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Research paper

Citizen science reveals sharp decline of the Mediterranean tree frog *Hyla meridionalis* over 40 years in southeastern Iberia: potential causes and proposed solutions to prevent regional extinction

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Abstract

Citizen science reveals sharp decline of the Mediterranean tree frog Hyla meridionalis *over 40 years in southeastern Iberia: potential causes and proposed solutions to prevent regional extinction*. Several previous studies have suggested that the Mediterranean tree frog *Hyla meridionalis*, a species highly sensitive to habitat alterations, is currently threatened in this region. Here, we provide an update on the distribution and conservation status of the hylid in this area, based on monitoring through nocturnal acoustic surveys and visual prospections in sites with favorable characteristics for the species during 2016-2019. Some of these points had historical occurrence data (1980s-1990s), allowing us to analyze temporal variations in the species' presence. Furthermore, we examined how habitat transformation and levels of protection were related to changes in the local presence of the anuran in the Adra River basin, an area where human-induced environmental modifications and frog persistence have been documented over the past 40 years. The Mediterranean tree frog was found at low numbers in only 17 of the 144 sampled points, mostly isolated, during 2016-2019, and had disappeared from 27 of the 43 points where it was previously present 40 years ago. We found that the loss of favorable habitat and the absence of legally protected areas were directly linked to the decline in sites inhabited by the species. Based on the changes observed and IUCN criteria, we classified the Mediterranean tree frog as 'endangered' in southeastern Iberia. Conservation measures are proposed to support the recovery of the hylid in the study area.

Key words: Agricultural impact, Conservation, Distribution, Greenhouses, *Hyla meridionalis*, Temporal evolution

Resumen

La ciencia ciudadana revela un marcado declive de la ranita meridional Hyla meridionalis *durante los últimos 40 años en el sureste de Iberia: posibles causas y soluciones propuestas para prevenir su extinción regional*. Diversos estudios previos señalan que la ranita meridional *Hyla meridionalis* una especie altamente sensible a las alteraciones de su hábitat, se encuentra actualmente amenazada en el sureste ibérico. Aquí aportamos una actualización sobre la distribución y el estado de conservación del hílido en esta área, basada en el seguimiento mediante sondeos acústicos y prospecciones visuales nocturnas en localidades con características favorables para la especie durante 2016-2019. En parte de estos puntos se disponía de datos históricos de presencia de la ranita meridional (décadas de 1980-1990), lo que permitió analizar cambios temporales en la presencia de la especie. Además, en la cuenca del Río Adra, donde se conocían las variaciones ambientales inducidas por el ser humano y la persistencia de la rana en los últimos 40 años, se estudió cómo las transformaciones del hábitat y el nivel de protección se relacionaron con cambios locales en la presencia del anuro. La ranita meridional fue detectada en números bajos en solo 17 de los 144 puntos muestreados durante 2016-2019, y había desaparecido en 27 de los 43 donde estaba presente hace 40 años. Se constató que la pérdida de hábitat favorable y la ausencia de espacios legalmente protegidos está directamente relacionada con el declive de lugares habitados por la especie. Teniendo en cuenta los cambios observados y los criterios de la UICN, catalogamos a la ranita meridional 'en peligro de extinción' en el sureste de Iberia. Proponemos medidas de conservación para favorecer la recuperación del hílido en el área de estudio.

Palabras clave: Conservación, Distribución, Evolución temporal, *Hyla meridionalis*, Impacto Agrícola, Invernaderos

Introduction

Global change processes, including climate change, pollution, the invasion of exotic species, and habitat loss and fragmentation, represent the primary current threats to biodiversity conservation (Duarte 2006, García and Jordano 2021). These drivers of negative change are notoriously evident for the most vulnerable amphibians, especially those dependent on aquatic habitats, and particularly at the edges of their distribution ranges (Duellman 1999, Collins and Crump 2009, Cordier et al 2021, Alves-Ferreira et al 2022, Mi et al 2023, Souza et al 2023). In this context, many species of the Hylidae family are expected to experience significant declines, as they are especially sensitive to human-induced environmental alterations (Dufresnes et al 2013, Hoskin et al 2013, Vasconcelos and do Nascimento 2016, Borzée 2022). In fact, hylids are often used as sentinels of the conservation status of the habitats they occupy (Semlitsch et al 2000, AbuBakr and Crupper 2010, Zazeri et al 2010, Gonçalves et al 2013, Sillero 2014, Santos et al 2015, Valdespino et al 2015, Borzée et al 2018, Button et al 2022).

The Mediterranean tree frog *Hyla meridionalis* was likely introduced to the Iberian Peninsula during prehistoric or historical periods (Dufresnes and Alard 2020). This species is abundant in coastal and inland temporary or permanent waterbodies with abundant marsh vegetation, such as waterlogged meadows, low-flowing streams, ponds, and wetlands (Sillero 2014). Given its habitat preferences, the impacts of global change on this hylid are expected to be bigger in arid and semi-arid regions where the lack of water is the main life constraint (Sillero 2014). While this frog species has been expanding to other regions or scales, likely as a result of global warming (Sillero 2009, 2010), several authors suggest it is threatened and at risk of extinction in the southeastern edge of Iberia, the driest region in Europe (175-300 mm/year in much of the territory, United Nations Environment Programme 1992). This is due to population reduction and fragmentation (Tejedo and Reques 2002, Torralva et al 2005, González-Miras and Nevado 2008, Alaminos 2013) and low habitat suitability (Sillero 2010, Reino et al 2016). In fact, the Mediterranean tree frog is currently included in the 'Spanish list of wild species needing special protection' (LESRPE), which mandates periodic reassessment of its conservation status under Spanish legislation (Executive order 139/2011).

To date, only one study has assessed the conservation status of Mediterranean tree frog populations in southeastern Iberia, showing evidence of declines in this region since 1980s-1990s (Paracuellos et al 2017), despite the recent discovery of a new population in the province of Alicante (Gilabert et al 2022). However, recent reports by Paracuellos et al (2017) may have underestimated the frog presence due to the fact that frog-singing activity was recorded on only a single day. Amphibian activity and, hence, detection are highly sensitive to fluctuating environmental conditions (Heyer et al 1994, Duellman 1999, Dodd 2010, Greenberg et al 2018, Moss et al 2021). Therefore, re-sampling across multiple years, covering a wide range of environmental conditions, is essential to avoid false absences (Gómez-Rodríguez et al 2012) and accurately assess the decline of the Mediterranean tree frog in southeastern Iberia.

The primary goal of the present study is to reassess the conservation status of the Mediterranean tree frog in southeastern Iberia, based on updated and multi-replicated records of frog-singing activity in localities with appropriate characteristics for the species. In some of these localities, historical (1980s-1990s) presence data exist, allowing us to investigate temporal variation in the species' presence. Furthermore, we aim to identify potential drivers behind changes in the species' geographical distribution in southeastern Iberia and to evaluate the effectiveness of potential conservation measures for planning future conservation actions. This last objective is addressed by studying the relationship between human-induced habitat transformations at the local level and frog persistence in the Adra River basin.

