

Citizen science reveals broad-scale variation of calling activity of the Mediterranean tree frog (*Hyla meridionalis*) in its westernmost range

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Abstract. Population monitoring is essential to determine different aspects of the ecology and conservation of the species. In anurans, recording the acoustic activity of choruses allows surveying populations. Therefore, knowing the timing of male calls is fundamental to achieve this goal. Here we monitored calling activity of the Mediterranean tree frog (*Hyla meridionalis*) at eight localities in southern Iberian Peninsula and western North Africa in the frame of a citizen science program. Subsequently, after summarizing call activity with Non-metric Multidimensional Scaling, we aimed to identify the geographic and environmental variables that associate with the calling activity of frogs. The results of the 258-hour census showed that male tree frogs called mainly from December to July, although the duration and intensity of choruses varied, depending on the elevation and seasonality of the water bodies. Males sang earlier and had more durable call activities at lower elevation sites, which are sites with higher and more stable ambient temperatures. Also, calling activity was lower in

sites where water fluctuates more over the annual cycle. Our results provide a first overview of the calling activity of the Mediterranean tree frog over a relatively large set of populations encompassing a wide variety of environmental conditions in its westernmost range of distribution. However, further studies relying on more intensive sampling, likely using automatic recorders, would be desirable to achieve a full understanding of the calling activity of tree frogs in the region.

Keywords: advertisement calls, breeding choruses, citizen science, environmental factors, phenology, population monitoring.

Introduction

Knowing the occurrence and size of populations is a key premise to understand the ecology of species and for assessing their conservation status. In vertebrates, the size of populations is estimated through censuses, which vary in methodology depending on the biology of target taxa (Davis, 1982; Sutherland, 2006; Eyre et al., 2018). Anurans mostly rely on vocalizations for communication, thereby call detection provides a relatively effective method for monitoring their populations (Dorcas et al., 2009). Hence, knowing when frogs are vocally active becomes fundamental, especially for anurans inhabiting temperate zones as the Mediterranean, where breeding and song activities occur in a narrow temporal window, and particularly so for species with flexible reproductive phenology, as the Mediterranean tree frog (*Hyla meridionalis*) (Díaz-Paniagua, 1992; Jakob et al., 2003; Sillero, 2014), the target of the present study.

Different environmental factors such as ambient temperature and water availability have been identified as predictors of anuran calling activity (Richter-Boix et al., 2007; Márquez et al., 2014; Sillero, 2014). The vocal activity of Mediterranean tree frog has been analyzed in several study sites with varying ecological conditions (Gerhardt and Schneider, 1980; Crovetto et al., 2019). In Iberia, previous studies were done comparing frog vocal activity between localities with extreme temperature (either high or low) or covered a very small geographical scale (Llusia et al., 2013a, b, c; Márquez et al., 2014). This might be unfortunate because factors influencing frog calling activity might differ between localities due to microscale variations (Brooke et al., 2000; Pérez-Granados et al., 2020), then urging for multi-population

approaches to achieve a better understating of frog calling activity.

The Mediterranean tree frog is included within the “least concern” category in the Red List of the IUCN (2021). However, according to Spanish law the species is in the “List of wild species under special protection” (Real Decreto 139/2011, de 4 de febrero, BOE, 46, de 23 de febrero de 2011), needing a periodic evaluation of their state of conservation. In this context, the present work aims to broaden the knowledge of the calling activity of this species in the southern Iberian Peninsula and northwestern Maghreb, its main area of distribution on a global scale (Schleich et al., 1996; Arnold, 2003). Specifically, here we adopted a multi-population approach to study the phenology of frog vocalizations over a broad geographical scenario encompassing huge environmental variation within the species’ distribution area in its westernmost range of distribution. Our main goal is to help clarifying the activity thresholds of breeding choruses in this region, for application in future studies for detection and monitoring of this species. On a more global scale, this study will enable comparisons of projected future expansions and regressions of the Mediterranean tree frog within its distribution area (Sillero, 2009, 2010). Finally, the obtained data will be useful to refine distribution models of this species in relation to climate change (Rodríguez-Rodríguez et al., 2020a, b).

Materials and methods

Study sites

We studied breeding choruses of the Mediterranean tree frog at eight localities comprising southern Iberian Peninsula and northwestern Maghreb (see fig. 1).

Whereas the North African populations of the Mediterranean tree frog are considered native, the Iberian and

