Long-term monitoring of an amphibian community after a climate change- and infectious disease-driven species extirpation

Running head: amphibian monitoring and global change

Jaime Bosch[1,2*], Saioa Fernández-Beaskoetxea[1], Trenton W. J. Garner[4] & Luis María Carrascal[1]

[1]Museo Nacional de Ciencias Naturales-CSIC, José Gutiérrez Abascal 2, 28006 Madrid, Spain

[2]Centro de Investigación, Seguimiento y Evaluación, Parque Nacional de la Sierra de Guadarrama, Cta. M-604, Km. 27.6, 28740 Rascafría, Spain

[3]Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK

*Author for correspondence (+34677772402; bosch@mncn.csic.es)

Abstract

Infectious disease and climate change are considered major threats to biodiversity and act as drivers behind the global amphibian decline. This is to a large extent based on short term studies that are designed to detect the immediate and strongest biodiversity responses to a threatening process. What few long term studies are available, although typically focussed on single species, report outcomes that often diverge significantly from the short term species responses. Here we report the results of an 18 year survey of an amphibian community exposed to both climate warming and the emergence of lethal chytridiomycosis. Our study shows that the impacts of infectious disease is ongoing but restricted to two out of nine species that form the community, despite the fact all species can become infected with the fungus. Climate warming appears to be affecting four out of the nine species, but the response of three of these is an increase in abundance. Our study supports a decreasing role of infectious disease on the community, and an increasing and currently positive effect of climate warming. We caution that if the warming trends continue, the net positive effect will turn negative as amphibian breeding habitat becomes unavailable as water bodies dry, a pattern that already may be underway.

Introduction

The current mass extinction stands apart from previous events due to the role of a single species in the reduction of global biodiversity (Ceballos *et al.*, 2015). Human activities that modify habitats, harvest wildlife, and alter ecosystem composition through biological introductions are behind contemporary biodiversity loss: evidence for this comes from decades of investigations into the causes of the global decline of amphibian biodiversity (Stuart *et al.* 2004, 2008). Habitat destruction, pollution and climate change render swathes of

environments unsuitable for native amphibians, amphibian trade reduces amphibian abundance, alters the composition of and vectors novel pathogens into amphibian communities, while introduced species and pathogens cause mass mortality or catastrophically impair recruitment (Berger *et al.* 1998; Davidson *et al.*, 2002; Finlay & Vredenberg 2007; McMenamin *et al.*, 2008; Johnson *et al.*, 2011; Fisher *et al.*, 2012; Faruk *et al.*, 2013; Price *et al.*, 2014; Martel *et al.* 2014). More worrisome is that many amphibian population declines or species extinctions have occurred in protected or relatively undisturbed areas (Collins & Crump, 2009; Becker & Zamudio, 2011), illustrating the point that habitat protection offers no barriers to threats like climate change and infectious diseases.

Despite the increasing abundance of reports of amphibian declines, it is likely that these are inaccurately informing us as to the impacts of climate change, infectious diseases or their combination. This is because publications generally report worst case scenarios, where impacts are immediate, overt and measurable over short time scales (Berger *et al.*, 1998, Price *et al.* 2014; Bosch *et al.*, 2001; Lips *et al.* 2006; Stegen *et al.*, 2017). While some of these studies report taxonomically broad impacts, others describe demographic declines of single species in multi-species amphibian communities, or spatially heterogeneous population responses (Price *et al.* 2014; Bosch *et al.*, 2001; Lips *et al.* 2006, Lips *et al.* 2008; Vredenburg *et al.* 2010; Walker *et al.*, 2010; Puschendorf *et al.*, 2013; Price *et al.*, 2016). These inconsistencies suggest that the threat posed by climate change and infectious diseases are not comprehensively described by short term studies, which long term monitoring projects are starting to reveal. For example, in some communities impacts are likely being underestimated, as cryptic, subtle, indirect or post-decline spill-over or knock-on effects that cause population declines downstream from the initial mortality event are now being reported (Clare *et al.*, 2016; Scheele *et al.*, 2017). Alternatively, some populations that have

experienced climate- and/or disease-driven declines appear to be recovering, suggesting that in some cases threat may be overestimated (Scheele *et al.*, 2017; Knapp *et al.*, 2016).

