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# Calling Phenology and Call Structure of Sympatric Treefrogs in Eastern Texas

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**Anurans often call in acoustically complex choruses, which can consist of multiple closely related species. Closely related sympatric species often differ in phenology and the acoustic properties of male advertisement calls, which, in some species, are phenotypically plastic. The tetraploid Eastern Gray Treefrog (*Hyla versicolor*) originates from multiple, separate hybridization events of the diploid Cope's Gray Treefrog (*Hyla chrysoscelis*) with now-extinct diploid anurans. The two species are visually indistinguishable, exhibit highly similar ecology and behavior, and co-occur frequently across their range. Males of these species can be distinguished from each other by the structure of their advertisement calls. Daily audio recordings and weather data were collected for five consecutive years from eight breeding sites of sympatric *H. versicolor* and *H. chrysoscelis*. We examined spatial and temporal patterns in calling phenology and responses of both species to environmental cues. Additionally, we measured three advertisement call properties to evaluate differences in call structure based on community composition. The phenologies of the two gray treefrog species were nearly identical, and the presence of the other species did not significantly affect call pulse rate or dominant frequency in either species. Chorus composition affected call duration in both species, but in *H. chrysoscelis,* this effect was temperature dependent. Our results indicate that while calling phenology does not contribute to reproductive isolation in sympatric gray treefrogs, modulation of phenotypically plastic call properties could affect male mating success and prezygotic reproductive isolation.**

ANURAN choruses are often dense and acoustically complex. A single chorus can consist of hundreds of individuals and commonly include multiple species. Chorus noise may affect the ability of female anurans to receive and interpret male advertisement calls (Schwartz et al., 2001; Marshall et al., 2006; reviewed in Bee, 2015), which can result in decreased fitness if females mate with heterospecifics or low quality conspecifics (Leary et al., 2008; Hettyey et al., 2014). In general, the spectral and temporal properties of calls may be masked by other sounds, absorbed by media through which the sound must travel, or otherwise degraded over distance (Forrest, 1994; Brumm and Slabbekoorn, 2005; Bee, 2007; Bee and Swanson, 2007). These effects may be exaggerated in high density, mixed species choruses (Wollerman and Wiley, 2002; Wong et al., 2009; Tanner and Bee, 2019); however, a number of mechanisms may mediate the costs of complex choruses. Chorus noise may be minimized by breeding at different times of year (Blair, 1961; Saenz et al., 2006; Heard et al., 2015) or using different breeding sites (Dayton and Fitzgerald, 2001). Within a species, male call timing is important to conspecific interactions, and males dynamically adjust the timing and properties of calls in competitive scenarios (Reichert and Gerhardt, 2013; Ward et al., 2013). Additionally, sympatric species often reduce the temporal overlap of calling (Obert, 1975; Oseen and Wassersug, 2002; Saenz et al., 2006; Heard et al., 2015; Neelon and Höbel, 2019), which may further decrease heterospecific chorus noise and alleviate the associated risks.

One aspect of breeding phenology that often varies among species is the response of anurans to particular cues, most notably rainfall and temperature. In many species, these weather variables affect both the stimulation and persistence of calling events (Obert, 1975; Dayton and Fitzgerald, 2001; Oseen and Wassersug, 2002). Additionally, different species may respond to different cues (Saenz et al., 2006). Even if the same environmental cue triggers a chorus response, species may have differential response times. This occurs on multiple time scales, resulting in some sympatric species calling at different times of year while others may vary only by a day or two in response time to cues, with much overlap in overall calling activity (Saenz et al., 2006).

One system that is well studied in the context of anuran calling behavior but not in comparative phenology is the gray treefrog species complex, *Hyla versicolor* (Eastern Gray Treefrog) and *H. chrysoscelis* (Cope's Gray Treefrog). The tetraploid *H. versicolor* originated from multiple, separate hybridization events between the diploid *H. chrysoscelis* with now-extinct diploid anurans (Ptacek et al., 1993; Holloway et al., 2006). The two species occur in both sympatry and allopatry across the eastern half of the United States of America and parts of southern Canada. Although they are visually indistinguishable, they can be distinguished by ploidy and by the pulse rate of male advertisement calls. Male *H. chrysoscelis* produce advertisement calls with a pulse rate twice that of calls produced by male *H. versicolor* (Gerhardt et al., 1994a), and females of both species prefer advertisement calls with conspecific pulse rates over those with heterospecific pulse rates (Gerhardt and Doherty, 1988; Bush et al., 2002; Schrode et al., 2012). In addition to these prezygotic isolating barriers between the species, postzygotic isolation plays a role in isolating the species as well. Mating between female *H. chrysoscelis* and male *H. versicolor* does not produce viable offspring, while mating between female *H.*

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*versicolor* and male *H. chrysoscelis* produces offspring that are sterile (Johnson, 1959; Bogart and Wasserman, 1972). Taken together, these results suggest that hybridization between gray treefrog species is costly, as natural hybrids between *H. chrysoscelis* and *H. versicolor* are not commonly observed (Gerhardt et al., 1994b).

