Artificial water bodies as amphibian breeding sites: the case of the common midwife toad (Alytes obstetricans) in central Spain

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Abstract. Natural breeding sites for amphibians are decreasing in quantity and quality in temperate regions, resulting in local extinctions and increasing population fragmentation. Artificial water bodies (e.g., water tanks or cattle troughs) can represent suitable reproductive habitats for some amphibians, but demographic data are required to assess this assumption. We evaluated the role of artificial water bodies in the persistence of a species of population concern, the common midwife toad, Alytes obstetricans (Laurenti, 1768), at local and regional scales. We surveyed 275 water bodies to characterize the distribution of the species and detected 63 breeding populations of A. obstetricans where we estimated larval abundance. In addition, we monitored two populations for three consecutive breeding seasons using capture-mark-recapture methods based on photo-identification, assessing abundance, breeding success and the use of space of adult individuals captured on multiple occasions. Our results show that artificial sites are preferentially used as breeding sites in the region compared to natural aquatic habitats, providing key habitat for the species and hosting much larger numbers and densities of larvae than natural sites. At local scale, populations of A. obstetricans in artificial sites were abundant and characterized by high male breeding success. However, adults are spatially aggregated around breeding sites, with small home ranges, implying high vulnerability to population fragmentation. Our results suggest artificial breeding sites can sustain viable populations of A. obstetricans, provided measures promoting connectivity among breeding nuclei are considered.

Keywords: abundance, breeding success, capture-mark-recapture, conservation, habitat fragmentation, home range, photo-identification.

Introduction

Habitat degradation and loss are among the main causes of global amphibian declines (Cushman, 2006; Campbell Grant, Miller and Muths, 2020). In the Mediterranean region, amphibian breeding habitats (mostly temporary ponds; Gómez-Rodríguez et al., 2009) are in continuous decline in quality and numbers, due to a combination of factors including pollution, climate warming, the introduction of invasive species, and changes in land uses (Ferreira and Beja, 2013). For associated amphibian communities, this results in local extinction events and increasing population fragmentation at regional scale (Cruz, Rebelo and Crespo, 2006), calling for actions to mitigate these effects.

In the context of the massive, ongoing loss of aquatic habitats, artificial water bodies (e.g., water troughs for cattle or irrigation tanks) can provide alternative breeding sites for amphibians (Buono, Bissattini and Vignoli, 2019; Caballero-Díaz et al., 2020; Valdez, Gould and Garnham, 2021). These elements
are usually associated with traditional agriculture and livestock in rural areas and thus represent a valuable cultural heritage that is enriched with their added value as ecological refugia for a diversity of taxa (Brand and Snodgrass, 2010). While these artificial sites do not usually host diverse amphibian communities (but see García-González and García-Vázquez, 2011), some species can successfully exploit them, thus representing important targets for conservation actions.

Previous studies have shown that artificial water bodies can serve as important refugia for amphibian populations (Valdez, Gould and Garnham, 2021, and references cited therein) and may partly provide them with substitute habitats in the absence or scarcity of natural ones (Martínez-Abraín and Galán, 2018). However, there is little information about the role of artificial water bodies in amphibian population dynamics at regional scale. These sites, while successfully exploited, may offer low-quality habitats, insufficient to sustain viable populations, thus functioning as ecological traps (Battin, 2004; Robertson and Hutto, 2006; Clevenot, Carré and Pech, 2018). Alternatively, artificial sites may represent keystone habitats with an important role in patterns of regional connectivity and contributing to the long-term persistence of amphibian populations. Robust assessment of the importance of artificial sites for the resilience of associated amphibian communities should integrate regional population surveys with abundance estimates and data on key demographic parameters like breeding success.

We conducted population surveys of the common midwife toad, *Alytes obstetricans* (Laurenti, 1768) in the southeast of Comunidad de Madrid (central Spain), a rural area where artificial water bodies seem to represent an important resource for the survival of the species (Caballero-Díaz et al., 2020). We updated the distribution of *A. obstetricans* in the region and quantified the relative importance of natural and artificial habitats for breeding populations.

We also assessed inter-annual patterns of population abundance in two populations breeding in artificial sites using photo-identification of adults. This methodology has been successfully applied to the monitoring of amphibian communities (Reyes-Moya, Sánchez-Montes and Martínez-Solano, in press), but no studies have been conducted on our target species. Finally, we estimated breeding success and quantified movement patterns in these sites based on capture histories of photo-identified individuals. In light of these results, we discuss the role of artificial water bodies as keystones proving critical breeding sites for the long-term persistence of *A. obstetricans* populations in rural areas of central Spain.

