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A BEHAVIORAL AND NEUROBIOLOGICAL STUDY OF THE RESPONSES OF GRAY TREEFROGS, *HYLA VERSICOLOR*, TO THE CALLS OF A PREDATOR, *RANA CATESBEIANA*

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ABSTRACT: The bullfrog, *Rana catesbeiana*, is a predator of the gray treefrog, *Hyla versicolor*. Recent research with a variety of taxa has demonstrated that females may be sensitive to changes in their risk of predation and may respond behaviorally during mate choice in ways that reduce this risk. In a three-speaker female choice experiment, we tested whether gravid female treefrogs would avoid approaching a source of conspecific advertisement calls when it was adjacent to a speaker broadcasting bullfrog calls. We also assessed the hearing sensitivity of female gray treefrogs to bullfrog calls with neurophysiology and tested whether calling male bullfrogs would attack a simulated treefrog. A final playback experiment tested whether males would reduce their calling in response to bullfrog calls. Because calling male bullfrogs often attacked our model frog, the calls of this ranid might be a useful indicator of predation risk to *H. versicolor*. Nevertheless, we found that neither female phonotaxis nor male calling was influenced by broadcasts of bullfrog vocalizations. While auditory thresholds of *H. versicolor* were about 17 dB SPL higher to the heterospecific than to the conspecific call, thresholds to the bullfrog call were well below sound pressure levels used in the tests of phonotaxis. Possible explanations for our behavioral findings are discussed.

Key words: Anura; Behavior; Female choice; Predation risk; *Hyla versicolor*; *Rana catesbeiana*

SEARCHING or advertising for a mate may expose an animal to a variety of hazards (Lima and Dill, 1989; Magnhagen, 1991). Potentially potent sources of mortality are predators, and their impact shaping reproductive behaviors of prey has received considerable attention in discussions of sexual selection (Andersson, 1994). For example, the use of conspicuous visual or acoustic displays exposes males of many species to the eavesdropping of illegitimate receivers (Otte, 1974) with potentially lethal consequences (Cade, 1975; Endler, 1988; Ryan, 1985; Thornhill and Alcock, 1983; Wagner, 1996). Searching for, assessing, and mating with members of the opposite sex also may impose considerable risks (Belwood and Morris, 1987; Heller, 1992; Sih, 1994). If this is so, then selection may act on female behaviors to reduce predation-related costs of these reproductive activities. Indeed, the findings of recent theoretical (Crowley et al., 1991; Pomiankowski, 1987; Real, 1990) and empirical (Forsgren, 1992; Forsgren and Magnhagen, 1993; Gong and Gibson, 1996; Hedrick and Dill, 1993) studies are consistent with this expectation.

One way for females to reduce their susceptibility to predation is to reduce their time and movements in a dangerous microhabitat. This might be achieved through behaviors that operate without or prior to detection of cues provided by a potential predator. For example, in the context of choosing a mate, Grafe (1997) speculated that female painted reed frogs, *Hyperolius marmoratus broadleyi*, prefer closer males or those with higher call rates to expedite searching in a chorus when the risk of predation is high. Rand et al. (1997) reported that females of *Physalaemus pustulosus* were more likely to engage in phonotaxis in the dark than in dim light. This may be because females are less vulnerable to visually orienting predators when ambient light levels are lower. Accordingly, Rand et al. suggested that ambient light levels could influence the number of calling males that a female Tungara frog will consider as a potential mate.

Another way females might reduce their exposure to predators is by being less choosy of mates in dangerous environments. For example, Hedrick and Dill (1993) found that the tendency of female crickets, *Gryllus integer*, to move towards males with longer calling bouts could be counteracted in two-stimulus playback tests by the presence of cover in half of the testing arena. Furthermore, female preference for the long-bout stimulus could be reduced gradually by adding increasing amounts of cover on the short-bout side of the arena. Sensory information provided by a predator may also alter the strength of female mate preferences. Female guppies, *Poecilia reticulata,* from a Trinidadian population, reduced their preference for the more colorful male in the presence of a predatory cichlid (Godin and Briggs, 1996). Male pipefish, *Syngnathus typhle,* copulated randomly with respect to female size when a predatory cod was visible (Berglund, 1993). Female sand gobies, *Pomatoschistus minutus,* also reduced their choosiness (for male color and size) in the presence of a predatory fish (Forsgren, 1992).

