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The Role of Artificial Breeding Sites in Amphibian Conservation: a Case Study in Rural Areas in Central Spain

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Abstract.—**Amphibians in rural landscapes often utilize various types of artificial constructions originally designed for irrigation, livestock supply, or other purposes (e.g., water tanks or cattle troughs) as breeding sites. These sites potentially function as local refugia; however, their importance for amphibian communities has yet to be widely assessed. Here we evaluate the role of artificial constructions in the persistence of amphibian populations in rural areas of central Spain, focusing on two species of conservation concern: the Common Midwife Toad, *Alytes obstetricans* (Laurenti 1768), and the Parsley Frog, *Pelodytes punctatus* (Daudin 1802). We surveyed 130 water bodies at 113 localities in an area of 1,450 km^2 during the breeding season of 2018 and documented the type of breeding site, species abundance, amphibian community structure, and any detectable threats. We found non-random patterns of breeding site selection and amphibian species co-occurrence in which *A. obstetricans* tended to inhabit artificial water tanks with simpler amphibian communities, whereas *P. punctatus* tended to co-occur in naturalized ponds in abandoned quarries with complex amphibian community structures. We discuss the relevance of artificial breeding sites for the resilience of amphibian populations and propose conservation measures to improve their efficiency in the face of detected threats, including trap effects, alien species, and chytridiomycosis.**

Key Words.—*Alytes obstetricans*; chytridiomycosis; habitat loss; invasive species; *Pelodytes punctatus*

Introduction

Amphibians are declining globally, with 43.2% of species experiencing population reductions and only 0.5% showing positive trends (Stuart et al. 2004). According to scientific consensus, habitat loss is the main factor contributing to the extirpation of species and entire communities (Stuart et al. 2004; Cushman 2006; Becker et al. 2007; Bickford et al. 2008; Sodhi et al. 2008). Most amphibians have biphasic life cycles and depend on adequate terrestrial and aquatic habitats for long-term demographic stability. Due to the highly philopatric behavior and low dispersal capacity of many species (Rittenhouse and Semlitsch 2007; Gutiérrez-Rodríguez et al. 2017), adequate management of both wetlands and surrounding terrestrial habitats is critical for amphibian conservation (Semlitsch and Bodie 2003).

Whereas the loss of terrestrial habitats is of major concern, aquatic habitats are also subjected to many threats and have rapidly declined in number and quality globally (Beebee and Griffiths 2005). The decline in wetland habitat can be illustrated by the Mediterranean wetlands, a preferred habitat for many amphibian species in southern Europe. These wetlands and accompanying biota are in a continuous decline due to a combination of factors including changes in land use (Beja and Alcazar 2003), the introduction of invasive species (Green et al. 2016), increasingly frequent droughts associated with climate change (Lehner et al. 2006), the spread of infectious disease like chytridiomycosis (Bosch et al. 2018), and the use of high concentrations of pesticides and agrochemicals (Marco 2002; Ortiz-Santaliestra and Egea-Serrano 2013). Furthermore, the long-term persistence of Mediterranean amphibian communities requires the maintenance of networks of ponds with different hydroperiods. These networks provide a wide range of breeding habitat characteristics that accommodate the preferences of species in the face of inter-annual climatic variation (Beja and Alcazar 2003; Gómez-Rodríguez et al. 2009).

In addition to natural wetlands, amphibians in rural areas often use various artificial constructions originally designed to hold water for irrigation, livestock supply, or other purposes (including fountains, water tanks, or cattle troughs) as breeding sites (García-González and García-Vázquez 2011; Buono et al. 2019). These sites are potentially critical for the resilience of diverse amphibian communities, yet their importance has not been widely assessed (Gálvez et al. 2018). Here we evaluate the role of artificial breeding sites in the persistence of amphibian populations in rural areas of central Spain.

We focus on two species of conservation concern in this region: the Common Midwife Toad, *Alytes obstetricans* (Laurenti 1768), and the Parsley Frog, *Pelodytes*

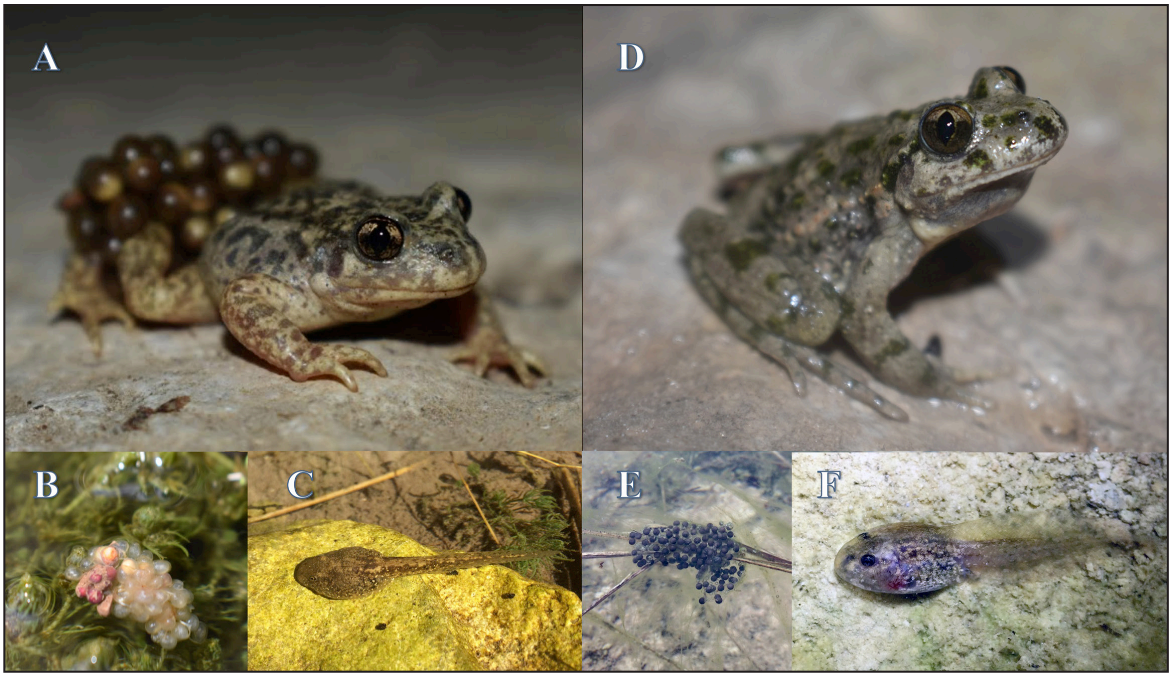

Figure 1. The two target species of the study: the Common Midwife Toad (*Alytes obstetricans*; A = adult with eggs, B = eggs, C = larva) and the Parsley Frog (*Pelodytes punctatus*; D = adult, E = eggs, F = larva). (A, B, D photographed by Carlos Caballero-Díaz and C, E, F by Íñigo Martínez-Solano).

punctatus (Daudin 1802; Fig. 1). *Alytes obstetricans* has a wide distribution that ranges from the northern half of the Iberian Peninsula through continental France and up into central Germany (Bosch et al. 2009), whereas *P. punctatus* occurs in Iberia, France, and northwestern Italy, with subspecies *P. p. hespericus* being endemic to Iberia (Díaz-Rodríguez et al. 2017; Dufresnes et al. 2020). *Alytes obstetricans* often requires permanent waters to complete its long larval period, with a portion of the larval population overwintering prior to metamorphosis (Bosch, J. 2014. Sapo partero común - *Alytes obstetricans*. In: Enciclopedia Virtual de los Vertebrados Españoles. Salvador, A., and I. Martínez-Solano. (Eds.). Museo Nacional de Ciencias Naturales, Madrid. http://www.vertebradosibericos.org/ [Accessed 28 November 2018]), whereas *P. punctatus* prefers temporary ponds suitable for their short larval period (Escoriza, D. 2017. Sapillo moteado mediterráneo - *Pelodytes hespericus*. In: Enciclopedia Virtual de los Vertebrados Españoles. Salvador, A., and I. Martínez-Solano (Eds.). Museo Nacional de Ciencias Naturales, Madrid. http://www.vertebradosibericos.org/ [Accessed 7 January 2020]). Both species can be found in syntopy within the study area, where habitat fragmentation and scarcity of adequate breeding sites represent the main threats for their persistence (Martínez-Solano and García-París 2001; Martínez-Solano 2006; Paños et al. 2011).