**Materials and methods**

**Study species and area**

The common midwife toad (*Alytes obstetricans*) (fig. 1) is widespread in the northern half of the Iberian Peninsula and in Western Europe. The species is listed as Least Concern (LC) by the IUCN, but its populations are decreasing due to the combined effect of multiple threats, including habitat loss caused by changes in land use, and emerging infectious diseases like chytridiomycosis (Bosch et al., 2009). In central Spain, at the southern end of the species range, populations are fragmented and seem to depend on the availability of artificial water bodies for breeding (París et al., 2002; Martínez-Solano, 2006; Caballero-Díaz et al., 2020). Adults are mostly terrestrial and mate on land; after the amplexus, males carry the eggs for several weeks before releasing free-swimming tadpoles in streams, ponds, and artificial water bodies. Larval development is slow and may take over a year before metamorphosis (Bosch and Montori, 2022).

The study area is located in the southeast of Comunidad de Madrid (Central Spain; fig. 1). It is delimited by the rivers Tajo in the south, Henares in the north and Jarama in the west, and elevation ranges from 463 to 902 m.a.s.l. According to Koppen’s classification, the climate in the region is Csa Mediterranean, with an average annual temperature of 16°C and annual precipitation of 312.51 mm in the 2014-2021 period (Meteorological Station of Arganda del Rey; Comunidad de Madrid, 2022). Mining, hunting, livestock and agriculture are the main land uses, and the landscape is dominated by calcareous soils with limestones, clays and gypsum. The vegetation comprises Mediterranean maquis of holm oak (*Quercus ilex ballota*) and kermes oak (*Q. coccifera*), calcicolous scrubland with common thyme (*Thymus vulgaris*), scorpion broom (*Genista scorpius*), thyme rock rose (*Fumana thymifolia*) and esparto grass (*Stipa tenacissima*),
Artificial breeding sites in *Alytes*

Figure 1. A) Location of the study area in the SE of Comunidad de Madrid (Central Spain). The inset (red box) shows the location of our study area (C). B) *Alytes obstetricans* male carrying an egg clutch. C) Distribution of *A. obstetricans* in the study area. The main rivers in the study region are marked in blue, while the white line represents the border of Comunidad de Madrid. Circles of different sizes represent larval abundances (in three categories: small: 1-100, medium: 101-1000, large: >1000), and colors represent natural (purple) and artificial (yellow) breeding sites (for details, see table 1). Numbers in red color refer to localities of Valtierra (28) and Fuente del Valle (29). Photo credit: Carlos Caballero-Díaz.
Table 1. Water bodies, either of natural or artificial origin, with recorded breeding activity of *Alytes obstetricans* in the study area, estimates of larval abundances and number of adult individuals detected in each site.

