

# Use of a light-dependent magnetic compass for y-axis orientation in European common frog (*Rana temporaria*) tadpoles

Francisco J. Diego-Rasilla · Rosa M. Luengo ·  
John B. Phillips

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**Abstract** We provide evidence for the use of a magnetic compass for y-axis orientation (i.e., orientation along the shore-deep water axis) by tadpoles of the European common frog (*Rana temporaria*). Furthermore, our study provides evidence for a wavelength-dependent effect of light on magnetic compass orientation in amphibians. Tadpoles trained and then tested under full-spectrum light displayed magnetic compass orientation that coincided with the trained shore-deep water axes of their training tanks. Conversely, tadpoles trained under long-wavelength ( $\geq 500$  nm) light and tested under full-spectrum light, and tadpoles trained under full-spectrum light and tested under long-wavelength ( $\geq 500$  nm) light, exhibited a  $90^\circ$  shift in magnetic compass orientation relative to the trained y-axis direction. Our results are consistent with earlier studies showing that the observed  $90^\circ$  shift in the direction of magnetic compass orientation under long-wavelength ( $\geq 500$  nm) light is due to a direct effect of light on the underlying magnetoreception mechanism. These findings also show that wavelength-dependent effects of light do not compromise the function of the magnetic compass under a wide range of natural lighting conditions, presumably due to a large asymmetry in the relatively sensitivity of

antagonistic short- and long-wavelength inputs to the light-dependent magnetic compass.

**Keywords** Anuran · Magnetic compass · Light-dependent magnetoreception · *Rana temporaria* · y-axis orientation

## Introduction

Tadpoles exhibit compass orientation along an axis perpendicular to their home shoreline (so-called y-axis orientation, Ferguson and Landreth 1966), which enables them to follow the most direct route to and from shore (Stebbins and Cohen 1997; Russell et al. 2005; Wells 2007). These movements are ecologically relevant because shallow water may provide them with food, whereas deep water may afford a refuge to larvae from predators (Stebbins and Cohen 1997; Zug et al. 2001). Also, tadpoles in temperate ponds usually stay at warmer and deeper water at night, and often move to shallow water during the daytime as water temperature increases due to solar heating (Ultsch et al. 1999), presumably because selection of warm microhabitats would be advantageous as temperature is a major factor determining larval differentiation and growth patterns (Harkey and Semlitsch 1988; Ultsch et al. 1999).

Tadpoles are myopic with poor acuity and it is unlikely that they, even in clear water, rely on vision to steer a course using familiar visual landmarks (Sivak and Warburg 1983; Mathis et al. 1988). Even though tadpoles could rely on a light intensity gradient along the shore-deep water axis that would not require high acuity, they would also be limited by turbidity, and/or by material (aquatic plants, debris, etc.) that obstructs their field of view. Therefore, the importance of compass cues for y-axis orientation in

F. J. Diego-Rasilla (✉)  
Departamento de Biología Animal, Universidad de Salamanca,  
Campus Miguel de Unamuno, Edificio de Farmacia 5ª planta,  
37007 Salamanca, Spain  
e-mail: fjdiego@herpetologica.org

R. M. Luengo  
Gabinete de Iniciativas Socioculturales y de Formación S.L.,  
Calle Nicaragua 7, 37003 Salamanca, Spain

J. B. Phillips  
Department of Biological Sciences, Virginia Tech,  
Blacksburg, VA 24061, USA

tadpoles may well be a consequence of poor visibility in aquatic environments like ponds, where turbidity and suspended material often limit visibility to a few centimeters.

Tadpoles are an excellent model system to study the sensory inputs involved in  $y$ -axis orientation because they will spontaneously learn the compass direction of the  $y$ -axis in a training tank with an artificial shore. Moreover, previous experiments have demonstrated that frog tadpoles are able to learn the direction of an artificial shore within a few days, e.g., 7-day training experiments (Freake et al. 2002; Freake and Phillips 2005) and 5-day training experiments (Diego-Rasilla and Phillips 2007; Diego-Rasilla et al. 2010).

