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Long-distance homing in the Iberian Green Frog, *Pelophylax perezi* (López-Seoane, 1885), in central Spain, documented via photo identification

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Amphibian movement ecology remains poorly understood compared to other vertebrate groups, such as birds and mammals (Pittman et al., 2014; Cayuela et al., 2020). In particular, amphibian movement patterns remain understudied in human-modified landscapes, where habitat loss and fragmentation severely impact dispersal, population connectivity, and genetic flow among populations (Cushman, 2006; Becker et al., 2007; Thompson et al., 2016). Connectivity across landscapes can be enhanced by understanding the nature and distance of amphibian movements, which is key for implementing effective conservation (Bailey and Muths, 2019; Reyes-Moya et al., 2022). Moreover, insights into movement behaviour can elucidate the interaction of amphibians with other organisms and the environment (Clobert et al., 2009).

Amphibian displacement can be categorised into migration (the seasonal and bidirectional movements adults perform between terrestrial habitats and their breeding site; Semlitsch, 2008) and dispersal, the unidirectional movement of individuals among breeding sites, including natal dispersal, and the main process that contributes to colonization and gene flow among populations (Smith and Green, 2005; Clobert, 2012). Generally, movement in amphibians is guided by complex mechanisms of orientation that rely on magnetic, olfactory, visual, and mechanical cues (Sinsch, 1990, 2006). These mechanisms enable individuals to return to their natal breeding sites or to colonise new ones (Sinsch, 2006; Semlitsch, 2008). However, little is known about the specific routes amphibians take to reach waterbodies, particularly after translocations, which may alter natural movement patterns but also provide a valuable opportunity to study these poorly understood displacement behaviours (Jreidini and Green, 2022).

Amphibians have limited movement capacities, which directly affects their dispersal distances. As a result, both migrations and dispersal events are generally short (Semlitsch and Bodie, 2003). This pattern is evident in European and Iberian amphibians, for which average movement distances rarely exceed 300 m and maximum recorded distances reach approximately 1 km (Semlitsch and Bodie, 2003; Kovar et al., 2009; Reyes-Moya et al., 2022; Sánchez-Montes and Martínez-Solano, 2023; Caballero-Díaz et al., 2024). However, dispersal and migration movements often go unnoticed and therefore are underestimated, especially during the dispersive juvenile stage, which often is poorly documented and understood (Pittman et al., 2014; Cayuela et al., 2020). Acknowledging this information is essential for conservation planning, as it guides the protection of migration corridors and the design of restoration actions, such as the construction of artificial ponds or wildlife road crossings.

The limited understanding of amphibian movement ecology is partly due to the difficulty of recapturing individuals far from their breeding sites. Although GPS devices and radio telemetry offer valuable data, these methods are expensive and may pose risks – such as stress or physical injuries – to certain species (Rowley and Alford, 2007; Altobelli et al., 2022). As a cost-

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effective alternative, Capture-Mark-Recapture (CMR) studies using Passive Integrated Transponders (PIT tags; Ousterhout and Burkhart, 2017; Muths et al., 2018; Sánchez-Montes and Martínez-Solano, 2023) or photo-identification techniques (Pereira and Maneyro, 2016; Caballero-Díaz et al., 2022a; Reyes-Moya et al., 2022) have provided important insights into amphibian movement patterns. Photo identification is a non-invasive monitoring method that relies on the detection of unique natural patterns in individuals and is becoming a useful tool for CMR programmes (Sannolo et al., 2016; Gould et al., 2021). It has proven to be reliable and effective, with minimal bias (Renet et al., 2019). Another strength is its cost-effectiveness, which facilitates conducting short-term monitoring programmes with minimal financial resources (Cruickshank and Schmidt, 2017). Thus, photo identification is often the only practical option to study conservation-sensitive species. We here employ this technique to report on two individuals of the Iberian Green Frog, *Pelophylax perezi*, that returned to their main breeding site after translocation.

Materials and Methods

The study area is located in the southwest of the Community of Madrid, central Spain (Fig. 1). According to the Köppen Classification, the climate is "Mediterranean Csa", with an average annual precipitation of 462 mm and a mean annual temperature of 11.5°C (period 1970–2000; Fick and Hijmans, 2017; Chazarra-Bernabé et al., 2022). The study area is situated on a granite-dominated hill. The main land uses in the area include forestry management (wood, acorns, chestnuts), livestock grazing, and hunting. The vegetation is heterogeneous, combining pastures, scrublands (*Cistus ladanifer*, *Cytisus scoparius*, *Rubus ulmifolius*), and forests (*Quercus ilex*, *Castanea sativa*, *Pinus pinaster*).

