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Meredith Anne Schlenner
Minnesota State University - Mankato

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Sun-Compass Orientation During Natal Dispersal in Hatchling Blanding’s Turtles (*Emydoidea blandingii*)

By

Meredith Anne Schlenner

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Meredith Anne Schlenner

This thesis has been examined and approved by the following members of the student's committee.

Advisor Dr. John D. Krenz

Committee Member Dr. Christopher T. Ruhland

Committee Member Dr. Robert Sorensen

Date of Approval

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(*Emydoidea blandingii*)
Meredith Anne Schlenner
Master of Science in Biology
Minnesota State University, Mankato
Mankato, Minnesota

Abstract

Blanding's turtle hatchlings emerge from their natal nests naïve to environmental stimuli and primarily sense visual cues on the horizon as a dispersal target. During a period of hours or days, hatchlings develop a compass mechanism that allows them to maintain a direction of travel, even when the target is not visible. We examined the dispersal directions of Blanding's turtle hatchlings captured during dispersal by translocating them into a circular arena in a field of corn in order to measure their dispersal direction guided by a compass mechanism. To test for use of a sun compass, a magnetic compass, or both, we observed dispersal direction of hatchlings released at the center of the arena. Hatchlings were released in an initial trial, treated with normal (no-shift) or 6-hr clock-shifted photoperiods for 4 to 10 days, and released into the arena for a second trial with magnets (or non-magnetic controls) adhered to their carapaces. We predicted that clock-shifting would reduce dispersal angle 90° and disruption of magneto-reception would disorient hatchlings. All four treatment groups dispersed directionally during first trials (Rayleigh's Z-tests; all $p < 0.001$) and in second trials dispersal angles were unchanged in hatchlings with magnets (Watson's $U^{2;}$ both $p > 0.50$); they were not using a geo-magnetic compass. Hatchlings that were not clock-shifted maintained their initial heading but clock-shifted hatchlings reduced dispersal angle a mean of 111°, not significantly different than the experimental prediction of 90° (Rayleigh's $Z = 22.217$, $p < 0.001$, no-shift; Rayleigh's $Z = 19.286$, $p < 0.001$, shift; Watson's $U^2_{55,58}$ $p < 0.001$). An analysis of dispersal angles using only daily means of groups of turtles each released on different days also showed significant directionality, no magnet effect, and a significant clock-shift effect (two-sample Hotelling test, $p < 0.002$). Hatchlings were using a sun compass exclusively.

Keywords: Sun-compass orientation, geomagnetic-reception, Blanding's turtles hatchlings, clock-shift, magnet, natal dispersal, Minnesota

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Chapter 1. Literature Review

Cycles caused by the tilt of the Earth's rotational axis have profound influences on the behavior of many living organisms. Most organisms exhibit rhythms in their physiology and behavior that endure under constant conditions (Hart, 1964; Menaker, 1969; Sharma, 2003a & 2003b; Phillips, 2005). These rhythms are guided by an endogenous mechanism called a biological clock (Sollberger, 1962; Aschoff, 1963; Menaker, 1969). Most measurable activities exhibited by organisms bear a synchronous relationship to the 24-hr daily cycle (Hart, 1964). These activities are termed circadian rhythms. Circadian rhythms have been widely observed in plants, animals, fungi, and cyanobacteria (Sharma, 2003b; Phillips, 2005).

Daily rhythms behave in much the same way as do many physical oscillators (Menaker, 1969). Therefore, it is important to become familiar with the language of physical oscillations and its application to biological cycles. Menaker (1969) provides a review of the relevant vocabulary. He considers the behavior of a nonlinear spring with a mass attached to one end and the other attached to a rigid surface. Upon initiation, and ignoring the effects of friction, oscillations will continue unless disturbed by an external force. These recurring oscillations comprise a *rhythm*. The repeating unit of a rhythm is referred to as a *cycle*. The length of time required to complete one cycle is referred to as the *period*. Rhythms can be displaced in time without changing the basic character of the oscillation. This is termed a *phase shift*. Phase shifts can occur in either direction and in any amount. The oscillator will exhibit a certain natural period for a given displacement in the absence of external forces. In this condition, the oscillator is said to be *free-*

running. If a sufficient external force with a different period is applied to the oscillator, it will then exhibit the period of the applied force. This phenomenon is termed *entrainment*. The biological clock is considered to be a collection of coupled oscillators (Beersma *et al.,* 2011).

In mammals, the circadian clock resides in the suprachiasmatic nucleus (SCN); (Ralph *et al.,* 1990; Young, 2000; Hastings & Herzog, 2004). The SCN is a neuronal hypothalamic tissue located just above the optic chiasm. The SCN consists of approximately 10,000 interconnected neurons and pacer cells (Moore & Eichler, 1972; Moore, 1973; Ralph *et al.,* 1990; Hastings & Herzog, 2004; Beersma *et al.,* 2011).

The specific genes that individual clocks use to generate circadian clocks can differ considerably among taxa (Rosato & Kyriacou, 2001; Phillips, 2005). Standard models for circadian clocks are generated and sustained by a transcription-translation negative feedback loop (Reppert & Weaver, 2002; Hastings & Herzog, 2004). In groups as diverse as mammals and fruit flies, a set of three primary genes produces proteins that interact with one another to regulate the activity of certain other genes on a cycle lasting approximately 24 hours. The period gene *(per),* codes for a protein (PER) that gradually accumulates inside and outside of the cell nucleus. Another key gene called *tau* in mammals and *dbt* in flies, codes for an enzyme, casein kinase epsilon (CKIe), that helps break down PER. Consequently, slowing the rate of accumulation in the cell. However, during peak periods of production of PER, more *per* is available to bond with another protein (TIM), coded for by the third gene *(tim)*. When the PER protein is bound incomplexes with TIM, (and another protein, chryptochrome (CRY), in the case of mammals), it cannot be broken down as quickly by CKIe. Therefore, more intact PER is carried back into the nucleus, where it blocks the activity of the gene that produces it. Subsequently, a new cycle of *per* gene activity and PER protein production begins (Young, 2000). A more detailed description of the process can be found in Ralph *et al.* (1990), Forger & Peskin (2003), Hastings & Herzog (2004), and Beersma *et al* (2011).

Not only are daily rhythms widespread phylogenetically, but they also occur at all levels of organization within species (Pittendrigh, 1960a & 1960b; Menaker, 1969). The ubiquity of circadian rhythms implies its strong adaptive significance. It is generally thought that organisms possessing a circadian clock gain fitness advantages in two ways: (1) coordinating internal metabolic processes and (2) allowing organisms to synchronize their behavior with environmental cues.

Circadian clocks provide organisms a fitness advantage by coordinating internal metabolic processes (Sharma, 2003b). It is thought that incompatible metabolic processes, requiring different physiochemical conditions for their successful functioning, appear to be separated temporally in an efficient way by means of the circadian clock (Moore-Ede *et al.,* 1982; Sharma, 2003b). Circadian clocks also provide organisms a fitness advantage by synchronizing behavioral and physiological processes to reliable, cyclic environmental changes (Sharma, 2003b). Although it remains unclear how and why clocks with an approximately 24-hour period evolved, a vigilant analysis would suggest that it provides ultimate stability to its expression under consistent environmental cues (Beersma *et al.,* 1999; Roenneberg *et al.,* 2003; Sharma, 2003a & 2003b). Organisms that possess circadian clocks are able to prepare their metabolic processes and behaviors appropriate for a specific time of day before the time actually arrives, as opposed to meeting daily cycles unprepared (Phillips, 2005).

