

The importance of naturalized quarries as amphibian breeding sites: a case study in central Spain

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Naturalized quarries can host rich amphibian communities, potentially playing an important role in local and regional population dynamics. Despite their importance for conservation, few studies have evaluated their potential to host large, viable populations and to connect breeding nuclei in neighboring areas, promoting long-term demographic resilience at the regional scale. We used photo-identification as a capture-mark-recapture method to estimate the population sizes and dispersal patterns of two amphibians of regional conservation concern (*Pleurodeles waltl* and *Pelodytes punctatus*), which breed in temporary ponds in an abandoned, naturalized quarry in SE Madrid (central Spain). We also surveyed all water bodies potentially harboring amphibian populations in an 80 km² area centered in our study site to detect additional breeding nuclei of the two species. Both species are abundant in the core study site, with estimated population sizes of around 300 individuals in *P. waltl* and about 800 in *P. punctatus*. We recorded 23 accumulated displacements, ranging from 80 to 840 m, performed by 20 individuals of *P. waltl* captured on multiple occasions, but were unable to detect spatial displacements of *P. punctatus* during the study. Regional surveys confirmed the presence of *P. waltl* and/or *P. punctatus* in 7 additional locations, mostly quarries, at distances of 0.6-4.4 km from the study site. Our results show that temporary ponds in naturalized quarries can represent key assets for the long-term resilience of amphibian populations in areas where human action has resulted in extensive habitat fragmentation and loss.

Key words: capture-mark-recapture; demography; habitat fragmentation; metapopulations; *Pelodytes punctatus*; photo-identification; *Pleurodeles waltl*; spatial ecology.

Amphibians are globally threatened, with 41% species in risk of extinction (HOFFMANN *et al.*, 2010). Demographic trends of amphibians are a cause of concern, with 43.2% species undergoing population declines and only 0.5% with positive population trends (STUART *et al.*, 2004). Major causes of declines include climate

change, pollution, infectious diseases and invasive species (BLAUSTEIN & KIESECKER, 2002; COX *et al.*, 2006; WAKE & VREDENBURG, 2008; TEMPLE & COX, 2009; BLAUSTEIN *et al.*, 2011; BOSCH *et al.*, 2018; WAKE & KOO, 2018), but habitat loss and fragmentation are considered the main global threats for the long-term survival of amphibian popula-

tions (STUART *et al.*, 2004; CUSHMAN, 2006).

Habitat loss reduces overall availability of favorable environments and results in the isolation of the remaining suitable patches (WILCOX & MURPHY, 1985), thus decreasing landscape-scale connectivity (TAYLOR *et al.*, 1993). Amphibians are particularly sensitive to local disturbances in aquatic and terrestrial habitats and to disruptions in connectivity, because most species have a biphasic life cycle with an aquatic larval period and terrestrial juvenile and adult stages, and perform seasonal migrations from breeding sites to foraging and refuge areas. Structural fragmentation hinders the potential of amphibians to move across the landscape and colonize habitat patches, because many species show limited dispersal capacity in unfavorable environments (SMITH & GREEN, 2005; RITTENHOUSE & SEMLITSCH, 2007; GUTIÉRREZ-RODRÍGUEZ *et al.*, 2017a, 2023). Consequently, preserving natural patches of well-connected terrestrial and aquatic habitats is a major focus of amphibian conservation programs (NORI *et al.*, 2015). However, in areas where natural habitats have been strongly modified and fragmented, habitat protection needs to be complemented with the restoration and creation of artificial breeding sites, which have been shown to represent adequate breeding habitats for some species (BRAND & SNODGRASS, 2010; CABALLERO-DÍAZ *et al.*, 2020, 2022).

A well-known example of amphibian breeding sites of artificial origin are ponds formed in naturalized quarries, which can host rich amphibian communities (RANNAP *et al.*, 2007; FLAVENOT *et al.*, 2015). While in exploitation, quarries for the industrial

extraction of materials used in construction cause massive alterations in the local topography, with important impacts on biodiversity, including air pollution, soil disturbance, and habitat destruction and fragmentation (CLEMENTS *et al.*, 2006; LAMEED & AYODELE, 2010). But when abandoned, these areas can re-naturalize, promoting the formation of new habitats (aquatic and terrestrial) that are potentially suitable for pioneer species, including pond-breeding amphibians. When ponds with different characteristics (hydroperiod, depth, substrate) are formed, these modified habitats can provide the only alternative to the natural amphibian breeding areas destroyed by human activities (GALÁN, 1997). Thus, these re-naturalized areas may play an important role in regional amphibian population dynamics by hosting large, viable populations and helping to interconnect breeding nuclei in neighboring areas, altogether promoting long-term demographic resilience. However, despite their potential importance for regional amphibian conservation, few studies have evaluated the role of naturalized quarries in local and regional population dynamics.

