

Frequency information in the vibration-cued escape hatching of red-eyed treefrogs

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SUMMARY

Incidental acoustic and vibrational cues generated by predators are a potential source of information for prey assessing risk. Substrate vibrations should be excited by most predators, and frequency, amplitude or temporal properties could allow prey to distinguish predator from benign-source vibrations. Red-eyed treefrog embryos detect egg predators using vibrations excited during attacks, hatching rapidly and prematurely to escape. We recorded vibrations in egg clutches during attacks by five species of predators and three common types of benign physical disturbance. We analyzed their frequency distributions to assess if and how frequency properties could be used to discriminate between vibration sources and used vibration playbacks to examine the effects of frequency properties on the escape hatching response. Vibrations produced by predators and benign disturbances generally have broad and overlapping frequency distributions, and all frequencies excited by attacks are also excited by benign disturbances. Decision rules based on the frequency distribution of vibrations alone would therefore result in either high levels of hatching in response to benign vibrations (false alarms) or common failures to hatch in response to predators (missed cues). Nevertheless, embryos hatch in response to predator and not benign disturbances in nature, and our playback results show that vibration frequency information is an important component of their hatching decision. Embryos combine frequency with temporal information to refine their hatching response. Moreover, comparing frequency spectra of predator and benign vibrations suggests that the presence of energy in frequencies outside the range characteristic of attacks might serve as an indicator of benign disturbance.

Key words: frequency, cue, decision rule, predator detection, discrimination, *Agalychnis*, embryo.

INTRODUCTION

Prey need information to assess and respond to variation in predation risk. A considerable literature documents the sources and sensory modalities of risk cues (Hill, 2008; Kats and Dill, 1998; Lima and Dill, 1989); however, we are just beginning to understand the specific information available to prey and how they use it to make behavioral decisions about defense.

Most of our detailed knowledge about animal information use comes from studies of communication (Bradbury and Vehrencamp, 1998). Because communication signals have evolved for efficacy in information transfer, they are stereotyped in form and distinct from their background (Endler, 1992; Guilford and Dawkins, 1991). These characteristics improve signal detection and allow receivers to extract useful information from relatively small variations in signal properties, such as duration, frequency or amplitude. Prey also exploit signals generated by predators, such as the echolocation calls of foraging bats and dolphins (Mann et al., 1997; Miller and Surlykke, 2001; Moiseff et al., 1978). Indeed, much of our knowledge of how prey use specific stimulus properties in risk assessment comes from such cases (Blumstein et al., 2008). The many predators that do not signal while hunting, however, deny their prey this opportunity for eavesdropping.

By contrast, incidental cues generated by foraging predators, including sounds and substrate vibrations, are likely more common, and some prey use such cues to assess risk (Bacher et al., 1997; Catania, 2008; Clegg and Barlow, 1982; Gnatzy and Kämpfer, 1990; Hergenröder and Barth, 1983; Tautz and Markl, 1978). Prey that use incidental, non-stereotyped predator cues to make defensive decisions face detection tasks that differ in important ways from

those of prey that exploit predator signals. Predator cues have not evolved to facilitate detection; instead, selection on predators often favors crypsis (Bradbury and Vehrencamp, 1998). Thus, although animals can often extract information from a short sample or a single issuance of a stereotyped signal, larger samples may be required to exploit statistical regularities of variable cues. Similarly, information obtained from a single signal property is often sufficient to separate that signal from its background, whereas overlap between the property distributions of a cue and its background may necessitate the use of multiple properties (Macmillan and Creelman, 2005; Warkentin and Caldwell, in press; Warkentin et al., 2007).

The frequency distributions of incidental predator cues are one potential source of information for prey. Eavesdropping prey attend to frequency properties of predator signals (Macedonia and Yount, 1991; Mann et al., 1997; Miller and Surlykke, 2001; Moiseff et al., 1978; Swaisgood et al., 1999). A few studies have examined prey use of frequency properties of incidental predator cues in defensive decisions (Djemai et al., 2001; Gnatzy and Kämpfer, 1990; Hergenröder and Barth, 1983; Tautz and Markl, 1978). However, the possibility that prey use frequency properties of acoustic and vibrational cues to assess predation risk remains relatively unexplored.

Compared to cues in other sensory modalities, vibrational cues may be particularly useful across animal taxa as a source of information in predator–prey interactions. Terrestrial and arboreal animals are generally mechanically well-coupled to their substrata, so substrate vibrations are inevitably excited by any movements they make. Vibrational silence or crypsis is therefore more difficult to achieve than acoustic silence or visual crypsis (Bacher et al., 1996;

Hill, 2008; Wilcox et al., 1996). Sensitivity to substrate vibration is taxonomically widespread (Cocroft and Rodriguez, 2005; Hill, 2008), and vibrational cues are used in both predator foraging and antipredator defense (Bacher et al., 1997; Catania, 2008; Clegg and Barlow, 1982; Djemai et al., 2001; Evans et al., 2005; Hergenröder and Barth, 1983; Lawrence, 1981; Narins et al., 1997; Pfannenstiel et al., 1995; Warkentin, 2005).

It is, nonetheless, unclear how informative the frequency properties of incidental predator vibrations are to prey. Frequencies excited by predator movements are determined by mechanical properties of both the predator and the vibrating substrate (Ginsberg, 2001). Because frequency properties depend on predator biomechanics, and therefore morphology, they may be more consistent and informative than temporal or amplitude properties of the same vibrational cue, which vary with predator behavior. However, any direct mechanical forcing of an object excites the fundamental vibration frequencies of that object, regardless of whether the forcing is caused by a predator or some other benign disturbance. Thus, incidental cue frequencies may be more indicative of the substrate than of the vibration source. Moreover, plants and other natural substrates are complex, variable filters of vibration frequency (Bell, 1980; Casas et al., 2007; Cocroft et al., 2006; Michelsen et al., 1982). These characteristics suggest that vibration frequency properties may be relatively uninformative to prey; indeed, much of the information in intraspecific vibrational communication is encoded in temporal properties (Schüch and Barth, 1985; Virant-Doberlet and Cokl, 2004).