Previous studies of this system have focused on comparisons between sympatric and allopatric populations of the two gray treefrog species (Ptacek, 1992; Gerhardt, 1994), whereas we aim to examine species interactions across sympatric populations. Specifically, we ask how different chorus environments among sympatric populations might influence calling phenology and advertisement call structure of both *Hyla chrysoscelis* and *H. versicolor*. To address this gap in our understanding of this system, this study examines: 1) how gray treefrog chorus composition varies spatially and temporally across calling sites in eastern Texas ponds; 2) whether the two species have similar calling phenology in response to environmental cues; and 3) if call duration, pulse rate, and dominant frequency of male advertisement calls differ when both gray treefrog species chorus together.

## MATERIALS AND METHODS

We collected ambient audio recordings every day from 1 January 2010 to 31 December 2014 at eight known gray treefrog breeding sites in eastern Texas (Fig. 1). Four of the breeding sites, designated ponds 01–04, were located in the Stephen F. Austin Experimental Forest (SFAEF) in Nacogdoches County, Texas, while the remaining four sites, designated ponds 05–08, were located in the Davy Crockett National Forest (DCNF) in Houston County, Texas. Additional details of these sites are described in Saenz et al. (2006), as the recordings examined in this study were made in continuation of that work.

***Audio recording.***—One SM1 Wildlife Acoustics Song Meter (Wildlife Acoustics, Maynard, MA) with dual-channel, nondirectional microphones was placed within 5 m of each pond, facing the center of the pond. Each song meter recorded a one-minute audio sample every hour, on the hour, from 2100 to 0200 CST, within which time gray treefrogs are known to call. We then analyzed each sample for the following variables: no detections of either *Hyla versicolor* or *H. chrysoscelis*, detections of only *H. versicolor*, detections of only *H. chrysoscelis*, or detections of both *H. versicolor* and *H. chrysoscelis*. Species were identified by pulse rate, which does not overlap between species (Jaslow and Vogt, 1977). The presence/absence of frogs of each species was used because the data collection methods did not allow us to determine the population/chorus size or proximity of individuals to each other.

***Environmental analyses.***—Abiotic variables measured at each site included air temperature (to 0.1°C); daylength (hrs); precipitation (to 0.01 cm) the day of a calling event; daily precipitation (to 0.01 cm) 1, 2, and 3 days prior to a calling event; cumulative precipitation (to 0.01 cm) 1–2 days prior to a calling event; and cumulative precipitation (to 0.01 cm) 1–3 days prior to a calling event. In addition to abiotic variables, the presence or absence of the other gray treefrog species was included in environmental analyses.

To determine relationships between each species' calling activity, measured as presence or absence of a given species, and all environmental cues, we used logistic regression with generalized estimating equations (see Schalk and Saenz, 2015). The subset of data used in these analyses included samples collected from 26 February through 29 September (the maximum recorded gray treefrog breeding season) for each of the five years. Ponds 07 and 08 were excluded due to low to absent populations. Two sets of models were created for each species: 1) effects of abiotic variables only and 2) effects of abiotic variables and presence of heterospecific calls. We used quasi-likelihood under the independence model criterion, adjusted for the number of parameters in

