

RESEARCH ARTICLE

Evidence for the use of a high-resolution magnetic map by a short-distance migrant, the Alpine newt (*Ichthyosaura alpestris*)

Francisco J. Diego-Rasilla^{1,*} and John B. Phillips^{2,*}**ABSTRACT**

Newts can use spatial variation in the magnetic field (MF) to derive geographic position, but it is unclear how they detect the 'spatial signal', which, over the distances that newts move in a day, is an order of magnitude lower than temporal variation in the MF. Previous work has shown that newts take map readings using their light-dependent magnetic compass to align a magnetite-based 'map detector' relative to the MF. In this study, time of day, location and light exposure (required by the magnetic compass) were varied to determine when newts obtain map information. Newts were displaced from breeding ponds without access to route-based cues to sites where they were held and/or tested under diffuse natural illumination. We found that: (1) newts held overnight at the testing site exhibited accurate homing orientation, but not if transported to the testing site on the day of testing; (2) newts held overnight under diffuse lighting at a 'false testing site' and then tested at a site located in a different direction from their home pond oriented in the home direction from the holding site, not from the site where they were tested; and (3) newts held overnight in total darkness (except for light exposure for specific periods) only exhibited homing orientation the following day if exposed to diffuse illumination during the preceding evening twilight in the ambient MF. These findings demonstrate that, to determine the home direction, newts require access to light and the ambient MF during evening twilight when temporal variation in the MF is minimal.

KEY WORDS: Homing, Map information, Migration, Navigation, Amphibians, Orientation

INTRODUCTION

The homing ability of newts (Salamandridae) has been studied in both North America and Europe (Diego-Rasilla, 2003; Diego-Rasilla et al., 2005, 2008; Phillips, 1986a, 1987; Phillips and Borland, 1994; Twitty et al., 1964, 1966, 1967). Although there is considerable variation among species and populations, all newts lay eggs in aquatic habitats and have aquatic larvae; as adults, most populations spend the summer and/or winter months (depending on species and local environment) on land as inhabitants of the forest floor. Seasonal breeding migrations to and from aquatic habitats occur in the spring and/or autumn. Gill (1979) found that eastern red-spotted newts that were displaced 400 m to a new pond, remained in the pond until the following breeding season when two-thirds homed back to their

original pond. Seasonal migrations between terrestrial and aquatic habitats are associated with physiological changes, i.e. in buoyancy, skin cornification, photoreceptor spectral sensitivity and use of magnetic cues (Phillips, 1986a; Pough et al., 2004; Russell et al., 2005; Wells, 2007; and see below).

Newts exhibit a remarkable ability to determine their home direction after artificial displacement over distances 10 or more times their normal range of movement (Diego-Rasilla, 2003; Diego-Rasilla et al., 2005, 2008; Fischer et al., 2001; Phillips, 1987; Phillips and Borland, 1994; Phillips et al., 2002a); homing ability has been demonstrated in field and laboratory studies. Twitty (1966) showed that western newts (*Taricha rivularis*), displaced and released up to 8 km from their natal stream, returned by a direct route to the same stretch of stream in which they were captured. Eastern red-spotted newts (Fischer et al., 2001; Phillips, 1986a, 1987; Phillips and Borland, 1994; Phillips et al., 1995, 2002a), palmate newts (Diego-Rasilla et al., 2008) and Alpine newts (Diego-Rasilla, 2003; Diego-Rasilla et al., 2005) displaced ~4 km to as much as ~42 km from their breeding ponds exhibited homing orientation and reliance on magnetic cues in arena tests carried out both indoors and outdoors under semi-natural field conditions (i.e. newts tested in outdoor arenas under natural diffuse illumination in four symmetrical magnetic field (MF) alignments; see Diego-Rasilla, 2003; Diego-Rasilla et al., 2005, 2008). Moreover, newts exhibit accurate homing orientation even when deprived of information about the displacement route to an unfamiliar testing site (route deprivation experiments; Phillips et al., 1995), indicating they can use site-based 'map' information to determine the home direction, i.e. 'true navigation'. These studies provided the first evidence that true navigation can be studied in amphibians under controlled laboratory conditions (Fischer et al., 2001; Phillips, 1986a, 1987; Phillips et al., 1995, 2002a).

Both olfactory and magnetic cues have been proposed to provide map information in other vertebrates (Gould, 1998; Lohmann, 2007; Lohmann et al., 2008; Wallraff, 2005) and invertebrates (Boles and Lohmann, 2003). As a consequence of variation in wind direction at ground level resulting from turbulence in the understory of forested habitat (Baldocchi, 1989; Hutchinson and Hicks, 1985), as well as eddies above woodland ponds (Rodda, 1984; F.J.D.-R. and J.B.P., unpublished observations), however, the direction of arrival of wind-borne odors could not be used by newts to 'map' the spatial distribution of remote odor sources or large-scale odor gradients proposed to underlie an olfactory map in birds (Wallraff, 2005). Other potential sources of map information proposed for birds (sun-arc hypothesis: Matthews, 1955; infrasound hypothesis: Hagstrum, 2013) are not tenable for small animals that move slowly over short distances.

The Earth's MF plays dual roles in animal orientation, providing both directional ('compass') and spatial ('map') information (Freake et al., 2006; Wiltschko and Wiltschko, 2005). The magnetic compass can guide directional movements over a wide range of distances, from

¹Departamento de Biología Animal, Universidad de Salamanca, 37007 Salamanca, Spain. ²Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061-0406, USA.

*These authors contributed equally to this work

†Author for correspondence (fjdiego@herpetologica.org)

 F.J.D.-R., 0000-0002-7682-014X; J.B.P., 0000-0002-0585-6540

local movements of a few centimeters to long-distance migration and homing over hundreds or even thousands of kilometers (Deutschlander et al., 2000; Diego-Rasilla et al., 2015; Dommer et al., 2008; Durif et al., 2013; Muheim and Deutschlander, 2010; Walker et al., 2002). In contrast, the utility of a magnetic map depends, at least to some degree, on distance. Magnetic maps have been shown to play an important role in long-distance movements (>75–100 km; e.g. by salmon, sea turtles, migratory birds and spiny lobsters; Chernetsov et al., 2017; Freake et al., 2006; Heyers et al., 2017; Kishkinev et al., 2015; Lohmann, 2007; Lohmann and Lohmann, 2006; Lohmann et al., 2007; Munro et al., 1997; Putman et al., 2020; Scanlan et al., 2018). Over distances of ~10–50 km (at least in terrestrial environments), however, local irregularities in the MF caused by iron-containing minerals in the Earth's crust may make spatial variation in the MF an unreliable indicator of geographic position (Courtillot et al., 1997; Lednor, 1982; Phillips, 1996; Vargas et al., 2006). But surprisingly, at distances less than 5–10 km, local gradients in the MF can be relatively uniform, raising the possibility that at some locations the MF can provide useful spatial information (map location) for short-range movements, although the impact of microscale magnetic anomalies at ground level has yet to be characterized (Phillips, 1996).

To use a magnetic map for homing orientation, an animal must be able to detect spatial variation in the MF. This requires comparison of the local value of one or more MF components at an unfamiliar site with the values and alignments of the local magnetic gradient(s) in the vicinity of their home (Phillips, 1996). Consistent with the MF being used to derive both directional (i.e. compass) and spatial position (i.e. map) information, newts have dual magnetoreception systems (Phillips, 1986a,b). One mechanism mediates a light-dependent, photoreceptor-based 'inclination' magnetic compass mediated by extraocular photoreceptors in the pineal organ (Deutschlander et al., 1999a,b; Phillips and Borland, 1992a). [Although newts and other amphibians do not exhibit goal-directed magnetic compass orientation in total darkness (Phillips and Borland, 1992b), Diego-Rasilla et al. (2005) showed Alpine newts can orient in the home direction using the magnetic compass at night under total overcast.] The second mechanism is a non-light-dependent, polarity-sensitive, presumably magnetite-based mechanism (Brassart et al., 1999), used exclusively during seasonal migrations to and from breeding ponds when newts rely on map information (Phillips and Borland, 1994; Phillips et al., 2002a). In theory, an array of directional ('compass') detectors used en masse could provide the sensitivity necessary to detect spatial variation in, for example, the intensity of the MF (Taylor, 2016; Walker, 2008). However, the differences in functional properties of the sensors that newts use for the 'compass' and 'map' components of homing suggest that fundamentally different biophysical mechanisms are involved.

Previous work on eastern red-spotted newts has shown that when newts are taking map readings, the magnetic compass is used to position the putative magnetite-based receptor in a fixed alignment relative to the MF, presumably to increase the accuracy of MF measurements necessary to detect spatial variation in the MF (Phillips and Borland, 1994; Phillips et al., 2002b). During the migratory season, newts held in outdoor tanks under long-wavelength (>500 nm) light, which causes the directional response of the magnetic compass to be rotated by 90 deg (Phillips and Borland, 1992a), were unable to obtain map information (Phillips and Borland, 1994). When subsequently tested in an indoor arena, instead of orienting in the home direction, newts exposed to both full-spectrum and long-wavelength (>500 nm) light exhibited 'fixed' (i.e. independent of shore and home directions) bimodal

orientation along the magnetic northeast/southwest axis (Phillips et al., 2002b), similar to spontaneous magnetic alignment (SMA) observed during non-goal-directed behaviors of other vertebrates (Begall et al., 2008, 2013; Červený et al., 2011; Diego-Rasilla et al., 2017; Hart et al., 2012; Obleser et al., 2016). Spontaneous alignment with respect to the Earth's MF in the dark has also been shown in Alpine newts (Schlegel and Renner, 2007) and eastern red-spotted newts (Schlegel, 2007).

Whole-body natural remanent magnetism (NRM) was obtained from a subset of newts exhibiting the fixed bimodal response (Brassart et al., 1999; Phillips et al., 2002b). Newts that were held in outdoor tanks under long-wavelength light, and then tested under full-spectrum light, exhibited a non-random distribution of their NRM relative to the external MF, consistent with a magnetoreception mechanism involving an ordered array of single-domain or super-paramagnetic magnetite particles and use of the light-dependent magnetic compass to position the non-light-dependent, magnetite-based detector relative to the MF (Phillips et al., 2002b). Newts that had been held under long-wavelength light and subsequently tested under long-wavelength light (i.e. without a normally functioning magnetic compass), appeared to systematically sample different alignments of the putative map detector relative to the magnetic field, possibly relying on a trial-and-error strategy in an attempt to align the putative map detector to derive accurate map information (Fig. S1B; Phillips et al., 2002b). Finally, the inability of newts tested in the indoor arena to orient in the home direction after being held in outdoor tanks under long-wavelength light provides further evidence that accurate homing orientation (compass component) requires newts to have access to map information in the outdoor tanks prior to testing (map component).

