

Perception of Airborne Chemosensory Cues by Sea Turtles

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ABSTRACT

COURTNEY STANTON ENDRES: Perception of Airborne Chemosensory
Cues by Sea Turtles
(Under the direction of Kenneth J. Lohmann, Ph.D.)

For sea turtles, an ability to locate nesting regions and foraging areas is vital. Turtles probably rely on several sensory cues to find these areas, some of which may be chemical in nature. Sea turtles can detect chemical cues in water, and because they surface to breathe, they potentially also have access to olfactory cues in air. To determine whether sea turtles can detect airborne chemical cues, loggerhead turtles (*Caretta caretta*) were exposed to air that had passed across a cup containing their food or distilled water. Food odors elicited increased searching behavior only after turtles surfaced to breathe, implying that turtles could detect airborne food cues.

During long-distance migrations, many sea turtles feed on invertebrates that are abundant in productive ocean regions. An ability to distinguish these regions from other areas might be adaptive. The volatile compound dimethyl sulfide (DMS) accumulates in the air above productive areas, and might serve as an indicator of high prey density for turtles. To determine whether turtles perceive DMS, loggerheads were exposed to air scented with DMS, distilled water, or several non-oceanic odors. Turtles exposed to DMS spent more time at the water surface than did turtles exposed to other odors, implying that turtles can detect DMS and might use this odor as a foraging cue.

An ability to detect land masses from some distance away, and to distinguish coastal areas from the open sea, might also be adaptive for turtles. In additional experiments, turtles exposed to coastal mud were shown to spend more time at the

water surface than did control turtles, suggesting that they can perceive an odor associated with land.

Turtles navigating to natal areas have been hypothesized to rely on a combination of magnetic and olfactory cues. To determine whether such a strategy is plausible for turtles that nest on islands, I modeled airborne and waterborne odor plumes emanating from Ascension Island (a remote island in the South Atlantic) and overlaid these on maps of magnetic intensity. Results indicated that turtles can plausibly reach the vicinity of the island using magnetic cues, then locate the island using chemical cues.

To Josh and my parents, who have learned more about sea turtles and
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CHAPTER ONE: INTRODUCTION

Numerous marine species undertake long-distance migrations. For example, tuna (Rooker et al., 2008), salmon (Dittman and Quinn, 1996), great white sharks (Bonfil et al., 2005), sea turtles (Carr et al., 1982; Allard et al., 1994; Encalada et al., 1998), and elephant seals (Stewart and DeLong, 1994; 1995) migrate extraordinary distances, particularly when they reach maturity and return to their natal regions to reproduce. The cues these animals use to navigate to natal areas remain largely enigmatic, but research over the last several decades has begun to illuminate some of the sensory abilities that may be involved. Similarly, little is known about how marine animals locate foraging areas during their migrations. Because all oceanic regions are not equally favorable in terms of foraging, an ability to rapidly identify favorable areas, and to concentrate foraging in such locations, might be adaptive. Thus, for migratory marine animals, the ability to home to natal sites, as well as the ability to locate productive oceanic foraging areas, are vital to survival and likely depend on several sensory cues.

Most species of sea turtle migrate long distances throughout their lives. The first migration typically begins when hatchlings emerge from nests buried on sandy beaches, crawl to the sea, and swim offshore. Young turtles rely on several sensory cues to accomplish this sequence of events. Visual cues guide them from the nest to the sea (Mrosovsky, 1978; Limpus, 1971; Witherington and Bjorndal, 1991; Salmon et al., 1992; Salmon and Wyneken 1990; 1994), and wave cues allow them to swim away from shore (Salmon and Lohmann, 1989; Lohmann et al., 1990; Wyneken et al., 1990; Lohmann and Lohmann 1992). Once turtles enter the water, they begin their first long-distance

migration, which may take them across entire ocean basins (Carr, 1987; Bolten et al., 1998; Bowen et al., 1995; Lohmann et al., 2012).

Young loggerhead turtles from the east coast of the United States spend a number of years in the North Atlantic Gyre system, the circular current system that flows around the Sargasso Sea, before eventually returning to North America (Carr, 1987; Bolten et al., 1998). Previous experiments have shown that young turtles are able to remain within the North Atlantic Gyre by exploiting positional information in the earth's magnetic field (Lohmann and Lohmann, 1994; 1996; Lohmann et al., 2001; 2012; Putman et al., 2011).

Magnetic field lines intersect the earth at a specific angle of inclination that varies with latitude. In addition, the intensity or strength of the earth's field also varies predictably over the surface of the globe. Distinct combinations of inclination angle and intensity exist along the turtles' migratory route. Hatchling turtles have shown an ability to detect both magnetic intensity (Lohmann and Lohmann, 1996) and inclination angle (Lohmann and Lohmann, 1994), and thus might use these to derive positional information during their migration around the gyre. Hatchlings subjected to magnetic fields that exist at the edges of the gyre were shown to swim in directions that enabled them to remain within the gyre (Lohmann et al., 2001; 2012; Putman et al., 2011), implying that hatchling turtles have a 'magnetic map', in which specific magnetic fields initiate changes in swimming direction at particular locations.

Older turtles are also known to use the earth's magnetic field to navigate (Lohmann et al., 2004; 2007). Juvenile green sea turtles captured from a foraging area along the east coast of Florida and placed in a simulated magnetic field that existed north or south of this area swam in the appropriate direction to take them back to their foraging area (Lohmann et al., 2004). In addition, green turtles attempting to home to Mayotte Island with magnets on their heads took longer and more convoluted routes to

the island than did turtles without magnets (Luschi et al., 2007), suggesting that an inability to use magnetic cues made homing difficult for these turtles.

After up to several decades in distant oceanic locations, most sea turtles return to their natal region to reproduce (Meylan et al., 1990; Bowen et al., 1993; 1994; 1995). The cues female turtles use to return to their natal beach are not fully understood, but it has been hypothesized that as hatchlings, they imprint on some feature of the beach in order to return to it later (Lohmann et al., 1999; 2008c; 2013). Olfactory cues and the magnetic field that exist at a turtles' natal site are the most likely candidates for this purpose (Owens et al., 1982; Lohmann et al., 2008c; 2013). After the initial migration to breeding areas, turtles make regular trips between these and foraging areas throughout their lives.

Although both hatchling and adult turtles can exploit the Earth's magnetic field as a navigational cue, the existence of magnetic anomalies and the gradual change of the earth's magnetic field over time (secular variation) probably make the magnetic map too imprecise to locate highly specific nesting or foraging areas (Lohmann et al., 1999; 2008a; 2008b). Thus, turtles likely rely on additional sensory cues once they are in the vicinity of their target area (Lohmann et al., 1999; 2008b; Hays et al., 2003). They might use a multi-modal navigational strategy, in which different sensory cues are important at different spatial scales (Lohmann et al. 2008a). For instance, a turtle attempting to locate a specific nesting area or foraging ground may use magnetic cues to arrive in the vicinity of this area, but because the magnetic field may not be useful at a fine scale, other cues are probably necessary to locate a destination precisely. The nature of these additional cues is unknown, but a number of possibilities exist, including long range visual landmarks (Carr 1967), the sounds of waves breaking (Mrosovsky, 1972; Luschi et al. 1996), and windborne or waterborne chemical cues (Koch et al. 1969; Akesson et al. 2003; Hays et al. 2003).

The ability of turtles to detect waterborne chemical cues has been studied to some degree. Turtles have demonstrated an ability to perceive a number of underwater chemical cues (Manton et al., 1972), including prey items such as jellyfish (Constantino and Salmon, 2003). Turtles have also been shown to detect chemical cues from sand and water obtained from areas to which they had been 'artificially imprinted' (Grassman and Owens, 1987; Grassman et al., 1984), although whether this response was attributable to imprinting remains unresolved (Lohmann et al., 1997; 2013).

The ability of turtles to perceive airborne chemical cues, however, has not been previously studied. There are at least three good reasons to suspect that turtles possess this ability. First, while sea turtles spend most of their lives in water, they require air to breathe. Thus they are semi-aquatic and might be expected to detect both waterborne and airborne chemical cues. This is true for the American alligator (*Alligator mississippiensis*), which is able to detect chemicals under water (Weldon et al., 1990; Hansen, 2007), and the odor of meat in the air (Weldon et al., 1990; 1992). Harbor seals (*Phoca vitulina*) can also detect chemical cues under water (Sticken and Dehnhardt, 2000), and in the air (Kowalewsky et al., 2006). The African clawed frog (*Xenopus laevis*), primarily aquatic but also seen on land, has been shown anatomically to have some olfactory epithelia that come into contact with only air, and others that contact only water, suggesting that these frogs have the ability to detect chemicals in both air and water (Frietag et al., 1995). Similarly, anatomical studies of sea turtles indicate that they may also have separate olfactory epithelia for waterborne and airborne chemical cues (Schwenk, 2008).

A second consideration suggesting that sea turtles might detect airborne odorants is that they possess a highly-developed olfactory and vomeronasal system (Saito et al., 2001; Schwenk, 2008). And third, turtles possess a wide range of functional olfactory receptor (OR) genes (Kishida et al. 2007). Compared to other

secondarily-adapted marine animals, this large number of functional OR genes suggest that turtles have retained the ability to detect airborne odors; this may, in part, reflect the importance of the terrestrial habitat to turtles, as they must return to land to lay their eggs (Kishida et al., 2007).

The second chapter of my dissertation explores whether sea turtles are able to detect airborne odors. I demonstrate that they can in fact perceive the airborne odor of their food. This ability may be important to turtles in two major ways – foraging and navigation.

As sea turtles migrate through the open ocean, they must find food. Not all oceanic areas are equally favorable in terms of foraging, and an ability to locate favorable foraging areas quickly might be adaptive. The third chapter of my dissertation investigates whether an airborne odor, dimethyl sulfide (DMS), might be a chemical cue that turtles could use to locate productive foraging areas at sea. DMS is produced by phytoplankton and is present in the air above productive areas of the ocean (Andreae, 1990). I demonstrate for the first time that sea turtles can perceive DMS in the air, and it is thus possible that they use this chemical to locate foraging areas at sea.

Airborne chemical cues may also play a role in the navigation of sea turtles. One particularly important use of chemical cues might be to detect nearby land masses. This might be critical for young turtles, which would benefit from avoiding predator-rich near-shore waters, as well as adults, which must locate land masses on which to nest. The airborne odors of animal feces, terrestrial vegetation, or soil might signal to turtles that land is nearby. The fourth chapter of my dissertation describes an experiment in which turtles were presented with the airborne odor of coastal mud. This study shows that sea turtles can perceive an airborne odor associated with land and thus, in principle, might be able to use this odor to determine when land masses are nearby.

The use of magnetic or olfactory cues alone probably does not allow turtles to locate specific destinations, but together, these cues might permit a turtle to locate a small island or specific nesting beach. The fifth chapter of my dissertation explores the concept of dual-cue navigation by examining how a combination of olfactory and magnetic cues might allow turtles to locate Ascension Island. Green turtles migrate over 2000 km from Brazil to this tiny island in the southeast Atlantic Ocean to nest. I modeled the overlap between magnetic intensity isolines and both airborne and waterborne olfactory cues emanating from Ascension Island. The results demonstrate that a multi-modal approach to natal homing, in which turtles rely on magnetic cues over large spatial scales until they encounter secondary, olfactory cues, would allow turtles to reliably reach Ascension Island. Thus, in this case, the use of magnetic and olfactory cues sequentially would allow turtles to reach their nesting grounds. I speculate that similar mechanisms may account for other instances in which turtles migrate long distances to return to specific nesting areas.

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CHAPTER TWO: PERCEPTION OF AIRBORNE ODORS BY LOGGERHEAD SEA TURTLES¹

Abstract

Sea turtles are known to detect chemical cues, but in contrast to most marine animals, turtles surface to breathe and thus potentially have access to olfactory cues both in air and in water. To determine whether sea turtles can detect airborne chemical cues, captive loggerhead turtles (*Caretta caretta*) were placed into a circular, water-filled arena in which odorants could be introduced to the air above the water surface. Air that had passed across the surface of a cup containing food elicited increased activity, diving, and other behavior normally associated with feeding. In contrast, air that had passed across the surface of an identical cup containing distilled water elicited no response. Increases in activity during food odor trials occurred only after turtles surfaced to breathe and peaked in the first post-breath minute, implying that the chemical cues eliciting the responses were unlikely to have been detected while the turtles were under water. These results provide the first direct evidence that sea turtles can detect airborne odors. Under natural conditions, this sensory ability might function in foraging, navigation, or both.

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Introduction

Chemoreception is ubiquitous among animals and is used for diverse purposes, including foraging (Nevitt et al., 1995; Derby et al., 2001; Clark, 2004), predator detection (Dielenberg and McGregor, 2001; Lukowiak et al., 2008), kin recognition (Todrank et al., 1998; Mateo, 2003), communication (Waldman and Bishop, 2004), and navigation (Hasler et al., 1978; Vickers, 2000; Lohmann et al., 2008a). Chemical cues may be particularly important to aquatic species, which often inhabit environments where visibility is poor and the availability of other sensory cues is limited (Wisenden, 2000; Lohmann et al., 2008a).

Most fish and many aquatic invertebrates spend their lives submerged in water and are rarely if ever above the air-water interface. As a consequence, the chemical senses of these animals have evolved to detect chemicals in water, the only medium that they normally encounter. In contrast, some aquatic and semi-aquatic animals surface to breathe and thus have access to chemical cues that exist not only in water, but also in air.

At least a few species are known to detect chemical cues both in water and air. For example, American alligators (*Alligator mississippiensis*) can detect chemical cues while swimming under water (Weldon et al., 1990; Hansen, 2007) and can also perceive airborne odors from meat (Weldon et al., 1990; 1992). Similarly, harbour seals (*Phoca vitulina vitulina*) can detect chemical cues related to salinity while submerged (Sticken and Dehnhardt, 2000), and can also detect airborne odorants potentially useful in foraging (Kowalewsky et al., 2006).

Sea turtles are another group of air-breathing, aquatic animals that might, in principle, detect chemical cues both in water and in air. Several lines of evidence indicate that aquatic turtles in general, and sea turtles in particular, have good olfactory

abilities (reviews by Bartol and Musick, 2003; Southwood et al., 2008; Schwenk, 2008). For example, electrophysiological recordings from cells in the olfactory and vomeronasal epithelia of several turtle species have revealed cells that respond to chemical cues (Shoji et al., 1993; Hatanaka and Matsuzaki, 1993; Brann and Fadool, 2006). Anatomical studies have indicated that aquatic turtles, including the loggerhead sea turtle (*Caretta caretta*), have well-developed olfactory and vomeronasal organs (Parsons, 1971; Saito et al., 2001). Sea turtles are also known to open their nostrils and engage in a rhythmic “throat-pumping” behavior while underwater, a process which floods the nasal cavities and may move water over the chemoreceptive organs (Walker, 1959; Manton, 1979; Schwenk, 2008). Several behavioral studies have provided evidence that sea turtles can perceive chemical cues (Manton et al., 1972; Grassman and Owens, 1982; Grassman et al., 1984; Constantino and Salmon 2003; Piovano et al. 2004).