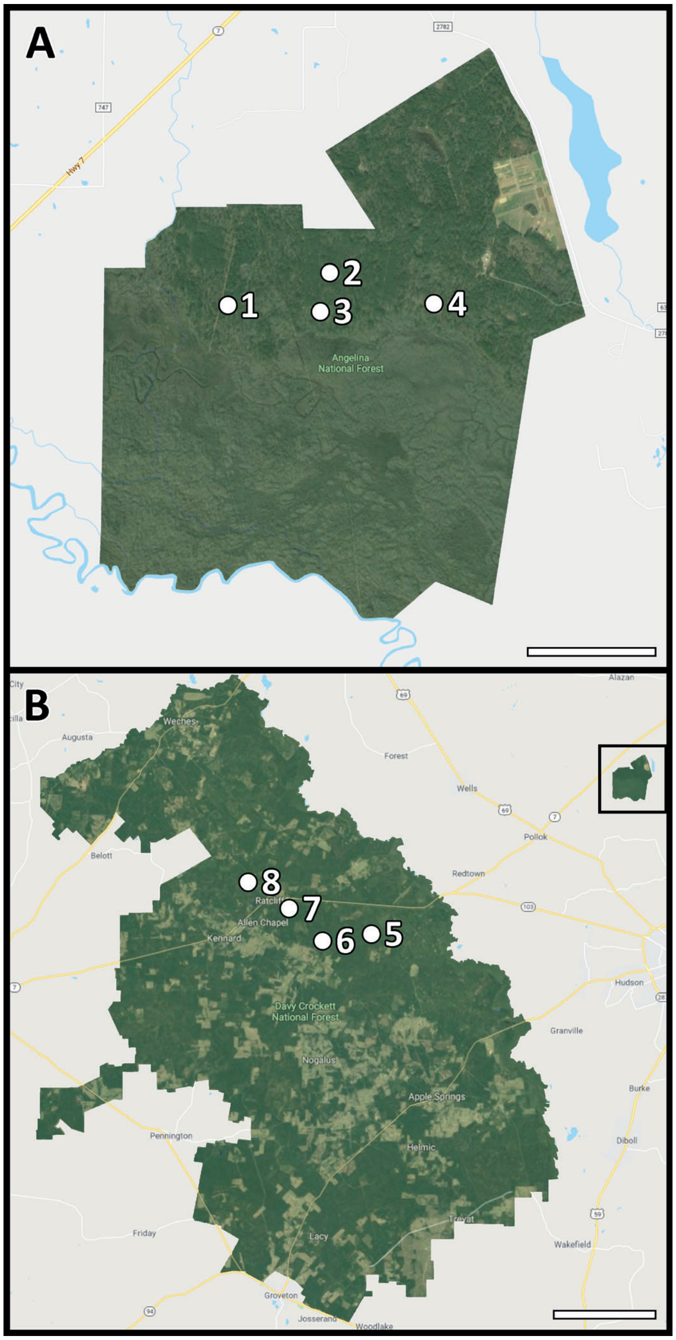

**Fig. 1.** Map of study sites in the Stephen F. Austin Experimental Forest (A) and Davy Crockett National Forest (B). The SFAEF is highlighted with a box in 1B showing relative size and distance compared to the DCNF. Scale bar is 1 km in 1A and 10 km in 1B.

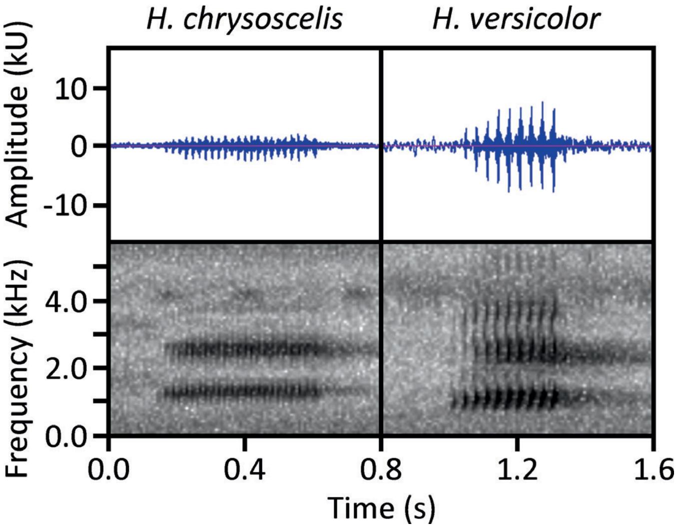

**Fig. 2.** Representative oscillograms and spectrograms of *Hyla chrysoscelis* and *H. versicolor* from this study.

the model (QICu) to compare models. We considered the model with the lowest QICu to be the best model (Pan, 2001). All statistical analyses were conducted in SAS 9.4 (SAS Institute, Inc., Cary, NC; https://www.jmp.com).

***Call analyses.***—The two species never called at more than six of the eight study ponds at one sample time. Thus, audio recordings used to measure call variables were limited to nights during which both species of gray treefrogs called simultaneously at five or six ponds. Additionally, to allow for comparisons between choruses of different species composition, each sample time had to include at least one pond each in which only *H. chrysoscelis*, only *H. versicolor*, and both species were detected. Based on these criteria, we sampled up to 3 calls/species from 109 recordings (Fig. 2). We then measured each call's duration (s) from the first pulse to the last, pulse rate (pulses/s), and dominant frequency (Hz). We selected these variables based on their potential importance in call propagation and female reception and assessment (Gerhardt, 1991). All call variables were measured in Raven Pro 1.5 (The Cornell Lab of Ornithology Bioacoustics Research Program, Ithaca, NY; https://www.birds.cornell.edu/raven).

We compared the call parameters of groups of frogs that had only *H. chrysoscelis* calling, only *H. versicolor* calling, and both species calling. When appropriate, ANOVA was used to compare between these groups. However, call variables may be influenced by abiotic variables. Temperature is known to affect the calls of *H. chrysoscelis* and *H. versicolor* in several ways. As temperature increases, the call duration of both species of gray treefrog decreases, while dominant frequency and call pulse rate increase (Gayou, 1984; Gerhardt, 2005). In consideration of these effects, we also used analysis of covariance (ANCOVA) to test for differences between the species, with temperature as the covariate. In analysis of call duration for *H. chrysoscelis*, assumptions of ANCOVA were not met. In this case, we compared the slopes from the regression of temperature and call duration when calling alone and together. To test whether slopes were significantly different, we used a full factorial model in JMP® Pro 10 (SAS Institute Inc., Cary, NC) to test for an interaction effect between temperature and calling condition (alone vs. together). Alpha was set at 0.05, and all analyses, unless stated otherwise, were conducted using SAS 9.4 (SAS Institute, Inc., Cary, NC; https://www.jmp.com).