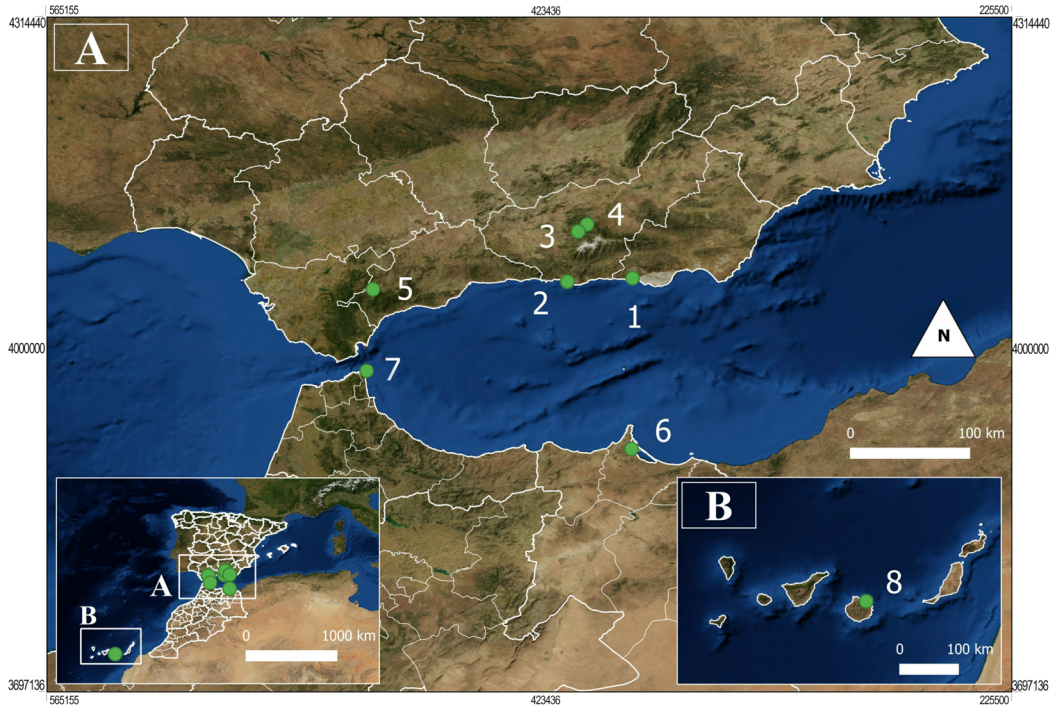


Figure 1. Geographical location of the study sites: 1, Lagoons of Adra (Adra river delta, Almería province, Spain); 2, Suárez pond (Guadalfeo river delta, Granada province, Spain); 3, lagoon of Güéjar Sierra (Sierra Nevada mountains, Granada province, Spain); 4, lagoon of Tocón de Quéntar (Sierra Nevada mountains, Granada province, Spain); 5, backwater of Jímera de Líbar (Guadiaro river of the Serranía de Ronda mountains, Málaga province, Spain); 6, Kola pond (massif of Gurugú, Nador province, Morocco); 7, Ceuta pond (on the Tingitana peninsula, autonomous city of Ceuta, Spain); 8, lagoon of San Lorenzo (Canary islands, Las Palmas province, Spain).

Canary ones were probably introduced during historical or, even earlier, prehistoric times (Dufresnes and Alard, 2020). Regardless of their origin, all sampled localities were selected in order to encompass as much as possible the range of environmental conditions where the species inhabit (table 1). The altitude ranges of the eight localities varied from sea level (Adra lagoons and Suárez pond) to the high-mountain, reaching the elevational limit for the species in Iberia (Fernández-Cardenete et al., 2000) in Tocón de Quéntar (maximum elevation of 1279 m a.s.l.; table 1).

Survey of calling activity

Nocturnal estimations of male calling activity at each site were made once a month by hearing between February 2018 and February 2019. 17 researchers made estimations, each one counting in one single site throughout the study period. Although it would have been preferable that the same researchers estimated frog calling activity in all localities to minimize inter-observer variability, this was not possible, as this is a citizen science study in which travel expenses were limited. Nonetheless, all the researchers were familiar with the calls of the species and were previously trained with call recordings. To this end, all researchers estimated the number of males calling in 12 recordings of tree

frog choruses of different size (ranging between 1 and 1000 males) obtained by experienced researchers that had estimated the number of males calling in the field. Estimations were blind with respect to the number of males estimated in the field, and revealed that the degree of correlation between the 17 researchers was high and significant ($r > 0.5$ in all cases, see table A2 in the Appendix). Moreover, estimates of number of males calling by the 17 researchers were highly and significantly correlated with those made by the experienced researchers ($r > 0.7$ in all cases, see table A2). This suggests that the 17 researchers participating in this study had a similar ability to estimate differences in number of the males calling in the field and, hence, that differences between researchers should not have greatly affected the patterns detected in our study.

The period of the day with the greatest likelihood of calling, i.e. the two hours after sunset (e.g., Márquez et al., 2014), was selected for the censuses, for a total of 258 h of sampling time. We avoided sampling those days with strong wind, rain or anthropogenic noise that could have interfered with call detection. Moreover, we avoided sampling during nights with clear, moonlit skies having more than 50% brightness, as moonlight may influence the breeding and detection probability of anuran, as *Hyla* species (Dorcas et al., 2009; Vignoli and Luiselli, 2013; Onorati and Vignoli,

Table 1. Geographical and environmental characteristics of the study sites, with geographical coordinates in ETRS89. For the description of the variables, see Materials and methods.

Locality	Geographic coordinates	Province	Distance to the climatic station (km)	Latitude (km)	Elevation (m a.s.l.)	Water seasonality (%)	Mean ambient temperature (°C)	Mean ambient temperature range (°C)	Mean annual precipitation (mm)	Bibliography
Lagoons of Adra	36°45'19"N 2°57'18"W	Almería	5	4092	4	24.40	18.28	12.97	324.60	Casas et al., 2003; Paracuellos, 2006
Suárez pond	36°43'28"N 3°32'24"W	Granada	13	4088	1	8.29	17.49	13.17	465.97	Tarragona, 1999
Lagoon of Gütejar Sierra	37°10'24"N 3°26'41"W	Granada	25	4027	1244	18.76	13.31	19.68	345.94	Molero et al., 1992
Lagoon of Tocón de Quéntar	37°14'13"N 3°21'47"W	Granada	19	4145	1279	22.66	13.31	19.68	345.94	Molero et al., 1992
Backwater of Jinera de Líbar	36°39'20"N 5°17'16"W	Málaga	13	4080	389	25.64	14.18	18.07	1813.03	Blanco and Herrera, 2009
Kola pond	35°13'25"N 2°57'52"W	Nador	6	3921	543	41.03	19.42	13.43	317.00	Yus et al., 2013
Ceuta pond	35°53'54"N 5°17'30"W	Ceuta	2	3996	125	26.58	18.93	12.27	758.45	Mateo et al., 2003
Lagoon of San Lorenzo	28°5'12"N 15°28'39"W	Las Palmas	20	3127	200	42.25	21.62	7.49	113.48	Bueno and Cárdenes, 2015