We surveyed 130 water bodies at 113 localities in an area of 1,450 km² during the breeding season of 2018. We documented the type of breeding site, species abundance, amphibian community structure, and any detectable threats at these sites. We discuss the relevance of artificial breeding sites for the conservation of amphibian communities in rural Mediterranean areas and propose conservation measures to improve their efficiency.

Materials and Methods

Study area.—The study area was located in the southeast of Comunidad de Madrid (central Spain) and includes 32 municipalities (see Appendix 1). It is a 1,450 km² area delimited by the Tajo River in the south, the Henares River in the north and includes the basins of the Jarama and Tajuña rivers (Fig. 2). Three nature reserves, the Regional Park Cursos Bajos del Río Jarama y Manzanares, and two Natura 2000 areas (Cortados y cantiles de los ríos Jarama y Manzanares, ES0000142; and Vegas, cuestas y páramos del sureste de Madrid, ES3110006; https://ec.europa.eu/environment/nature/natura2000/data/index_en.htm) provide legal protection for different sections of the study area. According to Koppen's classification the climate is Csa - Mediterranean, with a dry season extending for four months, an annual average precipitation of 325 mm and an average temperature of 15.3º C in the period 2007–2017 (http://gestiona.madrid.org/azul_internet/run/j/InformExportacionAccion.icm?ESTADO_MENU=8). Livestock, agriculture, hunting, and mining are

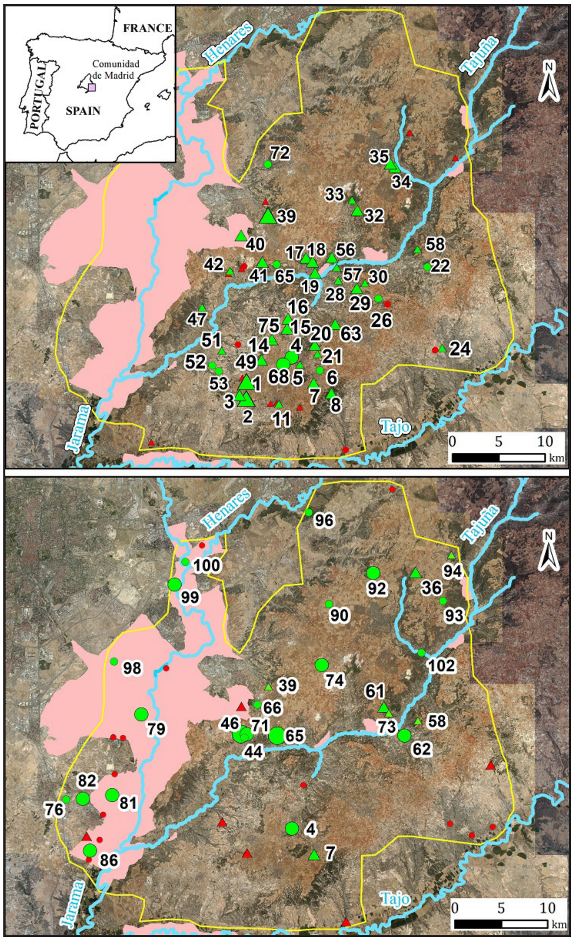

Figure 2. The location of the study area in Comunidad de Madrid, central Spain (inset), and the distribution of the Common Midwife Toad (*Alytes obstetricans*; top) and the Parsley Frog (*Pelodytes punctatus*; bottom) in the study area. Colors represent localities where the species was detected in the present study (green) or with previous reports that were not confirmed in the present study (red). Symbol sizes are proportional to recorded abundances (Appendix 2); locality numbers correspond to Appendix 1. Rivers (blue), study area outline (yellow), and protected areas (pink) are also shown. Triangles represent artificial sites, and circles represent natural water bodies.

representative of the main land uses. Natural vegetation includes calcicolous scrubland with Common Thyme (*Thymus vulgaris*), Scorpion Broom (*Genista scorpius*), Thyme Rock Rose (*Fumana thymifolia*), and Esparto Grass (*Stipa tenacissima*), Mediterranean Maquis Shrubland of Holm Oak (*Quercus ilex ballota*) and Kermes Oak (*Q. coccifera*), and Aleppo Pine (*Pinus halepensis*) plantations.

Site categorization.—We compiled a list of 130 water bodies in 113 localities that could potentially be used as breeding sites by amphibians. Some localities include more than one water body within a 100-m radius; we considered these separately in the analyses due to their different typologies, but because these water bodies are within the dispersal potential of each species, each of these localities represents a potentially panmictic breeding population (Fig. 2). We determined a preliminary list of localities from previous records regarding the two target species from Martínez-Solano (2006), Paños et al. (2011), and personal communications from local administrations and landowners. We completed this list with additional localities showing *a priori* favorable conditions for the presence of amphibians. First, we inspected satellite images using geographical information system tools to select locations within the study area that had water troughs, puddles, ponds, or streams. We then visited selected locations to confirm the presence of adequate habitats for amphibians and subsequently included them in the list of sampling localities (Fig. 2; Appendix 1). The final list of localities represents the most comprehensive catalogue of water bodies with breeding site potential for the two target species in the study area to date. We classified the types of aquatic water bodies in four categories (Fig. 3): (1) water tanks for livestock, agriculture and traditional uses with vertical walls (troughs, fountains); (2) artificial, semi-naturalized ponds; (3) natural water sites such as swamps, puddles, ponds or streams; and (4) artificial ponds in abandoned quarries (Appendix 1). We used Kruskal-Wallis' test to assess whether there were differences in the average number of amphibian species breeding in each type of aquatic water body and Chi-square tests ($\alpha = 0.05$) to investigate breeding site preferences in *A. obstetricans* and *P. punctatus.*

Field methods.—During 2018 we visited each locality at least once every three weeks (range, 1–11 visits per locality and water body) from January to June, which is the main period of activity for amphibians in the region (Martínez-Solano and García-París 2001). Detectability for each species was defined as the number of visits required to find the species at each water body for the first time. Surveys included larval counts and nocturnal transects to record the number of adult individuals of the two target species in water bodies and the surrounding terrestrial habitat. We sampled larvae with dip nets and the total abundance at each site was calculated in two ways. If possible, abundance was calculated by complete larval census with exhaustive visual counts. Alternatively, in ponds with high larval densities, abundance was estimated by extrapolation as follows: we selected two sampling sections accounting for habitat heterogeneity and counted all tadpoles visible in each sampling section. Extrapolation of the number of tadpoles counted in each of these sampling sections to the whole water body resulted in two estimates of larval abundance in each water body for each visit. We

Figure 3. Examples of the types of water bodies surveyed in the study area in Comunidad de Madrid, central Spain. (1) Water tanks with vertical walls (troughs, fountains; locality 57), (2) Artificial, semi-naturalized ponds (locality 54), (3) Natural water sites (swamps, puddles, ponds or streams; locality 22), and (4) Artificial ponds in abandoned quarries (locality 74). (Photographed by Carlos Caballero-Díaz).

report the two estimates from the visit with the highest recorded abundance (Appendix 2).

