Extreme weather event disrupts reproduction of an isolated western spadefoot toad population, *Pelobates cultripes* (Cuvier, 1829), at its southern range limit

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Abstract

Extreme weather event disrupts reproduction of an isolated western spadefoot toad population, Pelobates cultripes *(Cuvier, 1829), at its southern range limit*. *Pelobates cultripes* can be considered among those amphibians most affected by climate change in Spain. Its long larval period and predicted shorter pond hydroperiods in its temporary breeding ponds may ultimately reduce population recruitment. We conducted surveys during its breeding season 2021–2022 in order to analyse one of the last remaining urban populations in its southern limit (coastal Malaga province). We recorded a decrease in rainfall and an increase in temperature compared to previous years. Although the species shortened its larval period in comparison with that previously reported, no post–metamorphic juveniles were observed. This interruption in reproduction highlights the need for improved monitoring of these isolated amphibian populations as it could be an early warning sign of global changes in the Mediterranean region.

Key words: Biodiversity crisis, Early warning, Bioindicator amphibians, Habitat fragmentation, Global decline, Urban wildlife populations

Resumen

Los fenómenos meteorológicos extremos afectan a la reproducción de una población occidental aislada del sapo de espuela, Pelobates cultripes *(Cuvier, 1829), en su límite de distribución más meridional. Pelobates cultripes* (Cuvier, 1829) se puede considerar como uno de los anfibios más afectados por el cambio climático en España. Su largo periodo larvario y la reducción prevista de la duración de las charcas temporales donde se reproduce podrían reducir en última instancia el reclutamiento demográfico de sus poblaciones. Realizamos estudios durante su período de reproducción (2021–2022) para analizar una de las últimas poblaciones urbanas en el límite más meridional de su distribución (la costa de la provincia de Málaga). Detectamos una disminución de las precipitaciones y un aumento de la temperatura en comparación con años anteriores. Aunque el periodo larvario de la especie fue más corto que el determinado en estudios anteriores, no se observaron juveniles posmetamórficos. Esta interrupción de la reproducción pone de relieve la necesidad de mejorar el seguimiento de estas poblaciones aisladas de anfibios, ya que podría ser una alerta temprana de cambios globales en la región del Mediterráneo.

Palabras clave: Crisis de biodiversidad, Alerta temprana, Anfibios bioindicadores, Fragmentación del hábitat, Reducción mundial, Poblaciones silvestres urbanas

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In recent decades there has been a global decline of amphibians worldwide (Houlahan et al., 2000). Forty–one percent of known amphibians are threatened with extinction. Specifically in Spain, 35 % of amphibians are threatened and thus susceptible to disappearance in coming years (IUCN, 2021). Worldwide, the main cause of amphibian decline is the loss of natural environments. Besides affecting terrestrial habitats of adults this results in the loss of breeding ponds for larval growth and development (Houlahan et al., 2000). Furthermore, the emergence of barriers caused by urban development and road construction also limits the dispersal of these species, fragmenting populations and disrupting one of the main mechanisms for colonising new environments and maintaining metapopulations.

In this context, climate change may have a negative synergistic effect and further aggravate the situation (Blaustein et al., 2011), globally threatening many of these populations with extinction. According to climate change projections for 2050, several authors foresee particularly adverse conditions for amphibians due to globally increased temperatures and locally decreased precipitation (Romero et al., 2019; IPCC, 2021). In many parts of the globe, rainfall shortages, extreme drought, and shortened hydroperiods have been specifically linked to decreased breeding call activity in anurans, reproductive failure through mass larval mortality by premature drying of breeding water bodies (Taylor et al., 2006), decreased body sizes during metamorphosis (McMenamin and Hadly, 2010), and even local extinctions (Scheele et al., 2012).