2017). Samplings were initially scheduled for the first week-end of each month, but moved to the following weekend if climatic conditions or moonlight might influence call detection. For more details about the census method, see e.g., Dorcas et al. (2009).

Researchers counted the maximum number of males calling in each locality. When more than one researcher sampled in a locality, the highest estimate was used. From the compiled data, we calculated the (i) start (i.e., months elapsed from October, that is the month of the annual cycle in which we detected the earliest vocalization in any locality, to the month of the first vocalization), (ii) maximum intensity (i.e., months elapsed from October to the month of the maximum number of calls), (iii) end (i.e., months elapsed from October to the month of the latest vocalization), and (iv) duration (i.e., number of different months with detected call activity in the study annual cycle) of the choruses in each locality. Hence, all localities were evenly represented in our sample by one value of start, maximum intensity, end and duration of call, excluding potential noise due to variation within locality.

Predictors of geographical and environmental variation in calling activity

It has been described that the spatial location and water availability of the species are key factors that determine the breeding of the Mediterranean tree frog (Díaz-Paniagua, 1992; Schleich et al., 1996; Richter-Boix et al., 2007; Llusia et al., 2013a, b, c; Márquez et al., 2014; Sillero, 2014). Specifically, for each locality we retrieved information on (1) latitude, (2) elevation, and (3) water seasonality, to analyze its relationship with the calling activity of the anuran. The water seasonality was estimated visually in the field as the mean monthly value of relative fluctuation of water level with respect to the maximum annual flooding at each locality (expressed as $((\sum |prf_i - (\sum prf_i/m)|/m) \times 100)/prf_{max}$, where “ prf_i ” is the maximum monthly depth of the water in the month “ i ”, “ prf_{max} ” is the maximum annual depth of water, and “ m ” is the n° of months sampled at each locality).

On the other hand, variation in climatic parameters such as precipitation and temperature may lead to changes in the daily pattern in the breeding activity of anurans between consecutive days or between similar periods in different years with contrasting climate (Richter-Boix et al., 2007; Llusia et al., 2013a, b, c; Márquez et al., 2014). However, the low sampling effort of our work did not allow us to find causal relationships between climate parameters varying on a daily basis and the calling activity of frogs estimated on a monthly basis. Instead, our study allows us to evaluate the differences in the seasonal pattern of calling activity at the biogeographical scale and hence to study large-scale geographical determinants of frog call activity. For this reason, climatic variables varying on a daily basis were not considered at the first glance in the statistical analysis. However, we still calculated for each locality large-scale climatic conditions during the last 10 years (i.e., mean ambient temperature, mean ambient temperature range and mean annual precipitation) based on daily data from 2009

to 2019 retrieved at the closest climatological station of the Junta de Andalucía (<https://www.juntadeandalucia.es/agriculturaypesca/ifapa/ria/servlet/FrontController>) and the Ministerio para la Transición Ecológica y el Reto Demográfico (<https://datosclima.es/Aemethistorico/Estaciones.php>). This allowed us to study how large scale geographical variation covaries with large scale climatic variation and enriched our discussion on the causes of patterns of calling activity. The values of each of the variables for the different localities are in the table 1.

Data analyses

As a first step, we described variation in frog call activity by applying a Non-metric Multidimensional Scaling (NMDS) approach, using Vegan package (Oksanen et al., 2014) in R (version 3.6.0., R Core Team, 2019), on the four call variables. The NMDS yield low-dimensional geometric space from the proximities between a set of objects, and it is a suitable ordination method for data that are not normal or that are on a discontinuous or arbitrary scale, such as the four call variables from the tree frog. This analysis was made from the Bray-Curtis dissimilarity matrix, with two dimensions and a maximum of 1200 iterations. In a second step, aiming to preliminary identify major drivers of variation in calling activity, we studied covariation between emerging NMDS axis and latitude, elevation and water seasonality, by running Pearson correlations (applying the *Envfit* function with 999 permutations).

Closer frog populations are more likely to share common geographical and climatic features affecting frog call activities than more distant ones, urging for a test of spatial independence in the patterns of frog calling activity. We studied the association between the matrix of pair-wise differences in geographical distance between frog populations and the Bray-Curtis dissimilarity matrix obtained from the NMDS analysis describing dissimilarity in the calling activity (Legendre et al., 2005). This was done using the Mantel test with the “Mantel” function of the Vegan package in R (Oksanen et al., 2014).

Finally, we explored covariation between latitude, elevation and water seasonality, and climatic variables (mean ambient temperature, mean ambient temperature range and mean annual precipitation) across the localities by mean of Pearson correlations, setting the statistical significance in the correlation tests at $P \leq 0.05$ (Betensky, 2019).