We explored patterns of co-occurrence of the two focal species and other amphibian species in the study area. We inspected all water bodies and surrounding areas and recorded presence/absence of individuals (adults or larvae) of other amphibian species and identified non-random patterns of co-occurrence with Chi-square tests ($\alpha = 0.05$) using function chisq.test in R (R Development Core Team 2019). We also recorded the main threats detected at each aquatic water body and classified them as: (1) road mortality (water bodies < 20 m away from the nearest road), (2) full desiccation due to decreased groundwater levels precluding breeding activity or causing tadpole mortality, (3) presence of invasive predator or competitor species (e.g., alien species of fish and crayfish), (4) water turbidity (visibility limited to 10 cm deep or less), (5) structural damage (leaks in pipes or walls of water tanks, including loss of connection with water source), (6) chytridiomycosis (assessed by visual inspection of oral disks of tadpoles, and confirmed by quantitative Polymerase Chain Reaction [qPCR] in a sample of 30 tadpoles from five sites, see below), (7) trap effects (mortality associated with structural barriers in water bodies, including high vertical walls), (8) water pollution (presence of litter in the water body and/or indirect evidence of water contamination due to the presence of empty containers of pesticides, fertilizers, paint, or chloride tablets), and (9) disturbances caused by anthropogenic activities potentially affecting amphibians, including illegal collection of tadpoles, vandalism, and inadequate management practices in water bodies (tadpole mortalities associated with cleaning activities or water extraction).

For the detection by qPCR of *Batrachochytrium dendrobatidis* (*Bd*), the causal agent of chytridiomycosis, we swabbed the mouth parts of each tadpole for about 60 s. A new pair of gloves was used on each tadpole to decrease the chance of contamination of zoospores across individuals. After swabbing, we broke the tips of the swab off into screw cap vials and stored the vials at < 4° C and shipped them to the Vredenburg Lab at San Francisco State University, California, USA, for processing. We stored and processed swabs in 1.5 mL microcentrifuge tubes. We extracted DNA from swabs using 40μL of Prepman Ultra (Applied Biosystems, Carlsbad, California, USA) and diluted 1:10 with 0.25 × TE Buffer and assessed the presence of *Bd* by real-time PCR, following previously described methods (Boyle et al. 2004). As a reference, we used negative controls (H_2O, TE Buffer) and positive controls at dilutions of 100, 10, 1, and 0.1 zoospore equivalents (ZE). We considered a sample *Bd*-positive if the amplification curve was sigmoidal with a ZE value greater than zero.

Results

We detected eight amphibian species in the study area: *A. obstetricans* (44 localities, corresponding to 52 breeding sites), *P. punctatus* (28 breeding sites in 28 localities; Fig. 2), *Epidalea calamita* (Natterjack Toad; 49 localities), *Pelophylax perezi* (Perez's Frog; 23 localities), *Bufo spinosus* (Iberian Common Toad; 21 localities), *Discoglossus galganoi* (Iberian Painted Frog; 15 localities), *Pleurodeles waltl* (Iberian Ribbed Newt; three localities) and *Pelobates cultripes* (Iberian Spadefoot Toad; two localities; Appendix 1). Detectability was high across species, ranging from an average of 1.80 to 3.74 visits required to confirm their presence (*P. waltl* = 2.33; *A. obstetricans* = 2.37; *D. galganoi* = 3.74; *Pelodytes punctatus* = 1.89; *P. cultripes* = 2.00; *B. spinosus* = 2.68; *E. calamita* = 1.80; *P. perezi* = 2.65; all values below the average number of visits per water body = 3.84). We did not find any amphibian species in 21 localities (18.6%, Appendix 1).

Populations of *A. obstetricans* were mainly distributed around the Tajuña and Tajo river basins (Fig. 2). We found high larval abundances in the municipalities of Chinchón, Valdelaguna, Belmonte de Tajo and Colmenar de Oreja (e.g., between 900–2,500 tadpoles in localities 01, 02, and 08; Appendix 2), where breeding sites were mainly artificial ponds and water tanks (Appendix 1). Populations around the Tajuña river (municipalities of Tielmes, Morata de Tajuña, and Perales de Tajuña) occupied more diverse types of breeding sites, including an abandoned quarry (Appendix 1). We found some geographically isolated populations with a high number of breeding adults, for example, in localities 39 (Arganda del Rey) and 63 (Perales de Tajuña; Appendix 2). By contrast, other isolated populations showed low numbers of larvae and breeding adults (< 10, e.g., localities 22, 24 and 58, Appendix 2). We found overwintering larvae in at least 25 localities (01, 02, 03, 05, 08, 11, 16, 17, 18, 19, 20, 22, 32, 35, 39, 40, 41, 42, 49, 52, 53, 56, 58, 63, 75). Males were calling from February 19 (locality 01) to June 6 (locality 39), and we observed empty egg clutches and newly hatched larvae from early April in locality 32.

We found *P. punctatus* to be mostly distributed in the Jarama and Tajuña river basins (Fig. 2). Populations in the Jarama basin bred in natural ponds, with high larval abundances (e.g., 980–1,200 tadpoles in locality 79 and 1,000–1,500 in locality 86, Appendix 2). In the central part of the study area, there were large populations occupying ponds in abandoned quarries in Morata de Tajuña, Perales de Tajuña, and Arganda del Rey (locality 46: 40–45 adults; locality 65: 2,000–3,000 larvae; Appendix 2). In the municipality of Carabaña, *P. punctatus* used a wide range of breeding sites (Appendix 1). The breeding period of *P. punctatus* in the study area extended from mid-March (locality 46) to early May (localities 46 and 63). We observed the first metamorphs on May 22 at locality 98.

Of the 130 water bodies surveyed, 40.0% and 36.2% were classified as categories 1 (water tanks) and 3 (natural ponds), respectively, followed by categories 2 (artificial ponds, 17.7%) and 4 (abandoned quarries, 6.2%). Almost two-thirds of the water bodies detected in the study area were of artificial origin. We found evidence of amphibian breeding activity (mating calls, eggs, tadpoles of any amphibian species) in 104 water bodies (92 localities). There were significant differences in the average number of species among the different types of water bodies ($H = 13.38$, df = 3, $P = 0.004$), with a higher average number of species in abandoned quarries (2.5 species), followed by artificial ponds (two species), natural ponds (1.53 species), and water tanks (1.17 species). We found *A. obstetricans* mainly in artificial breeding sites (84.6%: 50% water tanks, 32.7% artificial ponds and 1.9% abandoned quarries; Appendix 1). Only 15.4% of the breeding sites used by *A. obstetricans* were natural ponds. *Pelodytes punctatus* showed preference for breeding in natural ponds (50%), whereas they used artificial breeding sites such as abandoned quarries (25%) and water tanks (21.4%) less frequently. The distribution of *A. obstetricans* and *P. punctatus* was not random regarding the type of breeding site ($\chi^2 = 16.2$, df = 3, $P = 0.002$, and $\chi^2 = 22.9$, df = 3, $P = 0.001$, respectively). *Alytes obstetricans* occurred more often than expected in artificial ponds and less often than expected in natural ponds, whereas *P. punctatus* was found more often than expected in abandoned quarries and less often than expected in artificial ponds (Table 1).