Material and methods

Study area

The study was conducted in southeastern Iberia, across the provinces of Almeria, Murcia and Alicante, covering nearly 26,000 km^2 (fig. 1) in the driest region of Europe. As a result, water scarcity is the primary limiting factor for biotic activity in the area, leading to sparse vegetation cover, typically dominated by small shrubs and grasses (Gil Olcina 2009). Despite these conditions, historical records (from 1980s to 1990s) indicate the presence of Mediterranean tree frog in different localities within the study area (Paracuellos et al 2017). In these, several points

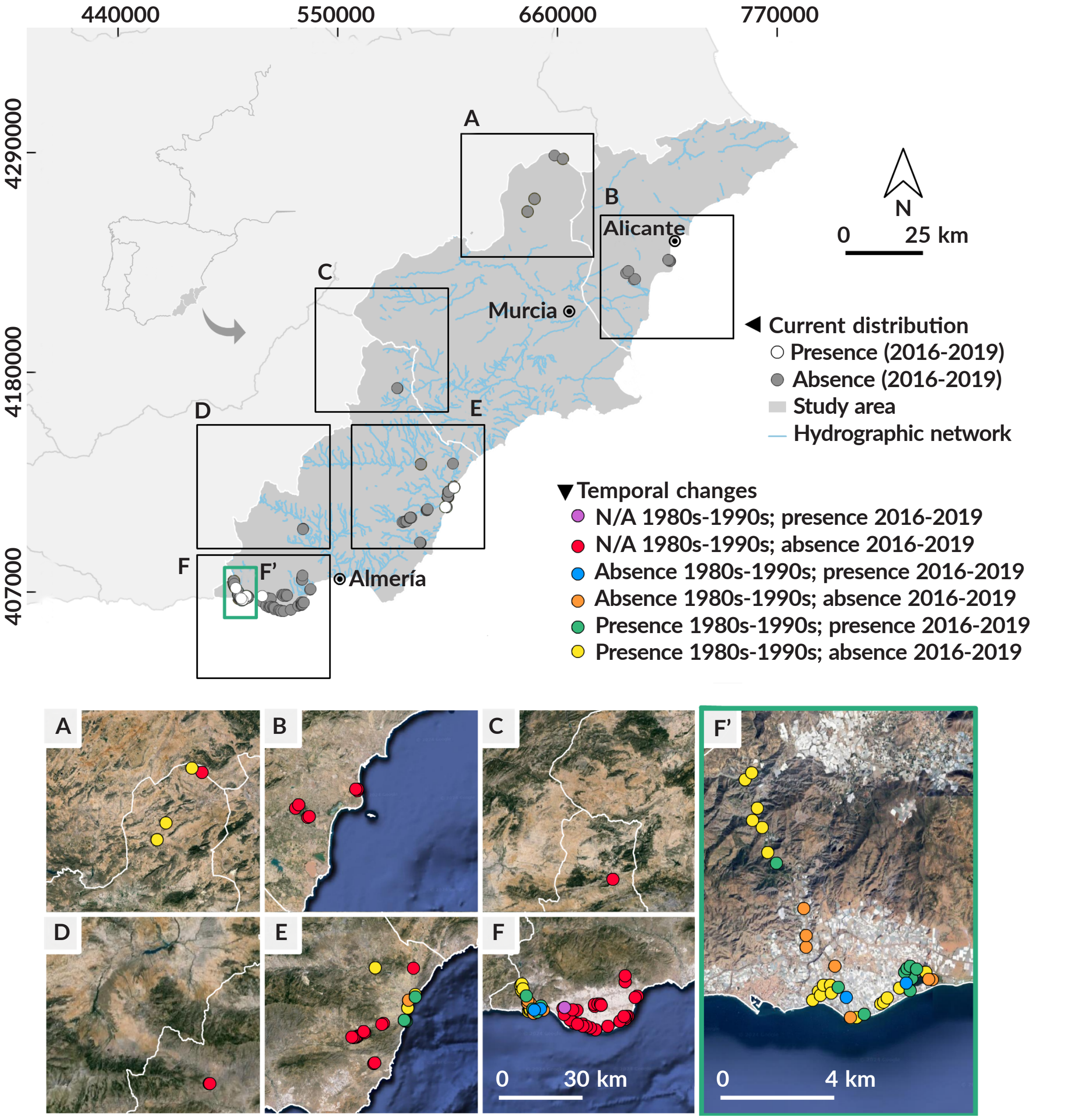

Fig. 1. Geographic distribution of the study area, sampling points and historical presence of the Mediterranean tree frog *Hyla meridionalis* in southeastern Iberia. Squares indicate the different regions surveyed: A, High plateau of Murcia; B, southern Alicante; C, Los Vélez; D, the Andarax River basin; E, East of Almería; and F, West of Almería. F', the Adra River basin, located within West of Almería, is shown in a separate inset panel. Colors at the sampling points represent temporal changes in species presence. All panels A-F share the same spatial scale, indicated in panel F.

***Fig. 1.** Distribución geográfica del área de estudio, puntos de muestreo y presencia histórica de la ranita meridional* Hyla meridionalis *en el sureste de la península Ibérica. Los cuadrados indican las diferentes regiones donde se realizaron los muestreos y los paneles corresponden a: A, Altiplano murciano; B, sur de Alicante; C, Los Vélez; D, cuenca del Río Andarax; E, Levante Almeriense; y F, Poniente Almeriense. F', la cuenca del Río Adra en el Poniente Almeriense se muestra en un panel aparte. Los colores de los puntos de muestreo indican los cambios temporales en la presencia de la especie. Todos los paneles A-F comparten la misma escala especial indicada en el panel F.*

were resampled, along with other additional locations showing favorable environmental conditions for this hylid. Considering the species' ecology (Sillero 2014), sampling points were generally located in backwater zones of both permanent and ephemeral rivers and wetlands, ranging from sea level on the coast to 1,081 m a.s.l., across six distinct regions (fig. 1). These study regions included: (i) West and (ii) East of Almería, (iii) the Andarax River basin and (iv) Los Vélez in Almería province, (v) the High plateau of Murcia province, and (vi) southern Alicante province (fig. 1, table 1s). West of Almería consists of an extensive plain of approximately 33,000 ha, now almost entirely covered by intensive greenhouse agriculture, with a few remaining waterbodies that persist surrounded by crops under plastic. East of Almería is also heavily transformed by land use, particularly along the coast, where the remaining ponds are surrounded by intensive agriculture and increasing urban development. In the Andarax River basin and Los Vélez, sampling sites included river stretches that, despite increasing pressure from water usage, still maintained streams and pools with natural riparian vegetation. In the High plateau of Murcia, the sampled sites exhibited greater environmental variability, ranging from agricultural landscapes, reforested areas, an old fountain, a stream, and a marshy pool. Finally, the surveyed areas in southern Alicante were also embedded within agricultural and urban surroundings. However, wetlands in this region were more favorable for the species due to lower water salinity. For more details about the study area and sampling locations, see Martínez et al (2000-2009), Box Amorós (2003), Casas et al (2003), Marco Molina (2005), Salinas and Casas (2007), López Martos et al (2010) and Navarro and Rodríguez Tamayo (2011).

Field surveys

To detect the presence of Mediterranean tree frogs, nocturnal surveys were conducted at several points in each locality during the years 2016 (with data from Paracuellos et al 2017, 2018 and 2019). These controls involved monitoring the calling activity of males and performing intensive visual searches of specimens. Such methodology is widely used to assess the local presence of anurans (Dodd 2010, Márquez et al 2014). All individuals visually detected were adults, which were easily identifiable without the need for capture. Sampling was performed within the framework of a citizen science program, in which volunteers were coordinated by expert herpetologists at each locality. All volunteers were familiar with the appearance and vocalizations of the study species and were previously trained using audio recordings of their calls (see a similar case in Paracuellos et al 2022).

