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Celestial orientation in the marbled newt (*Triturus marmoratus*)

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Abstract Orientation toward breeding ponds plays an important role in the seasonal movements of amphibians. In this study, adult marbled newts were tested in a circular arena to determine sensory cues used to locate breeding ponds. Animals were collected from a temporary pond situated in northern Spain, taken to the experimental site 340 m distant, and tested for orientation under a variety of conditions (i.e., orientation under a clear night sky, orientation under an overcast night sky, and orientation under a clear night sky in the presence of an altered geomagnetic field). These investigations have demonstrated that the marbled newt is able to orient using celestial cues. Animals chose a compass course in the direction of their breeding pond only when celestial cues were available. Conversely, the ambient geomagnetic field does not seem to be relevant to orientation of marbled newts since they were unable to orient themselves using the ambient geomagnetic field in the absence of celestial cues.

Key words Homing behaviour · Newts · Orientation · Orientation cues · *Triturus marmoratus*

Introduction

Amphibians use a variety of orientation cues to find their way around their home ranges or to locate breeding ponds (Sinsch 1991, 1992). Orientation toward aquatic breeding sites probably involves several sensory cues, including responses to celestial cues (Landreth and Ferguson 1967; Tracy and Dole 1969; Adler 1976; Taylor and Auburn 1978), geomagnetic field (Phillips et al. 1995), visual recognition of local landmarks (Dole 1965; Plasa 1979; Adler 1980), identification of odours from ponds (Grubb 1973a, b, 1975), and responses to auditory cues (Ferguson and Landreth 1966). Experimental studies in which these cues were manipulated suggest that the absence of one type of cue can reduce orientation ability, but that no single cue is absolutely essential for the animals to locate breeding sites (Sinsch 1990).

Some studies have demonstrated that amphibians can orient using celestial cues. Most of these works tested amphibians for *y*-axis orientation (Landreth and Ferguson 1967, 1968; Alder 1976). The *y*-axis is the axis perpendicular to the shoreline (Ferguson and Landreth 1966). Animals were trained to a particular shoreline direction and then tested for *y*-axis orientation in a circular arena. Species tested under a clear sky usually showed strong orientation to the trained direction but exhibited random orientation when tested under an overcast sky (Ferguson and Landreth 1966; Adler 1970; Taylor and Auburn 1978). Similar results have been obtained with migrating animals placed in arenas; thus, most species choose a compass course in the direction of migration under a clear sky, but not always under an overcast sky (Pough et al. 1998). In addition, it has been demonstrated that amphibians are capable of detecting polarized light through extraoptic photoreceptors, using it as a directional cue (Adler 1970; Taylor and Ferguson 1970; Taylor 1972; Adler and Taylor 1973; Taylor and Adler 1973, 1978; Justis and Taylor 1976; Taylor and Auburn 1978). Polarization is concealed by complete overcast, which explains why many amphibians cannot orient under a completely cloudy day or night sky (Adler 1976). Also, some amphibians appear to be capable of true navigation based on Earth's magnetic field, and the ability to detect changes in the magnetic field has been demonstrated in *Notophthalmus viridescens* (Phillips 1986a, b; Phillips and Borland 1994; Phillips et al. 1995; Deutschlander et al. 2000).

Although the orientation ability of *Triturus marmoratus* has been demonstrated (Fontanet 1990, 1991a, b), little is known about the orientation cues that they use. The pur-

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pose of this study was to determine whether *T. marmoratus* could use several sensory cues in orientation toward breeding ponds. In orientation tests, we checked the role of celestial cues and geomagnetic field on orientation of the marbled newt.