Despite this considerable body of work, an unanswered question is whether sea turtles detect chemical signals carried by water, air, or both. The behavioral experiments conducted so far have all involved presenting chemical cues to turtles while they were swimming in water, but such studies do not resolve the matter because turtles were free to surface and some or all of the chemicals might have partitioned into air.

The question is important because the ability to detect airborne odorants might be useful to sea turtles in at least two naturally occurring situations. First, turtles navigating into the vicinity of remote islands used as nesting sites might be able to perceive the targets from considerable distances downwind if they are able to detect volatile chemicals associated with the island (Luschi et al., 2001; Hays et al., 2003; Lohmann et al., 2008b). In addition, volatile chemicals such as dimethyl sulfide (DMS) emanate from oceanic regions in which productivity is high (Andreae and Raemdonck, 1983). Some seabirds use DMS to identify areas favorable for foraging (Nevitt et al.,

1995); thus, turtles might also be able to exploit such cues as markers of promising foraging grounds. At present, however, whether sea turtles are able to exploit chemical signals transmitted through air has not been studied. Here I report the first direct experimental evidence that sea turtles can detect airborne odors. Under natural conditions, this ability may play a role in navigation, foraging, or both.

Materials and Methods

Animals

The 8 loggerhead turtles used in the study were obtained as hatchlings from nests deposited on beaches at Bald Head Island and Cape Lookout, North Carolina, U.S.A. Turtles were taken to the North Carolina Aquarium at Pine Knoll Shores, where they were raised for 4 months before being transferred to the University of North Carolina at Chapel Hill. In Chapel Hill, each turtle was maintained in a separate tank in re-circulating artificial seawater maintained at a temperature of 26° to 30°C. Lights in the facility were on for 12 h and off for 12 h each day. At the time of the experiment, turtles were between 25.3 and 31.6 cm curved carapace length (ccl) and approximately 1.5 years of age.

At both locations where the turtles were raised, they were fed the Mazuri Omnivore Aquatic Gel-based diet (www.mazuri.com). During the time of the experiments, turtles were fed every other day. Experiments were conducted on days when the turtles were not fed.

Preliminary Observations

Preliminary observations of the turtles in their home aquaria during feeding revealed a characteristic pattern of behavior that provided the foundation for this study. I

observed that within about 60 seconds after food was dropped into the aquarium, the turtles typically began to dive and crisscross the bottom of the tank repeatedly. When a piece of food was encountered, a turtle paused briefly to eat it, then quickly resumed crisscrossing the tank. This elevated level of activity often continued for a period of minutes even after all food in the aquarium had been consumed. Although little is known about this behavior, it seems likely that chemical cues from the food (dissolved in water under these conditions) elicited increased activity and searching behavior in the turtles.

I reasoned that if loggerhead turtles detect airborne food odors, then such odors might elicit searching behavior similar to that which normally occurs when food is introduced into the home aquarium. My experiment was designed to investigate this possibility.

Experimental set-up

Experiments were conducted at the University of North Carolina in a laboratory located near to where the turtles were housed. The testing apparatus was a circular arena (48 cm in diameter) filled with artificial seawater to a depth of 28 cm. An opening in the side of the arena above the water line provided a portal through which airborne food odors could be delivered (Fig 2.1). The portal was connected to a length of PVC pipe (74 cm long and 5 cm in diameter), which extended through the wall of the room to an adjacent room from which the experiments could be monitored. A small fan at the far end of the pipe continuously moved air through the pipe and into the arena (Fig 2.1). A t-joint at the end of the PVC pipe was arranged so that one opening was directed downward into a plastic cup and one opening was located 2 cm from the fan (Fig 2.1). During experiments, the cup held either distilled water (as a control) or distilled water and a small amount (2 g) of gel food. When the fan was on, a gentle stream of air

moved steadily into the t-joint, past the top of the cup and into the arena, presumably picking up airborne odors from the contents of the cup along the way.

The top of the arena was covered with a transparent Plexiglas lid. A small gap was left between the Plexiglas and the top of the arena on the side away from the odor portal. Thus, air entered the arena on one side, flowed across the surface of the water, and escaped on the opposite side. A video camera was mounted on the ceiling and positioned above the arena, permitting the behavior of the turtle to be observed and videotaped from the adjacent room.

Experimental protocol

The behavioral responses of each turtle were monitored under 2 different conditions: (1) following exposure to airborne food odors; (2) following exposure to air that had passed over a cup of distilled water (as a control). The two test conditions were presented one after the other, separated by an interval in which airborne odors presented in the first trial were allowed to dissipate from the arena. To ensure that the order in which the odorants were presented did not affect the outcome, half of the turtles were subjected to the food odor first, while the other half were subjected first to distilled water trials.

At the start of experiments each day, the arena was filled with artificial seawater. An empty plastic cup was attached to the PVC pipe (Fig 2.1) and the fan was turned on. After air had blown through the PVC pipe for several minutes, the turtle to be tested was placed into the arena. Upon release, each turtle typically circled the arena rapidly for several minutes, sometimes splashing vigorously. Within 10 min, however, these rapid movements subsided and the turtle instead began to swim steadily in one or another part of the arena. At this time, the video recording system was turned on and either the

stimulus or control cup was affixed to the PVC pipe by gently pressing it onto the downward opening of the t-joint.

Once the cup had been put in place, observers recorded when the turtle surfaced and took its first breath; this moment presumably represented the point at which the turtle first had an opportunity to detect airborne odorants. For all turtles tested, this occurred between 3 and 202 sec after a stimulus cup was presented (mean = 104 sec). Videotaping continued for an additional 5 min after the time when a turtle took its first breath.

At the conclusion of the trial, the turtle remained in the arena while the stimulus cup was replaced with the empty cup. To disperse lingering odorants from the arena, the fan was left on to blow air continuously through the PVC pipe over the empty cup and into the arena. To further facilitate odor dispersal, the Plexiglas cover was removed from the arena and the door to the room was opened for at least 10 min to allow the test area to air out. A fan in the ceiling of the room connected to vents further enhanced air exchange.

After the arena and room were aired out for at least 10 min, the Plexiglas cover was replaced, all observers left the room, and the turtle was given several min to resume its normal swimming behavior. Once it did, videotaping resumed and the turtle was presented with the second stimulus.

Measurement of Activity

Videotapes of the 16 trials (8 with the food odor, 8 with distilled water) were analyzed blindly by 2 observers who were unaware of the purpose of the study and did not know what stimuli had been presented to the turtles. To provide a simple, objective measure of activity, the circular arena was divided into 4 equal quadrants on the video screen. When the turtle surfaced to breathe for the first time at the start of the trial,

observers recorded the quadrant in which the turtle's nose broke the surface of the water. Activity was measured in terms of traversals of the arena (i.e., the number of times that a turtle moved from one side of the circular arena to the other). Thus, when the turtle moved around the arena so that its nose reached the quadrant opposite the one in which it had surfaced, this was considered one traversal. Every subsequent time the location of the turtle's nose moved from one of these two quadrants to the other, an additional traversal was counted. Each traversal signified that the turtle had actively moved a considerable distance around the arena, an action consistent with the increased movement previously observed when turtles are searching for food (see "Preliminary Observations").

I also analyzed, in the same way, the behavior of the turtle during the interval which began when the airborne stimulus was introduced to the arena and ended when the turtle took its first breath. During this time, turtles were submerged and presumably did not have access to airborne cues. For the purpose of these measurements, the starting quadrant of the turtle was considered to be the quadrant in which the turtle's nose was located (below water) when the cup was put in place and the airborne odor was first introduced to the tank. Because it was impossible to predict when a turtle would surface to breathe, pre-breath intervals were of different durations for different turtles, (mean = 104 sec). For each of the 16 trials, a rate of traversals (traversals/min) was calculated for the period preceding the first breath and for the 5-min period after the first breath. This permitted a direct comparison of behavior immediately before and after the turtle gained access to airborne odors.

Statistical Analysis

For each turtle, the total number of traversals that occurred during the control and food odor trials was determined. Results in the two treatments were compared using the Wilcoxon signed-ranks test.

To determine whether traversal rates changed after turtles took their first breath (as would be expected if turtles detected airborne odors), traversal rates (traversals per min) were calculated for the control and food odor trials and for the corresponding pre-breath intervals. The traversal rates for pre-breath and post-breath intervals for food odor trials were then compared using the Wilcoxon signed-ranks test. The same analysis was done for pre-breath and post-breath intervals in control trials.

If turtles detect airborne odors, then traversals would be expected to increase immediately after turtles first surface to breathe; in contrast, if airborne odors slowly diffuse into water and are detected below the surface, then responses would be expected to increase over time as the concentration of odorants in the water gradually increases. To investigate the time course of the response of turtles during the period after the first breath, the number of traversals by each turtle was determined for each minute during the course of each trial.

Results

All 8 turtles crossed the arena more times when exposed to the food odor than they did when exposed to the odor of distilled water alone (Table 2.1). The difference in responses to the two treatments was significant (Wilcoxon test, $T=0$, $p=0.005$, one-tailed), implying that turtles increase activity in the presence of air that has passed across food.

For control trials in which the odor source was distilled water, the mean traversal rate was 0.26 traversals/min during the pre-breath period and 0.40 traversals/min during

the post-breath period (Fig 2.2). No significant difference existed between the traversal rate during pre-breath and post-breath periods (Wilcoxon test, $T=9$, not significant).

For trials involving food odors, the mean traversal rate during the pre-breath period was 0.62 traversals/min and 2.85 traversals/min for the post-breath period (Fig 2.2). The traversal rate for the post-breath period of the food odor trials was significantly higher than that of the corresponding pre-breath period (Wilcoxon test, $T=0$, $p=0.005$, one-tailed).

Analysis of traversals over the course of the 5-min trials indicated that, for turtles exposed to the food odors, the number of traversals peaked in the first minute after the first breath and then subsequently declined (Fig 2.3). In contrast, the traversal rates of control turtles remained relatively constant throughout the 5-min trial period (Fig. 2.3).

Discussion

The activity of juvenile loggerhead turtles, as measured by the number of times they traversed the arena, increased significantly in the presence of air that had passed over a cup containing food submerged in distilled water (Table 2.1; Fig 2.2). No such increases in activity occurred during control trials in which turtles were exposed to air that had passed over a cup containing distilled water alone. These results imply that loggerhead turtles can perceive food odors carried through the air and respond to them by increasing activity that is normally associated with searching for food.

In principle, chemical cues emanating from turtle food might have been detected in several different ways. One possibility is that turtles perceived airborne odorants from the food. Alternatively, chemical cues emanating from the food might have gradually dissolved into the water of the arena as the scented air passed along the water surface. In the latter case, turtles might potentially have detected chemicals using gustation or underwater olfaction without surfacing to breathe.

An analysis of behavior before and after turtles breathed provides evidence consistent with detection of airborne odors. In trials involving food odors, traversal rates did not increase until turtles first surfaced to breathe (Fig 2.2), implying that turtles had to sample the air to perceive the odor. Moreover, the peak number of traversals occurred in the minute immediately after the first breath and then declined in subsequent minutes (Fig. 2.3). This pattern of behavior is consistent with aerial olfaction but difficult to reconcile with detection of chemical cues underwater. In the latter case, the concentration of chemicals in the water should have steadily increased over the entire trial, and the first breath (which occurred at variable times between 3 and 202 sec after the food odor was presented) should not have been followed by an immediate increase in activity.

The behavior elicited by the airborne food odor closely resembled behavior observed when food was added to the home aquaria of the turtles. All turtles used in this study had been raised in captivity and received the gel food at least several times a week throughout their lives. During feeding sessions, food was typically brought into the turtle facility and kept there for several minutes before it was placed in the aquaria. Thus, one possibility is that the captive turtles used in these experiments learned to associate airborne odors of their food with the experience of being fed. An alternative possibility, however, is that all loggerhead turtles respond with increased activity to the odors that emanate from the gel food, regardless of whether they have encountered the food before. Additional experiments with wild-caught turtles unfamiliar with the gel food will be needed to resolve this issue. Regardless, the results imply that sea turtles can detect airborne odorants.

The way in which this ability is used under natural conditions is not known. One interesting possibility is that the presence of certain, specific airborne odors might signal favorable feeding areas. An odor that might be particularly useful in oceanic foraging is

dimethyl sulfide (DMS), a scented compound that has been studied in the context of global climate change (Kettle et al., 1999). DMS is the hydrolysis product of dimethylsulfoniopropionate (DMSP), a compound produced by phytoplankton (Kirst et al., 1991; Karsten et al., 1992). High concentrations of DMS are often associated with coastal upwelling areas and other oceanic regions with high productivity (Andreae and Raemdonck, 1983; Kettle et al. 1999). Some seabirds detect DMS and use it to identify areas that are likely to be favorable for foraging (Nevitt et al., 1995; 2008). Harbour seals are also capable of detecting DMS (Kowalewsky et al., 2006). If sea turtles can perceive DMS (or any other airborne chemical associated with food), then this ability might function in helping them locate productive oceanic areas for foraging.

Additionally or alternatively, airborne odors might play a role in the navigation of sea turtles under some conditions. Sea turtles of many populations and species migrate long distances and are thought to rely at least partly on the Earth's magnetic field for guidance (e.g., Lohmann et al., 2004; 2007; 2008a; Luschi et al., 2007). Additional cues are also likely to be involved, however, especially when turtles have arrived in the general vicinity of target areas and need to pinpoint islands, nesting areas, or other specific locations (Lohmann et al., 1999; Putman and Lohmann, 2008). Airborne odorants have been proposed to play a role in helping turtles locate islands at the end of long migrations (Luschi et al., 2001; Hays et al., 2003; Lohmann et al., 2008b). My results confirm for the first time that sea turtles can indeed perceive airborne odors, suggesting that the use of airborne chemical cues in navigation might be plausible. For example, turtles migrating through open ocean might detect nearby land by perceiving odorants from coastal vegetation or soil; similarly, on a smaller spatial scale, airborne odors from decaying turtle eggs laid in previous seasons, or of other turtles nesting on land, might signal the existence of nearby nesting areas.

