Behavioral Ecology and Sociobiology (2026) 80:1
https://doi.org/10.1007/s00265-025-03672-7

RESEARCH

Where did she go? Changes in social context alter calling strategies of eastern gray treefrogs

Olivia S. Feagles[1,2] · Alexander A. Sweet[1] · Kane D. Stratman[1] · Madison A. Rittinger[1] · Angela M. E. Mish[1] · Luke Nicol[1] · Gerlinde Höbel[1]

Received: 22 August 2025 / Revised: 31 October 2025 / Accepted: 6 November 2025
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Abstract
Displaying to attract mates often requires substantial energy investment and can increase the risk of eavesdropping from rivals and predators. To modulate the costs while maximizing the likelihood of attracting a mate, signalers are often attuned to social cues and adjust displays accordingly. Male Eastern Gray Treefrogs emit energetically expensive advertisement calls until a female approaches them and initiates amplexus (mating embrace). Females prefer longer calls and calls produced at higher rates, but males are energetically constrained from maximizing both call properties. Previous studies have shown that vocal performance is not maximized during the whole night, but only up-regulated during periods of male-male competition or in the last stage of courtship. In both contexts males increase call duration relative to baseline calling. Here we explore plasticity in calling behavior in another context - how males respond when the pair becomes separated. Across *within-male* (pre- and post- separation call data from same males) and *between-male* (calls from different sets of males) data sets we consistently found the same pattern: males decreased call duration, increased call rate, while maintaining vocal effort (duty cycle) relative to baseline calling. Control trials showed that those changes cannot be attributed to handling or differences in recording time. We suggest that males emphasize different call properties because initial courtship is best facilitated by the property females have the strongest preference for (call duration), while re-initiation of amplexus is best facilitated by allowing for fast female phonotaxis via frequent calls (increase call rate).

Significance statement
Many animals show plasticity in signaling behaviors and adjust signal properties in response to changes in behavioral context. For example, chorusing male treefrogs switch to longer calls when encountering a rival male and they also produce longer calls when a mate-searching female is nearby. Hence, although social contexts are different (competition versus courtship), males respond with similar changes in calling behavior. Here we examined plasticity in calling behavior in another courtship context - after becoming separated from their embraced female and needing to re-initiate pair formation. Surprisingly, males shorten calls and increase call rate instead. We suggest this is adaptive because females only move after hearing a call, and more frequent calls should result in quicker re-establishment of pair formation. Our data show that similar contexts do not predict similar behavior, and that males may use more nuanced behavioral adjustments.

Keywords Advertisement call · Amplexus · Behavioral plasticity · Displays · Hyla versicolor · Vocal performance

Introduction

Displaying to attract mates is a challenging and high stakes endeavor. Displays are often energetically demanding and cannot be sustained for extended periods of time (Wells and Taigen 1986; Reichert and Gerhardt 2012; Podos 2022). Elaborate displays also increase the risk of signal exploitation (Bailey and Haythornthwaite 1998; Bernal and Page 2023), which is employed by a variety of eavesdroppers. These include conspecific rivals (Beletsky et al. 1986;

Communicated by A. T Baugh.

Extended author information available on the last page of the article

Published online: 06 December 2025

McGregor and Peake 2000; Dunlop and Noad 2016; Trillo et al. 2019) and natural enemies (Zuk et al. 2006; Akre et al. 2011; Gomes et al. 2017; White et al. 2022). Not surprisingly, individuals often modify signal expression and use information gathered from the social environment to maximize mate attraction while also minimizing costs due to energy expenditure and eavesdropping risk (Wilde et al. 2023).

The presence, proximity, and behavior of potential mates are another common cause of adjustments in display strategies. For example, male chameleons target courtship towards females that are more receptive (Kelso and Verrell 2002) and male zebra finches target courtship towards females that are more fecund (Jones et al. 2001).Males may also modify their display behavior when they detect the presence or interest of a female. For instance, male greater sage grouse display at low levels when females are farther away and at higher levels as females approach (Patricelli and Krakauer 2009) and male túngara frogs respond to female "elicitation" behavior by producing more complex calls (Akre and Ryan 2011).

The ability to adjust the rate and intensity of costly displays is particularly relevant for chorusing species such as frogs, where males call for hours each night to allow for receptive females to approach them guided by their calls (a process called phonotaxis). The primary call type of male anurans is the advertisement call (Toledo et al. 2015). This dual-function display attracts females and mediates competitive interactions with neighboring males (Fellers 1979; Wells 2007). The properties of the advertisement call broadcast within a chorus setting, but in the absence of social cues indicating the close proximity of females or rivals (see below), serve as the baseline calling behavior against which socially modified signal variations can be compared.