Circadian rhythms allow organisms to anticipate and prepare for regular environmental changes through entrainment. Several cyclic features of the environment such as light-dark (LD) cycles, temperature, and humidity are examples of cues that are used to entrain circadian rhythms (Phillips, 2005). The natural LD cycle is arguably the most reliable of the environmental signals and is, consequently, the primary zeitgeber for resetting the clock (Phillips, 2005). However, circadian rhythms persist even in the absence of zeitgebers (Menaker, 1969; Sharma, 2003b). Organisms held under constant conditions typically exhibit rhythms different than but resembling a 24-hour cycle (Menaker, 1969). Thus, the period is said to be self-generating or endogenous and is referred to as the free-running period (FRP); (Menaker, 1969).

Circadian rhythms can be subjected to a phase shift upon the application of a periodic signal. The phase of the rhythm with respect to the signal will continue to adjust until the signal is positioned in such a manner that its daily effect compensates for the difference between the FRP of the rhythm and the period of the signal (Pittendrigh & Minis, 1964; Menaker, 1969). This practice is also commonly referred to as clock-shifting. Clock-shifting experiments can be utilized to assess navigation and orientation mechanisms present within organisms. The following publications are exemplary illustrations of clock-shifting experiments on pigeons, fresh water turtles, lizards, sparrows, and sea turtles, respectively: Schmidt-Koenig, 1960; DeRosa & Taylor, 1978, 1980, & 1982; Muheim & Aakesson, 2002; Foà *et al.,* 2009; Mott & Salmon, 2011.

Navigation is movement from a starting point to a specific goal location. To orient means to determine and keep a compass direction. Organisms with compass orientation, but without knowledge of a specific geographic goal, maintain their direction of travel even when displaced laterally. There are a number of ways in which environmental cues may be used to guide organisms during these movements. Multiple compass systems exist in vertebrates (sun, star, polarized light and magnetic compasses); (Wiltschko & Wiltschko, 1972 & 1996; Emlen, 1975; Able, 1980; Wiltschko, 1983; Phillips, 1986; Moore, 1987; Schmidt-Koenig *et al.,* 1991; Phillips & Moore, 1992; Lohmann & Lohmann, 1996 & 2006; Lohmann *et al.,* 2007). Compass mechanisms allow an organism to determine which direction to travel in a landscape and how to maintain that direction. This differs from a map sense, derived from a compass mechanism, which allows an organism to know where it is located within a landscape based on learned markers (Caldwell & Nams, 2006).

The utilization of the sun, as an aid in navigation and orientation, is referred to as a sun compass. This particular type of celestial orientation is accomplished via the determination of the position of the sun on the horizon (i.e., its azimuth) and the determination of local time (Adler, 1970). In order to sun-compass orient, an organism requires a natural or synthetic light stimulus and a biological clock in phase with the local time (DeRosa & Taylor, 1978). Utilization of a time-compensated sun compass for orientation is present in both invertebrate and vertebrate taxa (Avens & Lohmann, 2003). Due to the temporal component of this directional sense, use of a sun compass is typically demonstrated via the conduction of a clock-shift experiment.

Clock-shifting experiments are conducted by entraining the internal clock of an organism to a light-dark cycle that is equivalent to the natural cycle, except it is shifted in time a fraction of a cycle. Subsequent to this shift, orientation trials under regular conditions are observed and assessed for predicted shifts in orientation. The sun moves approximately 15° along its azimuth per hour (Schmidt-Koenig *et al.,* 1991). Thus, for each hour that an organism is clock-shifted from the local time, it should exhibit a 15° change in direction when released under natural conditions (Schmidt-Koenig *et al.,* 1991; Southwood & Avens, 2010). Specifically, clock-shifting experiments have demonstrated the use of a sun compass in a number of reptiles including but not limited to garter snakes (Lawson, 1994), water snakes (Newcomer *et al.,* 1974), and juvenile alligators (Murphy, 1981).

Sun-compass orientation has been well documented in sea turtles (Mott & Salmon, 2011) but insufficiently in freshwater and terrestrial turtle species. Sun-compass orientation experiments have been conducted on adult box turtles (*Terrapene carolina),* softshell turtles (*Trionyx spinifer),* painted turtles *(Chrysemys picta),* and eastern long-necked turtles (*Chelodina longicollis*); DeRosa & Taylor, 1978, 1980, & 1982; Graham *et al.,* 1996). We are not aware of any documentation of sun-compass orientation experiments conducted on hatchling, freshwater or terrestrial species. Few investigations of the behavior of freshwater hatchling turtles in general have been reported (Shine & Iverson, 1995; Southwood & Avens, 2010).

A second compass common to many varieties of organisms is the magnetic compass. Magnetic compasses are present in a wide variety of phylogenetic groups. They exist in several invertebrate groups as well as all major groups of vertebrates (Cain *et al.,* 2005; Lohman & Lohman, 2006). The exact mechanisms by which organisms detect magnetic fields are still poorly understood (Lohman & Lohman, 2006). Animals can evidently extract at least two different types of information from the earth's magnetic field: (1) compass information which allows an organism to maintain a consistent direction of orientation and (2) map information which enables organisms to assess geographic location and, in some cases, navigate to target areas (Cain *et al.,* 2005; Lohman & Lohman, 2006). Magnetic compasses are particularly adaptive to organisms by allowing them to establish a directional sense under environmental conditions where cues such as the sun, stars, or visual landmarks are not available for use, such as marine environments. Magnetic compasses also allow organisms to maintain a directional sense unaffected by seasons, winds, or the effects of day and night among other factors.

There does exist the possibility of redundancy among compasses. Having the ability to use two or more compasses simultaneously and/or non-synchronously would allow organisms to orient correctly under varying environmental conditions. If the cues used for one compass were indistinct or blocked, the organism could utilize a secondary compass mechanism in order to orient properly. The use of multiple compasses has been well documented in birds (Wiltschko & Wiltschko, 1976 & 2001; Wiltschko, 1983; Wiltschko *et al.,* 1987; Phillips & Moore, 1992). Wiltschko & Wiltschko (2001) reports that deflections produced by a 6-hr clock-shift in adult pigeons are markedly smaller than would be predicted on the basis of the difference of the sun azimuth (approximately 60% of the predicted deflection). The addition of small bar magnets to the clock-shifted birds increased the deflection to approximately 90% of that predicted. These findings suggest that the change in deflection in adult pigeons is to be attributed to the pigeons' use of a magnetic compass in the absence of accurate sun-azimuth cues; pigeons seem to combine directional information from the sun compass with information from the geomagnetic field.

Blanding's turtle, *Emydoidea blandingii*, is a medium-sized fresh water turtle that is native to the northeastern United States, the Midwest, and southeastern Canada. Blanding's turtles often occupy eutrophic habitats with abundant vegetation such as lakes, ponds, marshes, creeks, wet prairies, and sloughs (Ernst & Lovich, 2009). The species is listed as threatened or endangered in most of the locations in which it occurs. Within the literature, there exists extensive data pertaining to the natural history of Blanding's turtles (MacCulloch & Weller, 1988; Power, 1989; Ross, 1989; Ross & Anderson, 1990; Congdon & van Loben Sels, 1991; Rowe, 1992; Congdon *et al.,* 1993; Standing, 1997; Standing *et al.,* 1999; Congdon *et al.,* 2000; McNeil, 2002; Refsnider, 2005; Ruane *et al.,* 2008). Despite intensive research, however, the behavior of

Blanding’s hatchlings is still poorly understood (Congdon *et al.,* 1983; Butler & Graham, 1995; Standing *et al.,* 1997; McNeil *et al.,* 2000; Dinkelacker *et al.,* 2004; Smith, 2004; Refsnider, 2005; Carmaclang, 2007; Pappas *et al.,* 2009 & 2013; Congdon *et al.,* 2015).