We found differences between the amphibian communities co-occurring with *A. obstetricans* and *P. punctatus*. *Alytes obstetricans* was the only amphibian species present at 19 breeding sites (36.5%). Elsewhere, it co-occurred with one (n = 16 breeding sites, 30.8%), two (n = 11, 21.2%), or three or more species (n = 6, 11.5%). *Pelodytes punctatus* was the only species present in three breeding sites (10.7%). Elsewhere, it co-occurred with one (n = 13, 46.4%), two (n = 5, 17.9%), or with three or more species (n = 7, 25%).

Alytes obstetricans co-occurred often with *B. spinosus* (at 32.7% breeding sites), *E. calamita* (26.9%), *D. galganoi* (25%), and *P. perezi* (21.2%; Table 2). We did not find breeding sites where *P. cultripes* or *P. waltl* co-occurred with *A. obstetricans*. *Pelodytes punctatus* frequently co-occurred with *E. calamita* (79% breeding sites; Table 2). These two species coexist with *P. waltl* in the three breeding sites where the latter is found. *Pelodytes punctatus* also co-occurred with *D. galganoi*,

Table 1. Observed (Obs.) and expected (Exp.) frequencies of presence of the Common Midwife Toad (*Alytes obstetricans*) and the Parsley Frog (*Pelodytes punctatus*) in Comunidad de Madrid, central Spain, for each of the four breeding habitat categories (see text for details) and the total number of sites within each category. The distribution of *A. obstetricans* and *P. punctatus* was not random regarding the type of breeding site ($\chi^2 = 16.2$, df = 3, $P = 0.002$, and $\chi^2 = 22.9$, df = 3, $P = 0.001$, respectively).

Habitat category	Obs. *Alytes*	Exp. *Alytes*	Obs. *Pelodytes*	Exp. *Pelodytes*	Total sites
Type 1: water tanks with vertical walls (troughs, fountains)	26	20.4	6	11.0	51
Type 2: artificial, semi-naturalized ponds	17	9.2	1	5.0	23
Type 3: natural water sites: swamps, puddles, ponds or streams	8	19.2	14	10.3	48
Type 4: artificial ponds in abandoned quarries	1	3.2	7	1.7	8

B. spinosus, and *P. perezi* (17.9% in all cases), and less frequently with *P. cultripes* (4.5%). The two target species (*A. obstetricans* and *P. punctatus*) co-occurred in only five breeding sites (9.6% and 17.9% of the total number of breeding sites for each species, respectively; Table 2).

Patterns of co-occurrence of *A. obstetricans* and *P. punctatus* with other amphibian species in the study area were not random ($\chi^2 = 18.36$, df = 6, $P = 0.007$ and $\chi^2 = 18.7$, df = 6, $P = 0.014$, respectively). *Alytes obstetricans* co-occurred more often than expected with *D. galganoi* and *B. spinosus* and less often than expected with *P. punctatus* and *E. calamita* (Table 3). On the other hand, *P. punctatus* co-occurred more often than expected with *E. calamita* and less often than expected with *A. obstetricans* (Table 3).

Trap effects were the most frequent threat detected, affecting a large proportion of water tanks (n = 33, 63.4%), followed by road mortality, a threat associated with water tanks (n = 13, 25%), artificial ponds (n = 6, 26.1%), and natural ponds (n = 9, 19.1%; Table 4). We also detected high tadpole mortalities due to early desiccation of water tanks (n = 10, 19.2%) and some artificial ponds (n = 2, 8.7%; Table 4). Structural damage and inadequate cleaning habits in some water tanks (n = 5, 9.6%; Table 4) also caused tadpole mortality associated with early desiccation. Water turbidity was a problem in some sites, including water tanks (n = 4, 7.7%), artificial ponds (n = 3, 13%) and natural ponds (n = 2, 4.3%), whereas water pollution affected mostly water tanks (n = 8, 15.4%; Table 4). Alien invasive species (Table 5) were present in all types of water bodies and were especially abundant in abandoned quarries (n = 3, 37.5%; Table 4). We found one or more invasive species at 14 water bodies (Table 5, Appendix 1).

We are the first to detect chytridiomycosis in the study area (Table 6). All tadpoles of *A. obstetricans* sampled at localities 01, 02, 20, and 39, where we observed symptoms of pathogen infection (oral disk lacking keratin), tested positive for the presence of *Bd* (Table 6). An additional sample of asymptomatic tadpoles from a different locality (08) tested negative for the presence of the fungus (Table 6). We detected tadpoles symptomatic for chytridiomycosis in six other localities, but we did not test these via qPCR (Table 6). Most breeding sites with direct or indirect evidence of chytridiomycosis were artificial with permanent water, whereas only one is a puddle close to a water tank (locality 02; Table 6, Appendix 1). In addition, we detected disturbances associated with anthropogenic activity in localities 01, 33, 35, 37, 40, 44, 45, 48, 51, 53, 55, and 62 (Appendix 1).

Discussion

Amphibians in the Mediterranean region have adapted to breed in temporary ponds and streams, but these are some of the most fragile and threatened ecosystems on Earth (Gómez-Rodríguez et al. 2009). This fragility has led to both attempts to improve wetland conservation status by providing legal protection for these aquatic habitats and to an increased interest in

Table 2. Co-occurrence of amphibian species in the study region in Comunidad de Madrid, central Spain. Under the diagonal are the number of sites where each pair of species coexists. Abbreviations are N = number of breeding sites occupied by each amphibian species, Ao = Common Midwife Toad (*Alytes obstetricans*), Bs = Iberian Common Toad (*Bufo spinosus*), Dg = Iberian Painted Frog (*Discoglossus galganoi*), Ec = Natterjack Toad (*Epidalea calamita*), Pc = Iberian Spadefoot Toad (*Pelobates cultripes*), Ph = Parsley Frog (*Pelodytes punctatus*), Pp = Perez´s Frog (*Pelophylax perezi*), and Pw = Iberian Ribbed Newt (*Pleurodeles waltl*).

	N	Ao	Bs	Dg	Ec	Pc	Ph	Pp	Pw
Ao	52								
Bs	22	17							
Dg	19	13	9						
Ec	49	14	5	6					
Pc	2	0	0	0	2				
Ph	28	5	5	5	22	1			
Pp	26	11	5	5	9	1	5		
Pw	3	0	0	0	3	1	3	2	

Table 3. Observed (Obs.) and expected (Exp.) frequencies of co-occurrence of the Common Midwife Toad (*Alytes obstetricans*) and the Parsley Frog (*Pelodytes punctatus*) with other amphibian species in the study region in Comunidad de Madrid, central Spain, and the total number of sites where each accompanying species was found. Patterns of co-occurrence of *A. obstetricans* and *P. punctatus* with other amphibian species in the study area were not random (χ^2 = 18.36, df = 6, *P* = 0.007 and χ^2 = 18.7, df = 6, *P* = 0.014, respectively).

