MAGNETIC ORIENTATION IN ANURANS: A CASE STUDY ON RHINELLA ARENARUM (ANURA: BUFONIDAE)

Orientación magnética en anuros: un estudio de caso sobre Rhinella arenarum (Anura: Bufonidae)

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Resumen.— El campo magnético de la Tierra (EMF) es una de las fuentes de información direccional más uniformes y accesibles que los animales pueden usar en los movimientos de comportamiento. El presente estudio tuvo como objetivo determinar la capacidad de Rhinella arenarum para percibir los campos electromagnéticos. Para evaluar la influencia de los campos electromagnéticos en los anuros recolectamos cuarenta y cuatro especímenes adultos de R. arenarum durante la temporada de reproducción. Registramos el movimiento de cada sapo desde el centro hasta la periferia de un arenero circular utilizando una cámara de visión nocturna. Repetimos el experimento después de cinco minutos, con un campo magnético inducido adicional (IMF), que fue creado empleando dos bobinas de aire Helmholtz. Los movimientos de los sapos bajo la presencia de EMF y IMF fueron significativamente diferentes. Concluimos que R. arenarum podría usar el EMF como mecanismo de navegación y sistema de ubicación, para viajar largas distancias hasta los estanques de desove año tras año.

Palabras claves.— Navegación, migración de anuros, percepción magnética, ubicación.

Abstract.— The Earth’s Magnetic Field (EMF) is one of the most uniform and accessible sources of directional information that animals can use for behavioral movements. The present study aimed to determine the ability of Rhinella arenarum to perceive the EMF. To assess the influence of the EMF on anurans in situ we collected forty-four adult specimens of R. arenarum during the breeding season. We recorded the movement of each toad from the center to the periphery of the circular arena utilizing a night vision camera. We repeated the experiment after five minutes, with an additionally Induced Magnetic Field (IMF), which was created by two Helmholtz air coils. The movements of the toads under the presence of the EMF and IMF were significantly different. We concluded that R. arenarum could use the EMF as a navigation mechanism and location system, to travel long distances to spawning ponds year after year.

Key Words.— Navigation, anuran migration, magnetic perception, location.

INTRODUCTION

Animals exploit numerous sources of information while migrating, homing, or moving around their habitats (Freake et al., 2005; Lohmann et al., 2007; Shakhparonov et al., 2022). Among these, the Earth’s magnetic field (EMF) is a particularly pervasive environmental feature (Skiles, 1985). In contrast to other cues, the magnetic field is present both day and night, is mostly unaffected by weather and seasonality, and exists everywhere on the planet (Lohmann et al., 2007; Wiltschko &
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Wiltshcko, 2012). Some species can find their way back home after being displaced to an unknown location, despite the absence of geographical references, goal-emanating cues, and directional information obtained during the journey (Phillips, 1996).

Homing behavior in animals is defined as an individual's ability to navigate through a small or unfamiliar terrain back to its “home” place, its home range (Nothacker et al., 2018). Various advantages of such site fidelity have been discussed, particularly the maximization of resource exploitation, which is often linked to reproduction (Nothacker et al., 2018). This ability to navigate requires, in addition to a directional sense (compass), an understanding of the geographical position by processing the available spatial information in the place where the individual is located (Diego-Rasilla & Luengo, 2020; Kramer, 1953). This phenomenon is found in many arthropods and vertebrates, such as decapod crustaceans (Pittman & McAlpine, 2003), salmon (Quinn & Dittman, 1990), or pigeons (Walcott, 1996). Among amphibians, it is known that many species exhibit site fidelity (Sinsch, 2014).

During the spawning season, several species of terrestrial anurans return to water bodies for mating mainly type I and II reproductive modes sensu Haddad & Prado (2005). In this process, the females release eggs that are fertilized by males (Young et al., 2004). As summarized by Wells (2007), the homing behavior of salamanders and newts is relatively well understood compared to other amphibians. Mechanisms or processes leading to the ability to return to the same spawning location year after year are still poorly unknown. Several studies show that amphibians are able to use a magnetic compass for orientation and navigation to the breeding ponds (Phillips et al., 1995; Diego-Rasilla, 2003; Diego-Rasilla et al., 2005, 2008, Sinsch, 2006, Phillips et al., 2012; Landler, 2022). The use of the geomagnetic field would be essential in situations where other positioning sources (maps) and directional cues (compasses), such as olfactory signals and celestial cues are unavailable (Joly & Miaud, 1993; Diego-Rasilla et al., 2005).