The western spadefoot toad *Pelobates cultripes* (Cuvier, 1829) is a species restricted to the Iberian Peninsula and the south–eastern coast of France. It is considered 'Vulnerable' (VU) because of an estimated population decline of 30 % over the past 21 years (IUCN, 2022). In the meridional limit of the species' distribution, in particular in the province of Malaga (Andalusia, Spain), several authors have reported a strong decrease in the territory occupied by this species, from fourteen 10 x 10 km UTM squares before 2001 (Pleguezuelos et al., 2002) to only four (Jiménez et al., 2016), making it at risk of imminent local extinction. In the south of the Iberian Peninsula, this toad is an autumnal breeder. Its maximum reproductive activity usually coincides with the days of highest rainfall when the breeding ponds where they lay their eggs have filled up (Tejedo and Reques, 2002). The Western spadefoot toad adapts well in this rainfall pattern, with its breeding season period usually starting in November (Reques, pers. obs. for the provinces of Cordoba, Seville and Cadiz). Gomez–Mestre et al. (2013) described that under experimental conditions *Pelobates cultripes* larvae require a minimum of 89.77 (± 4) days to reach metamorphosis, being one of the Mediterranean amphibian species with the longest larval cycle. Because of this prolonged larval development, if breeding sites do not receive sufficient rainfall in early winter, the species may experience difficulties reaching metamorphosis. The Finca de la Cizaña population (fig. 1) (36.6487 ºN, –4.4782 ºE) is one of the most important remaining breeding sites for the species in Malaga province and the last urban population in the Malaga metropolitan area (Romero et al., 2022).

Isolation due to urban development is the main threat to this population. Despite the warnings described, the plot of land in question has no protection status. It is situated in an area of high touristic interest and is classified as developable land (fig. 1).

Analysis of the rainfall patterns in the south of the Iberian Peninsula reveals two important rain peaks, one at the end of autumn and one at the end of winter (REDIAM, 2022). The temporal rainfall pattern observed over a ten–year period in the study area, (fig. 1s in supplementary material) also presented two important rain peaks, one at the end of autumn and the other at the end of winter (REDIAM, 2022). Climate forecasts for the study area warn of a rainfall decline of 1.34 % by 2040, 4.25 % by 2070 and 9.37 % by the end of the century (REDIAM, 2022). This could alter the mentioned pattern.

Given this worrisome situation, we assessed the status of the population during the 2021–2022 breeding period. We conducted surveys of egg clusters, larvae and adults of the species between 28th February and 6th June 2022 (fig. 2C), extending the sampling period until the pond dried up and adult activity stopped. On 17th March, coinciding with the first rains of the year (fig. 2A, 2s in supplementary material), we began to observe adults. On 29th March, three days after the first rainfall that filled the temporary breeding pond used by the species, we detected the first egg clusters (fig. 2A). Once the pond is filled with water, its approximate dimensions are: a maximum depth of 35 cm, a maximum length of about 14 m, a maximum width of about 5 m, and a surface area of 44 m^2. On 13th April, we started monitoring *Pelobates cultripes*

Fig. 1. The blue line indicates the area occupied by *Pelobates cultripes* in Finca de la Cizaña (Malaga, Spain). The red dots indicate the distribution of adults, and the arrow indicates location of the breeding pond studied. The polygon outlined in red marks the most recently developed area, highly urbanised in the last five years. Source: Google Earth Pro.

Fig. 1. Área de distribución ocupada por Pelobates cultripes en la Finca de la Cizaña, en Málaga (España), delimitada por la línea azul. Los puntos rojos indican la ubicación de los adultos y la flecha señala el estanque de reproducción estudiado. El polígono delimitado en rojo es la última parcela de tierra cercana a la zona de estudio que ha sido fuertemente urbanizada en los últimos cinco años. Fuente: Google Earth Pro.

larvae (fig. 2B). Four larval surveys were carried out between April 25th April and May 17th May. Specifically, we sampled the breeding pond by dip–netting for 10 minutes (ranging shore, centre and opposite shore), obtaining an average of N = 78 larvae per sampling (minimum of 28 and maximum of 119); these were counted and returned to the pond. For larval sampling we used an amphibian net with a diameter of 20 cm, mesh size of 0.3 cm and net length of 20 cm. Sporadic larval surveys on previous years revealed that during the 2014 and 2015 seasons, both characterised by rainfall mostly concentrated in autumn, with values of 362 and 419 mm, respectively (fig. 1s in supplementary material), there was no reproduction. During the 2020–2021 season, rainfall was 421 mm but more evenly distributed over autumn and winter (fig. 2s in supplementary material). Although this facilitated reproduction, we obtained no record of individuals reaching metamorphosis.

