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Wind- and Rain-Induced Vibrations Impose Different Selection Pressures on Multimodal Signaling

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abstract: The world is a noisy place, and animals have evolved a myriad of strategies to communicate in it. Animal communication signals are, however, often multimodal; their components can be processed by multiple sensory systems, and noise can thus affect signal components across different modalities. We studied the effect of environmental noise on multimodal communication in the túngara frog (*Physalaemus pustulosus*). Males communicate with rivals using airborne sounds combined with call-induced water ripples. We tested males under control as well as noisy conditions in which we mimicked rain- and wind-induced vibrations on the water surface. Males responded more strongly to a multimodal playback in which sound and ripples were combined, compared to a unimodal sound-only playback, but only in the absence of rain and wind. Under windy conditions, males decreased their response to the multimodal playback, suggesting that wind noise interferes with the detection of rival ripples. Under rainy conditions, males increased their response, irrespective of signal playback, suggesting that different noise sources can have different impacts on communication. Our findings show that noise in an additional sensory channel can affect multimodal signal perception and thereby drive signal evolution, but not always in the expected direction.

Keywords: sexual selection, multimodal communication, vibrational noise, signal detection, *Physalaemus pustulosus*.

Introduction

Sensory systems process environmental stimuli and have evolved to aid animals in decision making, such as where to forage, when to fight, or with whom to mate (Dall et al. 2005; Bradbury and Vehrencamp 2011; Stevens 2013). Sensory systems detect a wide range of stimuli and are typically optimized to process relevant cues against a background of irrelevant stimuli, often referred to as noise (Moore 2003; Brumm and Slabbekoorn 2005; Klein et al. 2013). The hearing systems of mammals and birds, for example, have evolved narrowband frequency selective filters that improve cue detection in broadband acoustic noise (Dooling et al. 2000; Moore 2003). The impact of environmental noise has been extensively studied in the context of foraging and communication, in particular for the acoustic domain (Klump 1996; Langemann et al. 1998; Siemers and Schaub 2011; Francis and Barber 2013; Swaddle et al. 2015). However, animals use multiple senses to process their surroundings (Pettigrew et al. 1998; Von der Emde and Bleckmann 1998; Ben-Ari and Inbar 2014), and the impact of environmental noise should therefore be assessed in multiple sensory modalities (Halfwerk and Slabbekoorn 2015).

Assessing the impact of environmental noise across a range of sensory modalities is especially important for sexual signaling (Kunc et al. 2014; Halfwerk and Slabbekoorn 2015). Noise in one perceptual channel can affect overall signal production or perception and thereby impose selection on signal components in additional perceptual channels (Wilson et al. 2013; Kunc et al. 2014; Halfwerk and Slabbekoorn 2015). Furthermore, many sexual displays generate cues that can be perceived with multiple sensory systems (Partan and Marler 1999; Hebets and Papaj 2005; Higham and Hebets 2013). Sexual displays of birds can often be seen as well as heard, and frogs often call by inflating and deflating a vocal sac, thus simultaneously providing acoustic and visual cues (Narins et al. 2003; Stafstrom and Hebets 2013; Taylor and Ryan 2013). The multimodal cues of sexual displays are often tightly coupled through the production mechanism, thereby limiting selection pressures on individual components (Cooper and Goller 2004; Halfwerk et al. 2014*a*). Acoustic noise may hamper detection of acoustic signal components, for example, and will thereby indirectly affect selection pressures acting on visual components. Understanding selection pressures acting on multimodal signals thus requires knowledge of how animals process signal components under a variety of different sensory conditions.

We study a multimodal communication system that occurs at the boundary between air and water, namely, frogs

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calling from puddles. Calling from the water surface generates an airborne pressure wave (for convenience, referred to throughout as "sound") and at the same time creates water surface vibrations (or "call-induced ripples"). Water surface vibrations are used across the animal kingdom for foraging or communication and are processed through a variety of different sensory systems (Wilcox 1979; Bleckmann and Barth 1984; Elepfandt and Wiedemer 1987; Bleckmann 1994). Frogs have been shown to communicate with water surface waves by hitting the surface with their front legs, by kicking their hind legs, or through call-induced ripples (Walkowiak and Munz 1985; Halfwerk et al. 2014*a*). These ripples can be sensed through the lateral line system in some frog species and are known to play an important role in rival communication (Elepfandt and Simm 1985; Walkowiak and Munz 1985; Hobel and Kolodziej 2013; Halfwerk et al. 2014*b*). Wind and rain are the two most important abiotic sources of vibrational noise, but how these environmental factors shape perception and behavior of animals living on the water surface remains largely unknown (Bleckmann 1994).