Amphibian populations in rural areas of central Spain (including Iberian endemics and regionally endangered species) are severely fragmented and in rapid decline due to changes in land use that have resulted in the widespread loss of suitable aquatic breeding sites (MÁRQUEZ & LIZANA, 2002). Some species can successfully breed in artificial habitats (especially fountains, water tanks or cattle troughs), which may thus represent important refugia for the survival of amphibian populations in these

areas (GARCÍA-GONZÁLEZ & GARCÍA-VÁZQUEZ, 2011; BENÍTEZ *et al.*, 2017; GÁLVEZ *et al.*, 2018; BUONO *et al.*, 2019; CABALLERO-DÍAZ *et al.*, 2020, 2022; GUTIÉRREZ-RODRÍGUEZ *et al.*, 2023). Less attention has been devoted to the role of ponds forming in naturalized quarries as potential amphibian habitat. Quarries are part of an important economic sector in Spain, with > 2000 active quarries in 2019 (ESTADÍSTICA MINERA DE ESPAÑA, 2019). Previous studies have shown that naturalized quarries can host important ecosystems for amphibian communities (GALÁN, 1997; CABALLERO-DÍAZ *et al.*, 2020, 2022; CAPELLÁ-MARZO *et al.*, 2020; FERNÁNDEZ DE LARREA *et al.*, 2021). Nevertheless, there is little information about patterns of species richness and abundance or about the potential role of quarries in local population dynamics or in regional patterns of population connectivity. This information is critical to assess whether these habitats function as ecological traps (demographic “sinks”, with no leverage on long-term population viability), or if, alternatively, they can represent key areas for the resilience of amphibian communities, harboring populations of conservation interest.

In this study, we aim to contribute to our understanding of the function and value of naturalized quarries as amphibian breeding sites with a case study in central Spain. We used photo-identification as a capture-mark-recapture (CMR) method to estimate the population sizes of two amphibian species of regional conservation concern, the sharp-ribbed newt *Pleurodeles waltl* Michahelles 1830 and the common parsley frog *Pelodytes punctatus* (Daudin 1802), breeding in temporary ponds in a

naturalized quarry in the southeast of Madrid. We combine abundance estimates, dispersal records and species presence data from field surveys in the neighboring areas to assess the role of this artificial site in the regional population dynamics and the long-term viability of populations of both species.

MATERIALS AND METHODS

Study area and target species

The core study site is located near the village of Morata de Tajuña, in the southeast of Madrid (700 m.a.s.l., Fig. 1), with average annual temperatures between 12.5°C and 15°C, and yearly precipitations of 400-500 mm (dry Mesomediterranean climate). The vegetation is typical from calcicolous Mediterranean soils, and comprises *Quercus ilex* subsp. *ballota*, *Quercus coccifera*, *Populus* sp., *Retama sphaerocarpa* and *Thymus vulgaris*, with sparse plantations of *Pinus halepensis* and cultivars of olive trees (*Olea europaea*). We studied the amphibian community breeding in four temporary ponds (C1-C4) located in an abandoned, re-naturalized limestone quarry (40.244 °N, 3.433 °W) (Fig. 1). The four ponds are usually flooded from the end of autumn to spring, and dry out completely every summer. Maximum flooding surface (estimated from aerial images) and maximum depth (measured in the field) of the four water bodies were: C1 = 946 m² × 1.80 m; C2 = 1614 m² × 1.10 m; C3 = 314 m² × 0.50 m; C4 = 1196 m² × 1.30 m. We did not detect the presence of invasive species in any of the four study ponds.