Results

Mediterranean tree frog call activity usually began in December-March with few advertisement calls that augmented over the months reaching their maximums in February-May. Then vocalizations gradually declined and mostly ceased in June-July, before the summer-autumn period, when calling activity became

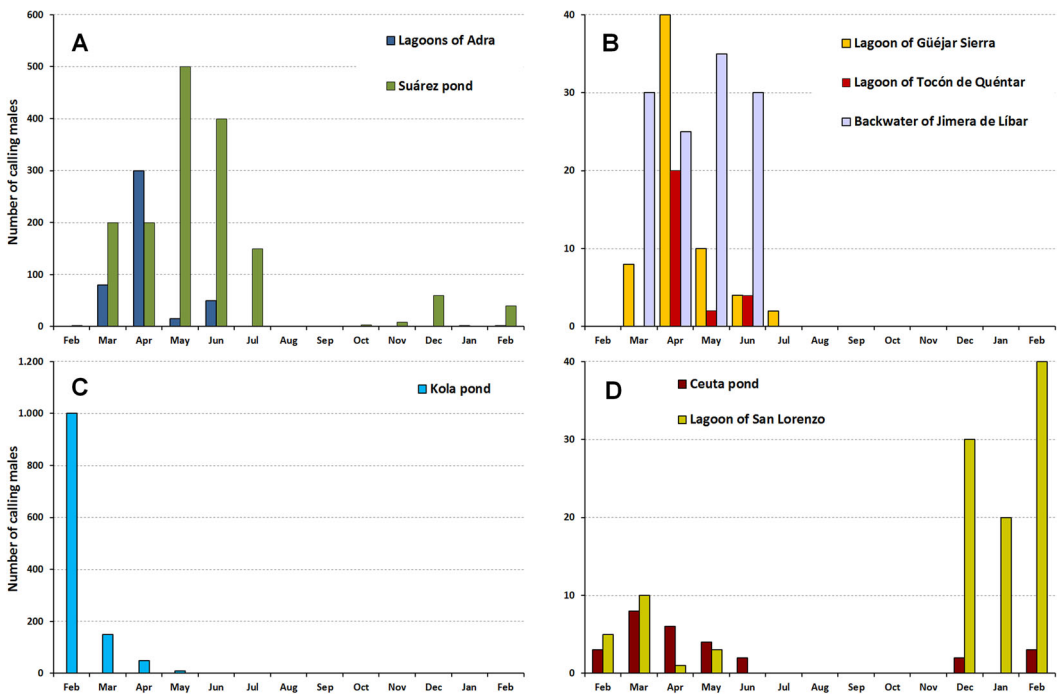


Figure 2. Calling intensity according to the estimated abundance of male frogs active in the breeding choruses over the annual cycle at the study sites sampled for the Mediterranean tree frog (*Hyla meridionalis*) by region. A, coastal localities of the Iberian Peninsula; B, inland localities of the Iberian Peninsula; C, North African locality on the Mediterranean; D, North African localities on the Atlantic and the Strait of Gibraltar.

null or minimum (mostly from August to November). Nevertheless, temporal patterns of calling differed among localities. In this sense, while the North African study sites registered their maximum and end of calling activity respectively in February-March and May-June, the South Iberian ones did so later, respectively in April-May and June-July (fig. 2).

Elevation and water seasonality, but not latitude, were significantly associated with the NMDS ordination axes summarizing calling activity (table 2). Specifically, the NMDS ordination illustrates an altitude gradient from the upper left corner to the lower right corner (fig. 3), with the main descriptors of the seasonal pattern aligning along this gradient. The calling activity started and reached its maximum activity earlier, and lasted longer, in the coastal and lower altitude localities, than in the mountainous and highest sites (fig. 3). On the

Table 2. Squared correlation coefficients (r^2) between the geographical and environmental variables, and the axes of the NMDS ordination for the parameters that characterized the temporal pattern of calling activity of the Mediterranean tree frog (*Hyla meridionalis*) at the eight study sites. Relationships have been assessed using the Envfit function in Vegan. The values in bold indicate the variables showing statistically significant correlations ($P \leq 0.05$).

Variables	r^2	P
Latitude	0.52	0.13
Elevation	0.65	0.05
Water seasonality	0.95	0.00

other hand, in the NMDS ordination water seasonality increased from the lower left corner of the figure (Suárez) to the upper right corner (Kola). The higher the seasonality, the earlier the maximum and the end of the vocalizations occurred (fig. 3). The duration of the choruses was also shorter in the localities with higher seasonality (fig. 3).