It has been demonstrated that magnetic field compasses are used in homing orientation by alpine newts (Diego-Rasilla, 2003) and in orientation towards the coast by eastern red-spotted newts (Phillips & Borland, 1992). Due to this, investigations on anuran migrations using the terrestrial magnetic field are important in order to clarify its effect. Studies on adult specimens of Pelophylax ridibundus showed the ability of anurans to use the Earth’s magnetic field for orientation preference (Shakhparonov & Ogurtsov, 2017). In migratory studies of adult anurans, Landler & Gollmann (2011) made observations on *Bufo bufo* and its ability to use the magnetic field. After capturing specimens migrating to spawning ponds, they tested their ability to correct their orientation toward the ponds in a circular arena in laboratory conditions. They demonstrated the influence of the Earth’s Magnetic Field on their orientation behavior. However, the specimens were not orient themselves towards the spawning ponds but rather maintained their orientation prior to capture (orientation of the “d” axis; see Endler, 1970). Landler et al. (2016) repeated this experiment and tested *B. bufo* specimens the same night of capture, and after keeping them in captivity for three days, presumably enough time for them to update their internal map. The specimens tested on the same night of capture maintained the migration direction prior to capture, while the specimens kept in captivity exhibited random orientation. Extending their studies on *B. bufo*, in a new experiment Pail et al. (2020), examined the true navigation response in an open-air setting after keeping the toads in quarantine four days. In addition to the magnetic field, they measured the effect of environmental variables and the lunar cycle on the orientation of *B. bufo*. Their results showed that the animals were not yet oriented towards the breeding pond, but rather toward their previous migration direction prior to capture.

This investigation aimed, firstly, to determine whether males of *Rinella arenarum* (Hensel, 1867) exhibit “homing” behavior toward the reproductive site from which they were displaced, and secondly, to determine the influence of the magnetic field on their orientation behavior towards the reproductive site in the arid region of San Juan, Argentina. We tested two hypothesis a) if we displace male frogs of *Rinella arenarum* from their spawning pond, they will maintain orientation to their initial spawning site, and b) if we modify the Earth’s Magnetic Field, adult males of *Rhinella arenarum* will change their orientation according to the new magnetic location of the spawning pond. The experiments presented in this study constitute a first approach to understand the orientation behavior in Neotropical anurans.

**MATERIALS AND METHODS**

The experiments were carried out under natural climatic conditions after sunset at 8:30 p.m., from September 3rd to September 10th, 2012, in the Sarmiento Provincial Park. This park is located in Zonda, 25 km west of the city of San Juan (3° 35’ 00.33” S, 68° 32’ 22.58” W; elevation: 800 m a.s.l.). The area is characterized by the presence of extensive wetlands (40 ha), which are irrigated by the subterranean watercourse of the San Juan River. During the winter season, the wetland is reduced to two small water bodies, while the flooded areas expand during the summer, until they become a large flooded area (Victoria, 1999). Study area is located in Monte phytogeographical
province (Cabrera, 1971), a region characterized by an arid climate with a mean annual temperature of 17.3 °C, a mean annual maximum of 25.7 °C, and a mean annual minimum of 10.4 °C. Rainfalls mainly occur during the summer season, with a mean annual precipitation of 84 mm (Camarillo-Naranjo et al., 2018; Poblete & Hryciw, 2017).

The common toad (R. arenarum) is a widely distributed species in north and central Argentina inhabits various types of environments, including anthropogenic ones such as residential yards and gardens (Sotomayor et al., 2012; Sanchez et al., 2014). In the Monte Desert region of central Argentina, R. arenarum breeds from late August until late November (Sanabria et al., 2005). During the reproductive process, the females of this species deposit their eggs in gelatinous strands, laying an average of 28,000 eggs per clutch (Quiroga & Sanabria, 2012). There is sexual dimorphism in this species, with females being larger than males (Quiroga et al., 2004). As for its diet, it primarily feeds on Hymenoptera, specifically Formicidae (ants) (Quiroga, 2006). Rhinella arenarum is primarily a thermoconforming organism, with ambient temperature and wind speed being the environmental variables that most affect its body temperature (Sanabria et al., 2012).