Adult anurans may use a variety of defenses following the detection of a predator. These include such behaviors as urination (Buchanan and Taylor, 1996), moving away, feigning death, biting, changing posture, alarm calling, and call cessation (Duellman and Trueb, 1986). Choice of an oviposition site by females may even be affected by the risk of predation on their eggs or larvae posed by fish (Resetarits and Wilbur, 1989) or conspecific tadpoles (Crump, 1991). In this study, we tested whether the simulated presence of a bullfrog, *Rana catesbeiana,* a common predator on frogs (Werner et al., 1995), would affect the mating behavior of female gray treefrogs, *Hyla versicolor.* Our main goal was quite modest relative to some of the studies that we have cited in that we wished to determine whether females would avoid approaching a male calling near a calling bullfrog. If we found this to be the case, future studies could explore the impact of predation risk on female mate discrimination and determine the relevant acoustic features of the bullfrog advertisement call. We also tested whether male gray treefrogs reduce or terminate calling if exposed to bullfrog calls. Our rationale was that if either response occurs in males, it would provide a possible explanation for a negative result in our test for female avoidance behavior.

The spectral structure of the bullfrog call (Capranica, 1965; Davis, 1988) contains energy that falls within the range of good hearing sensitivity of *H. versicolor* (see Fig. 7 in Gerhardt, 1993). Therefore, we expected that females of *H. versicolor* would be able to detect the calls of this predator at sound levels at which they would be vulnerable to attack. Nevertheless, we tested this by measuring neurophysiological thresholds from the auditory midbrain of females. For this purpose, we examined multi-unit activity in the torus semicircularis. This region is the largest auditory center in the brain of anurans and exhibits robust auditory responses (Hall, 1994); it has been used to estimate auditory thresholds in a number of species of frogs (e.g., Diekamp and Gerhardt, 1992; Ryan et al., 1990; Wilczynski et al., 1993).

As a final experiment, we tested whether calling male bullfrogs would attack gray treefrogs. Other workers have reported that bullfrogs will feed on *H. versicolor* (Hinshaw and Sullivan, 1990) as well as species of treefrogs similar in size to *H. versicolor* (e.g., *H. gratiosa*: Murphy, 1992), and we have observed bullfrogs with gray treefrogs in their mouths. One of us (S. Tanner) also detected a pit tag implanted in a gray treefrog inside a male bullfrog. Calling male bullfrogs have been observed preying on frogs (*H. regilla*: M. P. Hayes, personal communication) and feeding on crayfish (M. Bee, unpublished observation). Nevertheless, we attempted to quantify the propensity for calling males to attack a moving treefrog while advertising. If this propensity is low, selection on female treefrogs to avoid specifically the source of bullfrog calls, and so also avoid conspecific males that might be near a calling bullfrog, could be weak or absent. Of course, avoidance of bullfrogs could be related to a general tendency to stay away from any abiotic or heterospecific sources of detectable sound (for a test of the second possibility, see Gerhardt et al., 1994*a*).

MATERIALS AND METHODS

Acoustic Stimuli

In 1993, advertisement calls of *H. versicolor* and *R. catesbeiana* were digitally