We examined the role of vibration frequency properties in risk assessment by red-eyed treefrog embryos, *Agalychnis callidryas* (Cope), in the context of predator-induced premature hatching. This species inhabits wet forests from the Yucatan to northern Colombia (Duellman, 2001) and lays gelatinous egg clutches on vegetation over ponds. In Panama, undisturbed embryos hatch after 6–7 days, dropping into the pond below. Embryos, however, become hatching-competent at 4 days of age and will hatch rapidly if attacked by egg predators, including snakes and wasps (Warkentin, 1995; Warkentin, 2000). Escape hatching is cued by predator vibrations in snake attacks (Warkentin, 2005), and vibrations may cue hatching in attacks by other predators as well. The premature hatching response depends on at least two temporal properties of vibrations, the duration of individual vibrational events and the spacing between these events (Warkentin et al., 2006b). Also, embryos typically do not hatch as rapidly as they are able to in attacks but instead appear to sample a period of vibration prior to initiating their escape (Warkentin et al., 2007). Hatching early is costly because premature hatchlings are more vulnerable to aquatic predators (Warkentin, 1995; Warkentin, 1999). Selection by both arboreal and aquatic predators thus acts to refine the embryos' response to vibrational stimuli.

Vibrations excited by predator attacks on *A. callidryas* egg clutches are inherently stochastic, and both temporal and frequency properties overlap those of common benign disturbances such as rain and routine embryo movements. Moreover, rain can excite clutch vibrations of greater peak and root-mean-square (RMS) amplitude than those in predator attacks (Warkentin, 2005). Nonetheless, statistical differences in dominant frequency and the overall range of frequencies in snake and rain vibrations suggest that, given a sufficiently large sample of vibrations, frequency information could inform hatching decisions (Warkentin, 2005). To better assess the potential role of frequency information in risk assessment by red-eyed treefrog embryos, we recorded vibrations

from all common types of disturbances to egg clutches at our study site in Panama. We used discriminant function analyses to determine if predator and benign vibrations differ in their frequency distributions and to assess the potential accuracy of discrimination using such information. We then conducted vibration playback experiments to test if frequency properties affect the hatching response, constructing a frequency–response curve for vibration-induced hatching.

MATERIALS AND METHODS

Clutch collection and care

We collected recently laid *A. callidryas* egg clutches from Ocelot Pond, ~2 km southeast of Gamboa, Panama. We brought these to an open-air laboratory in Gamboa, where we removed any dead eggs, placed the clutches over water and misted them with rainwater several times daily to prevent desiccation. We returned all hatchlings to Ocelot Pond following our experiments. This research was conducted under permits from the Panamanian National Environment Authority (Autoridad Nacional del Ambiente). Research protocols were approved by the Smithsonian Tropical Research Institute and the Boston University Institutional Animal Care and Use Committee.

Vibration recordings

A. callidryas egg clutches are laid on many substrate types, ranging from rigid tree trunks to extremely flexible leaves, but for convenience we collected clutches on leaves. In nature, embryos must assess vibrational cues on all substrates, and in captivity escape rates in snake attacks do not differ between rigidly and flexibly mounted clutches (K.M.W., unpublished data). Therefore, to reduce substrate vibrations and better characterize vibrations in the clutch itself, we mounted most egg clutches used for recording on rigid support structures. We attached clutches on leaves to plastic support cards, and taped cards to jars of water or clay bricks (1–2 kg) with the long axis of the clutch perpendicular to the ground (Fig. 1) (Warkentin, 2005).

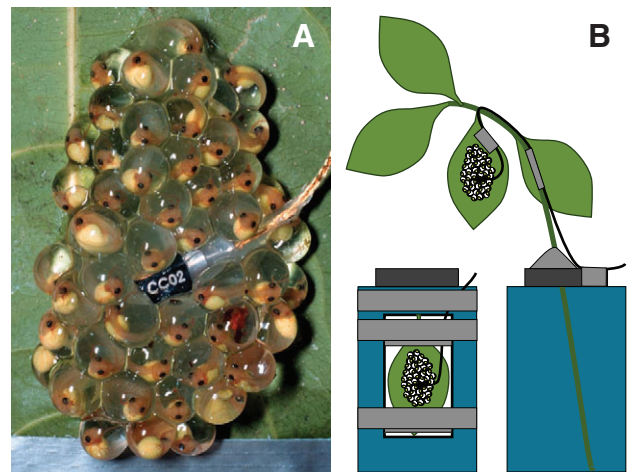


Fig. 1. Methods for recording vibrations within egg clutches of *Agalychnis callidryas*. (A) Hatching-competent egg clutch with embedded accelerometer. (B) Clutches were either (left) mounted on a rigid support by taping their leaf to a plastic card, then taping the card to a jar of water or brick or (right) collected on a larger plant cutting that was maintained in a jar of water. Accelerometer and wire are colored black, duct tape and Plasticine™ mounting materials are grey.

In tests with standardized mechanical excitations designed to mimic different natural disturbances, we found that clutch vibrations were only affected by support structure during wind-like disturbances. Wind produces low-frequency translational movements that are only detectable in plant-mounted clutches (M.S.C., K.M.W. and J.G.McD., unpublished). We therefore left clutches used for wind recordings attached to ~50 cm of the plant on which they were laid, affixing the base of the cutting in a jar of water (Fig. 1). Several snake, wasp, and rain recordings were also made from clutches on these larger plant cuttings; we have not detected any effects of mounting substrate with these types of forcing. The full analysis of egg clutch biomechanics and substrate effects will be presented elsewhere (M.S.C., K.M.W. and J.G.McD., unpublished).