To optimize male detection, the 2018-2019 surveys were carried out during periods of peak calling in each site. These time windows were identified based on data from Paracuellos et al (2022), which documented monthly calling activity throughout the 2018-2019 full annual cycle in multiple locations representative of the different environmental conditions across the study area. When a peak in calling intensity was detected in one of the reference sites, surveys then scheduled at all points of the current study with similar environmental conditions. To reduce the likelihood of false absences, nights with ambient temperature below 14 °C were excluded from sampling, as calling activity significantly decreases under such conditions (Llusia et al 2013a, 2013b, 2013c, Márquez et al 2014). Surveys were also avoided during periods of strong winds, rainfall, or anthropogenic noise that could interfere with acoustic detection. Additionaly, sampling was not conducted on clear, moonlight nights when lunar brightness exceeded 50 %, as this may affect both reproductive behavior and detection probability (Dorcas et al 2009, Vignoli and Luiselli 2013, Onorati and Vignoli 2017). Whenever possible, sampling was scheduled for dates when surface water was present at all the points, as water availability is closely linked to calling activity (Paracuellos et al 2022). Each point was surveyed at night during the two hours following sunset, which corresponds with increased nuptial chorus activity (Márquez et al 2014). Upon arrival at each sampling point, researchers spent approximately 15-30 minutes searching for individuals using both auditory and visual cues in and around waterbodies and adjacent marsh vegetation. If no individuals were detected during this initial search, pre-recorded male calls of the Mediterranean tree frog (from Márquez and Matheu 2004) were played for 15 minutes using Bluetooth speakers or smartphones, to stimulate calling and increase the chance of detecting males through vocal response (Heyer et al 1994). After 30-45 minutes of no frog detections, the sampled point was recorded as a negative detection for that visit.

The number of sampling points was proportional to the area surveyed within each locality and spaced at least 50 m apart (table 1, fig. 1). Surveys were conducted over 56 census days, from March 26 to July 14. Of all sampling points, 87 % were visited four or five times (typically one in 2016, and twice in both in 2018 and 2019), while the remaining 13 % were visited between one and three times during the same period. In total, 144 sample points (defined as environmentally favorable sites for surveys) were monitored across 27 localities (areas composed of spatially connected sampling points) over the years 2016, 2018 and 2019, resulting in 613 individual surveys (table 1s).

Statistical analyses

To assess changes in the distribution of the Mediterranean tree frog in southeastern Iberia, we focused on 54 sampling points in three regions, East and West of Almería and the High plateau of Murcia (fig. 1, table 1s), for which presence-absence data of the species were used in two time periods, 1980s-1990s and 2016-2019. We analyzed changes in the proportion of sampling points with the species presence between these periods in East and West of Almería using binomial generalized linear models. In the High plateau of Murcia, only visual data comparison between the two periods wase performed due to the small sample size.

Additionally, we conducted a more detailed analysis of the factors influencing the historical presence of the Mediterranean tree frog in the Adra River basin (fig. 1F'), traditionally the most important area for the species in southeastern Iberia (Paracuellos et al 2017). The Adra basin, located within the West of Almería region, constitutes a hydrological unit with interconnected waterbodies and a relatively high number of sampling points (N = 38). This area encompasses a wide range of environmental conditions affecting the frog, shaped by varying levels of agricultural and anthropogenic pressure, particularly over recent decades. We quantified historical and current values for five habitat features relevant to the Mediterranean tree frog (Lizana and Barbadillo 1997, Márquez and Lizana 2002, Sillero 2014, Smith and Sutherland 2014, Maceda-Veiga et al 2016) for each sampling point using aerial photographs from the 1980s-1990s and 2016-2019. Specifically, we measured: (a) distance to the nearest roads or paths as a proxy for human disturbance; within a 500 m radius, (b) area of favorable habitat, defined as the total surface of permanent or temporary waterbodies with shallow shores covered by dense marsh vegetation (sensu Sillero 2014), (c) area occupied by open-air crops, (d) area under intensive greenhouse

Table 1. Results of the general linear model assessing the disappearance of the Mediterranean tree frog *Hyla meridionalis* in the Adra River basin in relation to habitat changes. The model includes the first two axes of the principal component analysis (PCA), which summarize habitat variable changes between the 1980s-1990s and 2016-2019 across 38 sampling points. Corresponding PCA scores are provided in table 3s.

Tabla 1. Resultados del modelo lineal generalizado que describe la pérdida de la ranita meridional Hyla meridionalis *en la cuenca del río Adra en respuesta a los cambios en el hábitat. El modelo incluye los dos primeros ejes del análisis de componentes principales (PCA), relativo a los cambios en las variables de hábitat entre las décadas de 1980-1990 y 2016-2019 para los 38 puntos de muestreo. Los resultados del PCA se muestran en la tabla 3s.*

	Estimate	Z-value	P
(Intercept)	0.1241	0.33	0.73
Component 1	-1.8634	-1.99	0.05
Component 2	0.0316	0.03	0.98

cultivation, and (e) area invaded by the non-native giant reed *Arundo donax*. The giant reed is one of the world's worst invasive plant species (Lowe et al 2000), particularly in riverbeds, where it alters numerous ecosystem functions (Dudley 2000, Aguiar and Ferreira 2013), depleting invertebrate fauna and, thereby, likely reducing habitat suitability for the study frog (Paracuellos 1997, Maceda-Veiga et al 2016).

We explored potential drivers of change in Mediterranean tree frog distribution within the Adra River basin using both historical and contemporary data on species presence and key environmental variables. First, we assessed temporal changes in habitat features in the sampled points by using paired *t*-tests. Then, we used independent *t*-tests to evaluate whether current habitat variables differed between sampling points where the frog is present and absent.

Next, we calculated changes in each environmental variables f (current minus historical values) for each sampled point and tested whether these changes differed between points where the species has disappeared and those where it has persisted, using independent *t*-tests. We then conducted a principal component analysis (PCA) on the changes in the five habitat variables between the two periods, 1980s-1990s vs. 2016-2019. The first two PCA axes were used to examine the combined effect of habitat changes on frog loss. To this end, we fitted a binomial generalized linear model with species loss as the response variable and the PCA axes as predictors. All variables were standardized to a 0 and 1 scale prior to PCA.

Finally, using independent *t*-tests, we evaluated the potential role of habitat protection by testing whether the surface area of legally protected land within a 500 m radius around each sampling point differed: (1) between sampled points where the species is currently present vs. absent, and (2) between sampled points where the species has persisted vs. disappeared over the past 40 years.

Analyses were conducted using the *lme* (Bates et al 2015), *stats*, and *vegan* (Oksanen et al 2025) packages in R Statistical Software (v. 4.1.2., R Core Team 2021). Standard model validation plots (Zuur et al 2009) confirmed that assumptions of homoscedasticity and normality of residuals were met.