Materials and methods

Thirty-three marbled newts (*T. marmoratus*), 14 adult females and 19 adult males, were collected from a temporary pond of 270 m^2, situated in Valdeajos (Burgos Province, northern Spain; 42°44′ 32.1″N, 3°54′41.4″W), at an elevation of 1,040 m. Newts shared the pond with a population of *Bufo calamita*, and only one specimen of *Rana perezi* and one specimen of *Hyla arborea* were found. Our study was conducted during the aquatic period, when *T. mamoratus* exhibits nocturnal habits (Barbadillo et al. 1999). If compared with southerly populations of its closest relative, *T. pygmaeus* (García-París et al. 2001), the start of breeding is delayed in this northern area due to the extreme climatic conditions (Caetano et al. 1985; Díaz-Paniagua 1986; Díaz-Paniagua et al. 1996). Large numbers of newts were found in the pond in mid-March whereas they appeared to be absent earlier in the season. Test specimens were collected during spring in 2000 (13–14 and 19–20 May) between 2100 and 2230 hours, placed in rectangular plastic containers (31 × 21 × 17 cm) in which the water depth was 1 cm, taken to the terrestrial test arena by car, and tested. Animals were tested 2–4 h after being captured. The belly and dorsal patterns of each newt were photographed for individual identification, and animals were returned to their pond just after testing.

Each test animal was used in three consecutive experiments in an arena that afforded a view of the sky but not the horizon. The experimental site was an open area 340 m from the pond. The compass course toward the pond, measured with a 12-channel GPS receiver, was 288°. Tests were conducted under a starry sky, on a moonlit night with a slight southern breeze, between 2307 and 0206 hours. Air temperature during the tests was 8.91 ± 0.25°C, and relative humidity was 93.14 ± 1.28%. The test arena consisted of a circular plastic container (57 cm diameter, 28 cm high). Movements and behaviour of tested specimens were observed through holes in the plastic wall of the container. The floor of the arena was covered with brown wrapping paper that was changed after each newt's run; together with wiping of the floor and arena walls, this procedure presumably eliminated the use of odour trails for orientation (Adler 1980).

Marbled newts were taken separately from their rectangular plastic holding containers just prior to their individual testing session and put in the arena centre beneath an opaque, cylindrical plastic container (12.9 cm diameter, 12.7 cm high). Newts could not be seen through the container wall and they were kept in the container for 3 min to overcome effects of handling before the cover was lifted. Once the cylindrical plastic container was lifted, each newt was permitted to move about the arena, but animals remaining motionless in the arena centre for 1 min or more were excluded from the analyses. Criterion for direction of movement was recorded as the vector of the first point where an animal tapped against the wall. During testing, after an animal reached criterion for direction of movement it was re-run immediately in a new test condition, after replacing the paper floor and cleaning the arena. In the meantime animals were kept separately in their rectangular plastic containers in which the water depth was 1 cm. Three consecutive experiments were conducted according to the explained protocol and in the following sequence:

- Experiment 1. Orientation under a clear night sky: newts were placed in the arena and tested under a clear night sky. The ambient geomagnetic field was not altered.
- Experiment 2. Orientation under an overcast night sky or on very dark nights: we simulated heavily overcast conditions by covering the arena with a thick cardboard. Hence, we kept the sky out of the newts' sight. The ambient geomagnetic field was not altered.
- Experiment 3. Orientation under a clear night sky in the presence of an altered geomagnetic field: newts were placed in the arena under a clear sky. In this test we put two magnetic-base pots in the arena centre, altering the geomagnetic field. The magnetic base pots were made of neodymium and protected by epoxy resins (28.5 mm diameter, 10 mm high; residual induction = 11,700/12,100 gauss; coercive field = 10,800/11,200 oersted). The horizontal direction of the ambient geomagnetic field was altered by 200° using the neodymium magnets, which we could check with a compass.

We used a GARMIN GPS 12 Personal Navigator and a compass to estimate the compass course toward the pond, the distance from the pond to the experimental site, and the bearings exhibited by newts. Data were analysed using standard circular statistics (Batschelet 1981; Fisher 1995). Mean vectors were estimated and tested for significance using the Rayleigh test for a non-random distribution. Ninety-five percent confidence intervals were used to determine whether the mean for the distribution included the homeward direction (Batschelet 1981; Fisher 1995). We used method M (test for a common mean direction of two or more distributions; test statistic Y_r) for testing differences between the mean orientation of bearings obtained from newts in experiments 1 and 3 (Fisher 1995).