Consistent with newts relying on a magnetic map, in 'simulated magnetic displacement experiments' newts exhibiting homing orientation that were exposed to small increases or decreases in magnetic inclination – simulating magnetic values found at locations to the north or south of their home pond (respectively) – oriented as though they had been displaced to the sites where the altered values of magnetic inclination occurred (Fischer et al., 2001; Phillips et al., 2002a); similar changes in magnetic inclination had no effect on newts exhibiting shoreward magnetic compass orientation that does not require map information (Fischer et al., 2001).

Although evidence that newts derive map, as well as compass, information from the MF is compelling, from a theoretical perspective, use of a 'magnetic map' over such short distances is problematic; spatial variation in the MF (change in total intensity averages 3–5 nT km⁻¹, against a background intensity of 50,000–60,000 nT, and 0.01 deg km⁻¹ in inclination) is an order of magnitude weaker than the regular daily fluctuations in the MF, and can be two orders of magnitude weaker than the temporal variation associated with large magnetic storms. Because newts move slowly, variation in MF values resulting from the newt's change in location is likely to be much less than temporal variation in the MF. Moreover, long-term (secular) variation in the MF causes the baseline values of MF components at specific locations to change over time (Courtillot and Le Mouel, 1988). This raises a fundamental question: if newts (and other small vertebrates that home over distances of 1–2 km) use spatial variation in the MF to derive a short-range, high-precision map, what strategies do they use to sample the MF that make it possible to distinguish the 'spatial signal' from short-term temporal 'noise' and from long-term secular variation? Experiments reported here were carried out to determine whether newts use a sampling strategy that minimizes the errors in

magnetic map readings resulting from short-term temporal fluctuations in the MF.

A possible sampling strategy to minimize temporal noise in estimates of geographic position derived from the MF is suggested by an unlikely source: juvenile blue-fin tuna (*Thunnus maccoyii*; Willis et al., 2009). Bluefin tuna exhibit yearly roundtrip migrations of 5000–16,000 km between their feeding and spawning grounds (Bestley et al., 2008; Patterson et al., 2008, 2018; Willis et al., 2009). Willis et al. (2009) characterized an unusual behavior termed ‘spike dives’ exhibited by juvenile tuna, which occur at precise times during morning and evening civil twilight. Morning and evening spike dives are mirror image behaviors. As morning civil twilight approaches, tuna gradually descend to depths of ~100–600 m. Then, 30 min before sunrise, the fish swim up to the surface at full speed, where they spend 10–20 min in the top few meters of the water column. During evening civil twilight, tuna spend 10–20 min near the surface. At 30 min after sunset, they swim straight down at full speed to a depth of ~100–600 m, after which they gradually return to shallower depths (Willis et al., 2009).

Willis et al. (2009) argue that spike dives play a role in updating the tuna’s navigational systems. Celestial polarization patterns at sunrise and sunset can be used as a calibration reference for the sun and magnetic compasses, as shown previously in migratory birds (Muheim et al., 2006a,b), but would only be visible to tuna close to the water surface (Cronin and Shashar, 2001; Waterman, 2006). In addition, the precise timing of the maximum depth of the spike dives, 30 min prior to sunrise and 30 min after sunset, suggests that these components of the spike dives may also be involved in obtaining navigational information and, more specifically, magnetic map measurements. Magnetic map readings carried out at depth would be more accurate than comparable measurements taken near the surface because of temporal variation in the MF caused by wind-driven currents (Lilley et al., 2001) and orbital wave motion near the surface that would make it more difficult to obtain accurate MF measurements. Moreover, the greatest depths of the morning and evening spike dives coincide with the two times of day (30 min before sunrise and 30 min after sunset) when the total intensity of the MF tends to reach its maximum values and the regular daily pattern of temporal variation is effectively absent (Willis et al., 2009).

Because the timing of the two MF maxima is a world-wide phenomenon, other animals, including newts, could minimize the impact of the regular daily pattern of variation by taking map readings during morning and/or evening twilight. Moreover, as magnetic storms produce more rapid changes in the MF than the regular daily variation (Courtilot and Le Mouel, 1988; Skiles, 1985), comparison of MF values during morning and evening twilight could be used to help filter out the effects of magnetic storm activity; when values at these two times of day differ appreciably, map readings obtained from the MF would not be reliable (Willis et al., 2009).

The following experiments were carried out to test whether newts make magnetic measurements during morning and/or evening civil twilight when temporal variation in the MF is minimal.

MATERIALS AND METHODS

General methods

The Alpine newt, *Ichthyosaura alpestris* (Laurenti 1768), is a medium-sized newt, reaching an average total length of 8–12 cm, although males normally do not exceed 10 cm total length. This species inhabits aquatic habitats during the breeding season and spends the rest of the year on land in woodland habitats. The activity of adult Alpine newts is predominantly nocturnal during both the aquatic and terrestrial phases (Diego-Rasilla, 2014; Diego-Rasilla

and Ortiz-Santaliestra, 2009; Fasola, 1993), and they exhibit their highest levels of activity during evening twilight and the first hours of the night, especially during the terrestrial phase when homing behavior occurs (Diego-Rasilla, 2014; Montori and Herrero, 2004).

Adult male Alpine newts (experiments I, II and III) and male and female adults (experiments IV and V) were collected by dip netting from three breeding ponds, two 40 m apart (ponds B and C) and the third separated by ~500 m from the other two (pond A), during their seasonal migratory period (late April to early June; Diego-Rasilla, 2003, 2014). Ponds were situated in the Cieza Mountains, Natural Park of Saja-Besaya (Cantabria, northern Spain; 43°14′8″N, 4°9′59″W), where the vegetation mainly consists of thick forest of *Quercus robur* and *Fagus sylvatica*. The newts were carried from ponds to a transport vehicle. The vehicle was parked 1490 m (pond A), 615 m (pond B) and 655 m (pond C) southeast of ponds and was used to transport the newts to two testing sites (~4–9 km away from the ponds), and between testing sites (see ‘Specific methods’, ‘Experiment II: false testing site experiments’, below).

In experiments I and II, from immediately after capture at the breeding ponds (either in the afternoon on the day prior to testing or in the morning on the day of testing) until they arrived at the testing site, newts were displaced in opaque, plastic containers inside a black waterproof PVC bag. The PVC bag was placed inside a polyamide backpack, thus ensuring the animals were transported in total darkness and without access to visual, olfactory or light-dependent magnetic compass cues. At the holding and testing sites, animals were held in opaque plastic tanks, aligned on the north–south geomagnetic axis, that were open to the overhead sky under dense foliage, so the newts were exposed to diffuse natural illumination and wind-borne odors. Plastic mesh was placed over the tops of the plastic tanks during the night to prevent newts from escaping. Newts were tested under a clear daytime sky. Depending on the experiment, testing took place in an open area shaded by a stand of hazels, *Corylus avellana* (testing site 1; located ~9 km away from the ponds) or in a thick forest of *Fagus sylvatica* (testing site 2; located ~4 km away from the ponds); in both testing sites, vegetation formed a continuous canopy overhead, allowing diffuse light through the foliage.

In experiments IIIa–c and IV (see below), from immediately after capture in the afternoon until they arrived at testing site 1, the newts were displaced in transparent plastic containers inside a black waterproof PVC bag. The PVC bag was placed inside a polyamide backpack, ensuring that animals were transported in total darkness and did not have access to visual or olfactory cues. While held overnight at the testing site, newts were exposed to the ambient MF in total darkness in the transport containers, which were aligned on the north–south geomagnetic axis. However, during specific periods of time prior to testing, the newts held in the covered transparent plastic containers were exposed to diffuse light from the overcast sky in the ambient MF, without access to natural wind-borne odors. The following day, newts were tested at site 1 under a totally overcast sky. During testing, animals were held in opaque plastic tanks, aligned on the north–south geomagnetic axis, open to the overhead sky.

Because the amphibian magnetic compass is light dependent (Phillips and Borland, 1992a,b,c), dark transportation prevented newts from using the magnetic compass (or the sun or polarized light compasses) to determine the direction of displacement; total darkness has also been shown to eliminate the use of magnetic compass cues in both amphibians and birds (Phillips and Borland, 1992b; Wiltschko and Wiltschko, 1981). Furthermore, although newts attempting to home have been shown to use a non-light-dependent, presumably

magnetite-based, magnetoreception mechanism, the light-dependent magnetic compass is necessary to align the magnetite-based ‘map detector’ relative to the MF to obtain map measurements (Phillips and Borland, 1994; Phillips et al., 2002b).

Individual newts were tested only once, in a visually symmetrical terrestrial arena, located under dense vegetation, which blocked a view of the sun disk (and polarized skylight if present). The test arena consisted of a circular dark green plastic container (57 cm diameter, 28 cm high). The floor and arena walls were thoroughly wiped with a damp cloth between trials to eliminate directional olfactory cues (Fischer et al., 2001) and then wiped dry using paper towels. Newts were tested under a clear daytime sky (experiments I and II) or a totally overcast sky (experiments IIIa–c, IV and V). In all experiments except experiment IV, the ambient geomagnetic field was not altered.

Newts at this time of year are in the aquatic phase and were held in water from their home pond during transport to the holding or testing site, while held overnight and prior to testing. Therefore, in all the experiments, the newts were continuously exposed to their home pond water odors. In experiments I and II, the newts also had access to natural wind-borne odors during their exposure to the MF, whereas in experiments IIIa–c and IV they were exposed to the MF without access to natural odors (see above).

For testing, newts were placed individually in the arena center beneath an opaque, cylindrical plastic container (9.5 cm diameter, 14.5 cm high) that served as a release device. Once in the release device, each newt was held for 1 min to overcome the effects of handling. The release device was then lifted, and the newts were allowed to move freely about the arena. To minimize disturbance during experiments, the observers moved away from the arena, leaving each animal undisturbed for 5 min. A trial was discontinued if the newt did not leave the arena center after 5 min, and the newt was not tested again. A newt’s directional response was recorded by the moist trail it left on the arena floor. In all cases in which a newt left the center of the arena, it moved directly to the arena wall, and then, staying close to the wall, proceeded to circle around the arena. A newt’s directional response was recorded where the moist trail left by its movements first contacted the wall.

In all experiments, newts were returned to their pond after testing, and all applicable institutional and/or national guidelines for the care and use of animals were followed.

Data were analyzed using standard circular statistics (Batschelet, 1981). The mean vector bearing (MVB) was calculated by vector addition and tested for significance using the Rayleigh test. A 95% confidence interval was used to test whether the newts’ MVB differed significantly from the homeward direction from the holding (for the two groups held overnight at a different site) or testing (all four groups) site. The Watson U^2 -test was used to test for differences between two distributions of bearings and the Mardia–Watson–Wheeler test was used for differences between three distributions. Distributions of mean vectors were tested for significance using Hotelling’s test. Circular statistics were calculated with Oriana 4 (Kovach Computing Services).

To determine whether the newts’ motivation to orient differed between treatments, we compared the number of individuals that ‘scored’ (i.e. reached the arena wall) in each control or experimental group within the 5 min time limits using a Fisher’s exact probability test (Siegel, 1956).

