

Homing ability and sensitivity to the geomagnetic field in the alpine newt, *Triturus alpestris*

FRANCISCO JAVIER 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)

Received 25 June 2002, accepted 18 June 2003

Triturus alpestris has been found to be adept at homing, and previous studies have found that its orientation behaviour during breeding migration can be explained, at least in part, by the use of olfactory cues from the breeding pond. Nonetheless, the use of chemical cues from the pond probably does not support a guidance strategy when the newts are far from the pond. Hence, *T. alpestris* may rely on other orientation cues. In this study, alpine newts were tested in a circular arena to determine the sensory cues used to locate breeding ponds. Animals were collected from a permanent pond situated in northern Spain, taken to the experimental site 9020 m distant, and tested for orientation under a variety of conditions (i.e., orientation under a clear night sky, orientation under dark conditions, and orientation under a clear night sky in the presence of an altered geomagnetic field). The possibility of a non-homing directional bias was also tested. Newts chose a compass course in the direction of their breeding pond only when the ambient geomagnetic field and the celestial cues were simultaneously available. Conversely, animals failed to orient only when celestial cues or the geomagnetic field were the sole orientation cues available.

KEY WORDS: homing, magnetoreception, newt, orientation cues, *Triturus alpestris*.

Introduction	252
Methods	252
Results	254
First experiment: Orientation under a clear night sky	254
Second experiment: Orientation under dark conditions	254
Third experiment: Orientation under a clear night sky in the presence of an altered geomagnetic field	255
Fourth experiment: Testing the possibility of a non-homing directional bias	256
Discussion	257
Acknowledgements	258
References	258

INTRODUCTION

Homing ability seems to be well developed in newts (GILL 1979, JOLY & MIAUD 1989). Thus, the ability to return after displacement has been well established for

some species of newts (TWITTY 1959, TWITTY et al. 1966, GILL 1979). Many amphibians can orient in a homeward direction even when placed in situations where local cues are not available (SINSCH 1992) and they use a variety of senses for orientation (SINSCH 1991, 1992). The ability of homing requires, at least, a well-developed compass sense, and many amphibians can determine direction from celestial cues (LANDRETH & FERGUSON 1967, TAYLOR & AUBURN 1978, DIEGO-RASILLA & LUENGO 2002). Some amphibians seem to be capable of true navigation, which enables them to find their way home from an unfamiliar location without reference to familiar landmarks, to cues that emanate from the origin of displacement, or to directional information obtained during displacement (PHILLIPS et al. 1995). Navigational ability requires both a compass sense and a geographic-position sense derived from spatial information available at the release site (PHILLIPS 1996). The earth's magnetic field could be used for such navigation and it has been demonstrated that the earth's magnetic field provides a valuable source of directional information for the eastern red-spotted newt, *Notophthalmus viridescens* (PHILLIPS 1986, 1987; PHILLIPS & BORLAND 1994). The shoreward magnetic compass orientation of eastern red-spotted newts exhibits distinct properties from those exhibited when they use the magnetic field for homing (PHILLIPS & BORLAND 1994). Thus, displaced newts may be capable of homing using information about geographic position ("map" information) derived from subtle geographic gradients in the geomagnetic field (PHILLIPS 1996, FISCHER et al. 2001), as well as a compass to orient in the homeward direction (PHILLIPS 1986). Moreover, magnetic orientation has been shown to be light-dependent in the eastern red-spotted newt (PHILLIPS & BORLAND 1992a, 1992b, 1992c).

Magnetic compass orientation might be extended among urodeles. However, the use of the geomagnetic field by orienting urodeles has been only demonstrated in the eastern red-spotted newt (PHILLIPS 1986, 1987), and the cave salamander (*Eurycea lucifuga*) (PHILLIPS 1977).

Fidelity to a breeding site has been confirmed in the alpine newt, *Triturus alpestris* (Laurenti 1768) (JOLY & MIAUD 1989). Furthermore, its orientation ability has been confirmed (FONTANET 1991), and it has been demonstrated that olfactory cues play an important role in homeward orientation (JOLY & MIAUD 1993). FONTANET (1991) suggested that the alpine newt could use the ambient geomagnetic field as an orientation cue, since animals lacked auditory or visual cues during the tests conducted by him. Also, JOLY & MIAUD (1993) suggested an orientation mechanism based on perception of the earth's magnetic field when the alpine newt is far from the home pond. Nevertheless, this hypothesis has not been verified. Hence, the present experiments were designed to determine whether the alpine newt is capable of using celestial cues and the earth's magnetic field to orient homewards.

