

Climate Change Alters Sexual Signaling in a Desert-Adapted Frog

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ABSTRACT: Climate change is altering species' habitats, phenology, and behavior. Although sexual behaviors impact population persistence and fitness, climate change's effects on sexual signals are understudied. Climate change can directly alter temperature-dependent sexual signals, cause changes in body size or condition that affect signal production, or alter the selective landscape of sexual signals. We tested whether temperature-dependent mating calls of Mexican spadefoot toads (*Spea multiplicata*) had changed in concert with climate in the southwestern United States across 22 years. We document increasing air temperatures, decreasing rainfall, and changing seasonal patterns of temperature and rainfall in the spadefoots' habitat. Despite increasing air temperatures, spadefoots' ephemeral breeding ponds have been getting colder at most elevations, and male calls have been slowing as a result. However, temperature-standardized call characters have become faster, and male condition has increased, possibly due to changes in the selective environment. Thus, climate change might generate rapid, complex changes in sexual signals with important evolutionary consequences.

Keywords: climate change, mate signal, sexual selection, acoustic communication, spadefoot toad, desert.

Introduction

Climate change is altering habitats for species globally, and many are expected to undergo rapid evolutionary change as a result (Parmesan 2006). Much of the work on evolutionary responses to climate change has focused on traits that directly mediate survival in changing temperature, including traits involved in thermal tolerance, migratory patterns, or resource acquisition and competition regimes (Dunn and Møller 2014; Geerts et al. 2015; Miller-Struttmann

et al. 2015; Lindberg and Collins 2020). Less attention has been given to how climate change affects the evolution of fitness-related traits that do not directly impact survival but are nevertheless subject to novel selective regimes under climate change (Wong and Candolin 2015; Candolin 2019; Pilakouta and Ålund 2021).

One such set of traits are those involved in sexual signaling. The nature and expression of sexual signals are shaped by the physical environment, and shifts in the environment generally cause concomitant evolutionary change in sexual signals (Andersson 1994; Bradbury and Vehrencamp 2011). Sexual signals may be affected by changing environmental conditions through at least three general mechanisms, all of which can be driven by climate change.

First, climate change could directly alter sexual signals (and their perception) that are temperature dependent (sensu Endler and Basolo 1998; Wong and Candolin 2015; Conrad et al. 2017; Candolin 2019; Larson et al. 2019; García-Roa et al. 2020; Coomes and Derryberry 2021). Indeed, in many insects and anurans, the production and perception of temporal characteristics of acoustic signals are temperature dependent: signals increase in rate or intensity with increasing temperature, and the perception of those signals adjusts in parallel (reviewed in Gerhardt and Huber 2002). Moreover, the seasonal timing of sexual signaling can be temperature dependent. Indeed, climate change has altered breeding phenology for many species (Forchhammer et al. 1998; Parmesan 2006; Dunn and Møller 2014; Benard 2015; Green 2017; Larson et al. 2019). Whether climate change is causing direct changes in sexual signals over time is largely untested (but see Conrad et al. 2017; Coomes and Derryberry 2021). However, signals are unlikely to simply “speed up” in response to increasing global temperature. Temperature is becoming more variable with climate change (Easterling et al. 2000; Rummukainen 2012; Paaijmans et al. 2013), so temperature

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changes at the local level should be important to the timing and expression of sexual signals.

Second, climate change can alter sexual signals indirectly by changing body size and condition, which in turn affect the expression of size- or condition-dependent sexual signals (Mathis 1990; Doucet and Montgomerie 2003; Bradbury and Vehrencamp 2011; García-Roa et al. 2020). Specifically, climate change has been associated with reduced body size and condition across many taxa (Reading 2007; Gardner et al. 2011, 2018; Heide-Jørgensen et al. 2011; Sheridan and Bickford 2011; Aubry et al. 2013; Caruso et al. 2014; McLean et al. 2018; Tseng et al. 2018). Traits that are proportional to size or condition might therefore change with decreasing size or condition (Ryan and Brenowitz 1985; Hagman and Forsman 2003; Bonduriansky 2007; Han et al. 2020). For example, in acoustic signals, body mass is typically negatively correlated with dominant frequency (i.e., pitch; Gillooly and Ophir 2010), and body condition is generally positively correlated with more exaggerated expression of temporal features such as call rate (Mappes et al. 1996; Scheuber et al. 2003; Humfeld 2013). If mass and condition decline under climate change, dominant frequency of signals could increase and temporal features could “slow down” concomitantly. Yet although condition typically decreases in response to warming temperatures, these effects can be highly variable even for populations within species (McLean et al. 2018). As such, the effects of climate change on condition-dependent sexual signals might not be simple and need to be evaluated.

Third, climate change can alter patterns of sexual selection and mate choice that, in turn, alter selective patterns on sexual signals (i.e., climate change can alter the fitness landscape for sexual signals; Møller 2004; Spottiswoode et al. 2006; García-Roa et al. 2020; Vasudeva et al. 2021). In particular, climate change might drive changes to the environment that also alter the costs and benefits of particular sexual traits (Evans and Gustafsson 2017), the population sex ratio (Janzen 1994; Petry et al. 2016), mating system (Vasudeva et al. 2021), or population density (McClelland et al. 2018) and competition for mates (Møller 2004; Spottiswoode and Saino 2010; Berec 2019). If climate change induces changes in female preferences for sexual signals (e.g., Silva et al. 2007; Jocson et al. 2019; see also Leith et al. 2021), this also could change selection on those signals, particularly if signal production and perception are affected differently by temperature. To date, research has largely focused on how climate-change-induced shifts in the timing of migration and breeding phenology affect the strength of sexual selection in songbirds (reviewed in Spottiswoode and Saino 2010). Relatively little attention has been paid to nonavian species (but see Janzen 1994; Høye et al. 2009; Monteiro et al. 2017) or the effects of climate change on sexual signals per se.

We examined changes in sexual signals over time in natural populations of spadefoot toads (*Spea multiplicata*) that could be attributable to both direct effects of climate change on sexual signal production and indirect effects of climate change on body size, condition, and breeding phenology. To do so, we compiled local climate records of temperature and precipitation and combined them with long-term data on breeding phenology and measures of mating calls spanning a 22-year period.