| Id | Locality                     | Origin          | Typology          | Long  | Lat   | Larval abundance | Adults detected |
|----|------------------------------|-----------------|-------------------|-------|-------|------------------|-----------------|
| 1  | Valquejigoso                  | Artificial      | water tank        | −3.43 | 40.13 | 3500-4000        | 30              |
| 2  | Mingorrubio                  | Artificial      | water tank        | −3.43 | 40.11 | 1500             | 12              |
| 3  | Casa Dómène                  | Artificial      | water tank        | −3.43 | 40.11 | 330              | 0               |
| 4  | El Bosque                    | Natural         | pond              | −3.37 | 40.15 | 0                | 7               |
| 5  | El Rufo                      | Artificial      | water tank        | −3.36 | 40.14 | 40               | 5               |
| 6  | Arroyo Horcajuelo            | Natural         | stream            | −3.33 | 40.14 | 0                | 1               |
| 7  | Tierra del Agua              | Artificial      | water tank        | −3.34 | 40.13 | 400-500          | 7               |
| 8  | Fuente de los Perales        | Artificial      | water tank        | −3.32 | 40.12 | 900-1000         | 10              |
| 9  | Zacatín Este                 | Artificial      | water tank        | −3.39 | 40.1  | 4                | 3               |
| 10 | Valviesa                     | Artificial      | water tank        | −3.39 | 40.17 | 500-650          | 5               |
| 11 | Tejera Valdelaguna           | Artificial      | water tank        | −3.38 | 40.18 | 600-800          | 4               |
| 12 | Fuente María                 | Artificial      | water tank        | −3.37 | 40.19 | 900-1100         | 14              |
| 13 | La Gasca                     | Artificial      | water tank        | −3.35 | 40.25 | 550              | 5               |
| 14 | Barranco Oliver             | Artificial      | pond              | −3.34 | 40.24 | 950-1150         | 0               |
| 15 | Matagacha                   | Artificial      | water tank        | −3.34 | 40.23 | 700-900          | 5               |
| 16 | Fuente Vieja                 | Artificial      | pond              | −3.34 | 40.16 | 400-500          | 3               |
| 17 | Tejera Belmonte             | Artificial      | pond              | −3.34 | 40.15 | 0                | 2               |
| 18 | Valdelasierpe                | Natural         | stream            | −3.2  | 40.24 | 1                | 0               |
| 19 | Fuente Juan García           | Artificial      | water tank        | −3.18 | 40.16 | 20               | 0               |
| 20 | Olivar Villarejo             | Artificial      | pond              | −3.26 | 40.21 | 20               | 0               |
| 21 | Valdecañas                   | Artificial      | water tank        | −3.31 | 40.23 | 200              | 0               |
| 22 | Peña de la Cabrera           | Artificial      | pond              | −3.29 | 40.22 | 200-250          | 15              |
| 23 | El Horcajo                   | Artificial      | water tank        | −3.28 | 40.22 | 50               | 0               |
| 24 | Casasola                     | Artificial      | pond              | −3.29 | 40.29 | 400-450          | 5               |
| 25 | El Rejal                     | Artificial      | pond              | −3.29 | 40.3  | 50-100           | 5               |
| 26 | Pinar Villar                 | Artificial      | pond              | −3.24 | 40.34 | 300-400          | 11              |
| 27 | El Quemado                   | Artificial      | water tank        | −3.24 | 40.34 | 400-450          | 4               |
| 28 | Valtierra                    | Artificial      | water tank        | −3.4  | 40.29 | 1150             | 35              |
| 29 | Fuente del Valle             | Artificial      | pond              | −3.43 | 40.27 | 1500-1600        | 19              |
| 30 | Dehesa Morata                | Artificial      | pond              | −3.41 | 40.24 | 550-700          | 9               |
| 31 | Valdegatos                   | Artificial      | water tank        | −3.45 | 40.23 | 50-60            | 1               |
| 32 | Valgrande                    | Artificial      | pond              | −3.48 | 40.2  | 0                | 3               |
| 33 | Valdezarras                  | Artificial      | water tank        | −3.41 | 40.15 | 600-700          | 11              |
| 34 | La Rendija                   | Artificial      | pond              | −3.46 | 40.16 | 0                | 2               |
| 35 | Permiseba                    | Natural         | pond              | −3.47 | 40.14 | 200-250          | 8               |
| 36 | Fuente Valle Chinchón        | Natural         | stream            | −3.46 | 40.14 | 3                | 7               |
| 37 | Fuente de los ladrillos      | Artificial      | water tank        | −3.32 | 40.25 | 1500-2000        | 5               |
| 38 | Polideportivo Tielmes        | Artificial      | water tank        | −3.31 | 40.24 | 300              | 2               |
| 39 | El Cascón                    | Artificial      | water tank        | −3.21 | 40.26 | 10               | 2               |
| 40 | El Rey                       | Artificial      | water tank        | −3.31 | 40.18 | 600-800          | 18              |
| 41 | Valdecubillos                | Artificial      | abandoned quarry  | −3.39 | 40.24 | 0                | 2               |
| 42 | Valdericeda                  | Natural         | stream            | −3.38 | 40.14 | 90-100           | 14              |
| 43 | Barranco de la Vega          | Natural         | stream            | −3.4  | 40.34 | 0                | 2               |
| 44 | Charca Valviejo              | Artificial      | pond              | −3.39 | 40.17 | 2340             | 5               |
| 45 | La Cantarera                 | Artificial      | abandoned quarry  | −3.43 | 40.25 | 3                | 1               |
| 46 | Jardines Príncipe            | Artificial      | irrigation channel| −3.6  | 40.04 | 100-150          | 2               |
| 47 | Cortijo San Isidro           | Artificial      | irrigation channel| −3.56 | 40.06 | 0                | 5               |
| 48 | Balcón del Tajo              | Artificial      | pond              | −3.48 | 40.08 | 100-120          | 5               |
| 49 | Arroyo Valdepastores         | Natural         | stream            | −3.84 | 39.92 | 0                | 2               |
| 50 | Villamejor                   | Natural         | pond              | −3.77 | 39.94 | 2                | 0               |
| 51 | La Chimenea                  | Artificial      | irrigation channel| −3.54 | 40.07 | 0                | 3               |
| 52 | Mingovela                    | Artificial      | water tank        | −3.39 | 40.09 | 70-80            | 0               |
| 53 | Barranco Valdepozuelo        | Artificial      | pond              | −3.44 | 40.26 | 150-200          | 6               |
| 54 | Particular Orusco            | Artificial      | water tank        | −3.21 | 40.29 | 60-80            | 10              |
| 55 | Piscina Orusco               | Artificial      | swimming pool     | −3.2  | 40.29 | 0                | 4               |
Table 1. (Continued.)