## RESULTS

There was limited temporal variation between the two gray treefrog species at our study sites. Both species called at similar times of day and year. In each year, there was at least one pond where the first incidence of calling for both species was on the same night. Across all year/pond combinations, there were 19 instances of *H. versicolor* calling before *H. chrysoscelis*. When *H. versicolor* called before *H. chrysoscelis*, it was on average 23 days earlier. There were six instances of *H. chrysoscelis* calling at a pond before *H. versicolor*, and, on average, this was 16 days earlier. The date of last detection was more variable with the end of calling occurring on the same night for both species eight times, *H. versicolor* calling later in the year 12 times, and *H. chrysoscelis* calling later in the year nine times. Considering all ponds and all years, the calling season of *Hyla chrysoscelis* (mid-March to mid-August) was shorter than that of *H. versicolor* (early March to late September). Despite this, when only one species was found to be calling, it was more often *H. chrysoscelis* than *H. versicolor. Hyla chrysoscelis* was the only species detected in six year/pond combinations, whereas *H. versicolor* was detected calling alone for the whole season in only one pond (pond 4 in 2012).

The proportion of sample times in which *H. versicolor* and *H. chrysoscelis* were detected calling together varied both spatially, across ponds, and temporally, across years (Fig. 3). Eastern Texas experienced severe drought conditions during 2011, resulting in far fewer detections of both species in that year. The species exhibited some spatial variation, with a greater proportion of *H. versicolor* detected in ponds 01–04 in the SFAEF and a greater proportion of *H. chrysoscelis* detected in ponds 05–08 in the DCNF (Fig. 3). Recordings from ponds 01–04 included greater combined detections of gray treefrogs than those from ponds 05–08. Few gray treefrogs of either species were detected in ponds 07 and 08 throughout the study period (Fig. 3). Patterns of temporal change differed between ponds. Over the five-year period, detections of both species calling together decreased in ponds 01, 02, and 04, and increased in ponds 03 and 06. There was little change over time in pond 05. Some patterns in proportion of species calling together were seen between years. There was a decrease in detections of species calling together in all but pond 06 in 2012. In that year, only 6.8% of detections included calls of both species, with five of the eight ponds having no detections of both species calling together. In 2013, there was an increase in detections of *H. chrysoscelis* and *H. versicolor* calling together in all ponds with an average of 40% of all detections having calls of both species.

***Environmental analyses.***—Our analyses of the calling response of *H. chrysoscelis* and *H. versicolor* to only abiotic variables revealed that both species respond similarly to abiotic cues, and the model of best fit for both species was cumulative precipitation one, two, and three days prior to a calling event (Table 1A). Models that included conspecific calling produced similar results. The model of best fit in this set was detection of the other species, with precipitation the day of a