Spea multiplicata are small, desert-adapted frogs native to Mexico and the southwestern United States. They spend most of the year estivating belowground and emerge in the summer months only after monsoonal storms fill the ephemeral ponds in which they breed (Bragg 1965). Breeding takes place on a single night after a pond fills (Bragg 1965). To attract females, males call continuously throughout the evening or until they are mated (males and females mate once). Males produce simple pulsatile calls that can be characterized by dominant frequency (i.e., the frequency with the greatest power), which is often negatively correlated with size in anurans (Gerhardt and Huber 2002), and by the temporal features of pulse rate, call duration, and call rate (fig. 1).

These temporal characters are strongly impacted by temperature in anurans (Gayou 1984; Prestwich 1994), because as ectotherms, their basal metabolic rate during signal production is partly determined by their body temperature. (Whether spadefoot signal production is affected by temperatures experienced earlier in life—e.g., developmental or estivation temperatures—is unknown; however, there is strong support from work in other anurans that ambient temperatures have immediate effects on acoustic signal production.) Because spadefoots breed on a single night immediately after ponds rapidly fill with floodwater, males within each breeding aggregation experience the same temperature. Nevertheless, temperature varies across aggregations, and as with other anurans, spadefoot call duration decreases with increasing temperature, whereas pulse rate and call rate increase with increasing temperature (Pfennig 2000; Calabrese and Pfennig 2021b).

Although sensitive to temperature, temporal aspects of anuran male calls are important in mate choice (Gerhardt 1991, 1994, 1998) and exhibit variation independent of temperature. Call rate, pulse rate, and call duration can each indicate a male's species identity, size, condition, or ability to confer fitness benefits to females or their offspring (Gerhardt 1994; Welch et al. 1998; Forsman and Hagman 2006; Ziegler et al. 2016) and are used by female anurans in mate selection (Gerhardt 1994, 1998). In *S. multiplicata*, males with faster call rates or higher call effort (call rate $\times$ call duration) have higher fertilization success (Pfennig 2000) and better-performing offspring (Pfennig 2008; Kelly et al. 2019, 2021) than males with

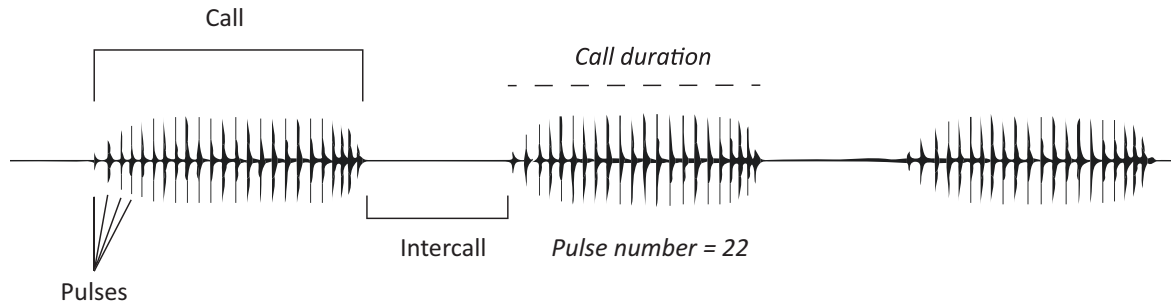


Figure 1: Temporal characters of *Spea multiplicata* calls. A waveform shows a series of three calls (made up of pulses) separated by silent intercalls. Call duration (s) and pulse number are measured directly; pulse rate = pulse number/call duration; call rate = (number of calls in series/duration of series) $\times$ 60. All else held equal, call rate and pulse rate are positively correlated (because squeezing pulses closer together allows more calls per unit time), while call rate and duration are negatively correlated (because shortening duration by increasing pulse rate or decreasing pulse number allows more calls per unit time).

slower call rates and lower call efforts calling at the same temperature, depending on population. Sexual selection on male calls within a breeding event is likely temperature independent, as females are choosing among males calling at the same ambient temperature and females themselves are experiencing the same ambient temperature as males. Moreover, male call rate can be indicative of species identity and thereby enable females to identify conspecifics in populations where congeners, *Spea bombifrons*, are present (Pfennig 2000; O'Brien 2017). Critically, females use call rate to choose mates, though the nature of their preferences for call rate can vary depending on the presence of *S. bombifrons* (Pfennig 2000; Pfennig and Rice 2014; Calabrese and Pfennig 2021b). Changes in call features over time that are independent of ambient temperature changes could be climate related but by a different mechanism than the effect of ambient temperature on signal production (e.g., if climate change is altering male condition, condition-dependent call features may change in response), or such trends could be unrelated to climate (e.g., a response to sexual selection that is itself unrelated to climate).

As desert-dwelling frogs, *S. multiplicata*'s sexual signals are potentially impacted by climate change, which is driving desertification of *S. multiplicata*'s habitat in the southwestern United States (Cayan et al. 2010; Seager and Vecchi 2010; Petrie et al. 2014). We sought to determine how climate change might be impacting these different features of male calls, especially call rate given its known significance in this system (Pfennig 2000, 2008). On the basis of regional climate trends, we expected to find increasing daily temperatures during the breeding season across years and, concomitantly, an increase in breeding pond temperatures across time. We predicted that as temperatures increase, call rate and pulse rate should also increase, whereas call duration should de-

crease (fig. 1). We further expected a decline in precipitation across years and therefore predicted that body size or condition should also decrease over time because drier conditions limit development time (Pfennig 1992; Pfennig and Simovich 2002), reduce resource availability, and increase the amount of time estivating versus acquiring resources. We further predicted that if we observed declining condition or body size, we would observe correlated changes in aspects of male calls separate from those associated with temperature. We found clear climatic changes over time, increasing condition, and concomitant changes in both temperature-dependent and temperature-independent features of male sexual signals. However, the relationship between changing temperature and male sexual signals was more complex than expected, pointing to the need for greater attention to climate change's impacts on mating behaviors.

Methods

Climate Data

We acquired temperature and precipitation data from the Portal, Arizona, weather station (31.8834°, -109.2056°) from the National Oceanic and Atmospheric Administration's National Centers for Environmental Information (<https://www.ncdc.noaa.gov/cdo-web/>). The Portal weather station is within 40 km of all spadefoot breeding sites in the study (mean distance: 16.4 ± 6.1 km) at an elevation of 1,643 m. Although it occurs at higher elevation than any of our breeding sites (range: 1,132–1,513 m), it is the only station in the area with data spanning the study period.