| Id | Locality             | Origin     | Typology           | Long | Lat | Larval abundance | Adults detected |
|----|----------------------|------------|--------------------|------|-----|------------------|-----------------|
| 56 | Arroyo1 Vía Verde    | Natural    | stream             | −3.2 | 40.28 | 0                 | 1               |
| 57 | Arroyo 2 Vía verde   | Natural    | stream             | −3.19| 40.31 | 0                 | 3               |
| 58 | Valdepinar           | Artificial | water tank         | −3.36| 40.1  | 50-60             | 0               |
| 59 | Fuente Pata          | Artificial | water tank         | −3.43| 40.13 | 85                | 2               |
| 60 | El Robledillo        | Artificial | pond               | −3.16| 40.35 | 40                | 0               |
| 61 | Entrada Belmonte     | Artificial | water tank         | −3.34| 40.14 | 0                 | 1               |
| 62 | Arroyo Valdelahiguera| Natural    | stream             | −3.41| 40.24 | 150-200           | 0               |
| 63 | Canal del Henares    | Artificial | irrigation channel | −3.29| 40.57 | 0                 | 10              |

plantedons of Aleppo pine (Pinus halepensis), and wetlands with white poplar (Populus alba) and salt cedar (Tamarix sp.). Farmlands of olive trees, vineyards and crops of wheat or corn are widespread in the region.

**Sampling and photo-identification**

We surveyed 275 water bodies in the study area to document the presence of breeding populations of *A. obstetricans* in the years 2018-2021. This list included 44 localities previously reported in Caballero-Díaz et al. (2020). Water bodies were categorized as artificial (water tanks, fountains, water troughs, reservoirs, artificial ponds) or natural (rivers, streams, puddles, natural ponds and storm pools) in order to assess their relative importance for the species. We visited the majority of localities with presence of *A. obstetricans* every year (2018-2021), during late spring and early summer, and several times each year; however, as we encountered various breeding sites for the first time during 2019 or 2020, these sites have been surveyed two or three years, with at least one visit per year. We conducted nocturnal surveys in all localities with presence of *A. obstetricans* to estimate larval abundances, following Caballero-Díaz et al. (2020). Briefly, we visually counted all tadpoles in water bodies whenever possible, and when this was not feasible due to pond size, we defined sampling sections accounting for heterogeneity, counted all tadpoles and then extrapolated to the entire pond area. All localities were surveyed at least once a year during the months of maximum larval abundance (May-September); for localities reported in Caballero-Díaz et al. (2020) this includes four yearly estimates; for localities newly reported in this paper, we obtained a minimum of two estimates from different breeding seasons. As multiple estimates from different visits were available, we report those with the highest recorded abundance (see Caballero-Díaz et al., 2020). We also estimated larval densities, measured in number of tadpoles/m² for water tanks and ponds, and number of tadpoles/m² of riverbed for streams, and counted the number of adult *A. obstetricans* detected at each site (again, when multiple adult count numbers were available for a given locality, we provide the maximum recorded value).

We selected two localities in the municipality of Arganda del Rey for the monitoring of populations of *A. obstetricans*: Valtierra (40.29N 3.40W, 722 m.a.s.l.) and Fuente del Valle (40.27N, 3.43W and 657 m.a.s.l.), separated 3.6 km from each other. The population in Valtierra breeds in a cattle trough (supplementary fig. S1a: width = 0.35 m, length = 6.5 m, depth = 0.30 m), while the population in Fuente del Valle breeds in an artificial pond (supplementary fig. S1b: width = 3 m, length = 6 m, and maximum depth = 0.45 m) and two water troughs (supplementary fig. S1c: surface = 1.2 m², maximum depth = 0.2 m; supplementary fig. S1d: surface = 1.2 m² and maximum depth = 0.4 m). A team of 2-6 persons visited each locality for a total of 81 nocturnal surveys from September 2018 to July 2021, including three consecutive breeding seasons (2019-2021), inspecting all water bodies and the surrounding areas for 1-3 hours. Each adult individual found was photographed with a smartphone (resolution: 20 Mpx), sexed, measured (snout-vent length, to the nearest 0.1 mm), weighted (to the nearest 0.1 g), and its position was georeferenced with a GPS device. Due to the lack of conspicuous secondary sexual characters distinguishing males and females, some individuals could not be unambiguously sexed (unless we recorded mating pairs, or eggs were visible through the ventral skin of gravid females, or males were detected carrying eggs). We recorded mating calls, males carrying eggs, and tadpoles as evidence of breeding activity of *A. obstetricans* in the study area. To avoid the transmission of pathogens, we used disposable gloves and disinfected field material using a bleach solution (1:10 bleach:water).

