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Behavioural Processes

Evidence of light-dependent magnetic compass orientation in urodele amphibian larvae

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ABSTRACT

Experiments were conducted to investigate whether larval palmate newts undertake orientation toward or away from the home shoreline (y-axis orientation) using the geomagnetic field to steer the most direct route, and if they accomplish this task through a light-dependent magnetoreception mechanism similar to that found in anuran tadpoles and adult newts. Larval palmate newts trained and then tested under full-spectrum light showed bimodal magnetic compass orientation that coincided with the magnetic direction of the trained y-axis. In contrast, larvae trained under long-wavelength ($\geq$500 nm) light and then tested under full-spectrum light displayed bimodal orientation perpendicular to the trained y-axis direction. These results offer evidence for the use of magnetic compass cues in orienting urodele amphibian larvae, and provide additional support for the light-dependent magnetoreception mechanism since they are in complete agreement with earlier studies showing that the observed 90° shift in the direction of magnetic compass orientation under long-wavelength light ($\geq$500 nm) is due to a direct effect of light on the underlying magnetoreception mechanism. This study is the first to provide evidence of a light-dependent magnetic compass in larval urodeles.

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1. Introduction

Larval amphibians have been shown to have a well-developed compass sense that enables them to orient efficiently between shallow and deeper waters (Pough et al., 2004; Wells, 2007). Landreth and Ferguson (1966) referred to this movement along an axis perpendicular to a shoreline as y-axis orientation; they referred to the shoreline as the x-axis. Larval amphibians orient towards or away from shallow water in response to abiotic and biotic factors affecting their growth and survivorship (Bancroft et al., 2008; Harkey and Semlitsch, 1988). For example, water temperature varies with depth, time of day, and cloud cover, and temperature is a major factor influencing larval differentiation and growth rate (Atlas, 1935; Bancroft et al., 2008; Castañeda et al., 2006; Lillywhite et al., 1973; Wells, 2007). Larval amphibians find optimal temperatures by moving along the y-axis (Ultsch et al., 1999). Furthermore, although shallow shores of ponds are warmer and have more abundant food

resources than deep waters (Zug et al., 2001), shallow margins are also the preferred microhabitat of aquatic insect predators, such as dragonfly larvae and dytiscid beetles (Bancroft et al., 2008; Fairchild et al., 2003; Holomuzki, 1986). Consequently, larval amphibians may reduce predation by being able to escape directly to deep water when a predator is encountered (Stebbins and Cohen, 1997).

Celestial cues seem to be of primary importance for y-axis orientation in many adult amphibians, both anurans (Ferguson, 1967, 1971; Ferguson et al., 1965, 1967, 1968; Ferguson and Landreth, 1966; Landreth and Ferguson, 1966, 1967) and urodeles (Adler and Taylor, 1973; Taylor and Adler, 1973, 1978). In addition, larval bullfrogs, *Lithobates catesbeianus* (Auburn and Taylor, 1979; Justis and Taylor, 1976), as well as larval salamanders, *Ambystoma* sp. (Taylor, 1972; Tomson and Ferguson, 1972) have been also shown to use celestial cues for y-axis orientation.

Use of the Earth's magnetic field information for compass orientation by amphibians (Diego-Rasilla, 2004; Diego-Rasilla and Rodríguez-García, 2007; Sinsch, 2006) can be advantageous because it can be used in contexts where visual cues are not available (Goodenough et al., 2010; Rozhok, 2008). However, evidence for y-axis magnetic compass orientation among adult amphibians has only been reported in Eastern red-spotted newts,

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Notophthalmus viridescens (Phillips, 1986a,b), while among larval amphibians it has been reported for three species of frogs (*Lithobates catesbeianus*, *Pelophylax perezi* and *Rana temporaria* (Diego-Rasilla et al., 2013; Diego-Rasilla and Phillips, 2007; Freake et al., 2002), and one species of urodele, *Lissotriton boscai* (Rodríguez-García and Diego-Rasilla, 2006).