Because toads breed during the summer months, we obtained data for each day from June 1 to August 15 for each year in the period spanning 1996–2018. We acquired

daily minimum temperature ($T_{\min}$; because *S. multiplicata* are nocturnal and breed at night), daily maximum temperature ($T_{\max}$), and daily precipitation. We excluded five observations each from $T_{\min}$ (1,675 observations remaining) and $T_{\max}$ (1,664 observations remaining) because data flags indicated that they failed consistency or quality checks, but all precipitation data passed such checks.

Male Calls

All animal procedures were approved by the institutional animal care and use committee at the University of North Carolina at Chapel Hill (current protocol 17-073.0-C). We recorded 526 male mating calls at 37 breeding aggregations between 1996 (the year field recording began) and 2018 (the last year field recordings were available at the onset of the study) in the foothills of the Chiricahua Mountains and surrounding San Simon Valley in Arizona and New Mexico (geographic center of study: 31.889235°, -109.102958°). A breeding aggregation is a unique population (i.e., breeding pond) $\times$ year combination; overall, there were 22 populations included in the study. Aggregations were sampled opportunistically because timing of breeding depends on local weather events and is unpredictable, and not every population breeds every year. Water temperature of the breeding pond was measured at each aggregation. Field sampling methods are further described in “Supplemental Methods” in the supplemental PDF. We measured mass and snout-vent length (SVL) and took tissue samples from those calling males that we were able to capture. Mass and SVL were subsequently combined using the scaled mass index to provide a measure of male condition (Peig and Green 2009, 2010; MacCracken and Stebbings 2012); see statistical analyses below. Recorded calls were measured for call rate, pulse rate, and call duration using Audacity version 2.1.3 and for frequency using Raven Pro version 1.5 (see “Supplemental Methods”). Male calls from this data set were used for different analyses in a separate study (Calabrese and Pfennig 2021b), and the data set is available in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.gtht76hmj>; Calabrese and Pfennig 2021a).

Hybridization between *S. multiplicata* and *S. bombifrons* potentially occurs in populations where both species are found, and hybrid male calls are intermediate between the two species (Pfennig 2000). We therefore excluded hybrid males from our analyses. In particular, early-generation hybrids can be identified by morphology or their calls (Simovich and Sassaman 1986; Pfennig 2000). Additionally, we genotyped those males for which we had tissue samples to identify and exclude advanced-generation hybrids from the study (Pfennig et al. 2012; see “Supplemental Methods”).

Statistical Approach

We performed all of the following analyses in R version 3.6.1 (R Core Team 2019). We first characterized climate patterns using weather station data. To evaluate changes in temperature and rainfall over time, we used generalized additive models (GAMs; package *mgcv*; Wood 2011) to model patterns of climatic data across the breeding season (intraspecific variation, from June 1 to August 15) and across years (interannual variation, spanning 1996–2018). Our analysis of intraseasonal variation was intended as an exploratory description of spadefoot breeding phenology, whereas our analyses of interannual variation in temperature and rainfall tested specific predictions (see the introduction).

For daily temperature data, we used linear models within the GAMs, with day of year and year of observation included as fixed effects. To account for temporal autocorrelation, we fitted autoregressive models to all GAMs using the *corARMA* argument to estimate the correlated error term. We fitted models with correlated error structures up to the third order and then used the highest-order model that significantly improved fit over the previous order. We assessed fit of the error terms with generalized likelihood ratio tests.

Because the daily precipitation data were zero inflated, we used hurdle models to separately model the probability of daily precipitation (following a binomial distribution) and the amount of precipitation (following a gamma distribution fit with package *fitdistrplus*; Delignette-Muller and Dutang 2015) on those days that received rainfall. We included day of year and year of observation as fixed effects in these models. To account for temporal autocorrelation, we fitted autoregressive models with correlated error structures as described above. Because likelihood ratio tests are not supported for binomial or gamma-distributed GAMs in *mgcv*, we determined the fit of error terms by comparing Akaike information criterion (AIC) scores (criteria of best fit: $\Delta\text{AIC} > 2$).

Smoothed terms estimated by the GAMs were assessed for statistical significance (i.e., whether they deviated significantly from zero) using a modified Wald test (Wood 2013). We report results from fitting all GAMs using restricted maximum likelihood to properly estimate correlation parameters. We fitted the same set of models using maximum likelihood, and the results did not differ qualitatively from the former method.

Having evaluated changes in precipitation and air temperature over time, we next tested whether day of year of breeding or temperature of breeding ponds changed over time. We used linear models (stats package; R Core Team 2019) in which each unique breeding aggregation was the unit of observation, and fixed effects included year

of observation and elevation. Significance of these linear models was assessed using *t*-tests.

We then tested whether temperature-dependent call characters changed over years in the study. Our specific predictions for how call characters would change with increasing temperatures are given in the introduction, and these were made a priori of our climate data analysis. More generally, we described changes in call characters over time and then evaluated the concordance between the observed climatic changes and call character changes in the context of the known relationship between ambient temperature and call features in spadefoots and other anurans (see the introduction).

Because call characters vary for reasons other than the direct effects of temperature (e.g., changes in body size or condition or in response to selection), we also evaluated whether call characters have changed after accounting for the water temperature at which the animals were recorded. Because temporal features of anuran calls are sensitive to temperature, a standard approach in the field of anuran communication is to standardize male calls to a similar temperature (typically via a simple linear regression of each call feature on the ambient temperature at which it was recorded). Although this approach assumes the same relationship across populations and across time, doing so enables one to better compare calls produced at multiple ambient temperatures and examine the factors—aside from temperature—that might generate variation in calls (Littlejohn 1965; Pfennig 2000; Smith and Hunter 2005; Holloway et al. 2006; Lemmon 2009; Reichert and Gerhardt 2012). To standardize our calls, we regressed each call character for individual males on water temperature of the breeding aggregation in which the call was recorded (males within a pond experience the same temperature). For relationships that were significant, we obtained temperature-standardized call characters by adding the residuals of the regression to the predicted value of the call character at 20.4°C for each male (consistent with past studies; Pfennig 2000; Calabrese and Pfennig 2021b).