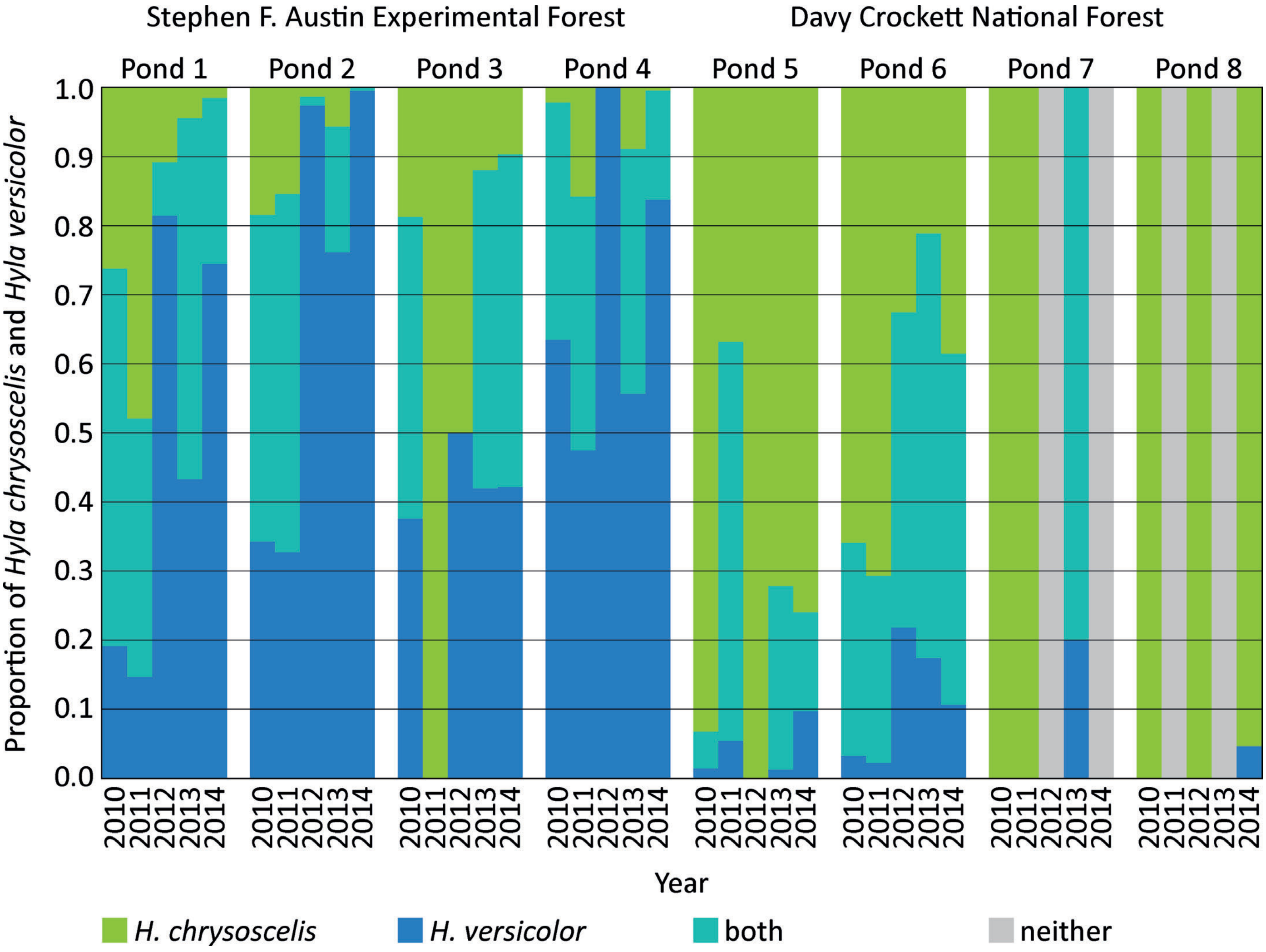

**Fig. 3.** Proportions of all gray treefrog (GTF) audio samples in which *Hyla chrysoscelis* (HC) and *H. versicolor* (HV) were detected.

calling event also of importance to *H. chrysoscelis* (Table 1B). Both species were roughly ten times more likely to call together than separately. These results indicate that the species call similarly in response to environmental cues and do not appear to avoid calling together.

***Call analyses.***—We most frequently detected both gray treefrog species calling together, and least frequently detected *Hyla chrysoscelis* calling in the absence of *H. versicolor*. Call pulse rate, dominant frequency, and call duration varied in how they were affected by abiotic factors and the presence of the other species (Table 2A). Temperature significantly affected pulse rate of both species, resulting in calls with greater pulse rates at higher temperatures. This result did not differ based on presence or absence of the other species. As expected, the pulse rates of the calls of *H. chrysoscelis* were roughly twice that of calls of *H. versicolor*. The presence of the other species did not significantly affect pulse rate in *H. chrysoscelis* or *H. versicolor* (Table 2B). The dominant frequency was not significantly affected by temperature in either species, and the presence of the other species did not significantly affect the dominant frequency (Table 2C).

Male *H. versicolor* produced calls with significantly longer duration in the presence of calling *H. chrysoscelis* than in their absence (Table 2B). Further, temperature significantly affected call duration in *H. versicolor* calling both with and without *H. chrysoscelis*, with shorter duration calls at higher temperatures. For *H. chrysoscelis*, the assumptions for ANCOVA were not met because temperature had different effects on the different groups. However, regression slopes of call duration and temperature were significantly different when calling alone versus together (interaction effect coefficient $= -0.0098$, $P = 0.027$), with a stronger effect of temperature on call duration when calling alone (Fig. 4).

## DISCUSSION

The extent to which *Hyla chrysoscelis* and *H. versicolor* overlap in calling has not been explicitly studied, and we offer the first evidence that both species respond similarly to environmental cues and suggest that environmental effects on calling may interact with community make-up (presence of the other species). How each species responds to environmental cues is an important factor in understanding the extent to which these species overlap in calling over the course of a breeding season, and it underlies the risk of costly mating choices in acoustically complex environments. This work builds on the extensive knowledge of calling in this

**Table 1.** Logistic regression results for the association of *Hyla chrysoscelis* (HC) and *H. versicolor* (HV) detections with temperature, daylength, and the model of best fit for (A) abiotic variables only; and temperature, daylength, and variables of significance in models including (B) both abiotic variables and presence of the other species.