The main breeding site (hereafter, Pond A) is an artificial pond called Presa de la Alberca (40.2792°N, 4.4699°W, elevation 926 m; Fig. 1), which used to be a small, dammed reservoir before October 2020 (maximum depth > 6 m, perimeter 215 m, surface area 5025 m^2). Due to structural weakness that posed potential risks to nearby villages, the government dismantled the dam on 26 October 2020. Restoration consisted of removal of the wall dike, creation of a new artificial pond for amphibians (maximum depth 2.5 m, perimeter 88 m, surface area 508 m^2), eradication of invasive Red-swamp Crayfish (*Procambarus clarkii*), and restoration of the surrounding terrestrial and aquatic vegetation (Caballero-Díaz et al., 2022b). The second breeding site (herafter, Pond B) is an artificial pond called Los Barrancos y el Palancar (40.2786°N, 4.4879°W, elevation 1114 m, maximum depth > 3 m, perimeter 80 m, surface area 254 m^2; Fig. 1), located 1610 m from Pond A at the top of a mountain pass. Between the two waterbodies, the landscape consists of a mosaic of public and private plots, including meadows, scrublands, and forests. A small stream runs between both sites, nearly connecting them (Fig. 1). However, only the last 400 m of the stream retains water for several months, while the remainder holds water for just 2–3 weeks following intense rainfalls.

Following a request from the regional administration, we conducted a rescue survey of frogs at Pond A on 25 October 2020, one day prior to the day restoration work began, to prevent injury or mortality during construction activities. We first surveyed the shoreline and surrounding terrestrial areas, then examined aquatic areas with floating and emergent vegetation up to 1 m in depth. Frogs were hand-captured and temporarily stored in fabric bags. Each individual was sexed using the presence of vocal sacs, measured (snout–vent length; SVL) to the nearest 1 mm using a ruler, weighed to the nearest gram using a digital jewellery scale, and photographed with a smartphone (Huawei P20 Lite). Photos were taken at a height of 15 cm above each frog to capture the dorsal pattern. All individuals were then promptly transported and released at the edge of Pond B, the nearest waterbody to Pond A.

Restoration was completed by February 2021 (Caballero-Díaz et al., 2022b). The first post-restoration sampling took place on 30 April 2021, when the aquatic vegetation had not yet become established. The second sampling session was conducted on 5 February 2022, by which time emergent and floating aquatic vegetation was present. The restored pond was more difficult to access due to large rocks and steep shorelines. During both sessions, we first examined the surrounding area (in a 20-m margin around the pond's edge) using flashlights, then recorded vocalizations and used flashlights to locate individuals. Frogs were subsequently hand-captured from the shoreline while wearing waterproof boots. They were temporarily stored in fabric bags and then sexed, measured, weighed, and photographed with a smartphone (Xiaomi Poco F3) before being released at the same location. After every sampling session, waders and boots were disinfected using a 1:10 bleach solution to prevent the transmission of pathogens. Measurements (in mm) and weights (in g) are provided as means ± SD.

Figure 1. Locations of two *Pelophylax perezi* study sites in central Spain. (A) Pond A (Presa de la Alberca). (B) Pond B (Los Barrancos y el Palancar). (C) Aerial image of the study area showing Ponds A and B and the stream network (in blue). The inset shows the Iberian Peninsula with the study area highlighted (orange). Photos by Carlos Caballero-Díaz.

To determine if any of the frogs captured in Pond A on 5 February 2022 were recaptures of individuals originally translocated to Pond B on 25 October 2020 (see below), we used WILD.ID v1.0 software (Bolger et al., 2012) for photo identification. Prior to analysis, dorsal photos were optimised using the software NOMACS v3.0 (http://nomacs.org) by cropping images to a standardised rectangular area (red rectangle in Fig. 2) extending from the posterior eye margins to the hind limbs (Caballero-Díaz et al., 2022a; Reyes-Moya et al., 2022; Gómez-Ramírez et al., 2023). Processed images were then uploaded to WILD.ID, which generates a ranking of the 20 most similar matches by comparing pixel values and spatial patterns of each photo against the database (Bolger et al., 2012). This software assigns a similarity score between 0 and 1 that quantifies how closely the spot pattern of a photo matches the individuals in the database. The higher score for each pair of photos has a higher probability of being a correct match. Potential matches were then confirmed by visual comparison of distinctive spot patterns across multiple body regions.