Mantel tests revealed that differences among localities in call activity were unrelated with

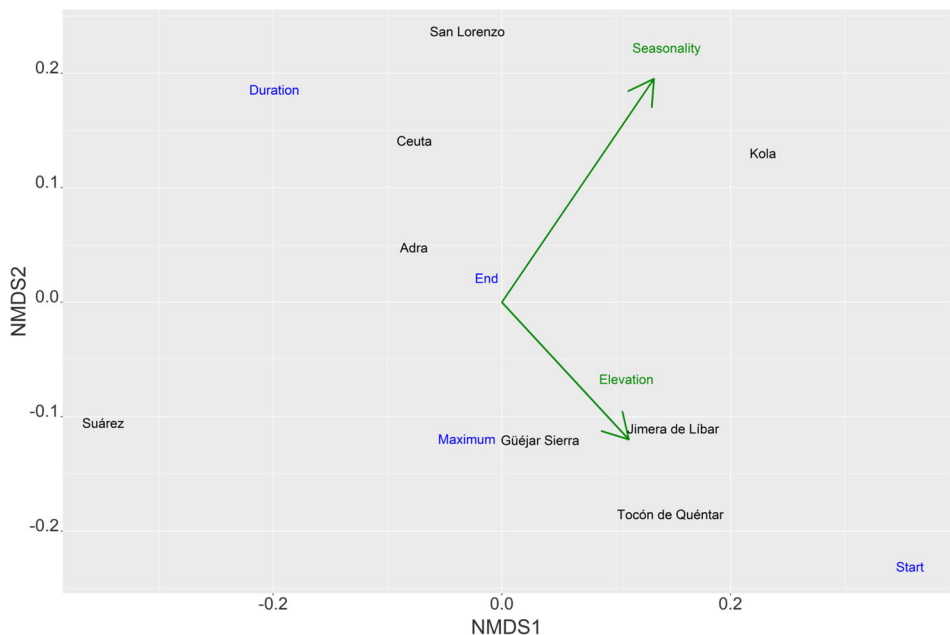


Figure 3. NMDS ordination analysis for the four parameters that determine the temporal pattern of calling activity of the Mediterranean tree frog (*Hyla meridionalis*) at the eight study sites (Start, onset of the choruses; Maximum, highest intensity of the choruses; End, termination of the choruses; Duration, length of time that the choruses last). Arrows indicate the direction of increase along a gradient of altitude (Elevation), and water seasonality (Seasonality), while the length is proportional to the correlation between the predictor variable and the ordination. Stress value = 0.005. For the description of the variables, see Materials and methods.

spatial distance among them ($r = -0.03$, $P = 0.50$) and could, therefore, be treated as independent samples. Moreover, correlation analysis between geographical and climatic features revealed that mean ambient temperature and mean ambient temperature range were significantly correlated with elevation. Temperature decreases with altitude whereas the range increases with it (see table A1 in the Appendix). The range of temperature was larger at higher latitude, whereas we did not detect any relationships between water seasonality, mean annual precipitation, and the other variables (see table A1).

Discussion

Our results revealed huge variations among Mediterranean tree frog populations in the temporal patterns of the calling activity. This variation mirrored an altitude gradient that may rep-

resent the response to changes in ambient temperature (Llusia et al., 2013a, b, c; Márquez et al., 2014). In this sense, our results revealed that the tree frog advertisement calls were predictably earlier and longer-lasting in localities close to the sea level, where temperatures are milder and more stable.

Breeding in the tree frog is triggered by rainfall and, as a result, by water availability in the ponds where it spawns. This is related to the possibility of adjusting its breeding cycles to the availability of suitable habitats (Díaz-Paniagua, 1992; Richter-Boix et al., 2007; Cayuela et al., 2012; Sillero, 2014). Flooding of the aquatic environment appeared to be important enough to obligate this species to reduce its duration of calling activity in wetlands that have greater water seasonality, e.g., Kola pond in the present study, before reaching the thresholds of dry-season drought that could limit breeding. By comparison, other sites with more stable

and persistent water cycles, and thus abundant water even late in the dry period, must allow longer breeding periods, e.g., Suárez pond in the present study.

Our results indicate that for effective monitoring of populations of the Mediterranean tree frog based on male vocalization in the study area and period, the surveying should have span March to June, period in which they sang in 91% of cases. Nevertheless, the coastal sites located near sea level should have been sampled earlier (preferably March to May in zones less than 600 m a.s.l.) than those others in inland localities at higher elevation (preferably April-June in zones over 600 m a.s.l.), since in these zones and dates they sang in 100% of the cases. Also, lowest elevation areas could have allowed longer intervals of suitable monitoring (4-9 months in zones less than 600 m a.s.l.) than those at higher altitude (3-5 months in zones over 600 m a.s.l.). Finally, appropriate monitoring should have required the presence of surface water in the reproduction sites. Such differences in calling activity between localities could even change in very short geographical distances due to the micro-environmental factors, according to the slight modifications in body conditions described to anurans (Brooke et al., 2000; Pérez-Granados et al., 2020).

Our sampling schedule summed up 258 h for the eight study sites, which allowed sampling with a low cost, where and when tree frogs inhabited and were active throughout a wide environmental gradient. However, this sampling schedule is likely insufficient to identify the relationship with climate and social variables, which are factors likely to vary in short time, which is a major limitation of our work. Hence citizen science programs, as this study, should be reinforced in the future through the implementation of more systematic methodologies of call recordings, such as the use of automatic recorders installed into the water bodies. This will allow improving our understanding of the calling activity of the species in the region while accounting for short term intra-local variation

in call activity (Akmentins et al., 2015; Willacy et al., 2015; Pérez-Granados et al., 2019) that could not be controlled for in this study.