Most of the studies of  $y$ -axis orientation in amphibians were performed in the late 1960s and 1970s and were predominantly focused on celestial cues, i.e. polarized light and sun position. Several species of newts and salamanders (Landreth and Ferguson 1967a, b; Tomson and Ferguson 1972), as well as frogs and toads (Ferguson et al. 1965, 1967, 1968; Ferguson and Landreth 1966; Ferguson 1967; McKeown 1968) were found to use the sun as a cue for  $y$ -axis orientation. Polarized light was also confirmed as an important cue for  $y$ -axis orientation in both anurans (Taylor and Ferguson 1970; Auburn and Taylor 1979) and urodeles (Taylor 1972; Adler and Taylor 1973; Taylor and Adler 1973; Taylor and Auburn 1978). But more recently, a number of studies have revealed that the Earth's magnetic field plays a role in the  $y$ -axis orientation of newts (Phillips 1986; Deutschlander et al. 2000; Rodríguez-García and Diego-Rasilla 2006) and frogs (Freake et al. 2002; Diego-Rasilla and Phillips 2007).

To date, only two species of anurans have been shown to rely on a magnetic compass for  $y$ -axis orientation, tadpoles of the bullfrog *Lithobates catesbeianus* (Freake et al. 2002) and tadpoles of the Iberian green frog *Pelophylax perezi* (Diego-Rasilla and Phillips 2007). There is also evidence that magnetic compass orientation is light-dependent in tadpoles of both two species of frogs (Freake and Phillips 2005; Diego-Rasilla et al. 2010). Interestingly, the light-dependent magnetic compass of frogs show similar features to that observed in the Eastern red-spotted newt *Nothophthalmus viridescens* (Phillips and Borland 1992a, b; Deutschlander et al. 1999a, b; Phillips et al. 2001).

The properties of the light-dependent magnetic compasses of both urodele and anuran amphibians are consistent with a magnetoreception that receives antagonistic input from a high-sensitivity short-wavelength sensitive mechanism and a low-sensitivity long-wavelength mechanism (Phillips and Borland 1992a; Phillips et al. 2010). The absolute sensitivity of the short-wavelength mechanism may be as much as two log units higher than that of the long-wavelength mechanism (Dodt and Heerd 1962); natural (i.e., full spectrum) lighting preferentially excites the

short-wavelength input, while a highly saturated long-wavelength stimulus (wavelengths  $>500$  nm) that rarely, if ever, occurs under natural conditions is necessary to preferentially excite the long-wavelength input (Deutschlander et al. 1999a; Diego-Rasilla et al. 2010).

Results from several experiments have demonstrated that Eastern red-spotted newts (Phillips and Borland 1992a, b) and Iberian green frogs (Diego-Rasilla et al. 2010) trained under full-spectrum light to orient in the direction of an artificial shore and tested under long-wavelength ( $\geq 500$  nm) light, or trained to orient in the direction of an artificial shore under long-wavelength ( $\geq 500$  nm) light and tested under full-spectrum light, oriented along an axis perpendicular to the trained axis. In contrast, those trained and tested under full-spectrum light, as well as those trained and tested under long-wavelength light, oriented along the trained axes. These findings confirmed that the  $90^\circ$  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 in both urodele and anuran amphibians (Diego-Rasilla et al. 2010; and see “Discussion”).

The aim of our work was to investigate whether tadpoles of the European common frog *Rana temporaria* are capable of using the Earth's magnetic field for orienting their movements along a learned  $y$ -axis. Furthermore, we investigated whether they accomplish this orientation task using a light-dependent magnetic compass similar to that found in the earlier experiments with other species of frogs and newts (Phillips and Borland 1992a; Freake and Phillips 2005; Diego-Rasilla et al. 2010). Finally, we show that despite differences in the directional response of the magnetic compass under highly saturated short- or long-wavelength light, the magnetic compass provides a reliable source of directional information under a wide range of natural lighting conditions.

## Methods

### Subjects and study site

European common frog (*R. temporaria*) tadpoles [stages 30–42 (Gosner 1960)] used in this study were collected from a permanent pond located at the Natural Park of Saja-Besaya (Cantabria, northern Spain;  $43^\circ 14' 8''$ N,  $4^\circ 9' 59''$ W; elevation 464 m). The pond, surrounded by a forest of *Quercus robur* and *Fagus sylvatica*, is devoid of aquatic vegetation and its bottom is covered with a thick layer of decomposing leaves.