Here we examine the impact of wind- and rain-induced vibrational noise traveling along the water surface on multimodal communication of the túngara frog (*Physalaemus pustulosus*). Males of this species gather at night in puddles and display to attract females and fend off rivals (Ryan 1985; Bernal et al. 2009; Halfwerk et al. 2016). A calling male produces a sound, the primary component of the signal that is necessary to elicit a response from receivers. Males also inflate and deflate a large vocal sac during calling, and this movement induces water surface waves, or ripples, that function as a secondary component (Halfwerk et al. 2014*a*). Males do not respond to these call-induced ripples when played to them in the absence of sound. Males respond to the multimodal signal only in the presence of sound and can use distance-dependent cues associated with the ripple signal to improve their assessment of a rival's location (Halfwerk et al. 2014*a*, 2014*b*). We recorded male multimodal signaling using a microphone and a laser Doppler vibrometer (LDV) and measured propagation of sound and call-induced ripples over different distances and for different water depths to assess detection ranges. We quantified male evoked vocal responses to a nearby rival that emitted a unimodal, sound-only signal or a multimodal signal, to which we added call-induced ripples to the sound. We compared male baseline call behavior and evoked response under control conditions as well as in the presence of simulated wind- and rain-induced vibrations.

Material and Methods

Field and Lab Recordings

We made several recordings of water surface vibrations at various breeding sites in Soberanía National Park, Panama, in September 2014. We recorded from puddles with and without calling males under different environmental conditions (e.g., during heavy rain). We did not experience much wind during these recording sessions, and we therefore based our wind treatment on wind speed data from a nearby weather station (see below). We also recorded males and carried out playback and transmission experiments in our labs in Gamboa, Panama, and Austin, Texas. We collected calling male túngara frogs as well as pairs in amplexus 1–3 h after sunset. Males and females were toe clipped for individual recognition after the experiment and released back to the field. All experiments with frogs were licensed and approved by the Smithsonian Tropical Research Institute (Institutional Animal Care and Use Committee permit 2014-0805-2017) and the Autoridad Nacional del Ambiente de Panamá (SE/A-82-14).

We recorded the sound of calling males with a directional microphone (Sennheiser ME66) connected to a field recorder (Marantz PMD660, sampling rate of 44.1 kHz, 16 bits, recording level fixed at 7). We recorded water surface vibrations using an LDV (Polytec PDV-100, set to 5 or 20 mm/s/V) connected to the same field recorder. Recordings were either mono (LDV only) or stereo (microphone and LDV). The laser was mounted on a tripod and pointed downward (90°) at a piece of retroreflective tape (0.5 cm × 0.5 cm) attached to a strip of aluminum foil floating on the water surface. The aluminum strip (3 cm × 1 cm) had a small slit (1.5 cm × 0.3 cm) at one side that was used to hold it in place by two metal wires that were attached to a heavy metal base. The laser dot was pointed at the reflective tape attached at the other end of the strip, at least 1 cm away from the two metal wires. The aluminum strip could be placed at different distances (2.5–40 cm) from a focal male. Laser output was always calibrated in the lab prior to (field) recording (see below for details on calibration using a vibrational reference signal and oscilloscope).

Prior to recording and testing, we placed individual males in small plastic containers with a receptive female to increase their motivation to call. The test male was then taken from the container after being with a female for 5–15 min and transferred to a small cage (hereafter "ripple cage") that was transparent to water surface waves. The ripple cage consisted of a circular plastic base (8-cm diam.) and a circular plastic top ring (8-cm diam. with a 4-cm-diam. hole in the center) that was supported by three metal rods (0.4-cm diam.). A ring of evenly spaced nylon monofilament (0.05-mm fishing line every 0.5 cm) kept males inside the cage. A camera (mini 1/4-inch CCTV camera, 2.8-mm lens) was mounted on top of the cage to monitor male behavior. A microphone (Sennheiser ME62) on a small tripod was placed at 50 cm from the ripple cage to record the acoustic component of a male's call. The LDV was used to record the water surface waves produced by calling males (also referred to as call-induced ripples for simplicity) at different distances (foil holding reflective tape placed at 2.5, 5, 10, 20, and 40 cm from the center of the cage) to assess attenuation rates. Additionally, we carried out a transmission experiment in which we generated an artificial call-induced ripple (details on playback methods below) and recorded it with the LDV over the same distances and in different water depths (0.25, 0.5, 1.0, 2.0, and 4.0 cm). Recordings were carried out in a large pool (150-cm diam.).

Playback Stimuli

We generated water surface waves based on an apparatus described by Branoner et al. (2012). We compressed and decompressed air in a tube held perpendicular to the water surface to generate vibrations, or ripples. A driver (Peerless 830855 SDS 4-inch woofer) was placed in a wooden box (12 cm × 12 cm × 10 cm) with a 2-m-long garden hose attached to the sealed compartment in front of the driver. The garden hose was attached to a smaller vinyl tube (1-cm diam., 10 cm long), which was supported by a metal platform that was standing on two poles in the water. The vinyl tube ended at the water surface to transfer the airborne vibrations to surface-borne vibrations. The tube and water surface formed a meniscus due to capillarity. The size of the meniscus was held at a constant width of approximately 1 cm. This apparatus is referred to hereafter as a "ripple generator."

We used call-induced ripples recorded from four males in the lab to assess overall waveform and spectral composition (fig. 1). We used these recordings as a benchmark to compare artificially created vibrational stimuli. Sending a single 5-Hz sawtooth pulse to the driver (compressing and decompressing air in the box once) resulted in waves with the best match to call-induced ripples (see fig. 1). We calibrated our playback setup by generating a continuous 20-Hz pure-tone signal of known velocity (as measured with a digital oscilloscope). For call-induced ripples, we placed the tube at a distance of 10 cm from the vibrational recording strip and set the maximum peak-to-peak velocity of the ripples to 5 mm/s (or 1 V as measured with the oscilloscope with the LDV set to 5 mm/s/V). This amplitude level was within the range of recorded males.