Y-axis magnetic compass orientation in amphibians has been shown to be mediated by a light-dependent magnetoreception mechanism (Deutschlander et al., 1999a,b; Diego-Rasilla et al., 2010; Phillips and Borland 1992a, 1999b; Phillips and Borland, 1999b; Phillips et al., 2001). Furthermore, the light-dependent magnetic compass exhibits similar features in larval frogs and adult newts (Deutschlander et al., 1999a,b; Diego-Rasilla et al., 2010; Phillips et al., 2001, 2010). In both groups, the light-dependent magnetic compass relies on a magnetoreception system receiving two antagonistic inputs, i.e., a high-sensitivity short-wavelength mechanism and a low-sensitivity long-wavelength mechanism (Phillips et al., 2010). *Y*-axis magnetic compass orientation of larval frogs and adult newts tested under natural light is indistinguishable from the orientation of those tested under short-wavelength light, showing that full-spectrum light preferentially excites the more sensitive short-wavelength input, whilst the long-wavelength input is excited by wavelengths >500 nm (Deutschlander et al., 1999a; Diego-Rasilla et al., 2010). The antagonistic inputs produce a wavelength-dependent 90° shift in the direction of *y*-axis magnetic compass orientation that has been shown to result from a direct effect of light on the underlying magnetoreception mechanism (Diego-Rasilla et al., 2010, 2013; Phillips and Borland, 1992a).

Previous experiments of palmate newts, *Lissotriton helveticus*, have also demonstrated that migrating adults use the calls of anurans as a guidance mechanism to locate breeding ponds, particularly for short-distance orientation under an overcast sky (Diego-Rasilla and Luengo, 2007), an ability also demonstrated in adult marbled newts, *Triturus marmoratus* (Diego-Rasilla and Luengo, 2004), and smooth newts, *Lissotriton vulgaris* (Pupin et al., 2007). Furthermore, palmate newts are capable of long-distance homing at night using the magnetic compass as the only source of compass information (Diego-Rasilla et al., 2008). However, no attention has been paid so far to the orientation behaviour of larval stages of this species.

Here, we investigate whether larval palmate newts are able of using the Earth's magnetic field to orient along a learned *y*-axis and, if so, whether this task is mediated by a light-dependent magnetoreception mechanism similar to that found in the earlier experiments with other species and developmental stages of amphibians (Diego-Rasilla et al., 2010, 2013; Freake and Phillips, 2005; Phillips and Borland, 1992a).

2. Material and methods

2.1. Subjects and study site

Palmate newt (*Lissotriton helveticus*) larvae were collected from a permanent pond located in Barros (Cantabria, northern Spain; 43°17′7″N, 4°4′41″W; 61 m a.s.l.). Twenty-five palmate newt larvae were collected in 2010 (19 August) and 56 in 2011 (27 July), and allowed to learn the direction of an artificial shore in an outdoor tank for four and five days, respectively. In 2010 one group of 13 larval newts was trained under long-wavelength light ($\lambda \geq 500$ nm) and a second group of 12 larvae was trained under natural (i.e., full-spectrum) skylight. Similarly, in 2011 one group of 28 larval newts was trained under long-wavelength light ($\lambda \geq 500$nm), whereas a second group of 28 animals was trained under natural skylight. In both experiments, all animals were subsequently tested under full-spectrum lighting conditions.

2.2. Procedure

2.2.1. Training protocols

Testing protocols, as well as training and testing apparatus have been described elsewhere (Diego-Rasilla et al., 2010; Diego-Rasilla and Phillips, 2007). Training and testing took place in an open area completely shaded by a group of hazels, *Corylus avellana* (43°17′07″N, 4°4′44″W; elevation 59 m). Larvae were trained under natural light–dark cycle in water-filled outdoor tanks and exposed to natural variation in light intensity. Animals tested in the experiments were returned to their home pond after testing.

The *y*-axis training tanks consisted of two 96 l all glass aquaria (length, 0.81 m; width, 0.31 m; height, 0.41 m), each one with a sloping bottom (~25° slope) providing a gradient of water depth from the deep end to the shallow end. Water depth at the shore end was 1 cm. The sides of each aquarium were covered with 1.5 cm thick wooden boards to prevent light penetration from the sides of the tanks, whereas the tops of the aquaria were covered with transparent window glass (transmission ≳370 nm). Training tanks were filled with tap water treated with aquarium water conditioner (TetraAcqua AquaSafe®, Tetra GmbH, Germany). Each day the aquarium water was partly replaced with fresh treated water; this was done at the time of maximum temperature (i.e., 14, 00–16, 00 GMT), thus maintaining the water temperature of the training tanks between 19 and 23 °C. Larval newts were fed frozen bloodworms, which were placed in the shallow end of the tank daily.