We created a database with all recorded photographs, organized by locality and sampling session, and used the software WildID (Bolger et al., 2012) for photo-identification (supplementary fig. S2). First, we used NOMACS (http://nomacs.org) to optimize photos for comparison by defining a dorsal rectangle from the posterior borders of the eyes to the vent. For each photo, WildID returned a ranking of the 20 most similar assignments by comparing size, shape and position of dorsal spots in individuals in the entire database. These assignments were subsequently checked by eye, to ensure robust identification, and then used to construct tables describing capture history records for all individuals in each population.

**Adult abundance, breeding success and use of space**

We estimated the number of *A. obstetricans* adults (Nₐ) for the breeding seasons of 2019-2021 in the localities of Valtierra and Fuente del Valle by analyzing individual capture history records using the POPAN formulation (Schwarz and Arnason, 1996) as implemented in program MARK v9.0 (White and Burnham, 1999). POPAN models open populations, that is, considering entrances (immigration or
recruitment) and exits (emigration or death), based on estimation of the following parameters: 1) population size, or the total number of individuals present in each locality during the study period \((N)\), 2) the probability of survival of individuals across sampling sessions \((\varphi)\), 3) the probability of capture of each individual at each sampling session \((p)\), and 4) the probability of entrance of individuals before each sampling session \((pent)\). We built a set of models by combining parameters \(\varphi\), \(p\), and \(pent\), which were allowed to vary according to time \((t)\), sex \((g\); male, female or unknown\), both factors and their interaction \((g^*t)\), or maintained as constant \((.)\). The resulting models were ranked based on Akaike’s information criterion corrected for small sample sizes \((AICc;\) Akaike, 1974\)). To check if the saturated model complied with POPAN assumptions – equal probabilities of survival and capture – we conducted two Goodness of fit tests, 3.SR and 2.CT, using the program U_CARE (Choquet et al., 2009). The test 3.SR controls for possible transience effects, while the 2.CT test assesses trap dependence.

As a proxy for breeding success, we counted the number of egg clutches carried by each \(A.\) obstetricans male during the entire study period in the populations of Valtierra and Fuente del Valle. We used pictures to count eggs and detect potential instances of multiple clutches carried by a single male, making a distinction between simultaneous multiple breeding (new clutch added within a month of the first observation of eggs, with near-simultaneous development of embryos from different females) and sequential multiple breeding (new clutch added between one and four months after the first observation of eggs, with non-overlapping development of embryos from different females).

Finally, in the locality of Valtierra we assessed fine-scale terrestrial habitat use and movement patterns separately for males and females of \(A.\) obstetricans in the breeding season of 2020-2021, based on the geographic coordinates of capture locations of individuals observed three or more times. For each individual, we calculated: 1) the accumulated distance across recapture events, 2) the maximum distance between consecutive recapture events, and 3) the area of the minimum convex polygon delimited by all geolocations. We also calculated the distances of capture locations of all identified toads to the cattle trough they used for breeding.

**Results**

**Breeding populations and larval abundance**

We detected 63 breeding populations of \(A.\) obstetricans in the study area (fig. 1, table 1). The species breeds disproportionately more in artificial sites \((n = 51)\) than in natural water bodies \((n = 12)\) \((\text{Chi Square test}, \chi^2 = 18.994, p < 0.001)\), considering their relative availability (artificial sites: 57%, \(n = 157\); natural sites, 43%, \(n = 118\)). Among artificial water bodies used as breeding sites by \(A.\) obstetricans, most \((n = 28, 54.9%)\) were water tanks or drinking troughs, 16 \((31.4%)\) were artificial ponds, and the rest included irrigation channels (four), ponds in abandoned quarries (two), and an unused swimming pool. Natural breeding sites comprised nine streams and three natural ponds (table 1).