We used the same setup for our rain-generated vibrational noise (hereafter "rain noise"). We recorded rain noise caused by raindrops hitting the water surface in several puddles around Gamboa during heavy rain with the LDV (see fig. A1 for an example; figs. A1, A2 are available online) and used these recordings to design our rain stimulus. We estimated that a water surface wave caused by a single raindrop was slightly longer in duration and greater in amplitude compared to a single call-induced ripple. We therefore used a double sawtooth pulse of 5 Hz and set the maximum amplitude of a single ripple to 12.5 mm/s (peak to peak of 0.5 V with the LDV set to 20 mm/s/V, measured at the center of the ripple cage). Rain stimuli were broadcast through three different ripple generators, with the tube openings placed around the ripple cage at 20–40-cm distance. Each ripple generator mimicked a raindrop hitting the water surface at a rate of one drop per 5 s. Start times of signals generated by the three ripple generators were randomly assigned (ranging from 0 to 5 s) prior to each rain noise trial.

Wind-generated vibrational noise (hereafter "wind noise") could not be reproduced reliably with our ripple generator. Instead, we used a fan placed at the edge of the pool that simulated natural wind at a maximum speed of 1.5 m/s (measured with a Voltcraft BL-30AN). This wind speed is representative of a light breeze, which commonly occurs at our study sites during the breeding season. Our wind treatment created vibrational noise with a maximum amplitude of 2.5 mm/s at the center of the cage. The fan also produced low levels (around 40 dB re: 20 μPa) of airborne sound, but these levels are well below levels experienced by frogs in the field and are unlikely to have an effect on their call behavior (see also Halfwerk et al. 2016).

We broadcast the sound of a calling male through a loudspeaker (Peerless 830984 2.5-inch full range) placed on the same platform that was used for the call-induced ripples. The loudspeaker broadcast an artificially created whine plus one chuck at 0.5 calls/s with a sound pressure level of 82 dB (re: 20 μPa at 50 cm, measured with Extech SPL-meter type 407764, set to C-weighted, fast, and max; see details in Rand et. al. 1992 for the creation of the synthetic call).

Experimental Procedures

We tested males in a large pool (150-cm diam.) containing 50 L of dechlorinated tap water. The pool contained the ripple cage to keep the test a male at a fixed location, a small tripod at 50 cm from the cage supporting a microphone, and four playback platforms to generate signals and noise. The platform supporting the speaker and tube for call-induced ripples was placed at 10 cm from the cage. We ensured that the test male always had at least a 2-cm water column inside the cage. The test male was placed in the ripple cage and provided a preliminary stimulation with the playback of a low-amplitude airborne acoustic chorus recording until they reliably called. A 5-min silent period was then provided prior to beginning the first experimental trial. Each trial started with 30 s of the vibrational noise treatment (rain/wind/control) in the absence of signal playback. After 30 s we started a 1-min signal playback while continuing the noise treatment. We used a 1-min period of silence in between trials. Signal treatment included a unimodal playback (consisting of a rival sound only) or a multimodal playback (consisting of a simultaneous playback of rival sound and call-induced ripples). Males do not respond to call-induced ripples in isolation, and we therefore left this