Co-occurring species	Obs. *Alytes*	Exp. *Alytes*	Obs. *Pelodytes*	Exp. *Pelodytes*	Total sites
Iberian Ribbed Newt (*Pleurodeles waltl*)	0	1.2	3	0.8	3
Common Midwife Toad (*Alytes obstetricans*)	--	--	5	13.9	52
Iberian Painted Frog (*Discoglossus galganoi*)	13	7.7	5	5.1	19
Parsley Frog (*Pelodytes punctatus*)	5	11.4	--	--	28
Iberian Spadefoot Toad (*Pelobates cultripes*)	0	0.8	1	0.5	2
Iberian Common Toad (*Bufo spinosus*)	17	8.9	5	5.9	22
Natterjack Toad (*Epidalea calamita*)	14	19.9	22	13.1	49
Perez´s Frog (*Pelophylax perezi*)	11	10.1	5	6.7	25

creating anthropogenic water bodies that can support viable populations of freshwater organisms (Chester and Robson 2013). Thus, assessing the importance of artificial habitats for the persistence of amphibian populations is vital in areas where original habitats have been degraded and/or lost. The value of artificial ponds and cattle troughs for sustaining amphibian populations in cool, humid areas in Atlantic Iberia has been highlighted by García-González and García-Vázquez (2011) and Martínez-Abraín and Galán (2018). Limited studies have focused on Mediterranean areas in Iberia (Beja and Alcazar 2003; Gálvez et al. 2018), however, where temporary ponds and streams are scarce and consequently the expected importance of artificial habitats for amphibian populations higher. Here we show that amphibians in our study region occupy both artificial and natural breeding sites, with the former, including abandoned and naturalized quarries, providing suitable breeding habitat for most amphibian species at high occupancy rates.

Among our target species, *A. obstetricans* showed preference for some types of artificial breeding sites. In particular, *A. obstetricans* showed high breeding success in well-preserved water tanks where it was often the sole amphibian species present. Water tanks also hosted larger population sizes than artificial ponds and were often associated with the presence of overwintering larvae. This developmental strategy may have both advantages (higher body size at metamorphosis) and costs (higher chytrid infection loads; see Fernández-Beaskoetxea et al. 2015), of which the relative importance should be addressed in future studies. Natural ponds, which are often temporary and thus inadequate for *A. obstetricans* due to its long larval development, were occupied much less frequently than expected.

In contrast, *P. punctatus* did not usually breed in artificial sites but rather used temporary ponds, especially those forming in abandoned quarries, where this species can be very abundant even in the presence of alien predatory species. In addition, *P. punctatus* also bred in natural water bodies like ephemeral puddles and ponds, albeit at lower abundance. Overall, the number

Table 4. Frequencies of threats detected in the four types of water bodies surveyed in Comunidad de Madrid, central Spain. The number of water bodies surveyed in each category is indicated in parentheses.

	Water tanks (52)	Artificial ponds (23)	Natural ponds (47)	Abandoned quarries (8)	Total
Road mortality	13	6	9	0	28
Desiccation	10	2	0	0	12
Invasive alien species	7	2	4	3	16
Turbidity	4	3	2	0	9
Structural damage	5	1	0	0	6
Chytridiomycosis	9	5	1	0	15
Trap effects	33	0	0	0	33
Water pollution	8	1	2	1	12
Other	6	3	2	1	12

Table 5. Invasive alien species found in the study area in Comunidad de Madrid, central Spain. Site numbers correspond to Appendix 1.

Species	Sites
Eastern Mosquitofish (*Gambusia holbrooki*)	08, 21, 35, 47, 65, 74
Red Swamp Crayfish (*Procambarus clarkii*)	33, 46, 50, 67, 100
Common Carp (*Cyprinus carpio*)	04, 12, 21, 70
Signal Crayfish (*Pacifastacus leniusculus*)	46, 100
Yellow-bellied Slider (*Trachemys scripta*)	46, 100
Crucian Carp (*Carassius carassius*)	47
Pumpkinseed (*Lepomis gibbosus*)	74
Black Bullhead (*Ameiurus melas*)	65
Florida Red-bellied Cooter (*Pseudemys nelsoni*)	100

Table 6. Sites in Comunidad de Madrid, central Spain, with (1) presence of chytridiomycosis confirmed by qPCR, (2) presence of tadpoles with symptoms of the disease but unconfirmed chytrid infection, or (3) presence of tadpoles that neither presented symptoms nor tested positive in qPCRs. Site numbers correspond to Appendix 1.

Confirmed	Unconfirmed but symptomatic	No symptoms or infection
01 Valquejigoso	03 Casa Dómine	08 Fuente de los Perales
20 Fuente Vieja	05 El Rufo	
02 Mingorrubio	16 Fuente María	
39 Valtierra	32 Casasola	
	40 Fuente del Valle Arganda	
	49 Valdezarzas	

of artificial sites used by *P. punctatus* was limited. This finding is unsurprising as it has been suggested that water tanks with hard substrates and little or no vegetation are not good breeding habitats for *P. punctatus* (Gálvez et al. 2018), which usually attach egg strings to stems and branches of aquatic or semiaquatic plants (Escoriza 2017, *op. cit.*).

We have shown that artificial habitats are important for our target species, albeit differences in breeding site selection between *A. obstetricans* and *P. punctatus* (both species coexisted in only five sites) imply that management actions need to target each species separately. An important consideration is to what extent *A. obstetricans* and *P. punctatus* can act as umbrella species to help protect populations of other taxa (Roberge and Angelstam 2004). Our analysis of co-occurrence patterns shows that other amphibian species in the study area could benefit from actions improving breeding habitat for *A. obstetricans* and *P. punctatus.* For instance, the association of *A. obstetricans* with *B. spinosus* and *D. galganoi* was rather frequent in water tanks, and thus actions directed towards correcting threats associated with these sites, like trap effects (see below), could benefit all three species. On the other hand, *P. punctatus* often co-occurs with several species breeding in temporary ponds, including some of the rarest in the study area, like *P. waltl* and *P. cultripes*. Our data on breeding habitat preferences could be used to create new ponds favoring connectivity among populations of these species, some of which are in decline (Martínez-Solano 2006).

Adequate management of artificial breeding sites requires addressing various detectable threats and is critical to the maintenance of viable amphibian communities in the study region. Some of these are specific to artificial breeding sites (trap effects, structural damage, or inadequate cleaning of water tanks causing early desiccation and failure of tadpoles to metamorphose) and are related to the abandonment of traditional farm practices in rural areas caused by intensification of agricultural practices (Rey Benayas and Bullock 2012). Multiple combined threats, like trap effects and road proximity, can lead to high mortalities in some species, especially *B. spinosus,* which appear to be declining in the study area (Martínez-Solano 2006; Vallvé and Sánchez-Iglesias 2018). Some of these threats can be corrected with active management practices, like the construction of ramps or the addition of rocks at the inner and outer walls of water tanks and fountains to facilitate the entrance and exit of adult and metamorphic amphibians. In addition, it is critical that the larval phenology of the different amphibian species present at each site be considered when planning the cleaning of water tanks and other artificial water bodies to facilitate recruitment and population resilience. Cleaning at inappropriate times causes massive larval mortalities every year and could be avoided or reduced by concentrating cleaning periods in the winter, when amphibian activity is lowest (Paños et al. 2011). For species with overwintering larvae like *A. obstetricans,* water tanks should not be fully emptied for cleaning and tadpoles could be extracted temporarily and released once the tank has been cleaned.