The amphibian community in the core study site comprises five species: *Pleuro-*

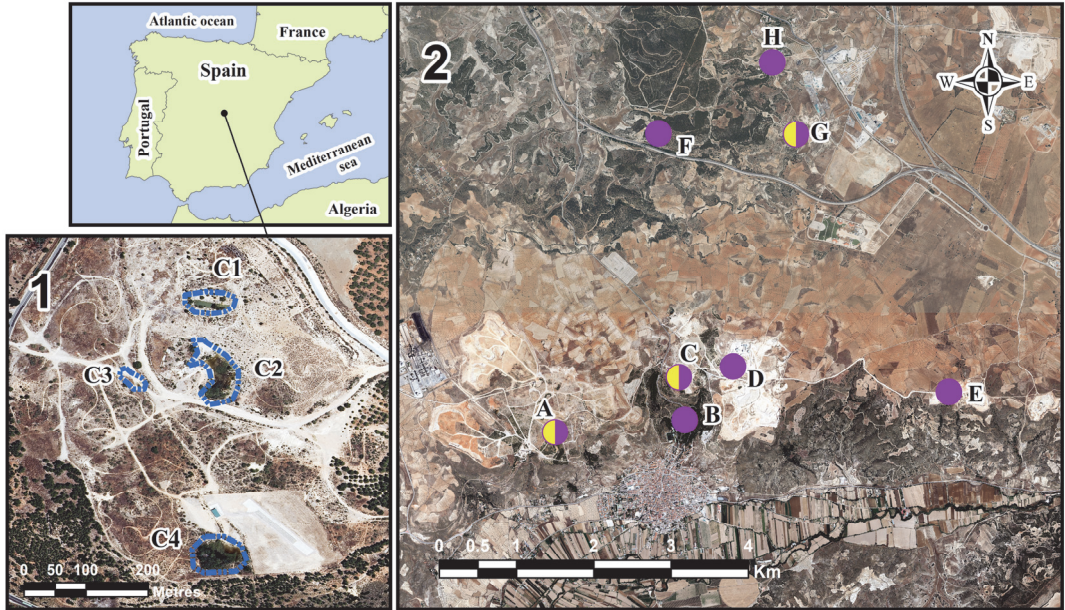


Figure 1: Study area: location of Morata de Tajuña (black dot) in central Spain (top left). Panel 1 shows the perimeter of the four temporary ponds (C1-C4, codes according to Table 1) sampled in the core study site. Panel 2 shows the nearest localities with breeding populations of *P. punctatus* (purple dots) or both *P. waltl* and *P. punctatus* (yellow and purple dots) in the vicinities of the core study site (marked as C, codes according to Table 3). For a proper visualization of the figure, the reader is referred to the online version of the article.

deles waltl, *Pelodytes punctatus*, *Epidalea calamita*, *Bufo spinosus*, and *Pelophylax perezi*. For the purposes of this study, we focused on *P. waltl* and *P. punctatus* because they are typical inhabitants of temporary ponds in naturalized quarries and present fragmented populations in the southeast of Madrid (MARTÍNEZ-SOLANO, 2006; CABALLERO-DÍAZ *et al.*, 2020). *Pleurodeles waltl* is endemic to the Iberian Peninsula and northern Morocco and is currently listed as Near Threatened in IUCN's global Red List (BEJA *et al.*, 2009) because its populations are decreasing due to habitat loss and the negative impact of invasive species (BEJA *et al.*, 2009; SALVADOR, 2015). *Pelodytes punctatus* is distributed in central and east-

ern Spain, most of France and in coastal northwestern Italy and is globally assessed as Least Concern (IUCN SSC AMPHIBIAN SPECIALIST GROUP, 2020), but as Vulnerable in the Regional Catalogue of Threatened Species of Madrid (BOCM, 1992). The main threats for *P. punctatus* are also habitat loss and fragmentation and the spread of alien invasive species (PAÑOS *et al.*, 2011; CABALLERO-DÍAZ *et al.*, 2020).

Surveys

In order to estimate the local abundance of both species in ponds C1-C4, we performed nocturnal sampling sessions focused on recording capture histories of adult individuals. The four ponds and

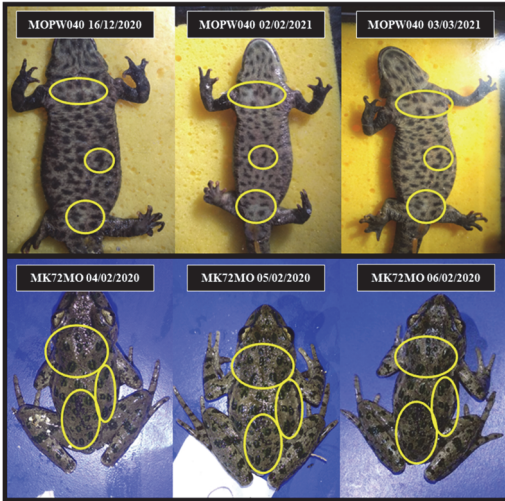


Figure 2: Pictures of the same individuals of *P. waltl* and *P. punctatus* (MOPW040 and MK72MO, respectively), captured in different sampling sessions. Ellipses highlight characteristic patterns facilitating photo-identification.