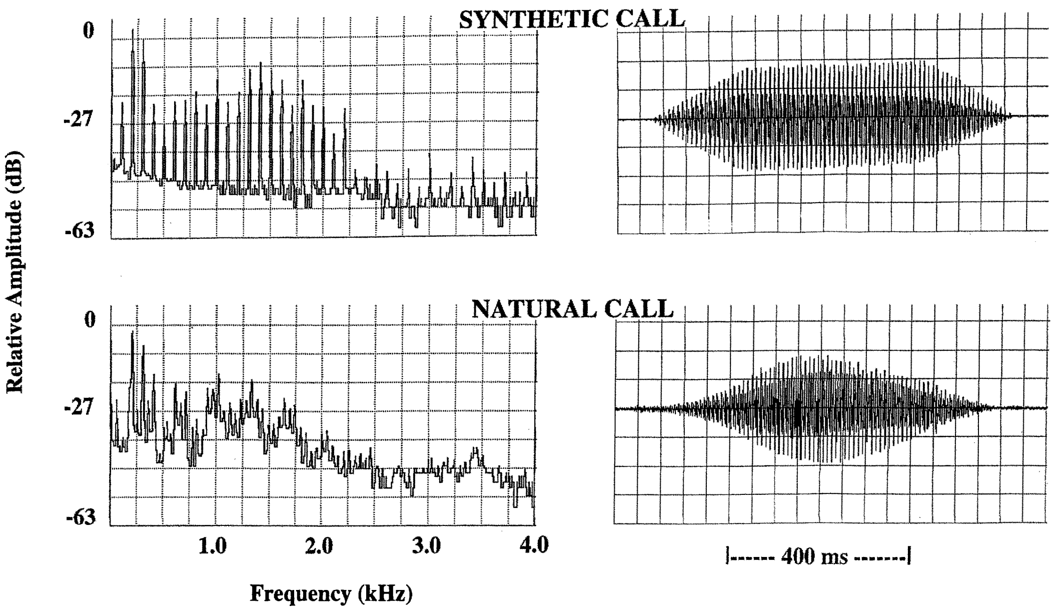

FIG. 1.—(Top) Oscillogram (right) and spectrum (left; 512 point FFT, frequency resolution = 29.5 Hz) of the synthetic bullfrog advertisement call used in the female choice tests and neurophysiology experiment. (Bottom) Oscillogram and spectrum of the natural bullfrog call used in the female choice test in 1997. Note that although the spectra of the two calls differ, both have a low frequency peak around 200–300 Hz, a dip near 500 Hz, and a broad region of increased energy from about 1000–1800 Hz. The periodicity of both calls is 100 Hz.

constructed using Fourier synthesis on an Amiga 2000 computer to resemble natural advertisement calls (sampling rate = 10 kHz, 8 bits/sample). The bullfrog call (Fig. 1, top) used in both tests of behavior and neurophysiology had 21 spectral components whose frequency and relative amplitude were derived from sonagrams presented by Capranica (1965). The call was 800 ms in duration with a linear rise and fall time of 200 ms. The call of *H. versicolor*, consisting of a train of 18 pulses, had two spectral components [1100 Hz (−6.0 dB) + 2200 Hz (0.0 dB)], and was 875 ms in duration with a 50 ms linear rise. The constituent pulses were 25-ms long with a 20-ms linear rise and a 5-ms nonlinear and concave down fall. In 1997, we repeated phonotaxis tests using a bullfrog call digitized from a recording of a Missouri male (Fig. 1, bottom). This exemplar was selected from recordings of a number of individuals based on the criterion of low background noise. Data based on tests with the natural vocalization increased our confidence in our previous results obtained with the synthetic bullfrog call. To conform to the hardware and software requirements of the systems used in 1997 (see below), the sampling rate of all calls was 16 kHz (16 bits/sample). In other respects, the synthetic call of *H. versicolor* was identical to the one synthesized in 1993.

Tests of Phonotaxis

In May 1993 and 1997, we captured female gray treefrogs in amplexus at a pond at the Baskett Wildlife Area near Ashland, Missouri. They were transported to a laboratory at The University of Missouri, refrigerated, and tested for phonotaxis the following day. Prior to testing, females were placed in an incubator and warmed to 20 C. We tested phonotaxis in a semi-anechoic chamber (Mr. Winter, Inc.; inside dimensions 7.0 m × 2.7 m × 3.7 m) floored with carpet and walled with wedges of acoustic foam (Soundcoat, Inc.). Temperature in the chamber was adjusted to 20 C. We observed phonotaxis of females on a television monitor located outside the chamber via a Sanyo VDC-2524 CCTV camera trained on the release point. The floor of the chamber was illuminated with an infrared light source (Panasonic model WV-CD810 CCTV).

During tests of female behavior in 1993, calls were output from the two audio ports of an Amiga 1000 computer running custom software. Two Analog-Digital-Systems (ADS) 200 speakers were used to broadcast calls of *H. versicolor* while a third speaker (Realistic Minimus 2.5) broadcast bullfrog calls. All calls, broadcast at a rate of 15/min, were amplified using two Realistic SA-10 stereo amplifiers. Sound levels were adjusted to 83 dB SPL (re 20 μPa, fast RMS setting) at the female's release point using a Gen Rad 1982 precision sound-level meter. This level falls within the range of variation for SPLs of the calls of both male bullfrogs and gray treefrogs at a distance of 1 m measured in nature (Gerhardt, 1975; Megala-Simmons, 1984; personal observations). A custom digitally controlled analog switch, attached to the parallel port of the computer, was used to direct the calls to the three speakers. In 1997, we used a Pentium chip based computer equipped with a SiliconSoft DacqPod 12B and running accompanying software to play treefrog and bullfrog calls. Calls of *H. versicolor* were amplified with two Nagra DSM amplifiers. Otherwise, procedures were the same as in 1993.

In Choice Test 1, the two ADS speakers were each placed 1 m from the central female release point. The third "bullfrog" speaker was placed adjacent to one of these. The computer was programmed to broadcast a call of *H. versicolor* followed by a bullfrog call from the adjacent speaker; these two calls alternated with broadcasts of the call of *H. versicolor* from the speaker on the opposite side of the arena. The arena layout and temporal arrangement of the stimuli are illustrated in Fig. 2. In 1997, 23 females were tested in the repeat of Test 1 using the natural bullfrog call.