We estimated a total larval abundance in the study area ranging from 22,618 to 26,005 tadpoles (minimum and maximum counts). Of these, artificial water bodies sustained between 22,172 and 25,449 tadpoles, an average of 435-499 tadpoles per site and with observed densities ranging between median (MED) minimum counts \(= 47.3\) tadpoles/m\(^2\), with an interquartile range (IQR) of 97.9 tadpoles/m\(^2\), and MED maximum counts \(= 47.3\) tadpoles/m\(^2\) (IQR \(= 116.7\) tadpoles/m\(^2\)). The relative importance of different types of artificial water bodies varied, with artificial ponds hosting a larval abundance of 7000-7640 tadpoles (average: 438-478 per site; density: MED minimum counts \(= 20\) tadpoles/m\(^2\) and IQR \(= 83.2\) tadpoles/m\(^2\), MED maximum counts \(= 21.9\) tadpoles/m\(^2\) and IQR \(= 84.2\) tadpoles/m\(^2\)) and water tanks/cattle troughs having the largest contribution with 15,172-17,808 tadpoles (average: 542-636 per site; density: MED minimum counts \(= 60\) tadpoles/m\(^2\) and IQR \(= 137.2\) tadpoles/m\(^2\), MED maximum counts \(= 75\) tadpoles/m\(^2\) and IQR \(= 156.8\) tadpoles/m\(^2\)). Natural breeding sites supported low larval densities, with total estimates of 446-556 tadpoles (average: 37-46 tadpoles per site; density: MED minimum counts \(= 14.3\) tadpoles/m\(^2\) and IQR \(= 14.3\) tadpoles/m\(^2\), MED maximum counts \(= 17.9\) tadpoles/m\(^2\) and IQR \(= 17.9\) tadpoles/m\(^2\); density in streams: MED minimum counts \(= 3.1\) tadpoles/m and IQR \(= 6.6\) tadpoles/m, MED maximum counts \(= 4.1\) tadpoles/m and IQR \(= 8.4\) tadpoles/m).
**Adult abundance**

During the period September 2018-July 2021, we identified 182 individuals of *A. obstetricans* (63 males, 33 females, and 86 of unknown sex) in Valtierra, of which 122 were recaptured at least once (recapture rate: 67.03%). The total number of captures in this locality was 630. In Fuente del Valle, the recapture rate was lower (50%), with 90 individuals identified (22 males, 16 females, and 52 of unknown sex), of which 45 were recaptured (total number of captures: 236). We did not detect individuals dispersing between both localities. POPAN analyses resulted in higher abundance estimates in Valtierra than in Fuente del Valle (fig. 2). The maximum estimate was obtained in Valtierra in 2021, with 151 individuals: 66 males (95% CI 51-81), 34 females (95% CI 22-46), and 51 of unknown sex (95% CI 37-68; fig. 2). In Fuente del Valle we estimated a maximum abundance of 51 individuals in 2020: 14 males (95% CI 11-17), 12 females (95% IC 9-15), and 25 of unknown sex (95% IC 20-30; fig. 2). The best models were similar for both localities and for the majority of the breeding seasons analyzed, and considered parameters \( p \) and \( pent \) as dependent on time \( t \), and the probability of survival dependent on sex \( g \), time \( t \) or constant \( . \) (supplementary table S1). Goodness of fit tests did not show consistent departures from model assumptions, but in Valtierra we found evidence of trap-dependence for females in 2019 and for males in 2021, and a significant (but weak) transience effect for males in 2020 (supplementary table S2).

**Breeding success**

The breeding period of *A. obstetricans* was mainly concentrated between April and June (76.5% of the egg clutches observed) in Valtierra and between June and August (61.5%) in Fuente del Valle. During the study period, we counted a total of 102 egg clutches in Valtierra (11 in 2019, 40 in 2020, and 51 in 2021; table 2, supplementary fig. S3), and 26 egg clutches in Fuente del Valle (four in 2019, seven in 2020, and 15 in 2021; table 2, supplementary fig. S3). The vast majority of males carrying more than one egg clutch in the same breeding season

### Table 2. Number of egg clutches of *Alytes obstetricans* detected each year and number of males carrying eggs along the study period.

| Locality | Year | Males with 1 clutch | Males with 2 clutches | Males with 3 clutches | Total males breeding | Total clutches |
|----------|------|---------------------|-----------------------|-----------------------|----------------------|----------------|
| Valtierra | 2019 | 9                   | 1                     | 0                     | 10                   | 11             |
|          | 2020 | 32                  | 4                     | 0                     | 36                   | 40             |
|          | 2021 | 25                  | 10                    | 2                     | 37                   | 51             |
| Fuente del Valle | 2019 | 4                   | 0                     | 0                     | 4                    | 4              |
|          | 2020 | 7                   | 0                     | 0                     | 7                    | 7              |
|          | 2021 | 11                  | 2                     | 0                     | 13                   | 15             |

![Figure 2](https://example.com/figure2.png) **Figure 2.** Abundance estimates \( (N_a, \) with their 95% confidence intervals) for *A. obstetricans* males, females and individuals of unknown sex in Valtierra and Fuente del Valle in the breeding seasons of 2019, 2020 and 2021, based on POPAN analyses.
(16 out of 19, table 2) were recaptured with a difference of more than one month and less than four months (sequential multiple breeding); only three males were observed to add egg clutches within a month (simultaneous multiple breeding).