their surroundings (up to 20 to 50 meters from the shore of each pond) were surveyed by a minimum of two persons during the breeding season of both species, capturing all observed adult individuals by hand or using dip nets. We sexed all captured individuals based on morphological characters with sexual dimorphism (nuptial pads in males of both species) and measured their length from the snout to the end of the cloaca (in mm) and body mass (to the nearest 0.1 g). Total length (= snout-to-vent length + tail length) was also recorded in *P. waltl*. Additionally, we took a photo of the dorsal (*P. punctatus*) or ventral (*P. waltl*) pigmentation patterns of all captured individuals for subsequent photo-identification (Fig. 2) (CABALLERO-DÍAZ *et al.*, 2022; REYES-MOYA *et al.*, 2022). We also recorded peculiar morphological features

of potential use in photo-identification (for instance, developmental or coloration anomalies, see CABALLERO-DÍAZ *et al.*, 2019). All individuals were released back in their place of capture immediately after pooled processing of all the captured individuals of the sampling session. The total sampling effort for *P. punctatus* comprised 29 sampling sessions from September 2019 to June 2020, whereas for *P. waltl* we performed 28 sampling sessions from October 2020 to May 2021. These broad study timeframes allowed us to characterize two main reproductive periods in *P. punctatus* (September-October and January-February) and *P. waltl* (November-December and February-April), during which we intensified the frequency of sampling sessions.

Additional surveys to detect nearby breeding nuclei of *P. waltl* and *P. punctatus* potentially connected with the core study populations were conducted in 2020 and 2021. All water bodies potentially harboring amphibian populations in an 80 km² area (Fig. 1) were surveyed at different times of the year (minimum four visits per water body) to detect the presence of both species at any developmental stage (eggs, larvae, adults). Visual transects in aquatic and terrestrial habitats and dip-netting to detect larvae of both species were combined with acoustic surveys to detect breeding choruses of male *P. punctatus*.

Photo-identification

We used the software packages APHIS (MOYA *et al.*, 2015) and WILD.ID 1.0 (BOLGER *et al.*, 2012) to aid the photo-identification of individuals of *P. punctatus* and *P. waltl*, respectively, encountered in

the core study site. Dorsal (*P. punctatus*) and ventral (*P. waltl*) pictures were compared and processed in order to identify all individuals across sampling sessions and to compile their capture histories (Fig. 2). In APHIS, we used algorithm I3S to obtain a similarity index. In WILD ID, we used algorithm SIFT for the same purpose. For each photograph analyzed, both programs provided a list of potential matching candidates from the photographic database in decreasing order of similarity. We inspected the characteristic spot patterns in all candidate images and used field-recorded information on sex and biometric measures when necessary to manually confirm individual identification in each case. After all photo-identification assessments, we recorded the final dataset of capture histories for all individuals.

Estimation of population sizes

We applied capture-mark-recapture analysis with software MARK v9.0 (WHITE & BURNHAM, 1999) to estimate the local abundance of both species in the core study site based on the individual capture histories datasets. We used the POPAN formulation, which was specifically developed for the study of open populations by accounting for the probabilities of sequential entrance, death, and/or permanent migration of individuals (SCHWARZ & ARNASON, 1996) to estimate the total number of adults of the population (N_a). The four parameters considered in POPAN are: " ϕ ", the probability of apparent survival of individuals between capture sessions (which accounts for both real mortality and permanent emigration rates indistinguishably); " p ", the capture probability of

individuals in each sampling session; " $pent$ ", the probability of entrance of individuals to the study area before each sampling session; and " N ", the total number of individuals that are present in the study area at some point during the sampling period. Our study system accommodates to the basic POPAN assumptions, namely: 1) marked and unmarked individuals have the same survival and capture probability (*i.e.*, no marking effect); 2) the study area is constant throughout the sampling period; 3) individuals of *P. punctatus* and *P. waltl* maintain the same dorsal and ventral patterns throughout the study; 4) patterns are correctly identified by image-processing software; and 5) surveys can be considered instantaneous compared to the intervals between sampling sessions (SCHWARZ & ARNASON, 1996). We further assessed the Goodness of Fit (GOF) of the datasets of both species to POPAN assumptions with software U-Care v3.3 (CHOQUET *et al.*, 2009). Specifically, we tested for two common causes of violation of capture-mark-recapture model assumptions: the presence of transient individuals in the sample (transience test 3.SR) and the possible effect of first capture and marking on the subsequent capture probability of marked individuals (trap-dependence test 2.CT).

