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Supplementary Materials for

Risky Ripples Allow Bats and Frogs to Eavesdrop on a Multisensory Sexual Display

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Published 24 January 2014, *Science* **343**, 413 (2014)
DOI: 10.1126/science.1244812

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Materials and Methods

<u>Frog experiments</u>

We collected calling male túngara frogs 1 - 3 hours after sunset in and around Gamboa, Panamá, in November 2012 and tested them at the Smithsonian Tropical Research Institute (STRI) laboratory. Males were toe-clipped for individual recognition after the experiment and released back to the field.

Frogs were tested in a pool (80 x 34 x 4 cm) filled with 4.5 L of rainwater in a hemi-anechoic chamber (ETS-Lindgren). We broadcast calls through a loudspeaker (Nanosat 5.0 connected to a NAD C316BEE amplifier) placed at the short side of the pool; a small metal tube attached to the pool's side created ripples by directing a puff of air onto the water surface (Fig. S1A). We positioned the metal tube in front of the speaker 11 mm from the surface and 10 mm from the side of the pool. The tube was connected to an electro-mechanical control unit that pushed a consistent volume (20 mL) of air. This machine was previously used to drive vocal sac inflation of a robotic frog, see (*13*) for design. We attached both loudspeaker and the machine that created ripples to a desktop computer outside the test chamber. We used a synthetic call consisting of a whine plus one chuck played at 0.5 calls/s and 82 dB SPL (re. 20 μPa at 50 cm, measured with Extech SPL-meter type 407764, set to C-weighted, fast and max; *13*). The ripple machine produced a series of 3-4 water waves with main frequencies ranging from 7.5 - 12 Hz (as measured from video and photos) at a speed of ~ 30 cm/s (as measured from videos and calculated using formula in *26*). Wave amplitude at the source was set to 1.0 mm and we estimated amplitude at different distances using the attenuation figure as given by (*29*). Additionally, we checked levels by holding fine-grain sand paper (which has low capillarity) perpendicular to the water surface and by measuring the water line with a digital calliper before and after ripple playback, at a distance of 7.5 cm from playback source. Starting with call onset, ripples were played at a height of ~ 0.3 mm at 7.5 cm, a natural amplitude of a similar-sized frog species (*17*), consistent with our observations of túngara ripple production in the field and in captivity.

We designed two experiments to test how male túngara frogs respond to ripples with and without adding sound. In the first experiment, males were exposed for 1 min to a unisensory (sound playback accompanied by ripple playback outside the pool) or a multisensory treatment (sound plus ripple playback within the pool). For the unisensory treatment, the ripple playback outside the experimental pool controlled for any noise that might have been generated by blowing a puff of air onto the water surface. These two treatments were followed by 30 s of either silence or ripple only playback. In the second experiment, we tested males at different distances from the playback source. Males were placed in the pool and constrained by wire mesh cage (20 x 13 cm; mesh 6 x 6 mm; Fig S1; Movie S2) with a transparent plastic top. Prior to each experiment, males were stimulated to call using a low-amplitude 5-min playback of a natural frog chorus. In the first experiment, we situated the focal male 15 cm from the playback site. In the second experiment, we placed the focal male either 7.5 or 30 cm from the playback site and stimulated with chorus playback in between the 1-min trials until they started calling.

Order of the trials was randomized. Trials with no acoustic response of the vocal male were repeated once.

We recorded males using an IR-sensitive camera (Everfocus, model EHD500), attached to a desktop computer and an omnidirectional microphone (Sennheiser ME62) attached to a Marantz recorder (PMD660, sample rate 44.1 kHz). We quantified the number of calls produced throughout the 1-min trials to calculate call rate (known to reflect the level of aggressive response *30*) and noted if males deflated during trials.

Bat experiments

We collected frog-eating bats (*Trachops cirrhosus*) from Soberanía National Park, Panamá between November 2012 and April 2013 (N = 10 adult bats, 6 males and 4 non-reproductive females, no clear differences observed between sexes). Bats were caught with mistnets set along streambeds, 0 - 2 hours after sunset, or collected with handnets from roosts during the day. We injected each individual bat with a subcutaneous passive integrative transponder (Trovan, Ltd.) for individual recognition, and released it in a large outdoor flight cage (5m x 5m x 2.5 m) for training and testing (see *31* for a more detailed description). All bats were released at their capture site after the experiment.