Sixty-two tadpoles were collected in 2009 (28 July) and 50 in 2010 (13 July). In 2009 two groups of 30 and 32 tadpoles were allowed to learn the direction of an artificial

shore for 3 days under natural (i.e., full spectrum) skylight conditions, and then tested under natural skylight conditions. In 2010 tadpoles were allowed 4 days to learn an artificial shore direction. One group of 25 tadpoles was trained under long-wavelength lighting conditions ( $\lambda \geq 500$  nm) and a second group of 25 tadpoles was trained under natural skylight conditions. The two groups were subsequently tested under full-spectrum and long-wavelength lighting conditions, respectively (see below for details).

## Procedure

### *Training protocols*

Testing protocols, as well as training and testing apparatus, have been described elsewhere (Diego-Rasilla and Phillips 2007; Diego-Rasilla et al. 2010). 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). Tadpoles were trained under natural light–dark cycle in water-filled outdoor tanks and exposed to natural variation in light intensity. Tadpoles used 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 ( $\sim 25^\circ$  slope) providing a gradual gradient from the deep end to the shallow end. Water depth at the shore end was 1 cm. In each aquarium sides were covered by means of 1.5 cm thick wooden boards to prevent light penetration from the sides of the tanks, whereas the top was uncovered. The tops of the aquaria were covered with transparent window glass (transmission  $> \sim 370$  nm). Training tanks were filled with tap water treated with aquarium water conditioner (TetraAcqua AquaSafe<sup>®</sup>, 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 24 °C. Tadpoles were fed boiled lettuce, which was placed in the shallow end of the tank daily.

Two different training configurations, with perpendicular shore directions, were used in these experiments. One training tank was aligned along the magnetic north–south axis, with shore facing South in 2009 and facing North in 2010, whereas the other training tank was aligned along the east–west axis, with shore to the East in 2009 and to the West in 2010.

In 2009, the two groups of 32 and 30 tadpoles were introduced into the shallow end of each training tank (N–S and E–W training tanks, respectively) on 28 July, and

allowed 3 days (i.e., from 28 to 31 July) to learn the y-axis direction before testing.

In 2010 tadpoles were introduced into the tanks on 13 July. This time, the top of the N–S training tank was covered with a  $\geq 500$  nm long pass filter, consisted of one layer of a spectral filter (Lee no. 101; Lee Filters<sup>®</sup>, Andover, Hants, UK) placed between two transparent methacrylate sheets (82 cm  $\times$  42 cm, 0.4 cm thick), whereas the E–W aquarium was covered with glass only. Two groups of 25 animals were introduced into the shallow end of each training tank and allowed 4 days (i.e., from 13 to 17 July) to learn the y-axis direction. After training, the tadpoles were tested individually in a water-filled outdoor arena for magnetic orientation. All tests were conducted on a single day during daylight hours.

### *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 1986) produced by means of a double-wrapped cube-surface coil (Rubens 1945) surrounding the outside of the arena. 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 a double-wrapped cube-surface 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, allowing only diffuse light to reach the tadpoles 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 temperature reached 21 °C.

Tadpoles were removed from the training tank one at a time with a small dip net and placed in the arena center beneath an opaque, cylindrical plastic container (9 cm diameter, 14.5 cm high) that served as a release device. Each tadpole remained in the cylindrical container for 1 min to overcome the effects of handling before the release device was lifted, and it was allowed to move freely within the testing arena. Tadpoles' movements were observed through tiny holes in the black curtain that covered the test apparatus. A tadpole'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 tadpoles that contacted arena wall in less than 10 s were not recorded. These individuals, which moved immediately as soon as the release device was lifted, were considered to exhibit a randomly oriented escape response and were excluded from the analyses. In addition, trials were abandoned if the tadpole failed to score within 10 min (in those cases, the next tadpole was tested under the same magnetic and lighting conditions) (Diego-Rasilla and Phillips 2007; Diego-Rasilla et al. 2010).