We modeled changes in raw and temperature-corrected call characters using linear mixed effects models, with the individual male as the unit of observation. Year and elevation were mean centered and scaled to 1 SD before model fitting. Call characters were modeled as the response variable, with population included as a random intercept, and year, elevation, and the year $\times$ elevation interaction included as fixed effects (package lme4; Bates et al. 2015). As a complementary approach to temperature standardization, we also modeled raw call rates as above but included pond temperature as an additional fixed effect. In all cases, results of these models agreed with temperature-corrected call rate models; therefore, we report only the temperature-corrected model results. Where

year $\times$ elevation interactions were neither significant nor marginally significant, they were dropped from models. Significance of predictor variables in all linear mixed effects models was evaluated using *t*-tests via the Kenward-Roger method for calculating denominator degrees of freedom with the lmerTest package (Kuznetsova et al. 2017). As a complementary approach to the *t*-tests, we used bootstrapping with 10,000 simulations to estimate *P* values for all fixed effects and interactions in our models (package pbkrtest; Halekoh and Højsgaard 2014).

Finally, we examined whether male body length (SVL) or condition has changed over time. We used the package smatr to calculate scaled mass index (Warton et al. 2012) as our metric of condition. We then fitted linear mixed effects models with either male SVL or condition as the response variable, population as a random intercept, and year, elevation, and their interaction as fixed effects. We also examined condition dependence of call characters by regressing call characters on scaled mass index, with population as a random intercept. We assessed significance of predictors as described in the previous paragraph. We used the following packages in plotting: ggplot2 (Wickham 2016), ggpubr (Kassambara 2019), emmeans (Lenth 2021), and gtools (Warnes et al. 2021).

Results

Climatic Patterns and Breeding Phenology

Within years, we found patterns of summer temperatures and rainfall as expected for this region of the southwestern United States. Specifically, daily minimum temperatures ($T_{\min}$'s; typically a nighttime temperature) at the Portal, Arizona, weather station increased through early summer, peaking around day 200 (July 19 in a nonleap year) before decreasing (fig. 2a; table 1). Similarly, daily maximum temperatures ($T_{\max}$'s; typically a daytime temperature) also increased, though they peaked earlier in the season than $T_{\min}$ (around day 175, June 24 in a nonleap year; fig. 2d; table 1). In terms of rainfall, the probability of daily precipitation peaked around day 208 (July 27 in a nonleap year; table 1; fig. S1a). For days that received precipitation, the amount of precipitation decreased as the season progressed, reaching its lowest amount around day 210 (July 29 in a nonleap year) before increasing again (fig. 2g; table 1).

The $T_{\min}$ and $T_{\max}$ have significantly increased across years (fig. 2b, 2e; table 1). Moreover, the seasonal pattern of temperature has shifted over time (fig. 2c, 2f; table 1). In particular, whereas the peak of $T_{\min}$ is occurring later in the year than in the past, $T_{\max}$ is peaking earlier in the season than in the past (fig. 2c, 2f). Rainfall has also shifted across years in both amount and pattern of occurrence.