We used miniature accelerometers [Endevco 25B (0.2 g), San Juan Capistrano, CA, USA; AP Technology AP19 (0.14 g), Oosterhout, The Netherlands] to record vibrations in 4- and 5-day-old hatching-competent clutches. We coated each accelerometer with a thin layer of silicone sealant and embedded it within the clutch jelly between eggs (Fig. 1). Each accelerometer was about the size of two eggs and moved with the elastic clutch jelly as it vibrated, much as eggs do. Accelerometers add approximately 5% of additional mass to each clutch, and clutches with an embedded accelerometer were still within the natural range of clutch mass variation (Warkentin, 2005). Although an embedded accelerometer may lower a clutch's fundamental free-vibration frequency slightly, it should have little effect on forced vibrations excited during clutch disturbances. Furthermore, any such changes affected all disturbance classes investigated in this study equally. Each accelerometer was inserted with its axis of sensitivity parallel to the long axis of the clutch. Accelerometers were powered by signal conditioners (Endevco 4416B and AP Technology APC7), signals were digitized with an Onkyo MSE-U33HB sound card (Onkyo USA, Saddle River, NJ, USA), and output was recorded using Canary bioacoustics software (v.1.2.4, Cornell University Laboratory of Ornithology, Ithaca, NY, USA) at 22.1 kHz on a Macintosh G4 notebook computer.

To record vibrations in egg clutches during snake attacks, we collected snakes [*Leptophis ahaetulla* (Linnaeus), *Leptodeira annulata* (Linnaeus), *Leptodeira septentrionalis* (Kennicott in Baird) and *Imantodes inornatus* (Boulenger)] from ponds near Gamboa and housed them in mesh cages or terraria, with water and vegetation, under ambient environmental conditions in the Gamboa laboratory. Snakes were offered *A. callidryas* clutches every few days. Once snakes were feeding regularly, we presented them with clutches containing accelerometers to record attack vibrations. Snake attacks were simultaneously videotaped under infrared illumination (Sony TRV120 and TRV350, New York, USA). After collecting several recordings we released each snake at its collection site.

To record wasp [*Polybia rejecta* (Fabricius)] attacks on egg clutches, we used established methods to recruit foragers to a feeding station in Gamboa (Warkentin et al., 2006a). Wasps were marked on the thorax with a dot of colored paint for individual identification. We presented wasps with clutches containing accelerometers and simultaneously recorded vibration and video of attacks.

To record rain, we placed clutches containing accelerometers outdoors, directly in the path of unobstructed falling rain. The rain recordings we analyzed from plant-mounted clutches may include low-amplitude vibration produced by wind but did not include periods of strong wind. To record wind, we placed plant sections with clutches containing embedded accelerometers outside during periods of particularly strong wind gusts, but without rain, and recorded clutch vibrations.

Sampling of vibrations

Video and vibration recordings of predator attacks were synchronized for analysis so that we could identify vibrations associated with specific behaviors of predators and embryos. We then sampled subsets of the recordings, associated with particular events, for analysis.

Snake attacks

Samples began when the snake first attacked the egg clutch and ended following the last bout of hatching not followed by additional hatching within 30 s or by any subsequent attack vibrations. At times, snakes bit and attempted to swallow accelerometers. We removed any periods of accelerometer biting from the samples and stopped sampling if the accelerometer was dislodged from the clutch. We also edited out any periods of vibrational silence lasting more than 2 min. Snake samples were 172±142 s in length (mean ± s.d.). Sample sizes per species were as follows: *L. ahaetulla*, seven individual snakes, 17 egg clutches attacked, 1–5 clutches per snake; *L. annulata*, seven snakes, 11 clutches, 1–4 clutches per snake; *L. septentrionalis*, five snakes, 13 clutches, 1–4 clutches per snake; and *I. inornatus*, two snakes, one clutch each. *I. inornatus* attacks are included in descriptions but excluded from statistical analyses due to insufficient sample size. A different analysis of recordings from two of the *L. ahaetulla* and three of the *L. annulata* used here was presented previously (Warkentin, 2005), and a mean *L. ahaetulla* spectrum is presented elsewhere (Warkentin and Caldwell, in press). All snake attacks sampled induced hatching. Vibrations from embryo movements and hatching, as well as snake contact with the clutch between bites, are an integral part of the overall vibrational pattern in snake attacks and were included in our analyses.

Wasp attacks

During visits to the feeding station, wasps interspersed attacks on eggs with extended periods of other behaviors that do not elicit hatching, including grooming, walking across the clutch and walking on the oviposition substrate. Moreover, even vibrations from direct wasp attacks were low amplitude. To better identify properties of attack vibrations and to avoid excessive vibrational and electronic background noise, we sampled only periods when a wasp was biting at or pulling on eggs. For each clutch used in our analysis, we sampled between 120 and 300 s of attack vibrations (203±125 s) depending on availability, in some cases digitally splicing these together from separate periods of wasp attack ($N=18$ clutches, 13 individual wasps, 1–3 periods of attack per wasp). All wasp attacks sampled induced hatching, and analyses include embryo vibrations.