Evaluation of conservation status

The regional conservation status of the Mediterranean tree frog was re-evaluated in southeastern Iberia using the categories, criteria, and application guidelines of UICN (2012a, 2012b), following a three-step process: (1) identification of the Mediterranean tree frog and its southeastern Iberian populations as the assessment targets, (2) evaluation of the species' conservation status within the study area to assign a preliminary category (UICN 2012a), and (3) consideration of the influence of neighboring populations of the same species, potentially adjusting the preliminary category accordingly (UICN 2012b). Based on field data collected, the relevant criterion applied was related to the species' geographic distribution in its area of occupancy (B2). The typical dispersal distance of the target species is generally used to define this parameter. While data exist for the spatial movements of other *Hyla* species in Europe (Vos and Stumpel 1996, Angelone and Holderegger 2009, Brandt et al 2018, Sánchez-Montes et al 2018, Martínez-Gil et al 2023), such information is currently lacking for the Mediterranean tree frog (e.g., Caballero-Díaz et al 2024). Although some studies suggest greater potential dispersal distances in related species (e.g., Vos et al 2000, Angelone and Holderegger 2009), we opted for a more conservative approach. In the absence of species-specific data, using the shortest known dispersal distances represents a more precautionary strategy from a conservation management perspective, as it helps ensure sufficient genetic flow and, consequently, population resilience. To this end, we relied on the geographically closest known and comparable case to our study, that of the Iberian tree frog *Hyla molleri* in central Spain. Based on existing data for such species, and taking into account the inherent uncertainty of this approach, we adopted a non-cumulative dispersal distance threshold of no more than 1 km, as longer distances have been reported to occur at markedly lower frequencies (Sánchez-Montes et al 2018, Martínez-Gil et al 2023, Sánchez-Montes and Martínez-Solano 2023). Using this standardized distance, we generated 1 km-radius buffers around those georeferenced sampling points with available information of the species jointly in 1980s-1990s and 2016-2019 where the frog was detected. The area of occupancy of the target hylid was then calculated as the total continental land surface encompassed by these presence buffers, for each time period separately.

Results

Current distribution of the Mediterranean tree frog in southeastern Iberia

Based on data collected through citizen science, the Mediterranean tree frog was recorded at 17 out of 144 sampled points during 2016-2019 (table 1s). The chorus size at these points was notably small, with no more than four calling males detected at most sites (table 2s). During the study period, the species appeared in the East and the West of Almería province, but not at sampled points

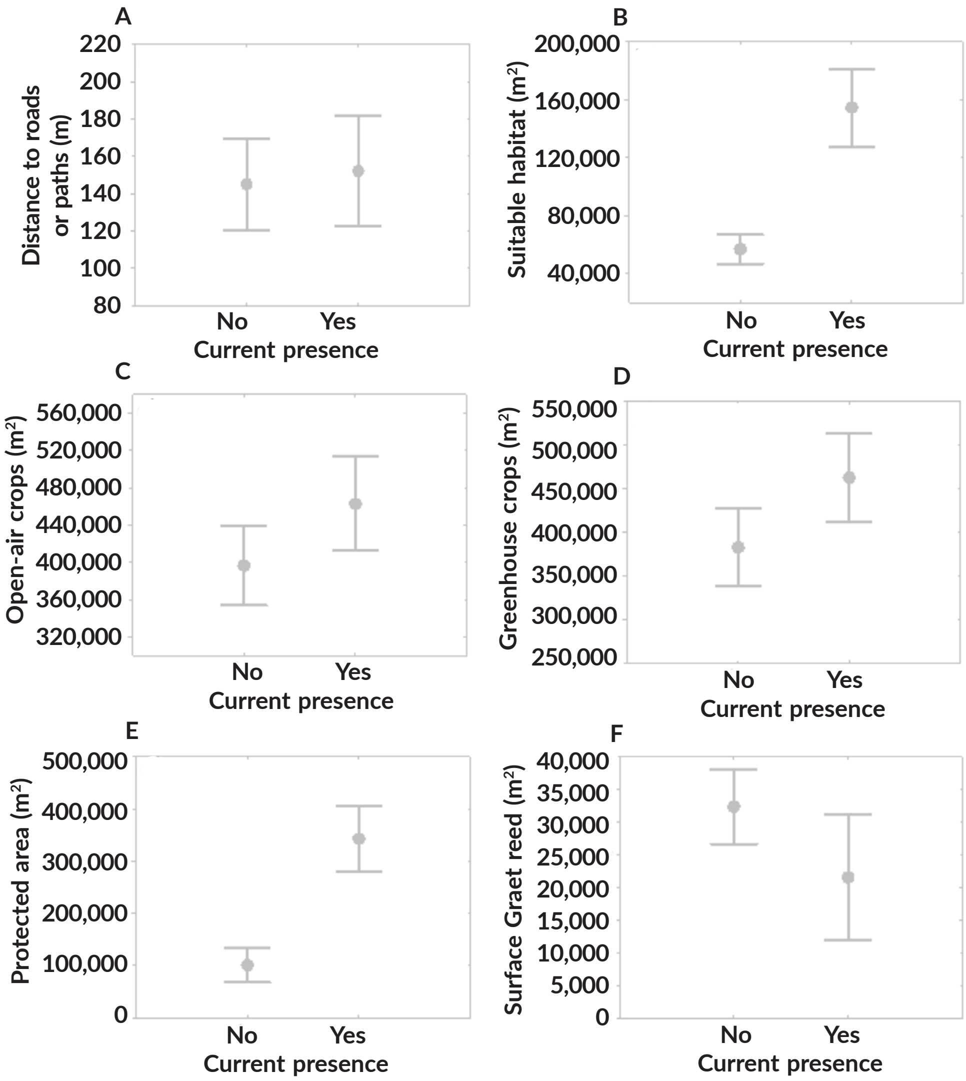

Fig. 2. Mean values (± SE) of habitat variables in relation to the presence of the Mediterranean tree frog *Hyla meridionalis* during 2016-2019 at 38 sampling points within the Adra River basin. A, distance to roads or paths; B, surface area of favorable habitat; C, surface area of open-air crops; D, surface area of greenhouse crops; E, surface area of giant reeds *Arundo donax*; and, F, surface area within legally protected zones.

Fig. 2. *Valores medios de las variables del hábitat (± ES) en relación con la presencia de la ranita meridional* Hyla meridionalis *en 2016-2019 para los 38 puntos muestreados dentro de la cuenca del río Adra. A, distancia a carreteras o caminos; B, superficie de hábitat favorable; C, superficie de cultivos al aire libre; D, superficie de cultivos en invernadero; E, superficie ocupada por las cañaveras* Arundo donax*; y F, superficie de zonas legalmente protegidas.*

in the provinces of Murcia or Alicante (table 1s, fig. 1). In West of Almería, most records originated from the Adra River basin (12 sampling points), with the exception of a single detection in a secluded ornamental garden (table 1s). In East of Almería, occurrences were limited to the Aguas and Almanzora rivers (four sampling points), with no detections in the Antas River (table 1s).

Temporal changes in the occurrence of the Mediterranean tree frog

Overall, the Mediterranean tree frog has become increasingly rare in southeastern Iberia. Forty years ago, the species was present in 43 of the 54 sampled points (79.63 %), whereas in the current survey it was detected at only 16 (29.6 %) (χ^2_1 = 27.23, $P < 0.001$) (fig. 1). Separate analyses for the three regions with historical data reveal the following patterns: (1) the species has disappeared entirely from all three sampled points where it was previously recorded in the High plateau of Murcia (fig. 1A), (2) in East of Almería, its presence declined from 11 of 13 sites (84.6 %) to only 4 (30.7 %) (χ^2_1 = 5.67, $P = 0.02$) (fig. 1E), and (3) in West of Almería, it declined from 29 of 38 sites (76.3 %) to only 12 (31.6 %) (χ^2_1 = 13.56, $P < 0.001$) (fig. 1F).