The present study showed that general threats like road mortality, alien invasive species, and chytridiomycosis are present in the study area. The negative effect of alien predatory species like fish and crayfish on amphibian species richness and abundance is widely documented (Cruz et al. 2008; 2015; Préau et al. 2017). In our study area, alien species were the main threat for populations of *P. punctatus*, but also affected all other amphibian species. At few sites where *P. punctatus* co-occurred with alien fish, the frog tended to occupy shallower, more vegetated areas to avoid predation. The eradication of alien, invasive species should be a management priority. We did not detect mass mortalities of larvae or post-metamorphs associated with chytridiomycosis; however, symptomatic tadpoles often showed poor body condition, suggesting potential negative effects on survival after metamorphosis. This contrasts with the mass mortalities reported just a few kilometers north in the Guadarrama Mountains, where this species is near local extinction (Bosch et al. 2018). Measures to avoid propagation of the fungal pathogen to other regions should be undertaken, including careful disinfecting of field equipment before and after working in these areas.

Artificial aquatic habitats like fountains, water tanks, newly constructed ponds, and naturalized quarries represent key breeding sites for amphibians in our study area, including national and regional red-listed species. Nevertheless, these habitats are associated with a variety of threats, which differ in severity between species and live stages. The adoption of the measures proposed (creation of new ponds, correction of trap effects, eradication of invasive species, cleaning practices respectful of amphibian phenology, prevention to avoid dissemination of infectious disease), which are simple and relatively inexpensive, will help preserve the biodiversity of this region.

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Appendices

Appendix 1. Relation of the 130 water bodies sampled with locality, municipality, Longitude and Latitude coordinates, amphibian species present, type of breeding site, and main threats detected. Species: Ao = Common Midwife Toad (*Alytes obstetricans*); Bs = Iberian Common Toad (*Bufo spinosus*), Dg = Iberian Painted Frog (*Discoglossus galganoi*); Ec = Natterjack Toad (*Epidalea calamita*); Pc = Iberian Spadefoot Toad (*Pelobates cultripes*); Ph = Parsley Frog (*Pelodytes punctatus*); Pp = Perez´s Frog (*Pelophylax perezi*); Pw = Iberian Ribbed Newt (*Pleurodeles waltl*). Types of water bodies: 1 = water tanks; 2 = artificial ponds; 3 = natural ponds; 4 = abandoned quarries. Main threats detected: ch = chytridiomycosis; d = desiccation; i = invasive alien species; o = other; p = water pollution; r = road mortality; sd = structural damage; t = trap effect; wt = water turbidity.

Code	Locality	Municipality	Longitude (W)	Latitude (N)	Species	Type	Threats
01	Valquejigoso	Chinchón	3.43	40.13	Ao	1	ch, o
02	Mingorrubio	Colmenar de Oreja	3.43	40.11	Ao	1	ch, d, t
					Ao, Ec	3	ch
03	Casa Dómine	Colmenar de Oreja	3.43	40.11	Ao	1	ch, t
					Ec	3	
04	El Bosque	Valdelaguna	3.37	40.14	Ao, Bs, Dg, Ec, Ph	3	i
05	El Rufo	Valdelaguna	3.36	40.14	Ao, Bs	1	ch, d, t
06	Arroyo Horcajuelo	Belmonte de Tajo	3.33	40.14	Ao	3	
07	Tierra del Agua	Belmonte de Tajo	3.34	40.13	Ao, Bs, Dg, Ph	1	r, sd
08	Fuente de los Perales	Belmonte de Tajo	3.32	40.12	Ao, Bs, Pp	1	d, i, r, t
09	Valdepuercos	Villamanrique de Tajo	3.30	40.06	Ec	3	
10	Albercas Zacatín 1(oeste)	Colmenar de Oreja	3.39	40.11		1	d, t
11	Albercas Zacatín 2(este)	Colmenar de Oreja	3.39	40.11	Ao	2	d
12	Fuente de los Huertos	Colmenar de Oreja	3.40	40.11		1	i, r, t
13	Pilones entrada Belmonte	Belmonte de Tajo	3.34	40.13		1	p, r
14	Valviejo	Valdelaguna	3.39	40.17	Ao, Dg, Pp	1	t
					Ao, Bs, Dg, Ec, Pp	2	
15	La Tejera	Valdelaguna	3.38	40.18	Ao, Dg	1	p, sd, t
16	Fuente María	Valdelaguna	3.37	40.19	Ao	1	ch
					Ao, Ec	2	ch
17	La Gasca	Perales de Tajuña	3.35	40.25	Ao, Dg, Pp	1	
18	Barranco Olivar	Perales de Tajuña	3.34	40.24	Ao	1	t
19	Matagacha	Perales de Tajuña	3.34	40.23	Ao, Dg	1	t
					Ao, Dg	2	
20	Fuente Vieja	Valdelaguna	3.34	40.16	Ao	1	ch, t
					Ao, Bs	2	ch
21	La Tejera	Belmonte de Tajo	3.34	40.15	Ao, Bs	1	i
22	Valdelasierpe	Valdaracete	3.20	40.24	Ao, Ec	3	

Appendix 1 (continued). Relation of the 130 water bodies sampled with locality, municipality, Longitude and Latitude coordinates, amphibian species present, type of breeding site, and main threats detected. Species: Ao = Common Midwife Toad (*Alytes obstetricans*); Bs = Iberian Common Toad (*Bufo spinosus*), Dg = Iberian Painted Frog (*Discoglossus galganoi*); Ec = Natterjack Toad (*Epidalea calamita*); Pc = Iberian Spadefoot Toad (*Pelobates cultripes*); Ph = Parsley Frog (*Pelodytes punctatus*); Pp = Perez´s Frog (*Pelophylax perezi*); Pw = Iberian Ribbed Newt (*Pleurodeles waltl*). Types of water bodies: 1 = water tanks; 2 = artificial ponds; 3 = natural ponds; 4 = abandoned quarries. Main threats detected: ch = chytridiomycosis; d = desiccation; i = invasive alien species; o = other; p = water pollution; r = road mortality; sd = structural damage; t = trap effect; wt = water turbidity.

