°W)
St. Johns	117	84 (30.25°N, 81.43°W)	151 (29.67°N, 81.21°W)
Flagler	165	151 (29.67°N, 81.21°W)	180 (29.43°N, 81.10°W)
Volusia	220	180 (29.43°N, 81.10°W)	260 (28.79°N, 80.73°W)
Brevard	316	260 (28.79°N, 80.73°W)	372 (27.86°N, 80.45°W)
Indian River	390	372 (27.86°N, 80.45°W)	408 (27.56°N, 80.32°W)
St. Lucie	425	408 (27.56°N, 80.32°W)	443 (27.26°N, 80.20°W)
Martin	460	443 (27.26°N, 80.20°W)	477 (26.97°N, 80.08°W)
Palm Beach	513	477 (26.97°N, 80.08°W)	549 (26.32°N, 80.07°W)
Broward	568	549 (26.32°N, 80.07°W)	587 (25.98°N, 80.12°W)
Miami-Dade	599	587 (25.98°N, 80.12°W)	611 (25.76°N, 80.13°W)

**Figure 3.5: A map of the Florida coastline showing the line along which distance was calculated and a table indicating the position of each county along the line.**

To determine coastal position, we used Google Earth to draw and measure a line parallel to the main coastline (shown in red). The northernmost point on the line (31.00° N, 81.42° W) lies just north of the Florida-Georgia border and was defined as 0 km. The southernmost point (25.75° N, 81.28° W) falls south of Miami and was calculated as 611 km. The table on the right indicates the position of the midpoint, northern boundary, and southern boundary of each county along the 611-km line. Counties are listed in order from north to south. For the northern and southern boundary of each county, the first number indicates the position of each location along the 611-km line; latitude and longitude are included in parentheses.



**Figure 3.6: Sample curves showing how convergence index is calculated.**

These are examples only and the exact form of each graph varies greatly among different time steps; thus, areas with converging isolines in one time step can have diverging isolines in another. On the x-axis, 0 km is near the northern Florida border and 600 km is in southern Florida (near Miami). (A) A graph showing the distance inclination isolines traveled during the 1993-1995 time step as a function of coastal position. Convergence index is calculated as the derivative, with respect to position, of this curve. (B) A graph from the same time step showing inclination convergence index as a function of coastal position. A positive convergence index indicates isoline convergence (situations when isolines to the north moved less than those to the south); a negative convergence index indicates isoline divergence (situations when isolines to the north moved farther than those to the south). A more positive convergence index indicates a greater degree of convergence while a more negative value indicates a greater degree of divergence. Convergence indices ranged from -0.0061 to 0.0014. For purposes of estimation, if these rates of convergence are assumed to exist uniformly over a hypothetical 100 km stretch of coastline, they would result in a maximum expansion of 0.61 km and a maximum compression of 0.14 km over a two-year time step. (In reality, such hypothetical extrapolations are likely to be inaccurate because convergence indices vary along the coast.)

## Tables

Inclination Analysis							
Fixed Effect	Random Effect	Slope	Intercept	Model Evaluation			p-value
				AIC	BIC	r <sup>2</sup>	
None	Intercept by county	N/A	-1.1 <sup>-7</sup>	2,040	2,050	N/A	N/A
None	Intercept by time step	N/A	-1.1 <sup>-7</sup>	1,954	1,964	N/A	N/A
Convergence index	None (OLS)	12,347	21.1	N/A	N/A	0.26	1.1 x 10 <sup>-14</sup>
Convergence index	Intercept and slope by county	12,490	21	1,970	1,990	N/A	2.1 x 10 <sup>-13</sup>
Convergence index	Intercept and slope by time step	9,017	12.4	1,891	1,911	N/A	3.7 x 10 <sup>-4</sup>

Intensity Analysis							
Fixed Effect	Random Effect	Slope	Intercept	Model Evaluation			p-value
				AIC	BIC	r <sup>2</sup>	
None	Intercept by county	N/A	-1.1 <sup>-7</sup>	2,040	2,050	N/A	N/A
None	Intercept by time step	N/A	-1.1 <sup>-7</sup>	1,954	1,964	N/A	N/A
Convergence index	None (OLS)	12,690	45.2	N/A	N/A	0.21	7.0 x 10 <sup>-12</sup>
Convergence index	Intercept and slope by county	12,690	45.1	1,983	2,003	N/A	8.7 x 10 <sup>-12</sup>
Convergence index	Intercept and slope by time step	10,890	36.7	1,897	1,916	N/A	8.2 x 10 <sup>-4</sup>

**Table 3.1: Regression statistics for the relationship between isoline movement and change in nesting density.**

For both inclination and intensity, we evaluated several linear regression models including ordinary least squares (OLS), mixed-effects models with random effects for county, and mixed-effects models with random effects for time step. All models showed a significant, positive relationship between convergence index and changes in nesting density. In both analyses the best-fit model included convergence index as a fixed effect and a random intercept and slope for time step.

Inclination Analysis					Intensity Analysis				
County	Slope	Intercept	r <sup>2</sup>	p-value	County	Slope	Intercept	r <sup>2</sup>	p-value
Nassau	16,617	17.6	0.16	0.110	Nassau	9,799	36.3	0.05	0.374
Duval	21,855	23.8	0.18	0.088	Duval	16,872	57.1	0.11	0.201
St. Johns	30,211	37.9	0.29	0.027	St. Johns	30,448	100.0	0.26	0.038
Flagler	10,130	8.5	0.13	0.160	Flagler	11,142	31.6	0.14	0.143
Volusia	12,423	11.8	0.18	0.090	Volusia	11,326	32.4	0.13	0.159
Brevard	16,580	20.6	0.49	0.002	Brevard	12,402	36.8	0.23	0.053
Indian River	15,670	26.6	0.40	0.007	Indian River	14,363	50.1	0.27	0.031
St. Lucie	11,661	19.5	0.39	0.004	St. Lucie	16,226	61.5	0.39	0.007
Martin	11,387	18.6	0.35	0.013	Martin	15,447	55.2	0.51	0.001
Palm Beach	14,019	32.6	0.55	0.0007	Palm Beach	14,274	57.5	0.45	0.003
Broward	9,989	22.1	0.51	0.001	Broward	9,708	37.3	0.38	0.009
Miami-Dade	14,209	36.8	0.53	0.001	Miami-Dade	13,175	54.9	0.36	0.011

**Table 3.2: Regression statistics for the relationship between isoline movement and change in nesting density for individual counties.**

Linear regression showed a positive relationship between convergence index and change in nesting density for each of Florida's Atlantic coast counties (n=17 time steps for each county). For inclination, the relationship is significant for 8 of 12 counties ( $p < 0.05$ ) and the trend is present in all. For intensity, the relationship is significant in 7 of 12 counties ( $p < 0.05$ ) and the trend is present in all. Counties are arranged in the table from north (top) to south (bottom).

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## CHAPTER 4: EVIDENCE THAT MAGNETIC NAVIGATION AND GEOMAGNETIC IMPRINTING SHAPE SPATIAL GENETIC VARIATION IN SEA TURTLES<sup>1</sup>

### Summary

The canonical drivers of population genetic structure, or spatial genetic variation, are isolation by distance and isolation by environment. Isolation by distance predicts that neighboring populations will be genetically similar, and geographically distant populations will be genetically distinct [1]. Numerous examples also exist of isolation by environment, a phenomenon in which populations that inhabit similar environments (e.g., same elevation, temperature, vegetation, etc.) are genetically similar even if they are distant, whereas populations that inhabit different environments are genetically distinct even when geographically close [2–4]. These dual models provide a widely accepted conceptual framework for understanding population structure [5–8]. Here we present evidence for an additional, novel process that we call isolation by navigation, in which the navigational mechanism used by a long-distance migrant influences population structure independently of either isolation by distance or environment. Specifically, we investigated the population structure of loggerhead sea turtles (*Caretta caretta*) [9], which return to nest on their natal beaches by seeking out unique magnetic signatures along the coast, a behavior known as geomagnetic imprinting [10–12]. Results reveal that spatial variation in Earth’s magnetic

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field strongly predicts genetic differentiation between nesting beaches, even when environmental similarities and geographic proximity are taken into account. The findings provide genetic corroboration of geomagnetic imprinting [10, 13]. Moreover, they provide strong evidence that geomagnetic imprinting and magnetic navigation help shape the population structure of sea turtles, and perhaps numerous other long-distance migrants that return to their natal areas to reproduce [13–17].

## **Results and Discussion**

Neither of the two classical drivers of population structure readily explains the enigmatic pattern of spatial genetic variation that exists within the largest sea turtle rookery in North America. Specifically, the genetic structure of the loggerhead turtle population in the southeastern U.S. appears inconsistent with isolation by distance, in that turtles nesting on beaches that are relatively close together are often genetically distinct, while those that nest on beaches that are farther apart (including some on the east and west coasts of Florida) are often genetically alike [9]. Similarly, isolation by environment cannot readily account for the pattern, inasmuch as nesting beaches that are close together, but used by genetically distinct populations, often appear to be physically identical.

An interesting possibility is that the unusual genetic structure arises through a mechanism involving navigation to natal beaches [9, 12]. After departing from their natal beaches as hatchlings and migrating across vast expanses of open ocean, loggerhead turtles return as adults to nest on the same stretch of coastline where they themselves hatched, a behavior known as natal homing [9, 12, 18–21]. Natal homing in sea turtles appears to be accomplished largely through the mechanism of geomagnetic imprinting, in which turtles learn the magnetic field of their home area when young and use this information to return

years later as adults [10–12]. Geomagnetic imprinting and magnetic navigation back to the natal beach are possible because Earth’s magnetic field varies predictably across the globe [22, 23]. Thus, most coastal areas are marked by different magnetic signatures (Figure 4.1) [10, 11], which turtles can detect because of their ability to perceive specific elements of Earth’s magnetic field such as intensity and inclination [24–26].

Geomagnetic imprinting and magnetic navigation have interesting but largely unexplored implications for the genetic structure of populations. In many parts of the world, the geomagnetic field varies more from north to south than it does from east to west (Figure 4.1). Consequently, the geographic distance between two nesting beaches is not a reliable predictor of the magnetic difference between them. Thus, if turtles do indeed locate their natal beaches by returning to the magnetic signature on which they imprinted, then the potential for navigational errors arises whenever two different nesting beaches have very similar magnetic fields. Under such conditions, geomagnetic imprinting predicts that within a given oceanic region, populations of turtles nesting on beaches with similar magnetic fields should be genetically similar and populations of turtles nesting on beaches with different magnetic fields should be genetically distinct. Moreover, this pattern of geomagnetically-mediated population structure might persist regardless of either the geographic distance between two nesting areas or their environmental characteristics.