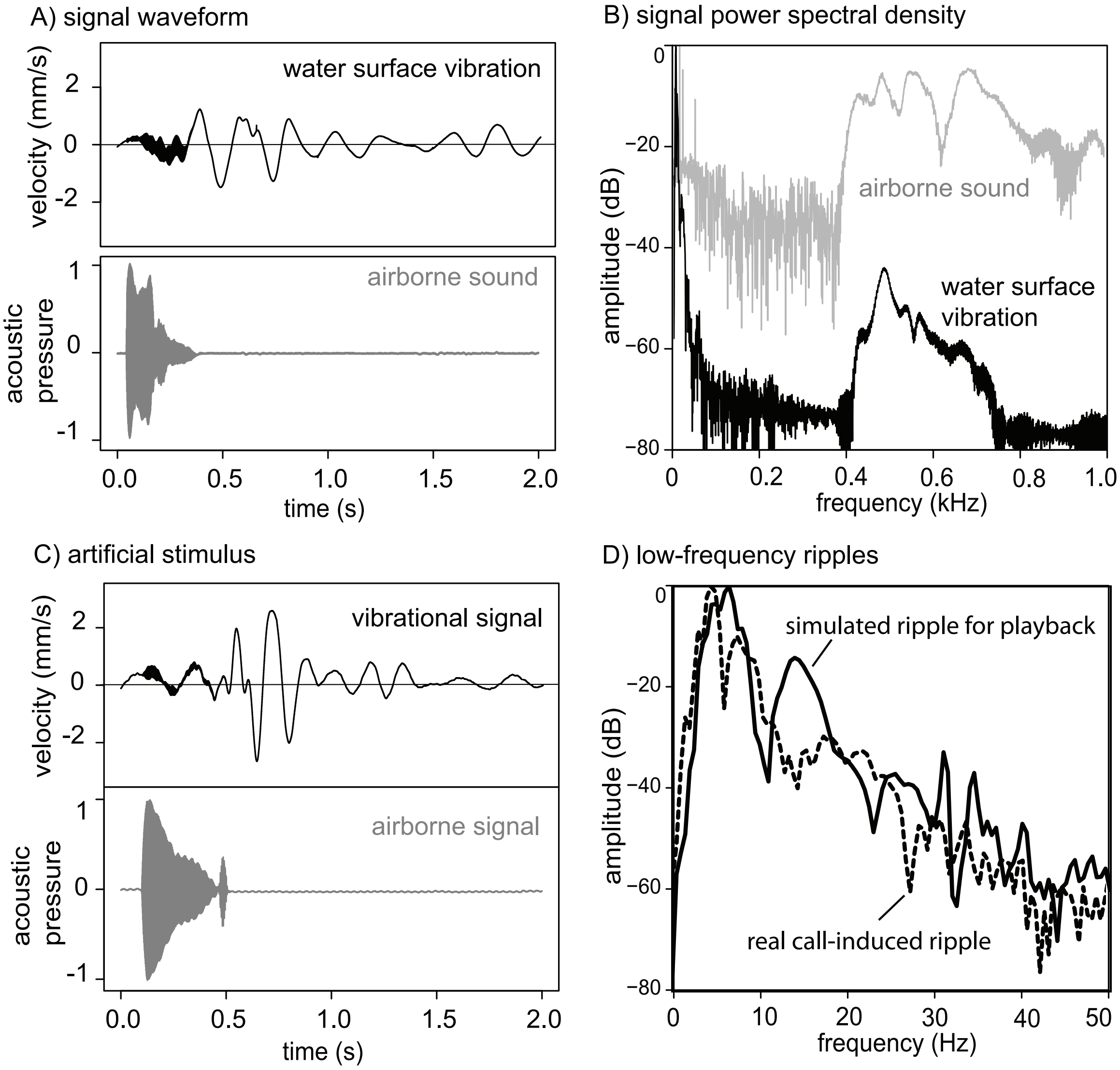

Figure 1: Characteristics of acoustic and vibrational call components. *A*, Waveforms of a male's multimodal call. The vibrational (top panel) and airborne sound (bottom panel) signals are shown. Only a whine was produced by this male. *B*, Power spectrum plots of the same vibrational (black lines) and airborne (gray lines) sound recordings. Note the presence of the whine in the range of 0.4–0.9 kHz for both airborne sound and water surface vibrations. *C*, Waveforms of our playback stimulus mimicking a male call of a whine plus one chuck and the call-induced ripples. *D*, Power spectrum plots showing the energy distribution of low-frequency water surface waves produced by a real male (dashed line) or our playback (solid line). Amplitude values on the *Y*-axis are normalized to 1 (for airborne sound only; *A*, *C*) or 0 (*B*, *D*) dB. The majority of energy of call-induced ripples is found between 5 and 20 Hz.

treatment out of the experiment. A full-factorial combination of the two signal and three noise treatments resulted in six different trials. Trial order was randomized between males.

Characterization of Call-Induced Ripples

Vibrational recordings of water surface waves were analyzed in the software program R using the package signal or seewave (Sueur et al. 2008). Call-induced ripples were analyzed by selecting for each male a 2-s time frame from the start of each call (using the microphone channel of the stereo recording as reference). We generated a 20-kHz continuous tone of known amplitude through our ripple generator, and the recorded peak-to-peak values from the Marantz were divided by peak-to-peak value obtained with the oscilloscope for calibration. We measured the maximum peak-to-peak amplitude value for 30 different calls per male per recording distance. We used a similar approach to measure the artificial stimuli in our transmission experiment. We used the amplitude values of our transmission experiment to fit regression lines across the different distances and between different water depths to assess the rate of attenuation of call-induced ripples.

Behavioral Analyses

Male calling behavior during the signal and noise playback experiments was analyzed in the program Audacity. We quantified male baseline vocal behavior by counting the number of calls produced by the focal male in the 30-s period prior to signal playback. We assessed evoked vocal response by counting whines and chucks produced during the 1-min signal playback. These counts were transformed to obtain call rate (number of whines/second) or call complexity (number of chucks/number of whines).

We analyzed male calling behavior with generalized linear mixed models from the package lme4 in R (R Core Team 2012). We explored optimal model structure by creating various models with different link functions and error distributions and by entering male identity and trial number as random effects (slope and/or intercept). We assessed quantile-quantile plots and used Shapiro-Wilcox tests on the residuals to test for violations of model assumptions. From these models we selected the best null model based on its Akaike information criterion corrected for small sample size (Zuur et al. 2009). Null models were compared to models containing noise treatment, signal playback, and/or their interaction as fixed effects using likelihood ratio tests.