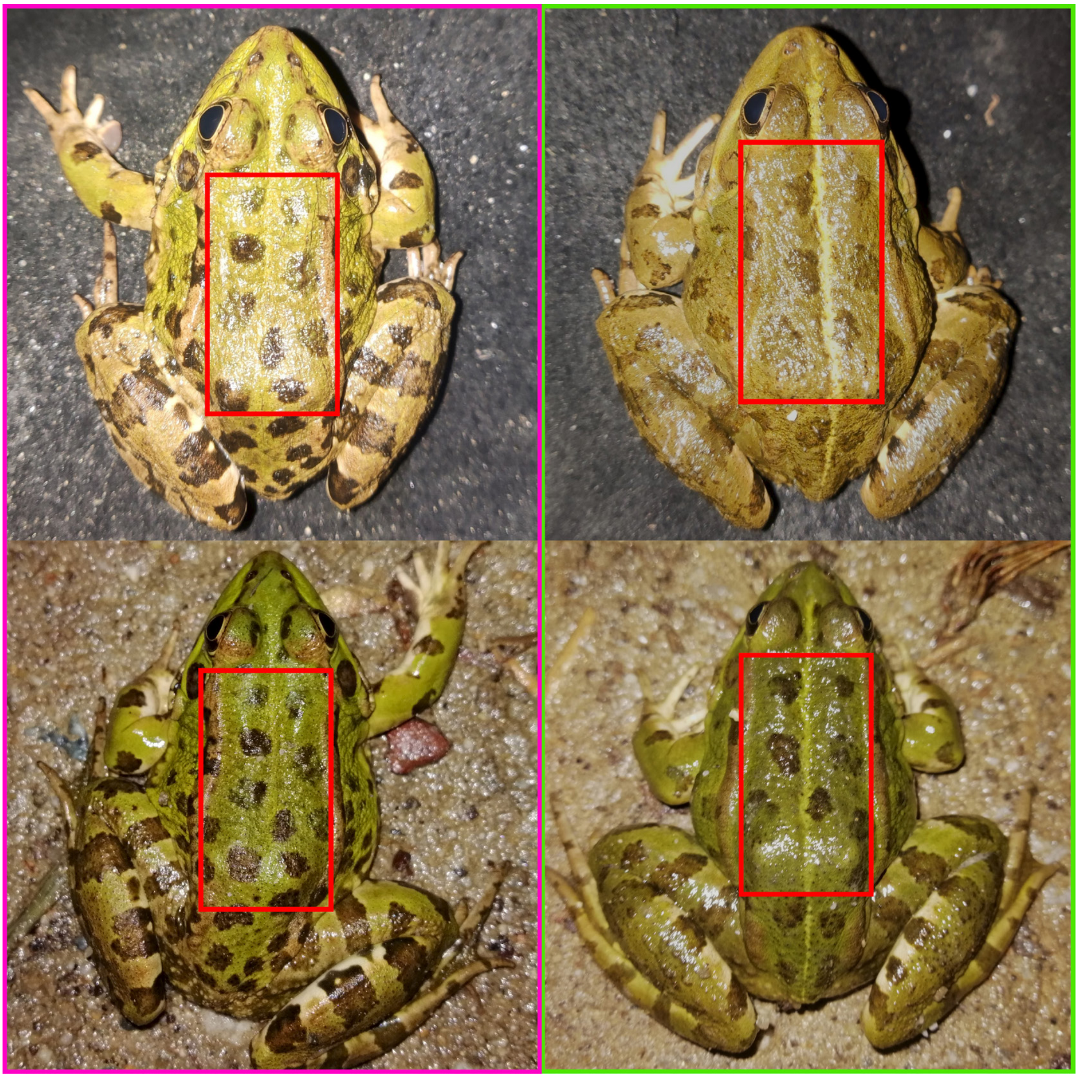

Figure 2. Dorsal views of two *Pelophylax perezi* males that moved from Pond B (bottom) to Pond A (top). Pink rectangle: Male 1. Green rectangle: Male 2. The red rectangle outlines the dorsal region cropped and standardised for photo-identification in WILD.ID. Photos by Carlos Caballero Díaz and Iria Labrado.

Results

We translocated 50 *P. perezi* from Pond A to Pond B on 25 October 2020. The translocated animals included 39 males (SVL 64 ± 11 mm; weight 29 ± 12 g), seven females (SVL 85 ± 10 mm; weight 61 ± 24 g), and four juveniles at a similar developmental stage (SVL 37 ± 9 mm; weight 6 ± 4 g).