We conducted POPAN analyses for each pond and species separately. In the case of *P. waltl* we also performed analyses for the global quarry population. We considered the following restrictions: for the global population of *P. waltl* and for *P. punctatus*, we excluded sampling sessions with less than five captures to avoid over-parameterization of models; for the indi-

Table 1: Total number of captures, number of different photo-identified individuals, percentage of individuals with more than one capture (recaptures), population size estimates (N_a) and their 95% confidence intervals (95% CI) and number of sampling sessions used to estimate N_a in male and female *P. waltl* and *P. punctatus* in each pond of the core study site.

Species	Pond	Sex	Total captures	Photoid. individuals	% of recapt.	N_a (95 % CI)	Sessions
<i>P. waltl</i>	C1	♂	9	7	14.29	-	-
		♀	6	5	20.00	-	-
	C2	♂	183	81	53.09	102 (84-120)	16
		♀	98	52	44.23	62 (49-78)	
	C3	♂	10	6	33.33	-	-
		♀	6	5	40.00	-	-
	C4	♂	81	44	52.27	89 (53-125)	10
		♀	13	12	8.33	55 (0-130)	
	Global quarry	♂	283	127	53.49	176 (151-201)	20
		♀	123	65	40.91	139 (91-186)	
<i>P. punctatus</i>	C2	♂	263	224	27.13	749 (485-1,013)	9
		♀	17	17	0.00	48 (12-83)	

vidual pond estimates in *P. waltl*, we analyzed only ponds C2 and C4, where we obtained sufficient number of recaptures, and excluded sessions with less than three and five captures, respectively, to avoid overparameterization. In ponds C1 and C3, given the low number of captures and recaptures obtained, we used instead the total number of photo-identified individuals in each pond as a proxy for their local abundance. Finally, when several sampling sessions were conducted in consecutive days, we excluded some of them from analyses in order to maintain homogeneous time intervals across capture sessions. The final datasets of *P. waltl* comprised 16 capture sessions in pond C2, 10 capture sessions in pond C4, and 20 sessions in the global analysis. For *P. punctatus* the final dataset comprised nine capture sessions. In each analysis, we built several models in

MARK by defining parameters φ , p and $pent$ as either: 1) constant (\cdot), 2) time-dependent (t), 3) sex-dependent (g), or 4) time-and-sex-dependent with interaction (g^*t). Then, we ranked models based on Akaike's Information Criterion corrected for finite sample sizes (AICs, AKAIKE, 1974). Finally, we computed N_a estimates for males and females of each species by weighted averaging N estimates for each sex from the different models based on their AICc weight.

Spatial displacements

Based on photo-identification, we quantified the number of displacements of individuals of the two target species among the ponds sampled in the core study site. We calculated a spatial displacement rate: m = number of individuals with at least one recorded displacement / number of

Table 2: Ranking of models used to estimate N_a in *P. waltl* and *P. punctatus* in the core study site with the POPAN formulation. Scores of the Akaike Information Criterion corrected for finite sample sizes (AICc), model weights, number of parameters and deviance are presented. “g” = sex-dependent, “t” = time-dependent, and “.” = constant.

Species	Pond	Model name	AICc	AICc weight	Num. of param.	Deviance	
<i>P. waltl</i>	C2	$\varphi(.) p(t) pent(t)$	804.662	0.804	33	-78.911	
		$\varphi(g) p(t) pent(t)$	807.491	0.195	34	-78.911	
		$\varphi(.) p(g^*t) pent(t)$	821.974	0.00014	49	-111.010	
		$\varphi(g) p(g^*t) pent(t)$	825.384	0.00003	50	-111.010	
		$\varphi(t) p(t) pent(t)$	827.645	0.00001	45	-92.104	
	C4	$\varphi(g) p(t) pent(t)$	266.166	0.972	22	-64.191	
		$\varphi(.) p(t) pent(t)$	273.256	0.028	21	-53.312	
		$\varphi(t) p(t) pent(t)$	282.330	0.00030	27	-69.107	
		Global quarry	$\varphi(.) p(g^*t) pent(t)$	1245.753	0.999	61	-231.456
			$\varphi(g) p(t) pent(t)$	1269.332	0.00001	42	-154.159
<i>P. punctatus</i>	C2	$\varphi(t) p(g^*t) pent(t)$	456.404	0.867	33	-704.746	
		$\varphi(.) p(t) pent(g^*t)$	461.722	0.061	27	-684.237	
		$\varphi(t) p(t) pent(g^*t)$	463.264	0.029	32	-695.302	
		$\varphi(.) p(.) pent(t)$	463.610	0.024	12	-647.420	
		$\varphi(.) p(g^*t) pent(t)$	464.225	0.017	28	-684.215	

individuals with two or more captures. We also calculated the total accumulated distance covered by each dispersing individual. For individuals with more than one registered displacement, we added up the distances of their recorded displacements (i.e., if an individual dispersed to another pond and then returned back to the first one, we added the distances of both displacements).