Blanding’s hatchlings emerge from the nest in late August through early October and migrate to a wetland habitat. Studies of post-emergent behavior of hatchlings show no significant orientation towards water (Standing *et al.,* 1997; McNeil *et al.,* 2000; Smith, 2004; Refsnider, 2005; Carmacling, 2007; Pappas *et al.,* 2009). Studies of post emergent behavior of hatchlings show a general modality toward dark horizons (Butler & Graham, 1995; Standing *et al.,* 1997; McNeil *et al.,* 2000; Carmacling, 2007; Pappas *et al.,* 2009; Congdon *et al.,* 2015). Pappas *et al.* (2009 & 2013) and Congdon *et al.* (2015) found that experienced hatchlings (those having a period of dispersal experience) were able to maintain their initial direction of travel upon translocation into experimental arenas. While the mechanisms used to maintain directionality in Blanding’s hatchling turtles is unknown, there is evidence suggesting that geotaxis, olfactory cues, humidity gradients, social facilitation, and geomagnetic cues are all unlikely (Noble & Breslau, 1938; Anderson, 1958; Burger, 1976; Ehrenfeld, 1979; Manton, 1979; Congdon *et al.,* 1983; Robinson, 1989; Butler & Graham, 1995; Standing *et al.,* 1997; McNeil *et al.,* 2000; Dinkelacker *et al.,* 2004; Smith, 2004; Refsnider, 2005; Carmaclang, 2007; Congdon *et al.,* 2015).

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Chapter 2. Use of sun compass during natal dispersal in hatchling Blanding's turtles

Contact address:

Department of Biological Sciences

Minnesota State University

Mankato, Minnesota 56001

Introduction

Orientation has been defined as a selective process in which certain environmental cues elicit a response that results in a non-random pattern of locomotion, a change in the direction of the body axis, or both (Adler, 1970). Movement in a specific direction, regardless of the homeward direction, in unfamiliar territory is known as compass orientation (Griffin, 1952). Organisms use external cues such as magnetic fields, prevailing winds, or celestial bodies to compass orient. If the position of the external cue is cyclic, an organism must use an internal biological clock to adjust its orientation relative to the cue depending on time of day (DeRosa & Taylor, 1976). The internal clock must be entrained to the external cycle. (Roenneberg *et al.,* 2003; Sharma, 2003a; Beersma *et al.*, 2011). If the stimulus used to entrain the clock cycles with a 24-hr period, it is referred to as a circadian rhythm (Menaker, 1969; Sharma, 2003a; Sharma, 2003b). If the stimulus used to reset the clock is the sun, an organism can use the sun's azimuth to maintain a constant compass orientation at any time of day (sun-compass orientation; Adler, 1970; DeRosa & Taylor, 1976).

Organisms have been experimentally caused to alter their orientation by subjecting them to entrainment by a light-dark (LD) cycle out of phase with the natural LD cycle (DeRosa & Taylor, 1976; Wiltschko *et al.,* 2000; Muheim & Aakesson, 2002; Giunchi *et al.,* 2003; Foa *et al.,* 2009; Nesbit *et al.,* 2009). This process is referred to as clock-shifting (Wiltschko & Wiltschko, 2001; Muheim & Akesson, 2002; Giunchi *et al.,* 2003). The sun moves across the horizon approximately 15° per hr (Schmidt-Koenig *et al.,* 1991). Thus, for each hour that an organism is clock-shifted from the natural cycle, it should exhibit a 15° change in direction when released under natural conditions (DeRosa & Taylor, 1976; Schmidt-Koenig *et al.,* 1991; Southwood & Avens, 2010; Mott & Salmon, 2011).

Sun-compass orientation has been well documented in sea turtles (Mott and Salmon, 2011) but insufficiently in freshwater and terrestrial turtle species. Sun-compass orientation experiments have been conducted on adult box turtles (*Terrapene carolina),* softshell turtles (*Trionyx spinifer),* painted turtles *(Chrysemys picta),* and eastern long-necked turtles (*Chelodina longicollis*) (DeRosa & Taylor, 1978, 1980, & 1982; Graham *et al.,* 1996). We are not aware of any sun-compass experiments on freshwater hatchling turtles.

Blanding's turtle, *Emydoidea blandingii*, is a medium-sized fresh water turtle that is native to the northeastern and mid-western United States, and southeastern Canada. Despite intensive research, the behavior of Blanding's hatchlings is poorly understood

(Congdon *et al.,* 1983; Butler and Graham, 1995; Standing, 1997; Standing *et al.,* 1997; McNeil *et al.,* 2000; McNeil, 2002; Dinkelacker *et al.,* 2004; Smith, 2004; Refsnider, 2005; Carmaclang, 2007; Pappas *et al.,* 2009 & 2013; Congdon *et al*., 2015).

Blanding's hatchlings emerge from the nest in late August through early October and are generally thought to migrate to wetland habitats. Pappas *et al.* (2009 & 2013) and Congdon *et al.* (2015) reported that experienced hatchlings (those having a period of dispersal experience) were able to maintain their initial direction of travel upon translocation into experimental arenas. While the mechanisms used to maintain directionality during dispersal in Blanding's turtle hatchlings are unknown, there is evidence suggesting that the use of geotaxis, olfactory cues, humidity gradients, and social facilitation are unlikely (Noble and Breslau, 1938; Anderson, 1958; Burger 1976; Ehrenfeld, 1979; Manton, 1979; Congdon *et al.,* 1983; Robinson, 1989; Butler and Graham, 1995; Standing *et al.,* 1997; McNeil *et al.,* 2000; McNeil, 2002; Dinkelacker *et al.,* 2004; Smith, 2004; Refsnider, 2005; Carmaclang, 2007). We suggest an initial dispersal toward dark horizons and the use of a sun compass in maintaining direction after a period of dispersal experience. A sun compass that allows individuals to maintain straight-line movements toward an identified goal in an efficient manner would be highly adaptive to hatchlings that are susceptible to desiccation and predation during neonatal dispersal (Zollner and Lima, 1999). Straight-line travel decreases the time and energy spent dispersing, therefore, preserving energy reserves and limiting time exposed to predators. The other advantage of straight-line travel (versus random or wandering) is the likelihood of finding an aquatic habitat; straight-line travel would always avoid the possibility of a turtle crossing its own path, which would reduce such likelihood.

The objective of this experiment was to determine the mechanisms by which hatchling turtles maintain compass orientation during natal dispersal. We hypothesized that once a hatchling has experienced visual horizon cues and begun to disperse toward one, it soon thereafter establishes a direction of travel and maintains it using a sun compass, a magnetic compass, or both. Field experiments were used to test the prediction that either clock-shifting or the blocking of magneto-reception would alter or disrupt compass orientation (table 1). Controlling for the possible use of each type of compass is necessary because some organisms are known to use two or more redundant compasses, each having utility under different environmental conditions. The use of more than one compass has been well-documented in birds (Wiltschko & Wiltschko, 1976 & 2001; Wiltschko, 1983; Wiltschko *et al.*, 1987; Phillips & Moore, 1992; Lohmann & Lohmann, 1996). Our experimental design accounts for the possibility that hatchlings have the ability to use both a sun compass and a magnetic compass redundantly in addition to testing for the use of each of the compasses independently.

Methods

Study Area

Fieldwork occurred from May 2012 to September 2013 in the Weaver Dunes area of southeastern Minnesota (44°15'N and 91°55'W). The Weaver Dunes area consists of 285 ha of gently rolling, sand dune topography interrupted by steep sloping, stabilized dunes and occasional blowouts. Some of the dunes are stabilized by sand prairie, oak savannah plant communities, or recently planted pine plantations, and some areas have been converted to agricultural fields. The dunes are located adjacent and on the east side of a 1500-ha wetland.

Obtaining hatchlings

Dispersing hatchlings were captured while dispersing in a westerly direction as we patrolled a 2-km paved north-to-south highway located between the dunes area and the wetland. Hatchlings were captured crossing the road throughout the day for several days during each of our field seasons; end of August through September. We transported each hatchling immediately to the field station laboratory, painted a unique number on the carapace, and maintained the animal under moist conditions with a natural light:dark cycle until released in an arena (1 or 2 days for most hatchlings).