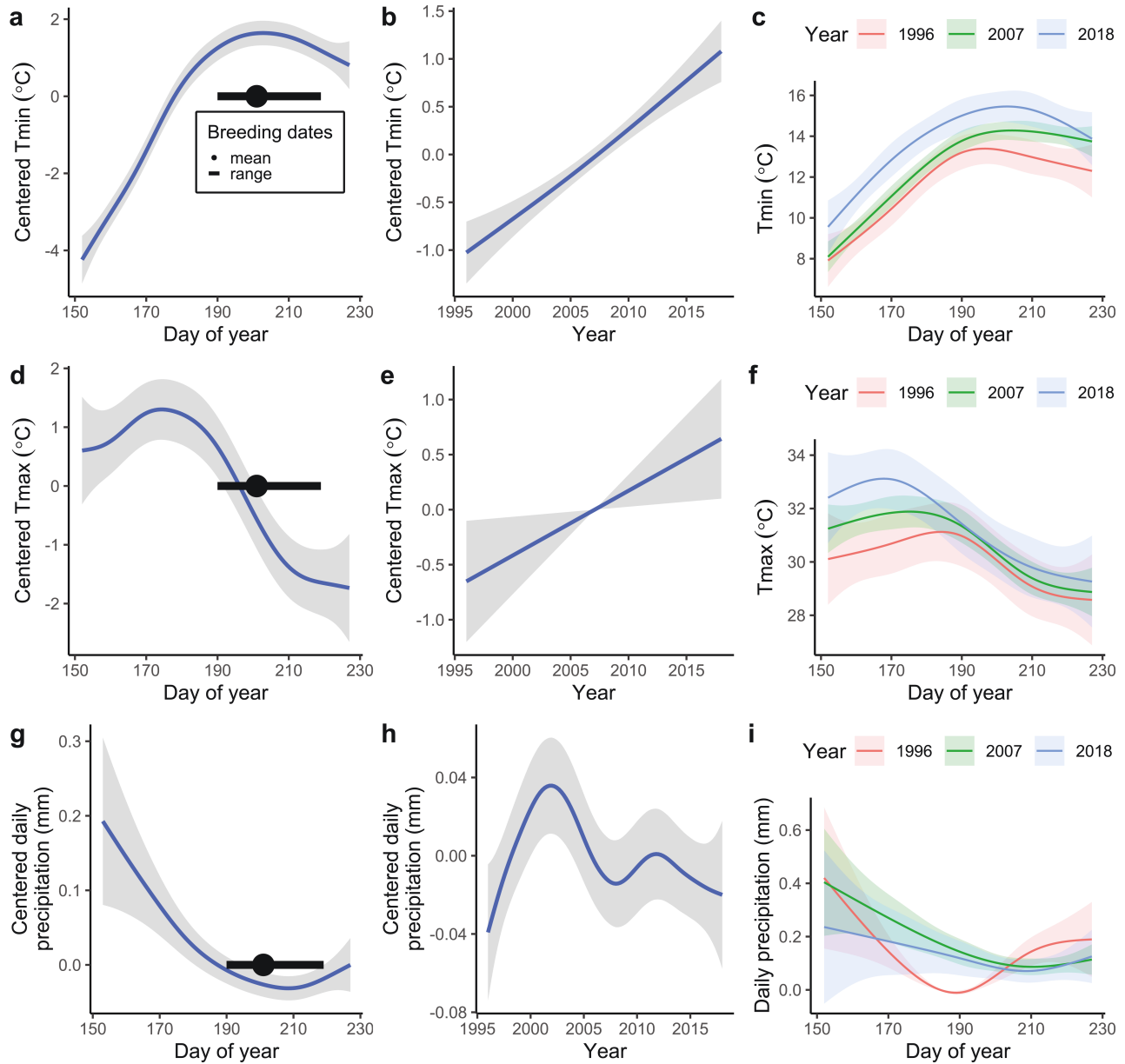


Figure 2: Temperature and precipitation within and across years in southeastern Arizona. Generalized additive model estimates of daily minimum temperature (T_{min} ; a–c), daily maximum temperature (T_{max} ; d–f), and precipitation (g–i) at the Portal weather station. Panels a, d, and g show seasonal patterns; b, e, and h show interannual variation; and c, f, and i show interaction of seasonal and interannual variation. Lines show predicted values (table 1), with 95% confidence interval shading. Black lines in a, d, and g indicate date range of observed spadefoot breedings (point shows mean breeding date). Day 150 is May 30, and day 230 is August 18.

For days that had nonzero precipitation, the amount of precipitation decreased across years in the study period (fig. 2h; table 1), with the probability of daily precipitation peaking earlier in the summer season than in the past (table 1; fig. S1c). Moreover, although the probability of daily precipitation has not increased overall across years (table 1; fig. S1b), the seasonal peak of probability of daily pre-

cipitation is higher than in the past (fig. S1c). Generally, rainfall amounts tend to decrease as the breeding season progresses, reaching a minimum around day 210 (July 29 in a nonleap year) before rebounding (fig. 2i). This seasonal pattern has shifted across years (table 1): rainfall amounts reach a higher minimum (difference of 0.15 mm between 1996 and 2018) than in the past but do so later

Table 1: Seasonal and interannual changes in climate in the study area

Response variable, smoothed term	edf	F	P
$T_{\min}$:			
Day of year	4.52	105.10	$<2 \times 10^{-16}$
Year	1.24	45.98	3.87×10^{-12}
Day of year $\times$ year	11	48.91	$<2 \times 10^{-16}$
$T_{\max}$:			
Day of year	3.99	13.18	1.73×10^{-10}
Year	1	5.62	.0179
Day of year $\times$ year	7.11	8.30	5.25×10^{-10}
Daily probability of precipitation:			
Day of year	2.83	31.65	$<2 \times 10^{-16}$
Year	1	.004	.95
Day of year $\times$ year	6.05	15.32	$<2 \times 10^{-16}$
Daily precipitation amount:			
Day of year	2.83	6.98	2.1×10^{-4}
Year	4.45	2.35	.04
Day of year $\times$ year	13.25	26.55	$<2 \times 10^{-16}$

Note: Seasonal variation and interannual changes in climate are modeled by generalized additive models for each of four response variables: minimum daily temperature ($T_{\min}$; °C), maximum daily temperature ($T_{\max}$; °C), daily probability of precipitation, and amount of daily precipitation (mm) for days on which precipitation is nonzero. Results of modified Wald tests (F , P) for statistical significance of model parameters are given, as are the estimated degrees of freedom (edf) of the smoothed terms. Statistical significance indicates that the slope of the model parameter does not equal zero.

in the season (about 20 days later in 2018 than in 1996) and do not rebound as high as in the past (fig. 2i).

When we evaluated patterns of spadefoot breedings, we found that within years, observed breedings clustered around the peak $T_{\min}$, as opposed to the peak $T_{\max}$ (average day of year of breedings = 201 ± 7 ; fig. 2a, 2d). Moreover, breedings clustered around the time just before highest probability of precipitation (fig. S1a) and just before the amount of precipitation reached a minimum (fig. 2g). We expected that changes in temperature and rainfall over time might impact the timing of breeding, but we found no significant changes in the timing of breeding across years (table 2). Interestingly, although day and nighttime air temperatures increased across years, changes in water temperatures at the observed breeding aggregations de-

pended on elevation. Pond temperature generally decreased across years, but this effect was strongest at low and middle elevations, and pond temperature was flat or slightly increasing across years at high elevation (table 2; fig. 3b).

Changes in Male Call Features across Years

Call rate, pulse rate, and call duration were all dependent on breeding pond temperature (table 3) in the expected direction (call rate and pulse rate increased with temperature, while call duration decreased with temperature). When we evaluated whether these characters changed over time, we found that raw call rates (i.e., those that were not temperature corrected) did not increase over time as would be expected under a warming climate (table 4). Instead,

Table 2: Changes in breeding pond temperature and breeding date across years

Model, parameter	Estimate	SE	t	df	P
Temp _{POND} $\sim$ year $\times$ elevation:					
Year	-.942	.290	-3.253	33	2.64×10^{-3}
Elevation	-1.313	.336	-3.914	33	4.29×10^{-4}
Year $\times$ elevation	.869	.378	2.299	33	.028
Breeding date $\sim$ year:					
Year	-1.764	1.089	-1.62	33	.115

Note: Linear models of changes in pond water temperature (°C) and breeding date (day of year) across years for the 37 unique breeding aggregations observed during the study period. For the model of breeding date, two aggregations were dropped because breeding day of year was missing. Year and elevation were mean centered and scaled to 1 SD before model fitting. Estimates $\pm$ SEs of the slope of parameters are given, as are results of t -tests for significance of each parameter.