In the 2009 experiment, European common frog tadpoles trained under full-spectrum natural skylight were tested for their directional preferences under full-spectrum natural skylight, whereas in the experiment carried out in 2010 one group of tadpoles was trained under long-wavelength ( $\geq 500$  nm) and tested under full-spectrum, and another group was trained under full-spectrum natural skylight and tested under long-wavelength ( $\geq 500$  nm).

Long-wavelength ( $\geq 500$  nm) testing conditions were produced by covering the top of the testing apparatus with filters consisting of a layer of Lee no. 101 spectral filter (Lee Filters<sup>®</sup>, Andover, Hants, UK) between two transparent methacrylate sheets (105 cm  $\times$  105 cm side). Since the sides of the training and the testing apparatus were opaque, only light passing through the spectral filters reached the animal in the testing arena. For full-spectrum tests, the top of the apparatus was uncovered exposing the animals to full-spectrum natural lighting. Light intensity in both lighting conditions varied over the course of the day due to normal changes in sun position and cloud cover, although sun position was not directly visible to tadpoles in the testing arena.

Each tadpole was tested only once in one of the four magnetic field alignments, and under only one of the lighting conditions. In both years, the order of the four magnetic field alignments (magnetic N = N, S, E or W) was determined using a random number sequence. In 2009 the first individual was tested in magN = S, the next individual in magN = W, followed by one in magN = E and one in magN = N, whereas in 2010 the first individual was tested in magN = E, the next individual in magN = W, 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 each tadpole 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 tadpoles 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.

Also, in the 2009 experiment, the first four tadpoles were taken individually from N–S 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 E–W training tank, and tested following the same sequence of magnetic field alignments. In 2010, the first four tadpoles were taken individually from E–W training tank and tested under long-wavelength light; the next four tadpoles were taken from the N–S training tank and tested under full-spectrum light. Then, this sequence was repeated.

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 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 the bearings from an approximately equal number of tadpoles 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 tadpoles’ orientation relative to the magnetic field (Phillips 1986; Diego-Rasilla and Phillips 2007; Diego-Rasilla et al. 2010).

#### Statistical analyses

The distributions of bearings were analysed using standard circular statistics (Batschelet 1981). 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 data value and reducing any greater than 360 using modulo arithmetic. 95 % 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 two different training directions and training/testing lighting conditions were analysed separately and the Watson  $U^2$  test was used to test the difference between the two distributions of magnetic bearings (Batschelet 1981; Mardia and Jupp 2000).

In the 2009 experiment training and testing lighting conditions were the same in both groups of tadpoles; all magnetic bearings were pooled with respect to the magnetic direction of the shore in training (combining the magnetic distributions from the two training tanks) prior to analysis.

In 2010, tadpoles experienced different wavelength conditions in training and testing (i.e., full-spectrum light in training and long-wavelength light in testing, or long-wavelength light in training and full-spectrum light in

testing). Again, magnetic bearings were pooled with respect to the magnetic direction of the shore in training (combining the magnetic distributions from the two training tanks) prior to analysis.

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 non-magnetic directional cues on the tadpoles’ orientation using the Rayleigh test.

## Results

### Same spectral conditions in training and testing

After training under natural (i.e., full spectrum) skylight, tadpoles were tested under full-spectrum light. The distribution of magnetic bearings from tadpoles held in the training tank aligned north–south with the shore toward the south exhibited a significant bimodal orientation that coincided with the magnetic direction of the trained y-axis (Table 1; Fig. 1a). Also, the 95 % confidence intervals for the mean vector bearing included the trained y-axis.

Tadpoles trained in the tank that was aligned along the east–west axis, with the shore toward the east, also exhibited a significant bimodal orientation that coincided with the magnetic direction of the trained y-axis (Table 1; Fig. 1b), and the 95 % confidence intervals for the mean vector bearing included the trained y-axis.

The two distributions of magnetic bearings (north–south and east–west training configuration) were significantly different

( $U^2 = 0.365, P < 0.002$ ; Watson  $U^2$  test; Fig. 1a, b) and their mean axes of orientation differed by approximately 90°.

The topographic bearings (i.e., absolute bearings, ignoring the alignment of the magnetic field in testing; see “Methods”) from the north–south alignment tank were non-randomly distributed (Table 1), but the 95 % confidence interval did not include the tank y-axis direction. The topographic bearings from the east–west alignment tank were randomly distributed (Table 1).