Batrachochytrium dendrobatidis (hereafter *Bd*), is a generalist, chytridiomycete fungal pathogen responsible for amphibian declines and extinctions at a global scale (Berger *et al.*, 1998; Fisher *et al.*, 2009). Climate change, predominantly differences in environmental temperatures, has been invoked as an important cofactor influencing the degree of impact chytridiomycosis caused by *Bd* may have on amphibian populations and communities (Walker *et al.*, 2010; Cohen *et al.*, 2017). The assumption that temperature dictates the virulence of *Bd* epidemics is predicated on *in vitro* studies of temperature-dependent *Bd* growth rates. Macroecological and other studies investigating the 'chytrid thermal optimum hypothesis' report ambiguous or contradictory findings, or suggest a more direct effect of climate change (Lips *et al.*, 2008; Pounds *et al.* 2006; Rohr et al. 2008, 2010). Indeed, climate change is likely behind many amphibian species population declines and range contractions, and changes in parasite incidence and prevalence, although affected by changes in environmental metrics, may be coincidental and not causative of changes in host abundance (Reading 1998; Bosch *et al.*, 2014). Nearly all studies of amphibian population declines and the impact of climate change and infectious disease invoke issues of scale and suggest the impact of climate, chytridiomycosis and their interactions will be elucidated through long term amphibian population and community monitoring projects.

Europe is home to several such long-term projects, including the Peñalara Amphibian Survey at the Peñalara Massif in the Sierra de Guadarrama National Park. That was the first project to detect European amphibian declines due to *Batrachochytrium dendrobatidis*, in this case affecting the common midwife, *Alytes obstetricans* (http://www.parquenacionalsierraguadarrama.es/es/blogs/sos-anfibios, Bosch *et al.*, 2001), subsequently described for this species in 3 other European countries (Walker SF *et al.*, 2010;

Tobler & Schmidt, 2010; Rosa *et al.*, 2013). The disease-driven near-extirpation of common midwives on the Peñalara Massif has since been attributed to a threshold effect of increasing environmental temperature on the emergence of chytridiomycosis (Bosch *et al.*, 2007). Two other amphibian species have been reported dying from chytridiomycosis caused by *Bd* and potentially declining as a result: *Salamandra salamandra* and *Bufo spinosus* (Bosch & Martínez-Solano, 2006; Garner *et al.*, 2009). However, population and *Bd* transmission dynamics of these two other species years after the initial disease emergence event suggest that early population responses do not fully describe demographic response to changing climate and disease in the region (Bosch & Rincón, 2008; Bosch *et al.*, 2014; Medina *et al.*, 2015; Fernández-Beaskoetxea *et al.*, 2016).

Here, we report the results of long-term monitoring of the amphibian community in Peñalara 18 years after the disease outbreak. We examine the relationship between fluctuating environmental temperature and changes in amphibian species abundance to determine the impact climate change is having on host community structure. In conjunction with this and to ascertain if *Bd* is a continued threat, we estimated prevalence of infection in the one remaining widespread and reasonably common amphibian species that is reported to be killed by lethal chytridiomycosis in Peñalara, the fire salamander *S. salamandra* (Bosch & Martínez-Solano, 2006; Medina *et al.*, 2015). Our objective was to determine if prevalence of infection had downstream effects on the abundance of larval salamanders.

Material and Methods

Study system

The Peñalara Massif (Guadarrama Mountains National Park, Central Spain, near Madrid, 41°N, 4°W; Fig. 1) is an alpine (1800-2430 m) habitat with 242 ponds above the tree line. Ponds lack vegetation along the shoreline, range in area from 0.3 to 6463.4 m^2 and are

clustered (1-24 ponds per cluster) within 29 basins (hereafter, sectors). The landscape consists mainly of granitic outcrops, alpine meadows, heathlands dominated by *Cytissus oromediterraneus* and *Juniperus communis nana*, and forests of *Pinus sylvestris* below the timber-line. Nine amphibian species breed regularly in the area, and the combination of lithology and scarcity of aquatic vegetation provides crystal-clear waters that are excellent for visual surveys of larval abundance (Martínez-Solano *et al.*, 2003).