Experimental arena

A circular arena approximately 95 m in diameter was constructed of solid aluminum flashing 15 cm tall and buried to a depth of 5 cm on relatively flat terrain in an agricultural field of mature corn plants. The arena was constructed in the same area of the same cornfield for both years of the study. The purpose of the cornfield site was to provide a uniform horizon and therefore eliminate horizon cues (Congdon *et al.,* 2015). Sixteen pitfall traps were placed at uniform intervals (22.5°) along the interior of the fence to prevent significant movement along the fence prior to capture. By patrolling the fence continually following each release, most hatchlings were captured before they fell into a pitfall trap, and we recorded their capture location using 160 evenly spaced marks on the fence (2.25° interval; figure 1).

Release protocol

Hatchlings were released in the arena on the first sunny or partly sunny day after initial capture following the protocol of Pappas *et al.* (2009). Prior to release, hatchlings were placed into a bucket with four compartments; each with a covered escape hole. Buckets were transported to the arena center between 0700 and 1000 hr; the release time depending on ambient temperature. We removed the escape hole covers and quickly left the arena. We removed the bucket after 1 hr. The arena fence was patrolled continuously starting approximately 30 min after hatchling release and continuing until 1 hr had elapsed with no hatchling captures. At capture, hatchling identification, time, and position along fence were recorded. A second release occurred after a 4 to 10-day clock-shifting period and magnet treatment and followed the same protocol as the first release. Following final captures, all hatchlings used in the study were released in wetlands near their original point of capture.

Clock-shifting protocol

Subsequent to the initial release, all hatchlings that were collected were transported to a light controlled room and maintained under constant conditions for 4 to 10 days. The length of light treatment varied due to weather constraints; turtles were released into experimental arenas only on warm, sunny days after at least 4 days of light treatment. The turtles were randomly divided into two groups and treated with indoor LD cycles that were either a natural mimic or clock-shifted 6 hr. The natural LD cycle (no-shift) was 0635 - 1950 hr (Central Daylight Time); the clock-shifted cycle was 0035 - 1350 hr. In each chamber, two 75-W fluorescent, grow-light bulbs were suspended approximately 2 m above the floor and the appropriate LD cycle was governed by automatic timers. Temperature in the light chambers was approximately 20° Celsius. The hatchlings were maintained in large plastic bins and provided moist soil and free water.

Disruption of magneto-reception

The two photoperiod treatment groups were further divided by assigning hatchlings randomly to a magneto-reception disruption treatment (magnet) or no-disruption control treatment (no-magnet), thereby creating an experimental design with four treatments. Disruption of magneto-reception was accomplished by the adhesion of neodymium disc magnets (6.3 mm diameter and 1.5 mm thick) with epoxy to the anterior portion of the carapace of hatchlings. Flux densities were approximately 25 and 9 gauss at 15 and 22 mm, respectively (part number D41-N52, K&J Magnetics, Inc.). For each hatchling in the no-magnet treatment, we glued an aluminum disc on their carapace; a small number of hatchlings in the no-disruption treatment received no metal. The four experimental groups described are illustrated in table 1.

To ensure the magnets would provide adequate disruption of hatchling magneto-reception, we estimated magnet strength relative to geomagnetism in the laboratory by placing a magnet and a compass on a level surface and moving the magnet toward the compass from the west and measuring deflection of the compass needle (total needle length 22 mm) at four distances. At each distance, we used four magnet orientations to simulate the slight variation in magnet position relative to the turtles' bodies during the field trials (figure 2). We also tested magnet strength by rotating the surface while the magnet and compass were in position to simulate changes in the angle of a turtle's line-of-travel during the field experiments. Although needle deflection was sensitive to magnet orientation at the longer distances, magnet distance had a significant effect on deflection: at 22 mm distance orientation was unimportant and needle deflection was maximal (west, 90°) and significantly greater than at 32 mm (64.5°, $t = 6.05$, $p < 0.001$). When the surface was rotated, the compass needle remained locked into position relative to the compass at a distance of 22 mm. During rotation of the surface with the magnet at 32 mm, the compass needle was not locked in place completely but was significantly affected by the magnet position. For the purpose of disruption of magneto-reception, these results demonstrate significant disruption at distances greater than those occurring during the field trials; hatchlings with magnets attached to their carapace could not possibly have navigated by magneto-reception of the geomagnetic field (figure 2).

Statistical analysis

Angles of dispersal were analyzed as circular data. We tested for nonrandom distributions using Rayleigh's Z-test and tested for the significance of differences in mean angles between treatment groups using Watson's U^2-test (Oriana software Version 4; Zar, 1999). Because of the possibility that hatchlings who were released together during second trials on the same day may have behaved non-independently, we conducted a second-order analysis with Hotelling tests comparing the daily mean dispersal angles by treatment. The non-random distribution of dispersal angles within each treatment was analyzed using the one-sample Hotelling test and treatment differences were analyzed using the two-sample Hotelling test (Zar, 1999).

Results

We collected complete data on 113 turtles over the course of two years; 2012 and 2013. 102 of those turtles were from the 2013 field season. Complete data refers to those turtles who were found moving WNW on a road, were translocated and released in the corn arena, captured within 12hrs, put into light treatment for 4-10 days, re-released in the corn arena, and re-captured within 12hrs. The main observation of interest is the difference before and after light treatments in dispersal angle in the corn arena.

Hatchlings captured while crossing the road in a westerly direction exhibited the same directionality when translocated and released into the corn arena. (Mean vector μ = 290.0°; Length of vector r = 0.5, Rayleigh's Z = 28.1, $p < 0.001$; figure 3).

Dispersal angles of the magnet and no-magnet treatments were significantly non-random for the no-shift light treatment (Rayleigh's Z = 10.31, $p < 0.001$, no-magnet; Rayleigh's Z = 12.49, $p < 0.001$, magnet) and for the clock-shift treatment (Rayleigh's Z = 10.34, $p < 0.001$, no-magnet; Rayleigh's Z = 9.09, $p < 0.001$, magnet). However, the effect of the magnet treatment on dispersal angle was not significant in either light treatment; Watson's $U^2_{26,29} = 0.05$, $p > 0.5$, no-shift; Watson's $U^2_{29,29} = 0.03$, $p > 0.5$, shift; figure 4). Hatchlings in the no-shift treatment consistently went he same direction in corn release one and corn release two, regardless of magnet treatment. Hatchlings in the shift treatment exhibited the expected change in dispersal angle between their first and second trials, regardless of magnet treatment. Because there was no effect of the magnet treatment, all data from magnet and no-magnet treatment groups were pooled to perform subsequent statistical tests of the effect of clock-shifting.

In 2012, dispersal angles of both no-shift and clock-shifted hatchlings significantly different from random (Rayleigh's $Z = 7.02$, $p < 0.001$, no-shift; Rayleigh's $Z = 4.88$, $p = 0.003$, shift). Change in dispersal angle from the first to second release of no-shift versus clock-shifted hatchlings was significantly different and in accord with the predicted change of 90° (mean change in dispersal angle = -3.2°, no-shift; -90.2°, shift; SE = 10,0, no-shift; 11.6, shift; Watson's $U^2_{6,9} = 0.33$, $p < 0.005$; figure 5).

Dispersal angles of all 2013 hatchlings in release four were not significantly different than random (Rayleigh's $Z = 0.83$, $p = 0.449$, shift). Change in dispersal angle from the first to second release of no-shift versus clock-shifted hatchlings was not significantly different (mean change in dispersal angle = -20.3°, no-shift; 28.7°, shift; SE = 27.4, no-shift; 33.0, shift; Rayleigh's $Z = 1.54$, $p = 0.220$, no-shift; Watson's $U^2_{6,9} = 0.16$, $0.1 > p > 0.05$; figure 6).