Benign-source vibrations

We analyzed three types of benign-source vibrations. From each rain recording ($N=19$ clutches, 19 different storms), we sampled the 300 s period that excited the highest peak amplitude of acceleration. Vibration amplitude varies over several orders of magnitude both within and between rainstorms. We chose periods of heavy rain because they are most likely to reach or exceed the intensity of snake attacks and therefore may present embryos with a more difficult discrimination task than light rain. Moreover, hard rain clearly exceeds the amplitude threshold for behavioral response based on playbacks of 0–100 Hz noise (M.S.C., J.G.McD. and K.M.W., unpublished). Although of above average intensity, these vibrations are well within the amplitude range commonly excited by rainstorms. A mean spectrum from these storms is also presented elsewhere (Warkentin and Caldwell, in press). From each wind recording ($N=7$ clutches), we also sampled the 300 s period that excited the highest

peak amplitude of acceleration. For vibrations caused by routine embryo movements (position changes within the egg), we sampled five position changes each from 16 clutches that were offered to snakes but had not yet been attacked. No hatching was associated with embryo movements or wind and at most a few eggs per clutch hatched during rain recordings.

Analysis of vibration samples

To correct for DC offset, we first highpass filtered all samples at 3 Hz using Canary. We then generated spectra using fast Fourier transform (FFT) in Canary (filter bandwidth, 2.69 Hz; frame length, 743 ms; grid resolution, 1.36 Hz). For some presentations and analyses, we standardized all individual sample spectra by peak power. We used individual sample spectra to calculate mean spectra and spectral confidence intervals for each class of benign and dangerous vibrational disturbance (Figs 2 and 3). We compared dominant frequencies excited by each class of disturbance using analysis of variance (ANOVA) in SPSS (v.16, SPSS, Chicago, IL, USA).

To compare the frequency distributions of vibrations from different sources, we conducted a series of discriminant function analyses (DFA). These are not based on *A. callidryas*' sensory physiology but simply assess statistical differences among the vibrations themselves. We produced spectra for each sample using FFT in Canary (filter bandwidth, 21.5 Hz; frame length, 92.9 ms; grid resolution, 10.8 Hz) and divided the output into equal-sized frequency bins. To test for discriminability between different predator types, between predators and wind, and between predators and routine embryo movements, we used 10.8 Hz-wide frequency bins, dictated by the grid resolution of our FFTs. Rain excited vibrations over a much larger frequency range, and the number of predictor variables was limited by the assumptions of DFA. Therefore, to test for discriminability between predator attacks and rain, we formed 43.1 Hz-wide frequency bins by taking the mean of every four power values output by FFT. We used the energy within each frequency bin as a predictor variable for DFA. We entered predictor variables into the model simultaneously and used cross-validated classification results to assess the efficacy of the discriminant functions generated. Due to sample size assumptions of DFA, the range of frequencies included in the wind vs predator discrimination analysis was limited by the number of wind recordings available. Analyses for all other vibration sources were conducted using all frequency ranges that, on average, had a power of at least 20% of that measured in the dominant frequency range for the same source. All DFA were conducted twice, with spectra standardized to match peak power and with unstandardized (absolute) power spectra. Standardization improves comparisons of the relative frequency content of each disturbance type, regardless of amplitude, whereas analyses of unstandardized spectra incorporate amplitude variation among recordings. Because DFA failed to discriminate between predator classes using either absolute (Wilks' $\lambda=0.481$, $\chi^2=37.00$, d.f.=33, $P=0.289$) or standardized spectra (Wilks' $\lambda=0.511$, $\chi^2=34.23$, d.f.=30, $P=0.272$), we included vibrations from all predator types in a single 'predator' class during all further analyses.

Vibration playback experiments

To test if, and begin to assess how, embryos use vibration frequency in their hatching decision, we conducted three series of playback experiments. Some of these playback data are discussed in a broader context elsewhere (Warkentin and Caldwell, in press). Eggs were collected from Ocelot Pond as above. Egg care and

playback protocol followed established methods (Warkentin et al., 2006b). Vibrational stimuli were played from Canary on a Macintosh G4 laptop computer, output *via* an external sound card (MSE-U33HB) and a custom-made amplifier designed to have a flat frequency response from DC to 5 kHz (E. Hazen, Boston University Electronic Design Facility) and transduced using an electrodynamic minishaker (Model 4810; Brüel and Kjær, Nærum, Denmark). For standard playbacks, individual clutches were rigidly mounted on a support stand and the shaker was mechanically coupled to clutches *via* a minishaker-clutch interface (MCI) consisting of a rigid stinger with blunt metal tines that were inserted into the clutch jelly. Only clutches with ≥ 20 eggs were used. Five minutes after any hatching induced during setup, we removed any hatched tadpoles. If $\geq 25\%$ of a clutch hatched during set-up we did not use it for the experiment. During playback, clutches were vibrated vertically, and any hatchlings fell into a tray of water. We recorded the number of hatched tadpoles every minute throughout the 5-min playback stimulus and for an additional 5 min thereafter. Following this, we assessed the hatchability of any remaining embryos using manual stimulation. We presented each clutch with only one stimulus.

Broadband playbacks

Analysis of our initial vibration recordings indicated that the energy in all common disturbance types was limited to below 1 kHz. In 2005, we tested embryo hatching response to frequencies in the 0–1000 Hz range, using four stimuli constructed from 250 Hz-wide bands of white noise (seven clutches per stimulus). Stimuli were 30 ms^{-2} in peak amplitude, within the range of amplitudes normally excited during snake attack (M.S.C., J.G.McD. and K.M.W., unpublished). To ensure that all frequency ranges were output at the same amplitude, prior to testing we re-recorded the output of each stimulus using accelerometers (Endevco 25B). Stimuli consisted of 1-s bursts of noise, with approximately rectangular amplitude envelopes, alternating with 1-s intervals of silence. This temporal pattern elicits high hatching in playbacks of 0–100 Hz noise (Warkentin et al., 2006b). To construct stimuli, we generated white noise in SoundEdit16 (v.2, Macromedia, San Francisco, CA, USA) and then bandpass filtered it in Canary. The first 30 one-second bursts of noise in each frequency range were unique, contiguous samples of the respective filtered noise file, with all edits made at zero crossings to avoid clicks during playback. This 1-min file was duplicated five times to produce a 5-min playback stimulus.