Field survey

We comprehensively surveyed all ponds across the Peñalara Massif during the amphibian reproductive and development period (May to September) each year from 1999 and 2016. For each species, we recorded number of clutches (for bufonids) or estimated larval abundances (all other species) up to 6 times per year and per pond, dependant on the hydroperiod and associated persistence of ponds. In larger ponds, or at high tadpole densities (roughly >100), we estimated the number of tadpoles through sub-sampling (stratified when required by habitat diversity) by counting all the larvae present in predetermined areas and extrapolating to the entire pond. To standardize variations in counting, and to minimize sampling error, we used six classes with different break points to score larval abundance (0, 1–10, 11–50, 51–100, 101–500, and 501-1000) and number of clutches (0, 1, 2-5, 6-10, 11-20, 21-50). For statistical analysis we first selected the maximum range value for each species at each pond as the best estimate of the maximum reproductive output for that year. We then converted maximum ranges to median counts and summed these across ponds within a sector, which resulted in 29 independent cumulative counts per year and per species. These values formed the data set we used in the TRIM analyses described below. Despite the relatively large number of pond x year abundance estimates (242 ponds x 18 years = 4356 different counts), due to highly heterogenous patterns of abundance across ponds and years only a small subset of the counts fell into the largest count class: *Alytes obstetricans*, 0.02%;

Epidalea calamita, 1.03%; *Bufo spinosus*, 0.23%; *Hyla molleri*, 0.67%; *Ichthyosaura alpestris*, 0.34%; *Pelophylax perezi*, 0.39%; *Rana iberica*, 0.00%; *Salamandra salamandra*, 0.53%; *Triturus marmoratus*, 0.00%.

To assess the relationship between temperature variation and amphibian abundance we used ambient minimum and maximum temperature data generated at the nearby meteorological station in Cotos mountain pass (provided by Guadarrama National Park, 1857 m a.s.l). We used data sets from 1991 to 2016 in order to estimate how time spans of up to seven years preceding the sampling year affected the annual abundance of each species. As monthly minimum and maximum temperature averages were highly and positively correlated throughout the whole study period (R^2 = 0.972, n = 312 months in 26 years), we focussed on two more distinct temperature measurements for analyses: yearly minimum temperature of the coldest month (January or February depending on the years) and yearly maximum temperature of the hottest month (July or August depending on the years; Fig. 2). These two variables represent the most extreme thermal conditions within the year but were weakly and negatively correlated across the 26 years of temperature records (R^2 = 0.270, n = 26). The yearly minimum and maximum temperatures were also separately averaged for an additional seven time spans of different length, from two (the census year plus the previous year) to eight consecutive years (from the year of the census to seven years before). Correlations between the minimum and maximum temperatures were non-significant across the eight time spans for the 18 study years (R^2 of 0.018 for single year estimates up to a maximum R^2 of 0.084 for average across eight years). We saw no evidence of temporal autocorrelation in the linear trend autocorrelation function plots of yearly minimum temperature and yearly maximum temperature, suggesting that the time series was stationary around a deterministic trend and that differencing was not required (i.e., the statistical properties of the time series of the minimum and maximum yearly temperatures in 1999-2016 were constant over time).

Data analyses

Annual population trends were estimated using the time-effects model of TRIM, statistical software developed specifically for time series of animal counts in monitoring programmes (Pannekoek & van Strie, 2005). Specifically, we developed log-linear Poisson regression models from species' counts using years (1999–2016) as the continuous predictor. These models accounted for over-dispersion and serial autocorrelation in the data to obtain population trends (±1) as the slope of the regression of the logarithms of the yearly indices. Standard errors of the trends were estimated as a measure of uncertainty in average linear population trends. We did not use non-linear models as our objective was to investigate overall change during the course of the sampling period and not annual, or other short-term fluctuations in abundance (see also Heldbjerg & Fox, 2008; Gregory *et al.* 2009; Reif *et al.* 2011 for a similar approach). Linear trend TRIM models were compared with their corresponding null TRIM models (not including the linear effect of year) using Akaike's Information Criterion (AIC). We estimated the overall additive change for each species by measuring the average inter-annual rate from the first to the last year of the study.

