Supplementary Materials for

Risky ripples allow bats and frogs to eavesdrop on a multisensory sexual

display

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    Movies S1 to S3

Materials and Methods

Frog experiments

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 15 - 24 Hz (as measured from video and photos) at a speed of ~ 25 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.

70 Order of the trials was randomized. Trials with no acoustic response of the vocal male
71 were repeated once.
72
73   We recorded males using an IR-sensitive camera (Everfocus, model EHD500),
74 attached to a desktop computer and an omnidirectional microphone (Sennheiser ME62)
75 attached to a Marantz recorder (PMD660, sample rate 44.1 kHz). We quantified the
76 number of calls produced throughout the 1-min trials to calculate call rate (known to
77 reflect the level of aggressive response [30]) and noted if males deflated during trials.
78
79 <u>Bat experiments</u>
80
81   We collected frog-eating bats (*Trachops cirrhosus*) from Soberanía National Park,
82 Panamá between November 2012 and April 2013 (N = 10 adult bats, 6 males and 4 non-
83 reproductive females, no clear differences observed between sexes). Bats were caught
84 with mistnets set along streambeds, 0 - 2 hours after sunset, or collected with handnets
85 from roosts during the day. We injected each individual bat with a subcutaneous passive
86 integrative transponder (Trovan, Ltd.) for individual recognition, and released it in a large
87 outdoor flight cage (5m x 5m x 2.5 m) for training and testing (see [31] for a more detailed
88 description). All bats were released at their capture site after the experiment.
89
90   The experimental setup consisted of two pools (80 x 34 x 2 cm, filled with 4.5 L
91 water) placed 1 m apart (Fig. S1B). Each pool had a model frog (~2 cm in length; [13]) on
92 the side of the pool furthest from the bat's perch. The frog model was attached to a
93 smooth-surfaced Plexiglas platform with a 5 cm radius, echo-acoustically mimicking a
94 small puddle ([24]). The platform (with holes drilled underneath the frog model to allow
95 air-borne sound transfer) was attached to a wooden box, placed above a speaker
96 (Peerless, 2.5 inch), 10 cm above ground level and partly covering the pool (Fig. S1C).
97 We generated ripples by blowing air through a small metal tube, attached to an
98 electronically-actuated piston. The metal tube was placed 11 mm above the pool's surface
99 (outside the bat's view). The tube was connected to a custom-made gas-relay station,
100 which would release 20 mL of air from a compressor tank upon receiving a 19 kHz
101 actuation signal from a laptop (Lenovo Thinkpad). Blowing air on a water surface
102 produces an air-borne sound that could be used as a cue by the bats as well. To control for
103 this as a potential cue, we placed a plastic cup, filled with 0.2 L of water, at the control
104 pool, underneath the wooden box and simultaneously blew air on both the water surfaces
105 of both ripple pool and control cup, using two gas-relay stations. Prior to each
106 experiment, the amplitudes of the sounds emanating from the control cup and ripple
107 machine were balanced using the SPL-meter.
108
109   Bats were first trained to fly to the testing area by offering small pieces of fish
110 placed on one of the frog models while broadcasting a synthetic frog call from the
111 speaker underneath the model. Playback side (left or right) was altered to avoid side
112 biases. After training, each bat was given (up to 24) two-choice tests between the ripple
113 and control pools. Trials started with bats on the perch 3 - 4 m away from the playback
114 site and the experimenter broadcasting identical calls from both speakers while driving
115 the gas-relay stations simultaneously. As soon as the bat left the perch, we turned off both

116 speaker and gas-relay systems (in nature, frogs often cease calling when a bat
117 approaches; *20*). To maintain motivation bats were rewarded with fish pieces on the frog
118 model on every third trial. Fish was presented on both models to prevent the bats from
119 learning to fly to the side where ripples were played.
120   In addition to the normal treatment, we tested bats on trials in which we impeded the
121 use of sonar cues by placing a screen partially covered with leaf litter over each of the
122 two pools (creating a highly cluttered environment). As above, one pool had ripples, the
123 other pool did not. Treatments (both pools cluttered and both pools uncluttered) were
124 presented in blocks of 6 trials and order plus side of playback were randomized and
125 balanced across trials.
126
127   The behavior of the bats was video recorded (Sony nightshot DCR-SR45 camcorder)
128 and observed using night vision goggles. The flight cage was illuminated only with
129 infrared lights (CMVision IR100). During trials a bat would fly to the platform, hover
130 over one of the models and occasionally attack. Bats almost always attacked the side over
131 which they hovered and we therefore used hovering as response measure (the subset of
132 trials with attacks did not show different results). Three bats developed a side bias during
133 the experiment (defined as choosing a particular side more than 6 times in a row) and
134 were subsequently rewarded only on the opposite side, until the side bias disappeared
135 (training trials conducted in the absence of ripple playback). Training trials and trials with
136 no clear choice were discarded from further analyses (38 discarded trials out of a total of
137 265).
138
139 <u>Assessment of detection limits and perceptual salience</u>
140
141   We recorded echolocation signals of 6 bats, emitted from their foraging perch
142 shortly before an attack flight. We used ultrasonic recording equipment (G.R.A.S.
143 microphone amplified by 40 dB by G.R.A.S. amplifiers connected to a Avisoft ultrasound
144 gate and Lenovo Thinkpad) to record calls on-axis at a sampling rate of 300 kHz and a
145 distance of 3 - 5 m. Calls were analysed in Avisoft SASLab Pro (FFT = 256, overlap =
146 98%) and the average call characteristics, such as signal duration (1.6 ms), start and end
147 frequency of the 1st harmonic (74.9 down to 49.8 kHz), lower and upper frequency limits
148 (47.2 - 100 kHz, minus 20 dB below peak amplitude) and peak frequency (72.3 kHz)
149 were used to create a synthetic call. The synthetic call was used for an ensonification
150 experiment and broadcast with ultrasonic playback equipment (Scanspeak ultrasonic
151 speaker connected to an Avisoft sound gate and a Lenovo Thinkpad) at a rate of 30 calls/s
152 to the experimental pool, at a distance of 30 cm from the water surface and at different
153 angles, varying from 45 - 90°, with the latter being perpendicular to the surface (see Fig.
154 2). Echoes were recorded with the ultrasonic G.R.A.S microphone placed 5 cm away
155 from the speaker directed at the water surface. The pool itself, plus part of the water
156 surface was covered with sound-absorbing foam to ensure that we only recorded
157 returning echoes from the playback angle of interest. Calls were played in bouts of 0.2 s
158 to the water surface during ripple and control playback. We selected the first three and
159 last three echoes from a call bout and measured the peak frequency and amplitude. We
160 estimated detection limits using measurements and methods described in Surlykke *et al*