Drivers of Mediterranean tree frog decline in the Adra basin

The Adra River basin has experienced significant environmental changes over the past 40 years (fig. 1s). Notably, the distance from each sampling point to the nearest road or path decreased by 260 meters (fig. 1s,

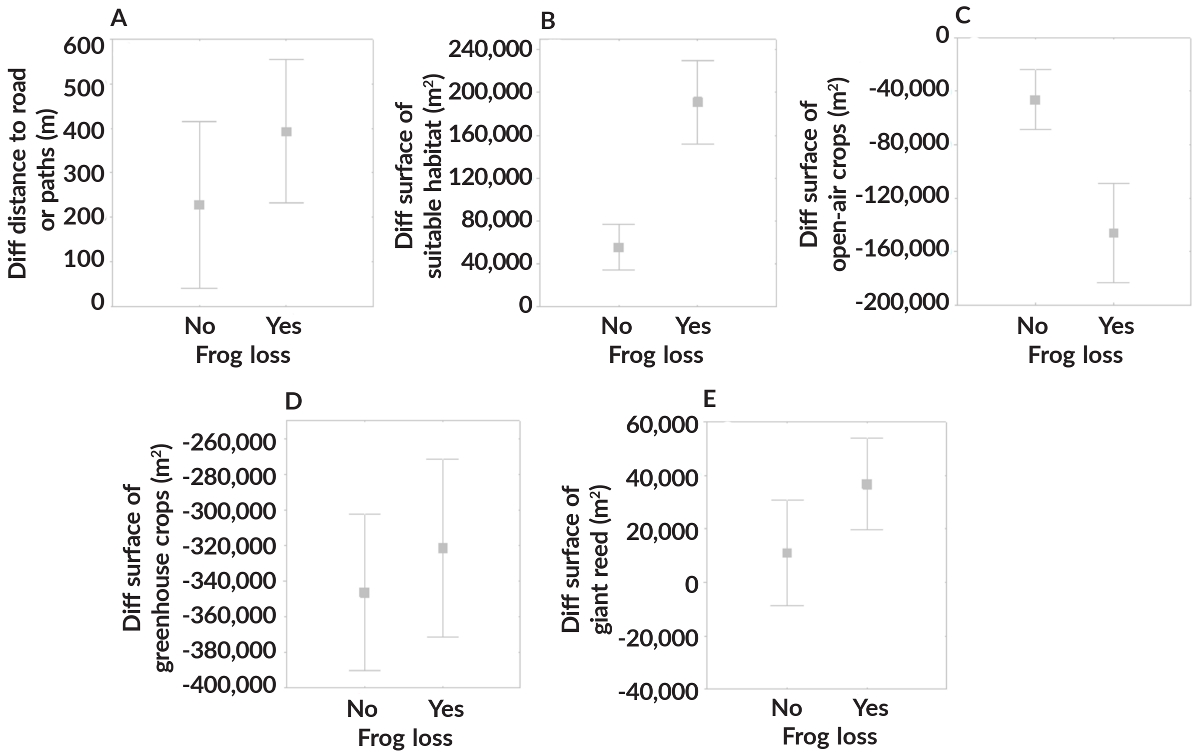

Fig. 3. Mean differences (± SE) between historical and current habitat values over the past 40 years at 29 sampled points within the Adra River basin, comparing sites where the Mediterranean tree frog *Hyla meridionalis* has either disappeared or persisted. A, distance to roads or paths; B, surface area of favorable habitat; C, surface area of open-air crops; D, surface area of greenhouse crops; and E, surface area of giant reeds *Arundo donax*.

Fig. 3. *Diferencias medias (± SE) entre históricas y actuales, en los valores de las variables del hábitat para los 29 puntos muestreados dentro de la cuenca del río Adra donde la ranita meridional* Hyla meridionalis *ha desaparecido o ha persistido durante los últimos 40 años. A, distancia a carreteras o caminos; B, superficie de hábitat favorable; C, superficie de cultivos al aire libre; D, superficie de cultivos en invernadero; y E, superficie ocupada por las cañaveras* Arundo donax.

t_{36} = 2.63, P = 0.012) and the extent of favorable habitat for the Mediterranean tree frog declined by over 57 % (fig. 1s, t_{36} = 4.91, P = 0.0001). These changes coincided with a marked 29% expansion of open-air crops (t_{36} = 4.37, P = 0.0001) and an almost 600 % increase in greenhouse crop area (t_{36} = 11.40, P < 0.0001). However, there was no statistically significant change in the area occupied by giant reed (t_{36} = 1.83, P = 0.075).

During the 2016-2019 survey period, sampled points where the frog was present had three times more favorable habitat (t_{35} = 4.19, P = 0.0001) and legally protected land (t_{35} = 3.77, P = 0.0005) than points where the frog was absent (fig. 2). In contrast, there were no significant differences between occupied and unoccupied sampled points in distance to roads or paths (t_{35} = 0.17, P = 0.86), or in the area covered by greenhouse crops (t_{35} = 1.28, P = 0.28), open-air crops (t_{35} = 0.94, P = 0.35), or giant reed (t_{35} = 1.02, P = 0.31) (fig. 2).

The magnitude of environmental change between the 1980s-1990s and 2016-2019 differed significantly between points where the frog has persisted and those where it disappeared. Sites where the species had been lost showed nearly four times greater reduction in favorable habitat (t_{27} = 2.39, P = 0.002), and a marginally greater increase in open-air crops area (t_{27} = 1.84, P = 0.07) (fig. 3). However, both site types experienced changes in roads/path proximity (t_{27} = 0.63, P = 0.53), greenhouse crop expansion (t_{27} = 0.32, P = 0.74) and loss of giant reed (t_{27} = 0.93, P = 0.36) (fig. 3). PCA analysis of the five environmental variables revealed that the first two components explained 84 % of the variance in environmental changes (table 3s). Of these, only the first component hard a significant effect on the probability of tree frog loss (table 1). Higher values of this component, indicating less habitat degradation and lower agricultural expansion, were associated with areas where the species persisted. Finally, sampling points where the frog had gone extinct had three times less protected area that those where the species persisted (t_{27} = 3.80, P = 0.0007, fig. 4).

Conservation status of the Mediterranean tree frog in southeastern Iberia

Using 1 radius buffers around the sampling points where the species was recorded, the estimated area of occupation of the Mediterranean tree frog in southeastern Iberia was approximately 43 km^2 during the 1980s-1990s and 16 km^2 in the 2016-2019 periods. This area was highly

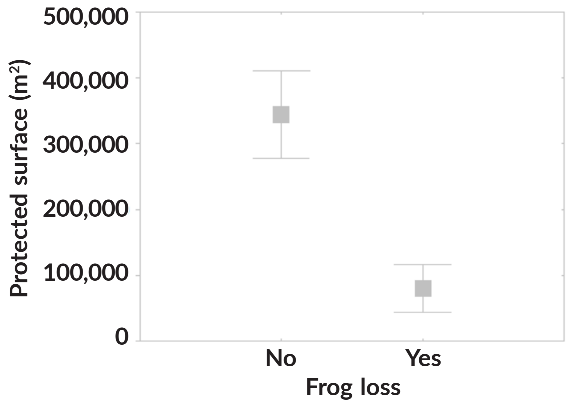

Fig. 4. Mean values (± SE) of surface area of legally protected zones at 29 sampling points in the Adra River basin where the Mediterranean tree frog *Hyla meridionalis* has either disappeared or persisted over the past 40 years.