RESULTS

Local and regional abundance of *P. waltl* and *P. punctatus*

We obtained a total of 280 captures of adults of *P. punctatus* (263 captures of males and 17 of females, all of them in pond C2), and 406 captures of adults of *P.*

waltl (283 captures of males and 123 of females) in the core study site. Captures of *P. waltl* were distributed unequally between ponds (Table 1), and some individuals were captured in more than one pond (see below).

Photo-identification allowed us to identify all individuals captured in the core study site, which corresponded to 241 different individuals (224 males and 17 females) of *P. punctatus* and 192 individuals (127 males and 65 females) of *P. waltl*. Among photo-identified individuals, 35 were captured more than once in the case of *P. punctatus* (recapture rate of 14.52%, 27.13% in males; 0% in females), and 96 in the case of *P. waltl* (recapture rate of 50%: 53.49% in males; 40.91% in females).

Table 3: List and location (latitude and longitude) of water bodies with breeding populations of *Pelodytes punctatus* (*Pp*) and/or *Pleurodeles waltl* (*Pw*) in a 6 km-radius buffer area from our core study site (Charcas Aeródromo, C).

Code	Locality	Municipality	Type of water body	Species	Lat	Long
A	Canteras Valdegatos	Morata de Tajuña	Quarry	<i>Pp, Pw</i>	40.24	-3.45
B	Túnel Ladrillos	Morata de Tajuña	Stream	<i>Pp</i>	40.24	-3.43
C	Charcas aeródromo	Morata de Tajuña	Quarry	<i>Pp, Pw</i>	40.24	-3.43
D	Cantera cordel Merinas	Morata de Tajuña	Quarry	<i>Pp</i>	40.24	-3.43
E	Valdecubillos	Perales de Tajuña	Quarry	<i>Pp</i>	40.24	-3.39
F	Fuente del Valle	Arganda del Rey	Artificial pond	<i>Pp</i>	40.27	-3.43
G	Cantera triturados	Arganda del Rey	Quarry	<i>Pp, Pw</i>	40.27	-3.41
H	Pilón vereda Valdecabañas	Arganda del Rey	Water tank	<i>Pp</i>	40.28	-3.42

According to MARK results, the best model in *P. punctatus* considers that the probability of entrance (*pent*) is time-dependent, the probability of capture (*p*) is time-and-sex-dependent and apparent survival (ϕ) is time-dependent (Table 2). In the case of *P. waltl*, the best model considers that ϕ is constant or sex-dependent whereas *p* is time-dependent or time- and sex-dependent, and *pent* is time-dependent (Table 2). We found no evidence of “transience” or “trap-dependence” effects in any dataset. Nevertheless, some tests could not be performed due to the low number of capture sessions with sufficient captures (females of *P. punctatus* and *P. waltl* in pond C4). The highest abundance estimates for both species were recovered

in pond C2 (> 160 individuals of *P. waltl* and > 750 of *P. punctatus*, Table 1). The global estimate for the *P. waltl* population in the quarry was > 300 individuals (Table 1).

Regional surveys revealed the presence of *P. waltl* and/or *P. punctatus* in 7 additional locations (Table 3), at distances of 0.6-4.4 km from the core study site (Table 4).

Spatial displacements

Twenty *P. waltl* individuals (20.83% of all photo-identified individuals with at least two captures) performed a total of 23 recorded displacements (13 by females, 10 by males) among the sampled ponds (Fig. 3). Only two individuals performed more

Table 4: Matrix of pairwise distances (in meters) between water bodies with breeding populations of *Pelodytes punctatus* and/or *Pleurodeles waltl* within a 6 km-radius buffer area from our core study site (Charcas Aeródromo, C).