Male anurans are highly attentive to changes in their social environment and modify their vocal performance in response to acoustic (e.g., Lopez et al. 1988Bosch and Márquez 2001; Schwartz et al. 2002, 2013; Reichert 2011a, b), visual (Akre and Ryan 2011; Reichert 2013), and vibrational (Lewis et al. 2001; Narins et al. 2018; Caldwell et al. 2022) cues. For example, when faced with nearby rival males, their first response (before switching to aggressive calling) is generally to modify the properties of their advertisement calls, including decreasing frequency (Lopez et al. 1988; Wagner 1989), increasing duration (Wells and Taigen 1986; Reichert 2011a), increasing rate (Schwartz and Wells 1985; Bosch and Márquez 1996), increasing amplitude (Lopez et al. 1988), or adding secondary notes (Wells and Schwartz 1984; Ryan 1985). Likewise, when males perceive a female nearby, they often increase the duration (Fellers 1979; Wells and Taigen 1986; Klump and Gerhardt 1987; Höbel 2000) or rate (Byrne 2008) of their calls, sometimes to the point where the call is sufficiently different from the advertisement call to merit a different call type designation (i.e., courtship call). Although the social context of rival repellence and close-range (courtship) mate attraction is radically different, the associated call modifications are often similar: producing longer, faster, louder, and generally more elaborate displays that are highly attractive to females (e.g., Wells and Schwartz 1984; Klump and Gerhardt 1987; Gerhardt et al. 1996).

To date, little attention has been given to assessing the type of close-range mate attraction that takes place in the time frame between the perception of a nearby female to the initiation of amplexus (the anuran mating embrace) (Byrne 2008; Akre and Ryan 2011; Reichert 2013). Yet, in anurans there is typically a several hour delay between amplexus formation and oviposition (Ritke et al. 1990). While the female carries the male towards her chosen oviposition site the pair may become accidentally separated because they need to evade predators or encounter mechanical resistance while moving through tight vegetation. We are not aware of any studies that have quantified the rate at which accidental amplexus separation occurs in nature but given the low male mating success typical for anuran amphibians even rare events should select for behaviors that quickly remedy the separation. Since male anurans generally do not pursue females but rely on the females to approach them and initiate amplexus, such a situation would require another round of close-range mate attraction. Here we add to the knowledgebase of how male frogs adjust their call properties to changes in their social environment by analyzing the calls males produce immediately after separation from their amplexed female.

Our study species is the Eastern Gray Treefrog, *Hyla versicolor*, whose acoustic communication system is exceptionally well studied. Males produce pulsed advertisement calls, and females prefer calls that are longer and calls that are produced at faster rates (Gerhardt et al. 2000; Reichert and Höbel 2015; Feagles and Höbel 2022; Stratman and Höbel 2024), but the preference for longer calls is stronger and more universally shared among females (Stratman and Höbel 2024). This species' advertisement calls are one of the most energetically expensive displays in ectothermic vertebrates (Taigen and Wells 1985). Both call duration and call rate are positively correlated with energy expenditure (Taigen and Wells 1985; Schwartz et al. 2008), setting an upper limit to vocal performance and forcing a trade-off between maximizing call duration or call rate when attracting potential mates (Wells and Taigen 1986; Ward et al. 2013; but see Reichert and Gerhardt 2012).

A wealth of previous research provides us with comparative data on socially mediated changes in calling behavior in *H. versicolor*. Males of this species increase call

duration with increasing proximity to competitive conspecifics (Reichert and Gerhardt 2012) and to females (Reichert 2013), as well as elevations in noise level (Schwartz et al. 2013). In general, the increase in call duration is compensated by a decrease in call rate such that the overall vocal performance remains stable (Schwartz et al. 2013; but see Reichert and Gerhardt 2012). In addition, female mate choice behavior has been examined in detail, including the locomotion pattern of female phonotaxis. Females move in short walking bouts (ca. 1 s) at the end of each call but remain immobile until they hear another call (Beckers and Schul 2004).

Drawing on this information on the natural history of *H. versicolor*, we tested two hypotheses related to socially mediated changes in calling behavior following an interruption of a previously formed amplexus. Our hypotheses are built upon the observations that unmated males increase the duration of their advertisement calls upon perceiving an approaching female (Reichert 2013) and that females move only for a brief period immediately after hearing a call (Beckers and Schul 2004). Consequently, in response to a separation from an amplexus, the *mate attraction hypothesis* predicts that males will produce longer calls to maximize attractiveness. By contrast, the *mate location hypothesis* predicts that males will produce calls at higher rates to hasten female phonotaxis. While these hypotheses have contrary predictions in terms of the emphasized call property (duration vs. rate), both are consistent with stable vocal performance (if the increase in one property is compensated by a decrease in the other property) or an increase in vocal performance (if one property increases and the other remains stable) after separation from amplexus.

Materials and methods

Study species and study site

Eastern Gray Treefrogs, *Hyla versicolor*, are a widespread species throughout eastern North America (Conant and Collins 1998). During the early-summer breeding season (May/June in Wisconsin), males gather in choruses in woodland ponds and emit advertisement calls to attract females. Females select males mainly based upon the features of their advertisement calls (Sullivan and Hinshaw 1992; Gerhardt et al. 2000; Reichert and Höbel 2015). It is the female that approaches the stationary calling male and initiates amplexus by touching the male; males do not pursue females. All frogs recorded for this study came from two adjacent ponds at the University of Wisconsin - Milwaukee (UWM) Field Station in Saukville, WI.