Specific methods

Experiment I: overnight versus morning map acquisition

Initial experiments were carried out to determine whether map information was obtained on the day that tests were carried out or,

instead, during the preceding overnight period spanning sunset to sunrise. One group of newts was collected on the day prior to testing, transported to the testing site in a sealed light-tight container, and held overnight under diffuse natural illumination in the ambient MF (see ‘General methods’, above, for a more detailed description). Because the amphibian magnetic compass is light dependent, dark transportation prevented newts from obtaining route-based information about the direction of displacement using magnetic, as well as celestial, compass cues. A second group of newts was collected from the same pond early the following morning, transported in the same manner, and held at the testing site under diffuse illumination for 2 h prior to the start of testing. Newts from the two groups were tested alternately in the ambient MF over a period beginning in the early morning and ending in the late afternoon to maximize their exposure to temporal variation in the MF.

Four replications of this experiment were carried out. In the first replication, newts were collected from pond B (30 on the day before testing and 27 the following morning) and transported to testing site 1 (home pond direction 232°, distance 9060 m).

In the second replication, newts were collected from pond C (31 on the day before testing and 30 the following morning), a pond 40 m distant from pond B, and displaced to testing site 2 (home pond direction 319°, distance 3380 m).

In the third replication, two groups of newts were collected from pond A (22 on the day before testing and 24 the following morning) and displaced to testing site 1 (home pond direction 235°, distance 9090 m).

In the fourth replication, two groups of newts from pond B (21 on the day before testing and 21 the following morning) were transported to testing site 2 (home pond direction 319°, distance 3390 m).

Experiment II: false testing site experiments

To confirm that newts obtain map readings overnight, and that the ‘map’ and ‘compass’ components of homing are independent (Rodda, 1984; Rodda and Phillips, 1992), newts ($n=21$) were held overnight at one site (site 1; false testing site) and the following morning after civil twilight transported to a second site (site 2; true testing site), which differed in home direction, where they were held in the ambient field for 2 h before being tested during daylight hours. In a second replication of this experiment, the true testing site and false testing site were reversed, i.e. newts were held overnight at site 2 ($n=64$) and then transported the following morning to site 1 for testing.

Experiment IIIa–c: timing of map acquisition

We investigated whether newts obtain map measurements during evening civil twilight, morning civil twilight, both evening and morning civil twilight, or the intervening night. The MF was not manipulated directly to avoid possible ‘after effects’, e.g. newts might respond to the altered field as a magnetic storm, regardless of the time of exposure (but see experiment V below). Instead, newts were held in the transport containers in complete darkness except for varying periods of time during which they were exposed to diffuse light from the overcast sky, without access to natural wind-borne odors, enabling them to use the light-dependent magnetic compass to align the putative map detector to obtain map readings (Phillips and Borland, 1994; Phillips et al., 2002b; and see earlier discussion). The following day, newts were tested under total overcast conditions, so any effect of dark exposure that might have altered the response of a time-compensated sun compass (Taylor, 1972; Taylor and Ferguson, 1970) would not affect the newts’ orientation.

Experiment IIIa: evening versus morning exposure including sunset and sunrise

The orientation of newts exposed to diffuse natural illumination to permit map measurements during a 75 min window spanning evening twilight including sunset ($n=21$) was compared with that of newts exposed to diffuse natural illumination during a 75 min window spanning morning twilight including sunrise ($n=21$).

Experiment IIIb: exposure during evening twilight versus the remainder of the night

The orientation of newts exposed to diffuse natural illumination during a 45 min window beginning at sunset and spanning evening twilight ($n=21$) was compared with that of newts exposed to diffuse natural illumination beginning just after evening twilight and continuing through the night until the end of morning twilight ($n=24$).

Experiment IIIc: evening twilight versus morning twilight exposure without sunset and sunrise

The orientation of newts exposed to diffuse natural illumination for 30 min during evening twilight beginning just after sunset ($n=27$) was compared with that of newts exposed to diffuse natural illumination for 30 min during morning twilight ending just before sunrise ($n=28$).

Experiment IV: disruption of the MF during evening twilight

Two groups of newts were exposed to the overcast sky at sunset for identical intervals spanning evening twilight (i.e. from 30 min before sunset to 45 min after sunset); one group received sunset exposure in the ambient MF (13 adult males and 12 adult females), while the other group (13 adult males and 12 adult females) was exposed to an array of permanent magnets ($47.7 \times 22.3 \times 10$ mm) surrounding the holding container to alter the MF. [Prior to these experiments, the array of bar magnets was aligned so that the long axes of the magnets were perpendicular to the geomagnetic field. Although the intensity of the altered MF varied considerably within the array of magnets, knowing the intensity of the horizontal component of the geomagnetic field made it possible to use the angle of rotation of a compass placed at the center of the magnet array to determine the distance between the two rows of magnets at which the intensity of the artificial field was roughly 10% of the geomagnetic field's horizontal component. In the experiments shown in Fig. 6, the array of magnets was aligned so the long axes of the magnets were parallel to the horizontal component of the MF, resulting in increases in intensity of the horizontal component, and decreases in the inclination of the MF, as well as smaller changes in declination. This information does not allow accurate characterization of the MF values newts were exposed to, which varied considerably within the holding container(s) as a result of the steep gradients that magnets produce, but rather is provided so that investigators attempting to replicate these experiments could use an array of bar magnets to expose newts to comparable MF conditions.] The changes in all three components of the MF produced by the array of magnets varied considerably within the holding container, eliminating any possibility that the newts could derive consistent map information from the MF. Both groups were then held in the ambient MF in total darkness for the remainder of the night until 30 min after sunrise. The following day, the orientation of the two groups of newts was tested under diffuse illumination in the ambient MF to determine whether manipulation of the MF during the preceding evening twilight period affected their ability to orient in the home direction.

The first time the experiment was carried out, newts exposed to the experimental treatment (i.e. magnets) were located 60 m away from controls. The distribution of bearings from controls in this experiment was indistinguishable from random; this was the only group of controls exposed to light during evening twilight from a total of 11 experiments that failed to show homeward orientation. Because equipment to measure the MF was not available, we were unable to determine whether there was a small change in the MF at the holding site of controls that could have disrupted the homing orientation. Instead, we repeated the experiment a second time with the holding sites for the control and experimental (i.e. magnet exposed) treatment groups separated by 100 m (control: 24 adult males; experimental: 26 adult males). Because the intensity of the MF generated by the magnets decreases as the cube of distance, this should have reduced any effect of the magnets on the MF at the control holding site by more than 4 times.

Experiment V: alignment relative to the MF during evening twilight

To obtain a preliminary look at the body alignment of newts during morning and evening twilight, a group of newts (13 adult males and 8 adult females) was photographed during one evening civil twilight (photographs taken at ~ 5 min intervals for 45 min beginning at sunset) and another group (12 adult males and 9 adult females) during one morning civil twilight (photographs taken at ~ 5 min intervals for 45 min ending at sunrise). Newts were captured and transported to testing site 1 as described in 'General methods' for experiment IV. At the testing site, the newts were exposed to the ambient MF in total darkness, except for the period of time during which they were photographed. At sunset, one group of newts was exposed under a totally overcast sky and photographed during evening civil twilight, while the other group was exposed under a totally overcast sky and photographed during morning civil twilight. From each photograph, a mean vector was calculated from the distribution of body alignments of newts that were at least 1 cm away from the walls of the holding container.

RESULTS

Newts showed strong motivation to orient under all experimental conditions, and there were no differences between treatment groups (Table 1).

Experiment I: overnight versus morning map acquisition

This experiment was carried out to determine whether newts obtain map information overnight or at the time of testing.

In all four replications of experiment I, newts captured on the day prior to testing and held overnight at the testing site exhibited significant clustering of bearings and the 95% confidence intervals for the mean vector bearing included the home direction (Fig. 1A–D, left circular diagrams). In contrast, in the four replications where the newts were captured and transported to the testing site on the day of testing, the newts failed to orient in the home direction. In three of the four replications (Fig. 1B–D, right diagrams), the distribution of bearings from newts captured on the day of testing was indistinguishable from random, while in one replication the distribution was non-random, but the 95% confidence intervals did not include the home direction (Fig. 1A, right diagram).

Experiment II: false testing site experiments

In both replications of experiment II, the bearings were non-randomly distributed, and the 95% confidence intervals for the mean vector bearings included the home direction from the false

Table 1. Motivation to home under all experimental conditions

Experiment	Details	Failed to orient	<i>n</i>	<i>P</i>
Experiment I				
Replication 1	Captured previous day	3	30	0.35
	Captured day of test	1	27	
Replication 2	Captured previous day	3	31	0.52
	Captured day of test	2	30	
Replication 3	Captured previous day	2	22	0.15
	Captured day of test	6	24	
Replication 4	Captured previous day	3	21	0.50
	Captured day of test	2	21	
Experiment II	Newts tested at true testing site 1	7	64	0.36
	Newts tested at true testing site 2	1	21	
Experiment IIIa	Exposure to light starting 30 min before sunset and ending 45 min after	1	21	0.76
	Exposure to light starting 45 min before sunrise and ending 30 min after	1	21	
Experiment IIIb	Exposure to light starting at sunset and ending 45 min after (i.e. spanning civil twilight)	0	21	
	Exposure to light starting 45 min after sunset and continuing until after sunrise	0	24	
Experiment IIIc	Exposure to light for 30 min starting at sunset	0	27	
	Exposure to light starting 30 min before sunrise	0	28	
Experiment IV				
Replication 1	Control	2	25	0.25
	Experimental	0	25	
Replication 2	Control	0	24	
	Experimental	0	26	

n, sample size; *P*, *P*-value for Fisher's exact probability test.

testing site where the newts were held overnight, not the home direction from the site where the newts were tested (Fig. 2; compare with Fig. 1A–D, left diagrams).

Experiment III: timing of map acquisition

Experiment IIIa

The bearings of newts exposed for 75 min to diffuse natural light spanning evening civil twilight, from 30 min before sundown to 45 min after sundown, were non-randomly distributed, and the 95% confidence intervals for the mean vector bearing included the home direction (Fig. 3A). In contrast, the bearings of newts exposed to diffuse light for 75 min spanning morning civil twilight, from 45 min before sunrise to 30 min after sunrise, failed to exhibit significant orientation relative to the home direction (Fig. 3B).

Experiment IIIb

The magnetic bearings obtained from newts exposed to diffuse natural light for 45 min during evening civil twilight, beginning at sundown when the sun disk was no longer visible, were non-randomly distributed, and the 95% confidence intervals for the mean vector bearing included the home direction (Fig. 4A). [Note, because of the location of site 2 on the north slope of a mountainous European beech (*F. sylvatica*) mature forest and the location of site 1 in a valley with hills to the east and west, even if the sun disk had not been obscured by overcast (experiments IIIa–c, IV and V) and/or dense overhead vegetation (experiments I and II), it was below the visual horizon for more than 30 min in the late afternoon before the official time of sunset and again for more than 30 min in the morning after the official time of sunrise.] In contrast, newts that were exposed to diffuse natural light beginning immediately after evening civil twilight (i.e. 45 min after sunset) and extending through the night, up to and including the time of testing (morning twilight) the following day, failed to exhibit significant orientation relative to the home direction (Fig. 4B).