To investigate the hypothesis of isolation by navigation, we analyzed data from an extensive study of loggerhead turtle population genetics [9] in which mtDNA samples were obtained from 834 nesting females across 20 different locations along the southeastern U.S. coast (Figure 4.1). We extracted  $F_{ST}$  values from the reported pairwise comparisons between each possible combination of nesting beaches.  $F_{ST}$  is a widely used metric of genetic

differentiation that ranges from zero to one, with low values indicating genetic similarity and high values indicating genetic differentiation.

The magnetic field at any location on earth can be described by a field intensity and an inclination angle (the angle that the field lines make with respect to Earth's surface), both of which turtles detect [24, 25]. We calculated a historical average of the magnetic intensities and the magnetic inclination angles that have existed at each of the 20 nesting beaches for the last 425 years and used this data to calculate the magnetic distance between each possible combination of nesting beaches. For the same combinations of beaches we also calculated: (1) the shortest possible oversea distance (i.e. the minimal distance a sea turtle would have to swim to travel from one location to the other); and (2) the environmental distance.

Environmental distance describes variation in the environment between nesting beaches and incorporates 21 environmental variables (Table 4.1), including sea surface temperature, ocean primary productivity, and 19 other standard bioclimatic variables (e.g. annual mean temperature, annual precipitation).

Analyses revealed a striking relationship between genetic differentiation, as estimated by  $F_{ST}$ , and spatial variation in Earth's magnetic field (Figure 4.2). Populations of turtles nesting at beaches with similar magnetic fields tended to be genetically similar; nesting populations at beaches marked by larger differences in magnetic fields had greater genetic differences. Indeed, multiple matrix regression with randomization [27–29] revealed a highly significant relationship between spatial variation in Earth's magnetic field and  $F_{ST}$ , but found no effect of geographic distance or environmental distance (Table 4.2). In other words, the difference between the magnetic fields at two nesting beaches was a strong predictor of the genetic differentiation between the turtle populations that nest in the two locations, regardless

of the geographic proximity of the nesting beaches or their environmental similarities. Moreover, bootstrap confidence intervals for each regression coefficient (see Methods) show that magnetic distance had a significantly stronger effect on genetic differentiation than did either geographic or environmental distance, when all three are considered together (Table 4.2).

These results provide strong evidence that spatial variation in Earth's magnetic field influences spatial genetic variation in loggerhead turtles, through a process most likely mediated by geomagnetic imprinting and magnetic navigation. A plausible interpretation of the findings is that, because some geographically separated beaches have similar magnetic signatures, adult females searching for the magnetic signatures of their natal beaches sometimes nest mistakenly on beaches located elsewhere that also have the 'correct' magnetic field. Consistent with this possibility, some loggerheads nest in widely separated locations during their lifetimes, including sites on both the east and west coasts of Florida [30].

The concept of isolation by navigation, in which a navigational process such as geomagnetic imprinting drives population genetic structure, is fundamentally different from isolation by environment. In the latter, the environmental characteristics associated with genetic differentiation are intrinsically coupled to physiology, survival, and fitness; for example, in sea turtles, air temperature and rainfall influence embryonic development [31–34], primary production in the ocean determines food availability [35], and water temperature influences nesting behavior [36, 37]. By contrast, slight differences in Earth's magnetic field, as occur in different geographic locations, have no known effects on either ecosystems or on physiology, with the single exception of the processes involved in magnetic navigation. For

this reason, the relationship we observe between spatial variation in Earth's magnetic field and genetic differentiation cannot be attributed to isolation by environment, but instead must be considered the result of a separate, independent driver of population structure arising from a navigational strategy. This concept may be important not only for sea turtles, but also for other animals that use magnetic positional information in navigation [38–41].

Although our results provide genetic evidence for geomagnetic imprinting, it is not yet possible to identify with certainty the exact magnetic parameter(s) that turtles use to identify their natal beaches. The most likely candidates appear to be intensity, inclination, or both together [10, 11]. We note that if intensity and inclination are considered separately and analyses are carried out in which each is used as the sole basis for magnetic distance between beaches, then strong relationships are found between each magnetic parameter and genetic differentiation (Figure 4.3). Although it is tempting to conclude that sea turtles imprint on both parameters, an important caveat is that intensity and inclination vary together across the globe, and particularly along the Florida coastline. For this reason, our multivariate analysis used a single metric of magnetic distance that incorporates both inclination and intensity in order to account for the collinearity between them. Moreover, due to the nature of Earth's magnetic field, intensity and inclination are also inherently coupled to other geomagnetic parameters such as horizontal and vertical intensity. Thus, no conclusion can be drawn yet about which parameters of Earth's magnetic field are of the greatest importance.

Similarly, our results do not imply that geomagnetic imprinting and magnetic navigation to natal beaches are the sole determinant of sea turtle population structure. The mechanisms that underlie spatial genetic variation are complex; thus, numerous factors are likely important. For example, even if two nesting beaches have similar magnetic fields,

strong ocean currents or other environmental barriers might impede movement between the two and lead to greater genetic differentiation than would be expected through magnetic navigation alone [42]. Conversely, if a population bottleneck or founder effect results in reduced genetic variation across a broad geographic region, then nesting beaches with distinct magnetic fields might harbor genetically similar populations even though the two locations should, in principle, be easily distinguished by magnetic signatures.

Additionally, although our multivariate analysis found no significant relationship between environmental distance and genetic differentiation (Table 4.2), we note that the trend is in the expected direction when environmental distance is considered alone. In other words, genetic differentiation between nesting beaches tends to increase with environmental distance (Figure 4.2C). Environmental factors are indeed critical to success at a nesting beach; both temperature and humidity are known to influence embryonic development [31–34]. Furthermore, at least some evidence suggests that thermal differences between nesting beaches might promote local adaptation under certain conditions [43]. Thus, the possibility remains that environmental distance might affect population structure of sea turtles in the southeastern U.S., even though our analysis failed to detect an effect.

Another intriguing aspect of using magnetic navigation to accomplish natal homing is that Earth's field changes over time; this can cause the magnetic signatures that mark natal locations to drift along the coast and might lead to navigational errors. Several studies, however, have revealed that typical rates of field change are compatible with geomagnetic imprinting [10, 11, 44].

Regardless of these considerations, our results provide a powerful, independent new line of genetic evidence for geomagnetic imprinting in sea turtles. In addition, the findings

reveal a previously undescribed process that can influence population genetic structure: isolation by navigation. The discovery that spatial variation in Earth's magnetic field shapes the population structure of a major sea turtle rookery, and the inference that magnetic navigation and geomagnetic imprinting can play a role in genetic differentiation, are likely relevant to numerous long-distance migrants including diverse fishes, reptiles, birds, and mammals [13–17, 40, 41, 45].

## **Method Details**

To estimate genetic differentiation we extracted previously reported  $F_{ST}$  values from pairwise comparisons between each possible pairing of 20 nesting beaches across the southeastern U.S.A. [9]. For the same combinations of nesting beaches we also calculated the geographic, environmental, and magnetic distances between each pair (see below) and scaled these distance metrics by dividing each observation by the mean of its group (i.e. geographic, environmental, or magnetic distance).

### *Geographic Distance*

To calculate the shortest possible oversea distance between nesting beaches, we used ArcMap 10.5.1 [46]. The goal was to determine the shortest distance that a turtle could swim in order to travel from one nesting beach to another, rather than the shortest distance a crow could fly. To accomplish this, we used the USA Contiguous Albers Equal Area Conic projection, which minimizes distortion for broader geographic areas, and implemented a processing mask over the continental United States to limit the analysis to marine locations. We then used the Path Distance tool with 500-meter grid cell resolution to calculate the shortest distance from one nesting beach to all 19 other beaches. We then iterated across nesting beaches to calculate the shortest oversea distance between all possible pairs of

beaches.

### *Environmental Distance*

To quantify the environmental differences between nesting beaches we compiled data for 21 environmental variables (Table 4.1) at each nesting beach. We then scaled each variable to have unit variance, centered it around zero, and incorporated all 21 into a principal components analysis (PCA). We then calculated the environmental distance between each possible combination of nesting beaches as the Euclidian distance between each pair along the resulting PCA axes. The analysis included 30-year averages (1970-2000) for 19 standard bioclimatic variables (Table 4.1) at each nesting beach, which we extracted from the WorldClim database [47]. We also included mean sea surface temperature during the nesting season (May, June, and July) averaged over 35 years of data (1981-2016) from NOAA's Optimum Interpolation Sea Surface Temperature database version 2 at locations just offshore from each nesting beach [48]. For the same offshore locations we included mean ocean productivity during the nesting season averaged over 13 years of data (2003-2016) from the Vertically Generalized Production Model, which incorporates MODIS data products to estimate net primary productivity [49].

### *Magnetic Distance*

Finally, we calculated the magnetic distance between nesting beaches using 425 years of magnetic field data from the *gufml* model (years 1590-1900) and the International Geomagnetic Reference Field model-12 (years 1900-2015) [50, 51]. First, we calculated the average inclination angle and the average magnetic field intensity at each nesting beach, centered and scaled the values as done previously with the environmental variables, and incorporated both magnetic parameters into a PCA. We then calculated the magnetic distance

between each possible combination of nesting beaches as the Euclidian distance between each pair along the resulting PCA axes. This method allowed us to account for the strong collinearity between inclination angle and intensity, and look for a relationship between genetic differentiation and Earth's magnetic field without regard to the specific magnetic parameters involved.

### **Quantification and Statistical Analysis**

All statistical analyses were done using R Version 3.3.2 [52].

#### *Multiple Matrix Regression with Randomization*

We used multiple matrix regression with randomization (MMRR) with 1,000 permutations to quantify any correlation between genetic differentiation ( $F_{ST}$ ) and each of the three distance metrics. MMRR is an extension of mantel analysis that uses randomization during significance testing to account for the non-independence that is inherent to distance matrices [27–29].

To account for potential collinearity between magnetic, geographic, and environmental distance, our findings are based on a multivariate analysis that includes all three together. We also considered the six other possible models including those that look at each possible combination of two distance metrics, and those that use each distance metric alone to predict genetic differentiation (Table 4.3). We then used the results from the entire suite of seven models to partition the variation in genetic differentiation that is explained by the full model [53], an approach that provides some insight into the relative importance of each distance metric (Table 4.4).