METHODS

Species and study site

Fifty-three alpine newts, *T. alpestris*, 17 adult females and 36 adult males, were collected from a permanent pond of 26 m², situated in the Cieza Mountains, Natural Park of Saja-Besaya (Cantabria, northern Spain; 43°14'8"N, 4°9'59"W) at an elevation of 464 m. The pond lacks of vegetation, but its bottom is covered with a thick layer of decomposing leaves. It is surrounded by a thick forest of *Quercus robur* and *Fagus sylvatica*, as well as less abundant and dispersed bushes of *Ilex aquifolium*.

In this area, alpine newts usually begin their migration to their breeding pond in mid-March, but large numbers of newts have not been found in the ponds until late April. Also, the males are the first to arrive at the ponds, whereas the females are not abundant until May (DIEGO-RASILLA personal observation).

Experimental design

Alpine newts were collected during spring in 2002 (19-20 May) between 18:30 and 20:00 hr, 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. Alpine newts were displaced from their home pond to the testing site in completely covered plastic containers kept inside a polyamide backpack that avoided access to directional cues en route, excepting magnetic cues, that could potentially have been used to determine the direction of displacement. Nonetheless, magnetic compass orientation in newts seems to be eliminated in the absence of visible light (PHILLIPS & BORLAND 1992a). Therefore, conducting the displacement in darkness might preclude the use of magnetic cues by the alpine newts. At the testing place, the rectangular plastic containers were aligned along the north-south geomagnetic axis. The dorsal pattern of each newt was photographed for individual identification, and newts were returned to their pond after testing.

The testing protocols described by DIEGO-RASILLA & LUENGO (2002) were employed. Each test animal was used in three consecutive experiments in a circular, visually symmetrical arena that afforded a view of the sky but not the horizon. The experimental site was an open area 9020 m from the pond. The compass course towards the pond, measured with a 12-channel GPS receiver, was 235°. Tests were conducted under a starry sky, on a moonlit night, between 22:10 and 02:35 hr. The air temperature during the tests was 7.53 ± 0.31 °C, and the relative humidity was $97.68 \pm 0.45\%$. The test arena consisted of a circular plastic container (57 cm diameter, 28 cm high). The floor and arena walls were thoroughly wiped with a damp sponge after each trial to eliminate directional olfactory cues (FISCHER et al. 2001), and then they were wiped dry using paper towels.

Alpine 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 (9.5 cm diameter, 14.5 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 were excluded from the analyses. To further minimize disturbance during the experiments, we left the testing site, leaving each animal alone for 1 min. The newt's directional response was later recorded by the moist trails it left on the floor of the arena. In all cases, the animals moved directly from their initial position to the arena walls, tapped against the wall, and then clinging to the wall, proceeded to circle around the arena. The criterion for the direction of movement was recorded as the vector of the first point where an animal made contact with the wall. During testing, after an animal reached criterion for direction of movement it was re-run immediately in a new test condition, after cleaning the arena. In the meantime animals were kept separately in their rectangular plastic containers, aligned along the north-south geomagnetic axis, in which the water depth was 1 cm. Three consecutive experiments were conducted in the sequence and according to the protocol described below. These three experiments were followed by a fourth experiment testing the possibility of a non-homing directional bias.

First experiment: 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.

Second experiment: Orientation under dark conditions. Covering the arena with a thick cardboard eliminated light from the arena. Also, light could not penetrate the walls of the arena. Thus the newts were unable to see the sky. The ambient geomagnetic field was not altered.

Third experiment: 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, shifting the polarity of the earth's natural magnetic field by 180°. 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).

Fourth experiment: Testing the possibility of a non-homing directional bias. Although unlikely, the possibility exists that the newts in this study were responding to a non-homing directional bias that just happened to coincide with the homeward direction. Hence, to test this possibility, during the next migration season (i.e., April 2003) two new tests were carried out according to the protocol explained below. These tests used newts captured in a different pond that was also situated in the Cieza Mountains (43°13'55"N, 4°09'46"W). Test 1: The home direction was varied by moving the arena to a new testing site, 3390 m from the pond, that was in a different direction from the home pond (i.e., 319°). At this site, a new group of alpine newts (19 adult males, and 8 adult females) were tested under a clear sky, and a non-altered geomagnetic field. Test 2: A new group of newts (16 adult males, and 3 adult females) were tested, following identical conditions, in a different testing site 9080 m from the pond for which the home pond direction was at 232°.