| Model set | Species | Variable | Estimate[a] | SE[b] | 95% LCL[c] | 95% UCL[d] | Z | P |
|---|---|---|---|---|---|---|---|---|
| (A) Abiotic variables | HC | intercept | −24.71 | 5.34 | −35.17 | −14.25 | −4.63 | $<0.0001$ |
| | | temperature (°C) | −0.09 | 0.01 | −0.11 | −0.06 | −6.84 | $<0.0001$ |
| | | daylength (hrs) | 1.78 | 0.40 | 1.01 | 2.56 | 4.51 | $<0.0001$ |
| | | cumulprecip (cm)[g] | 0.12 | 0.03 | 0.06 | 0.19 | 3.60 | 0.0003 |
| | HV | intercept | −21.09 | 4.03 | −28.99 | −13.18 | −5.23 | $<0.0001$ |
| | | temperature (°C) | −0.07 | 0.02 | −0.10 | −0.04 | −4.58 | $<0.0001$ |
| | | daylength (hrs) | 1.54 | 0.32 | 0.92 | 2.16 | 4.88 | $<0.0001$ |
| | | cumulprecip (cm)[g] | 0.09 | 0.03 | 0.04 | 0.14 | 3.64 | 0.0003 |
| (B) Abiotic variables + other species | HC | intercept | −18.46 | 5.10 | −28.45 | −8.47 | −3.62 | 0.0003 |
| | | other calling (yes/no)[e] | 2.32 | 0.37 | 1.59 | 3.05 | 6.25 | $<0.0001$ |
| | | temperature (°C) | −0.05 | 0.02 | −0.08 | −0.02 | −3.16 | 0.0016 |
| | | daylength (hrs) | 1.21 | 0.37 | 0.48 | 1.94 | 3.24 | 0.0012 |
| | | precipitation (cm)[f] | 0.14 | 0.08 | −0.01 | 0.29 | 1.89 | 0.0585 |
| | | cumulprecip (cm)[g] | 0.10 | 0.05 | 0.01 | 0.20 | 2.11 | 0.0347 |
| | HV | intercept | −17.68 | 3.41 | −24.37 | −10.99 | −5.18 | $<0.0001$ |
| | | other calling (yes/no)[e] | 2.25 | 0.36 | 1.56 | 2.95 | 6.34 | $<0.0001$ |
| | | temperature (°C) | −0.06 | 0.01 | −0.08 | −0.03 | −4.98 | $<0.0001$ |
| | | daylength (hrs) | 1.24 | 0.27 | 0.71 | 1.76 | 4.59 | $<0.0001$ |
| | | precipitation (cm)[f] | 0.08 | 0.03 | 0.02 | 0.14 | 2.47 | 0.0136 |
| | | cumulprecip (cm)[g] | 0.05 | 0.04 | −0.02 | 0.12 | 1.45 | 0.1460 |

[a] Estimate of explanatory slope ($\beta_x$).
[b] Standard error of slope estimate.
[c] Lower confidence limit.
[d] Upper confidence limit.
[e] Detection of the other gray treefrog species calling in the same sampling period.
[f] Precipitation day of calling event.
[g] Cumulative precipitation 1, 2, and 3 days prior to calling event.

species complex by incorporating an ecological component on a larger temporal and spatial scale.

In this study, the breeding seasons and sites of sympatric *Hyla chrysoscelis* and *H. versicolor* generally overlapped. With the exception of anurans in arid environments (Sullivan, 1989), closely related sympatric anurans with similar overlap of both breeding seasons and sites typically exhibit differences in calling phenology (Schalk and Saenz, 2015). Saenz et al. (2006) studied calling responses of 12 anuran species to the same environmental variables included in our analyses and found that no two species had the same calling phenologies. However, that study combined *H. chrysoscelis* and *H. versicolor* into one group. We separated the gray treefrog species and found that they exhibit near-identical calling phenology. Both species respond similarly to environmental cues and are ten times more likely to be detected calling in the presence than in the absence of the other species (Table 1).

In addition to the overlap in temporal patterns, the calling behavior of *Hyla chrysoscelis* and *H. versicolor* also overlapped spatially, although detections of the two species varied between the SFAEF ponds and the DCNF ponds (Fig. 3). This result may have been influenced by pond characteristics such as pond size, vegetation, or hydroperiod (Babbitt, 2005). Ponds in the DCNF are larger than those in the SFAEF and have slightly different vegetation, which may expose anurans to greater predation risk than small ponds. Differences in pond size or vegetation potentially make the DCNF ponds included in this study less suitable habitat for gray treefrogs than the SFAEF ponds, which could explain the fewer detections in DCNF ponds overall. While a generally greater proportion of *H. chrysoscelis* than *H. versicolor* were detected in the DCNF, population sizes and abundance of each species in chorus composition was not explicitly quantified. Both conspecific and heterospecific aggressive interactions may also influence calling behavior (Reichert and Gerhardt, 2013, 2014), and examining the abundance and spatial distributions between males of these species may provide additional insight to the patterns observed between locations. In general, the temporal and spatial range of detections of each species largely overlap (Fig. 1). The life history of these species is very similar (Ptacek, 1996), suggesting that differences in resource availability in the small ponds would not favor one species over the other. For example, the larvae of sympatric *H. chrysoscelis* and *H. versicolor* have similar hatch times, developmental periods, and growth rates (Ptacek, 1996). Thus, pond size, vegetation, and hydroperiod may have a similar effect on these species and may contribute to larger scale spatial patterns and population distribution of both species (Babbitt, 2005). These patterns result in frogs that will be calling at the same time and place as closely related species.