### *Bootstrap Confidence Intervals for Regression Coefficients*

To compare the effect sizes of each of the three distance metrics on genetic differentiation, we constructed bootstrap confidence intervals for each regression coefficient in the full model. To accomplish this and retain potential correlation structure among the residuals, we constructed a matrix of residuals from the model, organized by nesting beach. Then, within each of 10,000 iterations, we used the predicted  $F_{ST}$  from our model and a random permutation of the residual matrix to calculate simulated  $F_{ST}$  values before refitting the full model. The variation in the coefficient estimates across each of these simulations can be used to generate confidence intervals and evaluate the relative effect size that each distance metric has on genetic differentiation.

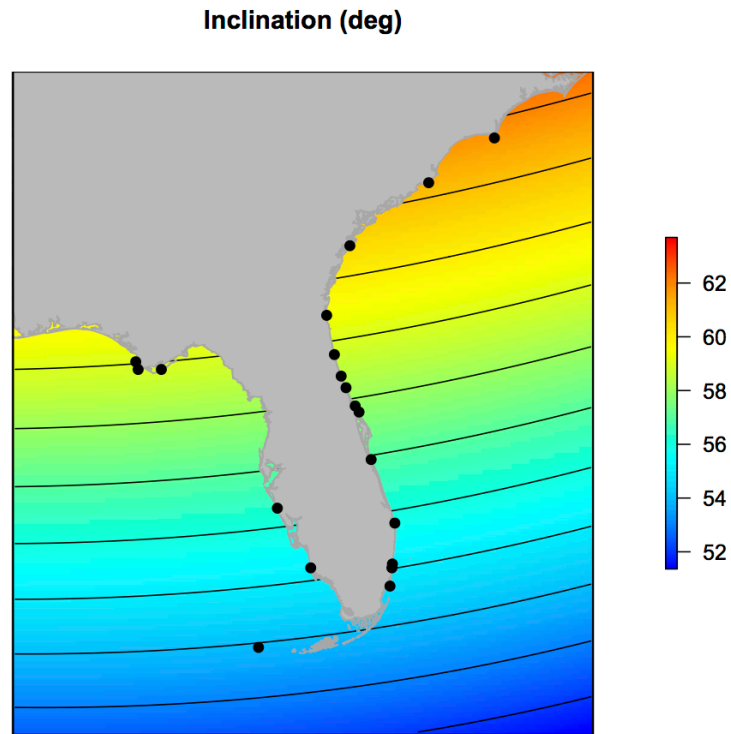
### **Data and Software Availability**

The three distance matrices (geographic, environmental, and magnetic) that we generated and used in our analyses have been deposited in the Mendeley Data repository (DOI: 10.17632/5kk6gzvvzr.1). The  $F_{ST}$  values that we used to estimate genetic differentiation are available in Shamblin *et al.*, 2011 [9]. All of the variables we included in both the magnetic and environmental distance calculations are from publicly available databases.

### **Acknowledgments**

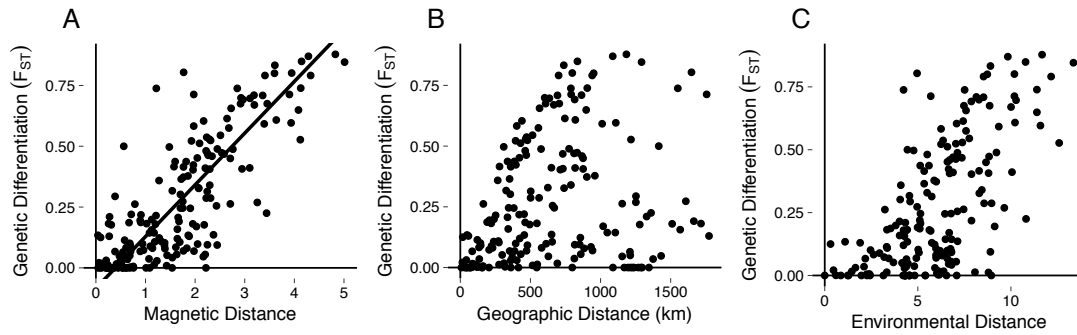
I thank Dr. James Umbanhowar for statistical guidance, Phillip McDaniel for assistance with ArcMap and Dr. Catherine Lohmann, Dr. David Steinberg, David Ernst, Vanessa Bézy, Kayla Goforth, and Lewis Naisbett-Jones for helpful conversations and feedback on manuscript drafts. This work was supported by National Science Foundation grant IOS-1022005 and Air Force Office of Scientific Research grant FA9550-14-1-0208.

## Figures



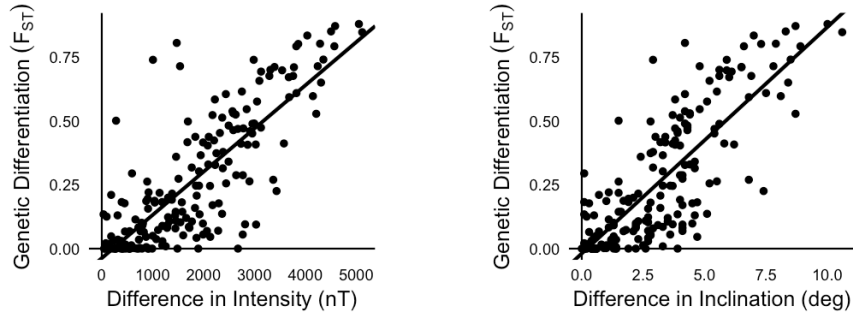
**Figure 4.1: A map showing isolines of magnetic inclination angle along the southeastern US coastline and the locations of the 20 nesting beaches included in the analyses.**

Inclination angle refers to the angle at which magnetic field lines intersect Earth's surface; it varies between  $0^\circ$  at the geomagnetic equator and  $90^\circ$  at the magnetic poles. In this map, each black line represents an isoline of inclination angle (i.e. a line along which inclination angle is constant). Adjacent isolines represent increments of  $1^\circ$ . Because the coastline trends north-south and magnetic isolines trend east-west, each area on the Atlantic coast has a different inclination angle and thus a different magnetic signature. Evidence suggests that sea turtles use these magnetic signatures to return to nest on their natal beaches, through a combination of geomagnetic imprinting and magnetic navigation [10, 11]. Intensity isolines are not shown, but the pattern is similar to that of inclination isolines [12]. Each black dot represents one of the 20 nesting beaches included in our analyses. Note that some nesting beaches on opposite sides of the Florida peninsula are close to the same isoline and therefore have similar magnetic signatures. As a result, a returning turtle might mistakenly nest on a beach that has the 'correct' magnetic field but is actually far from its natal location.



**Figure 4.2: Regression analyses showing the relationship between  $F_{ST}$  and the magnetic distance (A), the geographic distance (B), and the environmental distance (C).**

Each data point represents a pairwise comparison between two nesting beaches with the genetic differentiation between nesting beaches on the y-axis, and either the magnetic, geographic, or environmental distance between the nesting beaches on the x-axis. (A) There is a strong positive relationship between magnetic distance and genetic differentiation ( $p = 0.001$ ); nesting beaches with similar magnetic fields harbor populations of turtles that are genetically similar, while nesting beaches with different magnetic fields are home to populations of turtles that are genetically distinct. (B and C) By contrast, no significant relationship is observed between genetic differentiation and either geographic distance or environmental distance ( $p = 0.533$  and  $p = 0.185$ , respectively). Moreover, the 95% bootstrap confidence intervals of each regression coefficient indicate that magnetic distance has a significantly stronger effect on genetic differentiation than do geographic and environmental distance (Table 4.2). P-values were calculated with multiple matrix regression with randomization (MMRR) that incorporated all three distance metrics together and used 1,000 permutations (Table 4.2). In addition we used the results from all seven possible models (Table 4.3) to partition the variation in genetic differentiation explained by the full model (Table 4.4).



**Figure 4.3: Regression analyses showing the relationship between  $F_{ST}$  and the difference in intensity, and the difference in inclination.**

Each data point represents a pairwise comparison between two nesting beaches with the genetic differentiation between nesting beaches on the y-axis, and either the difference in intensity or the difference in inclination angle between the nesting beaches on the x-axis. Although both intensity ( $p=0.001$ ,  $r^2=0.650$ ,  $n=190$ , MMRR) and inclination ( $p=0.001$ ,  $r^2=0.646$ ,  $n=190$ , MMRR) are strongly related to genetic differentiation when they are considered separately, these two geomagnetic parameters vary together across the globe. For this reason, our multivariate analysis used a single metric of magnetic distance that incorporates both inclination and intensity in order to account for the collinearity between them. The linear regression that we have used fits the data quite well; nevertheless, a non-linear model, such as a logistic regression, might conceivably offer a marginal increase in goodness of fit, and the exact nature of the relationship between Earth's magnetic field and genetic differentiation is worthy of future study.

Environmental Variable	Database
Mean Sea Surface Temperature	NOAA OISST V2
Net Ocean Primary Productivity	Ocean Productivity, Oregon State University
Annual Mean Temperature	WorldClim
Mean Diurnal Range	WorldClim
Isothermality	WorldClim
Temperature Seasonality	WorldClim
Max Temperature of Warmest Month	WorldClim
Min Temperature of Coldest Month	WorldClim
Temperature Annual Range	WorldClim
Mean Temperature of Wettest Quarter	WorldClim
Mean Temperature of Driest Quarter	WorldClim
Mean Temperature of Warmest Quarter	WorldClim
Mean Temperature of Coldest Quarter	WorldClim
Annual Precipitation	WorldClim
Precipitation of Wettest Month	WorldClim
Precipitation of Driest Month	WorldClim
Precipitation Seasonality	WorldClim
Precipitation of Wettest Quarter	WorldClim
Precipitation of Driest Quarter	WorldClim
Precipitation of Warmest Quarter	WorldClim
Precipitation of Coldest Quarter	WorldClim

**Table 4.1: Environmental variables that comprise environmental distance and the database from which each was extracted.**

Multiple Matrix Regression with Randomization (1,000 permutations)			
Parameter	Estimate	Confidence Interval	p-value
Intercept	-0.008	-0.073 to 0.061	1.00
Magnetic Distance	0.351	0.280 to 0.421	0.001
Geographic Distance	0.014	-0.029 to 0.054	0.533
Environmental Distance	-0.090	-0.205 to 0.033	0.185
$r^2=0.670$ , $F=125.6$ , $p=0.001$ , $n=190$			

**Table 4.2: Results from the full model using magnetic, geographic, and environmental distance together to predict genetic differentiation.**

Multiple matrix regression with randomization (MMRR) revealed a significant effect of magnetic distance on genetic differentiation but failed to detect an effect of either geographic or environmental distance when all three were considered together. In addition, bootstrap confidence intervals for each regression coefficient indicate that magnetic distance had a significantly stronger effect on genetic distance than did either geographic or environmental distance. See methods for details on MMRR and about how bootstrap confidence intervals were calculated.