### Different wavelength conditions in training and testing

After training under  $\geq 500$  nm light, European common frog tadpoles tested under full-spectrum light exhibited bimodal orientation perpendicular to the magnetic direction of the trained y-axis (Table 1; Fig. 1c). Similarly, tadpoles trained under full-spectrum and tested under long-wavelength ( $\geq 500$  nm) light showed bimodal orientation perpendicular to the magnetic direction of the trained y-axis (Table 1; Fig. 1d). The two distributions of magnetic bearings were significantly different ( $U^2 = 0.453, P < 0.001$ ; Watson  $U^2$  test; Fig. 1c, d) and their mean axes of orientation differed by approximately 90°.

The topographic bearings from both training/testing configurations were randomly distributed (Table 1).

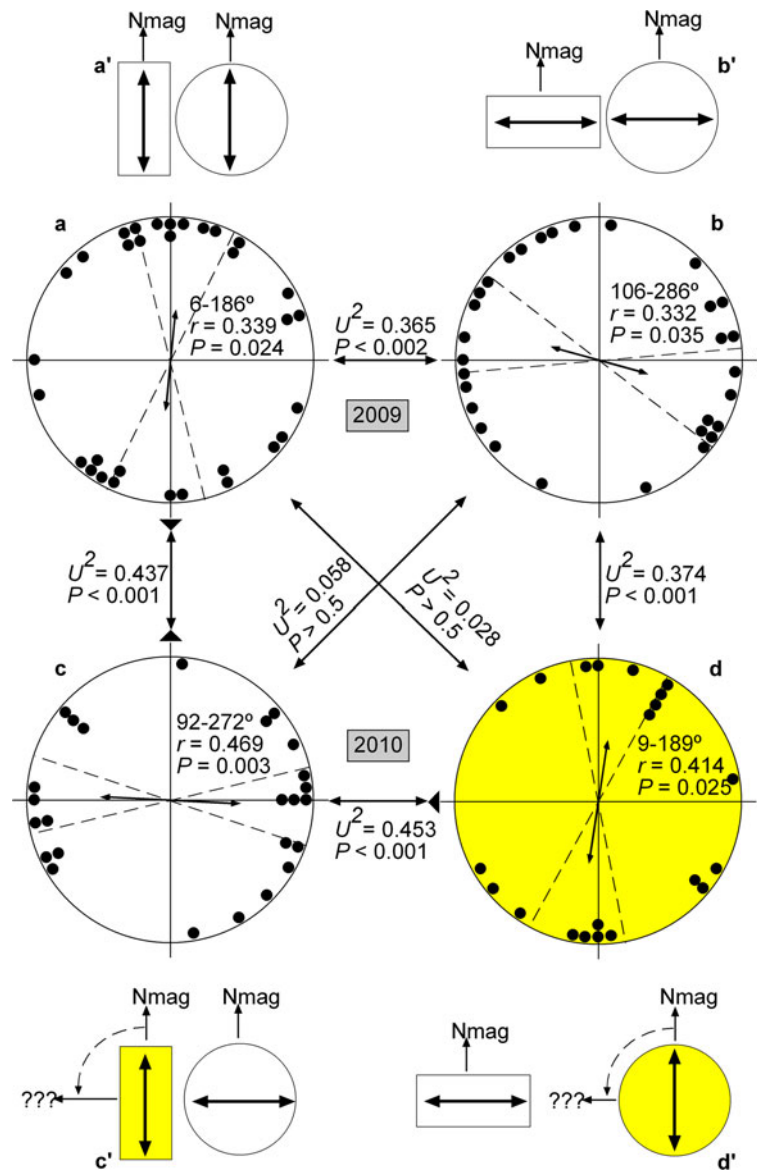
### Same vs. different spectral activation in training and testing

When the two distributions of magnetic bearings from tadpoles trained and tested under full-spectrum light were normalized with respect to the magnetic direction of shore