We constructed AutoRegressive Integrated Moving Average (ARIMA) models to estimate the influence of annual maximum and minimum temperatures on annual amphibian species abundance. Sixteen ARIMA models were built for each species using the same set of temperature values and using the command *auto.arima* of the package {forecast} run under R version 3.1.2 (R Core Team. 2014). We used the *auto.arima* command and the logarithm of annual species abundance estimates to obtain the optimum p, d and q parameters in ARIMA models. Second order Akaike's AICc for finite sample sizes were used to obtain the weights of these sixteen models. Model weights within species were added according to two variable categories: (1) minimum vs. maximum temperatures (sum of eight weights in each category), and (2) eight time spans (sum of two weights –maximum and minimum temperatures– per

time span from the census year to seven years before the amphibian counts). To illustrate the strength and significance of temperatures on species-level count data, we listed the ARIMA model with the lowest AICc value (Table 2). We tested for temporal autocorrelation using the residuals of the selected ARIMA models and autocorrelation function plots, the Kwiatkowski–Phillips–Schmidt–Shin (KPSS) test and the Ljung–Box test (up to lag six years), with the commands *Acf* of the package {forecast}, *kpss.test* of the package {tseries}, and *Box.test* and found no evidence of temporal autocorrelation. We used principal component analysis (PCA) of the logarithm of the abundance data for each species and each year to illustrate any general patterns of population trends across species. We employed a 5-fold cross-validation method for determining the optimal number of principal components, using Statistica 12 (StatSoft. Inc.).

Although all amphibian species inhabiting the Peñalara Massif can become infected with *Bd*, only 3 species have been reported to experience mortality due to chytridiomycosis (Bosch *et al.*, 2001; Bosch & Martínez-Solano, 2006; Garner *et al.*, 2009). Of these, *A. obstetricans* is for all intents and purposes extirpated in the area, while the distribution of *B. spinosus* is restricted to a few, permanent ponds located in different drainages of the massif. Because we were interested in the spatiotemporally broad impacts of *Bd* on resident species, we focussed on the remaining species that occupies a broad distribution in the massif and was most likely to exhibit changes in abundance associated with chytridiomycosis, *S. salamandra*. To do this we began collecting tissue samples of metamorphosed *S. salamandra* starting in 2001 and assigned infection status used qPCR following Boyle *et al.* (2004). We used generalized least squares regression models (GLS), taking into account any potential temporal autocorrelation of data. We calculated prevalence of infection per year and modelled the impacts of these estimates on larval abundance from 1 to a maximum of 7 years after sampling for infection was completed. In so doing, we could compare the short- and

long-term downstream effects of infection in the potentially breeding population on larval abundance. We used larval counts from 2001 to 2016, so as a result the number of comparisons per time span ranged from a maximum of 16 for the one year period to 9 for the eight year time span. Predictors for GLS models were the temperature measurement from the best-fit ARIMA model listed in Table 2 and abundance of larval salamanders in the time span that most strongly correlated with larvae counts. GLS models were carried out using the *gls* command of the {nlme} package and temporal autocorrelation structure of data was taken into account using the corARMA argument.

Results

Abundance of all nine amphibian species fluctuated significantly (see Fig. 2), and all goodness of fit likelihood ratio tests did not support the null model of stable abundance (p <<0.001 in all cases). Abundance estimates for *I. alpestris*, *S. salamandra* and *T. marmoratus* were not serially autocorrelated (serial r <0.3, see Table 1), but medium values for the remaining species (r = 0.35-0.53) denoted that abundance in a particular year depended to some degree on abundance in the previous year. LINEAR models fit better than NULL models for all species except *E. calamita* and *P. perezi* (Table 1). *Alytes obstetricans*, *R. iberica* and *S. salamandra* populations all exhibited significant, negative linear trends, but only the latter two suffered significant average declines of -11.2% and -3.5%, respectively. Although *A. obstetricans* experienced a sharp and non-recovering population decrease after 1999 and experienced an average yearly decline of -12.9%, the overall additive change was not significant (Table 1, Fig. 2). Conversely *H. molleri*, *I. alpestris* and *T. marmoratus* populations exhibited significant and positive linear trends that were associated with

significant average increases in abundance (Table 1; Fig. 2). Abundance trends for the other 3 species did not exhibit significant patterns of change (Table 1).