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References

- Akmentins, M.S., Pereyra, L.C., Sanabria, E.A., Vaira, M. (2015): Patterns of daily and seasonal calling activity of a direct-developing frog of the subtropical Andean forests of Argentina. *Bioacoustics* **24**: 89-99.
- Arnold, E.N. (2003): *Reptiles and amphibians of Europe*. Princeton University Press, Princeton.
- Betensky, R.A. (2019): The *p*-value requires context, not a threshold. *Am. Stat.* **73**: 115-117.
- Blanco, F., Herrera, T. (2009): Biodiversidad de los ecosistemas fluviales de la provincia de Málaga. *Jábega* **101**: 55-65.
- Brooke, P.N., Alford, R.A., Schwarzkopf, L. (2000): Environmental and social factors influence chorusing behaviour in a tropical frog: examining various temporal and spatial scales. *Behav. Ecol. Sociobiol.* **49**: 79-87.
- Bueno, A., Cárdenas, D. (2015): "Entre piedra y agua": estudio y propuestas para la Vega de San Lorenzo. 21-28. In: *Actas del II workshop celebrado en Las Palmas de Gran Canaria del 21 al 23 de enero de 2015. Estudio, aprovechamiento y gestión del agua en terrenos e islas volcánicas*, pp. 21-28. Cabrera, M.C., Cruz-Fuentes,

- T., Mendoza-Grimón, V., Palacios-Díaz, M.P., Eds, Instituto Geológico y Minero de España, Asociación Internacional de Hidrogeólogos (Grupo Español), Gran Canaria.
- Casas, J.J., Calvache, F., Delgado, S., García-Mayoral, J., Vivas, S., Bayo, M., López, D., Ortega, M. (2003): Inventario abierto de los humedales de la región semiárida almeriense: Consideraciones sobre su tipificación. In: Ecología, manejo y conservación de los humedales, pp. 171-186. Colección Actas, 49. Paracuellos, M., Ed., Instituto de Estudios Almerienses (Diputación de Almería), Almería.
- Cayuela, H., Besnard, A., Béchet, A., Devictor, V., Olivier, A. (2012): Reproductive dynamics of three amphibian species in Mediterranean wetlands: the role of local precipitation and hydrological regimes. *Freshwater Biol.* **57**: 2629-2640.
- Crovetto, F., Salvadio, S., Costa, A. (2019): Estimating abundance of the stripeless tree-frog *Hyla meridionalis* by means of replicated call counts. *Acta Herpetol.* **14**: 147-151.
- Davis, D.E. (1982): Handbook of Census Methods for Terrestrial Vertebrates. CRC Press, Boca Raton.
- Díaz-Paniagua, C. (1992): Variability in timing of larval season in an amphibian community in SW Spain. *Ecography* **15**: 267-272.
- Dorcas, M.E., Price, S.J., Walls, S.C., Barichivich, W.J. (2009): Auditory monitoring of anuran populations. In: Ecology and Conservation of Amphibians: a Handbook of Techniques, pp. 281-298. Dodd, C.K. Jr., Ed., Oxford University Press, New York.
- Dufresnes, C., Alard, B. (2020): An odyssey out of Africa: an integrative review of past and present invasions by the Mediterranean tree frog (*Hyla meridionalis*). *Biol. J. Linn. Soc.* **131**: 274-290.
- Eyre, T.J., Ferguson, D.J., Hourigan, C.L., Smith, G.C., Mathieson, M.T., Kelly, A.L., Venz, M.F., Hogan, L.D., Rowland, J. (2018): Terrestrial Vertebrate Fauna Survey Assessment Guidelines for Queensland. Department of Environment and Science (Queensland Government), Brisbane.
- Fernández-Cardenete, J.R., Luzón-Ortega, J.M., Pérez-Contreras, J., Tierno de Figueroa, J.M. (2000): Revisión de la distribución y conservación de los anfibios y reptiles en la provincia de Granada (España). *Zool. Baetica* **11**: 77-104.
- Gerhardt, H.C., Schneider, H. (1980): Mating call discrimination by females of the treefrog *Hyla meridionalis* on Tenerife. *Behav. Processes* **5**: 143-149.
- IUCN (2021): The IUCN red list of threatened species. Versión 2021-2. International Union for Conservation of Nature, Gland. Retrieved from <http://www.iucnredlist.org>. Accessed 22 November 2021.
- Jakob, C., Poizat, G., Veith, M., Seitz, A., Crivelli, A.J. (2003): Breeding phenology and larval distribution of amphibians in a Mediterranean pond network with unpredictable hydrology. *Hydrobiologia* **499**: 51-61.
- Legendre, P., Borcard, D., Peres-Neto, P.R. (2005): Analyzing beta diversity: partitioning the spatial variation of community composition data. *Ecol. Monogr.* **75**: 435-450.
- Llusia, D., Márquez, R., Beltrán, J.F., Benítez, M., do Amaral, J.P. (2013a): Calling behaviour under climate change: geographical and seasonal variation of calling temperatures in ectotherms. *Glob. Chang. Biol.* **19**: 2.655-2.674.
- Llusia, D., Márquez, R., Beltrán, J.F., Moreira, C., do Amaral, J.P. (2013b): Environmental and social determinants of anuran lekking behavior: intraspecific variation in populations at thermal extremes. *Behav. Ecol. Sociobiol.* **67**: 493-511.
- Llusia, D., Márquez, R., Moreira, C.N., Arias, A. (2013c): Seasonal and diel temperature variation of breeding microhabitat in *Hyla molleri* and *H. meridionalis*: comparison between Iberian populations at thermal extremes. *Bull. Soc. Herpétol. Fr.* **145-146**: 73-90.
- Márquez, R., Llusia, D., Beltrán, J.F. (2014): Aplicación de la bioacústica al seguimiento de anfibios. *Bol. Asoc. Herpetol. Esp.* **25**: 52-58.
- Mateo, J.A., Pleguezuelos, J.M., Fahd, S., Geniez, P., Martínez-Medina, F.J. (2003): Los anfibios y reptiles del estrecho de Gibraltar. Un ensayo sobre la herpetofauna de Ceuta y su entorno. Instituto de Estudios Ceutíes, Ceuta.
- Molero, J., Pérez, F., Valle, F., Eds (1992): Parque Natural de Sierra Nevada. Paisaje, fauna, flora e itinerarios. Rueda, Madrid.
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., Wagner, H. (2014): Package Vegan: Community Ecology Package. Retrieved from <http://CRAN.R-project.org/package=vegan>. Accessed 28 February 2021.
- Onorati, M., Vignoli, L. (2017): The darker the night, the brighter the stars: consequences of nocturnal brightness on amphibian reproduction. *Biol. J. Linn. Soc. Lond.* **20**: 1-16.
- Paracuellos, M. (2006): Las Albuferas de Adra (Almería, Sureste Ibérico) y su relación histórica con el hombre. *Farua Extra I*: 335-358.
- Pérez-Granados, C., Schuchmann, K.-L., Ganchev, T., Strüssmann, C., Dorado-Rodrigues, T., Tissiani, A.S., de Deus, F., Marques, M.I. (2019): Elucidating the diel and seasonal calling behaviour of *Elachistocleis matogrosso* (Anura: Microhylidae). *Journal of Natural History* **53**: 2699-2710.
- Pérez-Granados, C., Schuchmann, K.L., Ramoni-Perazzi, P., Marques, M.I. (2020): Calling behaviour of *Elachistocleis matogrosso* (Anura, Microhylidae) is associated with habitat temperature and rainfall. *Bioacoustics* **29**: 670-683.
- Richter-Boix, A., Llorente, G.A., Montori, A. (2007): Segregación espacial y temporal de una comunidad de anfibios en una región mediterránea. *Munibe* **25**: 120-128.
- Rodríguez-Rodríguez, E.J., Beltrán, J.F., El Mouden, E.H., Slimani, T., Márquez, R., Donaire-Barroso, D. (2020a): Climate change challenges IUCN conservation priori-