We first compared baseline vocal behavior with evoked vocal response using a null model with a cbind function, a binomial error distribution, a logit-link function, and male identity and trial number as random intercepts. We compared this null model with a model in which noise treatment was added as a fixed factor. Next, we assessed the influence of signal playback and noise treatment on evoked call rate and call complexity. For this analysis we used a null model with an identity link function, normal error distribution, and male identity and trial number as random intercepts. We compared this model with a model in which signal playback, noise treatment, and their interaction were added as fixed effects. Three males that responded during only one of six trials were removed from the analyses to improve model fit. All other males responded during at least four of six trials. We used post hoc independent contrasts to follow up on significant effects of noise treatment as well as the interaction with signal playback. Vocal response raw data has been made available in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.5q4sq (Halfwerk et al. 2016).

Results

Production and Transmission of Call-Induced Ripples

We recorded two types of ripples from four different calling males. Males produced low-frequency ripples with peak spectral energy in the frequency band from 3.02 ± 0.09 to 17.4 ± 4.60 Hz and a maximum velocity of 4.0 ± 1.0 mm/s at 10 cm (see also fig. 1). These low-frequency call-induced ripples were produced as a result of postural changes during calling, mostly related to inflation and deflation of the male's vocal sac. Males also produced high-frequency ripples on the water surface in the frequency band from 0.4 to 0.9 kHz. These high-frequency ripples were about 40 dB lower in intensity compared to the low-frequency ripples and were produced as a result of vibrating structures in the frog's larynx. Tissue vibrations in the larynx produce the airborne sound component of the mating call, but some of the mechanical energy is transferred to the water surface. In other words, a male's whines and chucks leave a signature on the water surface (as can also be seen on spectrograms in fig. A2), and that signal propagates away from the frog as a ripple.

Ripples propagate along the water surface at about one-thousandth the velocity of airborne sound waves, and their propagation speed and attenuation rates are frequency dependent (fig. 1). High-frequency components of call-induced ripples arrive earlier but at lower intensities compared to low-frequency components. As a result, call-induced ripples stretch out over time and space during propagation, a process known as dispersion (fig. 2). Furthermore, water surface wave propagation depends on water depth. Attenuation is inversely proportional to water depth, hence call-induced ripples attenuate more rapidly in shallow compared to deep water (fig. 2). Call-induced ripples thus provide unique distance information to receivers (cues such as wave amplitude, arrival time differences with sound, dispersion pattern, and spectral profile), but the reliability of this information seems to decrease rapidly after propagating between 10 and 30 cm from the calling male.

Rival Communication under Vibrational Noise

We found 17 out of 20 males to call spontaneously prior to the start of our experiment, and these males continued to call during the majority of experimental trials. Noise treatment did not significantly affect the baseline call rate, which was recorded in the absence of signal playback ($N = 17$; $\chi^2 = 3.81$, $\mathrm{df} = 2$, $P = .15$). Signal playback had a clear effect on male calling, as most males increased call rate in response, but this evoked vocal response was significantly affected by noise treatment ($N = 17$; $\chi^2 = 25.37$, $\mathrm{df} = 2$, $P < .001$; fig. 3). During the rain noise treatment, all 17 males responded to signal playback by increasing their call rates (fig. 3), and the average increase was significantly higher compared to the con-