Experiment IIIc

The bearings obtained from newts exposed to diffuse natural light for 30 min during evening civil twilight, beginning just after sunset,

were non-randomly distributed, and the 95% confidence intervals for the mean vector bearing included the home direction (Fig. 5A). Newts exposed to diffuse natural light for 30 min during morning civil twilight, ending just before sunrise, exhibited a non-random distribution of bearings, but the 95% confidence interval did not include the home direction (Fig. 5B).

Experiment IV: disruption of the MF during evening twilight

In the initial experiment, in which control and experimental (i.e. magnet-exposed) treatment groups were separated by 60 m, the distribution of bearings obtained from controls was indistinguishable from random (Rayleigh test; 243° , $r=0.18$, $P=0.49$, $n=23$; Fig. 6A). In contrast, the bearings of magnet-exposed newts were non-randomly distributed, but the 95% confidence did not include the home direction (Rayleigh test; $13\pm 36^\circ$, $r=0.42$, $P=0.01$, $n=25$; Fig. 6C). There was a significant difference between control and experimental distributions (Watson U^2 -test; $U^2=0.25$, $P<0.02$).

The second time this experiment was carried out, with the control and experimental (i.e. magnet-exposed) treatment groups separated by 100 m, controls tested the following day were significantly homeward oriented and the 95% confidence interval for the mean vector included the home pond direction of 235° (Rayleigh test; $233\pm 41^\circ$, $r=0.38$, $P=0.03$, $n=24$; Fig. 6B). The bearings of magnet exposed newts were randomly distributed (Rayleigh test; 40° , $r=0.24$, $P=0.23$, $n=26$; Fig. 6D), and there was a significant difference between controls and experimental distributions (Watson U^2 -test; $U^2=0.25$, $P<0.02$).

The distributions of bearings obtained from the two control groups were not significantly different (Watson U^2 -test; $U^2=0.05$, $P>0.05$; Fig. 6A,B). Likewise, the distributions of bearings from the two magnet-exposed groups did not differ significantly (Watson U^2 -test; $U^2=0.11$, $0.5>P>0.2$; Fig. 6C,D). When data were pooled from the two control groups, the distribution of bearings was non-randomly distributed, and the 95% confidence interval included the home direction (Rayleigh test; $236\pm 41^\circ$, $r=0.28$, $P=0.02$, $n=47$). When data were pooled from the two magnet-exposed groups, the distribution of bearings was also non-randomly distributed, but the

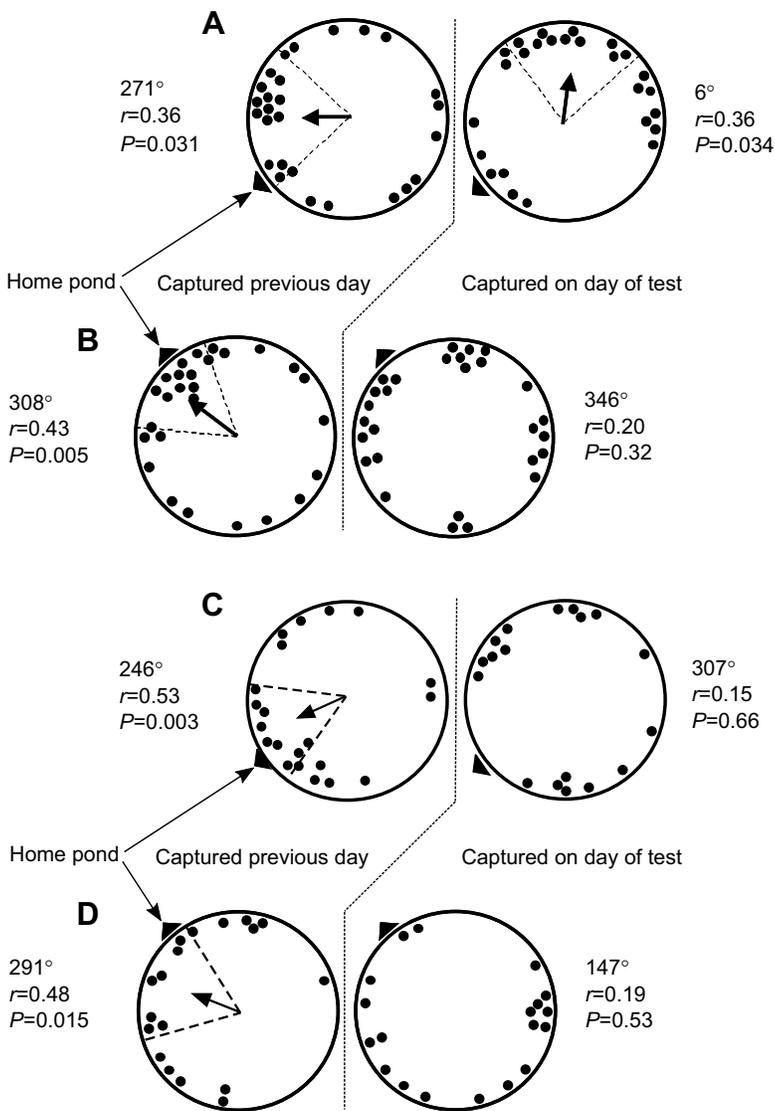


Fig. 1. Homing orientation is only exhibited by Alpine newts held overnight at the testing site prior to testing. One group was collected the day before testing and held overnight at the test site (left), while a second group was collected the following morning (right). (A) First replication of experiment I ($n=27$ and $n=26$ for left and right diagrams, respectively), (B) second replication ($n=28$ and $n=28$, respectively), (C) third replication ($n=20$ and $n=18$, respectively) and (D) fourth replication ($n=18$ and $n=19$, respectively). Filled circles indicate the direction of movement of individual newts. Arrows at the center of each circle are the mean vector bearings. The length of the arrows indicates the mean vector length (r), with the radius of the circle corresponding to $r=1$. Dashed lines are the 95% confidence intervals for the mean vector bearings. Filled arrowheads outside each circle indicate the magnetic bearing from the testing site to the newt's home pond.

95% confidence interval did not include the home direction (Rayleigh test; $23 \pm 34^\circ$, $r=0.32$, $P=0.006$, $n=51$). The pooled distributions from the control and magnet-exposed groups were significantly different (Watson U^2 -test; $U^2=0.45$, $P<0.001$). Also, the distribution of bearings from the two control groups in experiment IV was indistinguishable from that of newts given comparable exposure to diffuse natural illumination during evening twilight in experiment IIIa (Mardia–Watson–Wheeler test; $W=1.16$, $P=0.84$).

Experiment V: alignment relative to the MF during evening twilight

To determine whether characterizing the behavior of newts during the twilight periods would be a productive avenue for future research, mean vector bearings were obtained from newts that were at least 1 cm away from the walls of the holding container from photographs taken at ~ 5 min intervals from one group of newts during evening civil twilight and from a second group of newts during morning civil twilight (Table S1). Overall, the distribution of mean vector alignments obtained from successive photographs during evening twilight was bimodally distributed along approximately the north–south axis (Hotelling's test on doubled

angles; $164\text{--}334^\circ$, $r=0.43$, $P=0.0002$). In contrast, the distribution of mean vector alignments obtained from the second group of newts during morning twilight was indistinguishable from random (Hotelling's test on doubled angles; $24\text{--}204^\circ$, $r=0.22$, $P=0.08$). The two distributions were significantly different (two-sample Hotelling's test; $P=0.026$; comparing two distributions of doubled angles), suggesting that the newts were behaving differently during the two twilight periods (Fig. S1A).

A further indication that the behavior of Alpine newts differed during evening and morning twilight can be seen in a comparison of short-term changes in the alignment of individual newts. In an earlier study of eastern red-spotted newts (Phillips et al., 2002b), newts were tested in an indoor arena under diffuse white light in one of four symmetrical alignments of the MF, i.e. where magnetic north was north, south, east or west (each newt tested only once in one of the four fields). Eastern red-spotted newts that had been held in outdoor tanks under long-wavelength light prior to testing, which prevented them from obtaining map measurements, and then tested indoors under long-wavelength light exhibited differences in orientation that depended on the time taken by each newt to score (for details, see Phillips et al., 2002b). [Because the responses of eastern red-spotted newts in indoor arena tests were scored after they

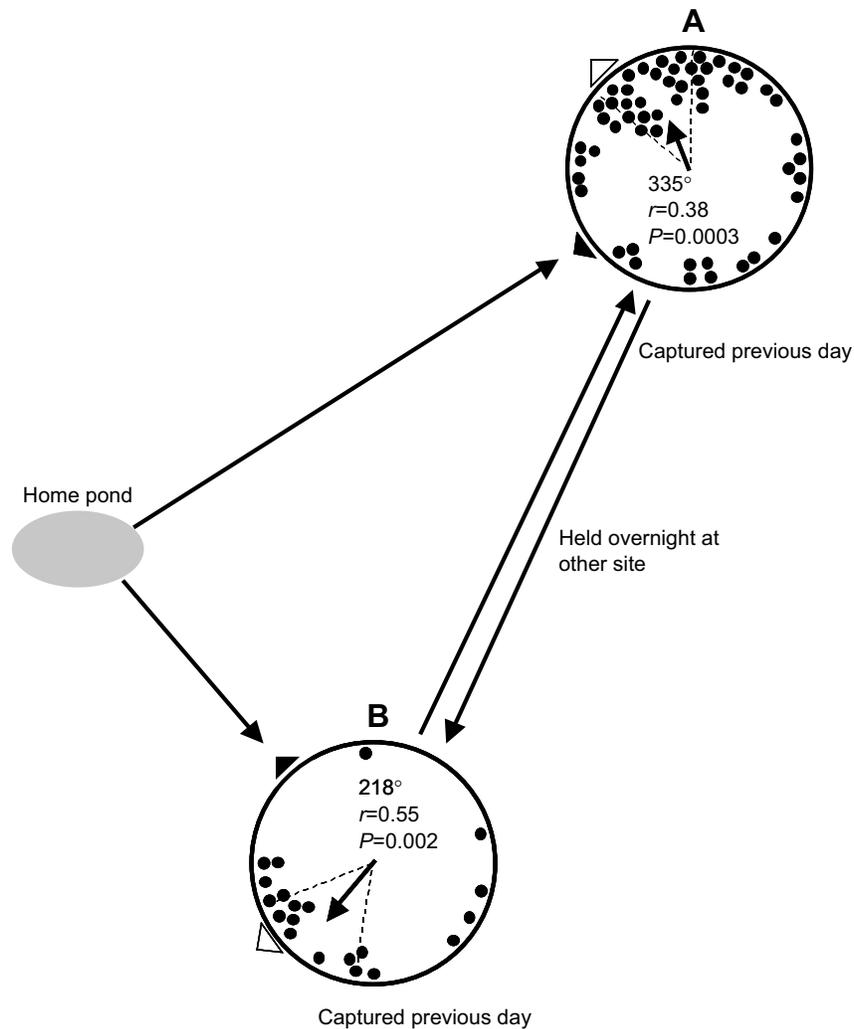


Fig. 2. False release site experiments. (A) Newts ($n=57$) were held overnight at site 2 (false testing site), and then transported the following morning to site 1 for testing (true testing site). (B) Newts ($n=20$) were held overnight at site 1 (false testing site), and then transported the following morning to site 2 for testing (true testing site). Filled arrowheads outside circles indicate the magnetic bearing of the home pond from the site where the newts were tested. Open arrowheads outside circles indicate the magnetic bearing of the home pond from the false testing site where the newts were held overnight. Other symbols are the same as in Fig. 1.