Dispersal angles of 2013 no-shift hatchlings in release six were not significantly different than random. Dispersal angels of 2013 clock-shifted hatchlings in release six were significantly different than random (Rayleigh's $Z = 1.50$, $p = 0.227$, no-shift; Rayleigh's $Z = 5.42$, $p = 0.003$, shift). Change in dispersal angle from the first to second release of no-shift versus clock-shifted hatchlings was significantly different and in accordance with the 90°shift prediction (mean change in dispersal angle = 29.1°, no-shift;

-101.0°, shift; SE = 30.2, no-shift; 15.9, shift; Watson's $U^2_{11,15} = 0.27$, $p < 0.020$; figure 7).

Dispersal angles of all 2013 hatchlings in release eight were significantly different than random (Rayleigh's $Z = 11.35$, $p < 0.001$, shift). Change in dispersal angle from the first to second release of no-shift versus clock-shifted hatchlings was significantly different and in accordance with the 90°shift prediction (mean change in dispersal angle = -11.9°, no-shift; -112.6°, shift; SE = 8.2, no-shift; 9.6, shift; Rayleigh's $Z = 14.64$, $p < 0.001$, no-shift; Watson's $U^2_{23,19} = 0.56$, $p < 0.001$; figure 8).

Mean dispersal angles were pooled for all release dates, and the distributions within both shift and no-shift treatments were non-random (Rayleigh's $Z = 19.29$, $p < 0.001$, no-shift; Rayleigh's $Z = 22.22$, $p < 0.001$, shift) and differed between the shift and no-shift treatments. Change in dispersal angle from the first to second release of no-shift versus clock-shifted hatchlings was significantly different and in accordance with the 90° shift prediction (mean change in dispersal angle = 1.2°, no-shift; -111.0°, Shift; SE = 7.9, no-shift; 9.9, shift; Watson's $U^2_{55,58} = 1.32$, $p < 0.001$; figure 9).

The distributions of daily mean dispersal angles by treatment were non-random (one-sample Hotelling test, $p = 0.031$, $n = 5$ releases, no-shift; $p = 0.037$, $n = 7$ releases, shift). The effect of clock-shifting was a significant reduction in dispersal angle of -99.4° (no-shift mean 2.3°, two-sample Hotelling test $p < 0.002$; figure 10). Neither mean was different than the experimental predictions of -90° and 0°.

Discussion

Soon after emergence from natal nests, Blanding's turtle hatchlings develop a compass mechanism that allows them to maintain a direction of travel towards a dispersal target. Possessing a mechanism that allows for straight-line travel during dispersal may have a number of benefits including but not limited to minimizing time and energy spent reaching the dispersal target, exposure to predation, and risk of desiccation. The mechanisms used to maintain directionality in Blanding's hatchling turtles are poorly understood. The use of a sun compass and/or geo-magnetic compass are likely the most practical means of maintaining directionality for hatchlings who exhibit a strong tendency to move on sunny, warm days during the daytime hours. The possibility of redundancy among compasses does exist. Having redundant compass mechanisms would allow an organism to maintain directionality under the circumstances that the cues for one compass were temporarily indistinct or unavailable. If hatchlings possess a magnetic compass, then their ability to maintain a previously established direction of travel should be disrupted by the attachment of a magnet to their carapace (magnet treatment). If hatchlings possess a sun compass, then their ability to maintain a previously established direction of travel should be disrupted by a shift in their natural LD cycle (clock-shift). If hatchlings possess both compasses, then their ability to maintain a previously established direction of travel should only be disrupted by both magnet treatment and a clock-shift. Under all other circumstances, they would be able to utilize their other compass mechanism as a means to maintain directionality.

Hatchlings in each of four treatments were not affected by the magnet treatment (table 2). Hatchlings maintained under natural LD conditions (no-shift) treatment maintained their direction of travel between releases (table. 3). Hatchlings exposed to a 6-hr clock-shift behind the natural LD cycle (clock-shift) exhibited a change of direction of approximately 90° counter-clockwise, commensurate with the magnitude of the clock-shift (table 3). Our experimental analysis (figure 2) in addition to existing data (Keeton, 1971; Lohmann & Lohmann, 1996) suggests that our manipulation for the magnet treatment was strong enough to impose a significant disruption of any existing magneto-reception. Natural experienced hatchlings captured moving approximately west in their natural environment provided compelling evidence for the use of a sun compass but not a geo-magnetic compass.

It is unclear how quickly sun-compass orientation is developed in individuals or how plastic orientation is once developed. Our data shows that 4 days of clock-shifting is sufficient to cause the expected change in orientation in hatchlings, but the necessary duration of clock-shifting may be more brief. Future studies should focus on the stimuli and mechanisms responsible for the formation of the sun compass in reptiles as well as the length of time and conditions required to develop it. It appears that our clock-shift treatment did not stimulate hatchlings to alter their intended direction of travel; orientation persisted despite captivity of more than 10 days for some batches of experimental hatchlings. Future studies could assess whether circumstances exist that would cause a hatchling to modify its original direction of orientation towards a target.

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Table 1. Predicted changes in compass orientation by natural-experienced, dispersing hatchlings in four experimental treatments including clock-shifting (clock-shift versus no-shift) and disruption of geomagnetism (magnet versus no-magnet). Natural experienced hatchlings are those that were captured while dispersing in a westerly direction across a paved road from a sand dunes area where females naturally nest. These captives have developed compass orientation during the hours and days prior to capture. The presence or absence of sun-compass orientation and magnetic compass orientation predicts that dispersing hatchlings will orient correctly (maintain their initial direction of travel) or be disoriented (orient in a direction other than their initial direction of travel). Disorientation is observed as a 90° error in direction of orientation (commensurate with a 6 hr clock-shift and indicative of the use of a sun compass) or random orientation (indicative of the use of a magnetic compass). Predictions assume that at least one compass orientation mechanism exists. a. outlines the results indicative of the exclusive use of a sun compass. b. outlines the results of the exclusive use of a magnetic compass. c. outlines the results of the use of both compasses.

a

Sun compass only		
	Clock-shift	No-shift
Magnet	90 degrees	None
No-magnet	90 degrees	None

b

Magnetic compass only		
	Clock-shift	No-shift
Magnet	Random	Random
No-magnet	None	None

c.

Using both compasses		
	Clock-shift	No-shift
Magnet	90 degrees	None
No-magnet	None	None

Table 2. Sample sizes; numbers of hatchlings completing experiment (dispersal to arena perimeter within 12hrs both before and after laboratory LD treatments).

Julian date of release	Treatment			
	No-shift LD cycle		Clock-shifted LD cycle	
	Magnet	Aluminum	Magnet	Aluminum
2012[a]	0	9	0	6
2013				
Release 3 (251)	0	0	0	5
Release 4 (245)	3	3	6	3
Release 5 (256)	3	3	1	1
Release 6 (257)	7	4	9	6
Release 7 (259)	0	0	1	1
Release 8 (265)	14	9	9	10
Total	27	28	26	32

[a] – Includes animals pooled from 4 experimental releases during fall 2012.

Table 3. Analysis of the effect of clock-shifting on angle of dispersal in natural-experienced Blanding's turtle hatchlings. Shown are the sample size (number of hatchlings), the mean change in direction in degrees, the standard error of the mean change in direction, and the Rayleigh's Z test statistic and corresponding p-value for each release; 2012 data represents all turtles for that field season. In addition, Watson's U^2 test value and corresponding p-value for each release are shown. Watson's U^2 test value is <0.001 for all data combined; indicating a significant result. The no-shift treatment resulted in a grand mean of -0.5°change in the dispersal angle while the clock-shift treatment resulted in a -83.8° shift in dispersal angle; not significantly different than the predicted shift of -90°.