Two different training configurations, with perpendicular shore directions, were used in experiments carried out in 2010. One training tank was aligned along the magnetic north–south axis, with shore facing North, and the other training tank was aligned along the east–west axis, with shore to the East. The top of the N–S training tank was covered with a ≥500 nm long pass filter, comprised of one layer of a spectral filter (Lee no. 101; Lee Filters©, Andover, Hants, UK) placed between two transparent methacrylate sheets (82 cm × 42 cm, 0.4 cm thick), while the E–W tank was covered with glass only.

In 2011 two training tanks were aligned along the magnetic north–south axis, with shore facing North. The top of one of them was covered with a ≥500 nm long pass filter, comprised of one layer of a spectral filter (Lee no. 101; Lee Filters©, Andover, Hants, UK) placed between two transparent methacrylate sheets (82 cm × 42 cm, 0.4 cm thick), while the other tank was covered with glass only.

In 2010, the two groups of 13 and 12 larvae were introduced into the shallow end of each training tank (N–S and E–W training tanks, respectively) on 19 August, and allowed four days (i.e., from 19 to 23 August) to learn the *y*-axis direction before testing. In 2011, two groups of 28 animals were introduced into the shallow end of each training tank on 27 July and allowed five days (i.e., from 27 to 1 August) to learn the *y*-axis direction.

After training, larvae were tested individually in a water-filled outdoor arena for magnetic orientation under natural (i.e., full-spectrum) skylight conditions. In both 2010 and 2011, all larvae were tested on a single day during daylight hours.

2.2.2. Testing apparatus and protocols

Each animal was tested only once in one of the four magnetic field conditions, the ambient magnetic field (magnetic north at North) and three altered fields (magnetic north rotated to East, South or West; Phillips, 1986a) produced by means of a double-wrapped cube surface coil (Rubens, 1945). The testing apparatus was a circular, featureless arena open only to the sky (circular plastic container, 43 cm diameter and 24 cm high) enclosed within the double-wrapped coil powered by a dual DC power supply (Protek 30158) used to alter the alignment of the Earth's magnetic field. The sides of the apparatus were covered with a black cotton

curtain. The testing apparatus was located under dense foliage that blocked a direct view of the overhead sky, preventing localization of the sun disk and allowing only diffuse light to reach the newts in the testing arena. The arena was filled with treated tap water to a depth of 1 cm. The temperature of arena water was maintained between 19 and 21 °C, by replacing some of the arena water with fresh water as soon as the temperature reached 21 °C.

Larval newts were removed from the training tank one at a time with a small dip net and placed in the arena centre beneath an opaque, cylindrical plastic container (9 cm diameter, 14.5 cm high) that served as a release device. Each newt remained in the cylindrical container for 1 min to recover from effects of handling before the release device was lifted and it was allowed to move freely within the testing arena. Larval newts' movements were observed through tiny holes in the black curtain that surrounded the test apparatus. A larva's directional response was recorded at the first point where it made contact with the wall. Directional bearings were scored with respect to symmetrical radial marks drawn on the exterior edge of arena walls.

Bearings of larvae that contacted arena wall in less than 10 s were not recorded. These individuals moved immediately when the release device was lifted, and were considered to exhibit a randomly oriented escape response and were excluded from the analyses. A similar criterion was used in experiments with adult newts tested in a terrestrial arena by Phillips (Phillips, 1986a; Phillips and Borland, 1994). In addition, trials were abandoned if the animal failed to score within 10 min (in those cases, the next larva was tested under the same magnetic condition) (Diego-Rasilla et al., 2010; Diego-Rasilla and Phillips, 2007).

The order of the four magnetic field alignments (magnetic N = N, S, E or W) was determined using a random number sequence. In 2010, the first individual was tested in magN = N, the next individual in magN = E, followed by one in magN = W and one in magN = S, whereas in 2011 the first individual was tested in magN = W, the next individual in magN = E, followed by one in magN = N and one in magN = S. These sequences were repeated until the tests were completed.

Testing was carried out double blind. One experimenter set the horizontal alignment of the field using remote switches, whereas the second experimenter carried in each larval newt from the tanks and recorded its directional response without knowing the alignment of the magnetic field. Thus, the same sequence of four magnetic field alignments was used for successive groups of four larvae without the observer being aware of the magnetic field alignment. The sequence of fields was not revealed to the observer until after the experiment was completed.