During the last study period (2021–2022), rainfall at the Finca de la Cizaña population was concentrated mainly during the month of March (see graphs in fig. 2s in supplementary material), accumulating a total of 304 mm. There was insufficient rainfall to fill the pond in the preceding months (see comparison over the last 10 years in the graphs in figure 1s in supplementary material, with an average of 503.3 mm). This concentrated rainfall event in March, together with the progressive increase in average temperature, implied that the breeding pond had water for only 71 days. Some of the larvae of *Pelobates cultripes* managed to reach the premetamorphic stage, Gosner Stage 42 (Gosner, 1960), in 39 days from the observation of the first egg clusters (fig. 1C); this amounted to half the minimum described under adverse conditions simulated in the laboratory (Gomez–Mestre et al., 2013). In this species and in other *Pelobatoidea*, Buchholz and Hayes (2002) described a great plasticity for accelerating or slowing down their larval period depending on the conditions within the ponds. Despite this potential plasticity, during the study period we found that most of the tadpoles died before completing metamorphosis because of the premature pond drying before they could complete full metamorphosis (fig. 2C). These late reproductive events that were associated with higher rainfall at the end of winter have also been reported in a Mediterranean population of natterjack toad (*Epidalea calamita*) (Tejedo and Reques, 1994; Tejedo, 2003). Larval development and metamorphosis occurred in only 40 days. This fast development could be linked

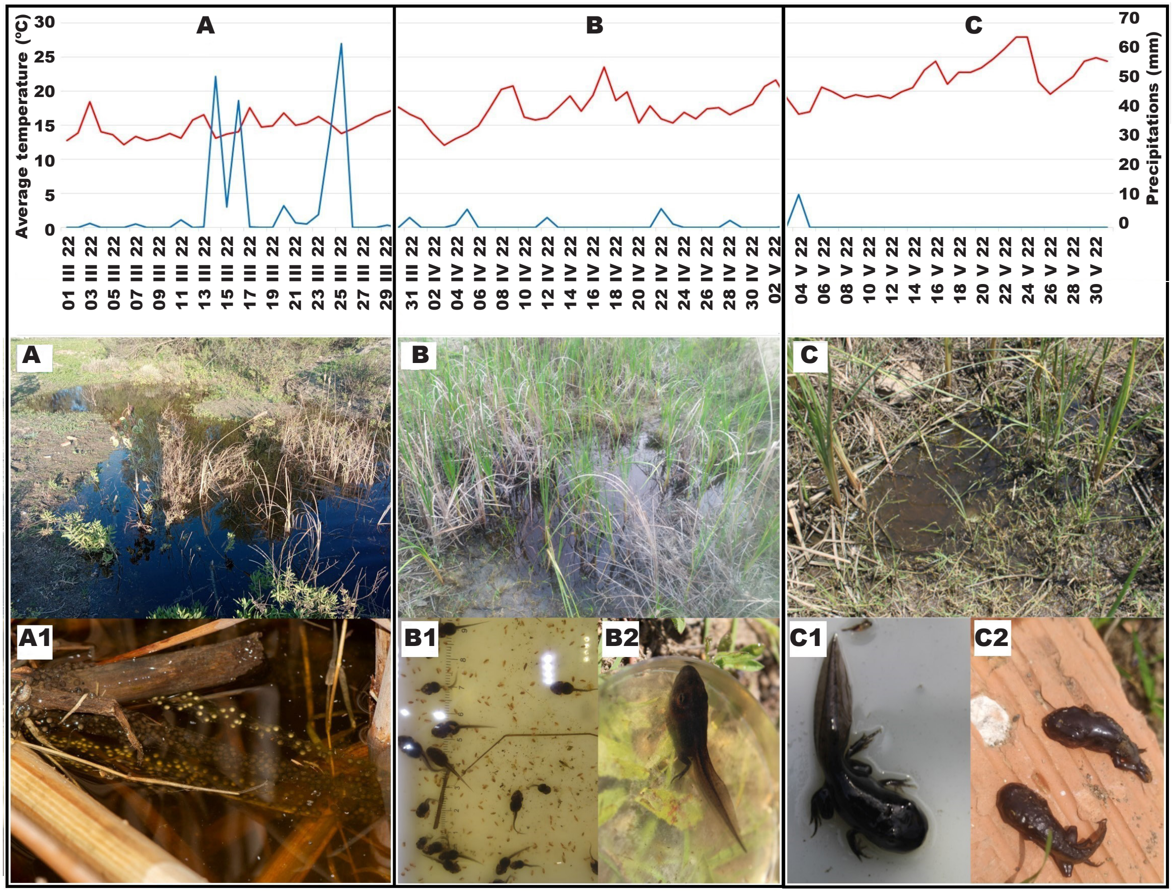

Fig. 2. Top row panel: climate diagram of the study period (March–May 2022) showing mean temperatures (red line) and precipitation (blue line). Middle row panels (A–C): series of pond images corresponding to the temporal period shown by the climate diagram above. Bottom row panels: stages of reproduction showing egg clusters (A1), larvae in early (B1), intermediate (B2), and advanced stages of development (C), live individual at metamorphosis climax (42–43 Gosner stage [Gosner, 1960]) (C1), and deceased individuals at metamorphosis (left 42 Gosner stage, right 43–44 Gosner stage) (C2). Pictures: Adrián Martín–Taboada (A1) and Raúl Arroyo–Morales (all other pictures). Data Sources from AEMET; http://www.aemet.es/ [Accessed on June of 2022].