	Light Treatment										Treatment effect	
	No-shift					Clock-shift						
Year and release number (Julian day)	n	Mean vector	r	Rayleigh's Z	p	n	Mean vector	r	Rayleigh's Z	p	Watson's U^2	p
2012[a]	9	-3.2	0.88	7.02	<0.001	6	-89.7	0.90	4.88	0.003	0.33	<0.005
2013												
3 (251)						5						
4 (245)	6	-15.1	0.51	1.54	0.222	9	108.3	0.30	0.83	0.449	0.16	<0.10
5 (256)	6					2						
6 (257)	11	75.6	0.37	1.50	0.227	15	-111.7	0.60	5.42	0.003	0.27	<0.02
7 (259)						2						
8 (265)	23	-9.81	0.80	14.64	<0.001	19	-115.4	0.77	11.35	<0.001	0.56	<0.001
Total	55	-1.0	0.64	19.29	<0.001	58	-111.0	0.58	22.22	<0.001	1.32	<0.001

n - sample size.

Mean vector refers to mean vector of dispersal for all hatchlings in a treatment group on their second release in the corn arena.

r – Mean length of vector

[a] – Includes animals released on 4 days during fall of 2012.

List of figures:

Corn field arena location for 2012 and 2013. Lines indicate the 16 approximate pitfall trap locations on perimeter for both years. Pitfall traps were placed by researchers guided by an observer located at the center of the arena using a mirror compass to identify azimuths.

Angles of deflection of a compass needle (22 mm total needle length) by a cylindrical magnet (6 mm diameter, 1.5 mm length) placed at four distances west of the compass in each of four orientations. The orientation treatments, which simulated the slight variation in tilt and elevation of the magnets relative to the turtle's bodies during the field trials, were level (magnet cylinder on end), level and elevated 5 mm above compass, nearly level but tilted at an angle of 10° toward east, and tilted 10° south. When the magnet was within 20 mm of the compass needle, there were no differences among treatments; any conformation of the magnet attracted the needle compass strongly (greater deflection than at 32 mm; t-test, $p < 0.001$). During the field trials, the magnets strongly disrupted potential reception of geomagnetism.

Figure 3……...……………..55

Dispersal angles of hatchlings released into the corn arena for the first release prior to LD treatments. Arrow indicates mean vector of dispersal (v). Length of the arrow indicates length of mean vector (r). Sample size (n), Rayleigh's z test statistic, and corresponding p-value are also shown.

Figure 4. ……...………….56

The effect of the magnet treatment on dispersal angle was not significant in either the no-shift treatment (magnet mean vector $\mu = 9.46°$ (SE = 9.9); Aluminum mean vector $\mu = -8.8°$ (SE = 11.1); Watson's test, $U_{26,29} = 0.053$, p-value > 0.5) or the clock-shifted treatment (magnet mean vector $\mu = -115.7°$ (SE = 12.214); no-magnet mean vector $\mu = -106.6°$ (SE = 11.25); Watson's test, $U_{29,29} = 0.025$, p-value > 0.5). Arrows indicate mean vector (v). Length of arrow indicates length of mean vector (r).

Figure 5. ……...………….57

Change in dispersal angle between first and second releases in the corn arena caused by shift and no-shift light treatments for all 2012 data pooled. The means of both treatment groups are significantly different. Distributions of both the no-shift and shift treatments are significantly directional, and mean vectors of dispersal (v) are not significantly different than the predicted shift of 0° for no-shift treatment and -90° for shift treatment (-3.2°, no-shift**;** -89.7°, shift). Arrows indicate mean vector (v). Length of arrow indicates length of mean vector (r). Sample size (n), Rayleigh's z test p-value, and Watson's U^2 test statistic and corresponding p-value are also shown.

Hotelling test comparing mean of all daily release means. The mean of means of both treatment groups were significantly different. Distributions of both the no-shift and shift treatments are significantly directional, and mean vectors of dispersal (v) are not significantly different than the predicted shift of 0° for no-shift treatment and -90° for shift treatment (2.3°, no-shift**;** -99.4°, shift). Arrows indicate mean vector (v). Length of arrow indicates length of mean vector (r). Sample size (n) and Hotelling test p-values are also shown. Points within figure represent means of each release for both treatment groups. Distance from center of figure to release mean points represent the length of mean vector for each individual release.

Figure 1.

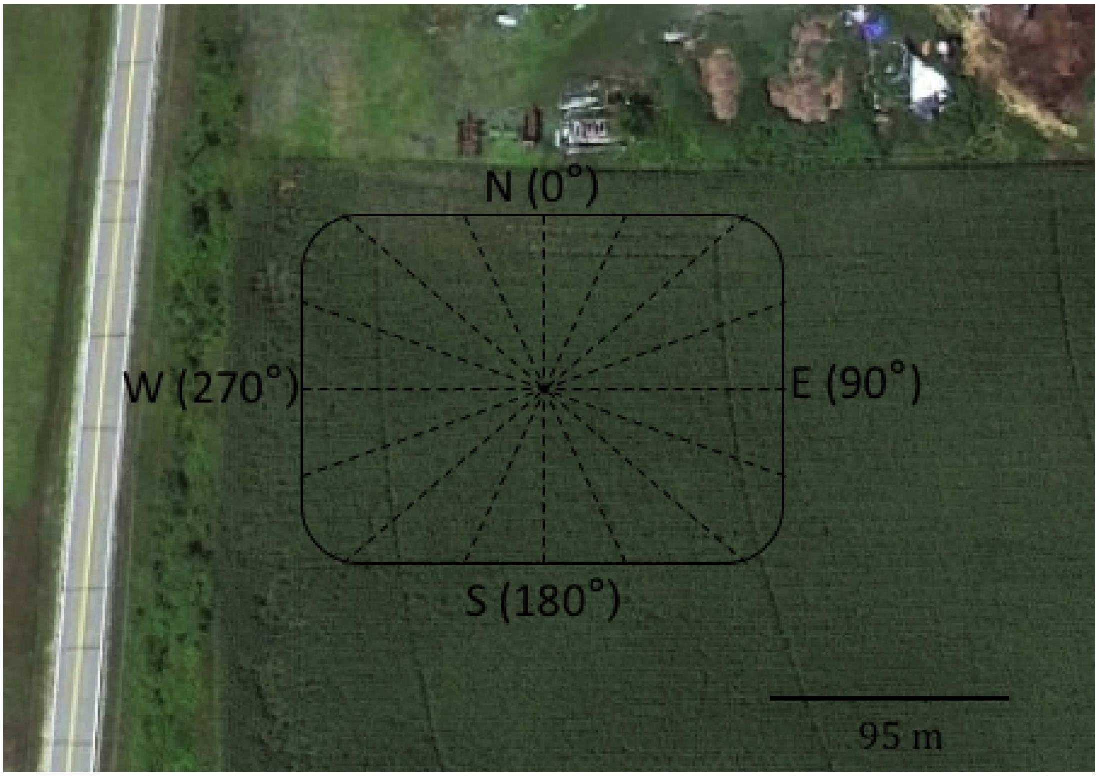

Figure 2.