We used a GARMIN eTrex Legend Personal Navigator, and a compass to estimate the compass courses toward the pond, the distance from the pond to the experimental sites, and the bearings exhibited by newts.

Statistical processing

Data were analysed using standard circular statistics (BATSCHULET 1981, FISHER 1995). Mean vectors were estimated and tested for significance using the Rayleigh test. Ninety-five percent confidence intervals around the mean vector bearing were used to determine whether the distribution was oriented with respect to the homeward direction; the Watson U²-test was used to test for differences between distributions (BATSCHULET 1981). Statistics for bimodal distribution were calculated by doubling each data value and reducing any greater than 360 using modulo arithmetic.

To determine whether the experiments affected the newts' motivation to orient, I compared the ability of individual newts to orient within the specified time limits under the three conditions using a Cochran's Q test (SIEGEL 1956). The numbers of individuals responding in one, two, or three of the consecutive experiments, or not responding to any of them were also compared using a Chi-square test (SIEGEL 1956).

RESULTS

First experiment: Orientation under a clear night sky

The mean vector bearing is $233.38 \pm 21.25^\circ$ (Rayleigh test; Fig. 1A) and the 95% confidence interval (191.72° , 275.04°) includes the homeward direction (235°), indicating that the newts can orient themselves toward the pond.

Second experiment: Orientation under dark conditions

Although the ambient geomagnetic field was an available cue for orientation, newts were unable to orient themselves homeward during this test. Thus, the distri-

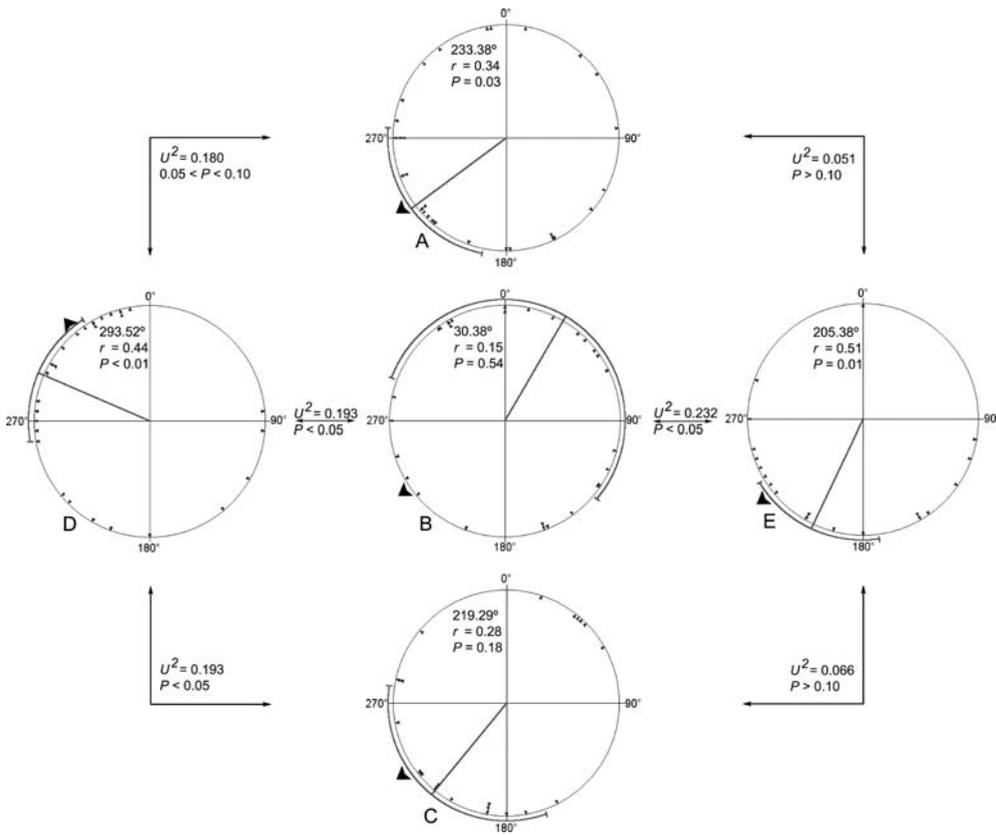


Fig. 1. — Orientation responses of the alpine newt, *T. alpestris*, during the experiments. A, First experiment: orientation under a clear night sky. B, Second experiment: orientation under dark conditions. C, Third experiment: orientation under a clear night sky in the presence of an altered geomagnetic field. D, Fourth experiment: testing the possibility of a non-homing directional bias (newts tested at a site in which the home pond direction is 319°). E, Fourth experiment: testing the possibility of a non-homing directional bias (newts tested at a site in which the home pond direction is 232°). Symbols indicate the direction of movement of each individual newt. The mean vector and its 95% confidence interval are also shown. Mean vector bearing, mean vector length (r), and probability (P) were estimated using the Rayleigh test. The arrowhead outside each circle indicates the home pond direction.

bution of bearings for newts tested under these experimental conditions is random (Rayleigh test; Fig. 1B).