Experimental design

We include three data sets to illustrate the robustness of pre- and post- amplexus calling plasticity. *Within-male field* and *within-male lab* data sets, respectively, are based on pre- and post-amplexus recordings from the same males but differ in the location where the post-amplexus recording was obtained (natural outdoor setting or acoustic lab setting, respectively). The *between-male* data set is based on recordings obtained from different males that were recorded either at the pond (pre-amplexus) or inside the acoustics lab (post-amplexus) (analogous to the *within-male lab* data set). Call recordings for each data set were taken over several nights (range: 5–11 nights/data set) spanning the duration of the respective breeding season, and between 1 and 16 (mode 6) males were recorded each night. Baseline calling (=pre-amplexus) recordings at the pond took place between 21:30 and 22:45, and post-amplexus recordings (field and lab) took place between 23:00–01:00. If a male that had been removed from the female did not call within five minutes of amplexus separation, we played a priming recording for ten minutes to encourage him to start calling. If the male still did not call, we removed him from the experiment. We did not mark males during the course of this study, however, the size of our study population and the short chorus tenure of males at our site make it unlikely that we recorded the same male more than once. It was not possible to record data blindly because our study involved focal animals in the field.

Within-male field and within-male lab data sets: Over the course of the 2023 breeding season, we recorded 51 calling males directly at their calling perch at the pond. Recorded frogs were not displaying aggressive calls or showed indications of being engaged in a contest with another male or approached by a potential mate (i.e. their call properties represent baseline calling). After recording the males, we collected and transported them either to a dry location adjacent to the pond ($n=29$; *within-male field*) or to a sound attenuated room at the UWM Field Station's research building ($n=22$; *within-male lab*).

Next, we placed each male individually inside a plastic terrarium (30 × 20 × 20 cm; ExoTerra Faunarium) and added a random female. Females were obtained from recently amplexed pairs that had been collected by some team members while the other team members had recorded the focal males. Males soon started calling in the terraria, and we observed the frogs until the pair entered in amplexus. We noted the time of amplexus and waited for one hour. Then we separated the amplexed pair. The female remained in the plastic terrarium that was occluded from the view of the male. The male was placed on a calling perch (wooden branch), either inside a 45 × 45 × 60 cm screen cage (to

prevent escape while maintaining an open sound field) near the pond (*within-male field*) or inside the sound chamber in the lab (*within-male lab*). With every recording (i.e., pre- and post-amplexus) we obtain data on the male's body temperature using an Extech IR270 IR thermometer. Three males required priming with a call playback, and of those, two never called after being removed from amplexus, resulting in a final sample size of $n = 27$ for the *within-male field* data set and $n = 22$ for the *within-male lab* data set (see Results).

Between-male lab data set: We used previously collected data, initially destined for unrelated studies, to compare between males and across years. The pre-amplexus portion of this data set contained calls of males recorded while naturally calling at the pond in 2017 ($n = 84$), 2018 ($n = 29$), and 2021 ($n = 28$). These recordings did not have temperature measures associated with them; hence data is not temperature-adjusted.

The post-amplexus portion of this data set contained calls of males recorded in 2019 ($n = 19$) and 2021 ($n = 78$). The males were collected while in amplexus at the pond and then transported to a sound-attenuated room (at Field Station Research building or UWM Campus in Milwaukee). Duration of amplexus was not controlled for and ranged from an estimated 1–3 h. Otherwise, the recording environment and methods were the same as described for the *within-male lab* data set. Two males required priming with a call playback, and of those, one never called, resulting in a final data set of $n = 18$ for 2019 and $n = 78$ for 2021.

Controls – In the above experiments, the pre-amplexus call data was derived from undisturbed males calling in the field. But in order to experimentally manipulate social context, the post-amplexus call data had to be obtained from males that had been handled, transported to a different location, placed with a female and then separated from that female after an hour relative to amplexus formation. To verify that the observed changes in calling behavior were due to manipulation of amplexus status, not the confounding factors listed above, we conducted two control experiments.

To assess the effect of being handled and transported to another location (*handling-control*), we sampled 48 males over the course of the 2025 breeding season. Males were recorded at their calling perch at the pond, captured, transported to a dry location adjacent to the pond, individually placed on a calling perch (wooden branch) inside a $45 \times 45 \times 60$ cm screen cage and recorded again. Location, recording enclosure, and recording procedure were identical to the ones used for the *within-male field* data set. With every recording (i.e., pond and cage) we measured the male's body temperature using an Extech IR270 IR thermometer. Optimally mirroring the previous recording procedure would have required waiting an hour between the two recordings. However, keeping the males for an hour without allowing them to call and then getting them to call thereafter was not feasible. Instead, we started the second recording shortly after arriving at the screen cage location (10–15 min after the pond recording). Although we had recorded 48 males at the pond, our final sample size of post-handling recordings was only $n = 12$ because most males did not call again (see Results).

To assess the effect of being recorded at a later time (*time of recording-control*), we used previously collected data destined for a study on nighttime chorusing behavior (KS et al., unpubl. data). Here, 28 males were continuously recorded for 60–90 min by placing EVISTR L357 mini audio recorders (affixed on metal stakes placed in the pond substrate) next to their calling location. Recording took place during 10 chorus nights in the 2021 breeding season. Recordings began around 21:00 (roughly a half hour into the peak chorus activity), were obtained at the same pond, and involved males that were calling at least 15 m apart from other calling males. From this we extracted call duration and call rate data from the first 10 calls and another 10 calls recorded an hour later.