Code	A	B	C	D	E	F	G	H
A								
B	2113							
C	1896	676						
D	2405	487	598					
E	5639	3531	3845	3265				
F	3806	3419	2799	3052	4992			
G	4987	3844	3429	3368	3967	1738		
H	5625	4922	4393	4479	5373	1837	1413	

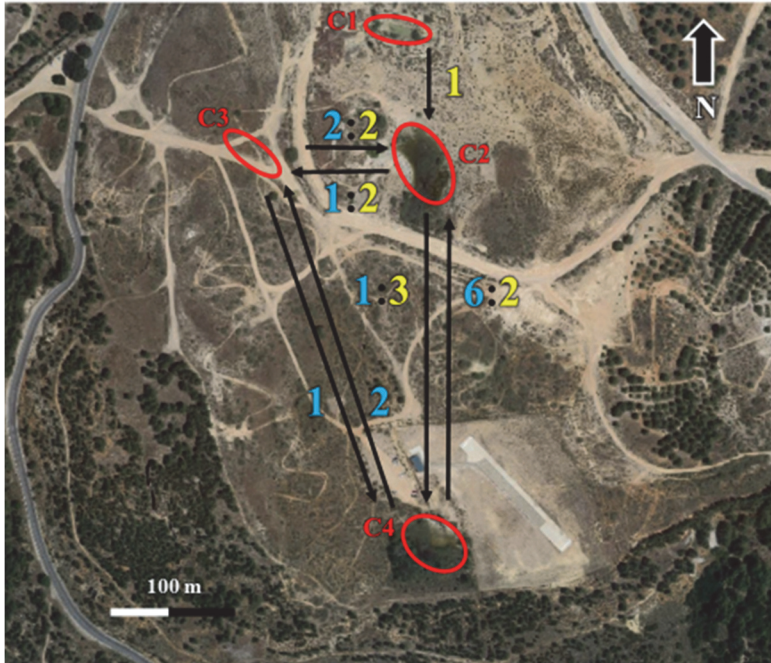


Figure 3: Map of the core study site showing recorded displacements among ponds (C1, C2, C3 and C4) of female (blue) and male (yellow) *Pleurodeles waltl*. For a proper visualization of the figure, the reader is referred to the online version of the article.

than one displacement. Individual accumulated distances ranged from 80 m to 840 m (Fig. 4). We did not record any spatial displacement of male or female *P. punctatus*.

DISCUSSION

Our results show that ponds in abandoned and re-naturalized quarries can host large amphibian populations, with estimates of several hundred adults of *P. punctatus* and *P. waltl*, which represent some of their highest recorded abundances in the region (CABALLERO-DÍAZ *et al.*, 2020). We also confirm the presence of additional breeding nuclei of both species in neighboring areas: two for *P. waltl* at 1.9 and 3.4 km of the core study site, respectively, and seven for *P. punctatus* at distances between 0.6 and 4.4 km from the core study site. These spatial distances are compatible with functional connectivity. Although we

recorded limited adult dispersal distances in *P. waltl* and found no evidence of dispersal in *P. punctatus* among the four ponds of the core study site, it cannot be ruled out that this abandoned quarry may be a potential source of migrant individuals to neighboring sites, especially mediated by juvenile stages, which were not accounted for in this study (see discussion below).

The relevance of local population nuclei for regional persistence of amphibian species must be evaluated on the basis of robust demographic assessments, for which accurate abundance estimates are cornerstone. In this line, the application of capture-mark-recapture approaches based on photo-identification holds great potential as an effective, non-invasive tool for the study of population dynamics in amphibians (CABALLERO-DÍAZ *et al.*, 2022; REYES-MOYA *et al.*, 2022). In our study, photo-

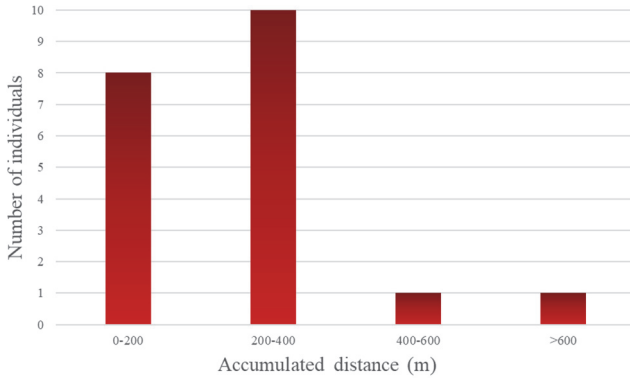


Figure 4: Maximum accumulated distances recorded for recaptured *P. waltl* individuals in the core study area.

identification was highly effective, allowing unambiguous identification of all captured individuals upon visual assessment of the natural marks of individuals in candidate images and aided by information on sex and biometric measures recorded in the field. However, the accuracy of population size estimates based on photo-identification also depends on recapture rates, which can be limited in explosive-breeding species like *P. punctatus*. Obtaining recaptures is especially challenging in species in which females have lower detectability, either because they have more cryptic habits or because they spend less time in ponds during the breeding season (SÁNCHEZ-MONTES *et al.*, 2017; GUTIÉRREZ-RODRÍGUEZ *et al.*, 2017b). In these cases, obtaining robust population size estimates requires monitoring during consecutive breeding seasons and active searching for females during their migrations to and from breeding ponds. These extended surveys have the additional value of providing data on inter-annual fluctuations in population sizes and female breeding stocks, which are key parameters in amphibian population ecology.