Temperature effects were inconsistent across species but on average affected amphibian species population dynamics across a 4-6 year lag period (Table 2). Minimum temperature had strongest effects (largest weight sum ≥ 0.7) on three (*B. spinosus*, *R. iberica*, *T. marmoratus*) of the nine species while maximum temperature was influential for *E. calamita* population dynamics. All other trends were weaker by comparison (greatest weight sums < 0.7, see table 2). Despite this, ARIMA models indicated significant temperature effects for six species after sequential Bonferroni adjustment to control for type I error in the nine significance estimations. Four of these six significant effects supported a role for maximum temperature (Table 2). The magnitude effects attributable to temperature (R^2, after controlling for the serial autocorrelation), were relatively low for all species (in brackets after the name of the species). The larvae counts for *H. molleri* (partial $R^2 = 0.150$ with minimum temperature), *I. alpestris* (0.040, maximum temperature), *S. salamandra* (0.133, maximum temperature) and *T. marmoratus* (0.165, minimum temperature) significantly increased with average year month temperature during the first six or eight years. Conversely, the number of clutches of *E. calamita* (partial $R^2 = 0.096$) and larvae of *R. iberica* (0.134) decreased with increasing, respectively, maximum and minimum temperatures during the six years before the counts.

Simple cross-validation of the PCA produced a single significant component that accounted for 31.8% of the information content of the correlation matrix among the nine species (eigenvalue = 2.861). Four species were responsible of 87% of the informative content of this component [loadings for *R. iberica* (0.718), *H. molleri* (-0.896), *T. marmoratus* (-0.854) and *I. alpestris* (-0.676), where the threshold for factor loadings was established arbitrarily at +/-0.590, the correlation coefficient at P = 0.01 and n = 18]. Three of

these four species shared a relatively similar pattern of covariation of population increase from 1999 to 2016, while *R. iberica* exhibited the inverse pattern of population decline.

Annual sample sizes for *Bd* detection in fire salamanders averaged 85.5 animals pa. Counts of *S. salamandra* larvae were increasingly negatively correlated with *Bd* prevalence across all time spans (Pearson correlations: one year, r=-0.12, n = 16; two years, r=-0.19, n = 15; three years, r=-0.26, n = 14; four years, r=-0.36, n = 13; five years, r=-0.46, n = 12; six years, r=-0.48, n = 11; seven years, r=-0.49, n = 10; eight years, r=-0.07, n = 9). A generalized least squares model with autocorrelation structure was built to specifically analyse the variation in salamander larvae counts from 2007 to 2016 (n = 10 years), using the *Bd* prevalence values corresponding to 7 years beforehand, and the average maximum temperature of the hottest month during the time span of 8 years (see Table 2). The model was significant (likelihood ratio test, p = 0.012) and explained 50.1% of the yearly variation in larvae counts (autocorrelation, Φ = -0.738). Larval counts were positively associated with average maximum temperature of the hottest month (standardized partial regression coefficient, beta = 0.468, se = 0.088, p <<0.001), and negatively with *Bd* prevalence (beta = -0.172, se = 0.085, p = 0.042). Nearly identical results were obtained using the time span of six years for average *Bd* prevalence from 2006 to 2016 and the time span of eight years for maximum temperature (n = 11 years): a highly significant model (p = 0.0005), explaining 52.3% of the variance, a positive effect of maximum temperature (beta = 0.350, se = 0.055, p <<0.001) and a negative effect of *Bd* prevalence (beta = -0.3180, se = 0.059, p <<0.001) on counts of larvae. Figure 3 depicts the partial relationships between the two predictors and the response variable (partial residual plots).