In the 2010 experiment, the first four larvae were taken from E–W training tank (i.e., full-spectrum training tank) and tested following the sequence of magnetic field alignments described previously. Once this first group of four alignments was completed, the next four larvae were taken from the N–S training tank (i.e., trained under long-wavelength light), and tested following the same sequence of magnetic field alignments. Testing of subsequent groups of 4 newts was alternated between the two tanks. In 2011, the first four larvae were taken individually from the natural skylight training tank and tested following the sequence of magnetic field alignments described previously; the next four larvae were taken from the long-wavelength ($\lambda \geq 500$ nm) training tank and tested following the same sequence of magnetic field alignments. As in 2010, testing of subsequent groups of 4 newts was alternated between the two tanks.

Absolute or "topographic" bearings (i.e., actual directions of movement ignoring the alignment of the magnetic field in testing) were recorded and then normalized with respect to the direction of magnetic North to which each newt was exposed during testing by rotating the distributions of bearings from each of the four magnetic alignments (magN = N, magN = E, magN = W, magN = S) so that the direction of magnetic North coincided at 0°. By pooling bearings from an approximately equal number of larvae tested in each of the four field alignments, we were able to factor out any non-magnetic bias from the resulting distribution, and retain only that component of the larval newts' orientation relative to the magnetic field (Phillips, 1986a).

2.3. Statistical analyses

The distributions of bearings were analysed using standard circular statistics (Batschelet, 1981; Fisher, 1995). Mean vectors were calculated by vector addition, and tested for departure from a random distribution using the Rayleigh test. Statistics for bimodal distributions were calculated by doubling each bearing and reducing any greater than 360° using modulo arithmetic. Ninety-five percent confidence intervals were used to determine whether the mean for the distribution included the shoreward direction. Values of $p < 0.05$ were considered statistically significant.

Data from the different training configurations were analysed separately. The Watson U^2-test carried out on doubled bearings was used to test for differences between the distributions of magnetic bearings (Batschelet, 1981; Mardia and Jupp, 2000).

The absolute or "topographic" bearings (i.e., the distribution of bearings with respect to topographic directions ignoring the alignment of the four test magnetic fields) were also examined for any evidence of an effect of nonmagnetic directional cues on the larval newts' orientation using the Rayleigh test.

In subsequent analyses, magnetic bearings from larval newts experiencing the same lighting conditions in training and testing (i.e., full-spectrum light in training and testing) were pooled, after first rotating the distributions so the shore directions were parallel and coincided with 0°. Similarly, magnetic bearings from larvae experiencing different wavelength conditions in training and testing (i.e., long-wavelength light in training and full-spectrum light in testing) were pooled with respect to the magnetic direction of the shore in training prior to analysis.

3. Results

In both sets of experiments [2010 and 2011], one group of larval newts experienced different wavelength conditions in training and testing (Fig. 1a,c), whereas the other group of larvae experienced the same spectral conditions in training and testing (Fig. 1b,d).

The distribution of magnetic bearings from larval newts experiencing different wavelength conditions in training and testing (i.e., trained under long-wavelength light and tested under full-spectrum light) exhibited significant bimodal orientation perpendicular to the magnetic direction of the trained y-axis (Rayleigh test; 2010 experiment, $p = 0.043$, $N = 13$, Fig. 1a, Table 1; 2011 experiment, $p = 0.033$, $N = 26$, Fig. 1c, Table 1), and the 95% confidence intervals for the mean vector bearing excluded the trained y-axis and included the 90° shifted axis in both cases.

The distribution of magnetic bearings from larvae trained and tested under full-spectrum light exhibited significant bimodal orientation that coincided with the magnetic direction of the trained y-axis (Rayleigh test; 2010 experiment, $p = 0.021$, $N = 12$, Fig. 1b, Table 1; 2011 experiment, $p = 0.021$, $N = 25$, Fig. 1d, Table 1), and the 95% confidence intervals for the mean vector bearing included the trained magnetic y-axis in both cases.