The finding that sea turtles can detect chemical cues in air extends the growing list of environmental cues that these animals are known to perceive, but also raises many additional questions. Further studies will be needed to determine the sensitivity of sea turtles to airborne odorants, which chemical cues can be detected, the physiological mechanisms that underlie aerial olfaction, and the purpose or purposes for which this sensory ability is used under natural conditions.

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Tables

Table 2.1: Summary of the total number of traversals for control trials (trials with distilled water alone) and food odor trials. Each turtle was tested under both conditions (see text for details). Each trial lasted 5 min. Turtles 1, 2, 4, and 6 were exposed to the control treatment first, whereas turtles 3, 5, 7, and 8 were exposed to the food odor first.

Turtle	Traversals during control trials	Traversals during food odor trials
1	3	10
2	1	15
3	3	22
4	3	9
5	2	8
6	1	18
7	0	16
8	5	16
total	18	114

Figures

Figure 2.1: Diagram of the experimental set-up (not to scale). The fan gently moved air through the PVC pipe, across the t-joint and opening of the cup, and into the arena where the turtle was tested. On the far side of the arena, a small opening between the cover and the top of the arena permitted air to escape. See text for details.

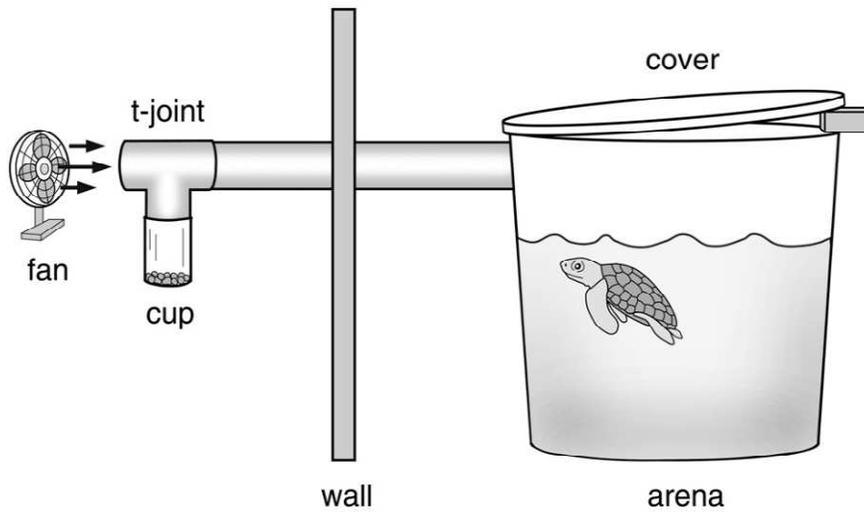


Figure 2.2: Summary of turtle traversal rates. For the control trials (those involving a cup filled with distilled water alone), no difference existed between the pre-breath rate and the post-breath rate (Wilcoxon test, $T=9$, not significant). For trials involving food odors, the asterisk indicates that the post-breath rate was significantly higher than the pre-breath rate (Wilcoxon test, $T=0$, $p=0.005$, one-tailed). Error bars indicate the 95% confidence interval. See text for details.

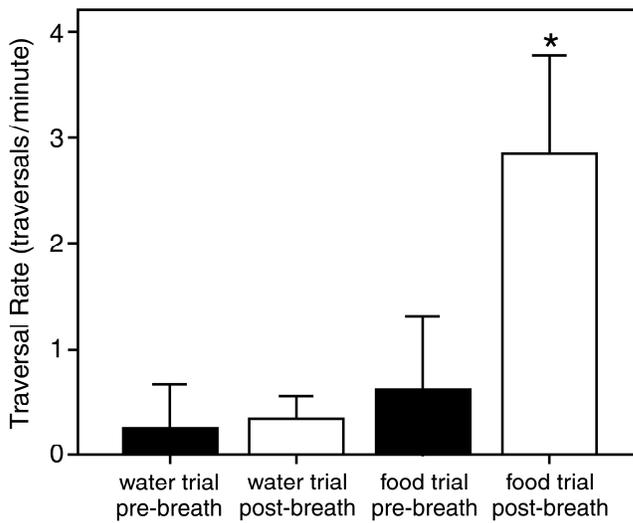
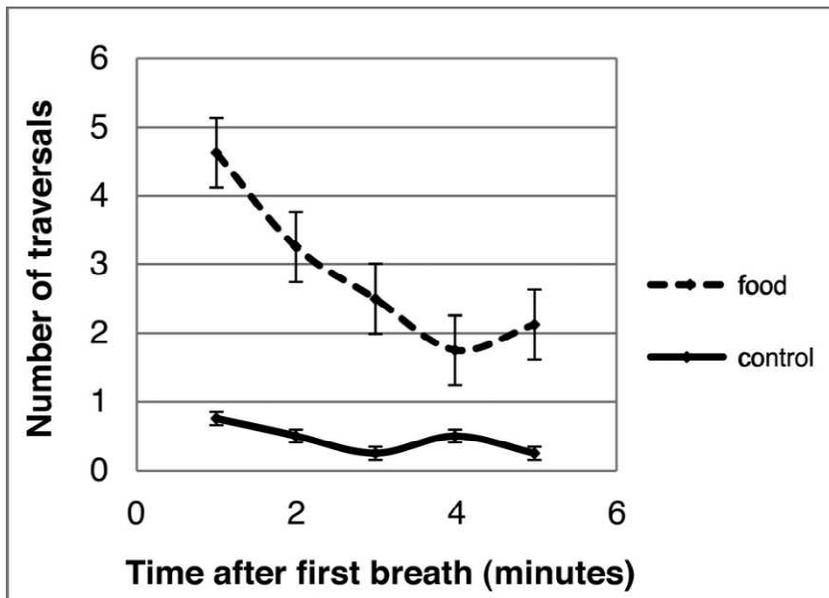


Figure 2.3: Number of traversals in relation to time after first breath. Each data point indicates the average number of traversals (for n = 8 turtles) that occurred during each min of the 5-min food trials and control trials. Error bars indicate the standard error of the mean. On the horizontal axis, zero indicates the time in the trial at which each turtle took its first breath (see text for details).



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**CHAPTER THREE:
PERCEPTION OF DIMETHYL SULFIDE (DMS) BY LOGGERHEAD SEA TURTLES: A
POSSIBLE MECHANISM FOR LOCATING HIGH-PRODUCTIVITY OCEANIC AREAS
FOR FORAGING²**

Abstract

During their long-distance migrations, sea turtles of several species feed on jellyfish and other invertebrates that are particularly abundant in ocean regions characterized by high productivity. An ability to distinguish productive oceanic regions from other areas, and to concentrate foraging activities in locations where prey density is highest, might therefore be adaptive. The volatile compound dimethyl sulfide (DMS) accumulates in the air above productive ocean areas such as upwelling and frontal zones. In principle, DMS might therefore serve as an indicator of high prey density for turtles. To determine whether turtles perceive DMS, juvenile loggerhead turtles (*Caretta caretta*) were placed into a water-filled arena in which DMS and other odorants could be introduced to air above the water surface. Turtles exposed to air that had passed over a cup containing 10 nM DMS spent more time at the surface with their noses out of the water than did control turtles exposed to air that had passed over a cup containing distilled water. Odors that do not occur in the sea (cinnamon, jasmine, and lemon) did not elicit increased surface time, implying that the response to DMS is unlikely to reflect a generalized response to any novel odor. The results demonstrate for the first time that sea turtles can detect DMS, an ability that might enable turtles to identify favorable foraging areas.

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Introduction

Numerous marine animals that migrate long distances, including fishes, sea turtles, seabirds, and marine mammals, periodically forage in unfamiliar oceanic regions along the migratory route. Because all oceanic regions are not equally favorable in terms of foraging, an ability to rapidly identify favorable areas, and to concentrate foraging in such locations, might be adaptive.

The loggerhead sea turtle (*Caretta caretta*) undergoes one of the longest and most spectacular marine migrations. Young loggerheads leave their nesting beaches and embark on transoceanic migrations that last a period of years and span entire ocean basins (Carr, 1987; Bolten et al., 1998; Bowen et al., 1995; Lohmann et al., 2012). During these migrations, juvenile turtles spend considerable time foraging in the open sea, particularly near fronts and upwelling areas (Carr, 1986; Polovina et al., 2000, 2001, 2004; Cardona et al., 2005; Etnoyer et al., 2006). Adult marine turtles of several species have also been observed foraging near fronts and other productive locations (Luschi et al., 2003, 2006; Lambardi et al., 2008; James et al., 2005; Eckert, 2006; Troeng et al., 2005).

Frontal areas, which typically occur in locations where water masses converge or diverge, are often characterized by high concentrations of phytoplankton (Belkin et al., 2009). When fed upon, phytoplankton release dimethylsulfoniopropionate (DMSP), which is cleaved to form the odiferous compound dimethyl sulfide (DMS) (Dacey and Wakeham, 1986). Because DMS volatilizes into the air (Andreae, 1990; Kettle et al., 1999), oceanic areas with an abundance of phytoplankton tend to have increased concentration of DMS in the air above them (Andreae, 1990). DMS and its precursor DMSP also attract some zooplankton and small fish (Steinke et al., 2006; DeBose et al., 2008), which in turn are eaten by other organisms. Thus, high concentrations of DMS in

the open sea tend to be found in high-productivity areas with abundant prey that can be exploited by large, mobile predators.

Several such predators, including procellariiform seabirds (Nevitt et al., 1995), African penguins (*Spheniscus demersus*) (Cunningham et al., 2008; Wright et al., 2011), chinstrap penguins (*Pygoscelis antarctica*) (Amo et al., 2013) and harbor seals (*Phoca vitulinis*) (Kowalewsky et al., 2006), can detect DMS. At least some of these animals appear to use the presence of DMS to identify areas with abundant prey (reviewed by Nevitt, 2008). Given that sea turtles can detect airborne odors (Endres et al., 2009), the possibility that they might also use DMS to locate favorable oceanic foraging areas appears plausible.

I investigated whether loggerhead sea turtles are capable of detecting DMS by presenting captive turtles with this compound, as well as with several other odors, and observing their behavior. I report that concentrations of DMS comparable to those found in high-productivity oceanic regions elicited an increase in the amount of time that turtles spent at the surface with their noses out of the water; no such change in behavior was elicited by the scent of distilled water or by several other odorants that do not occur in the ocean. These results demonstrate for the first time that loggerhead turtles can perceive DMS, giving credence to the possibility that turtles might use this volatile chemical as a foraging cue at sea.

Materials and Methods

Animals

The 11 loggerhead turtles used in the study were obtained as hatchlings from nests deposited on Atlantic Beach and Onslow Beach in North Carolina, USA. Turtles were taken initially to the North Carolina Aquarium at Pine Knoll Shores, where they

were raised for 2 months before being transferred to the University of North Carolina at Chapel Hill. In Chapel Hill, each turtle was maintained in a separate tank in recirculating artificial sea water maintained at a temperature of 26° to 30°C. Lights in the facility were on for 12 h and off for 12 h each day. At the time of the experiment, turtles were between 11.3 and 12.6 cm straight carapace length (scl) and approximately 5 months of age.

At both locations where the turtles were raised, they were fed Mazuri Omnivore Aquatic Gel-based diet (www.mazuri.com). During the time of the experiments, turtles were fed every day.

Experimental set-up

Experiments were conducted at the University of North Carolina in a laboratory located near to where the turtles were housed. Trials took place in a fiberglass arena identical to those in which the turtles were kept. The arena measured 99 x 53 x 46 cm. One of the short sides contained a clear Plexiglas® window (43 x 36 cm) (Fig 3.1). During all experiments the arena was filled with artificial sea water (Crystal Sea®) to a depth of about 30 cm. The salinity of the water was approximately 28 ppt. The top of the arena was open to the air.

Airborne odorants were delivered to the arena through a PVC pipe (length = 89 cm, diameter = 5 cm). One end of the pipe was angled down toward the surface of the water in the arena. The other was connected to a t-joint, which was arranged so that one opening was directed downward into a plastic cup while the other opening was located 2 cm from a small fan 13.3 cm in diameter (Fig 3.1). The fan gently blew air into the pipe, over the cup containing the stimulus, and into the arena. A video camera recorded turtles through the Plexiglas® window at the front of the arena (Fig 3.1). A

curtain prevented turtles from seeing when an observer approached to present an odorant.

Odorants

Although the primary focus of our study was to determine whether turtles perceive DMS, an additional question was whether behavioral responses elicited by DMS are specific to this chemical or instead reflect generalized responses to any novel, airborne odor. For this reason I used 5 different odorant presentations: DMS at a naturally occurring concentration (Nevitt et al., 1995) and essential oils of lemon, jasmine, and cinnamon (in 2 concentrations described below). The essential oils were chosen because they were likely to be completely novel; these odorants are not associated with food nor were turtles likely to have encountered them during their brief history in the wild before capture. Additionally, it is probable that the turtles could detect these odorants given that loggerheads have a large suite of functional olfactory receptor (OR) genes, suggesting that they have retained many of the olfactory capabilities of their terrestrial ancestors and can detect diverse odorants (Kishida et al., 2007).

The 5 odorants were prepared for the experimental apparatus as follows: (i) DMS: 3-4 drops of a solution of 10 nM DMS (Sigma-Aldrich, Milwaukee, WI, USA) mixed with 50 mL dH₂O; (ii) Cinnamon: 3-4 drops of cinnamon essential oil (Now Foods, Bloomingdale, IL, USA) in 50 ml dH₂O; (iii) Jasmine: 3-4 drops of jasmine oil (Now Foods, Bloomingdale, IL, USA) in 50 ml dH₂O; (iv) Lemon: 3-4 drops of lemon oil (Now Foods, Bloomingdale, IL, USA) in 50 ml dH₂O; (v) Concentrated Cinnamon: 25 drops of cinnamon oil (Now Foods, Bloomingdale, IL, USA) in 50 mL dH₂O.

Because detailed information on the chemical composition of the essential oil samples was not available, it was not possible to calculate a molarity for these

substances; all of these odorants, however, were easily detectable by human observers at the concentrations used in the study. To reduce the likelihood that any failure of turtles to respond to the non-DMS odorants (see Results) would be due to the use of concentrations below the detection threshold, an elevated concentration of one of these odorants (25 drops of cinnamon oil) was also tested.

Experimental protocol

For each odorant, paired trials were used to monitor the behavioral response of each turtle to: (1) the odorant itself; and (2) the odor of distilled water (as a control). To begin a trial, an empty plastic cup was affixed to the t-joint and the fan was turned on. A turtle was then placed into the arena and allowed to acclimate until it ceased rapid movements (characteristic of escape behavior) and began to swim steadily, a process that usually took about 10-20 min (Endres et al., 2009). Occasionally, a turtle failed to swim and instead floated motionless at the surface of the arena for the entire acclimation period. When this happened, the turtle was excluded from trials for that day but was subsequently tested again the next day. Those few turtles that failed to swim on two consecutive days were eliminated from the experiment.