Discussion

The Peñalara Amphibian Survey was established in response to the conservation threat chytridiomycosis was perceived to pose to *A. obstetricans*. This concern was validated at both local and regional scales, as the species is for all intents and purposes extirpated by disease-driven decline in Peñalara and collaborations spawned through the project have reported other high altitude declines of the common midwife caused by lethal chytridiomycosis in post-metamorphic cohorts (Walker *et al.*, 2010; Rosa *et al.*, 2013). This study adds further evidence of deleterious impacts of *Bd* in Peñalara, as chytridiomycosis appears to be reducing the abundance of *S. salamandra*. The historical impact of *Bd* on this species provides further evidence as to the reservoir role midwife tadpoles can play in spill-over transmission events (Clare *et al.*, 2016): the population trend line for *S. salamandra* suggests a sharp and rapid decline associated with the emergence of *Bd* caused by a year-on-year effect of high prevalence in larval *A. obstetricans* (Fig. 2). GLS models reveal additional, downstream impacts of prevalence of infection in fire salamanders that manifest a minimum of 6 years later. The contrasting effects of temperature (positive) and *Bd* (negative) on larval abundance (Fig. 3) suggests that warming temperature may mitigate the impacts of infection, either directly or indirectly, and if the warming trends reported at Cotos continue, climate change could conceivably release fire salamanders from the deleterious effects of chytridiomycosis. However GLS models examining the long term impacts of *Bd* and that controlled for the effect of warming temperature still showed that prevalence of infection explained the preponderance of change in larval abundance. Again referring to the trend line in Fig. 2, any evidence of short term recovery after the elimination of the reservoir appears to be followed by a pattern of persistent decline ongoing since 2011 (Fig. 2), which our models attribute to the delayed effects of infection on abundance.

Nevertheless, our findings support the conclusion the impact of *Bd* on the Peñalara Massif amphibian community as a whole is limited in scope and potentially declining. Only 3 of the 9 species in the area are in significant decline, and while two are *A. obstetricans* and *S. salamandra*, mortalities attributable to chytridiomycosis in the third, *R. iberica*, have never been detected in Peñalara despite the significant surveillance efforts. Transmission experiments have also shown that the species is at most weakly susceptible to infection (Fernández-Beaskoetxea *et al.*, 2016). These same experiments argue strongly for the role *A. obstetricans* plays as a reservoir from which infections are generated in Peñalara species other than fire salamanders, so the extirpation of midwives from the region should inevitably result in decreased impacts of chytridiomycosis in host species that do not sustain infections in the absence of this reservoir. In support of this, infection in 2005 was detected in more than 60% of occupied ponds (Walker *et al.*, 2007), while in 2016 infection was practically absent in amphibians occupying temporary water bodies and predominantly restricted to the few large and permanent ponds in the area (unpublished data).

In contrast, temperature has a more widespread, and when significant, predominantly beneficial influence on the abundance of amphibian species in Peñalara. Increasing temperatures affected counts of *H. molleri*, *T. marmoratus* and *I. alpestris*, all of which are increasing in abundance across the Peñalara Massif. Pearson correlations between the number of occupied water bodies and abundance estimates for these 3 species suggest that warming is causing demographic increases associated with expanded geographic ranges (*H. molleri*, *r* = 0.569; *T. marmoratus*, *r* = 0.673; *I. alpestris*, *r* = 0.432). For the former two, increasing minimum temperature was associated with demographic increases and expanded range, which likely indicates increasing pond availability through earlier thaws, and possibly increased time available for larval growth, development and post-metamorphic provisioning before overwintering. All of these metrics are known to improve survival of earlier life

history stages and recruitment to later stages for amphibians in general. Minimum temperature was not important for *I. alpestris*, which may not be surprising given that alpine newts are a common high altitude species with notable ability to modify life history strategies to meet the requirements of alpine environments (Miaud *et al.*, 2000). However increasing maximum temperature was associated with increased abundance, which may reflect a more specific impact on early life history. In Peñalara and other high altitude locations where the season for larval and post-metamorphic growth is short and cold, larval alpine newts commonly overwinter, a strategy that requires permanent water bodies. Increasing temperatures during the larval growth period should increase larval growth rate and speed more larvae through metamorphosis in the same year as hatching. If this is the case, water permanency is no longer obligatory for successful reproduction, an outcome that is supported by our detection of larval alpine newts in small, shallow streams and other impermanent water bodies.