Multiple Matrix Regression with Randomization (1,000 permutations)							
Model	Estimate				F	p-val	r <sup>2</sup>
	Intercept	Mag. Dist.	Env. Dist.	Geog. Dist.			
Full model	-0.008	0.351	-0.090	0.014	125.6	0.001	0.670
Full w/o Geog. Dist.	-0.002	0.351	-0.081	—	188.5	0.001	0.670
Full w/o Env. Dist.	-0.044	0.305	—	0.007	184.3	0.001	0.663
Full w/o Mag. Dist.	-0.123	—	0.379	0.011	69.7	0.001	0.427
Mag. Dist. Alone	-0.039	0.307	—	—	370.1	0.001	0.663
Env. Dist. Alone	-0.118	—	0.386	—	139.8	0.001	0.427
Geog. Dist. Alone	0.164	—	—	0.104	14.9	0.010	0.074
n=190 for all models							

**Table 4.3: Summary statistics for all seven possible MMRR analyses when each possible combination of distance metrics is used by itself to predict genetic differentiation.**

The table provides summary statistics for all seven possible models, including: (1) the full model, which uses all three distance metrics together to predict genetic differentiation; (2) three models, each of which removes one distance metric from the full model; and (3) three models, each of which includes one of the three distance metrics alone. Although these analyses provide some insight into the pattern of genetic differentiation, it is important to note that they should not be considered in isolation; given that potential collinearity exists between magnetic, geographic, and environmental distance, the significance of each metric as a predictor of genetic differentiation is likely to be overestimated if all three metrics are not considered simultaneously. For this reason, appropriate conclusions can only be drawn when all three distance metrics are evaluated together through multivariate analysis (Table 4.1).

Variation Partitioning	
Parameter	Variation Explained
Mag. Dist. alone	0.243
Env. Dist. alone	0.004
Geog. Dist. alone	0.000
Mag. or Env. Dist.	0.348
Mag., Env., or Geog. Dist.	0.072
Full model $r^2 = 0.670$	

**Table 4.4: Variation in genetic differentiation explained by the full model partitioned into variation explained by each distance metric.**

To the extent possible, the results from the entire suite of seven models (Table 4.3) were used to partition the variation in genetic differentiation explained by the full model (67%). Magnetic distance alone can definitively account for at least 24.3% of the variation in genetic differentiation. By contrast, 0.4% of the variation could be unambiguously assigned to environmental distance alone, and 0.0% to geographic distance alone. The analysis could not unambiguously partition all of the variation. Because magnetic, environmental, and geographic distances are not orthogonal, 7.2% of variation could not be partitioned at all and is instead explained equally well by any of the three distance metrics. Similarly, for 34.8% of the variation geographic distance could be eliminated as an explanation, but the variation could not be further partitioned between magnetic and environmental distance. No amount of variation was shared between only magnetic and geographic distance, or between only environmental and geographic distance.

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## **CHAPTER 5: ALIGNMENT BEHAVIOR OF LOGGERHEAD SEA TURTLE EMBRYOS**

### **Introduction**

Sea turtles detect Earth's magnetic field and use geomagnetic information to guide complex movements throughout all stages of their lives [1–3]. Young turtles undertake tremendous open ocean migrations that can sometimes traverse entire ocean basins [4, 5]. These impressive migrations are largely thought to be guided by an elaborate magnetic map [1]. Hatchling loggerhead turtles inherit a set of orientation responses to specific combinations of geomagnetic inclination and intensity that mark locations along the migratory pathway [1, 6–8].

Later in their lives juvenile sea turtles also use a magnetic map to determine their position along coastlines and relative to foraging areas [2]. When green turtles were captured at feeding grounds and subjected to simulated magnetic displacements, they responded with striking orientation. Turtles exposed to a magnetic field that exists north of the foraging area responded by swimming overwhelmingly to the south and turtles exposed to a field in the south swam to the north.

Still later, as adults, turtles use geomagnetic information to guide the nesting migration and return to their natal beach [3, 9]. This evidence is consistent with the idea that turtles accomplish natal homing through the process of geomagnetic imprinting [10], but one of the central tenets of the hypothesis remains largely unexplored. Geomagnetic imprinting proposes not only that adults use Earth's magnetic field to return home, but also that young

sea turtles imprint on the local magnetic field prior to leaving the natal beach. Because hatchling turtles exit the nest and leave the beach quite quickly, it seems reasonable that imprinting might occur as an embryo when turtles remain relatively still for a long period of time.

We do not yet know, however, if embryos detect Earth's magnetic field, as is required if sea turtles imprint prior to hatching. For two reasons, it seems likely that magnetoreception develops prior to hatching and that embryos can also detect the geomagnetic field. Firstly, an extensive body of behavioral evidence indicates that young sea turtles can derive complex navigational information from Earth's magnetic field immediately after hatching [1]. Secondly, the magnetic environment during incubation can alter the subsequent magnetic orientation of hatchling turtles [11].

To begin investigating these ideas more directly we looked for embryonic behavior that is consistent with magnetoreception. Specifically, we investigated whether late stage sea turtle embryos orient non-randomly inside the egg. Diverse vertebrates appear to spontaneously align their bodies along the geomagnetic north-south axis (i.e along magnetic field lines) [12–15]. Our findings provide evidence for magnetic alignment in loggerhead sea turtle embryos, with most embryos facing either to the north or south but not to the east or west. In addition, the results are consistent with the idea that embryos accomplish this alignment by detecting Earth's magnetic field and orienting relative to magnetic north.

## **Methods**

### *In Situ Embryos*

We analyzed the orientation of loggerhead embryos (*Caretta caretta*) from *in situ* clutches (n=50, 10 from each of 5 clutches) on Wabasso Beach on the Atlantic coast of

Florida, USA (latitude=27.76° N, longitude=-80.39° W), which trends from north to south.

We also assessed the heading of dead embryos from *in situ* loggerhead clutches (n=261 total from 5 complete clutches) on Cape San Blas on the panhandle of Florida, USA (latitude=29.68° N, longitude=-85.30° W), a coastline that trends from east to west.

### *Laboratory Incubation Procedures*

In addition to observing *in situ* embryos we also conducted two independent laboratory experiments. For an initial experiment that took place in Gainesville, Florida, USA (latitude=29.64° N, longitude=82.35° W) we collected 25 eggs from each of two nesting females on Melbourne Beach, Florida, USA (Atlantic coast; latitude=28.05° N, longitude=80.55° W). The eggs were collected at oviposition and placed in polystyrene egg cartons. The cartons were then placed on top of beach sand within polystyrene coolers and transported by car. In the laboratory the eggs from each clutch were divided evenly across two treatment groups (described below) and incubated in simulated egg chambers within polystyrene coolers full of sand. The eggs were placed under incubation conditions within 6 hours of oviposition. The room was temperature controlled with a target temperature of 27.5° C. The sand in which the eggs incubated was misted regularly with water to ensure appropriate humidity.

For the second experiment we collected one entire clutch (n=96) directly after it was deposited on the beach on Bald Head Island, North Carolina, USA (latitude=33.85° N, longitude=77.978° W) and incubated the eggs in the laboratory at the Bald Head Island Conservancy. The eggs were placed under laboratory conditions within 2 hours of oviposition and were incubated in polystyrene egg cartons (n=12 per carton) that were placed on top of beach sand within polystyrene coolers. The mean incubation temperature was 29.5°

C and we regularly misted the inside of the cooler and within the carton when necessary to ensure appropriate humidity.

### *Magnetic Manipulation*

Each of the two laboratory experiments was conducted independently, and the resulting data of the two experiments were analyzed separately. The treatment groups used in the two experiments, however, were similar. During each experiment the eggs from each clutch were divided evenly across two treatments. Half of the eggs, which served as controls, developed in the unaltered ambient geomagnetic field, and the other half developed at the center of a magnetic coil system [16] that was used to rotate the direction of magnetic north counter-clockwise by 90 degrees (i.e. magnetic north inside the coil pointed towards true west). One experiment (25 eggs per treatment) used a coil that measured 0.92 meters on each side, and the other experiment (48 eggs per treatment) used a coil that measured 1.8 meters on each side. For each experiment eggs were restricted to an area at the center of the coil that measured less than one third the size of the coil.

### *Embryo Observation*

For *in situ* eggs and one of the laboratory experiments we assessed embryo orientation through destructive sampling. We first used a compass to mark a reference line on each egg in the direction of north before cutting open the egg and estimating the direction of the longitudinal cranial midline (i.e. head direction) relative geomagnetic north. The orientation of embryos on Wabasso beach and in the lab were estimated to the nearest 10 degrees; for embryos on Cape San Blas, orientation was estimated to the nearest 45 degrees. We observed developing *in situ* embryos by cutting open the egg on incubation day 56.

Experimental embryos from the laboratory were cut open on incubation day 60 after first euthanizing and immobilizing the embryo by submerging the egg in liquid nitrogen.

For one of the laboratory experiments we non-invasively assessed embryo orientation. We monitored the eggs once per hour and photographed embryos as they first broke through the eggshell, or “pipped”. Two observers who were unaware of the assigned treatment groups analyzed the photos independently and estimated the head direction of each embryo (again along the longitudinal cranial midline). We then measured head direction relative to north using ImageJ software. In our analyses we used the mean estimate of each embryo’s head direction as it broke through the eggshell as a proxy for the embryo’s orientation prior to hatching. Because some embryos pipped during brief gaps in monitoring (e.g. between 2AM and 5AM) we excluded embryos from the analyses if more than the head had emerged from the egg at the time of observation (n=11). We also did not assess embryo heading if the two observers were unable to determine head direction from the photo (n=2)

### *Statistical Analyses*

All analyses were done in R Version 3.3.2 [17]. To test for non-uniformity of a distribution we used the non-parametric Rao’s spacing test. If a distribution appeared to be bimodal and axial, we continued with standard circular statistics for axial data, which involve doubling each angle in the dataset prior to canonical analyses (e.g. Rayleigh test, Watson test) [18]. When appropriate we constructed bootstrap confidence intervals for the mean axis of bimodal orientation (1,000 iterations).