Once a turtle was swimming steadily, the recording equipment was turned on and the empty plastic cup was replaced with one containing either dH₂O or the experimental odorant. A trial began after the turtle surfaced to take its first breath, which was presumably when it first had an opportunity to detect airborne odorants. The trial was videotaped for an additional 2 minutes after the first breath.

Upon completion of the trial, the stimulus cup was replaced with the empty cup and the fan was left on to allow lingering odorants to disperse. The door to the room was also left open to facilitate this dispersal. Once the room had been aired out for at

least 10 minutes, the door to the room was shut and the turtle was allowed to acclimate for another 10 minutes before it was presented with the second stimulus. Half of the turtles were exposed to the experimental odorant first; the other half were exposed to distilled water first.

Several days were usually required to subject all turtles to the same odorant. Once trials with each odorant had been completed, turtles remained undisturbed in their home aquaria for a minimum of 2 days before a new experiment involving a different odorant was begun. The sequence of odorants presented was DMS, cinnamon, jasmine, lemon, and concentrated cinnamon.

Data analysis and statistics

The videotape of each trial was analyzed by two observers who had no knowledge of the purpose of the study or which stimulus had been presented to the turtles. Observers recorded the total number of seconds each turtle spent with its head above the surface of the water during each trial. Surface time was selected as a metric because preliminary observations suggested that turtles spend more time at the surface when odors associated with food are present. Although the reason for this behavior is not known, one possibility is that turtles sample the air more when they encounter olfactory stimuli that may be biologically meaningful.

The Wilcoxon signed-ranks test was used to compare responses of turtles to each odorant with responses to corresponding dH₂O controls. The Kruskal-Wallis test was used to determine whether different responses were elicited by the 5 odorants tested (DMS, jasmine, lemon, cinnamon, and concentrated cinnamon). The Mann-Whitney U-test was used to analyze differences between specific pairs of odorants.

Results

Turtles spent more than twice as much time at the surface when DMS was present (mean = 10.0 sec) than they did during dH₂O control trials (mean = 4.5 sec) (Figs 3.2, 3.3). The difference in responses to these two treatments was significant (Wilcoxon signed-ranks test, $T=10$, $p<0.05$, two-tailed). By contrast, turtles did not show any difference in responses to any of the other odorants (cinnamon, jasmine, lemon, or concentrated cinnamon) relative to dH₂O controls (Wilcoxon tests, $P>0.1$ in all cases) (Figs 3.2, 3.3).

The amount of time turtles spent at the surface in the presence of the 5 odorants tested was significantly different (Kruskal-Wallis test, $H=13.50$, $p<0.01$). Pairwise comparisons (Mann-Whitney U-test) indicated that time spent at the surface during DMS trials was significantly greater than surface time during cinnamon trials ($p<0.01$), jasmine trials ($p<0.01$), lemon trials ($p<0.01$), and concentrated cinnamon trials ($p<0.001$) (Fig 3.2). These comparisons remain significant if the Bonferroni correction for multiple comparisons is applied (adjusted $\alpha = 0.0125$), a practice recommended by some, but not all, statisticians (Perneger, 1998).

Discussion

When DMS was present in the air, turtles spent significantly more time with their heads above water than they did when the air was scented with the odor of dH₂O. By contrast, air scented with cinnamon, jasmine, lemon, or concentrated cinnamon failed to elicit more surface time than did corresponding dH₂O controls. These data demonstrate that loggerhead turtles can perceive DMS.

Why turtles responded to DMS by spending more time with their heads above water is not known. One possibility, however, is that airborne odors of biological

importance, such as those associated with favorable foraging areas, attract the attention of turtles, leading them to linger at the surface while sampling the air more extensively than normal. This interpretation is consistent with preliminary observations suggesting that similar increases in surface time can be elicited in captive turtles by odors associated with their food (C. Endres, unpublished observations).

The failure of turtles to respond to cinnamon, jasmine, and lemon odors implies that the response elicited by DMS is not a generalized response to all novel airborne odorants. One possibility is that turtles detected some or all of these odors but failed to respond to them behaviorally, just as the chicks of some seabird species perceive diverse olfactory cues but respond with searching behavior only when presented with odors associated with food (Cunningham et al., 2003; 2006). Alternatively, because cinnamon, jasmine, and lemon are not odors that turtles are likely to encounter in the marine environment, turtles might not have evolved the olfactory receptors necessary to detect these odors, or might have lost the appropriate receptors if they were once present in terrestrial ancestors.

In principle, the ability of turtles to detect DMS might allow them to use this odor to identify favorable oceanic foraging areas in much the same way that procellariiform seabirds do (Nevitt et al., 1995). For seabirds, experiments have demonstrated that birds approach ocean areas to which DMS had been added (Nevitt et al., 1995); foraging in areas with high concentrations of DMS is presumably advantageous because such areas are likely to be high-productivity locations with abundant prey (Nevitt, 2008). For similar reasons, turtles might forage preferentially in oceanic areas with high concentrations of DMS.

The behavior of loggerhead turtles in the north Pacific is consistent with this possibility. These turtles travel as juveniles from Japan and Australia to foraging grounds near Baja California (Bowen et al, 1995). To reach this area, turtles travel

through the North Pacific Transition Zone, an area of convergent fronts and high productivity (Olson et al. 1994). Turtles frequently remain within these fronts, presumably to feed as they migrate (Polovina et al., 2000, 2001, 2004). Because these high-productivity areas are likely to have elevated concentrations of DMS, turtles might plausibly use DMS to help them remain in areas of high prey density.

Although my study was limited to loggerhead turtles, the close similarities in sensory abilities that exist among different species of marine turtles (e.g., Lohmann et al., 1990; Lohmann, 1991; Lohmann and Lohmann, 1993; Bartol and Musick, 2003; Southwood and Avens, 2010) suggest that other species might also have this ability. In principle, perception of DMS might be particularly useful for the leatherback turtle (*Dermochelys coriacea*), a species in which adults routinely forage in high-productivity areas such as frontal zones and upwelling areas (Luschi et al., 2003, 2006; Benson et al. 2007; Saba et al. 2008; Lambardi et al., 2008). Such areas frequently have elevated levels of DMS (Andreae et al., 1990) as well as high concentrations of jellyfish upon which leatherbacks feed. Leatherbacks are also commonly observed near shelf breaks (James et al., 2005; Eckert 2006), another topographic feature associated with elevated DMS (Andreae, 1990). The possibility that leatherbacks exploit DMS as a foraging cue thus appears plausible.

Finally, because coastal waters often have elevated concentrations of DMS (Andreae et al., 1990), turtles might be able to use DMS to detect nearby islands under some circumstances. A role of airborne odorants in island-finding has been suggested (Luschi et al., 2001; Hays et al., 2003), although no unequivocal evidence for this presently exists (Lohmann et al., 2008).

The finding that sea turtles can detect DMS adds to the list of environmental cues they are known to perceive. Many questions remain, however, about whether and how turtles exploit this chemical in the natural environment. Further studies will be needed to

examine whether turtles are attracted to DMS in the open ocean, and whether they can perceive other airborne chemical cues that may play a role in their long-distance migrations.

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Figures

Figure 3.1: Diagram of the experimental set-up. Turtles swam in a fiberglass arena with a window on one side, through which the behavior of the turtles was videotaped.

Airborne odorants were delivered to the arena by a gentle stream of air. A small fan moved air into the PVC pipe, across the top of the stimulus cup, and into the arena

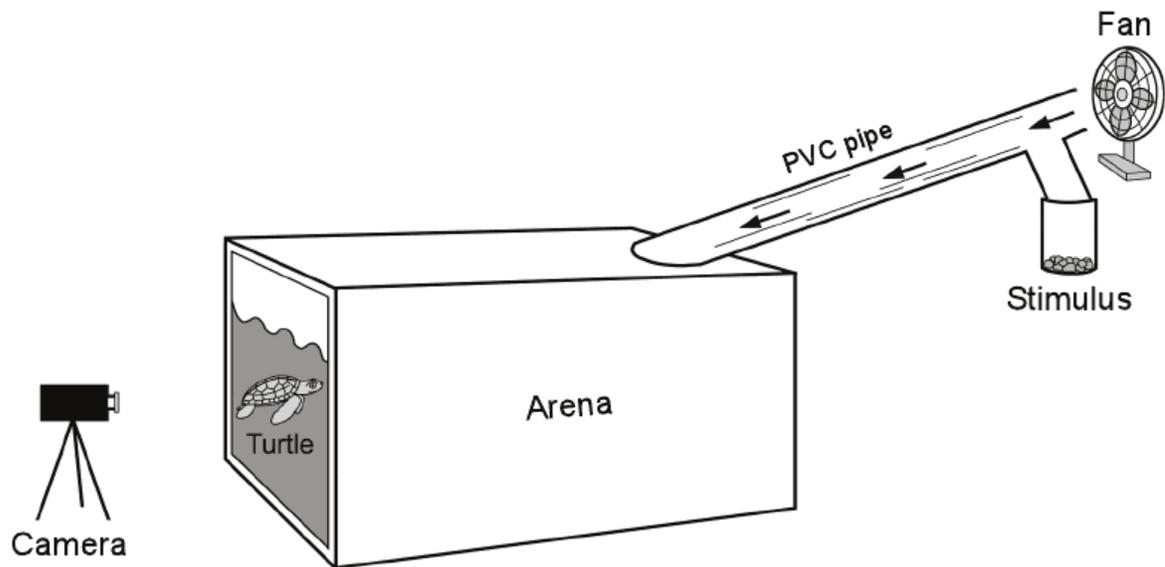


Figure 3.2: Time (in seconds) that turtles spent with their noses out of the water in response to airborne odorants. Values indicate group means; error bars represent standard error. Dark grey bars represent results from trials involving the odorant indicated below each set of bars. Light grey bars indicate the corresponding control trials involving air scented with dH₂O. 'DMS' indicates dimethyl sulfide, 'cinn' indicates cinnamon, 'jasm' indicates jasmine, and 'x-cinn' indicates concentrated cinnamon. Sample sizes were n = 11 turtles for DMS trials and controls and n = 10 for all other odors and controls. The asterisk denotes the only statistically significant pairwise comparison (see Results for details).

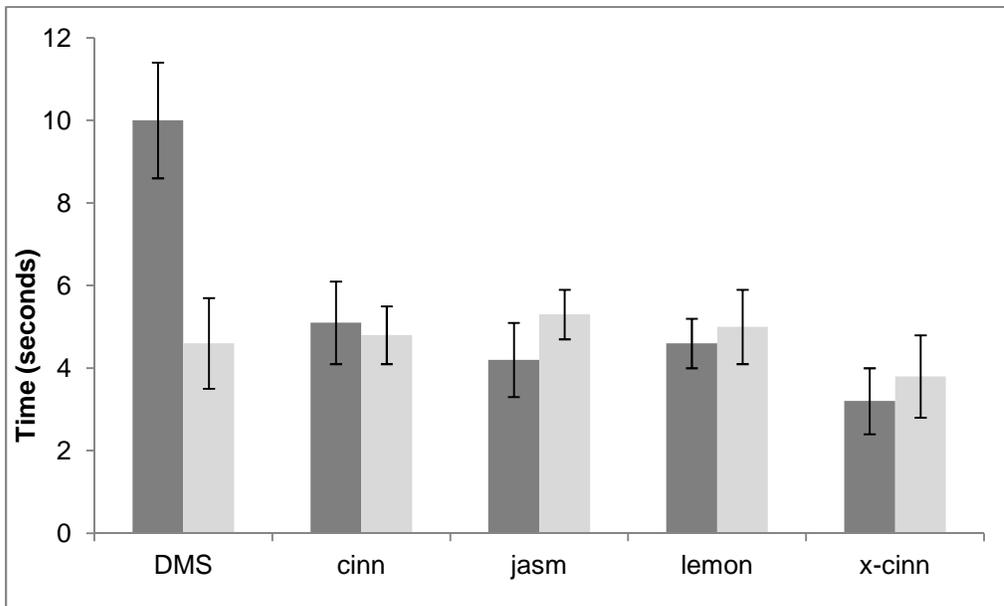
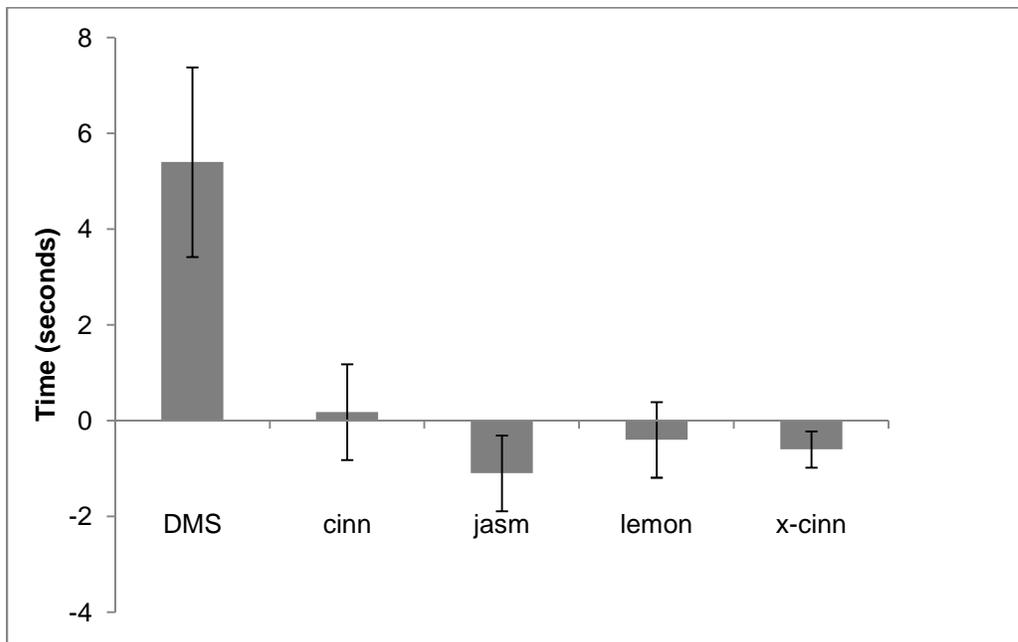


Figure 3.3: Mean change in surface time elicited by each odorant. Bars for each odor indicate differences in surface time observed in experimental trials (in the presence of the odor) versus the corresponding dH₂O controls. Error bars represent standard error. Conventions as in Fig. 2.