Climate changes on the Peñalara Massif are acting to the detriment of only a single species, *R. iberica*. Up until 2006, counts of Iberian frog tadpoles were consistently greater than 700, after which counts fell below this and as low as a few dozen individuals. The correlation between abundance and occupancy was far weaker than for species benefitting from increasing temperature ($r = 0.276$) and none of the ponds where this species breeds appears to have become unsuited to Iberian frog reproduction. The lack of a geographic component to the decline of this species instead argues for a more direct effect of warming on recruitment. Work on another brown frog species, *Rana temporaria*, has illustrated how shortening of the overwintering period caused by an earlier onset of spring directly impacted on both survival and reproductive output of potentially breeding females (Reading, 1998). We may only speculate, but the negative relationship between larval abundance and average minimum temperature may indicate that a similar process is dictating Iberian frog abundance

in Peñalara. If this is the mechanism, though, it is striking that only one of nine amphibian species occupying the Massif is demographically responding to climate warming in this manner. Shifts in breeding phenology, post-overwintering condition and reproductive outputs in anuran and caudate amphibians associated with shorter, warmer winters have been reported for over 15 years and are invoked as a potential cause for amphibian population declines (Reading, 1998, 2007; Gibbs & Breisch, 2001; Beebee, 2002; Corn, 2003). Instead, with a single exception, the amphibian species community occupying Peñalara are not responding demographically to warming trends or are more abundant in warmer years.

What remains to be seen is if warming trends will continue and if the relationships between warming temperatures and abundance hold. The Community of Madrid, within which Peñalara occurs, has experienced ever-increasing annual temperature increases. We observed unprecedented rates and speeds of pond-drying and high rates of larval mortality during the summer of 2017 associated with a record-breaking heatwave that swept across Southern Europe. Ultimately, any benefits warming proffers to Peñalara amphibians will be counteracted if warming trends begin to remove breeding sites rather than make them more available to *H. molleri*, *T. marmoratus* and *I. alpestris*. We predict if drying manifests as a significant threat to the amphibian community of Peñalara it will be seen first in *E. calamita*, a species that specializes in breeding in highly ephemeral ponds and utilizes an average of 20 breeding locations in the park. Given that all species other than *A. obstetricans* uses ephemeral or impermanent water bodies to some extent, drying will have far broader impacts on the community.

Acknowledgments

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	Serial r	AICc		LINEAR MODEL			ADDITIVE CHANGE		
		NULL	LINEAR	slope	se	P	parameter	se	P
Alytes obstetricans	0.422	6648	4294	-0.274	0.074	<0.001	-0.129	0.077	0.095
Epidalea calamita	0.430	2876	2873	-0.006	0.010	0.530	-0.005	0.009	0.594
Bufo spinosus	0.400	225	202	-0.030	0.016	0.066	-0.034	0.019	0.065
Hyla molleri	0.532	46686	42758	0.069	0.022	0.002	0.077	0.017	<0.001
Ichthyosaura alpestris	0.188	22602	20323	0.051	0.016	0.001	0.051	0.018	0.005
Pelophylax perezi	0.350	23050	23053	0.000	0.012	0.988	-0.002	0.014	0.877
Rana iberica	0.363	9019	7461	-0.086	0.029	0.003	-0.112	0.032	0.001
Salamandra salamandra	0.270	59588	57128	-0.039	0.009	<0.001	-0.035	0.011	0.001
Triturus marmoratus	0.185	2583	2354	0.063	0.023	0.006	0.092	0.030	0.003

Table 1. Results of TRIM models analyzing count data generated from 29 sampling sectors (for details see the Methods section). Two different models were constructed for each species; a no time effects models (NULL; testing for the absence of population trends) and LINEAR trend (assuming an increasing or decreasing monotonic trend). Serial r: serial correlation measuring the association of the counts in year *t* with year *t*-1. The AIC figures are provided for each of these models, as is the slope of the LINEAR trend model, its standard error and significance (against Ho: slope = 0). The ADDITIVE parameter defines the overall additive change in abundance for each species (se = standard error and the significance of deviation from the null hypothesis of 0 –P–). Populations counts remained constant if the additive parameter equaled zero; an additive figure of -0.112 denotes that from the first to the last year of amphibian counts the population has decreased at an average inter-annual rate of 11.2% (-0.112*100). All significant tests remain significant after Bonferroni sequential correction.