Fig. 4. Superficie media (± SE) de zonas legalmente protegidas para los 29 puntos muestreados dentro de la cuenca del río Adra donde la ranita meridional Hyla meridionalis *ha desaparecido o ha persistido durante los últimos 40 años.*

fragmented (minimum distance of 100 km between West and East of Almería, and High plateau of Murcia), embedded within a landscape extensively altered by human activities, primary agriculture and urban development. Overall, the species' area of occupation has declined by nearly two-thirds over the last four decades. However, accurate data on population sizes and trends remain unavailable. Based on the available information, the Mediterranean tree frog qualifies for the EN category under IUCN criteria B2ab (ii, iii, iv) (EN, 'endangered'; meeting criteria: B2, estimated area of occupancy less than 500 km^2; a, severely fragmented area of occupancy, with the species also known from no more than five locations; b, continued decline observed in (ii) area of occupancy, (iii) area, extent, and quality of habitat (iii), and (iv) number of locations or subpopulations). Furthermore, there was not current evidence of immigration from adjacent regions that could counterbalance local declines. Consequently, no adjustment to this preliminary conservation assessment was warranted.

Discussion

Several authors have highlighted that population size, distance between breeding waterbodies, and the nature of the intervening terrestrial habitat matrix are key factors regulating amphibian dispersal (Sánchez-Montes et al 2018, Reyes-Moya et al 2022). Average dispersal distances from breeding sites for species closely related to the Mediterranean tree frog typically range from 1-2 km, as observed in the Iberian tree frog and the European tree frog *Hyla arborea* (Vos et al 2000, Martínez-Gil et al 2023, Sánchez-Montes and Martínez-Solano 2023). Based on these expected distances, the Mediterranean tree frog populations recorded between 2016 and 2019 must have been highly fragmented within the study area, as the points where individuals were detected were relatively far apart (with a minimum separation exceeding 7 km), and also distant from the nearest known populations in Granada, Albacete or Valencia (with distances greater than 52 km; Tejedo and Reques 2002, AHT 2025, SIARE 2025; see fig. 1, table 1s). These factors, together with the low number of individuals typically recorded at sampled points, probably intensified isolation between occupied localities and may have been decisive in preventing recolonization of apparently favorable sites that were inhabited in the 1980s-1990s but are now unoccupied. This pattern was likely further exacerbated by the region's low habitat suitability due to climatic constrains, particularly aridity (Sillero 2010, Reino et al 2016), and by the expansion and intensification of agriculture in the environments currently occupied by the species in southeastern Iberia. These conditions have likely increased the barrier effect and further reduced connectivity between breeding sites.

The only exceptions to this pattern of decline observed in southeastern Iberia were an isolated garden in Almería (La Almunya del Sur, table 1s), and a recent detection near one of the sampling areas in Alicante (Gilabert et al 2022), where the species was recorded during and after the present study, respectively. It is possible that these occurrences resulted from intentional or unintentional human-mediated translocations (see related cases in Paracuellos et al 2018, Gilabert et al 2022), which, under favorable environmental conditions, can sometimes lead to the establishment of new stable populations in the colonized areas (Dufresnes and Alard 2020).

The detailed analysis of the Adra River basin suggests that the primary factor contributing to the decline of the Mediterranean tree frog is the degradation of favorable habitats caused by the intensification and expansion of agriculture. In particular, the loss of ponds and temporary waterbodies surrounded by marsh vegetation has become more pronounced in this basin, while intensive open-air and greenhouse farming has proliferated. Additionally, the construction of roads and paths has increased significantly over the past 40 years. Given the high sensitivity of hylids to environmental changes (Semlitsch et al 2000, AbuBakr and Crupper 2010, Zazeri et al 2010, Gonçalves et al 2013, Sillero 2014, Santos et al 2015, Valdespino et al 2015), our results indicate that the natural habitats of the Mediterranean tree frog in southeastern Iberia have been severely impoverished since the 1980s. This degradation, largely driven by the expansion and intensification of agriculture practices, has likely compromised the viability of local populations, in agreement with the findings of Paracuellos et al (2017).

Although agricultural intensification in southeastern Iberia was not quantitatively assessed in this study, it is widely associated with the extensive extraction and contamination of waterbodies (both streams and aquifers) by biocides, fertilizers, and other agricultural pollutans (Casas et al 2003, Amores et al 2013, Gil et al 2018, Castro et al 2019, García-Marín et al 2020, Arauzo et al 2024). Furthermore, the ongoing expansion and encroachment of greenhouse agriculture, buildings and infrastructure as roads and paths likely increases the risk of microplastic pollution. These factors may significantly degrade habitat quality through cascading adverse effects on frogs and their larvae, including impaired growth and body condition, altered pigmentation, histopathological, behavioral, and morphological changes, weakened immune responses and increased toxicity. Collectively, these impacts can lead to reduced survival rates and breeding success (Szkudlarek et al 2023).

Similarly to the Adra River basin, other areas in West of Almería with potentially favorable habitats for the Mediterranean tree frog have undergone severe landscape transformations due to the widespread expansion of greenhouses and urban development. This has resulted in significant habitat loss, fragmentation, and degradation of natural ecosystems (Casas et al 2003, Salinas y Casas 2007, López Martos et al 2010). Comparable processes likely occurred in other areas that were historically inhabited or potentially appropriate for the species during the 1980s-1990s, including the rest of Almería, the High plateau of Murcia, or southern Alicante, regions that have experienced intensive anthropogenic land-use changes in recent decades (Martínez et al 2000-2009, Box Amorós 2003, Casas et al 2003, Marco Molina 2005, Salinas and Casas 2007, López Martos et al 2010, Navarro and Rodríguez Tamayo 2011). This broader scenario of environmental degradation across the southeastern Iberia (Sánchez-Picón et al 2011, Quintas-Soriano et al 2016, Caparrós-Martínez et al 2020, Martín-Gorriz et al 2020, Castillo-Díaz et al 2021, Martínez-Valderrama et al 2024) suggests that the drivers identified as key to the decline of the frog populations in Adra are likely representative of the same forces operating in other areas with a historical presence of the species throughout the study area.

In addition to land-use changes driven by human intensification, habitat loss may also be exacerbated by increasing air temperatures and altered precipitation patterns associated with ongoing climate change (MedECC 2019). These climatic shifts further reduce both the availability and quality of water in the last refuges for the species. Moreover, the surviving populations of the Mediterranean tree frog in southeastern Iberia are located at the geographical limits of the species' distribution on the Iberian Peninsula. This peripheral positioning may heighten environmental and genetic stress, making them more vulnerable and less resilient to threats such as disease, pollutants, and abrupt habitat fluctuations (Shafer 1990, Duellman 1999, Dufresnes et al 2013).

Other amphibian species in the study area also appear to be negatively affected by habitat transformation and its indirect consequences (González-Miras et al 2003, Egea-Serrano et al 2005, 2006, González-Miras and Nevado 2008). For instance, agricultural chemical pollution has been shown to impact generalist species such as the Iberian green frog *Pelophylax perezi* (Egea-Serrano et al 2009a, 2009b), while emerging infectious diseases have contributed to declines in the Betic midwife toad *Alytes dickhlleni* (Bosch et al 2013, Thumsová et al 2021). Additionally, extreme weather events have been implicated in affecting populations of the Western spadefoot toad *Pelobates cultripes* (Arroyo-Morales et al 2023). These parallels suggest that the drivers of decline identified for the Mediterranean tree frog may reflect broader patterns of amphibian vulnerability to global change in southeastern Iberia.