As a control to check for attraction of females of *H. versicolor* to the bullfrog call, we conducted an additional set of choice tests in 1993. In these, either treefrog or bullfrog calls were broadcast from a single speaker (rate = 15/min). In Test 2a, phonotaxis of females was first tested with the treefrog stimulus. Then this stimulus was turned off, and females were returned to the release point. Following a 2 min rest period, they were given 5 min to approach the bullfrog stimulus from the same speaker. In Test 2b, females were tested with the treefrog stimulus both prior to and subsequent to exposure to the bullfrog stimulus. Exposure to conspecific calls was used to test whether or not females that failed to respond to the bullfrog call were inherently non-responsive or specifically non-responsive to the bullfrog calls. Test 2b is a more powerful test of this possibility because it tests whether females remain receptive throughout the exposure period to bullfrog calls.

For each test, a single female was placed in a small screen cage located at the release point. After playing stimuli for 30 s, we raised a lid on the cage using an attached cord that extended out of the chamber. We scored a positive response if the female approached to within 10 cm of a speaker within 5 min. Positions of the speakers and the side of the chamber from which the bullfrog call was broadcast were changed after every 2–3 tests. We used 25 females in Test 1 (1993), 10 in Test 2a, and five in Test 2b.

Bullfrog Behavior

In 1996, we tested whether calling male bullfrogs would attempt to feed on gray treefrogs at an artificial pond located at the Little Dixie Wildlife Area in Callaway County, Missouri. This site is within 17 km of our Ashland site, and has a large population of bullfrogs that are the focus of another study by one of us.

On the night of July 22, we tested the propensity of 10 calling male bullfrogs to attack a plastic fishing lure resembling a gray treefrog in color, size, and shape. Therefore, our experiment examined the response of bullfrogs to an approaching potential prey rather than an actual living female gray treefrog. We used a model frog rather than a gray treefrog to control

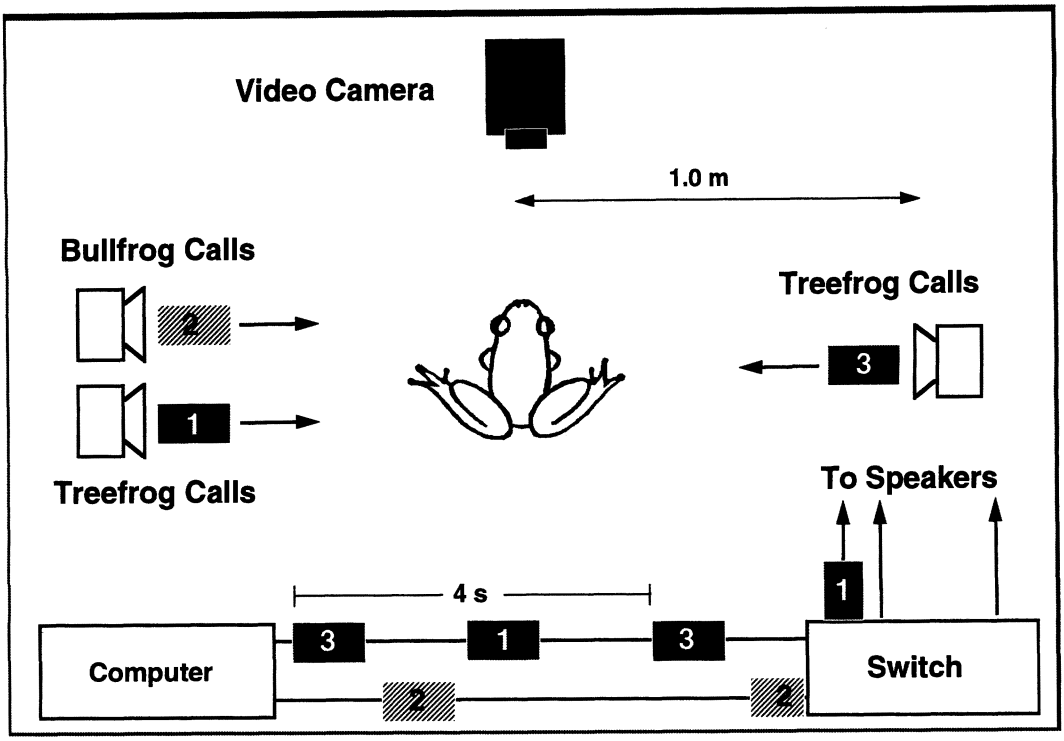

FIG. 2.—Diagram of the equipment, speaker arrangement, and stimulus timing used in Choice Test 1 in 1993. Amplifiers are not illustrated, and the female frog is drawn considerably larger than scale. Time goes from earlier on the right to later on the left for the stimulus sequence shown at the bottom of the figure. In 1997, the digitally controlled switch was not used (see text).

the movement of the experimental prey more effectively and to follow the recommendations of the Animal Care and Use Committee at The University of Missouri. The lure, which did not contain a hook, floated on the water surface. Using an angler's rod and reel, we cast the lure to the center of the pond (approximately 10 m from the shore) and retrieved the lure at a slow rate such that it passed within 30 cm of a bullfrog located at the edge of the pond. We recorded an attack if the bullfrog attempted to eat the lure. Each male was given three opportunities to strike. Following a successful attack, the lure was gently removed from the mouth of the male and he was returned to his calling site unharmed.