had moved only approximately one body length, the directions of orientation are likely to reflect the alignment of the newts in the release device prior to release (Phillips et al., 2002a).] Scoring times exhibited distinct clusters, i.e. less than 5 min, 5–9 min, and greater than 10 min (Fig. S1B). Newts that scored in less than 5 min exhibited bimodal orientation relative to the MF (Rayleigh test; $44\text{--}224^\circ$, $r=0.78$, $P<0.001$, $n=11$), newts that scored between 5 and 9 min exhibited unimodal orientation towards magnetic south (Rayleigh test; 183° , $r=0.82$, $P<0.05$, $n=5$), and newts that scored in greater than 10 min exhibited unimodal orientation towards magnetic north (Rayleigh test; 17° , $r=0.70$, $P<0.02$, $n=8$) (Fig. S1B). Newts that did not leave the center of the arena within 15 min were removed from the arena without scoring.

Remarkably, Alpine newts that had been held in total darkness to prevent them from obtaining map information (see Materials and Methods), exhibited similar changes in orientation when exposed to diffuse natural illumination during evening twilight in the first 15 min after sunset. In the first sample, taken 5 min after sunset, the alignment of individual newts was bimodally distributed (Rayleigh test; $1\text{--}181^\circ$, $r=0.51$, $P=0.05$, $n=11$). In the second sample, newts

oriented unimodally to the south (Rayleigh test; 151° , $r=0.68$, $P=0.02$, $n=8$) and in the third sample, the newts oriented unimodally to the north (Rayleigh test; 15° , $r=0.96$, $P=0.0005$, $n=6$) (Fig. S1C). Note that each bearing obtained in the earlier study of the eastern red-spotted newts was from a separate individual, and the alignment of the MF was changed between successive trials, while the distributions obtained from Alpine newts were from successive photographs of the same group of newts in the ambient MF, although the subset of Alpine newts in each group that were far enough away from the walls of the holding container to be counted differed to varying degrees in each sample. Comparable behavior was not exhibited by the second group of Alpine newts sampled during morning twilight, either during the 15 min immediately prior to sunrise or the 15 min immediately after sunrise (data not shown).

DISCUSSION

In 10 out of 11 experiments, newts transported from their home ponds and exposed to diffuse natural lighting during varying periods of time that included evening civil twilight (Figs 1–6) showed significant orientation in the homeward direction from the

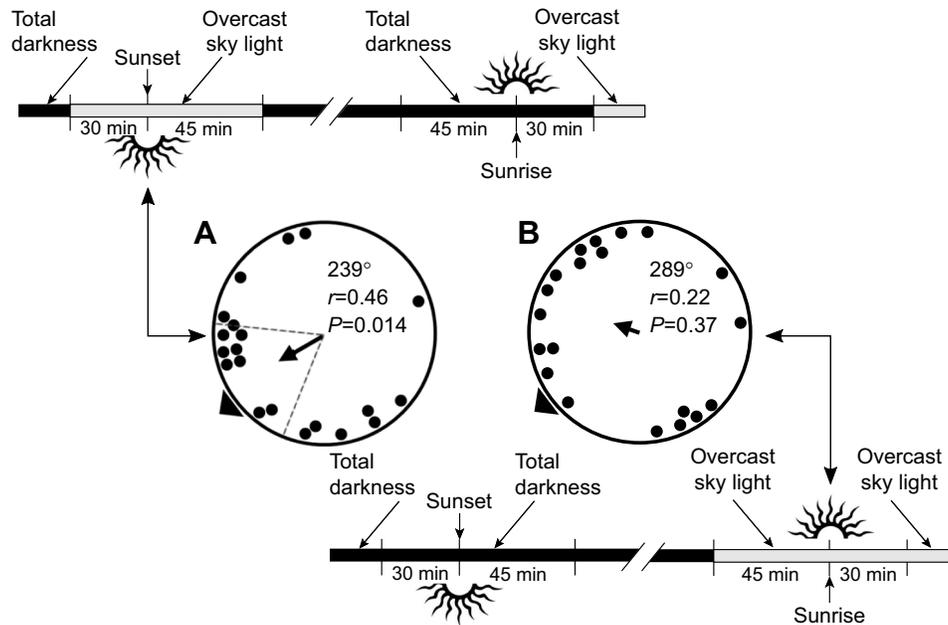


Fig. 3. Exposure to light starting 30 min before sunset and ending 45 min after sunset versus exposure 45 min before sunrise to 30 min after sunrise. (A) Newts exposed to diffuse natural light for 75 min around the time of sunset showed significant homeward orientation ($n=20$). (B) Newts exposed to sunrise were randomly oriented ($n=20$). Symbols are the same as in Fig. 1.

site at which the exposure occurred, i.e. either the true testing site (Figs 1 and 3–6) or the false testing (i.e. overnight holding) site (Fig. 2). In 9 experiments, newts captured and transported to the testing site on the morning of testing ($n=4$ experiments; Fig. 1), held at the testing site overnight in total darkness and then exposed to diffuse natural lighting for periods of time that did not include evening civil twilight ($n=3$ experiments; Figs 3–5), or exposed to diffuse natural lighting during evening twilight in the presence of

permanent magnets that disrupted the MF ($n=2$ experiments; Fig. 6) failed to exhibit homeward orientation.

These findings confirm that the newt's map and compass are separate, and separable, components of long-distance homing. Exposure to light in the ambient MF during civil twilight was necessary and sufficient for newts to determine the home direction (experiments I, II, IIIa–c, IV; and see below), and was independent of whether newts had access to another potential source of map

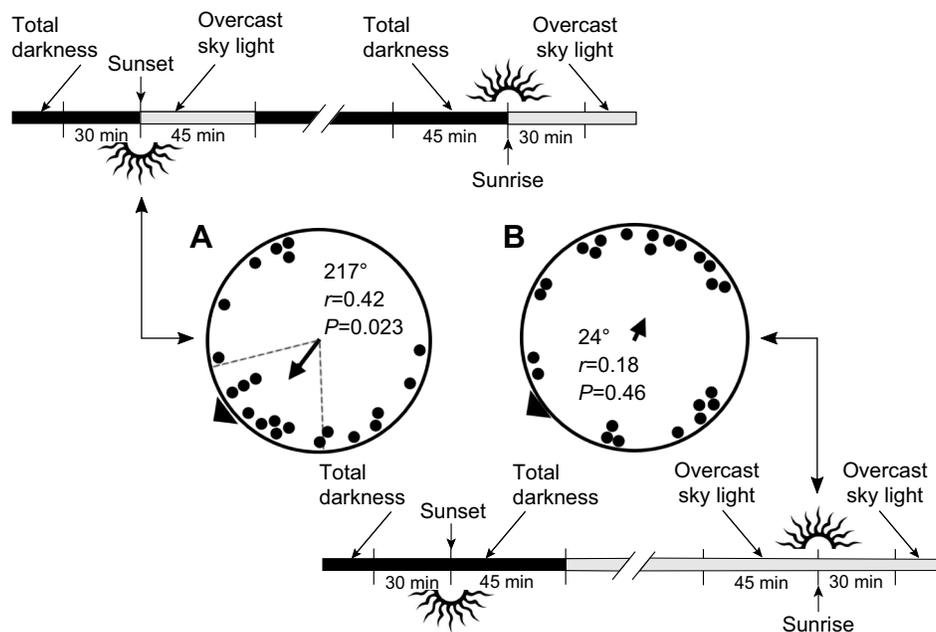


Fig. 4. Exposure to light starting at sunset and ending 45 min after versus starting 45 min after sunset and continuing till after sunrise. (A) Bearings of newts exposed to diffuse natural illumination from sunset to 45 min later (i.e. spanning civil twilight) were significantly homeward oriented ($n=21$). (B) Bearings of newts exposed to diffuse natural illumination from 45 min after sunset to sunrise (i.e. the remainder of the night) were randomly distributed ($n=24$). Symbols are the same as in Fig. 1.

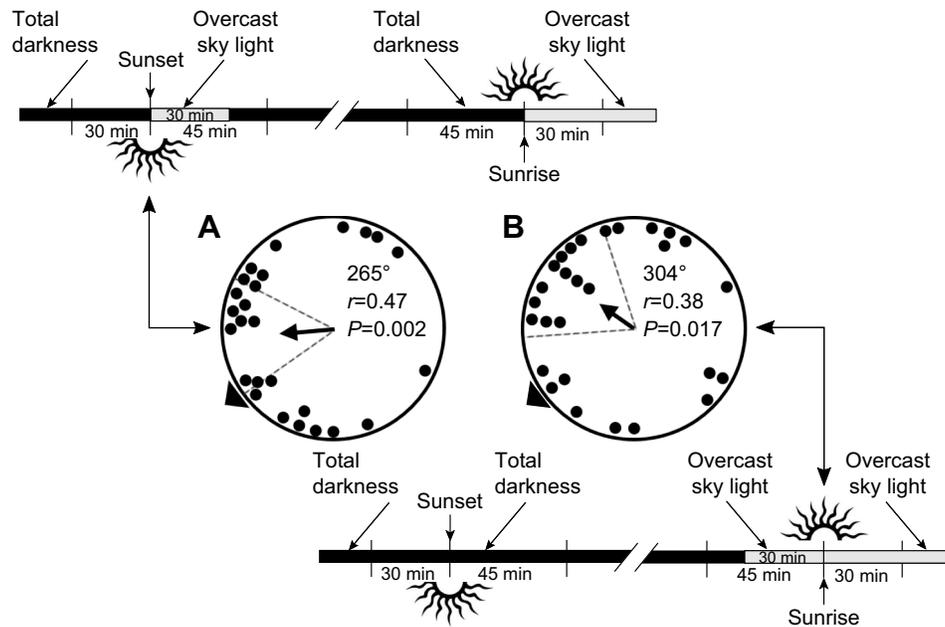


Fig. 5. Exposure to light for 30 min starting at sunset versus exposure starting 30 min before sunrise. (A) Newts exposed to 30 min of light immediately after sunset (i.e. evening civil twilight) were significantly homeward oriented ($n=27$). (B) Newts exposed to 30 min of light immediately before sunrise (morning civil twilight) were not homeward oriented ($n=28$). Symbols are the same as in Fig. 1.

information (i.e. natural olfactory cues) (experiments I and II) or not (experiments IIIa–c and IV). In the false testing site experiments (experiment II), newts were exposed to diffuse illumination overnight including evening civil twilight at one site and then transported the following morning to a site that differed in direction from the home pond for testing. The newts' orientation at the testing site (i.e. the compass component of homing) indicated that they had determined their geographic position relative to the home pond (i.e. the map component of homing) at the site where they were held

overnight. In these experiments, therefore, newts carried out the map and compass components of homing at different locations (i.e. false testing site where they were held overnight versus the site where they were tested the following day) and at different times of day (i.e. evening civil twilight versus daylight hours the following day; and see also Phillips and Borland, 1994; Phillips et al., 2002a).