Results

Experiment 1. Orientation under a clear night sky

We tested the null hypothesis of uniformity against the alternative of a single preferred direction. Data were not distributed uniformly and they showed evidence of a preferred direction ($\mu = 319.14°$, $r = 0.35$, $n = 25$, $P = 0.04$). The 95% confidence interval (275.49°, 2.79°) for the mean vector (μ) includes the direction of the pond ($\mu_0 = 288°$), indicating that the newts can orient themselves toward the pond (Fig. 1A, Table 1).

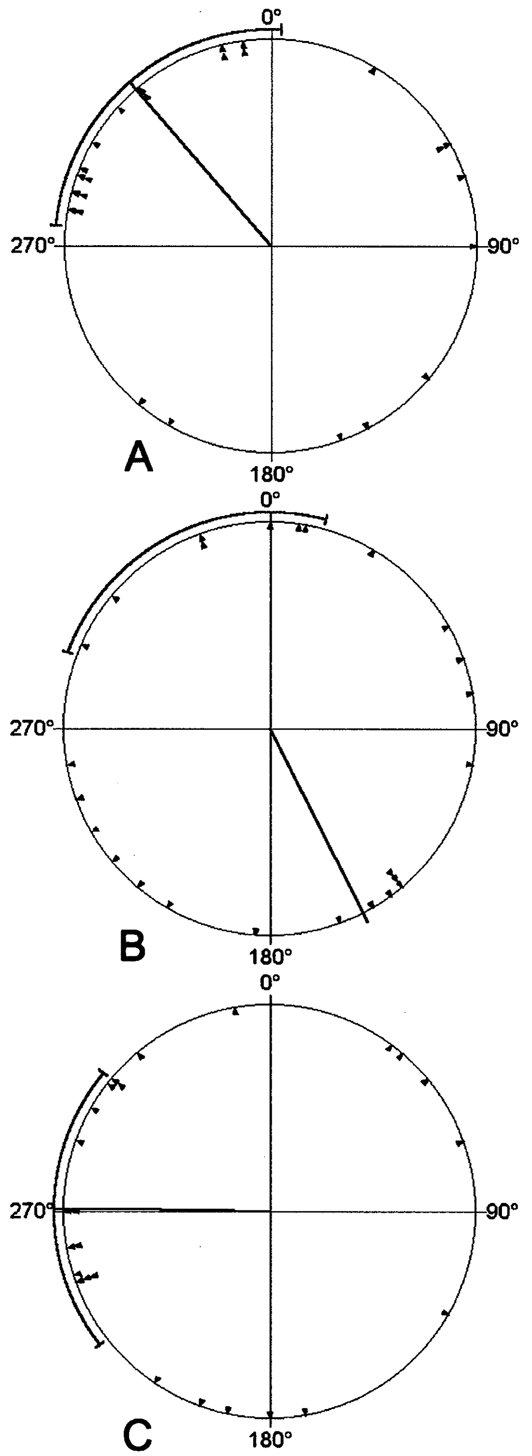

Fig. 1A–C. Orientation responses of marbled newts during the experiments. **A** Experiment 1. Orientation under a clear night sky. **B** Experiment 2. Orientation under an overcast night sky or on very dark nights. **C** Experiment 3. Orientation under a clear night sky in the presence of an altered geomagnetic field. These *circular diagrams* plot individual symbols for each datum in the sample to indicate the directionality of the data. *Mean vector* and the *95% confidence interval* for the mean vector are shown as well. Homeward orientation is 288°

Experiment 2. Orientation under an overcast night sky or on very dark nights

Although the ambient geomagnetic field was an available cue for orientation, newts were unable to orient themselves toward the pond during this test (Fig. 1B). Moreover, animals oriented randomly and we could not reject the null hypothesis of uniformity. So, there is no evidence that the newts can orient themselves on heavily overcast nights (μ = 152.89°, r = 0.07, n = 25, P = 0.88) (Table 1).