Neurophysiology

Between April and November of 1993, female gray treefrogs (n = 13) were anesthetized in a buffered (sodium bicarbonate, pH 7.0) 0.2% solution of 3-Aminobenzoic acid ethyl ester (MS-222; pH 7.2) for surgery. The midbrain was exposed using a dorsal approach through an aperture sliced in the fronto-parietal bone and a small tear in the surrounding membranes. A small drop of mineral oil was placed on the exposed brain tissue and lidocaine applied to the region around the wound.

Following a recovery period of at least 30 min, animals were immobilized with an intramuscular injection of 0.005 mg/g of tubocurarine chloride. The frogs were placed upright on a vibration isolation table (Kinetic Systems) in an air-conditioned (18–20 C), semi-anechoic chamber (Industrial Acoustics) lined with 10 cm thick acoustic foam (UNX4, NRC rating = 1.0, Illbruck, Inc.). Throughout each experiment, the animals were kept moist with a wet piece of paper towel draped over the back to aid cutaneous respiration. These procedures were approved by the Animal

Care and Use Committee of The University of Missouri.

Multi-unit activity was recorded with tungsten microelectrodes (0.5–1.0 MΩ; Micro Probe, Inc.) lowered into the torus semicircularis using a Burleigh 6000 microdrive. We used brain-surface landmarks and the range of microdrive depths that previous neurophysiological and histological studies of this species established as guides for placing a microelectrode in the torus semicircularis (Diekamp, 1990). Neural responses were first amplified (WPI DAM-5A Differential Preamplifier), high-pass filtered (150 Hz; Krohn-Hite 3202), and then amplified again (HP 461A). Neural responses were digitized (10 kHz) using an accelerated Amiga 500 computer equipped with a GVP A530 Turbo (40 MHz 68030) and an Applied Visions FutureSound™ sound digitizer (8 bits/sample).

Acoustic stimuli were amplified (Realistic SA-10 stereo amplifier) and broadcast from a Realistic Minimus-2.5 speaker mounted on a (0.78 m tall) pedestal 0.75 m from the subject. Prior to each recording session, sound levels were calibrated using the Gen Rad 1982 precision sound-level meter. The intensity level of all calls at the position of the subject was set to 83 dB SPL (re 20 μPa, fast RMS setting).

We determined the multi-unit auditory thresholds of 13 females to broadcasts of a 10-pulse version of the synthetic conspecific advertisement as well as the synthetic bullfrog call. Calls were presented at a rate of 15/min while a step attenuator (Leader Model LAT-45) was adjusted (with a resolution of 1 dB) until the multi-unit activity was no longer audible. The amplitude was then raised until auditory responses were just audible for at least three of five presentations of the stimulus. For some females, we also stored a copy of the digitized responses to both the bullfrog and conspecific call presented at 83 dB SPL, the same level used in the tests of female phonotaxis.

Behavior of Males of H. versicolor

On three nights in June and July of 1998, we tested the responses of six males of *H. versicolor* captured from the Ashland population to bullfrog calls. Males were placed on a cinderblock perch in an artificial pond in a greenhouse at The University of Missouri and initially stimulated to call with a low-amplitude broadcast of a recording of a natural chorus (70 dB SPL Fast RMS, Realistic 33–2050 sound level meter). The broadcast served also to simulate the natural sound environment at the Ashland pond. We recorded (Marantz PMD 360, Azden ECZ-660 unidirectional microphone) the vocalizations of each male for a 2 min no stimulus period and subsequently for 2 min during the broadcast of synthetic bullfrog calls (12/min; 82–83 dB SPL measured at the perch). The call was digitally looped using Applied Visions FutureSound™ software on an Amiga 600 computer, amplified (Realistic SA-10) and broadcast 1 m from the subject (tripod-mounted Heppner mid-range horn speaker). The male was simultaneously monitored with an A.M.T. M0-2 infrared night vision scope to check for movement away from the speaker. The call rate and pulses per call were determined from digitized recordings (Futuresound Software) and used to calculate the pulse effort of subjects (pulses per min) during the no stimulus and stimulus periods.