Code	Locality	Municipality	Longitude (W)	Latitude (N)	Species	Type	Threats
23	Valdecabra	Fuentidueña de Tajo	3.19	40.16	Ec	3	
24	Fuente Juan García	Fuentidueña de Tajo	3.18	40.16	Ao	1	sd, t
25	Cementerio Villarejo	Villarejo de Salvanés	3.27	40.18		1	t
26	Olivar Villarejo	Villarejo de Salvanés	3.26	40.21	Ao, Ec	3	
27	Las Pozas	Valdaracete	3.25	40.20		3	
28	Valdecañas	Tielmes	3.31	40.23		1	r, wt
					Ao, Dg, Ec	2	p, r, wt
29	Peña la Cabra	Tielmes	3.29	40.22		1	d, t
					Ao, Ec	2	d
30	El Horcajo	Tielmes	3.28	40.22	Ao	1	t
						2	sd
31	Fuente del Arce	Tielmes	3.30	40.24		1	sd
32	Casasola	Valdilecha	3.29	40.30	Ao, Bs, Dg	2	ch
33	El Rejal	Valdilecha	3.29	40.30	Ao, Bs, Dg	2	i, o, r
34	Pinar Villar del Olmo	Villar del Olmo	3.24	40.34	Ao	2	
35	El Quemado	Villar del Olmo	3.25	40.34	Ao, Pp	1	i, o, p, r, t
					Ao, Ec	2	r
36	Alberca Pezuela-Olmeda	Olmeda de las Fuentes	3.21	40.40	Ec, Ph	1	d, t
37	Área recreativa Olmeda	Olmeda de las Fuentes	3.22	40.37	Pp	1	o, r
					Pp	3	r
38	Abrevadero Pezuela	Pezuela de las Torres	3.19	40.41		1	t
					Ec	3	
39	Valtierra	Arganda del Rey	3.40	40.29	Ao, Bs, Dg, Ph	1	ch
40	Fuente del Valle	Arganda del Rey	3.43	40.27	Ao, Dg, Pp	1	ch, o, p, r
					Ao, Bs, Dg, Pp	2	ch, r, wt
41	Charca dehesa Morata	Morata de Tajuña	3.41	40.24	Ao	2	
42	Pilón Valdegatos	Morata de Tajuña	3.45	40.23	Ao	1	t
43	Registro agua Morata	Morata de Tajuña	3.45	40.23	Pp	1	t, wt
44	Túnel ladrillos	Morata de Tajuña	3.43	40.24	Ph	3	o, r
45	Fuente Parque Morata	Morata de Tajuña	3.43	40.24		2	o

Appendix 1 (continued). Relation of the 130 water bodies sampled with locality, municipality, Longitude and Latitude coordinates, amphibian species present, type of breeding site, and main threats detected. Species: Ao = Common Midwife Toad (*Alytes obstetricans*); Bs = Iberian Common Toad (*Bufo spinosus*), Dg = Iberian Painted Frog (*Discoglossus galganoi*); Ec = Natterjack Toad (*Epidalea calamita*); Pc = Iberian Spadefoot Toad (*Pelobates cultripes*); Ph = Parsley Frog (*Pelodytes punctatus*); Pp = Perez´s Frog (*Pelophylax perezi*); Pw = Iberian Ribbed Newt (*Pleurodeles waltl*). Types of water bodies: 1 = water tanks; 2 = artificial ponds; 3 = natural ponds; 4 = abandoned quarries. Main threats detected: ch = chytridiomycosis; d = desiccation; i = invasive alien species; o = other; p = water pollution; r = road mortality; sd = structural damage; t = trap effect; wt = water turbidity.

Code	Locality	Municipality	Longitude (W)	Latitude (N)	Species	Type	Threats
46	Charca aeródromo	Morata de Tajuña	3.43	40.24	Ec, Ph, Pp, Pw	4	i
47	Valgrande	Morata de Tajuña	3.48	40.20	Ao, Ec, Pp	2	i
48	Fuente Pata	Chinchón	3.43	40.13		1	o
49	Valdezarzas	Chinchón	3.41	40.15	Ao, Bs	1	ch, t, wt
					Ao, Bs, Ec	2	ch, wt
50	Valdelaspozas	Chinchón	3.44	40.16		1	i
						3	i
51	La Rendija	Chinchón	3.46	40.16	Ao	2	o
52	La Pernisteba	Chinchón	3.47	40.14	Ao	3	
53	Fuente el Valle	Chinchón	3.46	40.14	Ao, Ec	3	o
54	Fuente del Robledillo	Ambite	3.16	40.35	Bs, Ec	2	
55	Fuente del Arca	Ambite	3.19	40.34		1	d, o, wt
56	El Descubrimiento	Tielmes	3.32	40.25	Ao	1	t
57	Polideportivo Tielmes	Tielmes	3.31	40.24	Ao	1	r, t
58	El Cascón	Carabaña	3.21	40.26	Ao, Bs, Ph	1	
					Ec	3	
59	Los Lamaderos	Ambite	3.18	40.34		1	t
60	Fuente Óscar	Orusco	3.19	40.28	Bs	2	
61	Valdelabá	Carabaña	3.25	40.27	Ph	2	r
62	Pradejón	Carabaña	3.23	40.24	Ec, Ph	4	o, p
63	El Rey	Perales de Tajuña	3.31	40.18	Ao	1	p, sd, t
64	La Canaleja	Perales de Tajuña	3.38	40.24	Pp	1	d
65	Valdecubillos	Perales de Tajuña	3.39	40.24	Ao, Ec, Ph, Pp	4	i
66	Cantera triturados	Arganda del Rey	3.41	40.27	Ec, Ph, Pw	4	
67	Alberca Valtierra	Arganda del Rey	3.40	40.30	Bs, Dg	1	i, t
					Dg, Ec	3	i
68	Valdericeda	Valdelaguna	3.38	40.14	Ao, Bs, Pp	3	
69	Valdepinar	Colmenar de Oreja	3.36	40.10	Pp	1	p
70	Valdegredero	Colmenar de Oreja	3.37	40.10	Bs, Pp	1	d, i
71	Pelodycantera	Morata de Tajuña	3.43	40.24	Ec, Ph	4	

Appendix 1 (continued). Relation of the 130 water bodies sampled with locality, municipality, Longitude and Latitude coordinates, amphibian species present, type of breeding site, and main threats detected. Species: Ao = Common Midwife Toad (*Alytes obstetricans*); Bs = Iberian Common Toad (*Bufo spinosus*), Dg = Iberian Painted Frog (*Discoglossus galganoi*); Ec = Natterjack Toad (*Epidalea calamita*); Pc = Iberian Spadefoot Toad (*Pelobates cultripes*); Ph = Parsley Frog (*Pelodytes punctatus*); Pp = Perez´s Frog (*Pelophylax perezi*); Pw = Iberian Ribbed Newt (*Pleurodeles waltl*). Types of water bodies: 1 = water tanks; 2 = artificial ponds; 3 = natural ponds; 4 = abandoned quarries. Main threats detected: ch = chytridiomycosis; d = desiccation; i = invasive alien species; o = other; p = water pollution; r = road mortality; sd = structural damage; t = trap effect; wt = water turbidity.