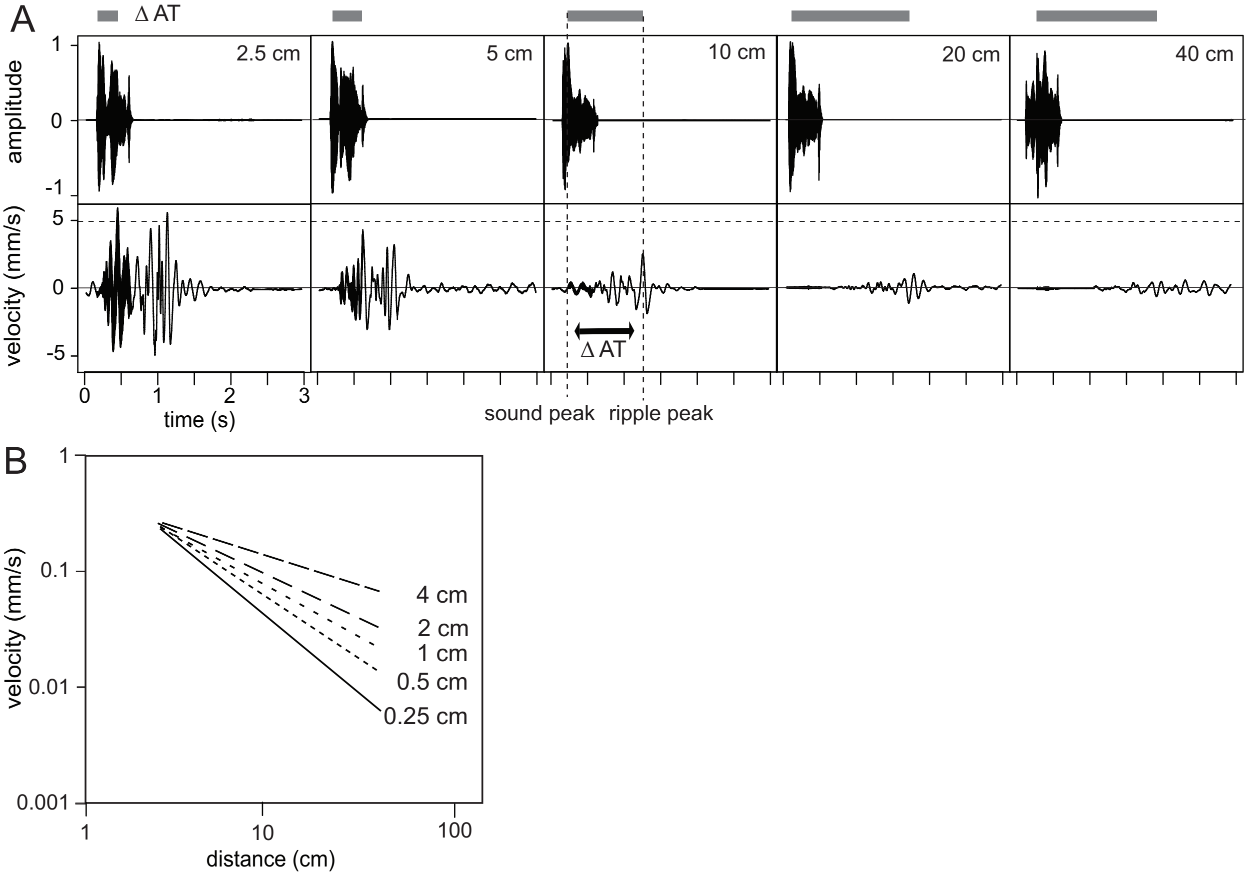

Figure 2: Transmission of call-induced ripples over different distances and for different water depths. *A*, Example of a male's multimodal signal recorded over different distances. Top panels show the airborne component of a male's call recorded with a microphone placed at 50 cm. Bottom panels show water surface vibrations, or call-induced ripples, recorded with the vibrometer at different distances from the male. Airborne sound levels shown on the top panels' *Y*-axes are normalized to 1. Values on the bottom panel's *Y*-axes depict velocity (millimeters/second) recorded from a reflective tape on the water surface. Note that the peak velocity values of the call-induced surface wave drop rapidly with distance. Furthermore, the difference in arrival time (Δ AT) between peak in sound amplitude and ripple velocity increases with distance (as illustrated by gray bars above the panels). Propagation speeds of water surface waves are frequency dependent, resulting in an increase in the passage of time between the front and end part of a single-wave pulse with increasing distance. As a consequence of this dispersion effect, the relative position of the peak velocity changes, making the assessment of arrival time differences between sound and ripples less reliable at larger distances (e.g., compare gray bars between 20 and 40 cm). *B*, Attenuation rates of call-induced ripples at different water depths.

trol treatment ($z = 4.62$, $P < .001$). During the wind noise treatment, we found an average increase in call rate in response to signal playback, but the pattern differed substantially between males, and the mean response did not differ from the control treatment ($z = 0.47$, $P = .64$).

In addition to a main effect of noise treatment on evoked vocal response, we found a significant interaction effect between noise treatment and signal playback on call rate ($N = 17$; $\chi^2 = 7.51$, df = 2, $P = .023$) as well as on call complexity ($\chi^2 = 11.02$, df = 2, $P = .004$). Males responded more strongly to the multimodal signal (playback of call-induced ripples together with the sound of a rival's call) compared to the unimodal signal (sound playback only), but only under control noise treatment (i.e., lack of wind or rain noise; fig. 4; multimodal vs. unimodal, call rate: $z = 2.98$, $P = .018$; complexity: $z = 3.82$, $P < .0001$). Under the rain noise treatment, signal playback had no impact on male vocal responses (multimodal vs. unimodal, call rate: $z = -0.64$, $P = .97$; complexity: $z = 0.73$, $P = .96$). Similarly, we did not find an effect of signal playback during wind noise conditions (multimodal vs. unimodal, call rate: $z = 0.025$, $P = 1.0$; complexity: $z = -2.1$, $P = 1.0$). Post hoc comparisons further revealed that evoked responses to the multimodal signal were decreased under wind noise treatment (wind noise vs. control, call rate: $z = -3.33$, $P = .006$; complexity: $z = -4.13$, $P < .001$) and increased or remained

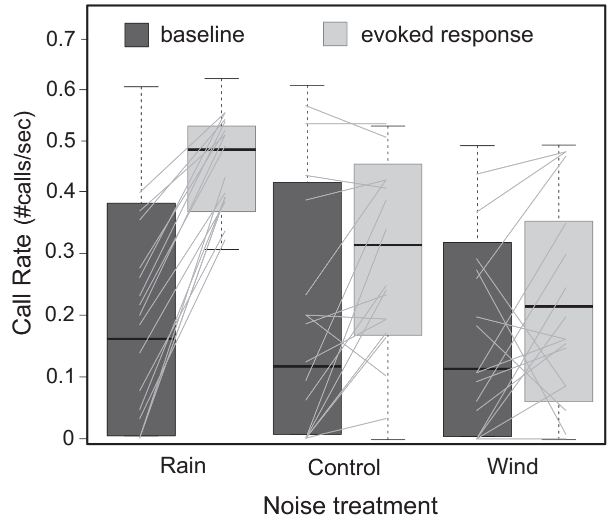

Figure 3: Effect of vibrational noise on evoked vocal response. The majority of males increased call rates in response to rival call playback, with rain noise inducing the strongest increase. There was no difference in baseline call rates across noise treatments. Shown are boxplots for both baseline calling and evoked response, with call rate on the *Y*-axis and noise treatment on the *X*-axis. Gray lines indicate individual responses.

the same under rain noise treatment (wind noise vs. control, call rate: $z = 2.90$, $P = .023$; complexity: $z = 1.80$, $P = .34$).