RESULTS

Female Choice Tests

In both 1993 and 1997, in Test 1, there was no significant difference in approaches to the speaker broadcasting conspecific calls adjacent to the source of bullfrog calls and the isolated speaker broadcasting conspecific calls (1993, 8:10, $P = 0.814$; 1997, 12:8, $P = 0.504$, two-tailed binomial test). The results from the 2 yr were not significantly different ($P > 0.250$, $G = 0.923$). Seven of the 25 females tested failed to respond in 1993, while in 1997, three of the 23 females tested failed to do so. If we are very conservative and count females that did not respond with females moving to the isolated speaker, the results from each year are still not significantly different from an expectation of a 50% approach probability to each source of conspecific calls (1993, 8:17, $P = 0.108$; 1997, 12:11, $P = 0.838$, two-tailed binomial test).

In Test 2a, all 10 females tested exhibited phonotaxis to a single speaker broadcasting conspecific calls. However, none of these females approached a single speaker broadcasting the bullfrog call, a significant difference in behavior ($P = 0.002$, two-tailed binomial test). Seven of the 10 females tested with the bullfrog call remained at the release site; three females wandered around the arena.

In Test 2b, all five females responded with positive phonotaxis to the conspecific calls both before and after broadcast of the bullfrog calls. None of the females oriented toward the source of the bullfrog calls ($P = 0.062$; two-tailed binomial test). Rather, when exposed to bullfrog calls, three females sat motionless and two wandered around the arena.

Bullfrog Behavior

Five of 10 calling bullfrogs attacked and attempted to swallow the lure resembling a gray treefrog. All attacks occurred on the first cast. The five males that did not attack the lure appeared to ignore it entirely.

Neurophysiology

Auditory thresholds to the bullfrog call were higher for all females ($\bar{x} = 55.5$ dB) than those to the conspecific call ($\bar{x} = 39.9$ dB, $P < 0.0001$, Wilcoxon test; Fig. 3; calculations of averages were performed after converting threshold values to μbars). The average difference in threshold was 16.8 dB SPL. Thresholds to the bullfrog call were well below the call intensity level of 83 dB used in the choice test and the multi-unit neural responses at this level were strong (Fig. 4).

Behavior of Males of H. versicolor

None of the subjects moved in response to the broadcasts of bullfrog calls. Nor did males reduce their pulse effort. Rather, all six males increased slightly the production of pulses during the stimulus relative to the no stimulus periods (by 2.1, 2.4, 2.7, 12.3, 14.4 and 25.1%). Additional information on calling behavior is provided in Table 1.

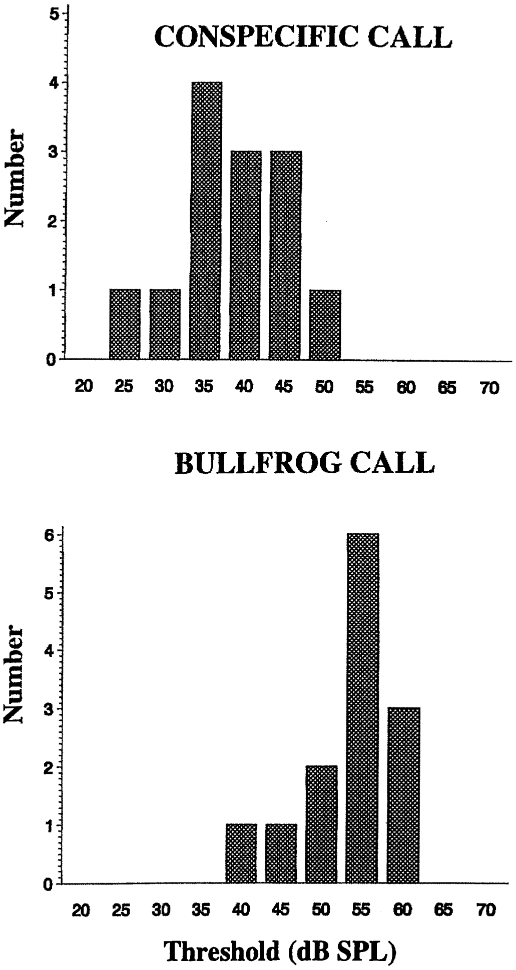

FIG. 3.—Histograms of multi-unit auditory thresholds measured to the bullfrog call and the gray treefrog call for 13 females.