Previous studies of eastern red-spotted newts have shown that the light-dependent magnetic compass is involved in both the map and compass components of true navigation. For the compass

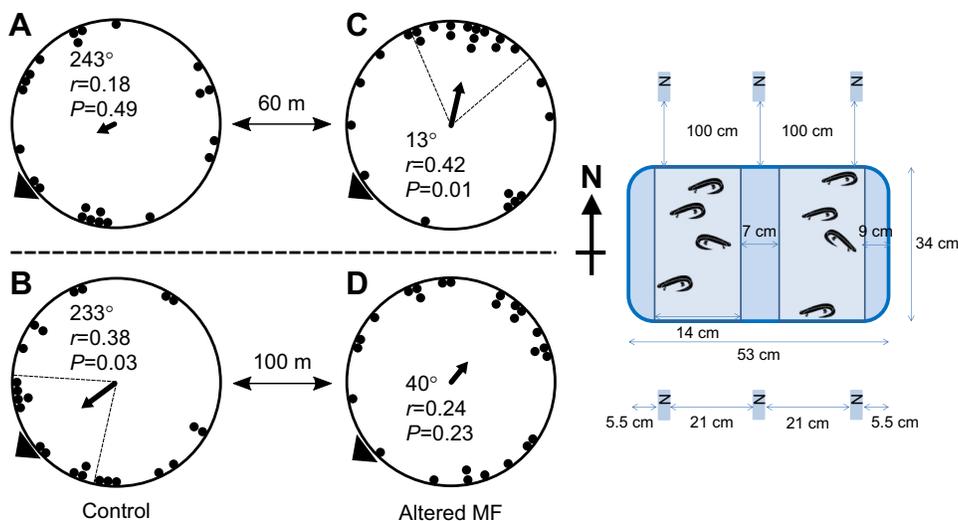


Fig. 6. Use of magnets to manipulate the magnetic field during evening twilight. Changes in the magnetic field (MF) were produced with magnets placed far enough away from the holding container to make a relatively small (<10%) increase in the intensity of the horizontal component of the MF, making magnetic inclination shallower and, correspondingly, total intensity greater. In the first experiment, control (A) and magnet-exposed (C) groups were separated by 60 m; in the second, control (B) and magnet-exposed (D) groups were separated by 100 m. (A,B) Bearings of newts tested the following day that had been exposed to the ambient MF during evening civil twilight, from 30 min before sundown to 45 min after sundown (A: $n=23$; B: $n=24$). (C,D) Bearings of newts tested the following day that had been exposed to an altered MF during evening civil twilight, from 30 min before sundown to 45 min after sundown (C: $n=25$; D: $n=26$). Symbols are the same as in Fig. 1.

component of homing, the light-dependent magnetic compass is used to determine the home direction, while for the map component of homing the magnetic compass is used as a reference to position a second magnetoreceptor in a fixed alignment relative to the MF (Diego-Rasilla et al., 2005; Fischer et al., 2001; Phillips, 1986a; Phillips et al., 2002a,b); holding newts under conditions that alter or eliminate input from the light-dependent compass (i.e. total darkness, or long-wavelength light) during times that include evening civil twilight (Figs 3–5; Phillips and Borland, 1994) eliminates homeward orientation even when newts are subsequently tested under full-spectrum light, i.e. conditions that enable the magnetic compass to function normally. It appears, therefore, that acquiring map information is constrained both to a narrow window of time (i.e. evening civil twilight; see below) and to natural (i.e. full spectrum) lighting conditions.

Newts are faced with another problem over longer periods of time. Secular variation in the MF would make the location of the map coordinates learned during a previous visit to the breeding pond a ‘moving target’, i.e. shifting in location by as much as 20–30 m day⁻¹. The resulting error could be minimized by using an estimate of the rate(s) of change in the relevant MF components, and/or by movement after arrival at the goal site in a fixed compass heading that compensates for the drift. In addition, migrating newts could use the calls of anurans (Diego-Rasilla and Luengo, 2004, 2007) and/or local olfactory cues (Grant et al., 1968; Joly and Miaud, 1993; McGregor and Teska, 1989; Sinsch, 2007) to increase the accuracy of orientation during the final approach to the breeding pond.

Our findings indicate that Alpine newts obtain map readings during civil twilight (Figs 1–6). The available evidence also suggests that this map information is derived from the MF; disruption of the MF during civil twilight prevented the newts from exhibiting homing orientation when tested the following day (Fig. 6). Previous studies of eastern red-spotted newts suggest that in order to take map readings, newts may position themselves in a fixed alignment relative to the MF to increase the accuracy of measurements necessary to derive geographic position from spatial variation in the MF (Phillips et al., 2002b). Preliminary observations suggest that Alpine newts exhibited a consistent axis of alignment during civil twilight (Fig. S1A). During morning civil twilight, the distribution of alignments approached significance and differed significantly from the distribution during evening twilight.

During evening civil twilight, beginning immediately after sunset, Alpine newts exhibited sequential changes in alignment (Fig. S1C), similar to responses observed in the earlier studies when eastern red-spotted newts were tested in an indoor arena under diffuse broad-band light after being held in outdoor tanks under long-wavelength light (Fig. S1B). In both cases, the conditions under which newts were held (i.e. total darkness and long-wavelength light, respectively) would have prevented access to map information prior to testing (see earlier discussion). The alignment responses exhibited by both species of newts may help to position the putative magnetite-based ‘map detector’ in a consistent alignment to the MF (Phillips et al., 2002b). Further research is needed to determine whether the mean axis of alignment during civil twilight and the short-term changes in alignment exhibited by Alpine newts (Fig. S1C) are reproducible.

Although the findings in Figs 1–6 support the prediction that newts should take map readings during civil twilight when temporal variation in the MF is minimal (Willis et al., 2009), we also predicted that newts would obtain map readings during morning

civil twilight; averaging morning and evening values would increase the accuracy of map measurements and/or could be used to avoid taking readings during magnetic storms that would make map measurements unreliable. It is noteworthy, therefore, that the prediction that newts should make use of map information obtained during morning twilight was not supported by the findings reported here (Figs 3–5). A possible explanation for reliance on map information obtained during evening civil twilight is that newts may rely on map measurements obtained during evening twilight because this coincides with the onset of nocturnal migratory activity (Diego-Rasilla, 2014; Montori and Herrero, 2004).

An alternative possibility is that under natural conditions, newts make use of information obtained during both evening and morning twilight periods, but were prevented from doing so in the present experiments. In the experiments shown in Fig. 1A–D (left circular diagrams) and Figs 2–5, newts were transported to the testing site between the morning and evening twilight periods on the day prior to testing. Under natural conditions, newts that migrate at night would remain at the same location during the daylight hours. Consequently, morning twilight readings obtained when a migrating newt first arrives at the location where it will spend the day could be used as a reference for evening twilight readings obtained at the same location prior to the resumption of migration, i.e. to avoid relying on magnetic map measurements when the MF was unstable. This might occur even if measurements obtained during the preceding morning twilight period were not used to obtain map information per se, and measurements obtained during evening twilight were sufficient to provide map information. If so, the role of magnetic measurements obtained during morning twilight may only be evident if newts are allowed to obtain magnetic measurements during morning twilight followed by measurements obtained during evening twilight at the same location (i.e. holding site).

Conclusion

The findings reported here show that if, and only if, newts were exposed to natural lighting during evening civil twilight in the ambient MF, they were able to orient in the home direction when tested under diffuse natural illumination the following day. When kept in total darkness during evening civil twilight or when the MF was disrupted during this time of day, newts tested the following day failed to orient in the homeward direction. Homeward orientation was not affected by whether or not newts had access to natural, wind-borne odors during evening civil twilight, or during the following day prior to testing, suggesting that they did not rely on olfactory map cues to determine the home direction. These findings are consistent with newts using a sampling strategy to derive high-resolution magnetic map information that minimizes errors resulting from short-term temporal variation in the MF. Without this type of ‘temporal filter’, reliance on a magnetic map sense for the short-range (1–2 km) movements that newts typically exhibit under natural conditions would not be possible.

Definitive evidence for a short-range, high-resolution magnetic map (to paraphrase Carl Sagan, the ‘extraordinary evidence’ necessary to provide support for this ‘extraordinary claim’) will require further research. Nonetheless, from what we now know, it appears likely that use of spatial variation in the MF for short-range homing requires behavioral and physiological adaptations that are comparable in sophistication and complexity to those underlying better known examples of long-range navigation in other vertebrates.