In 2010 experiment, the training tanks were aligned to coincide with perpendicular shore directions, although newts trained in the north–south tank were exposed to long-wavelength light, while newts trained in the east–west tank were exposed to full spectrum light. There was no difference in the 90° rotated axis of
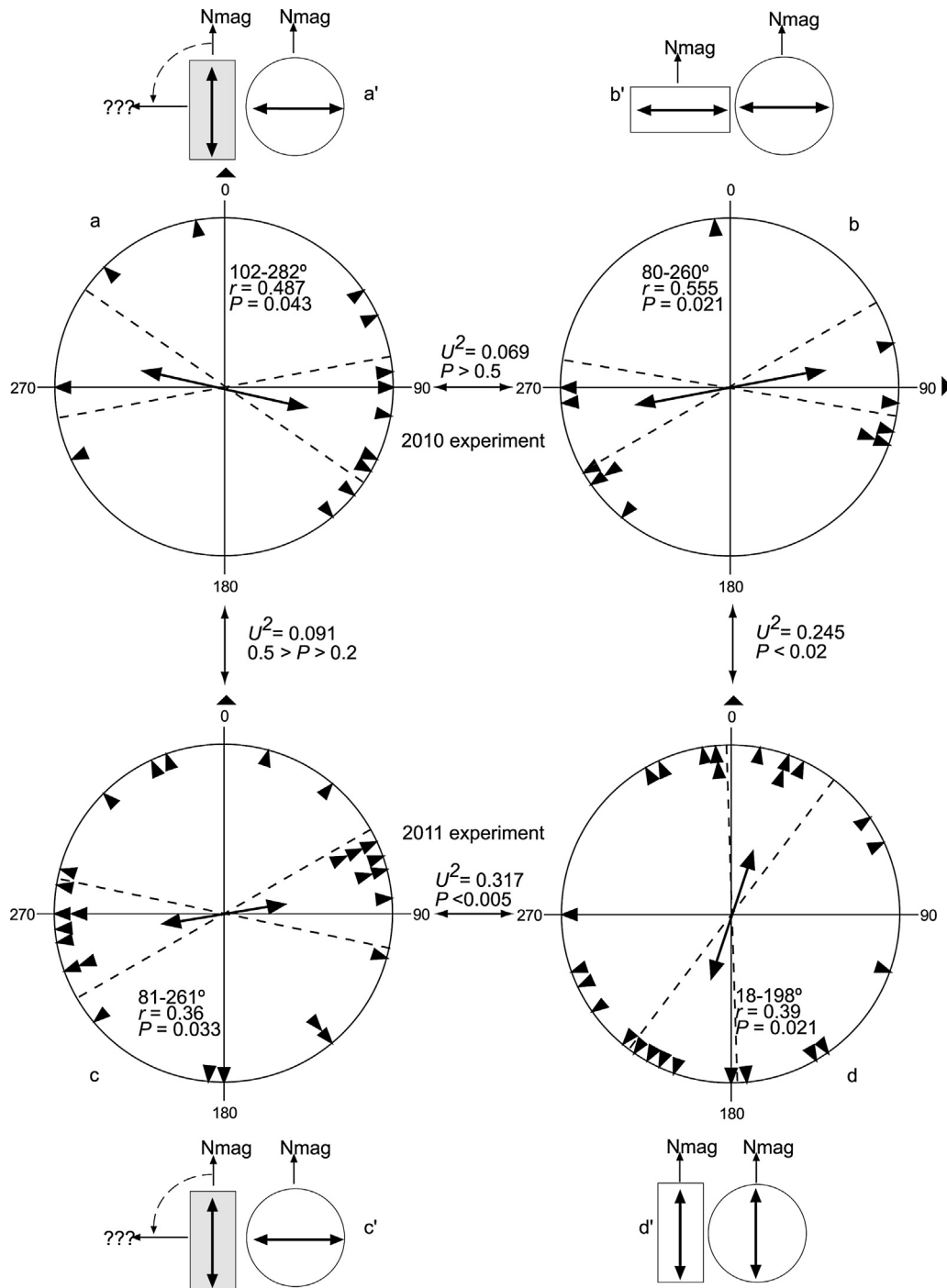
Fig. 1. (a′–d′) Predicted orientation of larval newts (double-headed arrow) and their perception of the direction of the magnetic north [based on Phillips and Borland (1992a)].

Schematic diagram of the training tank alignments are shown, as well as the training lighting conditions, full-spectrum natural skylight (white) and long-wavelength (≥500 nm) light (grey); circular test arenas show the predicted response of the larval newts under full-spectrum light (white). a′ & c′, long-wavelength training, full-spectrum testing. Based on previous work, larval newts' perception of the magnetic north under long-wavelength light should be rotated 90° relative to the actual field experienced during training. As a consequence, the direction of shore will appear to the newts to be towards magnetic east. When subsequently tested under full-spectrum light, this will cause the newt's orientation to be rotated 90° relative to the true shore direction. b′& d′, full-spectrum training and testing. Larval newts should show bimodal magnetic orientation along the shore-deep water magnetic axis. a–d, Results. Magnetic compass orientation of newts trained and tested under natural skylight (b & d) or trained under ≥500 nm long-wavelength light and tested under natural skylight (a & c). Symbols indicate the direction of movement of each individual larval newt. Double-headed arrows at the centre of each plot indicate the mean bimodal axis for each distribution. The length of each double-headed arrow is proportional to the mean vector length (r), with the diameter of the circle corresponding to r = 1. Dashed lines represent the 95% confidence intervals for the mean vector bearing. Triangles outside the plots indicate the magnetic direction of the shore end of the y-axis.