	minimum	maximum	one	two	three	four	five	six	seven	eight	TEMP	sign	P
Alytes obstetricans	0.34	0.66	0.12	0.06	0.06	0.06	0.09	0.29	0.22	0.10	max-6	+	0.060
Epidalea calamita	0.22	0.78	0.11	0.05	0.06	0.09	0.19	0.35	0.10	0.06	max-6	-	0.003
Bufo spinosus	0.71	0.29	0.09	0.13	0.11	0.20	0.11	0.10	0.12	0.15	min-4	+	0.044
Hyla molleri	0.54	0.46	0.05	0.20	0.06	0.04	0.05	0.27	0.23	0.10	min-6	+	0.005
Ichthyosaura alpestris	0.41	0.59	0.09	0.08	0.11	0.10	0.12	0.25	0.13	0.12	max-6	+	0.000
Pelophylax perezi	0.57	0.43	0.08	0.29	0.14	0.13	0.09	0.09	0.09	0.09	min-2	+	0.070
Rana iberica	0.81	0.19	0.05	0.03	0.03	0.05	0.73	0.05	0.03	0.03	min-5	-	0.001
Salamandra salamandra	0.42	0.58	0.04	0.12	0.06	0.08	0.10	0.14	0.17	0.29	max-8	+	0.000
Triturus marmoratus	0.80	0.20	0.14	0.11	0.03	0.10	0.09	0.37	0.05	0.10	min-6	+	0.003

Table 2. Results of ARIMA models testing for the influence of temperature on yearly trends of amphibian counts in Peñalara. Temperature measurements include the average minimum temperatures of the coldest month (usually January or February depending on the year) and average maximum temperatures of the hottest month (usually on July or August), with time lags of one to eight years before the year when the amphibians were censused (i.e., 1, 2, 3, 4, 5, 6, 7 and 8 years before). The figures for minimum and maximum temperatures are the addition of the AICc weights of the eight ARIMA models built, respectively, with the minimum and maximum yearly temperatures. The figures for one, two, … eight years before the amphibian counts refer to the addition of the AICc weights of the two ARIMA built with the minimum and maximum temperatures for each time span. TEMP: the model of highest evidence including a measure of temperature (i.e, lowest AICc and highest weight values). P and sign: significance and sign of the regression coefficient of temperature on the amphibian counts considering the corresponding time span. min-2, min-4, min-5 and min-6: average minimum temperature of the coldest month during the previous two, four, five and six winters before the year of the amphibian counts, respectively. max-6 and max-8: average maximum temperature of the hottest month during the previous six or eight years of the amphibian counts, respectively.

Figure 1. Location of the Peñalara Massif within Guadarrama National Park in the Iberian Peninsula.

Figure 2. Dynamics of abundance of nine Peñalara amphibian species estimated from 1999 to 2016. Time index 1 refers to the amphibian counts measured in 1999 (i.e., the baseline in first sampling year). Solid lines are annual abundance estimates, while the dashed lines were established by means of TRIM models using stepwise selection of change-points where changes in slope between consecutive periods of time attained significance. Yearly average temperatures from 1975 to 2016 and monthly average maximum temperatures of the hottest month (usually July or August of each year; black circles), and monthly average minimum temperatures of the coldest month (usually January or February of each year; open circles), from 1991 to 2016 are presented in the lowest panels.

Figure 3. Partial residual plot of the association between counts of *S. salamandra* larvae and the *Bd* prevalence and the average maximum temperature of the hottest month during the time span of 8 years in Peñalara (11 years from 2006 to 2016; p << 0.001 in both cases). Residual plots show the relationship between a given independent variable and the response given that the other independent variables are also in the model (generalized least squares model with autocorrelation structure), therefore partialling out their effects. The lines are the partial linear regression lines.

IBERIAN PENINSULA

100 Km

SEGOVIA

MADRID

GUADARRAMA NATIONAL PARK

10 Km

PEÑALARA MASSIF

1 Km

Laguna de los Pájaros (2170 m)

SEGOVIA

MADRID

Peñalara Peak (2428 m)

Laguna Grande (2017 m)

Alytes obstetricans • Salamandra salamandra • Bufo spinosus • Ichthyosaura alpestris • Hyla molleri • Triturus marmoratus • Epidalea calamita • Pelophylax perezi • Rana iberica