Considering that frogs did not avoid calling at the same time and place as the other species, we tested if they instead adjust advertisement call properties when calling with the other species. Many studies have provided evidence for phenotypic plasticity among anurans calling in dense and acoustically complex environments (Lopez et al., 1988; Gerhardt, 1994; Brenowitz and Rose, 1999; Sun and Narins, 2005; Bee, 2007; Bee and Swanson, 2007; Lemmon, 2009).

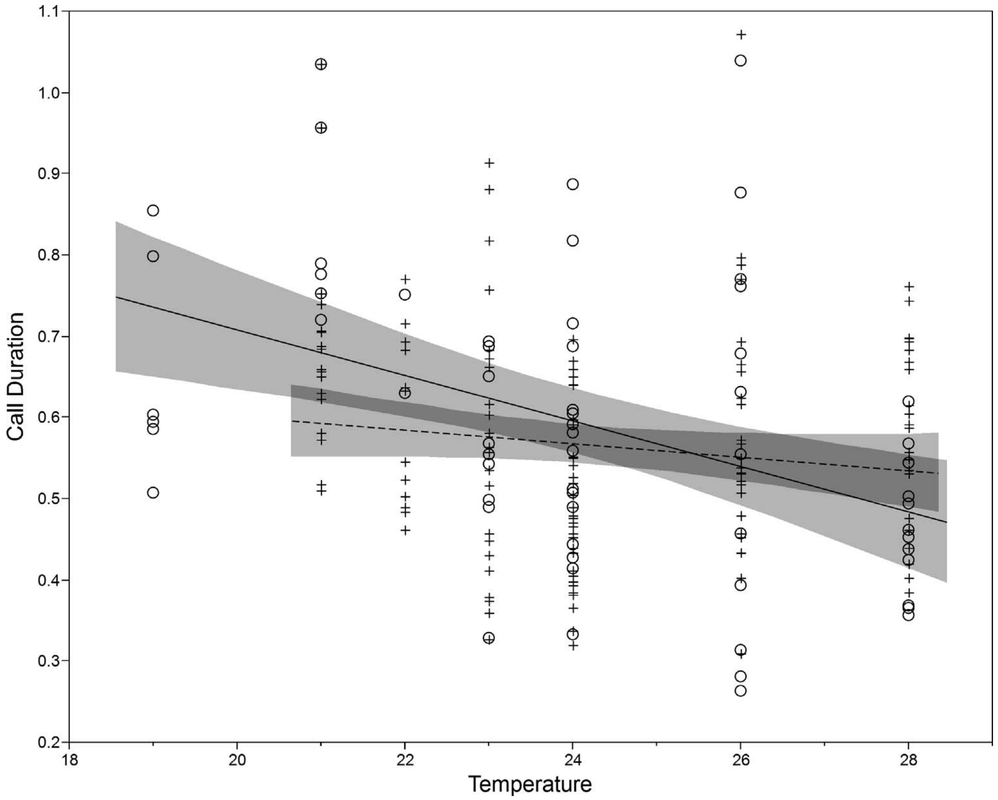

**Fig. 4.** Relationship between temperature and call duration for *Hyla chrysoscelis* showing effects of calling alone (o symbols, solid regression line) or with *H. versicolor* present (+ symbols, dashed regression line).

**Table 2.** (A) Means and (B) Analysis of Variance (ANOVA) and (C) Covariance (ANCOVA) for each measured advertisement call variable of *Hyla chrysoscelis* (HC) calling in the presence and absence ("group" effect) of *H. versicolor* (HV), and of *H. versicolor* calling in the presence and absence *H. chrysoscelis*.

(A) Means

| Species | Variable | Condition | *n* | Mean | SE |
|---|---|---|---|---|---|
| HV | duration (s) | no HC | 104 | 0.47 | 0.01 |
| | | with HC | 143 | 0.51 | 0.01 |
| | pulse rate (pulses/s) | no HC | 104 | 28.36 | 0.18 |
| | | with HC | 143 | 28.07 | 0.15 |
| | dom. freq. (Hz) | no HC | 102 | 2287.52 | 33.36 |
| | | with HC | 127 | 2275.86 | 29.90 |
| HC | duration (s) | no HV | 66 | 0.59 | 0.02 |
| | | with HV | 143 | 0.57 | 0.01 |
| | pulse rate (pulses/s) | no HV | 66 | 54.38 | 0.50 |
| | | with HV | 143 | 54.83 | 0.34 |
| | dom. freq. (Hz) | no HV | 55 | 2391.49 | 58.29 |
| | | with HV | 91 | 2288.42 | 45.32 |