Discussion

We assessed the effect of environmental noise on multimodal communication between rival male túngara frogs. We focused on two different signal components spanning the acoustic (airborne sound of the frog's mating call) as well as the vibrational domain (call-induced ripples) and tested for effects of two types of vibrational noise, caused by either raindrops or wind. We found no impact of either source of noise on baseline call behavior. Contrarily, stimulus playback of a rival call evoked a clear vocal response, but the effect depended on the type of signal (unimodal or multimodal) as well as the type of noise treatment. Vibrational rain noise had a significantly larger effect on evoked vocal response compared to control and wind noise conditions. Furthermore, males responded more strongly to the multimodal signal, but only during the control noise condition (i.e., in the absence of wind or rain noise).

Wind-induced noise had no effect on baseline call behavior compared to control conditions. Evoked vocal responses did differ slightly from baseline call behavior, but the increase in call rate did not depend on signal playback. In other words, we found similar responses to the unimodal (sound only) playback compared to multimodal (sound plus call-induced ripple) playback during wind noise treatment, whereas during control treatments we found a clear enhanced effect of the multimodal signal. These results suggest that wind-induced vibrational noise masks the detection and/or recognition of call-induced ripples. Due to the technical limitation of recreating vibrational wind noise, we had to use a fan blowing over the water surface. The associated airflow may also have disturbed calling males, but in this latter scenario we would expect to find differences in baseline calling between noise treatments. Furthermore, our findings are in agreement with studies on plant-borne vibrations (McNett et al. 2010; Gordon and Uetz 2012; Caldwell 2014). Male wolf spiders, for example, communicate with seismic signals, and exposure to filtered vibrational white noise can reduce female attraction, presumably through a masking impact of spectral overlap between signal and noise (Gordon and Uetz 2012).

Raindrops impacting the water surface make ripples and thereby create noise in the vibrational domain, but this type of noise had no effect on the baseline calling behavior of túngara frogs when compared to control conditions. Combining vibrational rain noise with signal playback, however, had a strong effect on male vocal responses. The males' evoked response tripled in this treatment compared to baseline calling and doubled compared to control conditions. Interestingly, when combined with rival sound playback, rain-induced ripples even evoked higher call rates compared to call-induced ripples. These results suggest that males associate ripples caused by rain with increased rival competition and imply that they lack the ability to discriminate between rain-induced and call-induced ripples.

The behavioral responses to the different noise and signal treatments can be explained by the amount of similarity in their physical characteristics. Rain-induced and call-induced ripples share many physical properties, such as a regular, cylindrical spread from the source, whereas wind-induced noise is much more erratic. The rain-induced and call-induced ripples that we used in our experiment did, however, also differ, most importantly in amplitude. We have previously shown that males respond more strongly to high-amplitude ripples from nearby rivals when compared to low-amplitude ripples from faraway rivals (Halfwerk et al. 2014*b*). Ripple amplitude may therefore provide a cue about rival distance, and rain-induced ripples may simply be perceived as a big competitive threat. If so, we would expect male evoked responses to be dependent on environmental factors such as rain intensity and raindrop size. Males may lack the ability to discriminate between call-induced and rain-induced ripples, but an alternative (not mutually exclusive) explanation could be that rain-induced environmental cues may provide information on optimal signaling (absence of eavesdroppers) or reproductive conditions (rain is highly favorable for breeding in puddles).