Our estimates show that both *P. puncta-*

tus and *P. waltl* are abundant in the temporary ponds in the core study area, in the order of hundreds of adult individuals. The role of this re-naturalized area in regional population dynamics will thus depend on its potential to exchange migrant individuals with neighboring populations. In this respect, our regional surveys show good potential for connectivity, especially with the close populations of *P. punctatus* in localities B and D (both within 700 m from the core study site). However, we did not detect any evidence of dispersal in our marked individuals. This could indicate strong site fidelity of breeding individuals of *P. punctatus*, but as a secretive, explosive breeding species, it cannot be discarded that undetected dispersal occurs, given the relatively low recapture rates obtained in this study and its limited timeframe (a single year). Indeed, adult dispersal up to 300 meters has been confirmed for *P. punctatus* in France (TOXOPEUS *et al.*, 1993). Furthermore, juvenile dispersal, which has been documented as an important factor for functional connectivity but remains challenging to address by mark-recapture methods, could represent an important contribution to inter-population connectiv-

ity in our study area. Thus, the potential for demographic inter-connection of *P. punctatus* between the core study site and neighboring areas should be further verified by integrating both direct (spatial displacements based on capture-mark-recapture over consecutive breeding seasons) and indirect (genetic) data.

On the other hand, our capture-mark-recapture results in *P. waltl* provide some insights about their dispersal capacity. The frequency of displacements observed in our surveys during a single year (20.83%) is notably higher than those recorded in previous multi-year studies: 0.51% during eight years (GUTIÉRREZ-RODRÍGUEZ *et al.*, 2017b), 1.3% during 12 years (FERNÁNDEZ DE LARREA *et al.*, 2021), and 2% during two years (REYES-MOYA *et al.*, 2022). This discrepancy can be partly explained by the shorter distances between ponds in our core study area as compared with other studies, but it could also reflect differences in the spatial ecology of *P. waltl* in drier Mediterranean ecosystems, like in SE Madrid, where the species is less abundant and populations are more fragmented. Supporting this latter scenario, other newt species have been shown to display altered dispersal behavior and even develop dispersal phenotypic syndromes in response to intrinsic and environmental factors (WINANDY *et al.*, 2017; DENOËL *et al.*, 2018). These results suggest that localities B and D could be potentially colonized by *P. waltl* from the core study site, and probably also locality H via locality G, because these potential breeding sites are within the range of dispersal distances recorded for *P. waltl* in this and other studies (GUTIÉRREZ-RODRÍGUEZ *et al.*, 2017b; FER-

NÁNDEZ DE LARREA *et al.*, 2021; REYES-MOYA *et al.*, 2022). Future studies should further characterize with molecular markers the extent of functional connectivity of *P. waltl* populations in this area, which is essential to maintain gene flow and avoid the risk of genetic erosion and inbreeding (FRANKHAM *et al.*, 2014).

The distribution of *P. punctatus* and *P. waltl* in SE Madrid is fragmented and populations are not abundant, especially in the latter (PAÑOS *et al.*, 2011; CABALLERO-DÍAZ *et al.*, 2020). The main threats are habitat degradation and loss due to mining activities and the construction of urban and road infrastructures, water pollution, and the introduction of alien invasive species (MARTÍNEZ-SOLANO, 2006; PAÑOS *et al.*, 2011; CABALLERO-DÍAZ *et al.*, 2020). In this context, artificial water points like ponds in naturalized quarries acquire a key role in conservation policies and actions (CABALLERO-DÍAZ *et al.*, 2020, 2022). These ponds represent important assets for the long-term persistence of amphibian populations, potentially hosting large populations and favoring connectivity with neighboring areas, altogether contributing to maintaining genetic diversity and reducing the risk of local extinctions (SHAFFER, 1981; FRANKHAM *et al.*, 2014). Management actions should ensure the protection of these sites and promote the creation of additional ponds to mitigate the negative effects of habitat fragmentation and loss on amphibian populations.

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