Third experiment: 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, newts oriented randomly (Rayleigh test; Fig. 1C). So, there is evidence that the

magnets affected orientation ability of newts. However, considering the data displayed in Fig. 1C, it seems reasonable to assume that there are two modal groups present. Thus, newts exhibited significant bimodal orientation ($35.08\text{--}215.08^\circ$, $r = 0.46$, $n = 22$, $P = 0.01$). The 95% confidence interval for the mean vector includes the direction of the pond ($55\text{--}235^\circ$) (Fig. 2).

Fourth experiment: Testing the possibility of a non-homing directional bias

When the home direction is varied to 319° , the mean vector bearing is $293.52 \pm 16.87^\circ$ (Rayleigh test; Fig. 1D) and the 95% confidence interval (260.45° , 326.59°) includes the new homeward direction. The distribution of bearings for newts tested in this site differs significantly from that obtained for the second and third experiments (Watson U^2 -test; Fig. 1D), although their orientation did not differ significantly from that of the first experiment, the difference approached significance ($0.05 < P < 0.10$, Watson U^2 -test) (Fig. 1D). Moreover, when a new control group of newts was tested at a new site for which the home pond direction was 232° , the mean vector bearing was $205.38 \pm 16.98^\circ$ (Rayleigh test; Fig. 1E), and the 95% confidence interval (172.10° , 238.66°) included the new homeward direction. In this test the newts' orientation did not differ significantly from that of the first and third experiments, but it differed significantly from that obtained for the second experiment (Watson U^2 -test; Fig. 1E). The distribution of bearings for newts tested in these two control tests differs significantly (Watson $U^2 = 0.304$, $P < 0.01$). Results

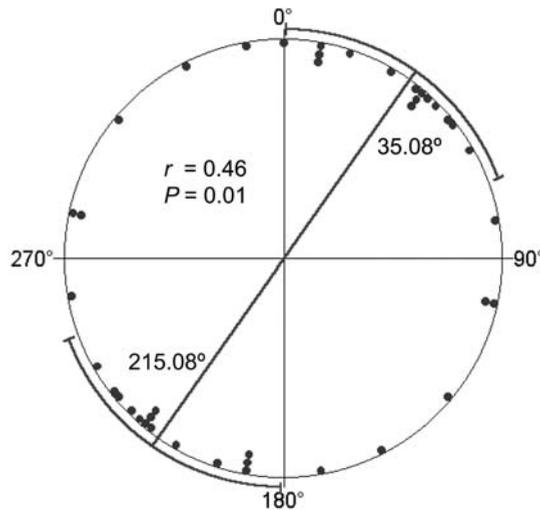


Fig. 2. — Bimodal orientation of the alpine newts, *T. alpestris*, during the third experiment (orientation under a clear night sky in the presence of an altered geomagnetic field). The distributions of the doubled bearings, used to calculate the bimodal statistics, are shown. Mean bimodal axis and its 95% confidence interval are also shown. Mean vector bearing, mean vector length (r), and probability (P) were estimated using the Rayleigh test. The homeward orientation, measured with a 12-channel GPS receiver, was 235° . The horizontal direction of the ambient geomagnetic field was altered by 180° using neodymium magnets.

obtained from these complementary tests have shown that the newts can orient in a homeward direction (Rayleigh test; Fig. 1D-E).

To determine whether the absence of visible light (second experiment) or the presence of an altered magnetic field (third experiment) affected the newts' motivation to orient, I compared the ability of individual newts to orient within the specified time limits under the three conditions (i.e., to achieve criterion for direction of movement). Twenty-three of 53 newts tested in the first experiment failed to orient compared to twenty-five of 53 newts tested in the second experiment, and thirty-one of 53 newts tested in the third experiment (Cochran's Q test; $Q = 4.95$, 2 df, $P > 0.05$). Furthermore, sixteen newts (30.19%) responded in the three consecutive experiments, whereas thirteen newts (24.52%) reached criterion for direction of movement in two tests, seven animals (13.21%) in only one of them, and seventeen newts (32.08%) did not respond in any of them (Chi-square test; $\chi^2 = 4.59$, 3 df, $P = 0.205$).