Call recording and analysis

For recording calls, we used a hand-held TASCAM DR-100MKII Linear PCM Recorder (TEAC Corporation, Tokyo Japan) and a Sennheiser K6+ME66 directional microphone. To analyze the recordings, we used Audacity (Audacity Team 2015) or RavenPro 1.5 (Cornell Lab of Ornithology) software. We recorded at least 20 consecutive calls for every recording.

We focused on three components of male calling behavior: call duration, call rate, and vocal performance (measured as duty cycle). We measured call duration as the time from beginning to end of a call (in ms). To obtain call rate (in units of calls/min), we first measured call period (time from the start of a call to the start of the subsequent call; in ms) and then divided 60,000 ms (=1 min) by the call period value. We calculated duty cycle (the relative amount of time occupied by sound) by dividing call duration by the call period (from average call duration and call period values of each male). For measuring we selected a 5–10 call sequence that was most consistent throughout the recording to be representative of a given male and report average call trait values per male per treatment.

For the *within-male field*, *within-male lab*, and *handling-control* data sets, recording temperatures ranged from 11.5 to 23.4 °C. Because temperature affects temporal call properties (Gerhardt and Huber 2002), we adjusted values of call duration and call rate to 18 °C (the average recording temperature) according to the equation: $\text{Value}_{18} = \text{Value}_T$

Table 1 Results of two standard least squares (REML) models examining the effect of social context (before and after amplexus) on call properties in the *within-male field* and *within-male lab* comparisons. The bolded values denote cases in which call properties significantly changed in response to changes in social context

Comparison	within-male, field				within-male, lab			
Call Property	df	F	*P*	Effect Size	df	F	*P*	Effect Size
Call Duration	1,26	128.26	**<0.0001**	0.91	1,21	55.35	**<0.0001**	0.85
Call Rate	1,26	24.28	**<0.0001**	0.69	1,21	50.31	**<0.0001**	0.84
Duty Cycle	1,26	2.29	0.14	0.28	1,21	0.09	0.77	0.07

- $m(T-18)$, where T represents the measured temperature, Value_T represents the value of the measured call property and m represents the slope of the regression of Value_T on T (Platz and Forester 1988). To obtain the slope m, we used the calls of the 49 males recorded in 2023. We then used these temperature-adjusted values to calculate duty cycle. Temperature data was not available for the *between-male* and the *time of recording-control* data sets, and we did not perform temperature correction on these calls.

Statistical analysis

We examined all hypotheses using standard least squares (REML) models performed in JMP 11 (SAS Institute Inc., Cary, NC). Before statistical analysis we verified that the data met normality assumptions by visually inspecting the quantile-quantile plots of duty cycle, call duration, and call rate. Only call rate did not meet normality assumptions and we subsequently used square root transformations for the within-male comparisons and log transformation for the between-male comparison to improve normality.

For the *within-male field* and *within-male lab* data sets, we entered temperature-adjusted male call properties (call duration, call rate, duty cycle) as response variables, context as the predictor variable, and male ID as a random term (since each male provided two data points). For the test of our hypothesis, the context term is the factor of interest because it tests whether males called differently before and after being in amplexus.

For the *between-male* data set, we entered male call properties (call duration, call rate, duty cycle) as the response variables, and context as well as replicate (indicating year of study) nested within context as predictor variables. For the test of our hypothesis, the context term is the factor of interest because it tests whether males engaged in baseline calling and males having been separated from their mate called differently. A significant replicate term indicates that call properties vary across the different years of the study.

For the *handling-control* data set, we entered temperature-adjusted male call properties (call duration, call rate, duty cycle) as response variables, context as the predictor variable, and male ID as a random term (since each male provided two data points). Here, the context under study is undisturbed baseline calling vs. handling/location change.

Table 2 Results of a standard least squares (REML) model examining the effect of social context (before and after amplexus) on call properties in the *between-male*, *lab* comparison. The bolded values denote cases in which call properties significantly changed in response to changes in social context

Call property	Factor	df	F	*P*	Effect size
Call duration	Context	1,232	267.72	**<0.0001**	0.73
	Repl.[Context]	3,232	0.47	0.70	0.08
Call Rate	Context	1,232	218.08	**<0.0001**	0.70
	Repl.[Context]	3,232	0.90	0.44	0.11
Duty Cycle	Context	1,232	1.42	0.24	0.08
	Repl.[Context]	3,232	1.71	0.17	0.15

For the *time of recording-control* data set, we entered male call properties (call duration, call rate, duty cycle) as response variables, context as the predictor variable, and male ID as a random term (since each male provided two data points). Here, the context under study is the difference in recording time.

We also calculated effect sizes for all comparisons. For this we first calculated partial eta square from the F statistics and degrees of freedom (Lakens 2013), and then converted those partial eta square values Pearson's r. Here, values below $r=0.10$ are considered small effect size, values around $r=0.30$ are medium effect size, and values above $r=0.50$ indicate large effect size (Cohen 1988).