Table 1
Statistical analysis of data.

Training light conditions	Testing light conditions						
	Experiment	Bearings	Tank alignment	$\alpha \pm$ IC 95%	r	p	N
Different wavelength conditions in training and testing				Full-spectrum			
Long-wavelength ($\geq$500 nm)	2010	TB	0–180°	116–296°	0.34	0.234	13
	2010	MB	0–180°	102–282 $\pm$ 22°	0.49	0.043	13
	2011	TB	0–180°	99–279°	0.23	0.245	26
	2011	MB	0–180°	81–261 $\pm$ 21°	0.36	0.033	26
Bearings pooled with respect to magnetic direction of shore (0°)				90–270 $\pm$ 16°	0.38	0.003	39
Same spectral conditions in training and testing				Full-spectrum			
Full-spectrum	2010	TB	90–270°	61–241°	0.32	0.303	12
	2010	MB	90–270°	80–260 $\pm$ 20°	0.56	0.021	12
	2011	TB	0–180°	40–220°	0.22	0.303	25
	2011	MB	0–180°	18–198 $\pm$ 20°	0.39	0.021	25
Bearings pooled with respect to the magnetic direction of shore (0°)				7–187 $\pm$ 16°	0.39	0.003	37

α, mean direction (degrees); r, mean vector length; p, probability; N, sample size; TB, Absolute ("topographic") bearings; MB, Magnetic bearings.

orientation between larval newts trained under long-wavelength light and tested under full-spectrum light when compared to the unrotated axis of orientation of those trained and tested under full-spectrum light, (Watson U^2 test; $U^2 = 0.069$, $p > 0.5$; Fig. 1). In 2011 experiment, when both training tanks were aligned along the magnetic north–south axis and both groups of newts were tested under full spectrum light, the axis of orientation differed about 90° between larval newts trained under long-wavelength light and tested under full-spectrum light (Fig. 1c) versus larvae trained and tested under full-spectrum light (Fig. 1d) (Watson U^2 test; $U^2 = 0.317$, $p < 0.005$).

The topographic bearings (i.e., absolute bearings, ignoring the alignment of the magnetic field in testing; see methods) were randomly distributed in all cases (Table 1).

3.1. Comparing same vs. different spectral conditions in training and testing

When the two distributions of magnetic bearings from larvae trained and tested under full-spectrum light in 2010 and 2011 were normalized with respect to the magnetic direction of shore (distributions of bearings in Fig. 1b rotated so the trained axis was aligned with the vertical axis, similar to Fig. 1d), there was no significant difference between the two distributions (Watson U^2 test; $U^2 = 0.103$, $0.5 > p > 0.2$; Fig. 2). Hence, the distribution of magnetic bearings relative to the trained y-axis was not different for larvae trained along the north–south and east–west axes.

Similarly, the distributions of magnetic bearings from larvae trained under long-wavelength light and tested under full-spectrum light in 2010 and 2011 (Fig. 1a,c) were aligned perpendicular to the y-axis and were not significantly different (Watson U^2 test; $U^2 = 0.091$, $0.5 > p > 0.2$; Fig. 1a,c).

When data were pooled from larval newts experiencing the same wavelength conditions in training and testing (i.e., full-spectrum light in training and testing; Fig. 1b and d combined), after first rotating shore direction to 0°, the newts showed bimodal magnetic orientation along the shore–deep water magnetic axis (Fig. 2a). In contrast, when data were pooled from newts experiencing different wavelengths of light in training and testing (i.e., long-wavelength light in training and full-spectrum light in testing; Fig. 1a and c combined), they showed bimodal magnetic orientation perpendicular to the shore–deep water magnetic axis (Fig. 2b). The distribution of magnetic bearings for larval newts trained and tested under full-spectrum light (Fig. 2a) was significantly different ($U^2 = 0.532$, $p < 0.001$; Fig. 2) from that of bearings for larval newts trained under long-wavelength light and tested under full-spectrum light (Fig. 2b), and their mean axes of orientation differed by 90°.