The experimental setup consisted of two pools (80 x 34 x 2 cm, filled with 4.5 L water) placed 1 m apart (Fig. S1B). Each pool had a model frog (~2 cm in length; *13*) on the side of the pool furthest from the bat's perch. The frog model was attached to a smooth-surfaced Plexiglas platform with a 5 cm radius, echo-acoustically mimicking a small puddle (*24*). The platform (with holes drilled underneath the frog model to allow air-borne sound transfer) was attached to a wooden box, placed above a speaker (Peerless, 2.5 inch), 10 cm above ground level and partly covering the pool (Fig. S1C). We generated ripples by blowing air through a small metal tube, attached to an electronically-actuated piston. The metal tube was placed 11 mm above the pool's surface (outside the bat's view). The tube was connected to a custom-made gas-relay station, which would release 20 mL of air from a compressor tank upon receiving a 19 kHz actuation signal from a laptop (Lenovo Thinkpad). Blowing air on a water surface produces an air-borne sound that could be used as a cue by the bats as well. To control for this as a potential cue, we placed a plastic cup, filled with 0.2 L of water, at the control pool, underneath the wooden box and simultaneously blew air on both the water surfaces of both ripple pool and control cup, using two gas-relay stations. Prior to each experiment, the amplitudes of the sounds emanating from the control cup and ripple machine were balanced using the SPL-meter.

Bats were first trained to fly to the testing area by offering small pieces of fish placed on one of the frog models while broadcasting a synthetic frog call from the speaker underneath the model. Playback side (left or right) was altered to avoid side biases. After training, each bat was given (up to 24) two-choice tests between the ripple and control pools. Trials started with bats on the perch 3 - 4 m away from the playback site and the experimenter broadcasting identical calls from both speakers while driving the gas-relay stations simultaneously. As soon as the bat left the perch, we turned off both speaker and gas-relay systems (in nature, frogs often cease calling when a bat approaches; *20*). To maintain motivation bats were rewarded with fish pieces on the frog model on every third trial. Fish was presented on both models to prevent the bats from learning to fly to the side where ripples were played.

In addition to the normal treatment, we tested bats on trials in which we impeded the use of sonar cues by placing a screen partially covered with leaf litter over each of the two pools (creating a highly cluttered environment). As above, one pool had ripples, the other pool did not. Treatments (both pools cluttered and both pools uncluttered) were presented in blocks of 6 trials and order plus side of playback were randomized and balanced across trials.

The behavior of the bats was video recorded (Sony nightshot DCR-SR45 camcorder) and observed using night vision goggles. The flight cage was illuminated only with infrared lights (CMVision IR100). During trials a bat would fly to the platform, hover over one of the models and occasionally attack. Bats almost always attacked the side over which they hovered and we therefore used hovering as response measure (the subset of trials with attacks did not show different results). Three bats developed a side bias during the experiment (defined as choosing a particular side more than 6 times in a row) and were subsequently rewarded only on the opposite side, until the side bias disappeared (training trials conducted in the absence of ripple playback). Training trials and trials with no clear choice were discarded from further analyses (38 discarded trials out of a total of 265).

<u>Assessment of detection limits and perceptual salience</u>

We recorded echolocation signals of 6 bats, emitted from their foraging perch shortly before an attack flight. We used ultrasonic recording equipment (G.R.A.S. microphone amplified by 40 dB by G.R.A.S. amplifiers connected to a Avisoft ultrasound gate and Lenovo Thinkpad) to record calls on-axis at a sampling rate of 300 kHz and a distance of 3 - 5 m. Calls were analysed in Avisoft SASLab Pro (FFT = 256, overlap = 98%) and the average call characteristics, such as signal duration (1.6 ms), start and end frequency of the 1st harmonic (74.9 down to 49.8 kHz), lower and upper frequency limits (47.2 - 100 kHz, minus 20 dB below peak amplitude) and peak frequency (72.3 kHz) were used to create a synthetic call. The synthetic call was used for an ensonification experiment and broadcast with ultrasonic playback equipment (Scanspeak ultrasonic speaker connected to an Avisoft sound gate and a Lenovo Thinkpad) at a rate of 30 calls/s to the experimental pool, at a distance of 30 cm from the water surface and at different angles, varying from 45 - 90°, with the latter being perpendicular to the surface (see Fig. 2). Echoes were recorded with the ultrasonic G.R.A.S microphone placed 5 cm away from the speaker directed at the water surface. The pool itself, plus part of the water surface was covered with sound-absorbing foam to ensure that we only recorded returning echoes from the playback angle of interest. Calls were played in bouts of 0.2 s to the water surface during ripple and control playback. We selected the first three and last three echoes from a call bout and measured the peak frequency and amplitude. We estimated detection limits using measurements and methods described in Surlykke *et al*

(*32*), complemented by target strengths (difference in dB) obtained from the ensonification experiment.