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**CHAPTER FOUR:
DETECTION OF COASTAL MUD ODORS BY LOGGERHEAD SEA TURTLES: A
POSSIBLE MECHANISM FOR SENSING NEARBY LAND³**

Abstract

For sea turtles, an ability to detect land masses from a considerable distance away, and to distinguish coastal areas from the open sea, might be adaptive. The loggerhead turtle, *Caretta caretta*, can detect airborne odorants associated with food. To investigate whether sea turtles can also detect odors associated with land, I studied the responses of juvenile loggerheads to odors from coastal mud. Turtles were tested in a water-filled arena in which odorants could be introduced to the air above the water surface. Turtles exposed to air that had passed over a cup containing mud spent more time with their noses out of the water than did control turtles exposed to air that had passed over a cup containing distilled water. The results demonstrate for the first time that loggerheads can detect airborne odorants associated with land, an ability that might play a role in foraging, navigation, or both.

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Introduction

Sea turtles migrate immense distances, sometimes crossing entire ocean basins before returning to their natal region to reproduce (Carr et al., 1978; Bolten, 2003; Musick and Limpus, 1997). Precisely how sea turtles navigate is not yet understood, but the ability to detect the Earth's magnetic field appears to play a critical role in guiding movements of turtles at several different life history stages (Lohmann et al., 2001; 2004; 2012; Luschi et al., 2007). Additional sensory cues, however, are also likely to be involved in sea turtle navigation (Avens and Lohmann, 2003; Lohmann et al., 2008a; 2008b).

Turtles have well-developed olfactory systems (reviews by Bartol and Musick, 2003; Southwood et al., 2008; Schwenk, 2008) and are known to detect both waterborne and airborne odorants (Manton et al., 1972; Constantino and Salmon, 2003; Endres et al., 2009; Endres and Lohmann, 2012). Chemical cues might therefore be used as a source of navigational information during migrations (Koch et al., 1969; Luschi et al., 2001; Lohmann et al., 2008a; 2008b).

In principle, one particularly important use of chemical cues might be to detect nearby land masses. Such an ability might be adaptive for turtles at several life stages. For example, because predators are particularly abundant in coastal areas, young turtles carried into coastal waters by ocean currents (Putman et al., 2012) might benefit by rapidly moving offshore. By contrast, adult turtles migrating to nesting areas on distant islands or continents might benefit from an ability to perceive airborne odorants from terrestrial vegetation, soil, or seabird feces, all of which might signal that land is nearby. Indeed, sea turtles might use magnetic cues to arrive in the vicinity of nesting beaches, and then use chemical cues to help pinpoint specific nesting areas (Lohmann et al., 2008a; 2008b; 2013; Putman and Lohmann, 2008).

As a first step toward determining whether turtles can perceive airborne odorants associated with land, I studied the responses of juvenile loggerheads (*Caretta caretta*) to air scented with coastal mud. The results indicated that turtles exposed to mud odors spent more time at the surface of the water than they did when exposed to air scented with distilled water or air scented with odors that do not occur in the ocean. Thus, loggerhead turtles can evidently perceive odors associated with land. In principle, migrating turtles might use this ability to determine when a land mass is nearby.

Materials and Methods

Animals and mud samples

The 10 loggerhead turtles used in the study were obtained as hatchlings from nests deposited on Atlantic and Onslow Beaches, North Carolina, USA. Turtles were taken to the North Carolina Aquarium at Pine Knoll Shores, where they were raised for 2 months before being transferred to the University of North Carolina at Chapel Hill. In Chapel Hill, each turtle was kept in a separate tank in re-circulating artificial seawater maintained at a temperature of 26° to 30°C. Lights in the facility were on for 12 h and off for 12 h each day. At the time of the experiment, turtles were between 11.8 and 13.0 cm curved carapace length (ccl) and approximately 5 months of age.

At both locations where the turtles were raised, they were fed the Mazuri Omnivore Aquatic Gel-based diet (www.mazuri.com). During the time of the experiments, turtles were fed every day.

The samples of coastal mud used in this study were obtained from Sage Bay in the Pamlico Sound (35.36°N, 76.11°W) North Carolina, USA. Samples were maintained in closed, Nalgene containers at room temperature until use within 10 days of collection.

Experimental setup

Experiments were conducted at the University of North Carolina in a laboratory located near the facility where turtles were housed. Trials took place in a fiberglass arena identical to those in which the turtles were kept. The arena measured 99 x 53 x 46 cm. One of the short sides contained a clear Plexiglas® window (43 x 36 cm) (Fig 3.1). During all experiments the arena was filled with artificial sea water (Crystal Sea®) to a depth of about 30 cm. The salinity of the water was approximately 28 ppt. The top of the arena was open to the air.

Airborne odorants were delivered to the arena through a PVC pipe (length = 89 cm, diameter = 5 cm). One end of the pipe was angled down toward the surface of the water in the arena. The other was connected to a t-joint, which was arranged so that one opening was directed downward into a plastic cup while the other opening was located 2 cm from a small fan 13.3 cm in diameter (Fig 3.1). The fan gently blew air into the pipe, over the cup (which contained either 50 ml of mud or 50 ml of distilled water; see *Experimental protocol*), and into the arena. A video camera recorded turtles through the Plexiglas® window at the front of the arena (Fig 3.1). A curtain prevented turtles from seeing when an observer approached to present an odorant.

Experimental protocol

The protocol for testing responses to odors of coastal mud was based on a protocol used previously to demonstrate that turtles can perceive dimethyl sulfide (DMS), a naturally occurring volatile odorant (Endres and Lohmann, 2012). In the present study, paired trials were used to monitor the behavioral response of each turtle to: (1) the odor of coastal mud; and (2) the odor of distilled water (as a control). To begin a trial, an empty plastic cup was affixed to the t-joint and the fan was turned on. A turtle was then

placed into the arena and allowed to acclimate until it ceased rapid movements (characteristic of escape behavior) and began to swim steadily, a process that usually took about 10-20 min (Endres et al., 2009). Once a turtle was swimming steadily, the video recording equipment was turned on and the empty plastic cup was replaced with one containing either dH₂O or mud. A trial began after the turtle surfaced to take its first breath, which was presumably when it first had an opportunity to detect airborne odorants. The trial was videotaped for an additional 2 minutes after the first breath.

Upon completion of the trial, the stimulus cup was replaced with the empty cup and the fan was left on to allow lingering odorants to disperse. The door to the room was also left open to facilitate this dispersal. Once the room had been aired out for at least 10 minutes, the door to the room was shut and the turtle was allowed to acclimate for another 10 minutes before it was presented with the second stimulus. Half of the turtles were exposed to the coastal mud first; the other half were exposed to distilled water first.

Data analysis and statistics

The videotape of each trial was analyzed by two observers who had no knowledge of the purpose of the study or which stimulus had been presented to the turtle. Observers recorded the time that each turtle spent with its head above the surface of the water during each trial. This metric was used previously in a study investigating responses of turtles to DMS (Endres and Lohmann, 2012), because turtles are thought to sample the air more, and thus spend more time at the surface, when they encounter olfactory stimuli that are biologically meaningful (Endres et al., 2009). In the present study, the Wilcoxon signed-ranks test was used to compare the surface time of

turtles exposed to mud odors with the surface time of turtles during corresponding dH₂O controls.

Although the primary focus of our study was to determine whether turtles perceive odors of coastal mud, an additional question was whether behavioral responses elicited by such odors are specific to mud or instead reflect generalized responses to any novel, airborne odor. As a first step toward investigating this, I carried out an additional analysis comparing the responses of turtles to mud odors (obtained in this study) with responses of the same turtles to essential oils of lemon, jasmine, and cinnamon (reported previously in Endres and Lohmann, 2012). As in the previous study (Endres and Lohmann, 2012), the oils are a useful point of comparison because: (1) the odors are almost certainly novel to the turtles; (2) it is likely that loggerhead turtles can detect these odors, given the large suite of functional olfactory genes in this species (Kishida et al., 2007). The odorants tested previously included 3-4 drops of cinnamon essential oil (Now Foods, Bloomington, Illinois, USA) in 50 ml dH₂O, 3-4 drops of jasmine oil (Now Foods) in 50 ml dH₂O, 3-4 drops of lemon oil (Now Foods) in 50 ml dH₂O, and 25 drops of cinnamon oil (Now Foods) in 50 ml dH₂O. For this analysis, the Kruskal-Wallis test was used to determine whether different responses were elicited by the 5 odorants (coastal mud, jasmine, lemon, cinnamon, and concentrated cinnamon). The Mann-Whitney U-test was used to analyze differences between specific pairs of odorants.

Results

Turtles spent more than twice as much time at the surface when exposed to odors from coastal mud (mean = 15.6 sec) than they did when exposed to odors from

dH₂O (mean = 6.1 sec). The difference in responses to these two treatments was significant (one-tailed Wilcoxon signed-ranks test, $t = 10$, $N = 10$, $P = 0.025$) (Fig 4.1).

The analysis of responses to mud odors and the 4 previously tested non-oceanic odors (cinnamon, jasmine, lemon, and concentrated cinnamon) revealed that differences existed in the amount of surface time elicited by the various odorants (Kruskal-Wallis test, $H_5 = 18.39$, $P < 0.002$) (Fig 4.2). Pairwise comparisons (two-tailed Mann-Whitney U-tests) indicated that surface time during mud trials was significantly greater than surface time during cinnamon trials ($U = 87.5$, $N_1 = 10$, $N_2 = 10$, $P < 0.005$), jasmine trials ($U = 89.5$, $N_1 = 10$, $N_2 = 10$, $P < 0.003$), lemon trials ($U = 89.5$, $N_1 = 10$, $N_2 = 10$, $P < 0.003$), and concentrated cinnamon trials ($U = 94.0$, $N_1 = 10$, $N_2 = 10$, $P < 0.001$). These comparisons remain significant if the Bonferroni correction for multiple comparisons is applied, a practice recommended by some, but not all statisticians (Perneger, 1998).

Discussion

When exposed to air scented with coastal mud, loggerhead turtles spent significantly more time with their heads above the water than they did when exposed to air scented with dH₂O (Fig 4.2). These results indicate that turtles can perceive airborne odors associated with mud. Increased surface time was not elicited by several non-oceanic odors (Fig 4.3), suggesting that the increased surface time elicited by mud odors is not a generalized response to all novel airborne odorants.

Why turtles responded to mud odors by spending more time at the surface is not known. A plausible explanation, however, is that turtles spend more time at the surface sampling the air when they encounter airborne olfactory stimuli that are biologically meaningful (Endres et al., 2009; Endres and Lohmann, 2012).

Although the results demonstrate that turtles can perceive mud odorants, how this ability is used during migrations, if indeed it is, remains to be determined. In principle, the ability to detect airborne odors associated with land might be advantageous to turtles at several life history stages. For example, such odors might signal young turtles in the open sea that they are drifting toward shallow, coastal waters. Because coastal areas often have dense concentrations of predatory birds and fish, young turtles might hypothetically respond to land odors by swimming offshore toward deeper, safer waters, in much the same way that young loggerheads respond to regional magnetic fields at the boundaries of their migratory route by adjusting their swimming direction (Lohmann et al., 2001; 2012). To my knowledge, this possibility has never been investigated.

Although shallow, coastal waters are potentially dangerous to small turtles, the same areas can serve as foraging areas for older turtles that have grown too large for most predators. Many juvenile loggerheads in the north Atlantic Ocean, for example, forage in the oceanic region surrounding the Azores Islands (Bolten et al., 1998). For such turtles, land odors might hypothetically signal the existence of both a nearby land mass and abundant benthic invertebrates that can be exploited as food (McClellan et al., 2010). Similarly, odorants associated with mud or land might be used by juvenile turtles to locate favorable foraging areas in sounds, bays, and coves along the east coast of the USA (Musick and Limpus, 1997).

Adult turtles might also use odors associated with land as a way to locate nesting areas at the end of a long migration (Lohmann et al., 1999; Luschi et al., 2001; Hays et al., 2003; Lohmann et al., 2008a). Although juvenile and adult turtles are thought to use the Earth's magnetic field to navigate over large spatial scales (Lohmann et al., 2004; 2007; Luschi et al., 2007), they may also use additional cues, possibly olfactory, to pinpoint nesting areas once they arrive in the correct geographic region (Lohmann et al.,

2008a; 2008b). A role of windborne odorants in island-finding has been suggested (Luschi et al., 2001; Hays et al., 2003), although no unequivocal evidence for this presently exists (Lohmann et al., 2008a).

An additional consideration is that turtles have been hypothesized to imprint as hatchlings on both chemical cues and on the magnetic field of their natal beach, and then use this information to return as adults (Lohmann et al., 2008c; 2013). Although discussions of chemical imprinting have typically focused on substances dissolved in water (Owens et al., 1982; Grassman et al., 1984; Grassman and Owens, 1987), my findings raise the possibility that turtles might imprint on unique airborne odorants that exist at a home beach or region. In principle, if airborne odors along different parts of a coastline differ sufficiently (due, for example, to different plant and microbial communities in different geographic areas), then turtles might be able to use airborne odorants associated with land not only to locate coastlines generally, but perhaps also to help locate specific nesting areas.

Although such speculation is appealing, it is important to recognize that my results do not actually demonstrate that turtles use airborne odorants to detect nearby land. An alternative interpretation is that the responses observed in my experiment do not reflect the migratory behavior of turtles but instead are related to feeding behavior. Because the mud used in this study is likely to contain both sulfur and organic matter, it is conceivable that these or other volatile components of the mud serve as feeding stimulants for turtles. I note that responses similar to those elicited by mud odors can be elicited by food odors (Endres et al., 2009) and also by DMS, a volatile odorant associated with high-productivity areas in the ocean (Endres and Lohmann, 2012). Of course, the possibility that mud odors elicit feeding or foraging behavior in turtles is not incompatible with the hypothesis that turtles can also, under at least some

circumstances, use such odors to detect the presence of nearby land masses, particularly after long migrations through the open sea.

In sum, my results demonstrate for the first time that loggerhead turtles can detect airborne odors associated with coastal mud, but the functional significance of this finding remains to be determined. Turtles in the open ocean might use this ability to detect nearby land masses; alternatively or additionally, the responses I observed might reflect feeding or foraging responses elicited by volatile components of the mud that turtles associate with food. Additional research will be needed to determine what role, if any, detection of airborne land odorants plays in the foraging and migratory movements of sea turtles.

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Figures

Figure 4.1: Time (in seconds) that turtles spent with their noses out of the water in response to odors of coastal mud and odors from dH₂O (as a control). Values indicate means; error bars represent standard error. Sample sizes were n = 10 turtles for both sets of trials.