DISCUSSION

Our three-speaker tests of female choice revealed no significant effect of bullfrog calls, either synthetic or natural, on the phonotaxis of *H. versicolor.* Furthermore, the results from both single speaker tests suggest that females ignored the heterospecific calls. The females used in Test 2a were responsive to conspecific calls just prior to testing with bullfrog calls. Females used in Test 2b were also responsive immediately after exposure to the bullfrog calls. Therefore, it is unlikely that a failure to orient towards the bullfrog call was due to an inherent lack of phonotactic responsiveness of our subjects.

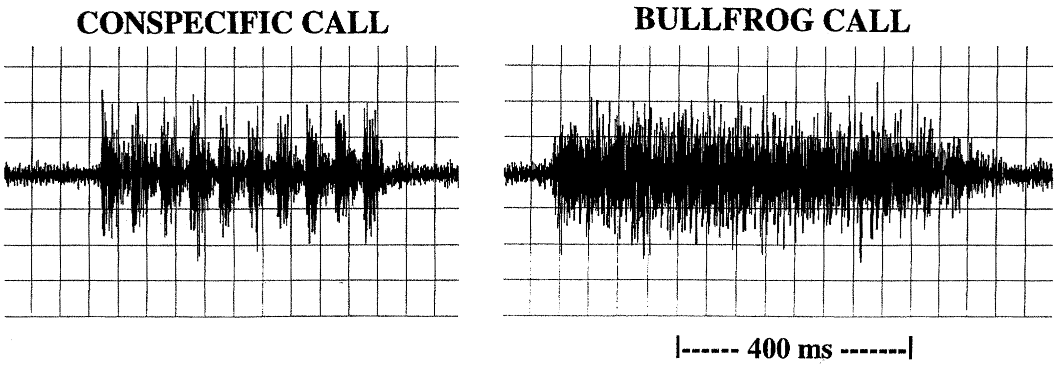

FIG. 4.—Oscillogram of a multi-unit auditory response to the bullfrog call and the gray treefrog call from the same female. For both calls, the stimulus SPL was 83 dB at the subject's position. The strength of neural responses of this female to the bullfrog call was typical of the frogs studied.

The behavior of males of *H. versicolor* suggests also that they perceived no threat during playbacks of bullfrog calls. In fact, their increase in pulse effort suggests that males were stimulated to call by bullfrog vocalizations. Whether this behavior increases the chances that males of *H. versicolor* on the ground or in the water will be preyed upon is unknown, although the bufonid, *Bufo marinus,* may use hearing to localize vocalizing anuran prey (Jaeger, 1976) as may bullfrogs feeding on *H. regilla* (M. P. Hayes, personal communication). Nevertheless, the hypothesis that females failed to respond to bullfrog calls because potential mates will not call near this predator was not supported by our data.

TABLE 1.—Vocal behavior of males of *H. versicolor* during no stimulus periods and during broadcasts of bullfrog calls. Pulse efforts and calls are for 2 min periods.

Male	Stimulus	Pulse effort	Calls	Pulses/call
258	None	347	23	15.1
258	Bullfrog	434	37	11.7**
332	None	433	31	14.0
332	Bullfrog	442	31	14.3
334	None	626	54	11.6
334	Bullfrog	643	57	11.3
338	None	627	49	12.8
338	Bullfrog	642	49	13.1**
365	None	464	38	12.2
365	Bullfrog	531	43	12.4
408	None	664	43	15.4
408	Bullfrog	746	60	12.4**

** $P < 0.01$, Wilcoxon two-sample test.

There are a number of other possible explanations for our results with females. One could argue that only seven of the 25 females used in Test 1 sensed danger and that their failure to show phonotaxis actually was a response to the bullfrog call. Because we did not test these females with single speaker broadcasts of the conspecific stimulus, we cannot rule out this possibility. However, we feel that it is an unlikely explanation for a number of reasons. The three speaker test was run on three different dates yet all seven females that failed to respond did so on the same date. In our extensive experience testing females of *H. versicolor* with conspecific calls, it has not been unusual to test a group of females that fail to show phonotaxis on a particular day. Five of the seven females that failed to respond hopped out of the release cage; however, only one of these moved to the side of the chamber opposite the speaker broadcasting the bullfrog calls. The results of the second single speaker test (Test 2b) also indicate that those females that remained in the release cage were probably not immobilized by exposure to the bullfrog call. When the conspecific stimulus was broadcast immediately after we stopped the broadcasts of the bullfrog calls, there was no unusual hesitation to respond, and all but one of the females moved to the speaker in <130 s ($\bar{x}$ = 62 s).