4. Discussion

The present study provides the first evidence for a light-dependent magnetoreception mechanism in urodele larvae. Thus, our results are in line with previous findings obtained in tadpoles of three species of frogs (Diego-Rasilla et al., 2013; Diego-Rasilla and Phillips, 2007; Freake et al., 2002), which have been shown to use directional information from the Earth's magnetic field to maintain a consistent heading toward or away from the home shoreline. These results substantiate previous results from larval Bosca's newt, *L. boscai* (Rodríguez-García and Diego-Rasilla, 2006), and widen our knowledge of sensory basis of orientation in larval urodeles.

Previous findings provided evidence that migrating palmate newts, *L. helveticus*, can use the geomagnetic field for the compass component of long-distance homing (Diego-Rasilla et al., 2008). However, prior to this study no information was available regarding orientation behaviour of larval stages in this species, which we show here are able to perform magnetic compass orientation along a previously learned y-axis. The adaptive significance of movement perpendicular to shorelines based on magnetic compass information is clear since this orientation mechanism enables larval amphibians to follow the most direct route to and from shore, particularly in turbid pond water with poor visibility (Diego-Rasilla et al., 2013). The ecological importance of these movements is clear because larvae development is controlled by abiotic and biotic factors varying between shallow and deep water (Mazerolle et al., 2005) including, for example, dissolved oxygen (Nie et al., 1999), water temperature (Álvarez and Nicieza, 2002; Bancroft et al., 2008; Harkey and Semlitsch, 1988; Nie et al., 1999; Ultsch et al., 1999), distribution of food resources (Álvarez and Nicieza, 2002; Zug et al., 2001) and predator-prey interactions (Morin, 1986; Relyea, 2002; Stebbins and Cohen, 1997; Werner and McPeek, 1994); see earlier discussion.

Although polarised light provides an axial cue that could have been used by larval palmate newts for y-axis orientation (Taylor and Adler, 1973), we can rule out this possibility because the distribution of topographic bearings (i.e., actual directions of movement ignoring the alignment of the magnetic field in testing) was indistinguishable from random. Also, since an approximately equal number of individuals were tested in each of the four magnetic field alignments, the resulting distributions retained only the component of the newts' orientation that was a consistent response to the magnetic field (Diego-Rasilla et al., 2013; Phillips, 1986a). Hence, the present study reinforces the usefulness of the Earth's magnetic field for y-axis compass orientation, and provides additional support for a light-dependent magnetic compass mechanism in amphibians, previously described in adult newts (Phillips and
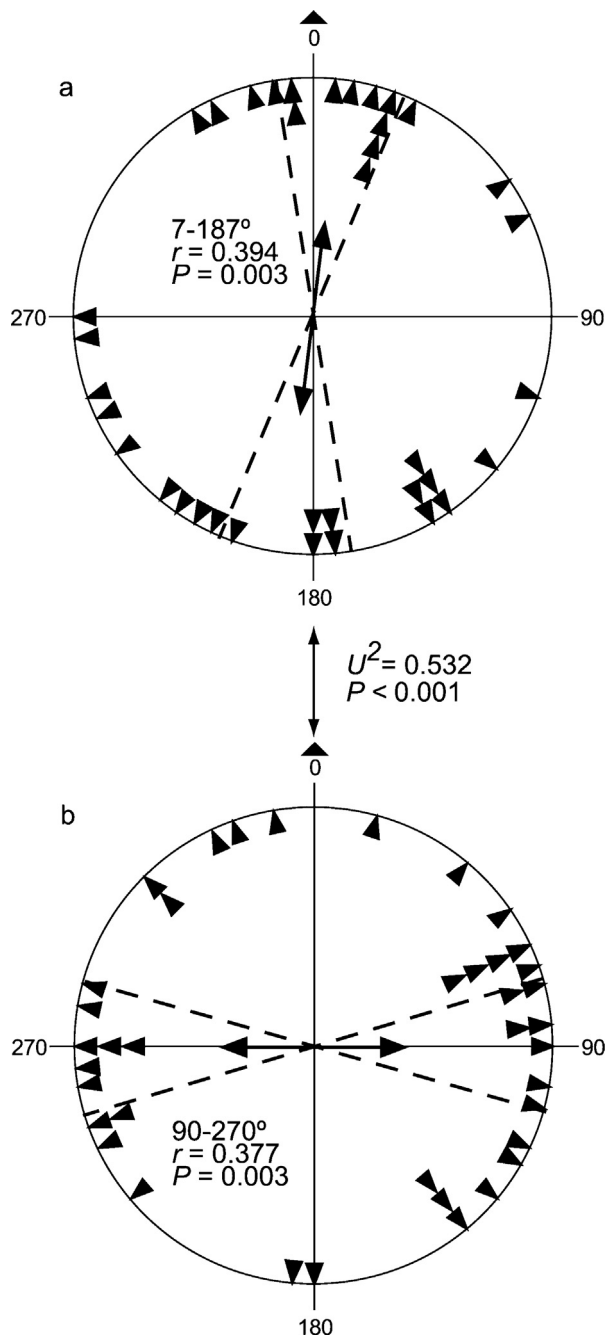
Fig. 2. (a) Bearings from larval newts trained and tested under full-spectrum light (data from Fig. 1b and d combined; bearings from Fig. 1b rotated by 90° so the trained axis coincided with that in 1d) vs. (b) Bearings from larval newts trained under long-wavelength light and tested under full-spectrum light (data from Fig. 1a and c combined). Each symbol represents the magnetic bearing of an individual newt tested only once in one of the four magnetic field alignments. Double-headed arrows at the centre of each plot indicate the mean bimodal axis for each distribution. The length of each arrow is proportional to the mean vector length (r), with the diameter of the circle corresponding to r = 1. Dashed lines represent the 95% confidence intervals for the mean vector bearing. Triangles outside the plots indicate the magnetic direction of the trained y-axis.