## Results

### *In situ embryos*

Striking non-random orientation was observed in developing *in situ* embryos from the Atlantic coast of Florida, USA ( $U=244.8$ ,  $p<0.0001$ ,  $n=50$ ; Rao's spacing test, Figure 5.1A). The majority of embryos faced either to the north or south but not east or west (i.e. the embryos were aligned approximately parallel to geomagnetic field lines). Similarly, we found non-random orientation of *in situ* dead embryos from the panhandle of Florida, USA ( $U=349.0$ ,  $p<0.0001$ ,  $n=261$ ; Rao's spacing test, Figure 5.1B). Moreover, a Rayleigh test for axial data revealed highly significant axial bimodal orientation along the north-south axis both for developing embryos (mean axis =  $1.8^\circ/181.8^\circ$ ,  $r=0.69$ ,  $p<0.0001$ ,  $n=50$ ; Rayleigh test) and dead embryos (mean axis =  $18.1^\circ/198.1^\circ$ ,  $r=0.19$ ,  $p<0.0001$ ,  $n=261$ ; Rayleigh test).

### *Laboratory Experiments*

When we brought embryos from the Atlantic coast of Florida, USA into the lab we found significantly different orientation between embryos that developed inside a magnetic coil that rotated the direction of magnetic north by  $90^\circ$  and control embryos that developed in the unaltered ambient geomagnetic field ( $U^2=0.35$ ,  $p<0.01$ ; Watson test). Both groups of embryos showed significantly non-random orientation (Coil:  $U=162.0$ ,  $p<0.05$ ,  $n=25$ ; Rao's spacing test. Control:  $U=163.7$ ,  $p<0.05$ ,  $n=23$ ; Rao's spacing test) and when all the data were analyzed together with each embryo's direction measured relative to its respective magnetic north we found significant bimodal orientation along the north-south axis (mean axis =  $4.5^\circ/184.5^\circ$ ,  $r=0.29$ ,  $p=0.019$ ,  $n=48$ ; Rayleigh test, Figure 5.2A).

Similarly, when we conducted a second laboratory experiment with embryos from North Carolina, USA and used "pipping" direction as a non-invasive proxy for embryo

heading, we found that embryos that developed in the rotated magnetic field within the coil showed significantly different orientation than control embryos that developed in the ambient geomagnetic field ( $U^2=0.24$ ,  $p<0.05$ ; Watson test). Additionally, embryos that developed inside the coil showed significant bimodal orientation consistent with the rotated magnetic field (mean axis =  $77.9^\circ/257.9^\circ$ ,  $r=0.40$ ,  $p=0.003$ ,  $n=35$ ; Rayleigh test). When both groups were analyzed together, relative to their respective directions of magnetic north, we found significant bimodal orientation along the magnetic north-south axis (mean axis =  $179.7^\circ/359.9^\circ$ ,  $r=0.21$ ,  $p=0.037$ ,  $n=75$ ; Rayleigh test, Figure 5.2B).

## **Discussion**

Taken together, our findings provide strong evidence that developing loggerhead sea turtle embryos align their bodies approximately along the geomagnetic north-south axis. In principle, there are several orientation cues that sea turtle embryos might use to achieve this alignment, but our findings are most consistent with the hypothesis that embryos align by detecting Earth's magnetic field. In two independent laboratory experiments, embryos showed significant alignment along the magnetic north-south axis regardless of whether they developed in the ambient geomagnetic field or within a magnetic coil system that rotated the direction of magnetic north by 90 degrees. It is not entirely surprising that late stage embryos might detect Earth's magnetic field in that sea turtles are capable of magnetoreception at all other life stages, including immediately after hatching.

Some possible alternative cues include temperature gradients, vibrations from wave-action on the beach, and light from the sun. Many of these cues, however, are correlated with the direction of the coastline, and we observed significant bimodal north-south orientation of embryos both on coastlines that trend north to south (Figure 5.1A) and also on coastlines that

trends east to west (Figure 5.1B). The vibrations that result from wave-action on the beach will always come from the seaward direction. Temperature gradients and light levels within the clutch might also be associated with the direction of the coastline inasmuch as they might be influenced by beach slope. In many cases, nesting beaches are on coastlines that trend north to south so these cues might coincidentally lead to alignment along the geomagnetic north-south axis. They do not, however, readily appear to be able to account for north-south alignment along an east-west coastline.

Additionally, many of these potential alternative cues are unlikely to be uniform across an entire clutch and, therefore, might not lead to consistent alignment of embryos. For example, there might be a temperature gradient on the beach, but the metabolism of the embryos themselves will likely cause these gradients to be inconsistent across the clutch and throughout the incubation period. Similarly, although light might filter through the sand the light that reaches embryos at the top, center, or bottom of the clutch is likely to come from different directions and be of varying intensities. By contrast, the direction of geomagnetic north would provide both a consistent and uniform orientation cue for the entire clutch and for all of development.

#### *Functional Significance of Alignment*

Although additional experiments are required to fully investigate the functional significance of the observed embryo alignment, numerous possibilities exist. For example, alignment might serve to ensure the appropriate calibration of the magnetic sense that sea turtles use to navigate throughout their lives. An additional possibility is that alignment might aid in natal homing by facilitating the precise detection of the magnetic signature that marks

the natal beach [19]. This is not intended to be an exhaustive list of the potential functions of embryo alignment and further studies are required to investigate the phenomenon.

Sea turtles derive navigational information from Earth's magnetic field as hatchlings, juveniles, and adults. Although the mechanism that turtles use to detect Earth's magnetic field remains a mystery, one hypothesis is that magnetic particles interact with the nervous system. In order for this mechanism to develop successfully and provide similar navigational information to all individuals it might be important that the position of these magnetic particles within the nervous system remains consistent across embryos. The embryo alignment that we observed might allow these magnetic particles to be positioned appropriately and incorporated successfully into the developing nervous system. If, on the other hand, embryos orient haphazardly and magnetic particles are positioned inconsistently, then each individual might assess the same magnetic stimulus differently and consequently derive unreliable navigational information.

An additional possibility is that alignment of embryos might facilitate geomagnetic imprinting and natal homing. Sea turtles migrate tremendous distances as young turtles before returning as adults to nest on the same stretch of coastline where they themselves hatched [20]. Turtles are thought to accomplish this natal homing behavior through a process called geomagnetic imprinting during which young animals learn the unique magnetic signature that marks their natal beach before using this information to return as adults [3, 10]. Because hatchling turtles emerge from the sand and enter the ocean quite quickly, a reasonable hypothesis is that imprinting might occur prior to hatching. In addition, certain theoretical considerations suggest that detection of magnetic field parameters might be simpler when animals face along geomagnetic field lines (i.e. north or south) [19, 21, 22].

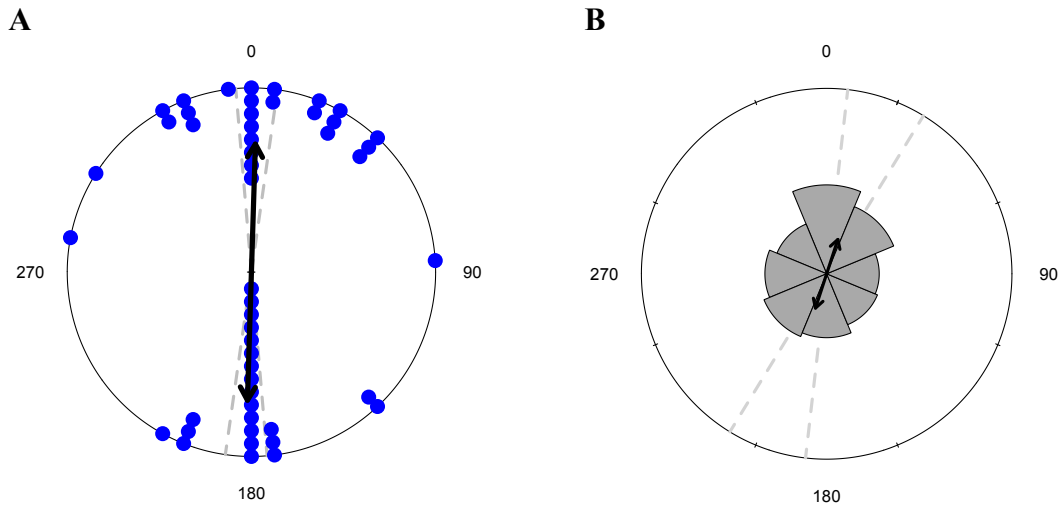
Therefore, it is possible that the observed north-south alignment of late stage sea turtle embryos might facilitate precise measurement of the local magnetic field and in turn play a role in geomagnetic imprinting and natal homing.

Regardless of these considerations our results provide strong evidence that developing loggerhead sea turtle embryos align their bodies inside the egg. The findings suggest that embryos might align along the north-south axis by detecting Earth's magnetic field prior to hatching and results provide evidence that the magnetic sense of animals exists in embryos.

### **Acknowledgements**

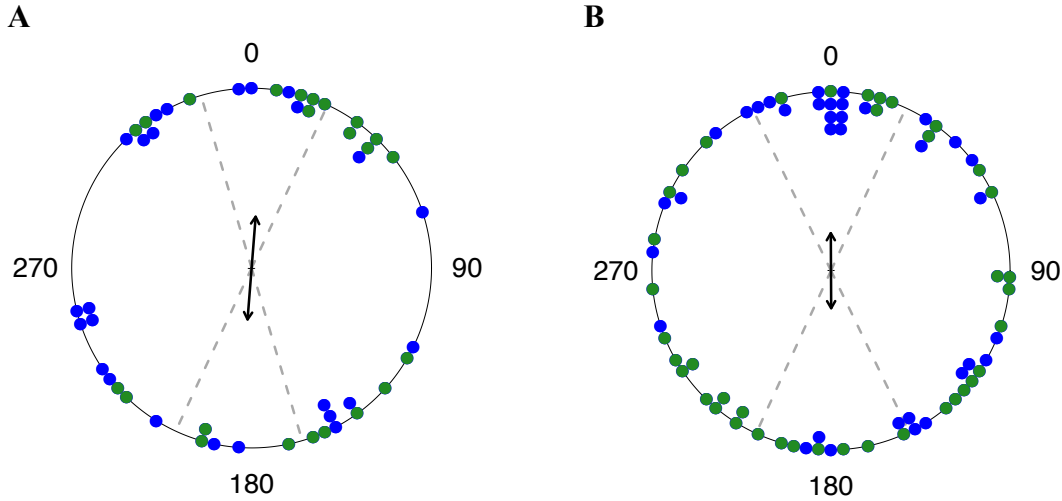
I would like to thank Ken Lohmann and Ray Carthy for their help conceptualizing, designing, and conducting the experiments. In addition, staff members and volunteers at the Bald Head Island Conservancy provided assistance throughout the project.