We collected adult males (R. arenarum) from ponds located 500 m in the SE direction of the experimental area. Then, we transport the individuals to the experiment area in dark plastic containers to avoid any type of signal during the movement (0.3 m in diameter filled with 0.8-1.0 l of pond water).

We tested the behavior of the toads one by one on a circular arena 1.2 m in diameter, made with a medium-density fiberboard (MDF) wall 0.6 m high. We painted the wall as well as the bottom black color, so the toads inside of the sandbox could not distinguish the line from the horizon. We made two Helmholtz-type coils with air cores and a diameter of 1.8 m's out of PVC (Landler & Gollmann, 2011). The coils were placed parallel 0.9 m's apart from each other. Every coil had 21 turns of aluminum wire (section = 0.5 mm²) wrapped in plastic. We located the coils at the ground level and made a wooden structure of the base to avoid the use of metal. We measured the magnetic field strength with a 3-D flow valve (FLC3-70, Stefan Mayer Instruments) and we used a mechanical combustion generator (Dowel, 6.5 HP, Japan) as an energy source (220 V). The combustion generator on we placed the generator at 200 m distances to the sandbox to avoid additional stress to the individuals. The generator was kept on throughout the experiment. We employed a current limiter (VATRANS, Argentina) for generating a constant magnetic field in the coils (at 12 V, 50 A).

We carried out the tests in the circular arena and placed an adult amphibian in a release device in total darkness at the center of the sandbox. We constructed the release device using a black plastic container (0.2 × 0.2 m). We released the individuals after five minutes. The release device was removed from the arena using a pulley system, which allowed the operator to be far from the terrarium, not disturbing the experiment. As a directional response, we took the first contact of the individuals with the wall of the circular arena (Landler & Gollmann, 2011). Individuals, which could not reach the wall of the arena within five minutes, were excluded from the analysis.

We tested each individual twice, the first time under conditions of the Earth’s Magnetic Field (EMF) and subsequently under the conditions of an added Induced Magnetic Field (IMF). To assess whether male Rhinella arenarum presents the “homing” behavior towards the reproductive site from which they were displaced, the displacement of each individual was first evaluated under the conditions of the Earth’s magnetic field. Subsequently, to determine the ability of R. arenarum to perceive the Earth's magnetic field, the same individuals were evaluated under the conditions of an induced magnetic field (IMF). We followed the protocol described by Diego-Rasilla & Luengo (2002), where each test animal was used in the experiments consecutively. After testing each animal, we cleaned the sandbox with a paper towel and ethanol 50% to eliminate chemical substances produced by the tested individuals. To inverse, the horizontal component of the terrestrial magnetic field 180°, the Helmholtz type air-cored, coils were switched on. We returned the individuals to the ponds from which they were collected after the all experiment finished.

Experiments were carried out in as much darkness as possible (minimal generation of artificial light). Therefore, we obtained the records by a video camera with night vision (Sony Handycam, Japan), which was placed in the upper part of the circular arena (3.10 m height positions determined by the focus of the image) and which operated remotely from a monitor (Super Sonic Deluxe 5” Portable FC-9100 - USA) to reduce the stress of the animal to the maximum during the experiment.

We analyzed the data using standard circular statistics (Batschelet, 1981). We estimated mean vector length (r); mean direction (μ), circular variance, and concentration. We used Rayleigh's test to determine whether a distribution exhibited significant orientation. The V test was used to test closeness to expected orientation (i.e., home pond direction) (Batschelet, 1981; Mardia & Jupp, 1999). For the comparisons between the direction of toads between treatments, we used Watson-Williams F-Tests. All the tests had a confidence interval of 95%. The Wilcoxon
RESULTS

The Earth's Magnetic Field in the study area at noon on September 23rd of 2012 had an average value of 0.21 ± 0.074 Gauss (range: 0.021 – 0.038), and the Induced Magnetic Field at the center of the coils gave a maximal value of 0.45 ± 0.048 Gauss (range: 0.017 – 0.098). From 44 studied anurans, only one individual, which did not move from the center of the arena, was excluded from the analyses. Under the influence of the Earth's Magnetic Field toads needed on average of 56.84 ± 35.56 s to touch the wall, and on average 48.49 ± 48.82 s, under an Induced Magnetic Field. No significant differences were found in the time of response among treatments (Wilcoxon test = -0.75, p = 0.45, n = 43).