wavelength (≤450 nm) light, but exhibited magnetic compass orientation perpendicular to the magnetic direction of the y-axis (i.e., rotated 90° to the shore direction) under long-wavelength (≥500 nm) light (Diego-Rasilla et al., 2010; Freake and Phillips, 2005; Phillips and Borland, 1992b). Additional experiments showed that Eastern red-spotted newts (Phillips and Borland, 1992a), as well as Iberian green frog tadpoles (Diego-Rasilla et al., 2010), trained outdoors under long-wavelength (≥500 nm) light and tested under full-spectrum exhibited orientation perpendicular to the magnetic direction of the y-axis whereas those individuals trained and tested under long-wavelength light exhibited orientation that coincided with the magnetic direction of the trained y-axis. These findings indicate that the 90° shift in the direction of magnetic compass orientation under long-wavelength light (≥500 nm) is due to a direct effect of light on the underlying magnetoreception mechanism (Diego-Rasilla et al., 2010; Phillips and Borland, 1992a). Subsequent experiments with European common frog tadpoles have corroborated this conclusion (Diego-Rasilla et al., 2013).

Furthermore, the studies of light-dependent magnetic compass orientation in Eastern red-spotted newts (Phillips and Borland, 1992a) and Iberian green frogs tadpoles (Diego-Rasilla et al., 2010) provide evidence that this response is caused by antagonistic short-wavelength and long-wavelength inputs, and that full-spectrum light preferentially activates the short-wavelength spectral mechanism, which has higher sensitivity than the long-wavelength spectral mechanism.

Our results are in agreement with earlier findings from larval frogs (Diego-Rasilla et al., 2013; Diego-Rasilla and Phillips, 2007; Freake et al., 2002). Thus, larval palmate newts experiencing the same spectral conditions in training and testing (i.e., trained and then tested under full-spectrum light) showed bimodal magnetic compass orientation that coincided with the magnetic direction of the trained y-axis. In contrast, as predicted, newts experiencing different wavelengths of light in training and testing (i.e., long-wavelength light in training and full-spectrum light in testing) displayed bimodal orientation perpendicular to the trained shore-deep water magnetic axis, confirming that the underlying magnetoreception mechanism is light-dependent (Diego-Rasilla et al., 2010, 2013).

As in previous studies of learned magnetic compass orientation by amphibians, there was no splitting of the responses of larval newts trained along either the north–south or east–west axis. This contrasts with adult flies and mice in which responses along the east–west, but not the north–south, axis were split into two clusters on either side (±45°) of the trained direction, consistent with subjects responding to different components of a complex pattern of magnetic input (Painter et al., 2013).

Future studies providing more detailed information on the spectral dependence of the larval response will help to identify the photopigments underlying this response.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

Borland, 1992a) and larval frogs (Diego-Rasilla et al., 2010, 2013; Freake and Phillips, 2005).

In y-axis orientation experiments, adult Eastern red-spotted newts, bullfrog tadpoles and Iberian green frog tadpoles trained outdoors to orient in the direction of an artificial shore under natural (i.e., full-spectrum) light exhibited y-axis magnetic compass orientation when tested under full-spectrum and short-

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