(B) ANCOVA

| Species | Variable | Effect | df | dfE | *F* value | *P* value |
|---|---|---|---|---|---|---|
| HV | duration (s) | Group | 1 | 244 | 5.80 | 0.0168 |
| | | Temperature (covariate) | 1 | 244 | 94.84 | <0.0001 |
| | pulse rate (pulses/s) | Group | 1 | 244 | 1.64 | 0.2017 |
| | | Temperature (covariate) | 1 | 244 | 229.82 | <0.0001 |
| HC | pulse rate (pulses/s) | Group | 1 | 206 | 0.56 | 0.4550 |
| | | Temperature (covariate) | 1 | 206 | 111.19 | <0.0001 |

(C) ANOVA

| Species | Variable | Effect | df | dfE | *F* value | *P* value |
|---|---|---|---|---|---|---|
| HV | dom. freq. (Hz) | Group | 1 | 227 | 0.07 | 0.7950 |
| HC | dom. freq. (Hz) | Group | 1 | 144 | 1.95 | 0.1649 |

Additionally, this has important fitness consequences as call parameters are important indicators for female preferences. In our study, the presence of the other gray treefrog species did not significantly affect call pulse rate or dominant frequency in either species (Table 2). It is unsurprising that dominant frequency was not significantly affected, as frequency preference in female gray treefrogs is usually weak (Gerhardt, 2005; Shrode et al., 2012). The presence of the other species did, however, affect call duration, which is a well-studied target for female preferences. Longer calls are preferred by females of both *H. versicolor* and *H. chrysoscelis* (Gerhardt et al., 2000; Bee, 2008), as call duration can be an indicator of genetic quality in *H. versicolor* (Welch et al., 1998), and the ability of females to receive and interpret this signal may impact offspring fitness (Welch, 2003). In our study, *H. versicolor* produced calls with significantly longer duration in the presence of calling *H. chrysoscelis* at all temperatures (Table 2B), which is consistent with the relationship between temperature and call duration in *H. versicolor* as described in Gayou (1984). Thus, male *H. versicolor* producing calls in mixed-species choruses may benefit from improved mating success. Additionally, in *H. chrysoscelis*, the effects of the other species on call duration may be temperature dependent. At higher temperatures, *H. chrysoscelis* in the presence of calling *H. versicolor* produced calls with longer duration than *H. chrysoscelis* calling alone. At lower temperatures, *H. chrysoscelis* calling in the presence of *H. versicolor* produced shorter calls than *H. chrysoscelis* calling alone. This pattern resulted from a reduced effect of temperature on call duration when calling together (Fig. 4). Reduced temperature effects in mixed choruses could also have effects on female mate choice. When call variables are temperature dependent, females may exhibit "temperature coupling" where they prefer call variables that correspond to those produced by males calling at the same temperature as that experienced by the female (Gerhardt, 2015). Temperature coupling, or lack thereof, can potentially result in heterospecific pairings in a mixed chorus with males calling at variable temperatures (Gerhardt and Mudry, 1980; Humfeld and Grunert, 2015). Reduced temperature effects on male call variables may help prevent such heterospecific pairings. Finally, an additional alternative is that females may alter their evaluation of different components of the call in mixed species choruses. Female *H. chrysoscelis* occurring in sympatry with *H. versicolor* show stronger preference for conspecific pulse rate and weaker preference for call duration than those occurring in allopatry (Gerhardt, 1994). Female *H. chrysoscelis* also have reduced preferences for long calls in noisy environments (Vélez et al., 2013); therefore, we predict that female preferences for call duration may be influenced by a temperature and community structure interaction as we found with male call duration.

Most sympatric anuran species exhibit different calling phenologies (Carter et al., 2018), which contributes to prezygotic reproductive isolation in closely related species in which hybridization is costly to fitness. Sympatric anurans that exhibit little variation in timing of breeding and breeding site selection could experience improved conspecific mating success by weighing these variables differently than heterospecifics, as in our study where the two species weigh abiotic and biotic variables differently. Although sympatric gray treefrogs exhibit clear postzygotic isolation, prezygotic isolation mechanisms not based on male call characteristics remain poorly understood. Our results indicate that that calling behavior, specifically spatial and temporal variation in calling phenology, does not contribute to reproductive isolation in gray treefrogs. In such a case, modulation of call properties such as phenotypically plastic call duration in response to the immediate calling environment could contribute to prezygotic reproductive isolation.

Temperature-dependent effects of mixed-species choruses on call properties may also have important evolutionary implications via mismating and hybridization between these two species. Our results specifically suggest interactions between how temperature and presence of heterospecifics are weighed, potentially leading to important differences between the species in call duration, which is known to be under sexual selection and determine male fitness. We did not explicitly compare the species to each other, and it will be important for future work to more fully explore the extent of plasticity in male calls (and female preferences) to understand their evolutionary implications. If the differences in male call parameters are associated with differences in female preferences, this co-evolution could be subject to strong female preference and may affect male mating success and prezygotic reproductive isolation in gray treefrogs.

## DATA ACCESSIBILITY

## ACKNOWLEDGMENTS

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