Across the study period, 40 males in Valtierra (63.5% of identified males) were recorded carrying only one egg clutch, while 20 males (31.7%) were observed carrying two or more different egg clutches, either in the same or in different years (2 clutches: eight males; 3 clutches: 10 males; 4 clutches: two males). In Fuente del Valle, 17 males (77.3%) were observed with one egg clutch across the study period, two males (9.1%) carried two different egg clutches, and only one toad was observed with egg clutches in three different years (4.5%).

Use of space

Thirty sexed individuals and five of unknown sex were captured at least three times during the breeding season of 2020-2021 in Valtierra (table 3, fig. 3). Of these, males (n = 24) averaged larger estimated home ranges, longer accumulated distances, and longer distances across consecutive recapture events than females (n = 6) (table 3). In addition, maximum values for all variables were also higher for males, especially home range estimates (table 3).

Average and maximum distances from the breeding site for the 69 sexed individuals captured in Valtierra (males = 46, females = 23) were similar across sexes (table 3). Two individuals of unknown sex were found at the farthest distances from the breeding site at 146 and 148 m, respectively.

Table 3. Descriptive statistics of spatial displacements of males and females of Alytes obstetricans in Valtierra during the 2020-2021 breeding season.

|                        | Male | Female |
|------------------------|------|--------|
| Number of adults captured once | 46   | 23     |
| Maximum distance from water body (m) | 137  | 135    |
| Average distance from water body (m) | 42   | 47     |
| Number of adults captured at least three times | 23   | 6      |
| Average accumulated distance (m) | 71   | 56     |
| Maximum accumulated distance (m) | 161  | 111    |
| Average distance across consecutive recaptures (m) | 38   | 33     |
| Maximum distance across consecutive recaptures (m) | 78   | 43     |
| Average estimated home range (m²) | 312  | 172    |
| Maximum estimated home range (m²) | 1442 | 403    |

Figure 3. Geolocations of Alytes obstetricans males (left) and females (right) in the locality of Valtierra during the breeding season of 2020-2021. White polygons approximate home ranges for individuals captured three or more times, white lines represent distances covered by individuals captured twice, and white dots show the locations of individuals captured only once. The orange line represents the minimum convex polygon including all geolocations of all toads (males, females and individuals of unknown sex combined).
Discussion

Our study provides evidence that artificial water bodies represent key habitats for *A. obstetricans* in the study region. They comprise the majority of breeding sites used by the species and host much larger larval abundances than available natural sites. However, artificial habitats have specific threats that, combined with the high philopatry and low dispersal capacity of the species, could compromise the viability of breeding populations of *A. obstetricans* at the regional scale.

In the study region, *A. obstetricans* preferentially use artificial over natural water bodies for breeding, in agreement with previous studies in other Iberian populations, which show the species can breed successfully and be locally abundant in these habitats (París et al., 2002; García-González and García-Vázquez, 2011; Gálvez, McKnight and Monrós González, 2018). The preference of *A. obstetricans* for artificial water bodies probably reflects both their suitability for the completion of the biological cycle of the species (because these habitats usually provide long hydroperiods) and the poor quality of natural habitats in large areas in their native range (Valdez et al., 2015). Most rivers and streams in our study area are polluted and/or host large populations of alien invasive species that prey on amphibian larvae, like the red crayfish *Procambarus clarkii* (Cruz, Rebelo and Crespo, 2006; Cruz et al., 2008), and natural ponds are scarce, making artificial water bodies essential for the long-term survival of the species.