ties: a test with western Mediterranean amphibians. *SN Appl. Sci.* **2**: 216.

Rodríguez-Rodríguez, E.J., Beltrán, J.F., Tejedo, M., Nicieza, A.G., Llusia, D., Márquez, R., Aragón, P. (2020b): Niche models at inter- and intraspecific levels reveal hierarchical niche differentiation in midwife toads. *Sci. Rep.* **10**: 10.942.

Schleich, H.H., Kästle, W., Kabisch, K. (1996): *Amphibians and reptiles of North Africa*. Koeltz Scientific Books, Koenigstein.

Sillero, N. (2009): Modelling a species in expansion at local scale: is *Hyla meridionalis* colonising new areas in Salamanca, Spain? *Acta Herpetol.* **4**: 37-46.

Sillero, N. (2010): Modelling suitable areas for *Hyla meridionalis* under current and future hypothetical expansion scenarios. *Amphib.-Reptil.* **31**: 37-50.

Sillero, N. (2014): *Ranita meridional – Hyla meridionalis*. In: *Enciclopedia virtual de los vertebrados españoles*. Salvador, A., Martínez-Solano, I., Eds, Museo Nacional de Ciencias Naturales (Consejo Superior de Investigaciones Científicas), Madrid. Retrieved from <http://www.vertebradosibericos.org>. Accessed 8 April 2021.

Sutherland, W.J., Ed. (2006): *Ecological Census Techniques*. A Handbook, 2nd Edition. Cambridge University Press, Cambridge.

Tarragona, F., Coord (1999): *Zonas húmedas de Motril. La charca de Suárez*. Monografías Ambientales de la Costa Granadina, 1. Concejalía de Medio Ambiente (Ayuntamiento de Motril), Granada.

Vignoli, L., Luiselli, L. (2013): Better in the dark: two Mediterranean amphibians synchronize reproduction with moonlit nights. *Web Ecol.* **13**: 1-11.

Willacy, R.J., Mahony, M., Newell, D.A. (2015): If a frog calls in the forest: bioacoustic monitoring reveals the breeding phenology of the endangered Richmond Range mountain frog (*P hyloria richmondensis*). *Austral Ecology* **40**: 625-633.

Yus, R., Tapia, M., García, H., Jerez, D., Gámez, S., González, J.A., Torres, M.A., Cabo, J.M., Coords (2013): VII. El macizo del Gourougou. In: *Historia natural de la región de Melilla (Guelaya, Alborán y Chafarinas)*. Yus, R., Cabo, J.M., Eds, Fundación Gaselec, Melilla.

Appendix

Table A1. Pearson's correlation coefficients (*r*) between the geographical and environmental variables, and the climatic variables. The values in bold indicate the variables showing statistically significant correlations ($P \leq 0.05$).