Results

Plasticity in calling behavior We found a consistent pattern of call variation across all three amplexus manipulation data sets: call duration and call rate differed significantly between contexts, but duty cycle did not (Tables 1 and 2; Fig. 1). On average, call duration decreased after separation from amplexus (within-male field changed from 964ms to 631ms; within-male lab 1001ms to 529ms; between-males 892ms to 434ms) while call rate increased (within-male field changed from 9 to 13 calls/min; within-male lab 10 to 17 calls/min; between-males 10 to 22 calls/min). No change in duty cycle indicates that males invest similar amounts of energy irrespective of calling context (Fig. 1, right column). However, the significant difference and opposite pattern

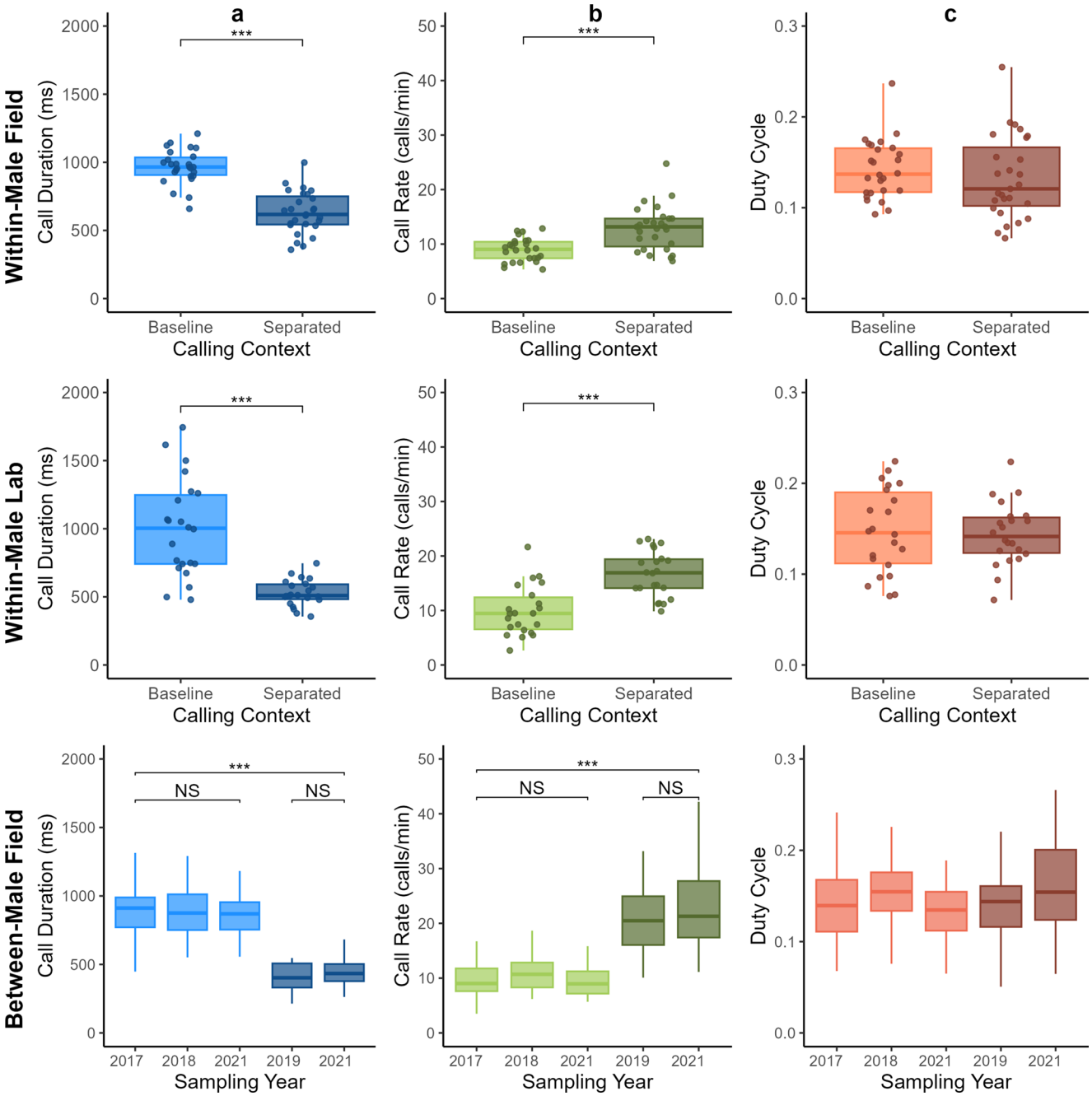

Fig. 1 Change in male calling behavior before and after having spent time in amplexus. Shown in data from three data sets: *within-male field*, *within-male lab*, *between-male*, respectively. Males decrease call duration (column a, blue), increase call rate (column b, green), but do not change duty cycle (column c, red). The two shades of each color note the call context: lighter shade = baseline calling (pre-amplexus), darker shade = separated calling (post-amplexus). Asterisks indicate significant comparisons (* $P<0.05$, ** $P<0.01$, *** $P<0.0001$); boxes depict the interquartile range (IQR) with the central line indicating the median and whiskers extend to 1.5*IQR

of variation in call duration and call rate (Fig. 1, left and center column) indicates that males emphasize call duration when engaged in baseline calling (pre-amplexus) but emphasize call rate after they have recently been separated from their mate (post-amplexus).