Acknowledgements

We sincerely thank R. M. Luengo for her invaluable assistance during the study and J. Webster for extensive help with editing the manuscript prior to submission. The Cantabria autonomous government granted the necessary permits for the study.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: F.J.D.-R., J.B.P.; Methodology: F.J.D.-R., J.B.P.; Validation: F.J.D.-R., J.B.P.; Formal analysis: F.J.D.-R.; Investigation: F.J.D.-R.; Resources: F.J.D.-R., J.B.P.; Data curation: F.J.D.-R., J.B.P.; Writing - original draft: F.J.D.-R., J.B.P.; Writing - review & editing: F.J.D.-R., J.B.P.; Visualization: F.J.D.-R., J.B.P.; Supervision: F.J.D.-R., J.B.P.; Project administration: F.J.D.-R., J.B.P.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

References

- Baldocchi, D. D.** (1989). Turbulent transfer in a deciduous forest. *Tree Physiol.* **5**, 357-377. doi:10.1093/treephys/5.3.357
- Batschelet, E.** (1981). *Circular Statistics in Biology*. New York: Academic Press.
- Begall, S., Červený, J., Neef, J., Vojtěch, O. and Burda, H.** (2008). Magnetic alignment in grazing and resting cattle and deer. *Proc. Natl. Acad. Sci. USA* **105**, 13451-13455. doi:10.1073/pnas.0803650105
- Begall, S., Malkemper, E. P., Červený, J., Němec, P. and Burda, H.** (2013). Magnetic alignment in mammals and other animals. *Mamm. Biol.* **78**, 10-20. doi:10.1016/j.mambio.2012.05.005
- Bestley, S., Patterson, T. A., Hindell, M. A. and Gunn, J. S.** (2008). Feeding ecology of wild migratory tunas revealed by archival tag records of visceral warming. *J. Anim. Ecol.* **77**, 1223-1233. doi:10.1111/j.1365-2656.2008.01437.x
- Boles, L. C. and Lohmann, K. J.** (2003). True navigation and magnetic maps in spiny lobsters. *Nature* **421**, 60-63. doi:10.1038/nature01226
- Brassart, J., Kirschvink, J. L., Phillips, J. B. and Borland, S. C.** (1999). Ferromagnetic material in the eastern red-spotted newt, *Notophthalmus viridescens*. *J. Exp. Biol.* **202**, 3155-3160. doi:10.1242/jeb.202.22.3155
- Červený, J., Begall, S., Koubek, P., Nováková, P. and Burda, H.** (2011). Directional preference may enhance hunting accuracy in foraging foxes. *Biol. Lett.* **7**, 355-357. doi:10.1098/rsbl.2010.1145
- Chernetsov, N., Pakhomov, A., Kobylkov, D., Kishkinev, D., Holland, R. A. and Mouritsen, H.** (2017). Migratory eurasian reed warblers can use magnetic declination to solve the longitude problem. *Curr. Biol.* **27**, 2647-2651.e2. doi:10.1016/j.cub.2017.07.024
- Courtilot, V. and Le Mouél, J. L.** (1988). Time variations of the Earth's Magnetic Field: from daily to secular. *Annu. Rev. Earth Planet Sci.* **16**, 389-476. doi:10.1146/annurev.ea.16.050188.002133
- Courtilot, V., Hulot, G., Alexandrescu, M., Moué, J.-L. and Kirschvink, J. L.** (1997). Sensitivity and evolution of sea-turtle magnetoreception: observations, modelling and constraints from geomagnetic secular variation. *Terra Nova* **9**, 203-207. doi:10.1111/j.1365-3121.1997.tb00013.x
- Cronin, T. W. and Shashar, N.** (2001). The linearly polarized light field in clear, tropical marine waters: spatial and temporal variation of light intensity, degree of polarization and e-vector angle. *J. Exp. Biol.* **204**, 2461-2467. doi:10.1242/jeb.204.14.2461
- Deutschlander, M. E., Phillips, J. B. and Borland, S. C.** (1999a). The case for light-dependent magnetic orientation in animals. *J. Exp. Biol.* **202**, 891-908. doi:10.1242/jeb.202.8.891
- Deutschlander, M. E., Borland, S. C. and Phillips, J. B.** (1999b). Extraocular magnetic compass in newts. *Nature* **400**, 324-325. doi:10.1038/22450
- Deutschlander, M. E., Phillips, J. B. and Borland, S. C.** (2000). Magnetic compass orientation in the eastern red-spotted newt, *Notophthalmus viridescens*: rapid acquisition of the shoreward axis. *Copeia* **2000**, 413-419. doi:10.1643/0045-8511(2000)000[0413:MCOITE]2.0.CO;2
- Diego-Rasilla, F. J.** (2003). Homing ability and sensitivity to the geomagnetic field in the Alpine newt, *Triturus alpestris*. *Ethol. Ecol. Evol.* **15**, 251-259. doi:10.1080/08927014.2003.9522670
- Diego-Rasilla, F. J.** (2014). Tritón alpino – *Ichthyosaura alpestris*. In *Enciclopedia Virtual de los Vertebrados Españoles* (ed. A. Salvador and I. Martínez-Solano). Madrid: Museo Nacional de Ciencias Naturales. <http://www.vertebradosibericos.org/>.
- Diego-Rasilla, F. J. and Luengo, R. M.** (2004). Heterospecific call recognition and phonotaxis in the orientation behavior of the marbled newt, *Triturus marmoratus*. *Behav. Ecol. Sociobiol.* **55**, 556-560. doi:10.1007/s00265-003-0740-y
- Diego-Rasilla, F. J. and Luengo, R. M.** (2007). Acoustic orientation in the palmate newt, *Lissotriton helveticus*. *Behav. Ecol. Sociobiol.* **61**, 1329-1335. doi:10.1007/s00265-007-0363-9
- Diego-Rasilla, F. J. and Ortiz-Santaliestra, M. E.** (2009). *Los Anfibios. Colección Naturaleza en Castilla y León*. Burgos: Caja de Burgos.
- Diego-Rasilla, F. J., Luengo, R. M. and Phillips, J. B.** (2005). Magnetic compass mediates nocturnal homing by the Alpine newt, *Triturus alpestris*. *Behav. Ecol. Sociobiol.* **58**, 361-365. doi:10.1007/s00265-005-0951-5
- Diego-Rasilla, F. J., Luengo, R. M. and Phillips, J. B.** (2008). Use of a magnetic compass for nocturnal homing orientation in the palmate newt, *Lissotriton helveticus*. *Ethology* **114**, 808-815. doi:10.1111/j.1439-0310.2008.01532.x
- Diego-Rasilla, F. J., Luengo, R. M. and Phillips, J. B.** (2015). Evidence of light-dependent magnetic compass orientation in urodele amphibian larvae. *Behav. Process.* **118**, 1-7. doi:10.1016/j.beproc.2015.05.007
- Diego-Rasilla, F. J., Pérez-Mellado, V. and Pérez-Cembranos, A.** (2017). Spontaneous magnetic alignment behaviour in free-living lizards. *Sci. Nat.* **104**, 13. doi:10.1007/s00114-017-1439-7
- Dommer, D. H., Gazzolo, P. J., Painter, M. S. and Phillips, J. B.** (2008). Magnetic compass orientation by larval *Drosophila melanogaster*. *J. Insect Physiol.* **54**, 719-726. doi:10.1016/j.jinsphys.2008.02.001
- Durif, C. M. F., Browman, H. I., Phillips, J. B., Skiftesvik, A. B., Vøllestad, L. A. and Stockhausen, H. H.** (2013). Magnetic compass orientation in the European eel. *PLoS ONE* **8**, e59212. doi:10.1371/journal.pone.0059212
- Fasola, M.** (1993). Resource partitioning by three species of newts during their aquatic phase. *Ecography* **16**, 73-81. doi:10.1111/j.1600-0587.1993.tb00060.x
- Fischer, J. H., Freake, M. J., Borland, S. C. and Phillips, J. B.** (2001). Evidence for the use of magnetic map information by an amphibian. *Anim. Behav.* **62**, 1-10. doi:10.1006/anbe.2000.1722
- Freake, M. J., Muheim, R. and Phillips, J. B.** (2006). Magnetic maps in animals: a theory comes of age? *Q. Rev. Biol.* **81**, 327-347. doi:10.1086/511528
- Gill, D. E.** (1979). Density dependence and homing behavior in adult red-spotted newts *Notophthalmus viridescens* (Rafinesque). *Ecology* **60**, 800-813. doi:10.2307/1936616
- Gould, J. L.** (1998). Sensory bases of navigation. *Curr. Biol.* **8**, R731-R738. doi:10.1016/S0960-9822(98)70461-0
- Grant, D., Anderson, O. and Twitty, V.** (1968). Homing orientation by Olfaction in Newts (*Taricha rivularis*). *Science* **160**, 1354-1356. doi:10.1126/science.160.3834.1354
- Hagstrum, J. T.** (2013). An infrasound-based avian navigational "map". *J. Acoust. Soc. Am.* **133**, 3499. doi:10.1121/1.4806216
- Hart, V., Kušta, T., Němec, P., Bláhová, V., Ježek, M., Nováková, P., Begall, S., Červený, J., Hanzal, V., Malkemper, E. P. et al.** (2012). Magnetic alignment in carps: evidence from the Czech christmas fish market. *PLoS ONE* **7**, e51100. doi:10.1371/journal.pone.0051100
- Heyers, D., Elbers, D., Bulte, M., Bairlein, F. and Mouritsen, H.** (2017). The magnetic map sense and its use in fine-tuning the migration programme of birds. *J. Comp. Physiol. A* **203**, 491-497. doi:10.1007/s00359-017-1164-x
- Hutchinson, B. A. and Hicks, D. D.** (ed.) (1985). *The Forest-Atmosphere Interaction. Proceedings of the Forest Environmental Measurements Conference held at Oak Ridge, Tennessee, October 23-28, 1983*. Springer.
- Joly, P. and Miaud, C.** (1993). How does a newt find its pond? The role of chemical cues in migrating newts (*Triturus alpestris*). *Ethol. Ecol. Evol.* **5**, 447-455.
- Kishkinev, D., Chernetsov, N., Pakhomov, A., Heyers, D. and Mouritsen, H.** (2015). Eurasian reed warblers compensate for virtual magnetic displacement. *Curr. Biol.* **25**, R822-R824. doi:10.1016/j.cub.2015.08.012
- Lednor, A. J.** (1982). Magnetic navigation in pigeons: possibilities and problems. In *Avian Navigation* (ed. F. Papi and H. G. Wallraff), pp. 109-119. Berlin Heidelberg New York: Springer.
- Lilley, F. E. M. T., White, A. and Heinson, G. S.** (2001). Earth's magnetic field: ocean current contributions to vertical profiles in deep oceans. *Geophys. J. Int.* **147**, 163-175. doi:10.1046/j.1365-246X.2001.00514.x
- Lohmann, K. J.** (2007). Sea turtles: navigating with magnetism. *Curr. Biol.* **17**, R102-R104. doi:10.1016/j.cub.2007.01.023
- Lohmann, K. J. and Lohmann, C. M. F.** (2006). Sea turtles, lobsters, and oceanic magnetic maps. *Mar. Freshw. Behav. Physiol.* **39**, 49. doi:10.1080/10236240600563230
- Lohmann, K. J., Lohmann, C. M. F. and Putman, N. F.** (2007). Magnetic maps in animals: nature's GPS. *J. Exp. Biol.* **210**, 3697-3705. doi:10.1242/jeb.001313
- Lohmann, K. J., Putman, N. F. and Lohmann, C. M. F.** (2008). Geomagnetic imprinting: a unifying hypothesis of long-distance natal homing in salmon and sea turtles. *Proc. Natl. Acad. Sci. USA* **105**, 19096-19101. doi:10.1073/pnas.0801859105
- Matthews, G. V. T.** (1955). *Bird Navigation*. Cambridge: Cambridge University Press.
- McGregor, J. H. and Teska, W. R.** (1989). Olfaction as an orientation mechanism in migrating *Ambystoma maculatum*. *Copeia* **1989**, 779-781. doi:10.2307/1445516
- Montori, A. and Herrero, P.** (2004). Caudata. In *Amphibia, Lissamphibia. Fauna Ibérica*, Vol. 24 (ed. M. García-París A. Montori and P. Herrero), pp. 43-275. Madrid: Museo Nacional de Ciencias Naturales, CSIC.
- Muheim, R. and Deutschlander, M.** (2010). Magnetic orientation in migratory songbirds. In *Encyclopedia of Animal Behavior* (ed. M. D. Breed and J. Moore), pp. 314-323. Oxford: Academic Press.