Fig 2. Hilera superior: diagramas climáticos del período del estudio (marzo–mayo de 2022) en los que se muestra la media de las temperaturas (línea roja) y de la precipitación (línea azul). Hilera media (A–C): serie de imágenes tomadas en el estanque durante el período mostrado en el diagrama climático superior. Hilera inferior: estadios de reproducción que muestran las agrupaciones de huevos (A1); las larvas en los estadios de desarrollo inicial (B1), intermedio (B2) y avanzado (C); ejemplares vivos en el clímax de la metamorfosis (estadio de Gosner 42–43 [Gosner, 1960]) (C1), y ejemplares muertos en la metamorfosis (izquierda: estadio de Gosner 42; derecha: estadio de Gosner 43–44) (C2). Imágenes: Adrián Martín–Taboada (A1) y Raúl Arroyo–Morales (el resto de las imágenes). Fuente de los datos: Agencia Estatal de Meteorología (AEMET), http://www.aemet.es/ [consultado en junio de 2022].

to high temperatures and possibly low larval densities that allow for faster growth and development in larval amphibians (Tejedo et al., 2010). Furthermore, rising temperatures are a limiting factor for many temperate species. Although it has been shown that tadpoles of this and other species can have a relative thermal tolerance, if reproduction occurs in spring they might be exposed to very high temperatures (Duarte et al., 2012). The rate of global warming predicted by the IPCC will be higher in temperate zones (IPCC, 2021; REDIAM, 2022). Thus, in coming decades, vulnerability of these species could increase considerably. Figure 2C shows the larvae that probably died due to a presumed increase in water temperature as recorded for air temperatures (fig. 2C top panel), with a maximum air temperature of 29.9 ºC recorded on 21st May (AE-

MET, closest weather station, at Malaga airport). Once metamorphosis is reached, juveniles of this species may bury themselves to avoid desiccation, thereby hindering detection. Although unlikely, it is possible that some individuals reached metamorphosis but were not detected. Considering the frequency of the monitoring, together with the early stage of development of the larvae detected before the water body dried up completely, we would expect that reproductive success, if any, was low.

This monitoring study coincided with the second driest winter in the Iberian Peninsula since 1961 (REDIAM, 2022). Our data indicate that the synergistic effect of decreased precipitation and increased temperatures led to adverse conditions for amphibians that, despite their plasticity in larval development (Gomez–Mestre et al., 2013), exceeded the response capabilities of this population. Becker et al. (2018) detected that in dry years, survival of adult *Capensibufo rosei* toads increased at the expense of reduced reproduction. Given the poor reproductive success of previous years, if this pattern of low rainfall in winter recurs for several consecutive years, the population could decline irreversibly.

We conclude that there is a need for continued monitoring of endangered amphibian populations in order to assess the mid– and long–term consequences of these breeding failures. We also recommend that conservation efforts be increased in the few remaining breeding shelters for *Pelobates cultripes* at its southern limit in view of the human modified environment where natural recolonization after a possible local extinction is not possible. As the effects of climate change are expected to continue and to worsen with the desertification of the southern half of the Iberian Peninsula, this case study could reflect what may already be happening in other threatened isolated amphibian populations.