Data analyses

We analysed response measures with generalized linear mixed models (GLMM) in R v. 3.0 (*33*) with error distribution structure and link-function depending on model fit. Male call rate was analysed with a Gamma error distribution and identity link-function (using Penalized Quasi-Likelihood and Wald-statistics for significance in the package MASS). Deflation was analysed with a binomial error distribution, a probit-link function and likelihood ratio tests for significance (in the package lme4). We added either ripple playback (yes or no for experiment I) or distance category (7.5 or 30 cm for experiment II) as fixed effect. We compared the bat attacks on the ripple pool (successes) with attacks on the control pool (failures) in a GLMM model with a binomial error structure and a logit-link function. Clutter treatment was added as fixed effect. We tested for a significant preference of ripples over control (deviance of 50%) and used a likelihood ratio test for the effect of clutter treatment on preference for ripples. All models included individual bat ID and playback order as random effects.

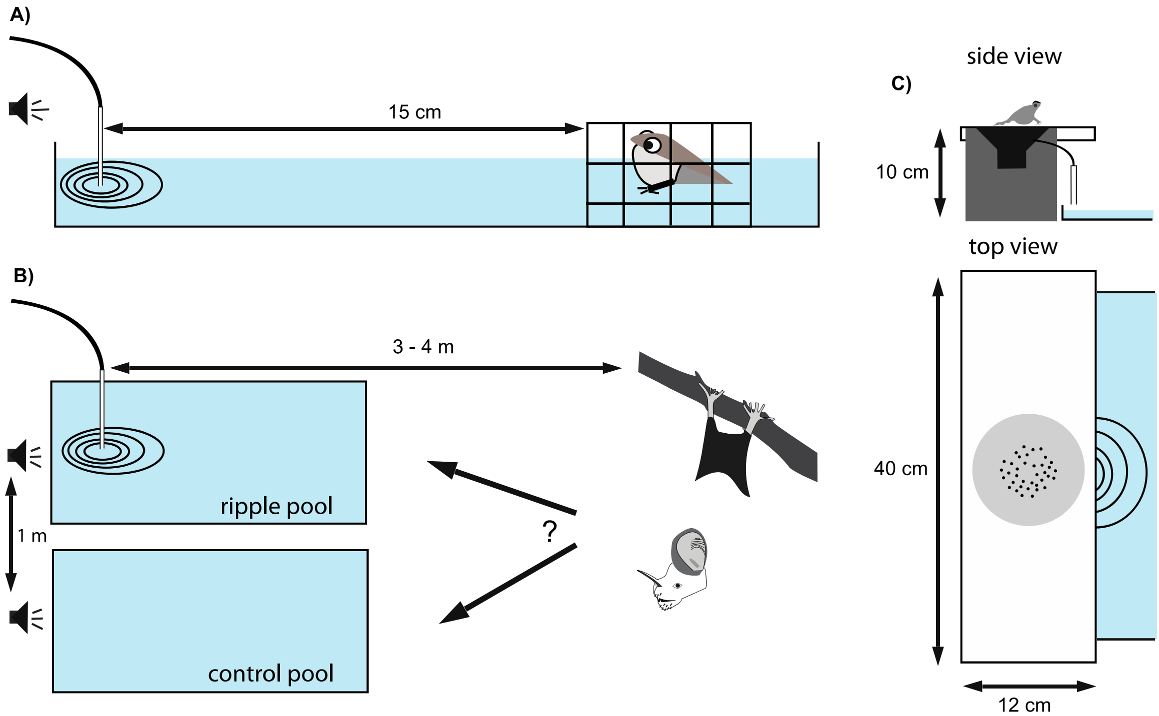

Fig. S1.

Experimental Setup. (**A**) frog experiment; (**B**) two-choice experiment with bats; (**C**) detail of ripple and sound playback setup used during bat experiments.

Movie S1. Calling male túngara frogs

Three clips of frogs recorded in the field. Notice vocal sac movement and associated water ripples.

Movie S2. Example ripple effect on male frogs

Males in mesh cage (with and without plastic top) respond as soon as ripples are added to sound.

Movie S3. Example trials of bat experiments

Bats (on perch outside video view at start of trial) prefer to attack frog models at ripple pools.

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