Narrowband playbacks

To construct a more detailed frequency response curve for vibration-induced hatching, we focused on the frequency range that elicited the most hatching in the broadband experiment. Our second experiment in 2005 used 17 stimuli constructed of 10 Hz-wide bands of white noise, ranging from 20 to 230 Hz as follows: 20–30, 30–40, 40–50, 50–60, 60–70, 70–80, 80–90, 90–100, 100–110, 110–120, 120–130, 130–140, 140–150, 160–170, 180–190, 200–210 and 220–230 Hz ($N=7-9$ clutches per stimulus, except for 220–230 Hz, where $N=4$). Stimuli were constructed as above, in the same temporal pattern, and were amplitude matched using re-recordings.

Our playbacks of 10 Hz-wide bands of vibrations revealed that embryos hatched strongly in response to very low frequencies. However, the displacements needed to reproduce frequencies below 20 Hz with acceptable fidelity at 30 ms^{-2} are beyond the capabilities of the Brüel and Kjær 4810 minishaker. Even the 20–30 Hz stimulus was only marginally acceptable, with inconsistent amplitude across its intended frequency range and a small amount of energy at higher

frequencies. In 2006, we conducted an additional playback experiment to explore hatching response to very low frequencies. Methods were as before, except we used a shaker and amplifier combination (51075e shaker and BAA 120 amplifier, TIRA, Schalkau, Germany) capable of producing extremely low-frequency stimuli at the amplitudes needed. We played seven stimuli in total. To assess the consistency of hatching response between the two playback series, using the different shakers, four of these stimuli [20–30, 70–80, 140–150 and 200–210 Hz ($N=4-6$)] overlapped with our 2005 experiment and were amplitude matched to those earlier playbacks. In addition, we included an amplitude-matched 10–20 Hz stimulus ($N=7$). We also included two stimuli designed to assess the response to very low frequencies and test whether tactile, rather than vibrational, cues are necessary for the hatching response to these high-displacement stimuli. These were a 0–10 Hz stimulus, played at an amplitude of 10ms^{-2} ($N=5$), the highest amplitude the TIRA shaker can produce in this frequency range, and a 0–10 Hz stimulus played to clutches mounted on the back of the MCI at the same amplitude ($N=6$). In acceleration-matched stimuli, lower frequencies entail greater displacements. With the vibrating MCI tines inserted into a clutch mounted on an immobile substrate, extremely low-frequency stimuli produced pronounced shearing as the tines moved through the clutch. By mounting clutches on the back of the MCI (i.e. on a vibrating substrate), the entire clutch moved as a unit, reducing shearing and removing tactile cues caused by MCI tines compressing or sliding between eggs; these clutches were simply shaken, not also stirred.

Statistical analysis of playback results

Changes in the playback equipment did not affect the hatching response to stimuli used with both shakers ($F_{1,49}=0.82$, $P=0.369$). We therefore pooled all narrowband playback trials in a single ANOVA, excluding clutches mounted on the back of the MCI, to test for effects of stimulus frequency. We compared hatching

resulting from the normal and back-mounted 0–10 Hz stimuli using Student's t -test.

RESULTS

Vibration frequencies in natural disturbances

There was substantial overlap between frequencies excited in *A. callidryas* egg clutches under predator attack and those excited during benign disturbances (Figs 2 and 3). Wind frequencies were extremely low, with most energy below 20 Hz. While there was some variation among vibration frequency in attacks by different types of predators, all excited a broad range of frequencies below ~ 130 Hz. This distribution was similar to that of routine embryo movements, although embryo movements did not excite the lowest frequencies present in predator attacks. Rain had by far the broadest frequency distribution, completely overlapping all other disturbance classes, and contained energy at frequencies extending to over 600 Hz. The intensity of vibrations produced by rainstorms also varied greatly at all of these frequencies, so the 95% confidence intervals around the absolute power spectrum of rain encompass the energy distributions of all other disturbance types (Fig. 3).

Dominant frequency varied significantly between different types of clutch disturbance ($F_{6,94}=3.12$, $P=0.008$) (Fig. 2). *Post-hoc* pairwise comparisons of class means using Tukey's HSD revealed significant differences between rain dominant frequencies and those of *L. ahaetulla*, *L. annulata* and wind (all $P<0.035$). None of the other disturbance classes differed in dominant frequency.

Discriminant function analyses (DFA) of absolute spectra identified significant functions for discrimination between predators and rain (Wilks' $\lambda=0.80$, $\chi^2=16.49$, d.f.=5, $P=0.006$) and between predators and wind (Wilks' $\lambda=0.78$, $\chi^2=14.96$, d.f.=7, $P=0.021$). However, the ability of these functions to correctly classify disturbances, as revealed by cross-validated classification results, was poor. They misidentified 74% of rain and 71% of wind spectra as predator vibrations. Moreover, the function discriminating