Post-restoration sampling at Pond A yielded four individuals that could not be captured (30 April 2021) and 14 individuals (5 February 2022), with ten captured from the latter survey (males; *n* = 9; SVL 79 ± 4 mm, weight 42 ± 6 g; females; *n* = 1; SVL 99 mm; weight 86 g). On 30 April 2021, we did not detect any frogs at Pond B, while on 5 February 2022, five males were acoustically detected, but dense growth of *Rubus ulmifolius* hindered their capture.

Two males were confirmed as recaptures (Fig. 2). In both cases, WILD.ID assigned them the highest similarity score of each pair of photos (i.e., indicating the most probable correct match), and their identities were further verified by comparing spot patterns on the snout, supraorbital region, and hindlimbs using the entire photographs. Male 1 measured 77 mm (SVL) and weighed 37 g, whereas Male 2 measured 74 mm and weighed 40 g (Fig. 2).

Discussion

Previous studies show that individuals of *Pelophylax perezi* frequently move between waterbodies and often return to previously used ones (Capellà-Marzo et al., 2020), which facilitates colonisation and gene flow (Sánchez-Montes and Martínez-Solano, 2011; Sánchez-Montes et al., 2018; Capellà-Marzo et al., 2020; Reyes-Moya et al., 2022). Documented displacements between ponds typically do not exceed 600 m, with the longest previously recorded terrestrial movement being 910 m (Reyes-Moya et al., 2022). The 1.60 km displacement reported here represents both the longest homing movement and the greatest terrestrial distance travelled ever documented for *P. perezi*.

At least two individuals returned to their original breeding site after translocation, demonstrating homing behaviour despite the long distance. Although we conducted only one post-restoration sampling session and cannot account for all translocated individuals, this finding confirms that *P. perezi* has considerable capacity for dispersal (Reyes-Moya et al., 2022; Sánchez-Montes and Martínez-Solano, 2023). The mechanisms underlying this navigation ability remain poorly understood, but similarly to other amphibians (Grover and Wilbur, 2002; Campbell Grant et al., 2010) these frogs may have traveled along the dry stream bed, which retains higher humidity and vegetation cover than surrounding areas. In addition, the upslope position of Pond B may have facilitated this movement (Sánchez-Montes et al., 2018). Further monitoring studies are needed to understand the spatial ecology and orientation mechanisms of this species.

Photo identification is a useful, non-invasive tool to recognise recaptured amphibians (Sannolo et al., 2016; Gould et al., 2021; Caballero-Díaz et al., 2022a; Reyes-Moya et al., 2022; Gómez-Ramírez et al., 2023). Some software products are facilitating the identification of individuals in different sampling sessions, such as WILD.ID (Bolger et al., 2012), which has been proven to be trustworthy for amphibians (Renet et al., 2019). In other studies, WILD.ID has yielded between 50–75% correct matches at the top rank, which is lower than the reported for other software (Nipko et al., 2020; Burgstaller et al., 2021; Civantos-Lobo, 2025). As a result, the verification of photos is required for each comparison, since the top-ranked image may not correspond to the correct match (Nipko et al., 2020; Civantos-Lobo, 2025). Manual verification often involves examining other parts of the body (e.g., spots in the snout or legs, scars, malformations), which may be not included within the cropped and standardised rectangular region (Caballero-Díaz et al., 2022a; Reyes-Moya et al., 2022; Gómez-Ramírez et al., 2023; see red rectangle in Fig. 2). Despite this limitation, as well as the time investment required for standardising images (Cruickshank and Schmidt, 2017; Civantos-Lobo, 2025), photo-identification software is becoming increasingly valuable for long-term studies involving thousands of photographs (Sannolo et al., 2016; Gould et al., 2021; Reyes-Moya et al., 2022). Ranking images by similarity score significantly reduces processing time compared to manual matching (Bolger et al., 2012; Civantos-Lobo, 2025), which is a limitation for monitoring large populations (Langtimm et al., 2004).

Our findings have direct implications for *P. perezi* conservation, including the design of habitat corridors and establishment of protected area networks that maintain population connectivity and gene flow. Furthermore, our study underscores the value of photo-identification as a cost-effective, non-invasive tool for monitoring individual movements. Expanding surveying efforts is necessary to address the substantial knowledge gaps in amphibian movement ecology, particularly in fragmented landscapes where maintaining connectivity is critical for population viability.

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