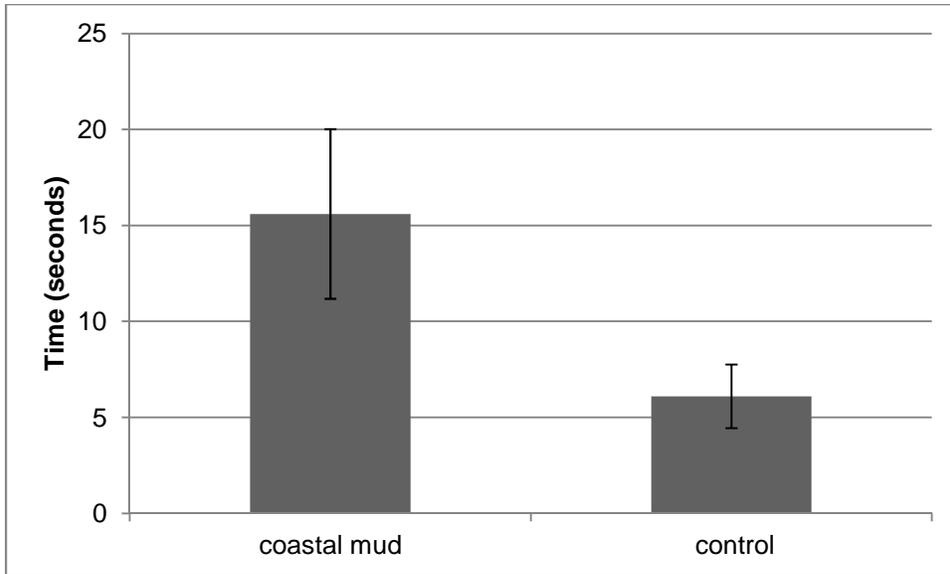
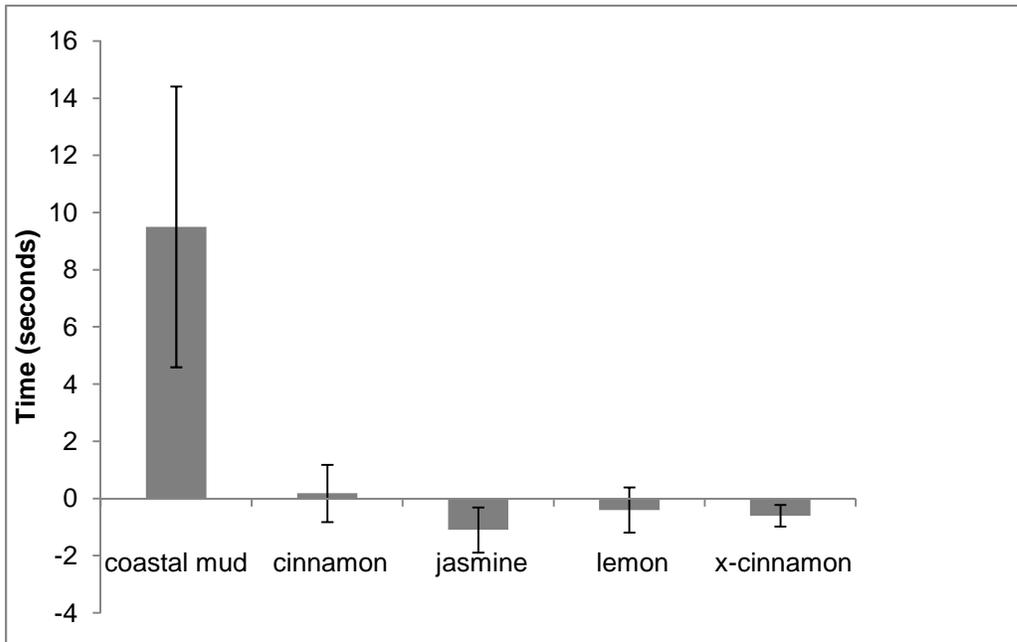


Figure 4.2: Mean change in surface time elicited by each of 5 odorants. Bars for each odor indicate differences in surface time observed in experimental trials (in the presence of the odor) relative to the corresponding dH₂O controls. Data obtained with coastal mud odors in this experiment are compared to data from cinnamon, jasmine, lemon and x-cinnamon obtained from the same turtles and reported previously (Endres and Lohmann 2012). Error bars represent standard error. See text for details.



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**CHAPTER FIVE:
POSSIBLE MULTI-MODAL HOMING IN SEA TURTLES: MAGNETIC AND CHEMICAL
CUES MAY PERMIT ISLAND-FINDING AT ASCENSION**

Abstract

Green sea turtles (*Chelonia mydas*) from Ascension Island forage over 2000 km away off the coast of Brazil. Before departing Ascension Island as hatchlings, turtles are thought to imprint on some feature of the nesting beach and use this information to home when they reach maturity. The sensory cues turtles use to relocate this small island are not fully understood, but they might involve a combination of magnetic and olfactory cues. To investigate whether these two cues might be used in a multi-modal homing strategy for green turtles, I modeled the trajectories of waterborne and airborne odor particles released from Ascension Island and overlaid these on maps of magnetic intensity. The results indicated that while magnetic or olfactory cues alone are usually insufficient to guide turtles from Brazil to Ascension Island, a navigational strategy that involves a combination of the two might plausibly be used. Specifically, one strategy that appears to be viable consists of following a magnetic isoline into the vicinity of Ascension Island, then encountering an odor plume emanating from the island and following the plume to its source. These results are consistent with the hypothesis that turtles use a multi-modal approach to natal homing.

Introduction

Numerous animals, both terrestrial and aquatic, are capable of natal homing. Some marine animals, such as tuna (Rooker et al., 2008) salmon (Dittman and Quinn, 1996) and sea turtles (Carr et al., 1978; Encalada et al., 1998; Bowen et al., 1995) may migrate extraordinary distances upon reaching maturity to locate their natal region for reproduction. The sensory cues these animals utilize to accomplish this navigational feat are not fully understood, but homing is likely a multi-modal process, in which the cues used by an animal vary based on the spatial scale over which it travels (Lohmann et al., 2008a).

Most species of sea turtles are long-distance migrants, traveling hundreds to thousands of kilometers between foraging and nesting areas. Some green sea turtles, for example, migrate over 2000 km from the coast of Brazil to the small, isolated island of Ascension in the Southeastern Atlantic Ocean to nest (Carr, 1975). These turtles hatch out of nests deposited on Ascension Island, spend years migrating and foraging in distant oceanic locations, and return to the island approximately 25 years later (Frazer and Ehrhart, 1985; Frazer and Ladner, 1986). It is hypothesized that adult turtles are able to return to Ascension Island because they imprinted on some feature of the island as hatchlings (Lohmann et al., 1999; 2008c; 2013). They might then rely at least partially on this cue to find the island when they reach maturity.

Turtles are able to detect the inclination angle and total intensity of the earth's magnetic field (Lohmann and Lohmann 1994, 1996; Lohmann et al., 2001, 2004, 2007). These two parameters vary in a predictable way over the earth's surface, and distinct combinations of them exist along the turtles' migratory route. Thus, turtles might be able to derive positional information from magnetic cues, and rely on these to navigate through the ocean. Further, it is possible that hatchlings imprint on the magnetic parameters that exist at the location where they hatch (Lohmann et al., 2008c, 2013).

If turtles do imprint on magnetic cues, however, then locating Ascension Island after 25 years is not as straightforward as returning to the area with the magnetic intensity and inclination angle that existed when the turtles emerged from nests. The earth's field is not completely stable but instead changes gradually over time (Skiles, 1985). The change in field elements, or secular variation, means that the field that exists in a particular natal area will gradually drift while turtles are away in distant oceanic regions (Lohmann et al., 1999). As a consequence, navigational errors may occur. For example, if a turtle returning to Ascension Island for the first time in 2010 were to try to find the island by swimming along a particular intensity isoline that intersected the island when the turtle was a hatchling 25 years earlier (Fig 5.1A), it would end up significantly south of the island because of how the magnetic field shifted during its absence (Fig 5.1B). Thus, a dependence on imprinted magnetic cues alone will probably not lead a turtle directly back to Ascension Island after it has been away for 25 years. Instead, a secondary cue, possibly olfactory in nature, could help turtles locate the island (Fig 5.1B). Alternatively or additionally, migrants approaching the island for the first time might find it by following other, more experienced turtles (Lohmann et al., 1999).

Female turtles return to Ascension Island to nest every 2-5 years after their initial migration (Carr, 1975; Mortimer and Carr, 1987; Mortimer and Poitier, 1989). During these short absences, the magnetic field typically shifts somewhat, but to a much lesser degree than occurs during 25 years. It is unknown whether turtles relearn the magnetic parameters of their nesting beach upon subsequent visits to Ascension Island, but even if they do, locating such a small island using magnetic cues alone might still be difficult.

Instead, perhaps female turtles employ a combination of magnetic and olfactory cues to locate Ascension Island. Sea turtles have a well-developed olfactory system (reviews by Bartol and Musick, 2003; Southwood et al., 2008; Schwenk, 2008) and can perceive both waterborne and windborne chemical cues (Manton et al., 1972; Grassman

and Owens, 1982; Grassman et al., 1984; Constantino and Salmon, 2003; Piovano et al., 2004; Endres et al., 2009; Endres and Lohmann, 2012). Because Ascension Island sits alone, thousands of kilometers from other land masses in the Southeast Atlantic Ocean, the chemical cues in the air and water near the island may be distinct from those farther from the island. Thus, in principle, a turtle might use the geomagnetic field to arrive in the vicinity of the island, where it would come into contact with land-based olfactory cues. In this way, odors emanating from the island would effectively enlarge the target, making it possible for turtles to find the island without encountering it directly, but instead by contacting secondary olfactory cues (Fig 5.1B). Once turtles come into contact with the chemical cues they could employ one of a number of search strategies to find the island.

To test whether green turtles might plausibly use a dual-cue strategy to relocate Ascension Island, I simulated the dispersal of waterborne and airborne odorants from the Island during the season in which adult green turtles migrate from Brazil. I plotted the locations of odorants on magnetic maps of intensity to assess whether magnetic cues and chemical plumes together might lead turtles to their natal site. The results show that over most of the last century there is a high degree of overlap between intensity isolines and both waterborne and airborne odorants, a finding consistent with the possibility that turtles rely on these two cues sequentially to locate Ascension Island.

Materials and Methods

The dispersal of waterborne odorants was simulated using the particle-tracking program ICHTHYOP v. 2 (Lett et al., 2008), which interpolates surface currents from the Global Hybrid Coordinate Ocean Model (HYCOM) (Bleck, 2002). Global HYCOM output has a spatial resolution of 0.08° (~5-7 km) and a daily time-step. HYCOM output

undergoes data assimilation to produce “hindcast” model output that better reflects *in situ* and satellite measurements. Global HYCOM thus resolves mesoscale processes such as meandering currents, fronts, filaments, and oceanic eddies (Bleck, 2002; Chassignet et al., 2006). For advection of particles through HYCOM velocity fields, ICHTHYOP implements a Runge Kutta 4th order time-stepping method (Lett et al., 2008). Additionally, I included horizontal dispersion in simulations to account for turbulent sub-gridscale processes not characterized by HCYOM (see Lett et al. (2008) for details).

Virtual particles were randomly seeded within an area 10 km from the coastline of Ascension Island. Particles were tracked at 5 vertical layers (0, 10, 20, 30, and 50 m from the surface). From December 15 - April 29 (the duration of the main nesting season at Ascension Island (Godley et al., 2001)), 500 particles were uniformly distributed among the 5 vertical layers and released. I assumed that the odorants being dispersed by currents maintained their integrity for 15, 30, or 45 d, after which they were removed from the simulation. For each odorant duration scenario, we plotted the location of all particles at 10 evenly spaced periods within the nesting season. We performed these simulations for the years 2004, 2005, 2006, and 2007.

Dispersal of airborne particles was modeled using the Hybrid Single-Particle Longrarian Integrated Trajectory (HYSPLIT) model (Draxler and Hess, 1997, 1998; Draxler, 1999). Virtual particles were released every hour from a height of 0 meters at the center of Ascension Island and tracked for 48 hours. Particle trajectories were plotted for 5 evenly spaced intervals between December 15 and April 29 during the 2009-2011 nesting seasons.

Maps of magnetic intensity isolines were derived from the International Geomagnetic Reference Field (IGRF-11) (MacMillan & Maus, 2005). Magnetic intensity was chosen because, in the geographic area near Ascension Island, it is a more stable parameter than inclination angle in terms of secular variation. Maps were produced

assuming that young green turtles imprint on the value of intensity at Ascension Island and after 25 years (i.e. upon reaching maturity) follow the isoline of this imprinted value (± 100 nT) from coastal Brazil to the open ocean. Similar maps were generated assuming that turtles returned after 5 years, as the case would be for a remigrating adult that had updated its memory of the intensity at Ascension Island during its last reproductive effort. The average remigration interval for Ascension Island turtles is 3-4 years, with the majority of turtles returning to Ascension between 2 and 5 years (Carr, 1975; Mortimer and Carr, 1987; Mortimer and Poirtier, 1989). Thus, a model for 5 years depicts a change in the magnetic field that is probably greater than what some turtles encounter, but may be realistic for others. The values for intensity were taken at the center of Ascension Island using the IGRF-11 model at 5 year intervals from 1900-2010, in accordance with changes in model coefficients (Macmillan & Maus, 2005). The isolines of the values were then plotted 25 years later (e.g. the intensity that existed at Ascension Island in 1900 was plotted in 1925; the intensity in 1905 was plotted in 1930, etc.). Likewise, isolines were also plotted 5 years later.

Maps of intensity isolines were then overlaid onto plots of waterborne and airborne particle trajectories (See Fig 5.2A, B). The goal was to assess how variability in ocean current and wind conditions would influence the two-part homing strategy over the past century. The particle distributions resulting from ocean current and wind conditions of each simulated year were overlaid on each magnetic map (1900, 1905, 1910, etc.) for both 25 and 5 year absences. We then determined the proportion of the time that conditions brought simulated particles (representative of windborne and waterborne odorants from Ascension Island) in contact with the intensity isoline.

Results

Maps depicting intensity isolines in combination with waterborne odor plumes from Ascension Island showed a high percentage of overlap between these two parameters, suggesting it is possible for a turtle to follow an intensity isoline from Brazil to the vicinity of Ascension Island, and then attempt to use waterborne odor cues to find the island directly. Waterborne odor plumes intersected 5-year isolines nearly 100 percent of the time, while they came into contact with 25-year isolines from 72 to 100 percent of the time, depending on the duration of the odorant. Simulations with odorant durations of 15 days intersected the intensity isoline in 72 to 84 percent of cases, a 30-day duration made contact in 96 to 99 percent of cases, and in the case of a 45-day duration, contact was made in 100 percent of cases (Fig 5.3). The means across all years showed that odor plumes contact the isoline an average of 78 percent of the time with a 15-day duration, 97 percent of the time for 30 days, and 100 percent of the time for 45 days (Fig 5.4).