The direction of *R. arenarum* under the Earth’s Magnetic Field was of μ=305.41° ± 121.4°, of the vector mean (r)= 0.107 and of the concentration of data= 0.215. The data exhibited a unimodal distribution (Test Rayleigh, Z= 0.492, p = 0.61). We not found significant differences in the orientation of *R. arenarum* respect of the spawning ponds (Test V (55.19°) = -0.036, p= 0.631).

The direction of *R. arenarum* under an Induced magnetic field, but respect at North magnetic of the earth, was of μ= 224.58° ± 94.79°, of the vector mean (r)= 0.254, and of the concentration of data= 0.526. The data show a different tendency of the unimodal distribution. In toads under the influence of the IMF showed a unimodal orientation that not coincided with the magnetic direction of the spawning ponds (Table 1). Concerning the magnitude of the mean vector and the concentration of the data, they were two times lower under EMF conditions than under IMF conditions (Table 1, Fig. 1) whereas the distribution patterns within the circular arena were significantly different among treatments (F = 14.637, p = 0.0002, n = 43 Watson-Williams F-Tests) and their orientation differed by 99° and 206°.

DISCUSSION

Our work studied the homing behavior of *Rhinella arenarum* towards the reproductive site and the influence of the magnetic field on their orientation to reorient their travel direction in response to an induced magnetic field were investigated. The results obtained demonstrate that, similar to other species of anurans, *R. arenarum* exhibits site fidelity (Roithmair, 1994; Neu et al., 2016; Nothacker et al., 2018). Ferguson et al. (1963) found that three species of anurans displayed homing behavior after returning to their site. Several studies show that male and female anurans can return to an initial capture site after experimental translocation (McVey et al., 1981; Crump 1986; Arcila-Pérez et al., 2020). The motivation for return might differ between sexes due to divergent selective pressures determining mating and reproductive success for males and females. The search motivation for territorial species’ males is likely attributed to reclaiming a site more favorable for attracting mates than the release sites (Schlupp & Podloucky, 1994; Matthews, 2003).

### Table 1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>u</th>
<th>r</th>
<th>Concentration</th>
<th>Median</th>
<th>s</th>
<th>s2</th>
<th>Rayleigh Test</th>
<th>V Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMF</td>
<td>3.573</td>
<td>0.107</td>
<td>0.216</td>
<td>332</td>
<td>0.893</td>
<td>121.07</td>
<td>1.012</td>
<td>0.007</td>
</tr>
<tr>
<td>IMF</td>
<td>220.408</td>
<td>0.265</td>
<td>0.556</td>
<td>232</td>
<td>0.735</td>
<td>93.36</td>
<td>6.183</td>
<td>0.002</td>
</tr>
</tbody>
</table>

α = 0.05. V Test; EMF: Earth magnetic field; IMF: Induced magnetic field.
In any case, males could return to the initial capture site due to the benefits associated with higher mating prospects. Perhaps a frog returns to its home spot because it is a good foraging site or perhaps a frog returns to its home spot because it is satisfactory, if not ideal, in terms of moisture, abundance of crevices in which to hide and sufficient overhanging vegetation to provide protection from the sun. Homing is clearly a strategy to minimize energy expenditure for locomotion, given the scarcity of suitable sites for reproduction, nutrition, and refuge.

Regarding the utilization of the amphibians’ magnetic field for orienting to spawning sites, the results found in the experiments provided evidence that supports the idea that *R. arenarum* has many more orientation mechanisms than just the use of magnetic compass signals. However, a higher concentration in the orientation found in *R. arenarum* under the influence of the Induced Magnetic Field could demonstrate the importance of Earth’s Magnetic Field, in the orientation in frogs (Fig. 1). Like the results obtained by Landler & Gollmann (2011), Landler et al. (2016), Pail et al. (2020), in adult specimens of *Bufo bufo*, we obtained that adult specimens of *R. arenarum* tend to returns to breeding pond after being moved from their site of origin. Our study reinforces the idea that the Earth’s Magnetic Field could allow anuran specimens to reorientate their migration direction. The main difference between our work on *R. arenarum* and those carried out on *B. bufo* is that the collection of specimens in the former was carried out during migration, and in our study, they were collected in spawning ponds.