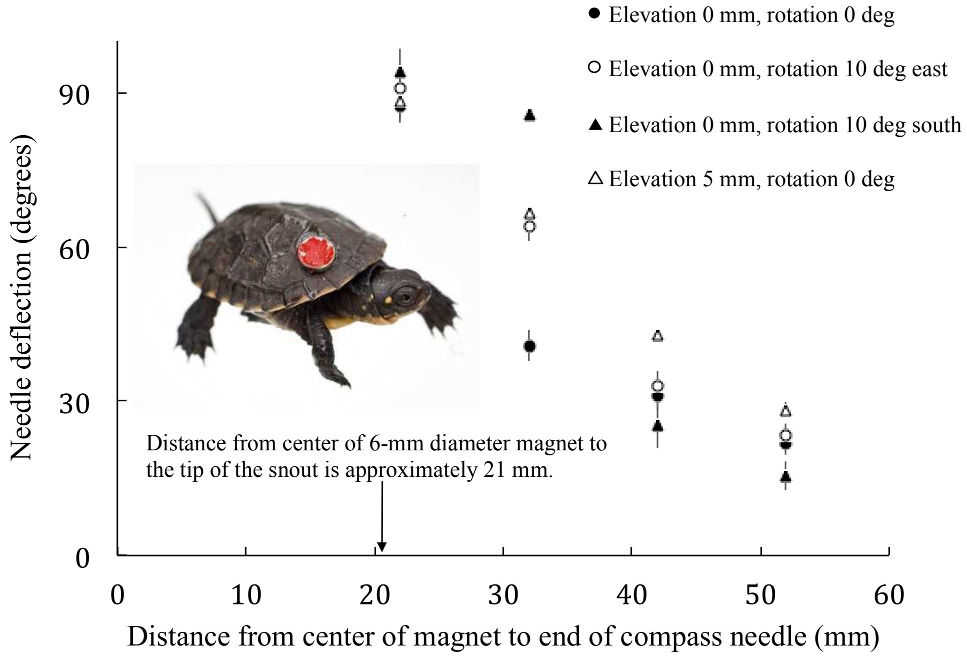
● Elevation 0 mm, rotation 0 deg
○ Elevation 0 mm, rotation 10 deg east
▲ Elevation 0 mm, rotation 10 deg south
△ Elevation 5 mm, rotation 0 deg
Needle deflection (degrees)
0
30
60
90
Distance from center of 6-mm diameter magnet to the tip of the snout is approximately 21 mm.
0
10
20
30
40
50
60
Distance from center of magnet to end of compass needle (mm)

Figure 3.

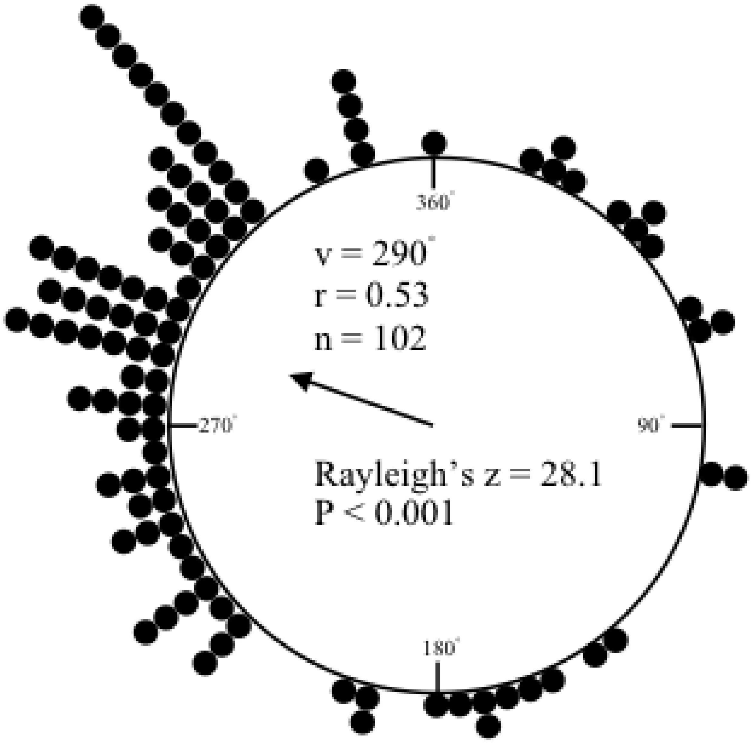

Figure 4.

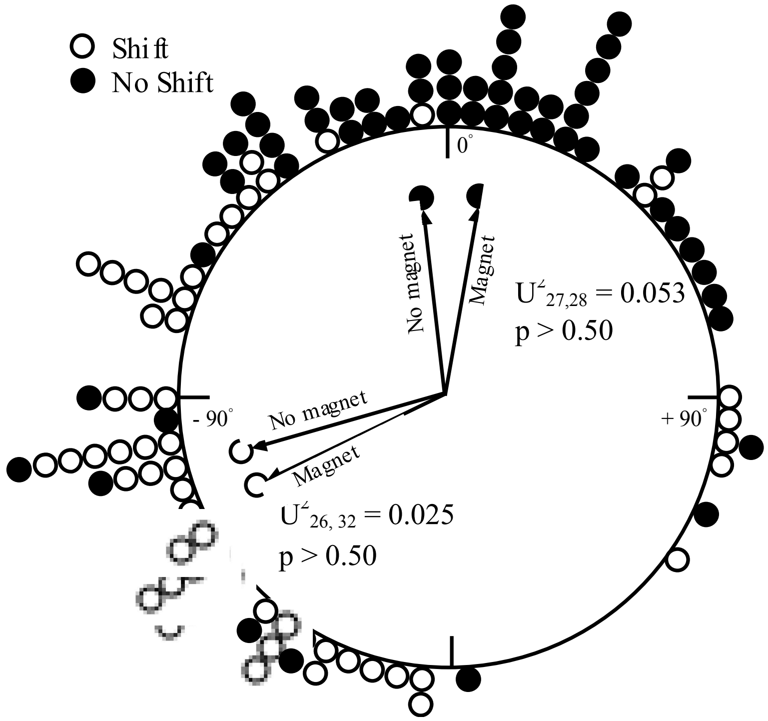
○ Shift
● No Shift
0°
- 90°
+ 90°
No magnet
Magnet
No magnet
Magnet
$U^2_{27,28} = 0.053$
$p > 0.50$
$U^2_{26,32} = 0.025$
$p > 0.50$

Figure 5.

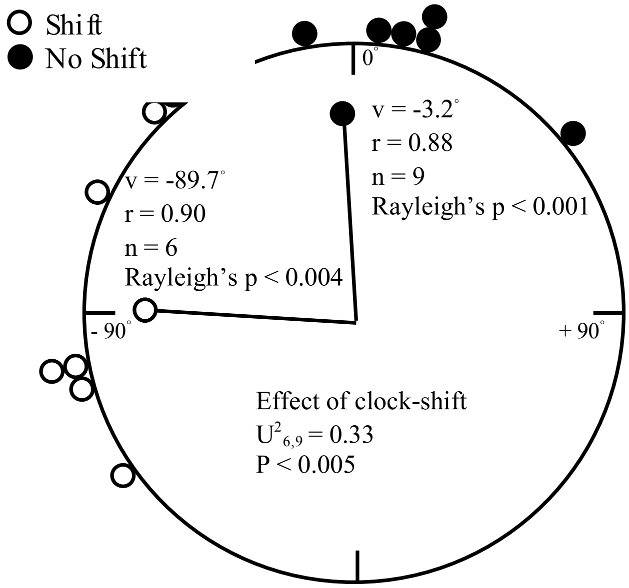
○ Shift
● No Shift
0°
v = -3.2°
r = 0.88
n = 9
Rayleigh's p < 0.001
v = -89.7°
r = 0.90
n = 6
Rayleigh's p < 0.004
- 90°
+ 90°
Effect of clock-shift
$U^2_{6,9} = 0.33$
P < 0.005

Figure 6

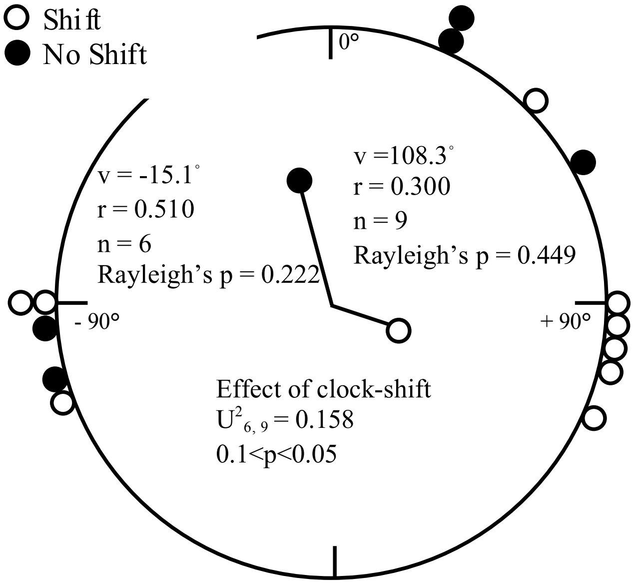
○ Shift
● No Shift
0°
v = -15.1°
r = 0.510
n = 6
Rayleigh's p = 0.222
v =108.3°
r = 0.300
n = 9
Rayleigh's p = 0.449
- 90°
+ 90°
Effect of clock-shift
U²₆, ₉ = 0.158
0.1<p<0.05

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Figure 7.