Maps depicting intensity isolines in combination with airborne odor plumes showed a lesser, but still frequent, overlap between these two parameters over the last century. Odor plumes intersected 5-year isolines between 82 and 91 percent of the time (Fig 5.5), while they came into contact with 25-year isolines between 63 and 71 percent of the time (Fig 5.6).

During the early part of the century, the overlap between isolines and waterborne odorants for an odorant duration of 15 days was not as frequent as it was later in the century (Fig 5.7). The greatest amount of movement of the 25-year intensity isoline occurred prior to 1940 (Fig 5.8).

Discussion

The results from my simulations and mapping indicate that over most of the past century, a navigational strategy involving sequential use of magnetic and olfactory cues appears sufficient to enable green turtles to find Ascension Island. A turtle that followed an intensity isoline from Brazil would always come into contact with odors associated with Ascension Island if remigrating to the island within 5 years, and the majority of the time if migrating to the island for the first time after 25 years. Thus, chemical cues may essentially enlarge the target area for turtles, allowing them to employ a search strategy (Moore and Crimaldi, 2004) to locate Ascension Island eventually even if they are not able to travel directly to it.

Because turtles are able to perceive both waterborne and airborne odors, I ran simulations for both types of cues. We do not know if turtles depend more on chemical cues in the air or water, but regardless, a strategy that employed chemical cues in natal homing would, in most cases, allow turtles to come into contact with Ascension Island. If turtles use both types of chemical cues equally, there is a greater chance that turtles will detect an odor that might provide them with information about the location of Ascension Island.

For waterborne chemical cues, the longer the odorant duration, the better this multi-modal navigational mechanism performs. We can only speculate as to how long a possible chemical cue would persist in the surface waters of the equatorial Atlantic due to turbulent mixing, diffusion, and degradation by microorganisms. In the case of airborne chemical cues, we assumed a 48-hour duration, although it is again difficult to predict how long a chemical cue is likely to last in this environment. Because the wind consistently pushed particles directly in a WNW or NW direction, longer durations would presumably yield identical outcomes in most cases (because the windborne particles

typically intersected magnetic isolines in less than 48 hours). Similarly, slightly shorter durations are likely to yield similar results, whereas ephemeral odorants (e.g., those lasting only minutes) are unlikely to endure long enough to reach the isolines.

The overlap between isolines and waterborne odorants that lasted for 15 days was not as frequent early in the century as it was later in the century (Fig 5.7). This is likely due to the fact that the greatest amount of movement of the 25-year intensity isoline occurred prior to 1940 (Fig 5.8). After 1940 the interaction between these two parameters increases.

Previous tracking studies have provided some evidence consistent with the idea that turtles might use olfactory cues to locate Ascension Island. Some green turtles taken from the island during the nesting season and relocated to various positions distant from the island were able to relocate it by following circuitous routes, presumably searching for sensory contact with the island, such as olfactory cues (Akesson et al. 2003; Luschi et al. 2001). Alternatively, the search elicited in the presence of waterborne or airborne odor cues could bring turtles in contact with tertiary cues, such as visual cues that would lead them to the island.

Contrary to earlier proposals (Carr, 1972; Koch et al., 1969), chemical cues emanating from Ascension Island would be unlikely to provide turtles with useful navigational information over most of their migration. An odor plume that emanates hundreds to thousands of kilometers away would probably be too diffuse for turtles to derive information about its source. Similarly, over most of the century, magnetic cues alone would not permit turtles to come within view of Ascension, particularly in the case of turtles returning to the island for the first time after 25 years. Together, however, it is possible that these cues might guide turtles to the island.

A multi-modal homing strategy could be used by turtles migrating to locales other than Ascension Island. A turtle navigating to any nesting beach, whether on an island or

the mainland, might benefit from the use of multiple sensory cues. This is particularly true if turtles imprint on olfactory cues at their natal beach as well as magnetic cues (Lohmann et al., 2008c, 2013). In this case, a turtle might use the magnetic information on which it imprinted to arrive in the vicinity of its nesting area, and then employ the olfactory cues unique to a particular nesting site to locate it precisely.

Our modeling results show that a multi-modal approach to natal homing is a plausible mechanism for sea turtles returning to Ascension Island and, potentially, for those migrating long distances to other nesting and feeding areas. Future studies should investigate the specific chemical cues that may aid turtles in natal homing, as well as whether this approach might work for turtles homing to other areas.

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Figures

Figure 1 – A. A map depicting a magnetic isoline that runs from Brazil to Ascension Island. Brazil is on the left, marked by hash marks, while Ascension Island is the small black dot. The black line represents the magnetic intensity isoline that runs from Brazil and intersects Ascension Island in 1985. **B.** Map as depicted in A but 25 years later, in 2010. The same isoline as in A has shifted so that it no longer intersects Ascension Island, but runs south of the island. However, a waterborne odor plume, depicted in grey, emanates from the island and intersects the intensity isoline, providing turtles that are swimming along the isoline with a potential way to find the island.

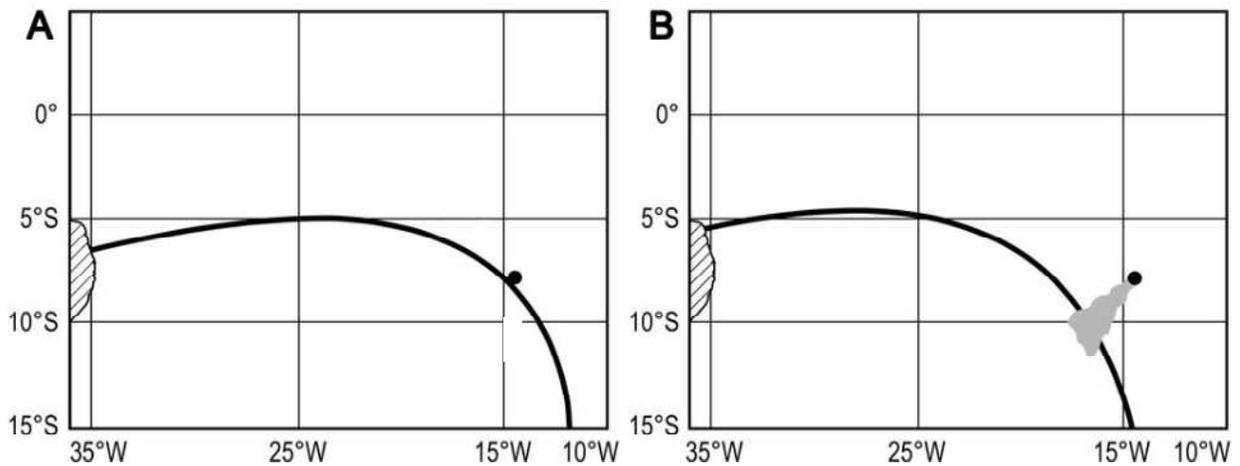
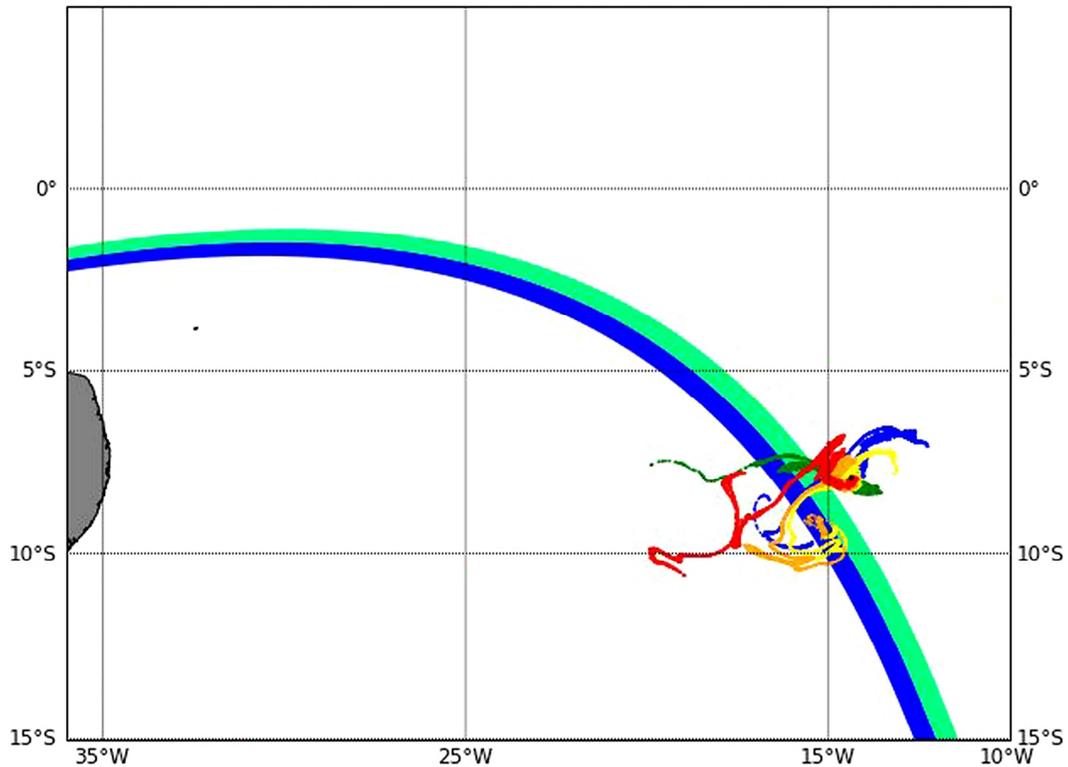


Figure 2

A. A map of a waterborne odor plume in combination with a magnetic intensity isoline. The small black dot at the right of the picture represents Ascension Island. The blue/green line represents the intensity ($\pm 100\text{nm}$) that existed at Ascension Island in the year 1940. Colored swirls emanating from the island represent the dispersal of waterborne odorant particles in the year 1965, when turtles that left the island in 1940 were expected to return for the first time. The red, orange, yellow, green and blue swirls represent odors at depths of 0, 10, 20, 30 and 50 m, respectively. Hypothetically, a turtle returning to Ascension Island in 1965 could follow an intensity isoline on which it imprinted in 1940 to arrive in the vicinity of the island, where it would then contact olfactory cues that may guide it the rest of the way to the island.

MagYear[1965.] Sim:2006 - Depth 0-50m: Particle Location at Day 303, Duration: 45 days



B. A map of airborne and waterborne odor plumes emanating from Ascension Island in combination with an isoline of magnetic intensity. The small black dot at the right of the picture represents Ascension Island. The blue/green line represents the intensity (+/- 100nm) that existed at Ascension Island in the year 1980. Colored lines headed in a WNW direction in pink, teal, light green, blue and red represent the trajectories of airborne particles released from Ascension Island in the year 2005, when turtles that left the island in 1980 would be expected to return for the first time. The red, orange, yellow, green and blue swirls are waterborne odor plumes as described in A. In the year 2005, both waterborne and airborne particles intersect the intensity isoline that contacted Ascension Island in 1980.

MagYear[2005.] Sim:2006 - Depth 0-50m: Particle Location at Day 333, Duration: 45 days

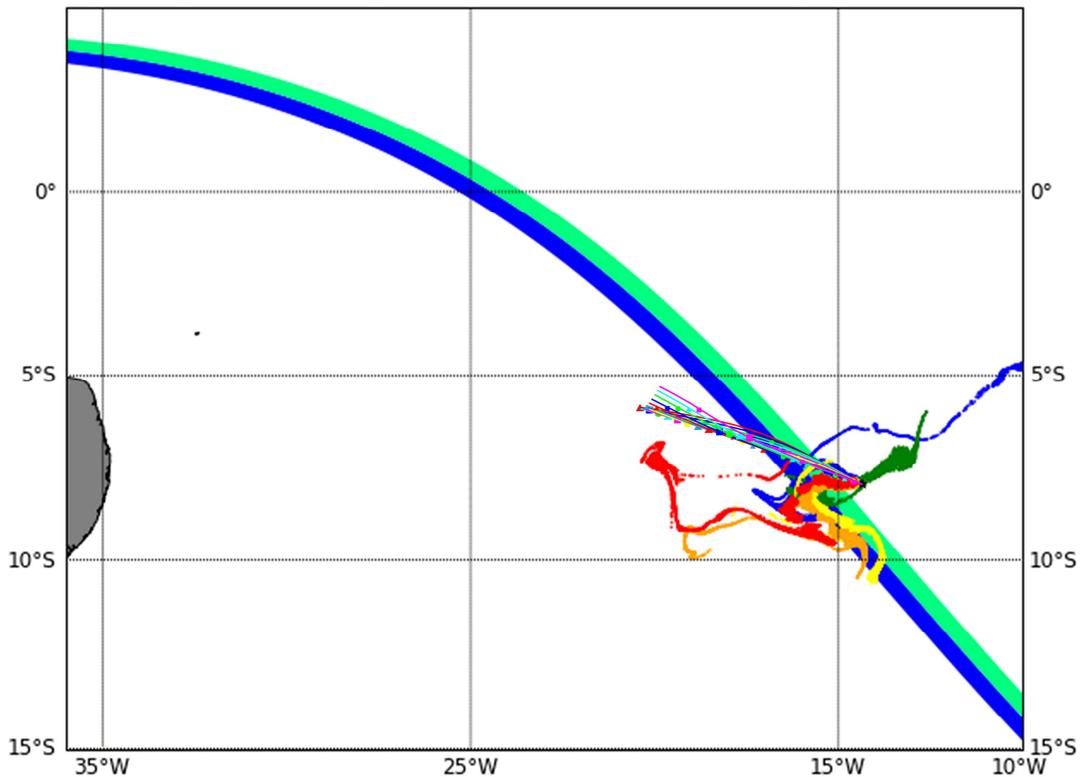


Figure 3

Proportion of time that particles intersect the 25-year intensity isoline for 15, 30 and 45-day odorant durations (yearly average). Error bars represent 95% confidence intervals

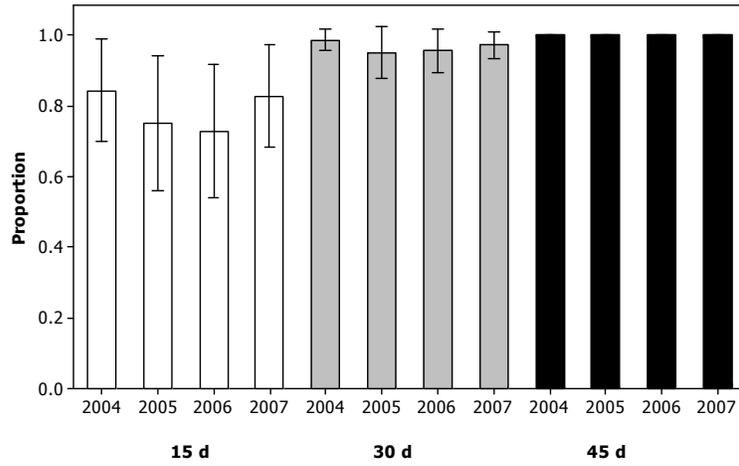


Figure 4

Proportion of time that particles intersect the 25-year intensity isoline for 15, 30 and 45-day odorant durations (averages from years 2004-2007). Error bars represent 95% confidence intervals.