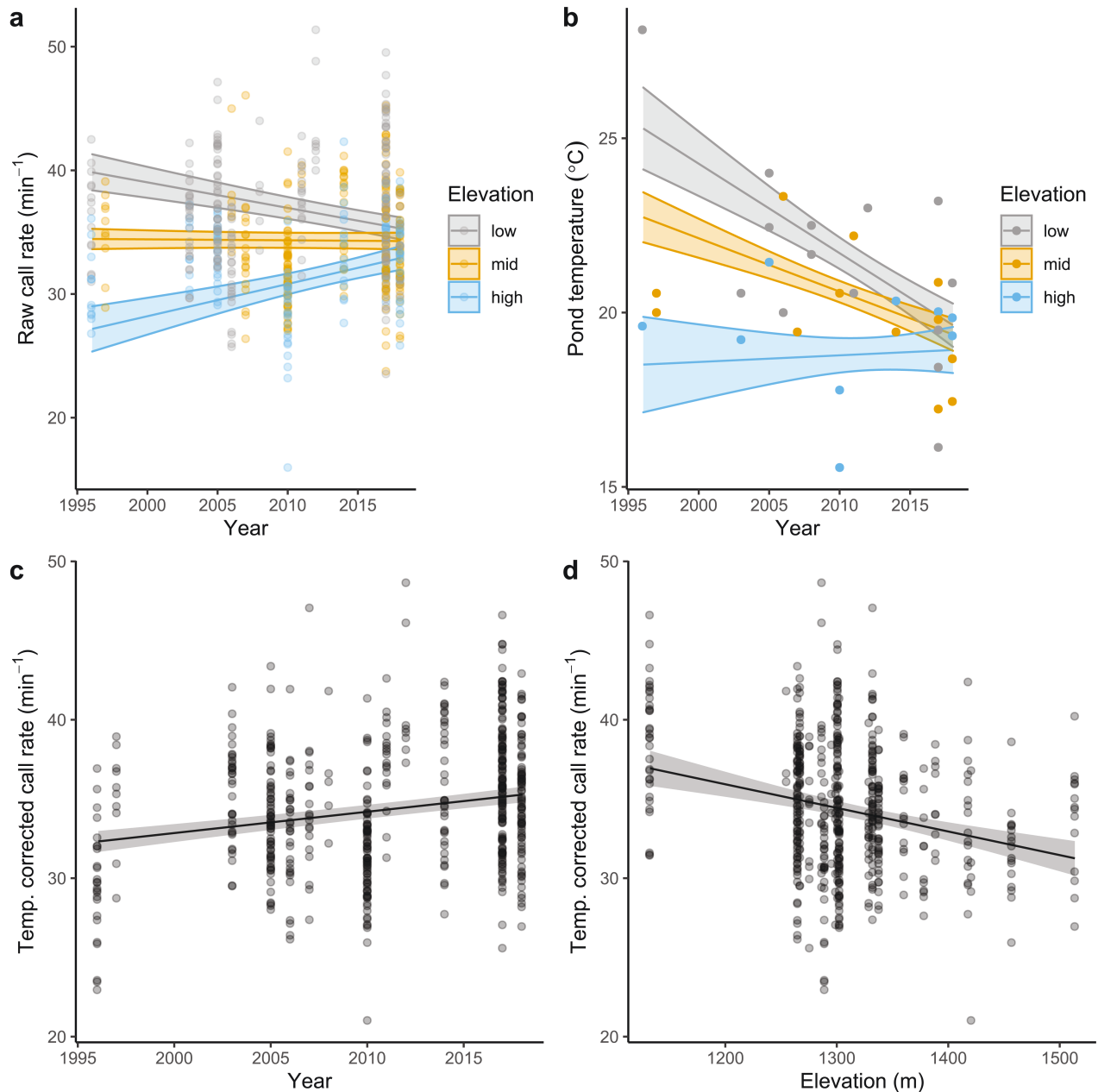


Figure 3: Call rate and breeding pond temperature over time and elevation. Raw call rates (a) and breeding pond temperatures (b) across years as a marginal interaction plot with three levels of elevation (corresponding to mean elevation of each tercile): low is 1,250 m, mid is 1,312 m, and high is 1,396 m (see also tables 2, 4). Call rate corrected to a common breeding pond temperature of 20.4°C over time (c) and across elevations (d). Lines show predicted slopes ± 1 SE shading; points are individual call rates (a, c, d) or breeding pond temperatures (b), colored by elevational tercile (a, b).

they decreased across years at low elevations and increased across years at high elevations (table 4; fig. 3a), similar to patterns in pond temperature (table 4; fig. 3b).

As with call rate, we found that raw pulse rate and raw call duration also changed over time (table 4). Pulse rate decreased across years and elevation, with the year effect

being strongest at lower elevations (significant year $\times$ elevation interaction; table 4). Call duration increased across years and elevation (table 4), again with stronger year effects at lower elevations (significant year $\times$ elevation interaction; table 4). These patterns are consistent with our results for call rate, as decreasing pulse rate

Table 3: Temperature dependence of call characters

Call character	Slope	SE	<i>t</i>	df	<i>P</i>
Call rate	1.04	.08	12.35	524	$<2 \times 10^{-16}$
Pulse rate	.65	.03	18.89	516	$<2 \times 10^{-16}$
Call duration	-.03	.003	-11.12	517	$<2 \times 10^{-16}$
Dominant frequency	.38	1.99	.19	516	.85

Note: All models are in the form of call character ~ temperature, where temperature is the breeding pond temperature (°C) at the time of call recording. Units are calls per minute (call rate), pulses per second (pulse rate), seconds (call duration), and Hertz (dominant frequency). Estimates $\pm$ SEs of the slope of temperature are given, as are results of *t*-tests for significance of the effect of temperature.

and increasing call duration are two mechanisms by which call rate may be reduced (fig. 1).

When call characters were corrected to a common temperature, we found that temperature-corrected call rates increased over time and were faster at lower elevations, but the effects of elevation and temperature were additive (table 4; fig. 3c, 3d). Temperature-corrected pulse rates

also increased over time, with a significant year $\times$ elevation interaction (table 4). Temperature-corrected call durations decreased across time and showed a marginally significant interaction between year and elevation (table 4).