Code	Locality	Municipality	Longitude (W)	Latitude (N)	Species	Type	Threats
72	Barranco de la Vega	Campo Real	3.40	40.34	Ao	1	
73	Alberca carretera Carabaña	Carabaña	3.25	40.26	Bs, Ph	1	r, t
74	Cantera Campo Real-Valdilecha	Campo Real	3.33	40.31	Ec, Ph	4	i
75	Charca Valviejo	Valdelaguna	3.40	40.17	Ao, Bs, Ec	2	
76	Club Tiro Valdemoro	Valdemoro	3.66	40.18	Ec, Ph	3	
77	Embalse Gózquez	San Martín de la Vega	3.60	40.24	Bs, Dg	3	
78	Arroyo de la Vega	San Martín de la Vega	3.58	40.24	Ec, Pp	3	
79	La Boyeriza	San Martín de la Vega	3.56	40.26	Ec, Ph, Pp	3	
80	Arroyo Espartinas	San Martín de la Vega	3.59	40.20	Ec	3	r
81	Charcas Valdemoza	San Martín de la Vega	3.60	40.18	Ec, Ph	3	
82	Charca cerca Camporroso	Ciempozuelos	3.63	40.18	Ec, Ph	3	
83	Arroyo Cañada	Ciempozuelos	3.61	40.16	Pp	3	p, wt
84	Pilón Palomero	Ciempozuelos	3.63	40.14		1	
85	Charcón Palomero	Ciempozuelos	3.63	40.13	Ec	3	
86	Altos de Palomero	Ciempozuelos	3.63	40.13	Ec, Ph, Pp,	3	
87	La Chimenea	Aranjuez	3.55	40.07	Pp	2	r
88	Salinas Espartinas	Ciempozuelos	3.63	40.12		3	
89	Arroyo Palomero	Ciempozuelos	3.61	40.14		3	
90	Encharcamiento Pozuelo	Pozuelo del Rey	3.32	40.37	Ec, Ph	3	r
91	Depuradora Pozuelo	Pozuelo del Rey	3.33	40.36	Ec, Pc	3	p
92	Fuente del Rey	Valverde de Alcalá	3.27	40.40	Ec, Ph	3	r, t
93	Monte Nuevo	Pezuela de las Torres	3.18	40.37	Ec, Ph	3	
94	Las Cruces	Pezuela de las Torres	3.17	40.42	Ph	1	t
95	Encharcamiento Santorcaz	Santorcaz	3.24	40.48		3	r
96	El Gurugú	Alcalá de Henares	3.35	40.46	Ec, Ph	3	
97	Charca la Aldehuela	Rivas Vaciamadrid	3.60	40.30		3	wt
98	Albergues de Macario	Rivas Vaciamadrid	3.60	40.31	Dg, Ec, Ph, Pp	3	
99	La Yesera	Rivas Vaciamadrid	3.52	40.39	Ec, Ph	4	
100	La Guindalera	San Fernando de Henares	3.51	40.41	Ec, Pc, Ph, Pp, Pw	3	i
101	Cantera Pozuelo-Valdilecha	Valdilecha	3.32	40.33	Ec	4	

Appendix 1 (continued). Relation of the 130 water bodies sampled with locality, municipality, Longitude and Latitude coordinates, amphibian species present, type of breeding site, and main threats detected. Species: Ao = Common Midwife Toad (*Alytes obstetricans*); Bs = Iberian Common Toad (*Bufo spinosus*), Dg = Iberian Painted Frog (*Discoglossus galganoi*); Ec = Natterjack Toad (*Epidalea calamita*); Pc = Iberian Spadefoot Toad (*Pelobates cultripes*); Ph = Parsley Frog (*Pelodytes punctatus*); Pp = Perez´s Frog (*Pelophylax perezi*); Pw = Iberian Ribbed Newt (*Pleurodeles waltl*). Types of water bodies: 1 = water tanks; 2 = artificial ponds; 3 = natural ponds; 4 = abandoned quarries. Main threats detected: ch = chytridiomycosis; d = desiccation; i = invasive alien species; o = other; p = water pollution; r = road mortality; sd = structural damage; t = trap effect; wt = water turbidity.

Code	Locality	Municipality	Longitude (W)	Latitude (N)	Species	Type	Threats
102	Encharcamiento V. del Olmo-Ambite	Ambite	3.21	40.32	Dg, Ec, Ph	3	r
103	Urbanización Pioz	Pioz	3.17	40.45	Ec	1	p, r, t, wt
104	Castillejos	Fuentidueña de Tajo	3.14	40.15		3	
105	Abrevadero	Estremera	3.12	40.21		1	r, t
106	Laguna	Estremera	3.11	40.11	Pp	3	
107	Encharcamiento Villarejo-Tielmes	Tielmes	3.29	40.20	Ec	3	r
108	Encharcamiento Ambite	Ambite	3.17	40.34	Ec	3	r
109	Albercas	Carabaña	3.24	40.26	Pp	1	d, t
110	Cañada real	Fuentidueña de Tajo	3.17	40.16		3	
111	Estremera WP322	Estremera	3.12	40.15		3	
112	El Chorrillo	Olmeda de las Fuentes	3.22	40.37		1	r, t
113	Arroyo de la Vega	Olmeda de las Fuentes	3.17	40.39	Ec	3	

Appendix 2. Abundance of larvae of the Common Midwife Toad (*Alytes obstetricans* = *A. obs.*) and the Parsley Frog (*Pelodytes punctatus* = *P. punc.*) in the study area. The abbreviation Larv. = counts or estimates of larvae and Ad. = Maximum number of breeding adults detected.

Locality	Larv. *A. obs.*	Ad. *A. obs.*	Larv. *P. punc.*	Ad. *P. punc.*
01	2,000–2,500	17	0	0
02	1,200–1,400	12	0	0
03	330	0	0	0
04	No estimate	7	300–350	0
05	25	5	0	0
06	0	1	0	0
07	400–500	7	700–800	0
08	900–1,000	7	0	0
11	4	3	0	0
14	500–650	3	0	0
15	600–800	4	0	0
16	900–1,100	12	0	0
17	280–330	0	0	0
18	950–1,150	0	0	0

Appendix 2 (continued). Abundance of larvae of the Common Midwife Toad (*Alytes obstetricans* = *A. obs.*) and the Parsley Frog (*Pelodytes punctatus* = *P. punc.*) in the study area. The abbreviation Larv. = counts or estimates of larvae and Ad. = Maximum number of breeding adults detected.

Locality	Larv. *A. obs.*	Ad. *A. obs.*	Larv. *P. punc.*	Ad. *P. punc.*
19	700–900	2	0	0
20	400–500	3	0	0
21	0	2	0	0
22	1	0	0	0
24	3	0	0	0
26	20	0	0	0
28	1	0	0	0
29	100	15	0	0
30	20	0	0	0
32	400–450	3	0	0
33	0	5	0	0
34	300–350	11	0	0
35	140–150	2	0	0
36	0	0	1,000–1,200	0
39	450–550	35	80–100	1
40	150–200	5	0	0
41	250–300	9	0	0
42	7	0	0	0
44	0	0	0	3
46	0	0	Thousands	40–45
47	0	3	0	0
49	170–200	3	0	0
51	0	2	0	0
52	7	8	0	0
53	3	7	0	0
56	350–400	5	0	0
57	80–100	0	0	0
58	1	2	1	0
61	0	0	500–550	15
62	0	0	400–500	1
63	100	18	0	0
65	0	2	2,000–3,000	0
66	0	0	120–150	0
68	40	14	0	0
71	0	0	1,000–1,500	0
72	0	2	0	0
73	0	0	60–70	0
74	0	0	100	0
75	1	2	0	0
76	0	0	6	0

Appendix 2 (continued). Abundance of larvae of the Common Midwife Toad (*Alytes obstetricans* = *A. obs.*) and the Parsley Frog (*Pelodytes punctatus* = *P. punc.*) in the study area. The abbreviation Larv. = counts or estimates of larvae and Ad. = Maximum number of breeding adults detected.

Locality	Larv. *A. obs.*	Ad. *A. obs.*	Larv. *P. punc.*	Ad. *P. punc.*
79	0	0	980–1,200	0
81	0	0	500–600	0
82	0	0	350–450	0
86	0	0	1,000–1,500	0
90	0	0	0	4
92	0	0	400–500	0
93	0	0	100	0
94	0	0	200–250	0
96	0	0	7	1
98	0	0	2	0
99	0	0	400–500	0
100	0	0	1	0
102	0	0	2	0