Although the Mediterranean tree frog appears to be expanding its distribution in other regions of Spain (Sillero 2014), the situation in southeastern Iberia is markedly different. The species has disappeared from 63% of the sites where it was recorded 40 years ago, and current surveys reveal a low number of individuals, high population fragmentation, and a deteriorated conservation status. These findings indicate that the anuran is now at serious risk of extinction in this part of its range. Accordingly, its classification as 'Endangered' at the regional level, following IUCN criteria (UICN 2012a, 2012b), is fully justified, a conclusion already supported by previous authors (Tejedo and Reques 2002, Torralva et al 2005, González-Miras and Nevado 2008, Alaminos 2013, Paracuellos et al 2017).

Finally, the present study underscores the value of citizen science programs in generating reliable data for monitoring and assessing the conservation status of species (Bonney et al 2009, Dickinson et al 2010, van Eupen et al 2021). Such programs have proven particularly useful in amphibian research and conservation efforts (Lee et al 2021, Petrovan et al 2022, Wangyal et al 2022).

Conservation implications

Based on our results, we recommend that restoration, creation, and maintenance of protected areas that enhance favorable habitat and curb agricultural expansion and intensification, as key strategies to preserve Mediterranean tree frog populations and reduce the risk of extinction of the species in southeastern Iberia. In particular, efforts should focus on restoring areas that have been degraded or transformed, making them once again favorable for colonization by the species (López-de Sancha et al 2025). These measures should be prioritized in zones located between currently occupied sites, to promote connectivity. Such interventions may include the removal of greenhouses and other landscape elements that act as ecological barriers, thereby facilitating habitat continuity and genetic exchange among fragmented populations.

The main population nucleus of the Mediterranean tree frog in southeastern Iberia is currently located within protected areas associated with the Adra River and its lagoons (Regional Government of Andalusia 1989, 2015). These sites also harbor other aquatic species classified as 'Endangered' or 'Critically endangered' at the global level (IUCN 2024), including the aquatic plant *Zannichellia contorta*, the European eel *Anguilla anguilla*, and the white-headed duck *Oxyura leucocephala* (Paracuellos et al 2019). Therefore, intensifying and expanding conservation measures within these protected areas and their surrounding environments where the Mediterranean tree frog still persists would not only benefit this species but also enhance the preservation of associated biodiversity of high ecological and conservation value.

In addition to restoring disturbed habitats, new breeding sites could be established through the creation of artificial waterbodies favorable for the hylid (Chester and Robson 2013, Caballero-Díaz et al 2020). Given the predominantly agricultural landscape in areas where the species still survives, abandoned irrigation ponds could be repurposed for this function, or even operational ponds could be adapted to support amphibian breeding (Peñalver et al 2015, Swartz and Miller 2019). This could be supported by the fact that several studies have highlighted the importance of farm ponds as complementary habitats to natural wetlands for maintaining biodiversity in southeastern Iberia (Casas et al 2011, 2012, Fuentes-

Rodríguez et al 2013, Juan et al 2012, 2013). In some cases, reintroductions from nearby, genetically related populations (such as those in Motril, Granada province; Recuero et al 2007), could be considered, particularly where habitat restoration alone is unlikely to ensure connectivity between breeding sites. However, such actions should be approached cautiously, with prior genetic assessments (e.g., Recuero 2020) to confirm compatibility, and strict biosafety protocols must be followed to prevent the spread of pathogens (Martínez-Silvestre et al 2023).

Finally, given the extinction risk currently faced by the Mediterranean tree frog, it is essential to implement long-term systematic monitoring of the species in southeastern Iberia. This should involve periodic repetition of population and distribution surveys over the coming years. Particular attention should be given to intensive larval monitoring using standardized protocols (e.g., AHE 2009, Ayllón and Gómez-Calmaestra 2014). Monitoring efforts should also be complemented by additional analyses focused on habitat connectivity (both structural and functional), population structure, and ecological niche modelling (Sillero 2010, Reino et al 2016, Rodríguez-Rodríguez et al 2020). These studies would provide critical insights into the species' current and potential future distribution, assess relative environmental impacts in aquatic or terrestrial habitats based on detected tadpole density relative to adult abundance. Collectively, such data could support a timely and evidence-based reassessment of species' conservation status of the Mediterranean tree frog in southeastern Iberia at any given time.

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M Paracuellos, conceived, designed and coordinated the study, coordinated and performed fieldwork, and wrote and edited the manuscript; **E Rodríguez-Caballero,** carried out the statistical analyses, and edited the manuscript; **E Villanueva,** designed the study, coordinated and performed fieldwork; **M Santa,** designed the study, coordinated and performed fieldwork; **JL Molina,** carried out fieldwork, and refined the figures; **E González Miras,** carried out fieldwork, and supervised the manuscript; **Daniel Alfonso,** carried out fieldwork; **B Aranega,** carried out fieldwork; **IM Arnaldos,** carried out fieldwork; **S Benavides,** carried out fieldwork; **FJ Canillas,** carried out fieldwork; **JM Díaz,** carried out fieldwork; **M Fernández,** carried out fieldwork. **GJ Gómez,** carried out fieldwork; **JM Gómez,** carried out fieldwork; **B González,** carried out fieldwork; **P López Acosta,** carried out fieldwork, **F López-de-Haro,** carried out fieldwork; **A Martín,** carried out fieldwork; **M Palmero,** carried out fieldwork; **M Sánchez,** carried out fieldwork; **H Schwarzer,** carried out fieldwork; **JM Avilés,** supervised the study, conducted statistical analyses, wrote and edited the manuscript, and refined the figures.

Conflicts of interest

The authors declare that they have no competing interests.

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Supplementary material

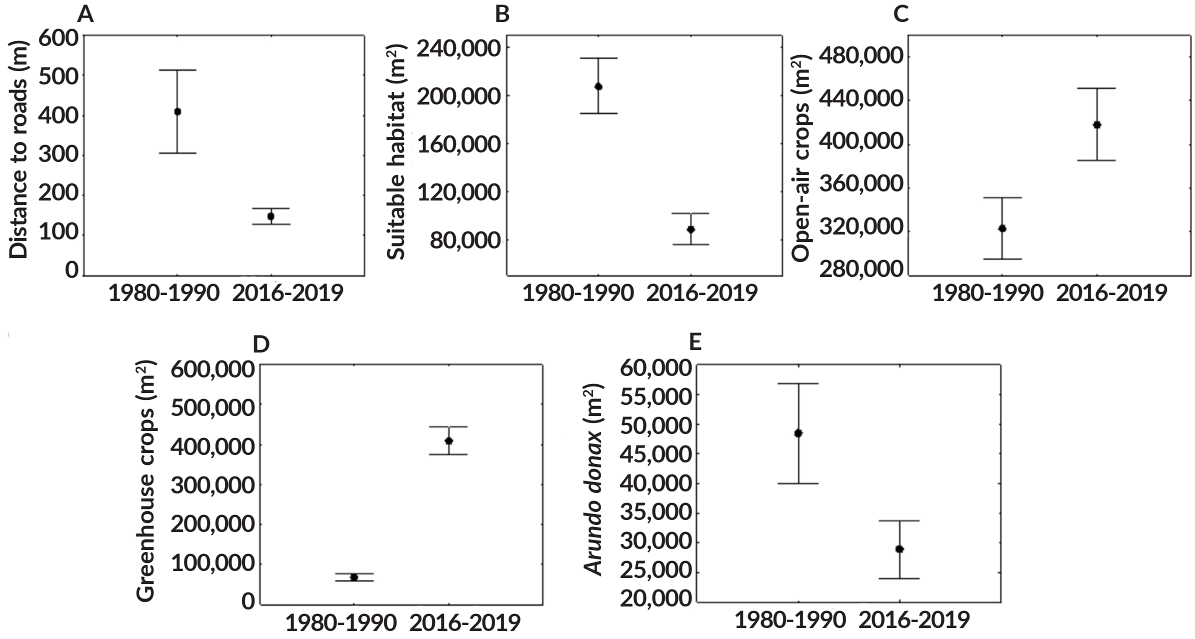

Fig. 1s. Temporal changes in habitat features associated with the Mediterranean tree frog *Hyla meridionalis* in the Adra River basin. Dates represent average values (± SE) for the 38 sampling points. A, distance to roads or paths; B, surface area of favorable habitat; C, surface area of open-air crops; D, surface area of greenhouse crops; and E, surface area of giant reeds *Arundo donax*.