Experiment 3. Orientation under a clear night sky in the presence of an altered geomagnetic field

When geomagnetic field was altered, testing the animals under a clear night sky, they showed a preferred direction (μ = 270.82°, r = 0.39, n = 25, P = 0.02). Once again, the 95% confidence interval (232.01°, 309.62°) for the mean vector (μ) includes the direction of the pond (μ_0 = 288°), indicating that newts can orient themselves toward the pond in spite of the fact that the geomagnetic field was altered (Fig. 1C, Table 1). Animals were not affected by the magnets in any way.

Mean bearings exhibited by the newts in experiments 1 and 3 were similar (see Fig. 1A, C) and we can state that there is no evidence for differences in orientation of the two data sets (method M for testing a common mean direction of two or more distributions; Y_2 = 0.0092, P > 0.05).

Discussion

Diverse studies have shown that some amphibians return to the same breeding pond in successive years (Heuser 1960; Wisniewski et al. 1980; Barbadillo et al. 1999; Matthews and Pope 1999; Marvin 2001). It may be reproductively advantageous for a marbled newt to return to a tested breeding place. When migrating to breeding ponds it is likely that several directional cues are simultaneously used (Sinsch 1990). The use of celestial cues as an orientation mechanism has been largely demonstrated in several species of anurans (Ferguson and Landreth 1966; Landreth and Ferguson 1966, 1968; Ferguson et al. 1967, 1968; Justis and Taylor 1976; Sinsch 1990) and urodeles (Landreth and Ferguson 1967; Taylor 1972; Adler and Taylor 1973; Taylor and Adler 1973, 1978). Its significance is probably important since celestial orientation is based on stable orientation cues that have gone unchanged for a longer time than other possible cues (Ferguson and Landreth 1966). Our results suggest that celestial orientation is the main orientation mechanism in this species, at least under the experimental conditions presented. Identification of odours from the pond could also be a sensory cue involved in orientation, and the same could happen with the visual recognition of local landmarks. In our experiments, these two types of cues were not tested, so we cannot confirm their possible role in orientation. However, it is hardly probable that olfactory orientation works

Table 1. Statistical analyses for orientation tests

	Experiment		
	1	2	3
Sample size	25	25	25
Mean vector (μ)	319.14°	152.89°	270.82°
Length of mean vector (r)	0.35	0.07	0.39
Concentration	0.75	0.14	0.85
Circular variance	0.65	0.93	0.61
Circular standard deviation	82.79°	131.64°	78.34°
Standard error of mean	22.27°	113.33°	19.79°
95% confidence interval for μ	275.49°, 2.79°	290.71°, 15.07°	232.01°, 309.62°
Rayleigh test of uniformity (P)	0.04	0.88	0.02

efficiently in terrestrial environments where presumably odours reach only those animals located downwind from the source (Ferguson and Landreth 1966). In any case, the reversal of wind direction within our experimental site excluded possible olfactory orientation.

When ambient geomagnetic cues were the only information available for orientation, animals failed to orient. Conversely, when the geomagnetic field was modified, but celestial cues were available, newts oriented successfully. These results suggest that the geomagnetic field is not relevant to orientation of *T. marmoratus*. Conversely, Fontanet (1991b) suggested that this species could use the ambient geomagnetic field as an orientation cue, since animals lacked auditory or visual cues during the tests conducted by him. Our results are contrary to this suggestion. Even so, orientation ability based on geomagnetic field has been demonstrated in the eastern red-spotted newt, *N. viridescens* (Phillips 1986a; Phillips and Borland 1994; Phillips et al. 1995; Deutschlander et al. 2000). Deutschlander et al. (2000) have found that *N. viridescens* can learn the direction of the *y*-axis with respect to the geomagnetic field within 12–16 h. However, it seems that geomagnetic field orientation is not widespread among amphibians (Landreth and Ferguson 1967; Sinsch 1990, 1991). Moreover, the use of this kind of cue has been criticized due to the small magnitude of the effects reported (Griffin 1982).

We can conclude that the present study has confirmed the role of celestial cues in orientation toward aquatic breeding sites. However, the marbled newt does not use directional information from the geomagnetic field.

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