Handling and transportation (*handling-control*) and variation in time of recording (*time of recording-control*) also changed calling behavior, but the patterns were different than the one observed for amplexus manipulation (above). Handling resulted in a (non-significant) increase in call duration and a significant decrease in call rate (Table 3, left columns), which is opposite to what we observed for amplexus manipulations. Being recorded at a later time resulted in shorter calls, slower call rate, and decrease in duty cycle (Table 3,

Table 3 Results of two standard least squares (REML) models examining the effect of being handled and transported to another location (*handling-control*) or of being recorded at an alternate time (*time of recording-control*) on call properties. The bolded values denote cases in which call properties significantly changed across context

Comparison	handling-control				time of recording-control			
Call Property	df	F	*P*	Effect Size	df	F	*P*	Effect Size
Call Duration	1,11	1.01	0.34	0.29	1,27	6.36	**0.02**	0.44
Call Rate	1,11	7.54	**0.02**	0.64	1,27	8.08	**0.01**	0.48
Duty Cycle	1,11	1.98	0.19	0.39	1,27	22.12	**<0.0001**	0.67

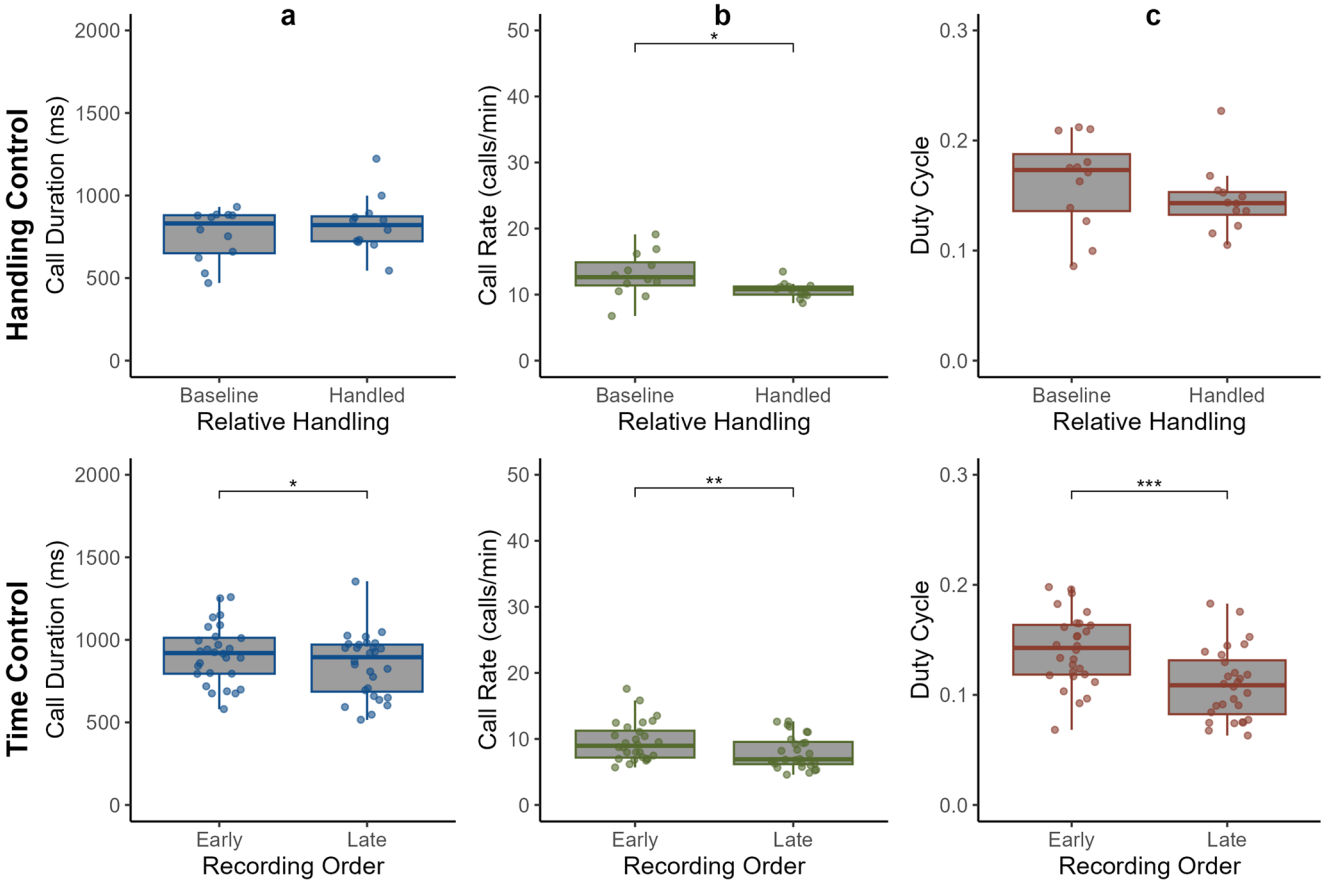

Fig. 2 Change in male calling behavior after having been handled (*handling-control*) and when being recorded at a later time (*time-control*). Shown is data for call duration (column a), call rate (column b), and duty cycle (column c). Even in cases where call properties change, the direction of the change is opposite to the pattern observed during amplexus separation treatments (see Fig. 1). Asterisks indicate significant comparisons (* $P<0.05$, ** $P<0.01$, *** $P<0.0001$); boxes depict the interquartile range (IQR) with the central line indicating the median and whiskers extend to 1.5*IQR

right columns). The changes in call rate and duty cycle are opposite from what we observed for amplexus manipulations, and although the change in call duration mirrors that of the amplexus manipulation males, the effect size was only of medium magnitude in the *time of recording-control* while effect size was always of large magnitude in the amplexus manipulation comparisons. Overall, the change in calling behavior in the *time of recording-control* is best interpreted as a general decrease in vocal performance over the course of the night.