The ability of *R. arenarum* to orient itself to spawning ponds was consistent with results reported in several amphibian studies. Deutschlander et al. (1999) carried out the first experiences of the influence of the geomagnetic field on the orientation of *Notophthalmus viridescens* (Urodela) by studying the direction of these organisms by changing the magnetic field by a deviation of 90°. Diego-Rasilla et al. (2005) found in

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**Figure 1.** Orientation of *R. arenarum* under the effect of the Earth's Magnetic Field (A) and Induced Magnetic Field (B) relative to geographic north. In each circular diagram, the direction of the samples, are grouped concerning the course to the edge of the arena. Each dots represents the orientation of a single toad. The arrows represent the mean vectors of the distributions (radius of the circle corresponds to a vector length of 1).
Triturus alpestris that the magnetoreception mechanisms subject to celestial signals mainly explained the choice of direction toward the spawning pond. In another similar work carried out by Diego-Rasilla & Luengo (2007), they found that the Lissotriton helveticus was able to orient itself utilizing acoustic signals at short distances. However, at great distances from their spawning ponds, direction selection could only be done using the magnetic compass. Shakhpardonov et al. (2017) studied adults of Pelophylax ridibundus and obtained similar results in comparison with the aforementioned investigations. Diego-Rasilla et al. (2010) and Freake et al. (2002) proposed that the existing mechanisms of anurans and urodèles are traces of the evolution of sensory capacity in amphibians. Consequently, the sensitivity function of magnetoreception could have been present in a common ancestor of Lissamphibia, originating in the Lower Permian Period (Zhang & Wake, 2009). Despite all these studies on amphibian localization, it is not clear whether they can return home from unknown sites (Pail et al., 2020). It has been questioned whether true navigation represents a general ability of amphibians or if it could be a restricted phenomenon only present in a few species or even populations (Pasukonis et al., 2014; Sinch & Kirst, 2016).

The common toad, R. arenarum could orient itself to reproductive sites, being able to use the magnetic compass as a source of directional information. In our experiment, the R. arenarum specimens were shown to maintain the previous direction of migration in the “d” axis after being subjected to a field of inverse magnitude to the Earth’s Magnetic Field. However, its geographical location seems to depend more on the information on the map than on the integration of the route, to determine the initial location direction. The most likely source of information on the map is the geomagnetic field, which has been shown to play a critical role in the search process (Freake et al., 2002; 2006). However, various studies show the complexity of sensory integration in anurans. In distances less than 10 km it is very difficult to use a magnetic map (Komolkim et al., 2017). While olfactory signals could play a key role in orientation (Wallraf, 2004; Vignoli et al., 2012; Pail et al., 2020). Therefore, studies that complement the evaluation of various signals will serve to clarify the source of the orientation of anurans.

Despite finding a trend in our data and the lack of statistical support (p = 0.061), our results in this contribution provide the first direct in situ experimental support of the detection capacity of the terrestrial geomagnetic field in a species of toads in South America. Our research provides valuable insights into R. arenarum is homing behavior and their utilization of the magnetic field for orientation towards reproductive sites. The species showcases remarkable site fidelity and demonstrates the intricate interplay of multiple orientation mechanisms, highlighting the complex nature of amphibian navigation. As in other amphibians, R. arenarum have a multisensory orientation and could use the terrestrial geomagnetic field as a navigation mechanism and location system to travel long distances to spawning ponds year after year. The orientation of amphibians is not a simple response to the environment but a complex process. We are confident that our work will enhance the understanding of amphibian orientation mechanisms. These findings contribute to a deeper understanding of anuran behavior and underscore the importance of Earth’s magnetic field in guiding their migrations and reproductive journeys. However, we believe that there is a need for a more comprehensive approach and a standardized methodology to identify the true mechanisms involved. Improved understanding will assist conservation efforts worldwide.

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Ethical approval: All handling procedures were international guidelines and national and institutional regulations that apply to the care and use of animals.

CITED LITERATURE


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