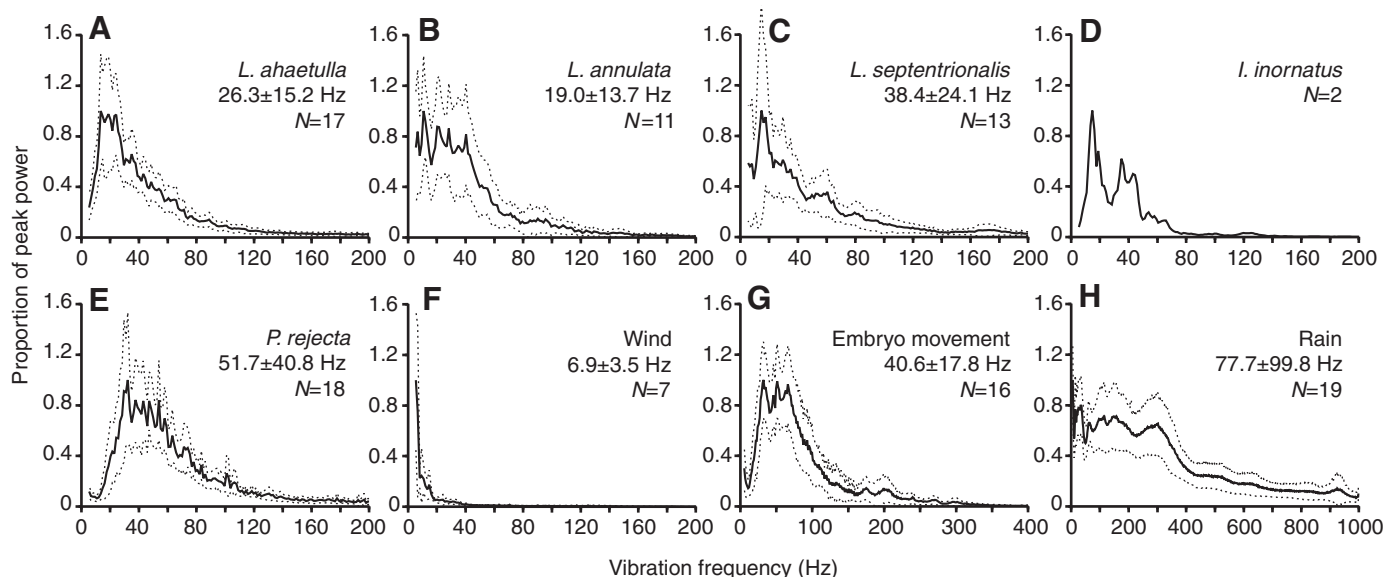


Fig. 2. Frequency spectra for eight common vibrational disturbances to *Agalychnis callidryas* egg clutches. Data are mean spectra and 95% confidence intervals calculated across individual spectra standardized to peak power. Dominant frequencies (means \pm s.d.) and sample sizes are listed for each type of disturbance. Egg predators include the snakes (A) *Leptophis ahaetulla*, (B) *Leptodeira annulata*, (C) *Leptodeira septentrionalis* and (D) *Imantodes inornatus* and (E) the wasp *Polybia rejecta*. Benign disturbances include (F) wind, (G) routine embryo movements and (H) rain. Note that both embryo movements and rain are plotted over broader frequency ranges than are the other disturbances. Vibrations were recorded using accelerometers embedded in hatching-competent egg clutches. Predator attacks induced substantial hatching; benign disturbances induced little or no hatching.

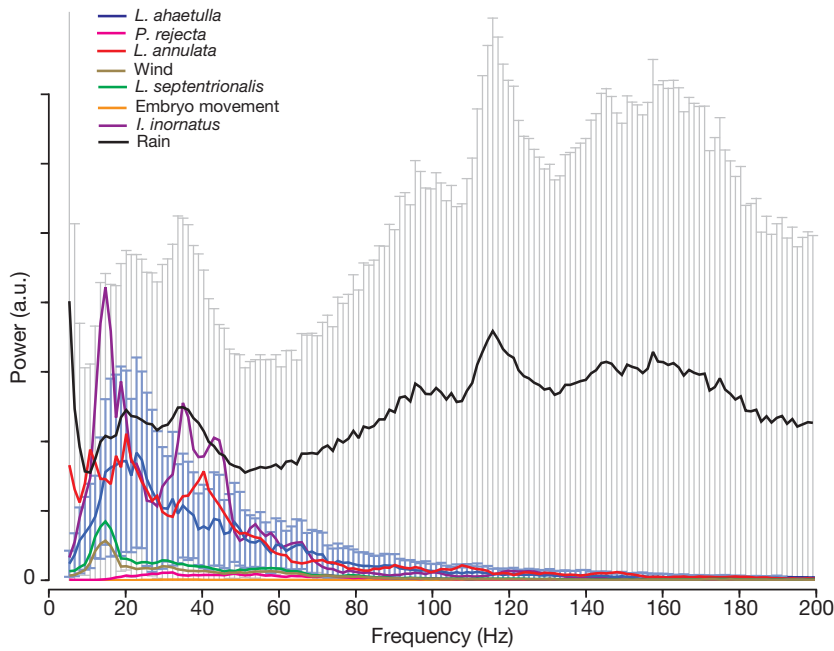


Fig. 3. Absolute frequency spectra for eight common vibrational disturbances to *Agalychnis callidryas* egg clutches, showing amplitude variation across predator and benign-source vibrations. Values are plotted on a quadratic scale in arbitrary units. Rain and wind sampled were of above-average intensity but were within the range commonly experienced by *A. callidryas* embryos. Note that, across all frequencies, hard rain excites higher intensity vibrations than do all other common types of vibrational disturbance. Included are 95% confidence intervals around the spectra for rain and for one typical predator class (the snake *Leptophis ahaetulla*).

predators from rain weighted all frequencies similarly, classifying high-amplitude recordings as rain, and recordings that were less intense overall as predators. The DFA of absolute spectra failed to discriminate between routine embryo movements and predator attack vibrations (Wilks' $\lambda=0.89$, $\chi^2=7.77$, d.f.=11, $P=0.734$).

The DFA based on standardized spectra identified significant functions for discrimination between vibrations in predator attacks and each of the three classes of benign disturbance and were better able to correctly classify recordings (Table 1). Discrimination between predators and rain was highly effective, with 92.3% of

disturbances correctly classified, a 46.3% improvement from results expected through random classification (Wilks' $\lambda=0.178$, $\chi^2=120.04$, d.f.=13, $P<0.001$). Frequencies above 130 Hz, not strongly excited during predator attacks (Fig. 2), were most useful for discriminating between predators and rain (Table 1).