Fig. 1s. *Cambios temporales en las características del hábitat de la ranita meridional* Hyla meridionalis *en la cuenca del río Adra. Los valores son la media (± SE) en los 38 puntos de muestreo. A, distancia a carreteras o caminos; B, superficie de hábitat favorable; C, superficie de cultivos al aire libre; D, superficie de cultivos en invernadero; y E, superficie ocupada por las cañaveras* Arundo donax.

Table 1s. Sampling points by region and locality showing the current (2006-2019) presence of the Mediterranean tree frog *Hyla meridionalis* in southeastern Iberia. The number of sampled points per locality is proportional to its surface area. Shown are the average geographic coordinates and altitude for each region and locality, calculated based on the sampling points. The current seasonality of the waterbodies is also indicated, distinguishing between temporary (T) and permanent (P) types. Localities with historical records of species presence (i.e., 1980s-1990s) are marked with an asterisk (*). Rows in bold indicate cumulative values for each region.

Tabla 1s. *Puntos muestreados por región y localidad para la presencia actual (2006-2019) de la ranita meridional* Hyla meridionalis *en el sureste de la península Ibérica. El número de puntos muestreados por cada localidad es proporcional a su superficie. Se muestran los valores medios de coordenadas geográficas y altitud por región y localidad calculados sobre los puntos de muestreo. También se muestra la estacionalidad actual de las masas de agua, diferenciando entre temporales (T) y permanentes (P). Las localidades con información histórica sobre la presencia de la especie (es decir, de las décadas de 1980 a 1990) están marcadas con un asterisco (*). Las filas en negrita muestran los valores acumulados por región.*

Region/locality	Geographical coordinate	Altitude (m a. s. l.)	Current seasonality	Sampled points with frog presence	Sampled points	Total num of visits	Sampling periods
West of Almería	36°45'N 2°49'W	70	P and T	13	78	332	06/04-14/07
Adra River and lagoons*	36°46'N 3°0'W	64	P and T	12	38	186	06/04-16/06
Cañada de las Norias	36°45'N 2°44'W	25	P	0	6	24	29/04-19/06
Punta Entinas-Sabinar	36°41'N 2°41'W	0	P and T	0	10	40	11/05-19/06
Ribera de la Algaida	36°47'N 2°35'W	2	T	0	2	8	05/05-13/06
Playa Serena golf course	36°43'N 2°38'W	1	P	0	4	16	28/04-11/07
Almerimar golf course	36°42'N 2°47'W	4	P	0	4	16	30/04-14/07
Guardias Viejas lagoons	36°42'N 2°49'W	4	P	0	3	12	22/04-12/07
Sotomontes pond	36°42'N 2°50'W	16	P	0	1	4	22/04-12/07
La Almunya del Sur garden	36°45'N 2°51'W	84	P	1	1	2	26/04-25/05
El Toril	36°45'N 2°52'W	84	P	0	1	2	26/04-25/05
Cañada de Ugíjar	36°44'N 2°48'W	43	P	0	1	2	26/04-25/05
Cañada de Onáyar	36°44'N 2°50'W	40	P	0	1	2	26/04-25/05
El Loco stream	36°43'N 2°51'W	29	P	0	2	4	26/04-25/05
El Cura ravine	36°50'N 2°38'W	300	P and T	0	4	14	06/04-12/06
East of Almería	37°7'N 1°55'W	172	P and T	4	39	174	26/03-18/06
Alías River	36°59'N 1°58'W	98	P	0	4	16	15/04-10/06
Aguas River*	37°8'N 1°57'W	104	P	3	24	104	26/03-16/06
Antas River*	37°12'N 1°48'W	6	P	0	4	20	26/03-14/06
Almanzora River*	37°17'N 1°52'W	81	P and T	1	5	24	26/03-18/06
Los Canos salt marsh*	37°13'N 1°48'W	4	P	0	2	10	26/03-09/06
Andarax River basin	37°03'N 2°38'W	449	P	0	10	38	07/04-09/06
Nacimiento River	37°03'N 2°38'W	449	P	0	10	38	07/04-09/06
Los Vélez	37°41'N 2°5'W	1.069	P	0	5	20	09/05-07/07
La Canastera ravine	37°41'N 2°5'W	1.064	P	0	2	6	10/05-22/06
La Fuente ravine	37°41'N 2°5'W	1.041	P	0	3	14	09/05-07/07
High plateau of Murcia	38°36'N 1°14'W	782	P and T	0	4	17	18/04-30/06
Tobarrillas stream*	38°43'N 1°8'W	831	T	0	2	7	18/04-30/06
El Pino source*	38°32'N 1°17'W	617	T	0	1	5	18/04-30/06
El Zorro pool*	38°28'N 1°20'W	509	P	0	1	5	18/04-30/06
Southern Alicante	38°12'N 0°39'W	18	P and T	0	8	32	08/04-11/06
El Hondo	38°10'N 0°45'W	0	P and T	0	4	16	08/04-11/06
Clot de Galvany	38°14'N 0°31'W	2	P and T	0	4	16	15/04-10/06

Table 2s. Maximum number of individuals recorded at the 17 sampling points with presence of the Mediterranean tree frog *Hyla meridionalis* during 2016-2019. Values represent the average (± SE) across the 17 sample points.

Tabla 2s. *Número máximo de individuos en los 17 puntos muestreados con presencia de la ranita meridional* Hyla meridionalis *durante 2016-2019. Los valores son la media (± SE) en los 17 puntos de muestreo.*

Sampled point code	N° of individuals
AA02	1
AA03	1
AA05	2
AA06	20
AA07	30
AA08	4
AA09	4
AA12	10
AG01	1
AG02	1
AG03	1
AS01	1
DT08	1
DT10	1
MZ01	3
RA02	1
RA06	1
Average	**4.88**
± SE	**1.96**

Table 3s. Principal component analysis (PCA) scores and explained variance for five habitat variables measured at 38 sampling points, representing changes between the 1980s-1990s and 2016-2019. Variables include distance to roads and paths, and surface area of favorable habitat, open-air crops, greenhouses crops, and giant reeds *Arundo donax* within a 500 m radius around each sampling point.

Tabla 3s. *Resultados del análisis de componentes principales (PCA) y varianza explicada para las cinco variables medidas en los 38 puntos de muestreo que representan la variación entre las décadas de 1980-1990 y 2016-2019. Las variables incluyen la distancia a carreteras y caminos, así como el área cubierta por hábitat favorable, cultivos a cielo abierto, cultivos en invernadero y el área ocupada por cañaveras* Arundo donax *dentro de un radio de 500 m alrededor de cada punto de muestreo.*

Variable	Component 1 (62.23 %)	Component 2 (84.26%)
Distance to roads and paths	0.113	0.934
Favorable habitat	0.542	-0.232
Open-air crops	-0.573	0.176
Greenhouses crops	-0.419	-0.179
Giant reeds	0.436	0.107