161 (*32*), complemented by target strengths (difference in dB) obtained from the
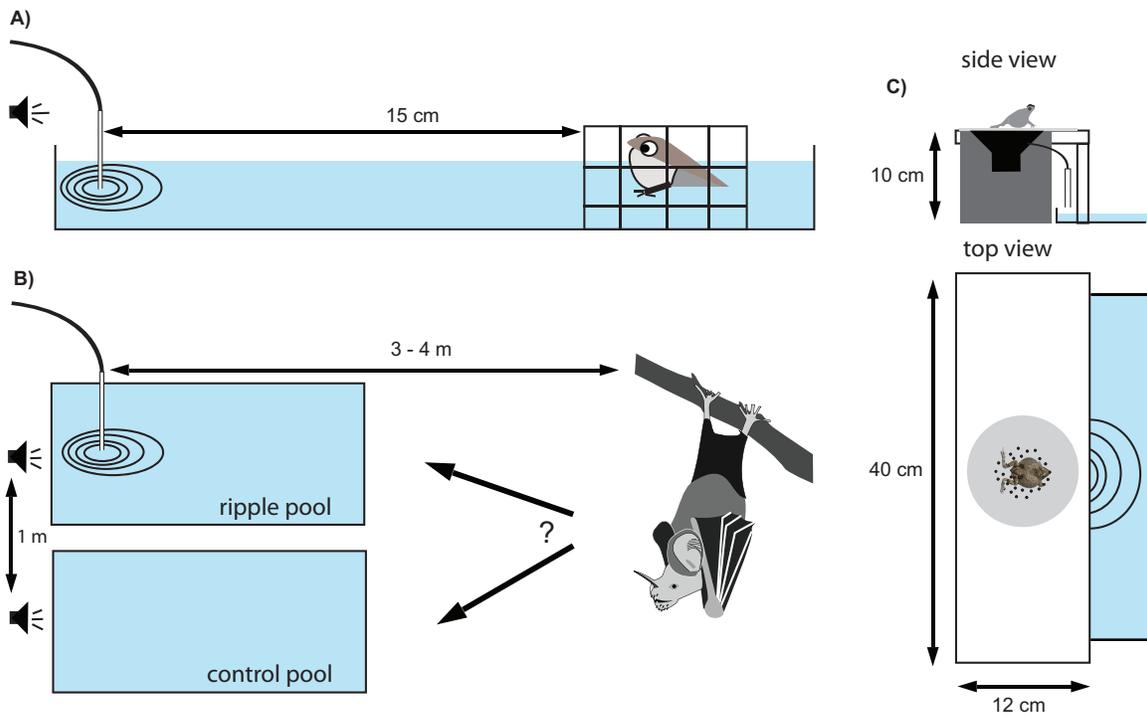162 ensonification experiment.
163
164 <u>Data analyses</u>
165
166   We analysed response measures with generalized linear mixed models (GLMM) in
167 R v. 3.0 (*33*) with error distribution structure and link-function depending on model fit.
168 Male call rate was analysed with a Gamma error distribution and identity link-function
169 (using Penalized Quasi-Likelihood and Wald-statistics for significance in the package
170 MASS). Deflation was analysed with a binomial error distribution, a probit-link function
171 and likelihood ratio tests for significance (in the package lme4). We added either ripple
172 playback (yes or no for experiment I) or distance category (7.5 or 30 cm for experiment
173 II) as fixed effect. We compared the bat attacks on the ripple pool (successes) with
174 attacks on the control pool (failures) in a GLMM model with a binomial error structure
175 and a logit-link function. Clutter treatment was added as fixed effect. We tested for a
176 significant preference of ripples over control (deviance of 50%) and used a likelihood
177 ratio test for the effect of clutter treatment on preference for ripples. All models included
178 individual bat ID and playback order as random effects.
179
180

181
182 **Fig. S1.**
183 Experimental Setup. (**A**) frog experiment; (**B**) two-choice experiment with bats; (**C**)
184 detail of ripple and sound playback setup used during bat experiments.

185 **Movie S1. Calling male túngara frogs**
186 Three clips of frogs recorded in the field. Notice vocal sac movement and associated
187 water ripples.

188 **Movie S2. Example ripple effect on male frogs**
189 Males in mesh cage (with and without plastic top) respond as soon as ripples are added to
190 sound.

191 **Movie S3. Example trials of bat experiments**
192 Bats (on perch outside video view at start of trial) prefer to attack frog models at ripple
193 pools.
194