All wind recordings had dominant frequencies and most of their energy in our lowest frequency bin, making DFA of standardized spectra poorly suited to statistically assess the discriminability of frequency distributions from predators and wind (Dillon and Goldstein, 1984). Thus, although this DFA identified a significant

Table 1. Classification results and function coefficients for discriminant function analyses based on the relative frequency distributions of vibrations in *Agalychnis callidryas* egg clutches during predator attacks and three common types of benign disturbance (rain, wind and routine embryo movements within the egg)

Predator attack vs:	Rain		Wind	Embryo
Significance of function	$P<0.001$		$P<0.001$	$P=0.004$
Correctly classified*	92.3%		92.4%	70.7%
Expected correct by chance*	63.1%		81.0%	66.5%
Missed cues†	1.6%		6.8%	13.6%
False alarms†	26.3%		14.3%	87.5%
Frequency range (Hz)	Structure coefficient	Frequency range (Hz)	Structure coefficient	Structure coefficient
5.4–48.5	0.17	5.4–16.2	–0.53	0.8
48.5–91.5	0.04	16.2–26.9	0.35	0.54
91.5–134.6	–0.3	26.9–37.7	0.6	–0.01
134.6–177.7	–0.54	37.7–48.5	0.59	–0.05
177.7–220.8	–0.49	48.5–59.2	0.51	–0.15
220.8–263.9	–0.41	59.2–70.0	0.45	–0.31
263.9–306.9	–0.46	70.0–80.8	–	–0.55
306.9–350.0	–0.48	80.8–91.6	–	–0.53
350.0–393.1	–0.49	91.6–102.3	–	–0.38
393.1–436.2	–0.54	102.3–113.1	–	–0.31
436.2–479.3	–0.53	113.1–123.9	–	–0.35
479.3–522.3	–0.48			
522.3–565.4	–0.44			

*The fraction correctly classified includes both correct detections of predators (hits in detection theory) and correct classifications of benign-source cues, out of all recordings analyzed. The fraction expected correct by chance is based on the number of each disturbance type in the analysis.

†The fraction of predator recordings incorrectly classified as benign (missed cues), and of benign disturbances incorrectly classified as predator attacks (false alarms), describes the sensitivity and specificity of the discriminant functions for predator detection.

discrimination function (Wilks' $\lambda=0.56$, $\chi^2=35.18$, d.f.=6, $P<0.001$), this measure of significance should be considered with caution. The discriminant function correctly classified 92.4% of samples; however, this represents only a 14.0% improvement on results expected from random classification. Nevertheless, examination of the structure coefficients proved informative. Frequencies below 16 Hz were highly negatively weighted, while all other frequencies were weighted positively (Table 1). Indeed, wind frequencies are mostly confined to a range below 16 Hz, a pattern not representative of any other common egg-clutch disturbance, including all predators (Fig. 2).

The function identified by DFA of standardized spectra to discriminate between predators and routine embryo movements was significant (Wilks' $\lambda=0.67$, $\chi^2=27.07$, d.f.=11, $P=0.004$) but ineffective. Only 70.7% of disturbances were classified correctly, a 6.4% increase from the proportion expected from random classification, and the level of false alarms was very high (Table 1). This is not surprising given the large overlap between frequencies excited by embryo movements and during predator attacks (Fig. 2). Low frequencies were weighted most strongly by the discriminant function (Table 1); these are somewhat more indicative of predator attack than embryo movement (Fig. 2).

Playback results

The hatching response of *A. callidryas* embryos to vibration varied significantly with vibration frequency in playbacks of 250 Hz-wide bands of white noise ($F_{3,28}=11.56$, $P<0.001$), with hatching limited to the 0–250 Hz range (Fig. 4). Narrowband playbacks demonstrated that the hatching response varies with frequency within this range as well ($F_{18,169}=13.65$, $P<0.001$). Embryos responded most strongly to the lowest frequencies, with nearly 100% hatching in response to vibrational stimuli below 20 Hz. There was a very strong hatching response to stimuli below ~90 Hz, and a substantial hatching response to frequencies below 150 Hz, but very little hatching in response to the highest frequencies within the range tested (Fig. 5).

The hatching response to the 0–10 Hz stimulus differed between clutches where MCI tines were inserted into the clutch jelly, among eggs, and clutches mounted on the back of the MCI ($t_{(9)}=2.65$, $P=0.026$). This is not surprising because inserting the MCI tines into a clutch changes its mechanical response, greatly improving playback fidelity (M.S.C., K.M.W. and J.G.McD., unpublished). Nonetheless, the clutches mounted on the vibrating substrate did show very high levels of hatching (Fig. 5).

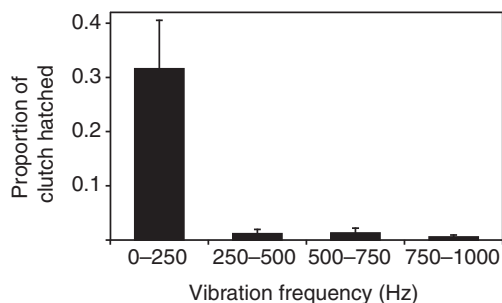


Fig. 4. Escape hatching response of *Agalychnis callidryas* embryos to vibration playbacks varying in frequency between 0 and 1000 Hz. Data are mean proportion hatched + s.e.m. Stimuli were 1-s bursts of 250 Hz-wide bands of noise, separated by 1-s periods of silence, played for 5 min to 5-day-old embryos. Embryos hatched in response to the lowest frequency range and showed little response to frequencies above 250 Hz.