	Latitude	Elevation	Water seasonality	Mean ambient temperature	Mean ambient temperature range
Elevation	0.23 $P = 0.58$				
Water seasonality	-0.68 $P = 0.06$	-0.07 $P = 0.87$			
Mean ambient temperature	-0.67 $P = 0.07$	-0.72 $P = 0.04$	0.58 $P = 0.13$		
Mean ambient temperature range	0.73 $P = 0.04$	0.77 $P = 0.03$	-0.49 $P = 0.22$	-0.98 $P < 0.01$	
Mean anual precipitation	0.35 $P = 0.39$	-0.13 $P = 0.76$	-0.17 $P = 0.06$	-0.39 $P = 0.34$	0.36 $P = 0.39$

Table A2. Reliability of inter-researcher estimates. Pearson's correlation coefficients (r) between numbers of calling males estimated on a sample of 12 recordings of advertisement choruses of variable size (ranging between 1 and 1000 males) by 17 researchers, and between the estimates of each of them and the estimate made by the experienced researchers directly in the field (Field estimation).

	Field estimation	Researcher 1	Researcher 2	Researcher 3	Researcher 4	Researcher 5	Researcher 6	Researcher 7	Researcher 8	Researcher 9	Researcher 10	Researcher 11	Researcher 12	Researcher 13	Researcher 14	Researcher 15	Researcher 16
Researcher 1	0.98																
Researcher 2	$P < 0.001$	0.76															
Researcher 3	$P = 0.004$	$P = 0.001$															
Researcher 4	0.99	0.98	0.80														
Researcher 5	$P < 0.001$	$P < 0.001$	$P = 0.002$														
Researcher 6	0.97	0.84	0.99	0.84													
Researcher 7	$P < 0.001$	$P < 0.001$	$P = 0.001$	$P < 0.001$													
Researcher 8	0.62	0.75	0.73	0.62	0.76												
Researcher 9	$P = 0.031$	$P = 0.005$	$P = 0.007$	$P = 0.031$	$P = 0.004$												
Researcher 10	0.99	0.99	0.79	0.98	0.99	0.72											
Researcher 11	$P < 0.001$	$P < 0.001$	$P = 0.002$	$P < 0.001$	$P = 0.008$												
Researcher 12	0.84	0.88	0.99	0.87	0.89	0.71	0.86										
Researcher 13	$P = 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.009$	$P < 0.001$											
Researcher 14	0.75	0.81	1.00	0.79	0.83	0.72	0.78	0.99									
Researcher 15	$P = 0.005$	$P = 0.001$	$P < 0.001$	$P = 0.002$	$P = 0.001$	$P = 0.009$	$P = 0.003$	$P < 0.001$									
Researcher 16	0.93	0.96	0.82	0.93	0.95	0.76	0.95	0.87	0.82								
Researcher 17	$P < 0.001$	$P < 0.001$	$P = 0.001$	$P < 0.001$	$P = 0.004$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.001$								
Researcher 18	0.99	0.99	0.79	0.98	0.99	0.72											
Researcher 19	$P < 0.001$	$P < 0.001$	$P = 0.002$	$P < 0.001$	$P = 0.004$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.001$								
Researcher 20	0.84	0.88	0.99	0.87	0.89	0.71	0.86										
Researcher 21	$P = 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.009$	$P < 0.001$											
Researcher 22	0.95	0.98	0.91	0.97	0.98	0.75	0.97	0.95	0.91	0.95	0.97						
Researcher 23	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.005$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$						
Researcher 24	0.76	0.82	0.97	0.77	0.82	0.77	0.79	0.97	0.96	0.84	0.80	0.90					
Researcher 25	$P = 0.004$	$P = 0.001$	$P < 0.001$	$P = 0.003$	$P = 0.001$	$P = 0.003$	$P = 0.002$	$P < 0.001$	$P < 0.001$	$P = 0.001$	$P = 0.002$	$P < 0.001$					
Researcher 26	0.81	0.90	0.88	0.84	0.93	0.91	0.88	0.89	0.88	0.90	0.87	0.92	0.87				
Researcher 27	$P = 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$				
Researcher 28	0.99	0.97	0.75	1.00	0.97	0.61	0.98	0.82	0.74	0.92	0.98	0.95	0.73	0.82			
Researcher 29	$P < 0.001$	$P < 0.001$	$P = 0.005$	$P < 0.001$	$P = 0.035$	$P < 0.001$	$P < 0.001$	$P = 0.001$	$P = 0.006$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.007$	$P = 0.001$			
Researcher 30	0.94	0.97	0.91	0.97	0.98	0.71	0.96	0.95	0.91	0.95	0.95	0.99	0.88	0.91	0.95		
Researcher 31	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.010$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$		
Researcher 32	0.89	0.88	0.74	0.90	0.88	0.55	0.90	0.80	0.75	0.82	0.85	0.86	0.67	0.73	0.88	0.87	
Researcher 33	$P < 0.001$	$P < 0.001$	$P = 0.006$	$P < 0.001$	$P < 0.061$	$P < 0.001$	$P < 0.001$	$P = 0.002$	$P = 0.005$	$P = 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.017$	$P = 0.007$	$P < 0.001$	$P < 0.001$	
Researcher 34	0.72	0.81	0.70	0.70	0.81	0.94	0.80	0.72	0.70	0.86	0.78	0.79	0.75	0.90	0.69	0.75	0.67
Researcher 35	$P = 0.009$	$P = 0.002$	$P = 0.011$	$P = 0.011$	$P = 0.001$	$P < 0.001$	$P = 0.002$	$P = 0.008$	$P = 0.011$	$P < 0.001$	$P = 0.003$	$P = 0.002$	$P = 0.005$	$P < 0.001$	$P = 0.012$	$P = 0.005$	$P = 0.018$