Acknowledgements

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Supplementary material

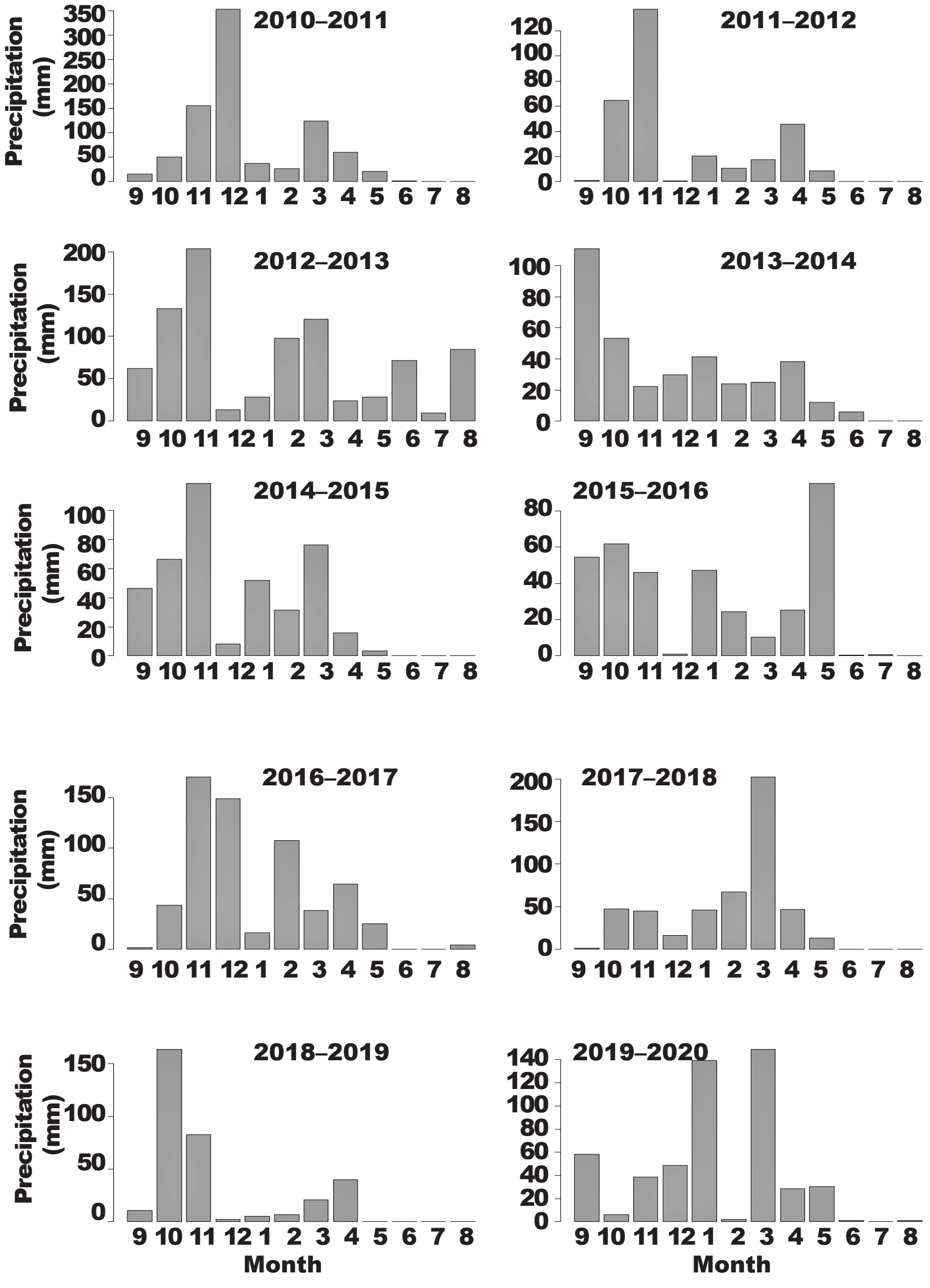

Fig. 1s. Monthly rainfall pattern in La Finca de la Cizaña for the ten years prior to the study period. Data extracted from raster format of monthly precipitation (1, January; 2, February; 3, March; 4, April; 5, May; 6, June; 7, July; 8, August; 9, Sptember; 10, October; 11, November; 12, December) from the meteorological stations of the Agencia Estatal de Meteorología (AEMET). Source: Red de Información Ambiental de Andalucía (REDIAM), Portal Ambiental de Andalucía: https://www.juntadeandalucia.es/medioambiente/portal/acceso-rediam [Accessed on July 2022].

Fig. 1s. Régimen de precipitaciones mensuales en la Finca de la Cizaña durante los 10 años anteriores al período del estudio. Datos extraídos del formato ráster de las precipitaciones mensuales de las estaciones meteorológicas de la Agencia Estatal de Meteorología (AEMET). Fuente: Red de Información Ambiental de Andalucía (REDIAM), Portal Ambiental de Andalucía: https://www.juntadeandalucia.es/medioambiente/portal/acceso-rediam [Consultado en julio de 2022]. (Para las abreviaturas de los meses, véase arriba).