By contrast with the temporal features of male calls, dominant frequency of calls was not temperature dependent

Table 4: Changes in breeding male call features across time and elevation

Response variable, parameter	Slope	SE	<i>t</i>	ddf	<i>P</i>	Bootstrapped <i>P</i>
Call rate _{RAW} :						
Year	-.12	.24	-.51	356	.609	.006
Elevation	-2.81	.58	-4.82	22	8.44×10^{-5}	9.99×10^{-5}
Year $\times$ elevation	1.47	.46	3.17	72	.002	.002
Pulse rate _{RAW} :						
Year	-.05	.10	-.50	381	.62	9.99×10^{-5}
Elevation	-1.63	.27	-6.01	21	5.31×10^{-6}	9.99×10^{-5}
Year $\times$ elevation	1.03	.20	5.06	86	2.36×10^{-6}	9.999×10^{-5}
Call duration _{RAW} :						
Year	.008	.007	1.127	282	.261	9.00×10^{-4}
Elevation	.073	.015	4.79	21	9.41×10^{-5}	1.00×10^{-4}
Year $\times$ elevation	-.048	.013	-3.71	55	4.83×10^{-4}	3.00×10^{-4}
Call rate _{TEMP COR} :						
Year	.87	.22	3.96	261	9.86×10^{-5}	3.00×10^{-4}
Elevation	-1.01	.38	-2.80	19	.012	.013
Pulse rate _{TEMP COR} :						
Year	.57	.08	6.75	213	1.35×10^{-10}	9.99×10^{-5}
Elevation	-.68	.15	-4.45	21	.0002	3.00×10^{-4}
Year $\times$ elevation	.37	.14	2.71	44	.01	9.60×10^{-3}
Call duration _{TEMP COR} :						
Year	-.018	.007	-2.56	205	.011	6.10×10^{-3}
Elevation	.032	.012	2.71	21	.013	.0265
Year $\times$ elevation	-.021	.011	-1.99	44	.053	.0662
Dominant frequency:						
Year	-2.73	5.47	-.50	170	.619	.653
Elevation	1.04	7.92	.13	18	.897	1.00

Note: All models are in the form of response variable ~ year $\times$ elevation + (1|population). Year and elevation were mean centered and scaled to 1 SD before model fitting. Interactions are included where significant or marginal. Units are calls per minute (call rate), pulses per second (pulse rate), seconds (call duration), and Hertz (dominant frequency). Estimates $\pm$ SEs of the slopes of parameters are given, as are results of *t*-tests for the significance of each parameter (using the Kenward-Roger method for calculating denominator degrees of freedom [ddf]) and parametric bootstrapped *P* values for each model parameter. RAW = temperature-dependent call features that have not been corrected to a common temperature; TEMP COR = temperature-dependent call features that are corrected to a common temperature of 20.4°C.

(table 3). Dominant frequency did not change across the study period or with elevation (table 4).

Male Size and Condition

When we evaluated male body size and condition over time, we found that male body size (SVL) did not change significantly over time or elevation (table 5). By contrast, male condition (measured as scaled mass index) did increase with year (table 5; fig. S2). However, none of the temperature-dependent call characters were predicted by individual males' condition (table 5) across the entire data set. Interestingly, although we found no change in dominant frequency over time, this call character did show a weak, marginally significant decline with increasing male condition (table 5).

Discussion

By combining climate data with a long-term data set on male sexual behaviors, we evaluated the hypothesis that climate change alters the expression of sexual behaviors that are either directly or indirectly (via effects on body size or condition) impacted by temperature. Using spadefoot toads, which occur in the desert southwestern United States, we documented local effects of climate change and concordant changes in male calling behavior. As described below, the findings are significant in that they highlight the broad—and potentially complex—impacts of climate change on trait expression and evolution.

When we examined summer climate patterns in our study area, we found that both daily maximum and daily minimum (i.e., overnight low) air temperatures have been increasing across years (fig. 2*b*, 2*e*; table 1), as expected given climate change's broad impacts on the western United States (Diffenbaugh et al. 2008; Cayan et al. 2010; Seager and Vecchi 2010; Gonzalez et al. 2018). Contrary to expectation, however, water temperatures of the pools in which the toads breed have actually declined across years at low and middle elevations and increased only at high elevation. This is surprising because breeding aggregations occur at night during the summer, when daily minimum temperatures reach their peak (fig. 2*a*). We had therefore expected changes in breeding pond temperatures to parallel increasing daily minimum air temperatures across years (see fig. 2*b*).

This surprising result that breeding pond temperatures are declining at low and middle elevations could be explained by changing rainfall temperatures. Ponds are freshly filled with rainwater at the time of breeding (recall that *Spea multiplicata* breed on the night that ponds are filled by monsoonal rains). Rainfall temperature is determined by a complex combination of climatic factors including rain intensity and cloud height (Gosnell et al. 1995), which could be changing with global warming. However, the upper limit of rainfall temperature is set by the wet-bulb temperature (Gosnell et al. 1995), which is itself determined by both air temperature and relative humidity (Bohren and Albrecht 1998). At 100% relative humidity, the wet-bulb temperature is equal to the air temperature, but as relative

Table 5: Male size and condition over time and condition dependence of call characters

Response variable, parameter	Slope	SE	<i>t</i>	ddf	<i>P</i>	Bootstrapped <i>P</i>
Snout-vent length:						
Year	5.72×10^{-4}	.247	.002	110	.998	1.00
Elevation	.271	.529	.513	12	.618	.709
Condition:						
Year	.478	.157	3.05	191	.003	.016
Elevation	−.313	.319	−.981	11	.348	.398
Call rate _{TEMP COR} :						
Condition	−.098	.135	−.723	242	.471	.473
Pulse rate _{TEMP COR} :						
Condition	−.006	.057	−.107	236	.915	.896
Call duration _{TEMP COR} :						
Condition	.007	.004	1.643	237	.102	.098
Dominant frequency:						
Condition	−7.362	4.094	−1.800	238	.073	.079

Note: All models are in the form of response variable ~ parameter(s) + (1|population). Year and elevation were mean centered and scaled to 1 SD before model fitting. Interactions are included where significant or marginal. Units are millimeters (snout-vent length), calls per minute (call rate), pulses per second (pulse rate), seconds (call duration), and Hertz (dominant frequency). Condition was measured by scaled mass index and is unitless. Estimates ± SEs of the slopes of parameters are given, as are results of *t*-tests for the significance of each parameter (using the Kenward-Roger method for calculating denominator degrees of freedom [ddf]) and parametric bootstrapped *P* values for each model parameter. TEMP COR = temperature-dependent call features that are corrected to a common temperature of 20.4°C.

humidity drops, the wet-bulb temperature (and thus the rainfall temperature) becomes colder than the air temperature (Bohren and Albrecht 1998).