## Figures



**Figure 5.1: Orientation of *in situ* loggerhead embryos from Florida, USA.**

Each data point represents the heading of one loggerhead sea turtle embryo relative to geomagnetic north. Late stage developing embryos from a north-south trending nesting beach on the Atlantic coast of Florida, USA (A) showed highly significant bimodal orientation with most embryos facing either north or south but not east or west (mean axis =  $1.8^\circ/181.8^\circ$ ,  $r=0.69$ ,  $p<0.0001$ ,  $n=50$ ; Rayleigh test). Similarly, dead embryos from an east-west trending nesting beach on the Gulf coast of Florida, USA (B) showed significant alignment along the geomagnetic north-south axis (mean axis =  $18.1^\circ/198.1^\circ$ ,  $r=0.19$ ,  $p<0.0001$ ,  $n=261$ ; Rayleigh test). Due to the large sample size in panel B we chose a circular histogram to represent the data. In each plot the arrow represents the mean axis of bimodal orientation and dotted lines indicate the 95% confidence interval for the mean axis; the length of the arrow is proportional to the magnitude of the mean vector ( $r$ ), with the diameter of the circle representing  $r=1$ .



**Figure 5.2: The headings of embryos (relative to magnetic north) from two independent laboratory experiments during which some embryos developed in the ambient geomagnetic field (green dots) and others developed inside a magnetic coil system (blue dots).**

One experiment was conducted in Gainesville, Florida USA and involved measuring the alignment of embryos (A). A second took place on Bald Head Island, North Carolina and used pipping direction of hatchlings as an indicator of late-embryo alignment (B). Each data point represents the heading of one embryo or hatchling measured relative to the respective magnetic north of that turtle's treatment group. In each experiment some embryos developed in the unaltered ambient geomagnetic field and others developed inside a magnetic coil system that rotated the direction of magnetic north counter-clockwise by 90° (i.e. inside of the coil magnetic north pointed towards true west). In these plots the data are normalized for magnetic north so that for embryos that developed under control conditions (green dots) 0° indicates geomagnetic north but for embryos that developed inside of the coil (blue dots) 0° indicates the direction of the rotated magnetic north. In an initial experiment embryos that developed both inside the coil and under control conditions showed significant non-random orientation (Coil:  $U=162.0$ ,  $p<0.05$ ,  $n=25$  Rao's spacing test; Control:  $U=163.7$ ,  $p<0.05$ ,  $n=23$ , Rao's spacing test). The two distributions were significantly different from each other when embryo heading was measured from true north ( $U^2=0.35$ ,  $p<0.01$ ; Watson test), but when the two groups were co-plotted with the data normalized for magnetic north (A) embryos aligned significantly along the magnetic north-south axis (mean axis = 4.5°/184.5°,  $r=0.29$ ,  $p=0.019$ ,  $n=48$ ; Rayleigh test). An additional experiment provided similar results; when embryo heading was measured from true north the orientation of embryos that developed inside of the coil was significantly different from the orientation of embryos that developed under control conditions ( $U^2=0.24$ ,  $p<0.05$ ; Watson test). When co-plotted relative to each treatment's respective magnetic north, however (B), embryos aligned significantly along the magnetic north-south axis (mean axis = 179.7°/359.9°,  $r=0.21$ ,  $p=0.037$ ,  $n=75$ ; Rayleigh test). In each plot the arrow represents the mean axis of bimodal orientation and dotted lines indicate the 95% confidence interval for the mean axis; the length of the arrow is proportional to the magnitude of the mean vector ( $r$ ), with the diameter of the circle representing  $r=1$ .

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## **CHAPTER 6: EVIDENCE FOR ORIENTATION OF IN VIVO OLIVE RIDLEY SEA TURTLES EMBRYOS**

### **Introduction**

There is now evidence that all life stages of sea turtle, including embryos, detect Earth's magnetic field. Hatchling, juvenile and adult turtles derive navigational information from Earth's field [1–3], and loggerhead sea turtle embryos appear to orient along the magnetic north-south axis during late stages of development. Investigation of embryonic orientation, however, has largely involved destructive sampling and the direct observation of embryos. Although these techniques are effective, they are limited to relatively small sample sizes, and are thus, not conducive to manipulative experiments with threatened species. Therefore, to further investigate this exciting phenomenon, we developed an objective, non-invasive approach for assessing the orientation of *in vivo* embryos. We used trans-illumination, or “candling,” to observe living olive ridley sea turtle embryos and investigate whether non-random orientation also occurs in embryos of this species.

Although we found more variability than with loggerhead turtles, we consistently observed non-random orientation in late-stage olive ridley embryos. In addition, we found a significant difference between the orientation of embryos that developed in the unaltered ambient geomagnetic field and those that developed inside a magnetic coil system. The findings corroborate previous evidence from loggerhead turtles and are consistent with the idea that embryos might orient by detecting earth's magnetic field.

## Methods

### *Animals*

We investigated embryo orientation in olive ridley sea turtles (*Lepidochelys olivacea*) from Ostional Beach, on the Pacific coast of Costa Rica. We collected 888 total eggs from 13 nesting females. The eggs were collected at oviposition and carried by hand to a hatchery that was on the nesting beach and no more than a few hundred meters away. All eggs were placed within the hatchery under incubation conditions within three hours of oviposition. We observed some embryos to examine natural orientation, but also conducted an experiment to investigate the effects of magnets and magnetic coils on the direction of embryo orientation.

### *Natural Incubation*

For eggs from three of the females (n=80 eggs for each clutch) we kept the clutch intact and incubated the eggs in three separate artificial egg chambers within the hatchery. We assessed embryo heading for one of these clutches on incubation day 36 and the other two on incubation day 41.

We also distributed 360 of the eggs (n=60 from each of 6 clutches) into 30 polystyrene cartons (n= 12 eggs per carton, 2 from each of the 6 clutches). The cartons were buried in 1m<sup>2</sup> plots within the hatchery (5 plots, n=72 eggs and 6 cartons per plot) at the depth of a natural egg chamber. We then assessed embryo heading for one entire plot each night beginning on incubation day 37 and ending on incubation day 41.

### *Magnetic Manipulation Experiment*

The remaining 288 eggs were used in an experiment that involved excavating eggs towards the end of development and reburying them in one of three treatments (n=96 eggs per treatment). Some eggs were excavated and reburied in the unaltered ambient

geomagnetic field, some eggs were reburied in the presence of magnets, and other eggs were reburied inside magnetic coil systems that were used to rotate the direction of north counter-clockwise by 90° [4]. We assessed embryo orientation after the eggs had remained under treatment conditions for 20 hours.

Immediately after collection the 288 eggs were distributed among 24 polystyrene cartons (n=12 eggs per carton) and buried in 1m<sup>2</sup> plots (4 plots, n=72 eggs and 6 cartons per plot). The eggs came from 10 different females and the eggs from each female were divided evenly across the three treatments. We did not, however, use an equal number of eggs from each female during this experiment (Table 1).

For the experiment, we excavated two 6 carton plots on incubation day 41 and one 6 carton plot on each of incubation days 42, and 43. Within 30 minutes of excavation the cartons were reburied under treatment conditions at a new location within the hatchery. The cartons were reburied in pairs, with one carton stacked on top of the other. The magnet and control treatments were spatially interspersed and their positions were switched for each of the three successive iterations (i.e. day 41, 42, or 43). We used two magnetic coil systems (details below) that were separated from each other and from the other treatments to ensure that the magnetic fields that they generated did not reach embryos in the other locations.

To ensure that the initial position of incubation did not elicit spurious results we evenly distributed the cartons from each of the excavated plots to each of the new treatment locations. In this way both the original location of incubation and the new location of the treatment conditions are interspersed across the treatments and, therefore, cannot be implicated in the results.

Each of two magnetic coil systems [4] measured 0.65m on a side and were used to rotate the direction of north counter-clockwise by 90° (i.e. magnetic north inside of the coils pointed towards true west). For the magnet treatment we buried three ceramic disk magnets between each pair of cartons and alternated whether the north pole of the magnet pointed up or down. As a result, the direction of magnetic north pointed in a different direction at each position within the carton.

#### *Trans-illumination or “Candling”*

To assess embryo heading we placed a bright LED light beneath each egg. This technique, known as candling, allows for visualization of embryos because light readily passes through the eggshell, yolk, and albumen, but not through more opaque structures such as the carapace, flippers, or skull. Specifically, we used the caudal and nuchal scutes, and the longitudinal mid-dorsal ridge along the carapace as anatomical landmarks.

Immediately after excavating an egg we first used a wax pencil to draw a reference arrow on top of the eggshell in one of the eight cardinal or ordinal directions (e.g. magnetic north, northeast, east, etc.). Next, an observer who was unaware of the alignment of the reference arrow candled the egg and drew a second arrow on the eggshell that followed the mid-dorsal ridge of the embryo. We then photographed the intersection of these two arrows and measured the angle between them in ImageJ. Using the angle of intersection between the two arrows and the known direction of the reference arrow we calculated the orientation of the embryo relative to geomagnetic north. Using this methodology we non-invasively and objectively estimated each embryo’s heading.

The time from excavation to candling was never more than 30 minutes. Some eggs had mold infestations, creases from dehydration, or contained undeveloped embryos; these eggs could not be candled and, therefore, were not included in analyses.

### *Statistical Analyses*

All statistical analyses were done in R version 3.3.2 [5] and included a combination of canonical circular statistics [6] and more recently developed maximum likelihood analyses for circular data [7]. To test for orientation we used the Rayleigh test and if data appeared to be axially bimodal we doubled each angle prior to analysis [6]. To test for a difference in circular dispersion we used Wallraff's rank-sum test, which uses the non-parametric Kruskal-Wallis H test and applies it to circular data. We also incorporated maximum likelihood analyses that do not assume that the data have unimodal or axially bimodal means. Instead, the likelihood-based approach uses AIC values to select the best fitting model from 12 different models for circular data. The set of potential models is described in detail elsewhere [7, 8], but briefly it comprises a uniform model (random data), a unimodal model (one mean direction), and various mixtures of two different models. These mixtures can describe axially bimodal data (i.e., a mixture of two unimodal models with mean angles that are directly opposite one another), bimodal data (i.e., a mixture of two unimodal models with means that are not opposite one another), or a mixture of a unimodal model and a uniform model (i.e., some data that are oriented and some data that are random).

### **Results**

When we observed embryos that developed as intact clutches from three different females we saw considerable variation in embryo orientation (Figure 6.1). The headings of embryos from a single clutch that were observed on day 36 of incubation were statistically

indistinguishable from random ( $r=0.2$ ,  $p=0.98$ ,  $n=64$ ; Rayleigh test, Figure 6.1A). By contrast, embryos from one clutch that was observed later in development (day 41, Figure 6.1B) oriented significantly northeast ( $36.8^\circ$ ,  $r=0.30$ ,  $p=0.016$ ,  $n=44$ ; Unimodal Rayleigh test); for the other clutch that was observed on day 41 (Figure 6.1C), however, we were unable to detect non-random orientation ( $r=0.12$ ,  $p=0.50$ ,  $n=47$ ; Rayleigh test).