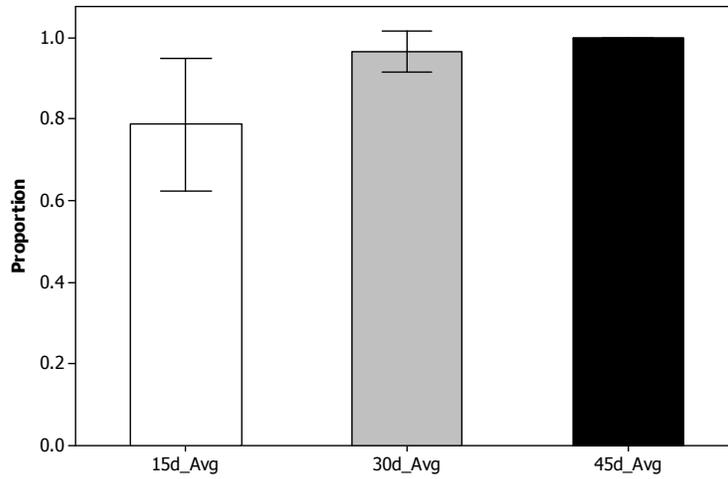


Figure 5

Proportion of time that airborne odor particles intersect 5 year isoline (yearly averages).

Error bars represent 95% confidence intervals.

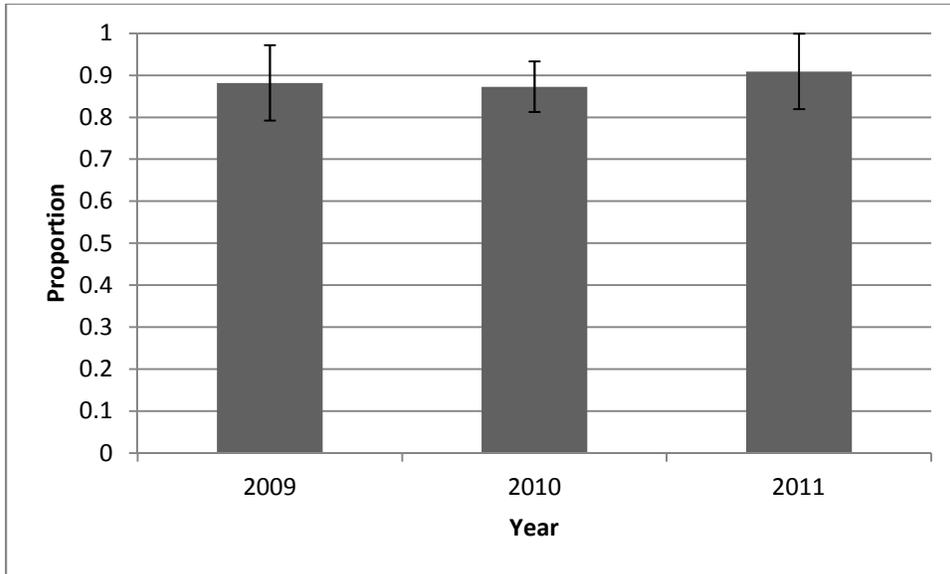


Figure 6

Proportion of time that airborne odor particles intersect 25-year isoline (yearly averages).

Error bars represent 95% confidence intervals.

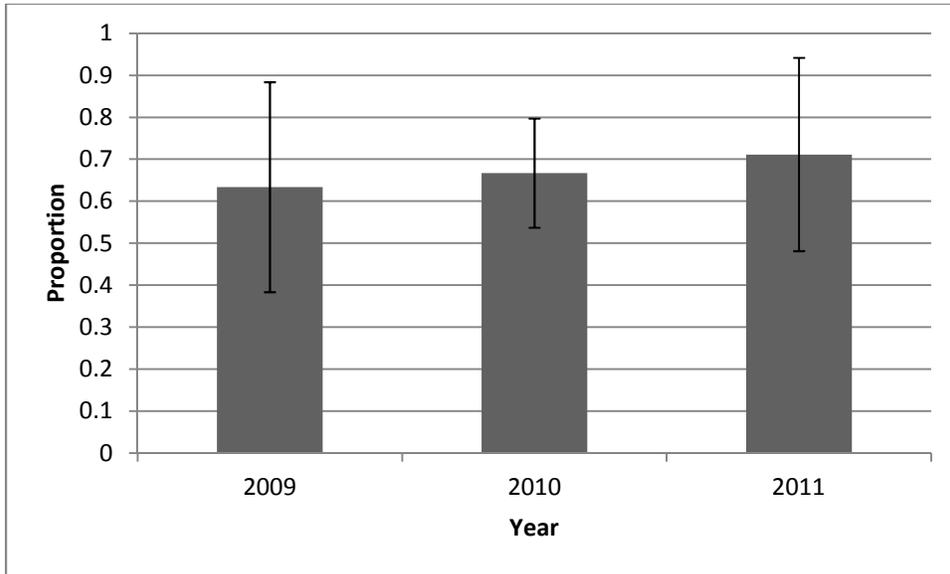


Figure 7

Proportion of time that particles intersect 25-year intensity isoline (4 year average) over the past century

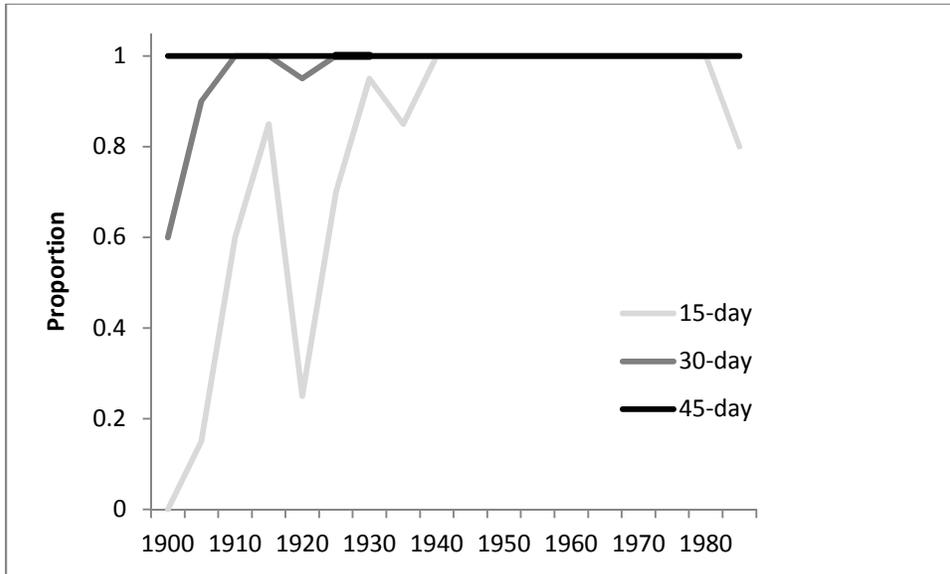
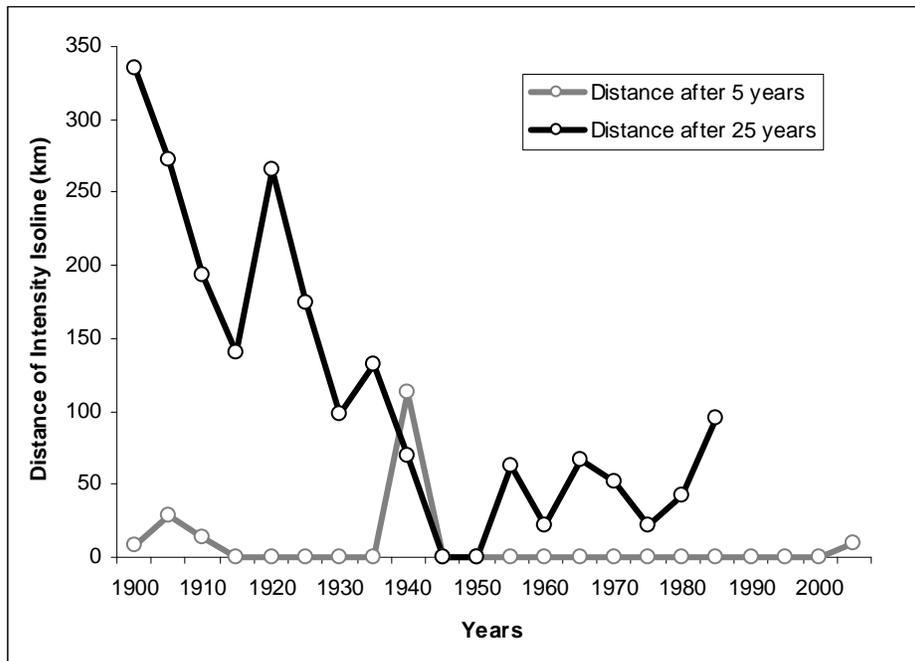


Figure 8

Distance an intensity isoline moves from Ascension Island over 5 and 25 years. For example, an intensity isoline in the year 1905 is nearly 350 km away from where it will be 25 years later in 1930.



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CHAPTER SIX: CONCLUSIONS

The objectives of this research were to investigate the chemical senses of sea turtles and to apply this knowledge to their ability to locate foraging and breeding areas at sea. I addressed these objectives through behavioral research and modeling, and was successful in demonstrating that sea turtles can detect airborne odors that might be useful in recognizing favorable foraging areas, as well as possibly enabling turtles to determine when they are in close proximity to land. In addition, I demonstrated that the ability to detect both waterborne and airborne odors, in combination with the use of magnetic cues, might function in a multi-modal homing strategy for sea turtles. These findings suggest that the detection of chemical cues might play an important role in turtle navigation.

In Chapter 2 I investigated whether turtles possess the ability to perceive airborne odorants. Turtles were presented with the airborne odor of their gel food and were found to increase their activity in its presence, and not in the presence of dH_2O , indicating that they could indeed smell their food. This ability is consistent with the fact that turtles breathe air and have well-developed olfactory systems (Schwenk, 2008) and olfactory gene repertoires (Kishida et al., 2007). The study with gel food, however, provided the first direct experimental evidence that sea turtles can indeed detect airborne chemical cues.

In Chapter 3 I began to explore specific odors that might be important to turtles migrating through the sea. Dimethyl sulfide (DMS) has been studied widely in the context of global climate change and can be detected by several animals (Cunningham

et al., 2008; Kowalewsky et al., 2006; Amo et al., 2013), including Procellariiform sea birds, which are likely to use it as a foraging cue (Nevitt et al., 1995; Nevitt, 2008). Like these birds, turtles need to locate widely distributed patches of prey in the ocean, so it seemed that they, too, might benefit from an ability to detect DMS. We found that turtles responded to DMS by spending more time at the water surface with their noses in contact with air, presumably sniffing the air. They did not spend as much time at the surface when presented with dH₂O, cinnamon, jasmine, or lemon scents, suggesting that they were particularly interested in DMS, and that it might be biologically meaningful. This experiment demonstrated that it is possible that turtles use DMS to locate productive oceanic foraging areas.

In Chapter 4 I explored the ability of turtles to detect another airborne odorant that might be of benefit in terms of navigation. An ability to detect land might be important to turtles throughout their lives. We performed an experiment to determine whether turtles could perceive airborne odors associated with coast mud. Again, turtles spent more time with their noses above the water in the presence of coastal mud than they did in the presence of dH₂O, cinnamon, jasmine, or lemon scents, suggesting that they were interested in this odor. The ability to detect land odors might aid turtles in navigation at several life stages. Young turtles might benefit from knowing that they are near land because such shallow, coastal areas tend to be predator-dense and turtles could move away from such areas. Adult turtles looking for remote islands or specific nesting beaches might also benefit from the ability to detect land.

Finally, in Chapter 5, I looked at how a combination of olfactory and magnetic cues might be used by turtles in a multi-modal approach to natal homing. With the use of oceanographic and atmospheric models, I created odorant trajectories that were then combined with maps of magnetic intensity to assess how a multi-modal homing strategy might work for turtles. The results indicated that, over most of the past century, a turtle

could follow the magnetic isoline that intersected Ascension Island 5 or 25 years previously to arrive within the vicinity of the island, and then encounter secondary olfactory cues emanating from the island that might allow it to find the island directly. These results suggest that using magnetic and olfactory cues sequentially is a reasonable multi-modal homing strategy.

This research on sea turtle olfaction has several important implications. First, because all species of sea turtles are endangered or threatened, it is of benefit to their conservation that we know as much as possible about their behavior and sensory systems. Knowledge of the cues they use to find food and navigate is important in helping determine why some nesting areas or populations of turtles may be in decline.

In addition, findings and concepts derived from studies on sea turtles may be applicable to other marine migrants. For example, an interesting parallel exists between sea turtles and salmon, inasmuch as both are likely to use a multi-modal homing strategy. Salmon hatch in streams, swim out to the ocean where they remain for several years, and then return to their natal stream to spawn (Dittman and Quinn, 1996). They are known to imprint on their natal stream using olfactory cues, and use these cues to locate their natal stream once they arrive in its vicinity (Johnsen and Haler, 1980; Doving et al., 1985; Dittman et al., 1996; Nevitt and Dittman, 1998). However, it is not known what cues they use to migrate through the ocean, although magnetic cues are one possibility (Lohmann et al., 2008b). Thus, the situation in salmon is the reverse of that in turtles, in which we have an idea of which sensory cues they use over large scales, but not which they use once they are in the vicinity of their goal. However, the idea that turtles might use olfactory cues in the last phase of their migration, and new data suggesting that salmon also use magnetic cues during their migration from the open sea to their natal rivers (Putman et al., 2013) demonstrates how these two species might use

similar navigational strategies, with magnetic cues and olfactory cues used sequentially to home (Lohmann et al., 2008b, 2008c).

Future research in this area might investigate whether sea turtles will respond to the scent of DMS or odors associated with land under natural conditions, and also whether turtles detect and respond to additional airborne odorants of biological importance. Additionally, it might be useful to test a magnetic and olfactory multi-modal approach to homing in a different locale. Another isolated island or coastal nesting area would be a good candidate for this, and could provide insights into whether this is a generally plausible mechanism.

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