Motivation to call Motivation to call was strikingly different between the three amplexus data sets (i.e., males that were handled, moved, and then handled again to be separated from their female) compared to the handling-control males that only experienced handing and transport (Fig 2). Almost all males separated from amplexus called again (98%; 145 of 148 males), most doing so quickly (modal latency to call was 20 s) and they rarely required being primed by a chorus recording before starting to call (only 2 of 145 males required priming before starting to call). [Within-male field: 93% called again (27 of 29 males); within-male lab: 100% called again (22 of 22 males), with one requiring priming;

between male: 99% called again (96 of 97 males), with one requiring priming.] By contrast, few of the males initially recorded in the handling-control treatment called again later (27%; 12 of 48 males), and of these many required priming by a chorus recording before they called (7 of 12 males).

Discussion

Male anurans are highly attentive to changes in their social environment, and *H. versicolor* have previously been shown to modify their vocal performance during male-male competition as well as close-range (courtship) mate attraction (Reichert and Gerhardt 2012; Reichert 2013). In both contexts, males increased their call duration relative to baseline calling and also increased duty cycle (Reichert and Gerhardt 2012). In the present study we examined short-range mate attraction in another context – how males respond after they have been separated from their mate. In this context, males shorten their calls but increased their call rate instead, without an overall increase in duty cycle. This pattern was robust across years, recording locations, consistent for within-male or between-male comparisons, and was not due to potential confounding factors like handling males for mate removal or recording post-amplexus calls at a later time in the night.

Our data supports the *mate location hypothesis* of post-amplexus call modification. We interpret these results as a function of the different goals the males have during distinct types of short-range mate attraction. When switching from baseline (i.e., long-range) to short-range mate attraction after perceiving a nearby female for the first time, the goal is to provide information on mate attractiveness. This is best accomplished by upregulating the call property which females have the strongest preferences for (i.e., call duration). By contrast, after accidental separation from a female the male had previously attracted, the main goal is to provide information on his location to allow for fast re-initiation of amplexus. Female *H. versicolor* approach sound sources by walking for a short period of time (1–2 s) immediately after hearing a call but stop walking before the start of the next call (Beckers and Schul 2004). Presumably, reunion with a previous mate is thus best accomplished by upregulating the call property that allows for faster phonotaxis (i.e., call rate).

Evaluating how common this pattern of call adjustment is in anurans is hampered by the lack of information on vocal behavior during close-range mate attraction. Courtship calls are known from a number of species (Toledo et al. 2015); they are often akin to exaggerated versions of advertisement calls (i.e., longer and/or faster), but frequently delivered at lower amplitude (e.g., Höbel 2000; Bourne et al. 2001; Owen and Tucker 2006). To our knowledge, this is the first study that directly focused on call adjustments after amplexus interruption, and even studies examining how the actual presence of a female changes male vocal behavior are scarce. For example, males of the dendrobatid frog *Colostethus beebei* increase call rate when perceiving an approaching female (Bourne et al. 2001). Male *Pseudophryne bibronii* toadlets (Byrne 2008) and *Hypsiboas rosenbergi* gladiator frogs (Höbel 2000) increase call rate and also produce longer calls with a higher number of pulses when presented with a female near their nests. Male Eastern Gray Treefrogs give longer calls when presented with a female, but do not increase call rate (Reichert and Gerhardt 2012; Reichert 2013), and male túngara frogs give more complex calls with more secondary chuck notes when females approach, especially if those engage in elicitation behaviors (Akre and Ryan 2011). Thus, although species vary in which or how many of their call properties they upregulate, the presence of a female generally elicits some increase in vocal performance.

An unexpected finding of our study was that males did not increase vocal performance (measured as duty cycle) after having been separated from their mate. Males from a Missouri population of *H. versicolor* did increase vocal performance when females were present (calling males were touched with a female by the experimenter) (Reichert and Gerhardt 2012). While recording post-amplexus males in our study, females were no longer present, which may explain why males did not increasing vocal performance. Likewise, males from the Missouri *H. versicolor* population increased vocal performance during male-male competition (Reichert and Gerhardt 2012), while in a closely related species, *Hyla chrysoscelis*, males did show dynamic shifts in calling behavior but did not increase vocal performance (they increase call duration but concomitantly decrease call rate) (Ward et al. 2013). The way in which competition was simulated in the two studies differed (movable platforms that brought live rival males in closer proximity vs. call playbacks), and Missouri *H. versicolor* males may have responded with increased vocal performance because they perceived live rivals as a more intense competitive environment.