With groups of eggs derived from multiple females, non-random orientation was not observed on incubation days 37 ( $r=0.05$ ,  $p=0.86$ ,  $n=62$ ), 38 ( $r=0.12$ ,  $p=0.41$ ,  $n=66$ ), or 39 ( $r=0.17$ ,  $p=0.20$ ,  $n=55$ ) (all Rayleigh tests, Figures 6.2A, 6.2B, and 6.2C respectively). We did, however, reliably find significant and axially bimodal orientation in embryos that we observed later in development (Figures 6.2D and 6.2E). Interestingly, the embryos that we observed on day 40 ( $170^\circ/350^\circ$ ,  $r=0.33$ ,  $p<0.005$ ,  $n=49$ , Rayleigh test, Figure 6.2D) oriented along a slightly shifted axis from those that we observed on day 41 ( $121.9^\circ/301.9^\circ$ ,  $r=0.25$ ,  $p=0.015$ ,  $n=67$ ; Rayleigh test, Figure 6.2E).

Lastly, when we conducted an experiment that involved manipulating the direction of magnetic north that the embryos experienced, the headings of embryos that spent time inside a magnetic coil were significantly more dispersed than the headings of embryos that remained in the ambient geomagnetic field ( $\chi^2=5.04$ ,  $p=0.02$ , Wallraff's test). Additionally, when we compared the orientation of embryos in the presence or absence of magnets that induced variation in the direction of magnetic north the dispersion in orientation between the magnet and control groups approached significance ( $\chi^2=3.18$ ,  $p=0.07$ , Wallraff's test). Control embryos that were excavated and reburied in the unaltered geomagnetic field aligned significantly but were offset from the geomagnetic north-south axis ( $151.3^\circ/331.3^\circ$ ,  $r=0.248$ ,  $p<0.01$ ,  $n=79$ ; Rayleigh test, Figure 6.3A). By contrast, as expected, embryos that were

reburied with magnets were statistically indistinguishable from random ( $r=0.049$ ,  $p=0.83$ ,  $n=79$ , Rayleigh test, Figure 6.3B). Interestingly, those embryos that were reburied inside a magnetic coil system for 20 hours failed to orient relative to the rotated axis of magnetic north and were also statistically indistinguishable from random ( $r<0.01$ ,  $p>0.99$ ,  $n=73$ ; Rayleigh test, Figure 6.3C)

In all cases the canonical circular statistics and the maximum likelihood approach led to identical conclusions. In other words, in cases that a Rayleigh test failed to detect significant orientation the maximum likelihood analysis selected the uniform model as the best fit. Similarly, in instances when a Rayleigh test for axial data detected significant bimodal orientation the maximum likelihood approach selected a symmetric axially bimodal model with a mean axis of orientation within one degree of that revealed by the Rayleigh test.

## **Discussion**

These results suggest that olive ridley sea turtle embryos orient non-randomly during the later stages of development. The findings corroborate initial studies with loggerhead turtles by providing evidence for embryo orientation in a second species of sea turtle. Additionally, the work demonstrates that orientation of sea turtle embryos can be detected and investigated through a non-lethal methodology.

The orientation results from when we exposed eggs to rotated directions of magnetic north suggest that developing embryos might detect earth's magnetic field and use it to orient prior to hatching. Specifically, we found a significant difference in the orientation of embryos that were excavated and placed inside a magnetic coil system relative to control embryos that were treated identically but placed back in the unaltered ambient geomagnetic

field. Moreover, this difference arose after only 20 hours under treatment conditions. Control embryos aligned significantly along the northwest-southeast axis (Figure 6.3A), but the headings of embryos placed inside the coil or in the presence of magnets were statistically indistinguishable from random (Figures 6.3B and 6.3C, respectively). It is interesting to consider why the embryos placed inside the coil did not rotate their alignment and orient significantly relative to the rotated magnetic north produced by the coil. This result does not, however, indicate that the direction of north is irrelevant and at least two possibilities are compatible with the data. Embryos might simply take longer than 20 hours to readjust their orientation, or, the late stage embryos might be large enough that their mobility inside of the egg is limited.

Although we consistently observed non-random orientation in late stage olive ridley embryos, the situation appears more complex than with loggerhead embryos. For example, observations with loggerhead embryos suggest that they exclusively align their bodies along the geomagnetic north-south axis. By contrast, ridley embryos appear to orient in a different direction and with more variation. Although we often observed axial bimodal orientation, the typical mean axis was offset from geomagnetic north-south and was more often closer to northwest-southeast (Figures 6.2D, 6.2E, and 6.3A). In one case, however, we also observed unimodal orientation to the northeast (Figure 6.1B), and on another occasion we observed random orientation in seemingly healthy late stage embryos (Figure 6.2C).

From the current data it is not yet possible to determine the cause of the variation between loggerhead and olive ridley embryos. It is interesting to note, however, that the coastline on the ridley nesting beach does not run directly north to south, but instead trends along the northwest-southeast axis. Therefore, although we found evidence that Earth's field

matters in both species, it is possible that ridley embryos are more influenced by additional environmental cues than are loggerhead embryos.

Similarly, it is not possible from our data to fully interpret why we consistently observed non-random orientation of embryos at later stages but not earlier in development. It is possible that only late-stage embryos are capable of orienting, potentially because they have a fully developed magnetic sense that does not yet exist in younger embryos.

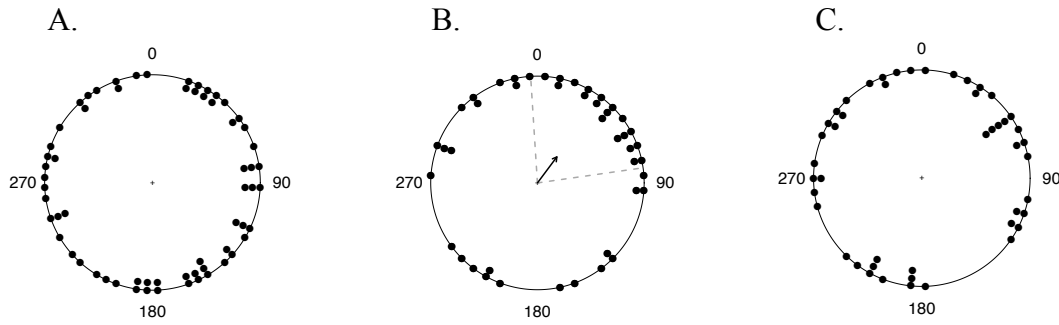
Alternatively, young embryos might be small enough that they are more mobile inside the egg and there is consequently more variation in their headings. Yet another possibility is that young embryos also orient but our methodology was not capable of detecting it.

Regardless of these considerations, our findings provide evidence that olive ridley sea turtle embryos orient non-randomly during the later stages of development. As in loggerhead embryos, orientation is typically axially bimodal and might represent magnetic orientation prior to hatching. The results are likely relevant to sea turtles generally in that we now have evidence for embryo alignment in two species of sea turtles and on four widely separated nesting beaches.

### **Acknowledgements**

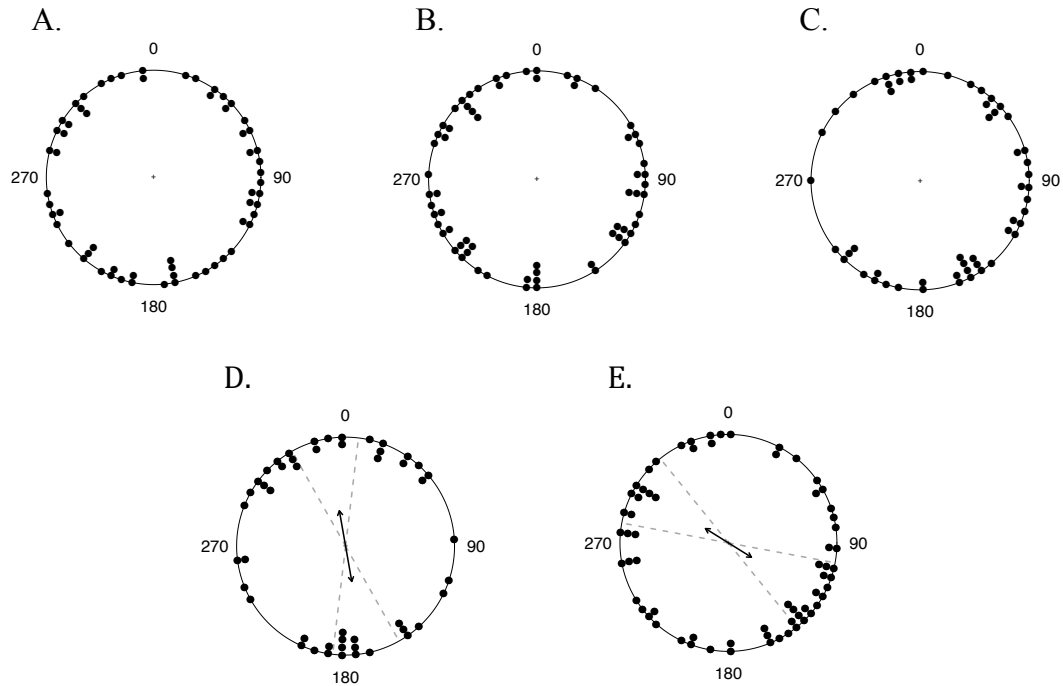
I would like to thank Ken Lohmann and Ray Carthy for their assistance conceptualizing and designing these experiments. Vanessa Bézy was instrumental in conducting the experiments and numerous volunteers and staff members at the Ostional National Wildlife Refuge research station provided additional assistance.