**Table 1** Statistical analysis of data

Training light conditions	Bearings	Tank alignment	Testing light conditions			
			$\alpha \pm \text{IC } 95\%$	$r$	$P$	$N$
<i>2009 experiment</i>						
Full-spectrum	TB	0–180°	84 ± 34°	0.395	0.006	32
		90–270°	83 ± 55°	0.258	0.136	30
	MB	0–180°	6–186 ± 20°	0.339	0.024	32
		90–270°	106–286 ± 21°	0.332	0.035	30
	Bearings pooled with respect to the magnetic direction of shore (0°)			11–191 ± 15°	0.33	0.001
<i>2010 experiment</i>						
Long wavelength ( $\geq 500$ nm)	TB	0–180°	234 ± 50°	0.336	0.058	25
	MB	0–180°	92–272 ± 16°	0.469	0.003	25
Full-spectrum			Long-wavelength ( $\geq 500$ nm)			
	TB	90–270°	90 ± 99°	0.174	0.533	21
	MB	90–270°	9–189 ± 20°	0.414	0.025	21
Bearings pooled with respect to the magnetic direction of shore (0°)			95–275 ± 13°	0.441	0.0000874	46

$\alpha$  mean direction (degrees),  $r$  mean vector length,  $P$  probability,  $N$  sample size, *TB* absolute (“topographic”) bearings; *MB* magnetic bearings



**Fig. 1** **a–d'** Predicted orientation of tadpoles (*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 ( $\geq 500$  nm) light (*yellow*); circular test arenas show the predicted response of the tadpoles under either full-spectrum (*white*) or long-wavelength (*yellow*) light. **a–b'** full-spectrum training and testing: Tadpoles should show bimodal magnetic orientation along the shore-deep water magnetic axis. **c'** long-wavelength training, full-spectrum testing: Tadpoles' perception of the magnetic north should be rotated  $90^\circ$  relative to the actual field during training. As a consequence, the direction of shore will appear to the tadpoles to be toward magnetic east. When subsequently tested under full-spectrum light, this will cause the tadpole's orientation to be rotated  $90^\circ$  relative to the true

shore direction. **d'** full-spectrum training, long-wavelength testing: tadpoles' perception of magnetic north in testing, and their orientation in the test arena, should be rotated  $90^\circ$  relative to the actual field during training. **a–d** Results. Magnetic compass orientation of tadpoles trained and tested under natural skylight (**a–b**), trained under  $\geq 500$  nm long-wavelength light and tested under natural skylight (**c**), or trained under natural skylight and tested under  $\geq 500$  nm long-wavelength light (**d**). Each dot represents the bearing of one tadpole, tested only once. *Double-headed arrows* at the center 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

(i.e., pooling all bearings so that shore direction was rotated to  $0^\circ$ ), we did not find a significant difference ( $U^2 = 0.025$ ,  $P > 0.5$ ; Watson  $U^2$  test). Therefore, the distribution of bearings relative to the trained  $y$ -axis was

not different for tadpoles trained along the north–south and east–west axes.

Similarly, the pooled distributions of magnetic bearings from the two shore directions (i.e., pooling all bearings so

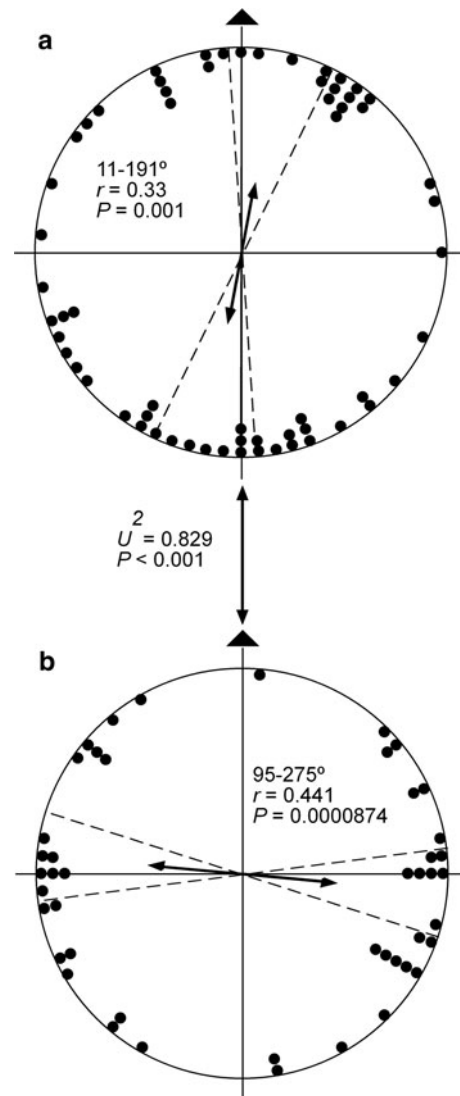
that shore direction is rotated to  $0^\circ$ ) were not significantly different between tadpoles trained under full-spectrum and tested under long-wavelength light, and those trained under long-wavelength and tested under full-spectrum ( $U^2 = 0.075$ ,  $0.5 > P > 0.2$ ; Watson  $U^2$  test), i.e., tadpoles experiencing activation of different spectral inputs in training and testing.