DISCUSSION

Orientation toward breeding sites is probably dependent to an assembly of several sensitive mechanisms (ABLE 1991; SINSCH 1991, 1992). *T. alpestris* has been found to be adept at homing (JOLY & MIAUD 1989) and its orientation behaviour during breeding migration can be explained, at least in part, by a mechanism based on chemical goal-emanating cues (JOLY & MIAUD 1993). However, the use of chemical cues from the pond probably does not enable a guidance strategy when the newts are far from the pond, although it may be efficient at a very limited distance from the pond (JOLY & MIAUD 1993). Likewise, although the red-spotted newts, *N. viridescens*, can use the earth's magnetic field for orientation, piloting based on the perception of chemical cues emanating from the breeding pond has been also found in this species (HERSHEY & FORESTER 1980). In any case, homing cannot be explained by the use of olfactory cues (JOLY & MIAUD 1993).

In the present study, alpine newts showed reliable homeward orientation in control tests (first and fourth experiments), which provide the reference for the response to the experimentally altered magnetic field, and for the test performed in the absence of celestial cues. Since newts were tested at three different sites that are in a different magnetic directions from the home pond (i.e., 232°, 235° and 319°), and they oriented in a homeward direction, the behavioural response in these experiments demonstrates homing. The results of the present study suggest that the newt's homing response is eliminated in the absence of celestial cues, as has been found in the marbled newt, *T. marmoratus*, which exhibits homeward orientation using celestial cues, although the ambient geomagnetic field does not seem to be relevant to orientation in this species (DIEGO-RASILLA & LUENGO 2002). Conversely, magnets affected the orientation ability of alpine newts, which suggests that when the alpine newts are far from the breeding pond a mechanism based on perception of the earth's magnetic field may be involved in homing. Also, alpine newts failed to orient only when celestial cues or the geomagnetic field were the sole orientation cues available. These findings suggest a light-dependent magnetoreception in *T. alpestris*. However, although magnets will change the net magnetic direction in an area of this size, other field components such as inclination and total intensity will change as well. Moreover, the magnets will produce steep gradi-

ents that are not present under natural conditions. Consequently, a change in the newts' behaviour may represent a response to intensity or inclination, or to the spatial variation in one or more of these components, rather than a response to the direction of the magnetic field. Nevertheless, the present results represent a first step in understanding the homing ability of the alpine newt. Future investigations using a doubly wrapped cube-surface coil (RUBENS 1945) should be performed, thus shifting the direction of the magnetic field in a controlled manner (i.e., without changing the inclination or total intensity) (PHILLIPS 1986). In fact, tests recently carried out using coil systems to change parameters of the geomagnetic field more homogeneously in a more natural intensity range have demonstrated a magnetic compass orientation in the alpine newt (DIEGO-RASILLA et al. in preparation).

Moreover, the ability of newts to orient within the specified time limits was similar under the three conditions; hence, the effects do not appear to result from a lack of motivation to orient, but rather from an inability to obtain directional information from the magnetic field. In any case, previous experiments using the same testing protocol revealed that most of the *T. marmoratus* tested were able to orient within the specified time limits (i.e., 1 min) (DIEGO-RASILLA & LUENGO 2002).

In summary, the findings reported here provide the first direct experimental support for the use of light (i.e., celestial cues), and the ambient geomagnetic field by homing *T. alpestris*.

ACKNOWLEDGEMENTS

I sincerely thank Rosa M. Luengo for assistance during this study and Domingo Matías for valuable discussions. Valentín Pérez-Mellado took special interest in this study. Thanks are also due to John B. Phillips, David H. Dommer, Nicole Edgar, and one anonymous reviewer for invaluable comments and suggestions regarding the manuscript. The Cantabria autonomous government kindly granted the necessary permits for the study. I am particularly indebted to F. Javier Manrique, director of the Natural Park of Saja-Besaya, for permitting the study to be carried out at the Natural Park.