Perhaps females of *H. versicolor* did not hear the bullfrog calls at the intensity level that we used. Although we cannot comment on the subjective nature of their auditory experience, this seems unlikely. Our neurophysiological data indicate that auditory thresholds are about 17 db lower to the conspecific call than to the bullfrog call. Because the neural audiogram of *H. versicolor* is better matched to the frequency distribution of energy in the conspecific call than the bullfrog call, this result meets with expectations. Nevertheless, the stimulus intensity used in our choice tests was well above the threshold to the bullfrog call for all females, and the multiunit responses at 83 dB were strong (Fig. 4). Assuming attenuation due only to spherical spread and no masking due to noise, the auditory system of a female with an average threshold to the heterospecific call would respond at a distance of up to about 24 m from a male bullfrog calling at our playback SPL.

Neither positive or negative motor responses in our subjects were activated possibly because critical temporal features were absent from the bullfrog call. For example, Gerhardt and Doherty (1988) found that female gray treefrogs would not show phonotactic orientation to an unmodulated stimulus composed of two tones of 1.1 and 2.2 kHz (the dominant frequencies of the conspecific call). The amplitude modulation rate of the advertisement call of *H. versicolor* (about 20 Hz at 20 C) is well below the waveform periodicity of the bullfrog call (100 Hz). In single-speaker tests of phonotaxis, females of *H. versicolor* have occasionally approached the source of calls of *H. chrysoscelis* (Gerhardt and Doherty, 1988), and heterospecific pairings in the field have been observed (Gerhardt et al., 1994*b*). However, the AM rate of the call of *H. chrysoscelis* at 20 C is only about twice that of conspecific calls and the spectral structure is nearly identical.

In a recent study, Gerhardt et al. (1994*a*) failed to observe avoidance by females of *H. versicolor* of the source of the calls of *H. chrysoscelis*. They offer two explanations that are certainly valid for our study. First, some element of our experimental protocol precludes observation of avoidance behavior. For example, it is possible that females are no longer responsive to signals indicating danger after they enter amplexus. If a long refractory period exists, perhaps until oviposition, our test subjects, which had paired in the field, would exhibit behavior different from a female making her initial phonotactic approach at the pond. However, the limited available data indicate that amplexus does not alter the mating preferences of female anurans for conspecific calls (Murphy and Gerhardt, 1996).

Second, the results of our phonotaxis tests are an accurate representation of female behavior in the field. If this is so, selection on females to avoid the source of bullfrog calls may have been very weak for females of *H. versicolor*, either in general or in our collection population. At our main collection pond for *H. versicolor* (about 0.25 ha), we have never observed more than four calling male bullfrogs present at the same time. We typically find >200 female gray treefrogs at the pond during the breeding season (from mid-May to mid-July), and on a reproductively active night, 20 females may be found in amplexus. Many of these frogs pair in vegetation along the edge of the pond, although before oviposition, some females are clearly out of striking range of bullfrogs. One important factor influencing survival of a prey organism is the probabilty that it remains outside the perceptive field of the predator (Brodie et al., 1991). During our tests with male bullfrogs, the lure had to be within a few centimeters of a male before he would lunge at it. Therefore, although real, the chance that a female will be taken by a calling bullfrog may be quite low on any given night at our pond.

Both the first and second explanations could be tested in the field, albeit with some difficulty, by observing female movements in the vicinity of calling bullfrogs or attempting to divert females during a phonotactic approach with broadcasts of bullfrog calls. If females did respond, it would indicate a problem with our testing protocol as described above. If they did not, the data would corroborate our laboratory results. It would also be interesting to test females from ponds with a much greater density of calling male bullfrogs than our study site in Ashland, Missouri. The possibility remains that gray treefrogs from other areas who differ genetically or experientially from our test subjects behave differently from them. For example, the sensitivity of females to the presence of a predator during mate choice is influenced by the risk of predation among different natural populations of guppies in Trinidad (Godin and Briggs, 1996).

Although we failed to see any effect of bullfrog calls on the behavior of females, females may behave in other ways that reduce predation risk while searching for a mate. Gerhardt et al. (1996) found that the willingness of females to increase travel distance to a speaker broadcasting longer (preferred) calls was sensitive to the distance they had to move during two-stimulus choice tests. While one explanation is that females will tolerate only a limited increase in their energetic costs of movement during mate choice, another is that females were attempting to limit risk. In addition to suffering predation by bullfrogs, *H. versicolor* is consumed by green frogs (*Rana clamitans*), water snakes (*Nerodia sipedon*), and ribbon snakes (*Thamnophis proximus*) at our pond site in Ashland. Giant water bugs (Belostomatidae) also may eat gray treefrogs (Hinshaw and Sullivan, 1990; our observations). Increased travel in or near a pond would subject a female to greater susceptibility to these and perhaps other predators.

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