When data were pooled from tadpoles experiencing the same wavelength activation in training and testing (full-spectrum light in training and testing, i.e., Fig. 1a, b combined), the tadpoles showed bimodal magnetic orientation along the shore-deep water magnetic axis (Fig. 2a). In contrast, when data were pooled from tadpoles experiencing different wavelengths of light in training and testing (full-spectrum light in training and long-wavelength light in testing, or long-wavelength light in training and full-spectrum light in testing, i.e., Fig. 1c and d combined), the tadpoles showed bimodal magnetic orientation perpendicular to the shore-deep water magnetic axis (Fig. 2b). The distribution of magnetic bearings for tadpoles trained and tested under full-spectrum light (Fig. 2a) was significantly different ( $U^2 = 0.829$ ,  $P < 0.001$ ; Watson  $U^2$  test; Fig. 2) from that of bearings for tadpoles trained and tested under different wavelengths of light (Fig. 2b) and their mean axes of orientation differed by  $90^\circ$ .

## Discussion

The present study demonstrates the use of a light-dependent magnetic compass for y-axis orientation. Although anurans can use celestial cues (i.e., sun compass, polarized light patterns) for y-axis orientation (Ferguson and Landreth 1966; Ferguson et al. 1967, 1968; Adler 1970, 1976; Justis and Taylor 1976), we can dismiss the use of these cues by European common frog tadpoles in our experiments because any consistent nonmagnetic bias, including any response to celestial compass cues, was factored out by pooling the magnetic bearings from an approximately equal number of tadpoles tested in each of the four magnetic field alignments. The resulting distributions retained only the component of the tadpoles' orientation that was a consistent response to the magnetic field.

Previous studies indicate that the wavelength-dependent  $90^\circ$  shift in the direction of magnetic compass orientation results from a direct effect of light on the underlying magnetoreception mechanism both in newts (Phillips and Borland 1992a) and frogs (Diego-Rasilla et al. 2010), and our study confirms this conclusion. Furthermore, the ability of *R. temporaria* tadpoles to learn a consistent magnetic direction relative to the magnetic field, despite all the natural variation in spectral composition and intensity of light over the 24 h day, indicates that the light-dependent



**Fig. 2** Same **a** (i.e., tadpoles trained and tested under full-spectrum light) vs. different **b** (i.e., tadpoles trained under full-spectrum and tested under long-wavelength light, and those trained under long-wavelength and tested under full-spectrum) spectral activation in training and testing. Each data point represents the magnetic bearing of an individual tadpole tested only once in one of the four magnetic field alignments. Double-headed arrows at the center 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 shore end of the y-axis

magnetic compass is extremely robust under a wide range of spectral lighting conditions. The findings of these studies show that light-dependent magnetic compass receives two antagonistic spectral inputs, the short-wavelength input with peak sensitivity  $<450$  nm and the long-wavelength input with peak sensitivity  $>500$  nm (Phillips and Borland 1992a; Diego-Rasilla et al. 2010).

Given the evidence for antagonistic short- and long-wavelengths to the light-dependent magnetic compass, our results suggest that these two inputs are not equally excited under natural conditions, which would prevent the magnetic compass from operating as occurs in the laboratory when newts are exposed to 475 nm light (see Phillips and Borland 1992a). This is consistent with earlier evidence from newts that the short-wavelength input to the light-dependent magnetic compass is substantially more sensitive than the long-wavelength input, and is preferentially activated by full-spectrum light (Deutschlander et al. 1999a, b; Phillips et al. 2001).