## Figures



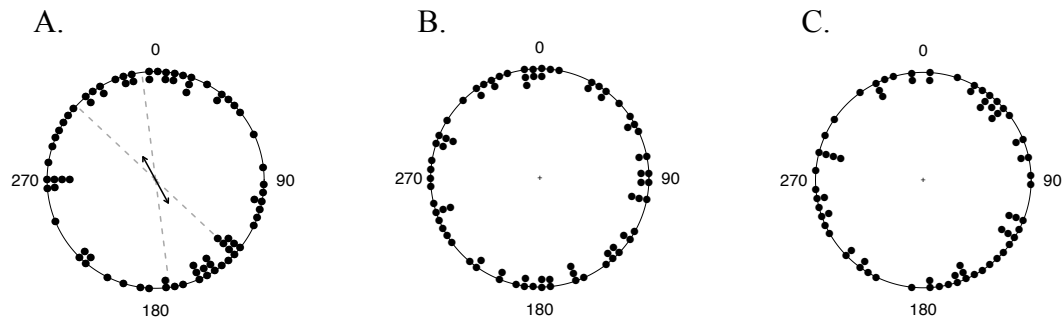
**Figure 6.1: Olive ridley embryo headings from three different clutches that were visualized on incubation day 36 (A) and 41(B and C).**

Each panel represents a clutch from a different nesting female and each data point represents the heading of one embryo relative to geomagnetic north. Embryos from three nesting females were incubated as intact clutches in three separate artificial egg chambers. We observed the heading of embryos from one clutch on incubation day 36 (A) and did not detect significant orientation of embryos. When we observed different embryos on incubation day 41, however, we found that one clutch had embryos that were oriented significantly to the northeast (B) and a second had embryos that were statistically indistinguishable from random (C). The arrow shows the mean angle of orientation and the grey dotted lines indicate the 95% confidence interval for the mean. The length of the arrow is proportional to the magnitude of the mean vector, with the radius of the circle corresponding to  $r=1$ .



**Figure 6.2: Orientation of olive ridley embryos visualized on incubation days 37-41.**

Each panel represents a group of embryos that were observed on the same incubation day and each data point represents the heading of one embryo relative to geomagnetic north. Eggs laid by six different females were divided equally across the five groups. Although we were unable to detect significant orientation when embryos were observed relatively early in development (on incubation days 37 (A), 38 (B), and 39 (C)), we observed significant axially bimodal orientation on both incubation day 40 (D) and 41 (E). The arrows show the mean axis of orientation and the grey dotted lines indicate the 95% confidence interval for the mean axis. The length of the arrow is proportional to the magnitude of the mean vector, with the diameter of the circle corresponding to  $r=1$ .



**Figure 6.3: Olive ridley embryo orientation during a three-treatment experiment that involved rotating the direction of magnetic north.**

Each panel represents a different treatment group and each data point represents the heading of one embryo relative to geomagnetic north. Embryos were excavated and reburied towards the end of development; some embryos were reburied in the unaltered ambient geomagnetic field (A), some embryos were reburied in the presence of magnets to induce variation in the direction of magnetic north (B), and other embryos were reburied inside magnetic coil systems used to rotate the direction of magnetic north by 90° (C). After 20 hours under treatment conditions we observed the embryos' headings. We found a significant difference in orientation between control embryos and those that were placed inside a magnetic coil ( $p=0.02$ , Wallraff's test). Control embryos oriented significantly along an axis slightly offset from geomagnetic north-south, but embryos placed in either the presence of magnets, or inside a magnetic coil were statistically indistinguishable from random. The doubleheaded arrow shows the mean axis of orientation and the grey dotted lines indicate the 95% confidence interval for the mean axis. The length of the arrow is proportional to the magnitude of the mean vector, with the diameter of the circle corresponding to  $r=1$ .

Female	1	2	3	4	5	6	7	8	9	10
Eggs per treatment	20	16	12	12	8	8	8	4	4	4

**Table 6.1: The number of eggs per treatment from each of ten clutches.**

This table shows how many eggs from each of 10 females were used per treatment in our magnetic experiment (Figure 6.3). For each female the same number of eggs was used in each treatment but we did not use the same number of eggs from each female.

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## **CHAPTER 7: CONCLUSIONS**

Natal homing represents a fascinating aspect of animal behavior that is both enigmatic and phylogenetically widespread [1–5]. For more than a century scientists have been amazed by the ability of animals to migrate long distances before returning to reproduce in relatively precise locations [6]. Only recently, however, have we begun to understand how animals accomplish natal homing and there is now strong evidence that diverse taxa use Earth's magnetic field to return to their natal areas [7–11].

For sea turtles, the findings of my research provide four distinct lines of inquiry, each of which is consistent with the idea that sea turtles return to their natal beach by seeking out unique magnetic signatures along the coast [1, 7, 8]. Behavioral evidence indicates that nesting females use earth's magnetic field to determine their position relative to the natal beach. Two separate population level analyses confirmed independent predictions of the geomagnetic imprinting hypothesis. And, an investigation of embryonic behavior suggests that developing embryos detect Earth's magnetic field, as is required if turtles learn the magnetic signature of their natal beach prior to hatching.

The results presented in chapter two provide evidence that nesting females use magnetic navigation to guide their nesting migration and return to the natal beach. In orientation trials nesting females responded to a simulated magnetic displacement by swimming in a direction that would take them back to the nesting beach, as though they had actually been geographically displaced. This finding suggests that adult turtles possess a

magnetic map and can use magnetic cues alone to determine their position relative to the natal beach.

Additionally, the fact that sea turtles use Earth's magnetic field to find the natal beach appears to have profound ecological implications. Chapters three and four investigate these implications and their findings provide strong evidence that sea turtles accomplish natal homing by seeking out magnetic signatures along the coast. The two complementary chapters both examine population level trends and reveal striking relationships between natural variation in Earth's magnetic field and sea turtle nesting ecology.

Chapter three investigates an interesting consideration for animals that use magnetic navigation to return to natal areas: earth's magnetic field naturally changes over time. As a result of this change the magnetic signatures that mark the location of nesting beaches can drift along the coast while turtles are gone. When we analyzed these changes, however, in combination with a long-term dataset that describes the nesting distribution of a major sea turtle rookery, we found a strong relationship between subtle changes in earth's magnetic field and where sea turtles choose to nest [7].

In addition, magnetic navigation to natal beaches can apparently help to shape the genetic population structure of sea turtles. When we examined the pattern of genetic differentiation across one of the largest loggerhead nesting aggregations in the world [12] analyses revealed a striking relationship between spatial variation in Earth's magnetic field and the genetic differentiation between nesting beaches. We found no evidence, however, for isolation by distance or isolation by environment when we also considered spatial geomagnetic variation. This result suggests that over evolutionary time sea turtles have

consistently accomplished natal homing by returning to the magnetic signature that marks their natal beach.

The findings of these two investigations are complementary because each study uses an independent approach to measure both the variation in earth's field (temporal vs. spatial) and the corresponding change in the sea turtle population (change in nesting density over time vs. genetic population structure). Each analysis is powerful on its own, but when considered in combination the findings provide strong evidence that nesting females turtles return to nest at the magnetic signature of their natal beach.

Chapters two, three, and four all provide evidence that adult turtles use earth's magnetic field to guide nesting migrations. Chapter five, however, begins to investigate whether sea turtle embryos also detect Earth's field, as is required for turtles to learn the magnetic signature of the natal beach prior to hatching. We consistently observed non-random orientation of late stage sea turtle embryos from two species and four widely separated nesting beaches. Additionally, evidence from multiple experiments that manipulated the magnetic field during incubation suggests that embryos detect Earth's magnetic field and use the direction of magnetic north to orient prior to hatching.

Taken together the findings from all four chapters provide strong evidence that sea turtles use earth's magnetic field to accomplish natal homing and are consistent with the geomagnetic imprinting hypothesis [8]. Imprinting refers to a specific form of learning that typically occurs early in an animal's life and is restricted to a critical window of time. The effects are long lasting and the learned information cannot usually be modified.

Although our results are consistent with the idea that turtles imprint on the magnetic signature of their natal beach before leaving, the timing of imprinting remains unclear.

Acquiring the information could hypothetically take place over a relatively long period of time, (e.g., the entire incubation period) or a short window, such as while the hatchlings leave the nest and crawl to the ocean. Determining the developmental period during which the magnetic signature of the natal beach is learned requires further investigation and would inform the conservation of sea turtles worldwide.

It is also unclear whether turtles imprint on other aspects of the natal beach in addition to the magnetic field. It is possible that sea turtles, as do salmon [2], learn the chemical signature of their natal area and use olfaction to refine homing after magnetic navigation brings them close to the natal beach [1]. This seems particularly relevant for island finding and mass nesting events; in each case turtles can return to beaches that span only a few kilometers [13, 14]. Magnetic navigation alone is unlikely to account for such precise homing and multimodal navigation that uses local cues (e.g., olfactory or otherwise) is possible, if not likely [13, 15]. In addition, the use of secondary local cues might also help to minimize navigational errors due to secular variation [15].

Similarly, learning mechanisms outside of classical imprinting might function in concert with geomagnetic imprinting to mitigate the effects of secular variation on natal homing. For example, turtles might periodically update their knowledge of the magnetic signature that marks the natal beach [1, 7, 8]. A turtle could return to an imprinted magnetic signature of its natal beach during its first nesting migration, but not nest reflexively at that signature each time she returns to nest. Instead, nesting females might retain the original and unmodified imprinted magnetic signature of the natal beach but also acquire the magnetic waypoints of favorable or unfavorable nesting beaches as they gain experience throughout their lives.

At least one additional possibility exists. It is interesting to consider the potential intersection of natal homing and the magnetic map that guides young turtles on their open ocean migration. Hatchling turtles inherit an elaborate set of orientation responses to numerous magnetic fields that mark locations along their migratory pathway [16]. One potential explanation is that turtles reflexively swim in a particular direction any time they encounter a certain magnetic field. Within the context of this framework, it is hypothetically possible that turtles do not imprint on, or even learn, the magnetic signature of their natal beach each generation, but instead inherit specific magnetic coordinates that describe where to nest.

Alternatively, the process of geomagnetic imprinting might organize the magnetic map of hatchling turtles. The set of magnetic coordinates that young turtles use to guide their open sea migration might not be inherited as fixed and absolute pairings of inclination and intensity. Instead, it is possible that turtles imprint on the magnetic field at the natal beach before they leave, and that this imprinted signature calibrates the entire magnetic map. In other words, the coordinates that elicit orientation responses and guide turtles throughout their oceanic migration might be encoded relative to magnetic field at the home beach. This organization of the magnetic map might be able to mitigate the effects of natural changes in earth's magnetic field. Although the inclination and intensity that mark locations around the migratory pathway can drift as a result of secular variation, this drift might be correlated geographically. Therefore, the change over time in the magnetic coordinates that mark a given location might tend to be larger if they are encoded as an absolute magnetic field than if they are encoded relative to the natal beach.

These considerations aside, the results of my research provide invaluable insight into an enigmatic mystery of animal behavior and provide strong evidence that sea turtles use Earth's magnetic field to accomplish natal homing. The findings are consistent with the geomagnetic imprinting hypothesis and are likely applicable to diverse long-distance migrants.

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