Although wet-bulb temperature is a good approximation of rainfall temperature in natural systems (Flament and Sawyer 1995; Gosnell et al. 1995; Anderson et al. 1998), neither wet-bulb temperature nor relative humidity measurements were available for the Portal station or other nearby stations during the study period. So we were unable to evaluate whether rainfall temperature might be declining in this region. Although air temperatures have increased over time (fig. 2*b*, 2*e*), it is possible that relative humidity has decreased sufficiently to cause both wet-bulb and rainfall temperatures to decrease at times of breeding, resulting in the observed decrease in pond water temperatures. However, more research is needed to better understand how climate change is impacting these types of climate variables (Sherwood et al. 2010; Dunn et al. 2017; Vicente-Serrano et al. 2018) and the resulting impacts on the microhabitats that organisms experience (Kleynhans and Terblanche 2011; Baldauf et al. 2021).

The elevational and year interaction in breeding pond temperatures parallels the pattern we observed in raw, temperature-uncorrected call rate, pulse rate, and call duration over the study period (fig. 3*a*, 3*b*). In particular, we found that raw call rate and pulse rate decreased and call duration increased at low and middle elevations (which is the expected direction of change under cooling temperatures) and showed the reverse pattern at high elevation. These results contrast with our initial prediction that a warming climate should cause call rates to increase (with associated changes in pulse rate and call duration). Our study therefore highlights the possibility that global warming will not simply result in direct increases of traits that are positively correlated with temperature. Instead, changes in temperature of local microhabitat (in this case breeding pond temperatures) might be more relevant to trait expression patterns (Scheffers et al. 2014; Brahim and Marshall 2020; González-del-Piiego et al. 2020).

In contrast to our results for the raw call characters, we found that temperature-corrected call rate and pulse rate increased and temperature-corrected call duration decreased over time. Thus, males are calling faster now (with faster pulse rates and shorter calls) at a given breeding pond temperature than in the past, even though raw call rates and pulse rates have declined (and call duration increased) with decreasing breeding pond temperatures at low and middle elevations. Why males might be calling faster now than in the past for a given temperature is unclear. One possible explanation is evolutionary compensation (Grether 2005; Ghalambor et al. 2007, 2015; Swaegers et al. 2020). If decreasing water temperatures slow male calls but the optimum call rate set by selection

remains constant, males would need to evolve faster calls at a given temperature to meet that optimum. Consistent with this hypothesis, the interaction effect of year and elevation on raw call rates disappears for temperature-corrected call rates.

Evolutionary compensation could arise when populations with the same call rate optimum vary in displacement from that optimum owing to differential temperature effects across elevation. For local adaptation to occur, populations would thus have to evolve different levels of compensation for temperature to achieve a similar optimum call rate (e.g., a greater increase in call rate would be required to reach the optimum in a low-elevation population relative to a high-elevation population). This possibility requires further evaluation, but it emphasizes the complex way in which sexual signals (and other traits more generally) might respond to climate change.

Another explanation for changes in calls for a given temperature is that patterns of sexual selection could have been changing over time, especially if sexual selection is indirectly impacted by variation in the physical environment owing to climate change (Spottiswoode and Saino 2010; Evans and Gustafsson 2017; Berec 2019; Candolin 2019; García-Roa et al. 2020; Pilakouta and Ålund 2021; Vasudeva et al. 2021). For example, in *S. multiplicata*, a male's call rate predicts his tadpoles' ability to express a morphology (via phenotypic plasticity) that enables them to rapidly develop and escape drying ponds (Kelly et al. 2019). Tadpoles die if they fail to metamorphose before ponds dry, so selection strongly favors rapid development (Pfennig and Simovich 2002). Given that air temperature has increased and precipitation has decreased (fig. 2*e*, 2*h*, 2*i*), breeding pond longevity could also have declined over time. If so, offspring of faster-calling males might have been more likely to survive pond-drying events, with the result that call rates could have increased. Future work is needed to evaluate this possibility in our system and others, because sexual selection could be an important factor impacting how traits evolve in response to climate change (Møller 2004; Cockburn et al. 2008; Spottiswoode and Saino 2010).

Regardless of why males call faster for a given temperature, this pattern suggests that males invest more in sexual signaling than in the past. Such changes in investment in sexual signals could have long-term consequences for other aspects of male phenotype and life history (e.g., Given 1988; Lemckert and Shine 1993; Peters et al. 2004; Sieferman and Hill 2005). Indeed, although male body size did not change over time, we found that male condition increased over the study period (table 5; fig. S2), as might be expected if increased call rates for a given temperature require greater energy investment by males (Pfennig 2000, 2008). If males are investing greater energy into their calls, we would have predicted that male condition predicts

male calls, but we found no such relationship. However, establishing such relationships can be difficult, especially when the data are derived from uncontrolled observations in natural populations. In particular, calling frogs that produce more energetically demanding calls can lose weight more quickly than those that produce less energetically demanding calls (Taigen and Wells 1985). Thus, variation in the timing of our recordings relative to the length of time a given male is calling can obscure relationships, if any, between male condition and call characters. Additional work is therefore needed to better understand how changes in calling impact male investment in sexual signals and the downstream consequences for other aspects of male phenotypes. More generally, our results are consistent with changes in sexual signals concomitant with climate change, and changes in the costs and benefits of producing those signals. Much more work is needed to understand climate change's impacts on sexual signaling and the resulting effects on other aspects of the phenotype, life history evolution, and population dynamics (Wong and Candolin 2015; Candolin 2019; Pilakouta and Ålund 2021; Woods et al. 2021).

In sum, climate change is altering the physical environment in ways that can both directly impact production of sexual signals and change the selective landscape acting on those sexual signals and their associated traits. Our work highlights the potential for such relationships to be complex and underscores the need for additional work to better evaluate how climate change is altering trait expression and evolution of mating and reproductive traits. Understanding how climate change affects sexual signals is crucial because such signals are frequently environment dependent, and they determine mating patterns that affect adaptation and demography, which are important for species persistence in a changing world.

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Statement of Authorship

G.M.C. and K.S.P. developed methods, acquired funding, collected and validated data, and contributed to writing and editing the manuscript. K.S.P. provided equipment and other resources. G.M.C. conceptualized the study, analyzed and visualized the data, and wrote the original draft.

Data and Code Availability

Data are available in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.0k6djhb1z>; Calabrese and Pfennig 2022); associated code (<https://doi.org/10.5281/zenodo.5866212>) and supplemental files (<https://doi.org/10.5281/zenodo.6678397>) are hosted by Zenodo and are also linked from the Dryad Digital Repository.

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