As predicted, our experiments show that *R. temporaria* tadpoles experiencing the same spectral conditions in training and testing (i.e., trained and then tested under full-spectrum light) displayed magnetic compass orientation that coincided with the trained shore-deep water axes of their training tanks. These results show that *R. temporaria* tadpoles use a magnetic compass for y-axis orientation. Tadpoles experiencing different wavelength conditions in training and testing (i.e., trained under  $\geq 500$  nm light and tested under full-spectrum light and vice versa) showed bimodal orientation perpendicular to the magnetic direction of the trained y-axis, indicating that the magnetic compass is light-dependent.

Earlier studies have shown that short-wavelength and long-wavelength inputs to the magnetic compass of amphibians are mediated by extraocular photoreceptors located in the pineal complex (Deutschlander et al. 1999a, b; Phillips et al. 2001). The pineal complex is directly sensitive to light and consists of the extracranial frontal organ (stirnorgan) present in anuran tadpoles and adults, and the intracranial pineal organ or pineal body (epiphysis) present both in anuran and urodele amphibians (Eakin 1961; Eakin et al. 1963; Adler 1970; Korf et al. 1981; Lannoo, 1999; Vígth et al. 2002; Bertolucci and Foà 2004).

The frontal organ of frogs is located on top of the head between the eyes (Adler 1976; Vígth et al. 2002). Similar to the pineal organ, the stirnorgan of tadpoles and adult frogs contains photoreceptor cells that exhibit many cytological features similar to those of retinal rods and cones (Eakin 1961; Kelly 1962; Kelly and Smith 1964). Projection neurons from the frontal organ exhibit chromatic antagonism, i.e., neural activity is inhibited by ultra-violet ( $\lambda_{\max} = 370$  nm) and excited by green ( $\lambda_{\max} = 515$  nm) light (Dodt and Heerd 1962; Eldred and Nolte 1978; Korf et al. 1981; Koyanagi et al. 2004). The peak sensitivity of the UV input is  $\sim 2$  log units more sensitive than that of the long-wavelength input, and the two inputs are equally sensitive at  $\sim 475$  nm (Dodt and Heerd 1962), consistent with the properties of the light-dependent magnetic compass found in newts (Phillips et al. 2010; Diego-Rasilla et al. 2010). Recent multiunit recordings from the frontal

organ nerve of bullfrog *L. catesbeianus* have provided evidence for an effect of magnetic field alignment on the response to light with properties consistent with the behavioral findings (Phillips & Borland in prep.).

The spectral sensitivity of the light-dependent magnetic compass in amphibians (Phillips and Borland 1992a; Deutschlander et al. 1999a, b; Freake and Phillips 2005; Diego-Rasilla et al. 2010; and present study) is consistent with the involvement of the photo-interconvertible fully oxidized and partially reduced (semiquinone) forms of cryptochrome photopigment (Biskup et al. 2009; Phillips et al. 2010). However, parapinopsin has been recently identified as a UV-sensitive photopigment in the pineal complex of river lamprey (*Lethenteron japonica*), one of earliest vertebrate lineages (Koyanagi et al. 2004), as well as of clawed frog (*Xenopus tropicalis*) (Koyanagi et al. 2004), rainbow trout (*Oncorhynchus mykiss*) (Koyanagi et al. 2004), and in the parietal eye of green iguanas (*Iguana iguana*) (Wada et al. 2012). Parapinopsin in lampreys occurs in two photo-interconvertible forms with peak sensitivities (370 and 515 nm; Koyanagi et al. 2004), matching those of the chromatic mechanism in the frog frontal organ (Dodt and Heerd 1962; and see earlier “Discussion”). However, although the peak absorption of the short-wavelength form of parapinopsin matches the peak of UV-sensitivity in neurophysiological recordings, there is no evidence for an antagonistic response mediated by the long-wavelength form of this photopigment (Koyanagi et al. 2004). Further work needs to be done to establish whether parapinopsin or cryptochrome, or an interaction of the two photopigments, is involved in magnetoreception (Phillips et al. 2010).

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