- Muheim, R., Moore, F. R. and Phillips, J. B.** (2006a). Calibration of magnetic and celestial compass cues in migratory birds - a review of cue-conflict experiments. *J. Exp. Biol.* **209**, 2-17. doi:10.1242/jeb.01960
- Muheim, R., Phillips, J. B. and Åkesson, S.** (2006b). Polarized light cues underlie compass calibration in migratory songbirds. *Science* **313**, 837-839. doi:10.1126/science.1129709
- Munro, U., Munro, J. A., Phillips, J. B., Wiltchko, R. and Wiltchko, W.** (1997). Evidence for a magnetite-based navigational "map" in birds. *Naturwissenschaften* **84**, 26-28. doi:10.1007/s001140050343
- Objeser, P., Hart, V., Malkemper, E. P., Begall, S., Holá, M., Painter, M. S., Červený, J. and Burda, H.** (2016). Compass-controlled escape behavior in roe deer. *Behav. Ecol. Sociobiol.* **70**, 1345-1355. doi:10.1007/s00265-016-2142-y
- Patterson, T. A., Evans, K., Carter, T. I. and Gunn, J. S.** (2008). Movement and behaviour of large southern bluefin tuna (*Thunnus maccoyii*) in the Australian region determined using pop-up satellite archival tags. *Fish. Oceanogr.* **17**, 352-367. doi:10.1111/j.1365-2419.2008.00483.x
- Patterson, T. A., Eveson, J. P., Hartog, J. R., Evans, K., Cooper, S., Lansdell, M., Hobday, A. J. and Davies, C. R.** (2018). Migration dynamics of juvenile southern bluefin tuna. *Sci. Rep.* **8**, 14553. doi:10.1038/s41598-018-32949-3
- Phillips, J.** (1986a). Two magnetoreception pathways in a migratory salamander. *Science* **233**, 765-767. doi:10.1126/science.3738508
- Phillips, J. B.** (1986b). Magnetic compass orientation in the Eastern red-spotted newt (*Notophthalmus viridescens*). *J. Comp. Physiol. A* **158**, 103-109. doi:10.1007/BF00614524
- Phillips, J. B.** (1987). Laboratory studies of homing orientation in the eastern red-spotted newt, *Notophthalmus viridescens*. *J. Exp. Biol.* **131**, 215-229. doi:10.1242/jeb.131.1.215
- Phillips, J. B.** (1996). Magnetic Navigation. *J. Theor. Biol.* **108**, 309-319. doi:10.1006/jtbi.1996.0105
- Phillips, J. B. and Borland, S. C.** (1992a). Behavioural evidence for use of a light-dependent magnetoreception mechanism by a vertebrate. *Nature* **359**, 142-144. doi:10.1038/359142a0
- Phillips, J. B. and Borland, S. C.** (1992b). Magnetic compass orientation is eliminated under near-infrared light in the eastern red-spotted newt *Notophthalmus viridescens*. *Anim. Behav.* **44**, 796-797. doi:10.1016/S0003-3472(05)80311-2
- Phillips, J. B. and Borland, S. C.** (1992c). Wavelength specific effects of light on magnetic compass orientation of the eastern red-spotted newt *Notophthalmus viridescens*. *Ethol. Ecol. Evol.* **4**, 33-42. doi:10.1080/08927014.1992.9525348
- Phillips, J. and Borland, S.** (1994). Use of a specialized magnetoreception system for homing by the eastern red-spotted newt *Notophthalmus viridescens*. *J. Exp. Biol.* **188**, 275-291. doi:10.1242/jeb.188.1.275
- Phillips, J. B., Adler, K. and Borland, S. C.** (1995). True navigation by an amphibian. *Anim. Behav.* **50**, 855-858. doi:10.1016/0003-3472(95)80146-4
- Phillips, J. B., Freake, M. J., Fischer, J. H. and Borland, S. C.** (2002a). Behavioral titration of a magnetic map coordinate. *J. Comp. Physiol. A* **188**, 157-160. doi:10.1007/s00359-002-0286-x
- Phillips, J. B., Borland, S. C., Freake, M. J., Brassart, J. and Kirschvink, J. L.** (2002b). 'Fixed-axis' magnetic orientation by an amphibian: non-shoreward-directed compass orientation, misdirected homing or positioning a magnetite-based map detector in a consistent alignment relative to the magnetic field? *J. Exp. Biol.* **205**, 3903-3914. doi:10.1242/jeb.205.24.3903
- Pough, F. H., Andrews, R. M., Cadle, J. E., Crump, M. L., Savitzky, A. H. and Wells, K. D.** (2004). *Herpetology*, 3rd edn. Upper Saddle River, New Jersey: Pearson, Prentice Hall.
- Putman, N. F., Williams, C. R., Gallagher, E. P. and Dittman, A. H.** (2020). A sense of place: pink salmon use a magnetic map for orientation. *J. Exp. Biol.* **223**, jeb218735. doi:10.1242/jeb.218735
- Rodda, G. H.** (1984). The orientation and navigation of juvenile alligators: evidence of magnetic sensitivity. *J. Comp. Physiol. A* **154**, 649-658. doi:10.1007/BF01350218
- Rodda, G. H. and Phillips, J. B.** (1992). Navigational systems develop along similar lines in amphibians, reptiles, and birds. *Ethol. Ecol. Evol.* **4**, 43-51. doi:10.1080/08927014.1992.9525349
- Russell, A. P., Bauer, A. M. and Johnson, M. K.** (2005). Migration in amphibians and reptiles: An overview of patterns and orientation mechanisms in relation to life history strategies. In *Migration of Organisms* (ed. A. M. T. Elewa), pp. 151-203. Springer-Verlag.
- Scanlan, M. M., Putman, N. F., Pollock, A. M. and Noakes, D. L. G.** (2018). Magnetic map in nonanadromous Atlantic salmon. *Proc. Natl. Acad. Sci. USA* **115**, 10995-10999. doi:10.1073/pnas.1807705115
- Schlegel, P. A.** (2007). Spontaneous preferences for magnetic compass direction in the American red-spotted newt, *Notophthalmus viridescens* (Salamandridae, Urodela). *J. Ethol.* **25**, 177-184. doi:10.1007/s10164-006-0016-x
- Schlegel, P. A. and Renner, H.** (2007). Innate preference for magnetic compass direction in the Alpine newt, *Triturus alpestris* (Salamandridae, Urodela)? *J. Ethol.* **25**, 185-193. doi:10.1007/s10164-006-0017-9
- Siegel, S.** (1956). *Nonparametric Statistics for the Behavioral Sciences*, New York: MacGraw-Hill Book Company.
- Sinsch, U.** (2007). Initial orientation of newts (*Triturus vulgaris*, *T. cristatus*) following short- and long-distance displacements. *Ethol. Ecol. Evol.* **19**, 201-214. doi:10.1080/08927014.2007.9522562
- Skiles, D. D.** (1985). The geomagnetic field: Its nature, history and biological relevance. In *Magnetite Biomineralization and Magnetoreception in Organisms. Topics in Geobiology*, vol 5 (ed. J. L. Kirschvink, D. S. Jones and B. J. MacFadden), pp. 43-102. Boston: Springer.
- Taylor, B. K.** (2016). Validating a model for detecting magnetic field intensity using dynamic neural fields. *J. Theor. Biol.* **408**, 53-65. doi:10.1016/j.jtbi.2016.08.010
- Taylor, D. H.** (1972). Extra-optic photoreception and compass orientation in larval and adult salamanders (*Ambystoma tigrinum*). *Anim. Behav.* **20**, 233-236. doi:10.1016/S0003-3472(72)80041-1
- Taylor, D. H. and Ferguson, D. E.** (1970). Extraoptic celestial orientation in the southern cricket frog *Acris gryllus*. *Science* **168**, 390-392. doi:10.1126/science.168.3929.390
- Twitty, V. C.** (1966). *Of Scientists and Salamanders*. San Francisco: W. H. Freeman.
- Twitty, V., Grant, D. and Anderson, O.** (1964). Long distance homing in the newt *Taricha rivularis*. *Proc. Natl. Acad. Sci. USA* **51**, 51-58. doi:10.1073/pnas.51.1.51
- Twitty, V., Grant, D. and Anderson, O.** (1966). Course and timing of the homing migration in the newt *Taricha rivularis*. *Proc. Natl. Acad. Sci. USA* **56**, 864-871. doi:10.1073/pnas.56.3.864
- Twitty, V., Grant, D. and Anderson, O.** (1967). Initial homeward orientation after long-distance displacements in the newt *Taricha rivularis*. *Proc. Natl. Acad. Sci. USA* **57**, 342-348. doi:10.1073/pnas.57.2.342
- Vargas, J. P., Siegel, J. J. and Bingman, V. P.** (2006). The effects of a changing ambient magnetic field on single-unit activity in the homing pigeon hippocampus. *Brain Res. Bull.* **70**, 158. doi:10.1016/j.brainresbull.2006.03.018
- Walker, M. M.** (2008). A model for encoding of magnetic field intensity by magnetite-based magnetoreceptor cells. *J. Theor. Biol.* **250**, 85-91. doi:10.1016/j.jtbi.2007.09.030
- Walker, M. M., Dennis, T. E. and Kirschvink, J. L.** (2002). The magnetic sense and its use in long-distance navigation by animals. *Curr. Opin. Neurobiol.* **12**, 735-744. doi:10.1016/S0959-4388(02)00389-6
- Wallraff, H. G.** (2005). *Avian Navigation: Pigeon Homing as a Paradigm*. Berlin Heidelberg: Springer-Verlag.
- Waterman, T. H.** (2006). Reviving a neglected celestial underwater polarization compass for aquatic animals. *Biol. Rev.* **81**, 111-115. doi:10.1017/S1464793105006883
- Wells, D. K.** (2007). *The Ecology and Behavior of Amphibians*. Chicago: The University of Chicago Press.
- Willis, J., Phillips, J., Muheim, R., Diego-Rasilla, F. J. and Hobday, A. J.** (2009). Spike dives of juvenile southern bluefin tuna (*Thunnus maccoyii*): a navigational role? *Behav. Ecol. Sociobiol.* **64**, 57-68. doi:10.1007/s00265-009-0818-2
- Wiltchko, W. and Wiltchko, R.** (1981). Disorientation of inexperienced young pigeons after transportation in total darkness. *Nature* **291**, 433-434. doi:10.1038/291433a0
- Wiltchko, W. and Wiltchko, R.** (2005). Magnetic orientation and magnetoreception in birds and other animals. *J. Comp. Physiol. A* **191**, 675-693. doi:10.1007/s00359-005-0627-7