REFERENCES

- ABLE K.P. 1991. Common themes and variations in animal orientation systems. *American Zoologist* 31: 157-167.
- BATSCHULET E. 1981. Circular statistics in biology. *London: Academic Press*.
- DIEGO-RASILLA F.J. & LUENGO R.M. 2002. Celestial orientation in the marbled newt (*Triturus marmoratus*). *Journal of Ethology* 20: 137-141.
- FISCHER J.H., FREAKE M.J., BORLAND S.C. & PHILLIPS J.B. 2001. Evidence for the use of magnetic map information by an amphibian. *Animal Behaviour* 62: 1-10.
- FISHER N.I. 1995. Statistical analysis of circular data. *Cambridge: Cambridge University Press*.
- FONTANET X. 1991. Estudio sobre la capacidad de orientación de *Triturus marmoratus* (Latreille, 1800) y *Triturus alpestris* (Laurenti 1768) (Amphibia, Salamandridae). *Revista Española de Herpetología* 6: 91-100.
- GILL D.E. 1979. Density dependence and homing behavior in adult red-spotted newt *Notophthalmus viridescens* (Rafinesque). *Ecology* 60 (4): 800-813.
- HERSHEY J.L. & FORESTER D.C. 1980. Sensory orientation in *Notophthalmus viridescens* (Amphibia: Salamandridae). *Canadian Journal of Zoology* 58: 266-276.

- JOLY P. & MIAUD C. 1989. Fidelity to the breeding site in the alpine newt *Triturus alpestris*. *Behavioural Processes* 19: 47-56.
- JOLY P. & MIAUD C. 1993. How does a newt find its pond? The role of chemical cues in migrating newts (*Triturus alpestris*). *Ethology Ecology & Evolution* 5: 447-455.
- LANDRETH H.F. & FERGUSON D.E. 1967. Newts: sun-compass orientation. *Science* 158: 1459-1461.
- PHILLIPS J.B. 1977. Use of the earth's magnetic field by orienting cave salamanders (*Eurycea lucifuga*). *Journal of Comparative Physiology* 121: 273-288.
- PHILLIPS J.B. 1986. Two magnetoreception pathways in a migratory salamander. *Science* 233: 765-767.
- PHILLIPS J.B. 1987. Homing orientation in the eastern red-spotted newt (*Notophthalmus viridescens*). *Journal of Experimental Biology* 131: 215-229.
- PHILLIPS J.B. 1996. Magnetic navigation. *Journal of Theoretical Biology* 180: 309-319.
- PHILLIPS J.B., ADLER K. & BORLAND S.C. 1995. True navigation by an amphibian. *Animal Behaviour* 50: 855-858.
- PHILLIPS J.B. & BORLAND S.C. 1992a. Magnetic compass orientation is eliminated under near-infrared light in the eastern red-spotted newt *Notophthalmus viridescens*. *Animal Behaviour* 44: 796-797.
- PHILLIPS J.B. & BORLAND S.C. 1992b. Wavelength specific effects of light on magnetic compass orientation in the eastern red-spotted newt. *Ethology Ecology & Evolution* 4: 33-42.
- PHILLIPS J.B. & BORLAND S.C. 1992c. Behavioural evidence for the use of a light-dependent magnetoreception mechanism by a vertebrate. *Nature* 359: 142-144.
- PHILLIPS J.B. & BORLAND S.C. 1994. Use of a specialized magnetoreception system for homing by the eastern red-spotted newt *Notophthalmus viridescens*. *Journal of Experimental Biology* 188: 275-291.
- RUBENS S.M. 1945. Cube surface coil for producing uniform magnetic field. *Review of Scientific Instruments* 16: 243-245.
- SIEGEL S. 1956. Nonparametric statistics for the behavioral sciences. *New York: MacGraw-Hill Book Company*.
- SINSCH U. 1991. The orientation behaviour of Amphibians. *Herpetological Journal* 1: 541-544.
- SINSCH U. 1992. Amphibians, pp. 213-233. In: Papi F., Edit. Animal homing. *New York: Chapman and Hall*.
- TAYLOR D.H. & AUBURN J.S. 1978. Orientation of amphibians to linearly polarized light, pp. 334-346. In: Schmidt-Koenig K. & Keenton W.T., Edits. Animal migration, navigation, and homing. *Berlin, Heidelberg: Springer Verlag*.
- TWITTY V.C. 1959. Migration and speciation in newts. *Science* 130: 1735-1743.
- TWITTY V.C., GRANT D. & ANDERSON O. 1966. Course and timing of the homing migration in the newt *Taricha rivularis*. *Proceedings of the National Academy of Science of the U.S.A.* 56: 864-869.