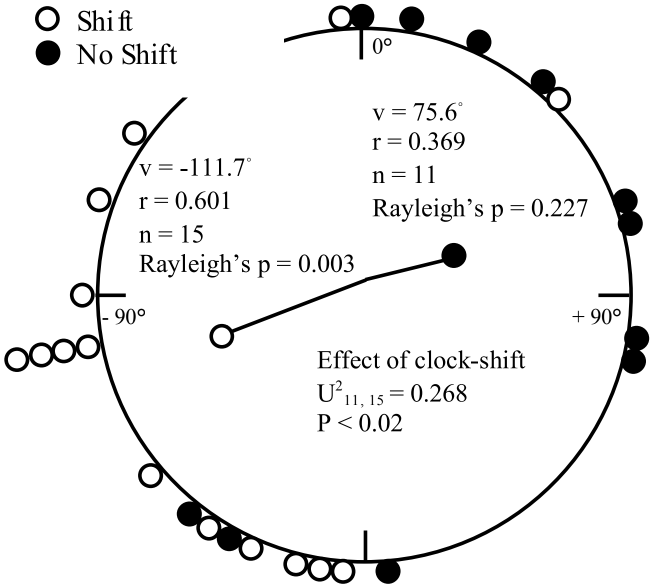

Shift
No Shift
0°
- 90°
+ 90°
v = 75.6°
r = 0.369
n = 11
Rayleigh's p = 0.227
v = -111.7°
r = 0.601
n = 15
Rayleigh's p = 0.003
Effect of clock-shift
$U^2_{11, 15}$ = 0.268
P < 0.02

Figure 8.

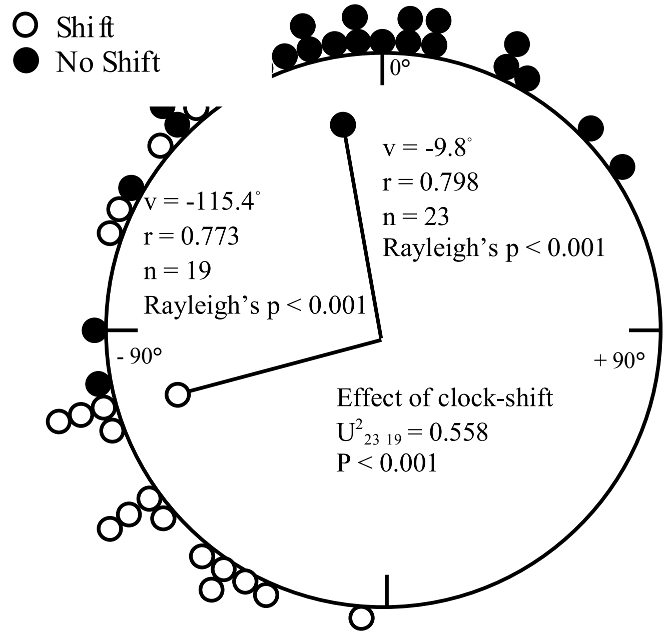

○ Shift
● No Shift
0°
- 90°
+ 90°
v = -9.8°
r = 0.798
n = 23
Rayleigh's p < 0.001
v = -115.4°
r = 0.773
n = 19
Rayleigh's p < 0.001
Effect of clock-shift
U²23 19 = 0.558
P < 0.001

Figure 9.

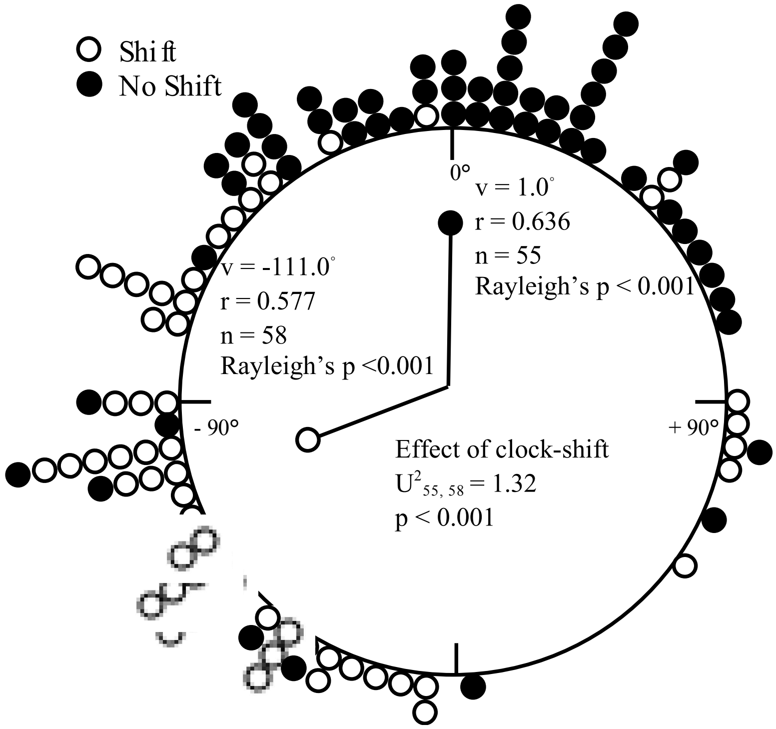
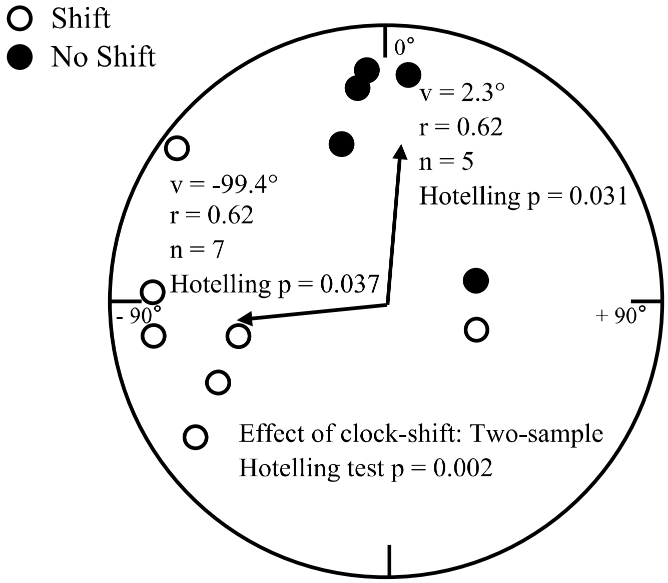

○ Shift
● No Shift
0°
- 90°
+ 90°
v = 1.0°
r = 0.636
n = 55
Rayleigh's p < 0.001
v = -111.0°
r = 0.577
n = 58
Rayleigh's p <0.001
Effect of clock-shift
U²55, 58 = 1.32
p < 0.001

Figure 10.


○ Shift
● No Shift
0°
- 90°
+ 90°
v = 2.3°
r = 0.62
n = 5
Hotelling p = 0.031
v = -99.4°
r = 0.62
n = 7
Hotelling p = 0.037
Effect of clock-shift: Two-sample
Hotelling test p = 0.002