The importance of artificial water bodies in the local dynamics of *A. obstetricans* populations is highlighted by the fact that they host approximately 10 times higher larval abundances and 20 times higher larval densities than natural sites, with estimates exceeding 100 tadpoles/m² in the case of water tanks. Despite their generally small size, we recorded 12 water tanks with abundance estimates of >500 tadpoles, five of which exceeded 1000 larvae (localities 1, 2, 12, 28, 37; table 1). Importantly, artificial ponds also contributed to host abundant populations, with four sites having estimates >500 tadpoles, of which three (localities 14, 29, 44; table 1) hosted >1000 tadpoles. These sites are generally small, associated with springs or fountains and maintain water at least eight or nine months a year, and thus represent important assets for the conservation of the species. Nevertheless, their continued use as amphibian breeding habitats requires active management to prevent their decay (Caballero-Díaz et al., 2020). In fact, while many artificial water bodies hosted healthy *A. obstetricans* breeding populations, some had low abundances, including localities 5, 19, 20, or 34 (table 1). These cases are largely related with the abandonment of traditional agricultural practices (Caballero-Díaz et al., 2020), which is part of a broader socioeconomic issue causing negative impacts on native amphibians in southern Europe (Canessa et al., 2013). Given the low number of natural breeding sites and the technical and economic difficulties involved in restoring stream and river habitats, conservation measures focusing on the creation and maintenance of artificial breeding sites as part of an extensive network of interconnected breeding nuclei for *A. obstetricans* are paramount.

Our capture-mark-recapture approach based on photo-identification proved useful to obtain robust estimates of adult population sizes and breeding success in our study populations. To our knowledge, this is the first study to estimate population sizes in *A. obstetricans* using photo-identification. Our results suggest that artificial water bodies can sustain viable populations of *A. obstetricans* in the long term, especially in Valtierra, which hosts a larger population than Fuente del Valle, with estimates exceeding 150 adult individuals and high larval counts (table 1, fig. 2). However, considering the extensive fluctuations in abundance that are characteristic of amphibian populations (Pechmann et al., 1991), further research is needed to better characterize...
temporal variation in abundance estimates. Our approach provides a simple, inexpensive framework to obtain robust estimates based on periodic surveys focusing specially on the months with higher reproductive activity (April-August, supplementary fig. S3).

Estimating breeding success in wild amphibian populations is challenging; we used the number of egg clutches carried by males throughout our study as a proxy. While we accounted for the possibility of multiple mating (Márquez, 1992; Schleich, Kastle and Kabisch, 1996) (both simultaneous and sequential), our results should be taken with caution because of imperfect detection. Alternative estimates of breeding success, for instance using molecular markers (Sánchez-Montes et al., 2017), should be considered in order to obtain more robust estimates of the magnitude of breeding stocks of *A. obstetricans* in artificial breeding sites. Provisionally, our results suggest high breeding success of *A. obstetricans* in our study sites, with over 60% and up to 75% of males mating at least once. However, the overall number of breeders (*N_b*) in *A. obstetricans* populations may be low, as shown by Tobler, Garner and Schmidt (2013) in Swiss populations of the species. High breeding success may be interpreted as a compensatory mechanism to avoid inbreeding depression in small, isolated populations; robust assessment of patterns of connectivity among breeding populations of *A. obstetricans* is thus critical to assess their long-term viability.

Functional connectivity depends both on the relative distances among breeding sites and the dispersal capacity of the species. Regarding the former, artificial breeding sites form an extensive well-connected network in the core of the study area (fig. 1), except at the northern and southern extremes, where isolated populations exist at high extinction risk (localities 49, 50, 63). On the other hand, there is little information about the spatial ecology of the species, although previous studies suggest low dispersal capacity (Gosá, 2003; Ryser et al., 2003). Our results during the monitoring of the Valtierra population are consistent with this idea and suggest high philopatry in *A. obstetricans*, with adults of both sexes concentrating close (<150 m) to the breeding site. Accordingly, estimated home ranges, albeit probably underestimated, are generally small, with minor differences between sexes. These results are consistent with regional estimates of gene flow based on microsatellites (Gutiérrez-Rodríguez et al., in press), which show strong, fine-scale genetic differentiation and suggest population fragmentation could represent an important threat for the regional persistence of the species. This could be counteracted by the creation of breeding sites in strategic locations informed by our extensive survey.

Artificial water bodies have been traditionally used for different purposes (irrigation, cattle, washing) and are usually close to villages in rural areas, including recreational facilities or gardens in historical monuments, like localities 38 and 54. The presence of breeding populations of *A. obstetricans* in these areas implies challenges and opportunities to make compatible use of artificial water bodies for people and biodiversity, with a fundamental role for education. Our study shows that artificial water bodies are key for the survival of *A. obstetricans* in rural areas in Central Spain, hosting locally abundant populations with high reproductive success that can represent pivotal nodes in dense breeding networks. However, these habitats have specific threats that should be addressed in addition to the restoration of their natural habitats, taking into account aspects of their biology like their phenology, philopatric behavior and low dispersal capacity.

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