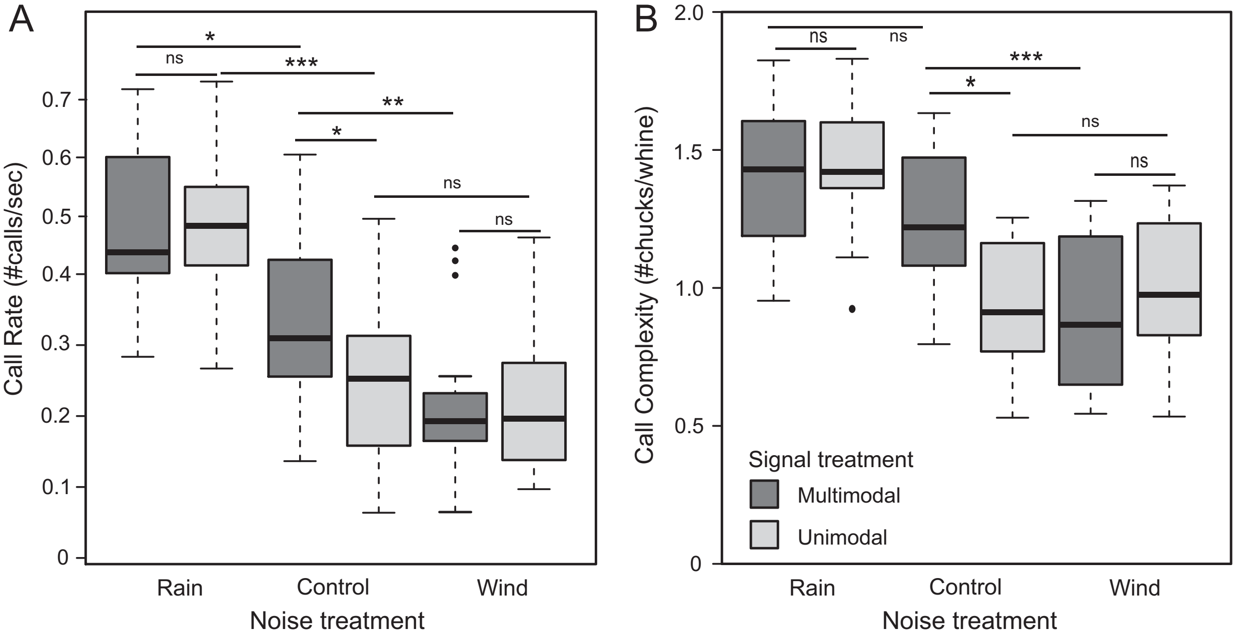

Figure 4: Differential effect of vibrational noise on multimodal rival communication. *A*, Effect of noise treatment and signal playback on evoked call rates. *B*, Effect of noise treatment and signal playback on call complexity. Shown are boxplots for unimodal (sound only) and multimodal (sound plus call-induced ripples) playback separately.

Signal Adjustment to Noise

Signaling animals have been found to adjust their signals in response to noise. Birds increase the amplitude of their songs during acoustic noise or switch song types to avoid spectral overlap (Brumm and Todt 2002; Halfwerk and Slabbekoorn 2009). Frogs have also been found to increase signal amplitude as well as other signal characteristics such as call rate, call duration, and call timing during acoustic noise exposure (Penna et al. 2005; Sun and Narins 2005; Love and Bee 2010; Halfwerk et al. 2016). The few studies that have addressed signaling plasticity outside the acoustic domain have found that signalers either increase signaling effort (e.g., increase signaling rate or signal amplitude) or avoid temporal overlap during noisy conditions (Ord et al. 2007; McNett et al. 2010). Wind-induced vibrational noise, for example, interferes with the detection of seismic signals in treehoppers (McNett et al. 2010). These insects are able to adjust their signal timing and tend to send out seismic signals during periods with low-wind conditions. Call-induced ripples likely function in territory defense in our study system, and selection may thus favor individuals that restrict their calling effort to times with low-wind conditions. Alternatively, individuals can increase signaling effort by hitting the water surface with their forelegs or by kicking their hind legs (Walkowiak and Munz 1985; Seidel 1999).

Sensing Water Surface Waves

We did find clear behavioral differences between noise treatments, which suggest that túngara frogs possess a sensory system that allows them to discriminate between different types of vibrational noise. How túngara frogs can do this, we do not know, as we have not identified the sensory structures that they use to detect and process water surface vibrations. Some distant amphibian clades retain their lateral line system or neuromast cells after metamorphosis (e.g., *Xenopus* and *Bombina*; Elepfandt and Simm 1985; Elepfandt and Wiedemer 1987). Using lateral line sensing, *Xenopus laevia* can, for example, detect differences in spectral, spatial, and temporal characteristics of water surface waves (Behrend et al. 2008; Branoner et al. 2012). Adult túngara frogs, however, lack clear morphological structures on their skin that can be associated with lateral line sensing, such as canals or pores. Alternatively, frogs may use neurological structures in their ears that are sensitive to substrate-borne vibrations (Lewis and Narins 1985; Lewis et al. 2001).

In conclusion, we tested evoked vocal responses of male frogs during multimodal rival communication in the presence and absence of different noise treatments. We found stronger responses to multimodal compared to unimodal rival signals, but only under the control condition. These findings suggest that vibration noise can potentially render the use of multimodal signals useless and may consequently select for unimodal signals. Alternatively, vibrational noise could select for a change in signaling strategy if there are clear functional benefits associated with multimodal signals. We also found that rain noise enhanced calling in túngara frogs, irrespective of signal composition. An enhanced response can alter both sexual and natural selection pressures, as we know that both female frogs and frog-eating bats respond more strongly to higher call rates and higher complexity (Tuttle and Ryan 1981; Lea and Ryan 2015). Future studies should aim for better characterization of wind-induced and rain-induced vibrational noise and test male and female frogs over a wide range of different habitats (e.g., puddles in open field vs. under canopy cover) and stimulus combinations (e.g., testing responses under rain-induced acoustic plus vibrational noise; Halfwerk and Slabbekoorn 2015). How signals should evolve under rainy conditions will ultimately depend on how intended receivers and unintended eavesdroppers respond to rain. Taken together, our findings show that noise in an additional sensory channel can affect multimodal signal perception and thereby drive signal evolution, but this may not always be in the same direction.

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