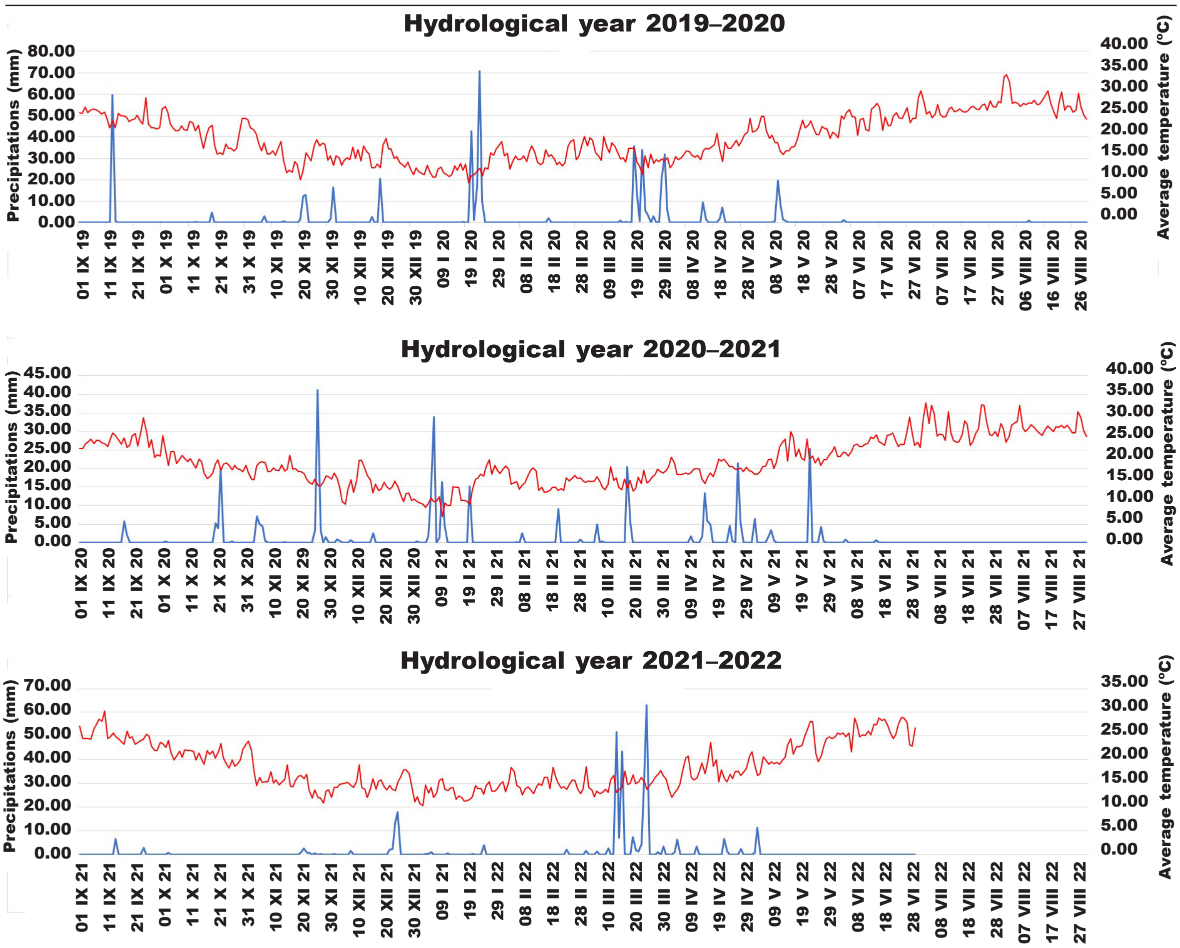

Fig. 2s. Climate diagram showing temperatures (red line) and precipitation (blue line) for the September–August hydrological year for the 2019–2020, 2020–2021 and 2021–2022 seasons. Data extracted from the nearest weather station listed in AEMET (Malaga airport). Source: http://www.aemet.es/

Fig. 2s. Diagrama climático en el que se muestran las temperaturas (línea roja) y la precipitación (línea azul) del año hidrológico (de septiembre a agosto) de las temporadas 2019–2020, 2020–21 y 2021–22. Datos extraídos de la estación meteorológica más cercana de la AEMET (aeropuerto de Málaga). Fuente: http://www.aemet.es/