Male frogs separated from their mates start calling quickly and reliably, while non-amplectant males rarely start calling again once handled or removed from the pond (*handling-control* data set; see also Garcia et al. 2019). While it may be tempting to take advantage of the increased motivation to call of amplectant males and use them to obtain call recordings, our data suggests that care should be taken when using them to collect population call data. For example, some studies have used a mix of baseline calling non-amplectant and amplectant males to obtain recordings for call descriptions

(i.e., Briggs 2010). Without prior examination of context-dependent variation in calling behavior, this may result in unrealistic data because males may emphasize different call characteristics after having their amplexus interrupted. As shown by our *between-male* data set, comparison between the calls obtained from these two groups of males can be used to rule out this potentially problematic result.

Amplexus is energetically demanding for the male anurans (McLister 2003), which may explain why there was no overall post-amplexus increase in vocal performance. Amplexus is also associated with elevated androgen and glucocorticoid levels (Orchinik 1988; Baugh 2024). For example, male *H. chrysoscelis* in amplexus show a 60% increase in testosterone and 350% increase in CORT levels compared to solo males (Baugh 2024), and experimentally inducing amplexus also increases testosterone in these frogs (M. Feiler et al., in prep.). Whether these changes in physiology translate to changes in calling behavior is less clear. In the treefrog *Hypsiboas faber*, testosterone did not correlate with calling behavior at all (De Assis et al. 2012). In the leptodactylid frog *Batrachyla taeniata*, testosterone was correlated with motivation to call (total number of calls given in response to call playback) but not with other call properties (Solís and Penna 1997). And in *H. chrysoscelis* there was no association between CORT levels and call properties, but elevated testosterone was weakly associated with more vigorous calling (A.T. Baugh, in prep.). Consequently, it is still puzzling how amplexus-associated hormonal changes could explain why calling males in our study emphasized call duration before amplexus but call rate thereafter.

Plasticity in calling behavior is well documented in anurans (i.e., Schwartz et al. 2002; Dapper et al. 2011; Reichert 2013), and here we have documented several additional sources of this plasticity. That handling and prolonged calling reduces motivation to call or vocal performance is not surprising, yet, to our knowledge, has never or only rarely been specifically quantified (see Runkle et al. 1994). But the most novel result of our study is that seemingly *similar* objectives (attracting a mate) involving the *same* receiver (receptive females) can lead to *different* behavioral adjustments (increasing call duration and vocal performance before amplexus but call rate after separation from amplexus), while seemingly *different* objectives (repel rival vs. courtship) involving *different* receivers (male rival vs. female mate) can lead to *similar* behavioral adjustment (increasing call duration and vocal performance; Reichert and Gerhardt 2012). Clearly, male frogs are capable of even more nuanced adjustments of calling behavior than currently appreciated.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00265-025-03672-7.

Acknowledgements We thank the staff at the University of Wisconsin – Milwaukee's Field Station for logistical support and fellow lab members H. Warner and J. Cannizzaro. We also thank our multiple funding sources: Animal Behavior Society's Student Research Grant awarded to OSF, the James and Dorathea Levenson Ecology and Field Biology Fellowships awarded to OSF, KDS, AMEM, and MAR, and the University of Wisconsin – Milwaukee's Student Undergraduate Research Fellowship (SURF) awarded to AAS and LN. We also thank the reviewers for their time and advice on improvements to this manuscript.

Author contributions Olivia S. Feagles and Gerlinde Höbel conceptualized, analyzed, and drafted the original manuscript. Olivia S. Feagles, Gerlinde Höbel, Luke Nicol, and Alexander A. Sweet extracted call data from recordings. All authors were involved in fieldwork and manuscript feedback.

Funding This study was funded by the Animal Behavior Society's Student Research Grant awarded to OSF, the James and Dorathea Levenson Ecology and Field Biology Fellowships awarded to OSF, AMEM, MAR, and KDS, and the University of Wisconsin – Milwaukee's Student Undergraduate Research Fellowship (SURF) awarded to AAS and LN.

Data availability All data supporting the findings of this study are available within the paper and its Supplementary Information.

Declarations

Ethics approval Frogs were collected with permission from local landowners and with permits obtained from the Wisconsin Department of Natural Resources. Experimental procedures were approved by the Animal Care and Use Committee of the University of Wisconsin-Milwaukee (IACUC # 22–23 #29) and adhered to the guidelines published by the Animal Behavior Society and Association for the Study of Animal Behavior.

Competing interests The authors declare no competing interests.

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Authors and Affiliations

Olivia S. Feagles[1,2] · Alexander A. Sweet[1] · Kane D. Stratman[1] · Madison A. Rittinger[1] · Angela M. E. Mish[1] · Luke Nicol[1] · Gerlinde Höbel[1]

✉ Olivia S. Feagles
olivia.feagles@gmail.com; ofeagles@usf.edu

Alexander A. Sweet
alexander.sweet.659@my.csun.edu

Kane D. Stratman
stratma8@uwm.edu

Madison A. Rittinger
ritting2@uwm.edu

Angela M. E. Mish
ammish@uwm.edu

Luke Nicol
luke_nicol@outlook.com

Gerlinde Höbel
hoebel@uwm.edu

[1] Behavioral and Molecular Ecology Group, Department of Biological Sciences, University of Wisconsin-Milwaukee, 3209 N Maryland Ave, Milwaukee, WI 53211, USA

[2] Integrative Biology Department, University of South Florida, 12037 USF Beard Dr, Tampa, FL 33620, USA