DISCUSSION

Selection by egg predators, favoring early hatching in response to predator vibrations, and by aquatic predators, against early hatching in response to benign-source vibrations, have presumably worked together to shape the specificity of *A. callidryas*' escape hatching response. The success of potential decision rules for vibration-cued hatching will, however, be limited by the distributions of cue properties in predator attacks and benign disturbances. We have previously demonstrated that two temporal properties, vibration duration and intervibration interval, are informative and used by embryos (Warkentin et al., 2006b). Nonetheless, using just these two properties is insufficient for rapid, error-free classification of snakes and rain (Warkentin, 2005).

Frequency properties of vibrations in egg clutches are also informative in distinguishing predator attacks from common benign vibrations, and embryos use this information. There is, however, considerable variation within, and overlap between, vibration frequencies excited by benign and dangerous sources. Thus, even the best possible strategy relying on frequency information alone would lead embryos to hatch in response to some rainstorms, wind gusts and routine embryo movements or to remain vulnerable within their eggs during some predator attacks. Rather than relying on any single property, *A. callidryas* embryos combine frequency information with temporal properties of vibrational cues to refine their escape hatching response.

Frequency information and constraints on effective decision rules

Although vibration amplitudes vary among the five predator species we recorded (Fig. 3), the frequency ranges they excite are largely consistent (Fig. 2). This may simplify *A. callidryas*' risk assessment task considerably. Embryos could employ decision rules based on a common template for predator attack and need not identify multiple distinct frequency distributions characteristic of different predators. Although wasps and snakes have dissimilar morphologies and sizes, they interact with clutches in some fundamentally similar ways, biting and pulling to remove eggs. This similar behavior may, in part, be responsible for the similarity of attack vibration frequencies. By contrast, the relative frequency distributions of different categories of benign vibrational disturbances to *A. callidryas* egg clutches are distinct from one another.

One simple strategy that embryos could employ would be to hatch in response to any cue exciting frequencies that are common in predator attacks, and not in response to other frequencies (Wytenbach et al., 1996). This, however, would result in many false alarms. All frequencies excited during predator attacks are also characteristic of rain, and vibrations generated by routine embryo movements excite frequencies common to predator disturbances as well (Fig. 2). Thus, embryos could use the absence of frequencies in the range excited by predators as an indicator that a disturbance is benign, but their presence is insufficient to indicate danger. Alternatively, if predator vibrations consistently contained more energy than benign vibrations in any frequency range, embryos could use a threshold response to those frequencies as an escape strategy. These conditions are, however, not satisfied; rain vibrations vary greatly in amplitude and are often more intense than predator vibrations across all frequencies (Fig. 3). Indeed, a discriminant function based on absolute spectra categorized intense vibrations as rain and weaker vibrations as predators, a strategy likely to lead to many false alarms given the wide variation in rain intensity in nature.

Another possible strategy would be to assess the dominant frequency of disturbances. Dominant frequency is commonly used

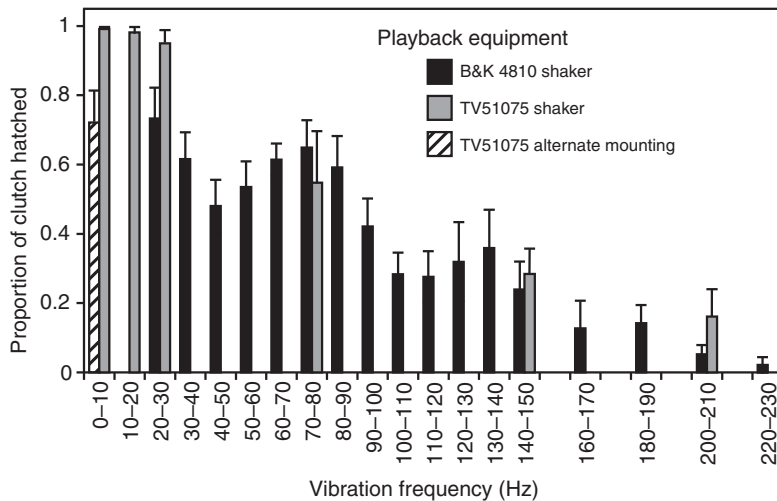


Fig. 5. Escape hatching response of *Agalychnis callidryas* embryos to variation in vibration frequency in the 0–250 Hz range that elicits hatching in broadband playbacks. Data are mean proportion hatched + s.e.m. Stimuli were 10 Hz-wide bands of noise, in a temporal pattern of 1-s noise:1-s silence, repeated for 5 min. Playbacks used two different shaker types, as indicated. All stimuli but one were delivered directly to clutches mounted on an immobile substrate via vibrating tines inserted among eggs. The lowest frequency stimulus (0–10 Hz) was also played to clutches mounted on a vibrating substrate. Embryos hatch more strongly in response to lower frequencies and in response to direct stimulation rather than stimulation transferred from the substrate.

by biologists to characterize acoustic communication signals and is used by many species to encode information (Bradbury and Vehrencamp, 1998; Gerhardt and Huber, 2002). This measure is particularly useful for describing or distinguishing between sounds or vibrations that are nearly tonal or have a few discrete harmonics. It is likely to be less useful for discriminating among broadband stimuli with overlapping frequency distributions, such as those generated both by predators and many benign disturbances to egg clutches. Rain dominant frequencies are, on average, higher than those of some, but not all, predator species (Fig. 2). However over half of rainstorms sampled (11 of 19) had dominant frequencies below the mean for predator attacks. Moreover, wind, embryo movement and predator cues did not differ in dominant frequency. Thus, hatching in response to all disturbances with dominant frequencies characteristic of predator attack would result in frequent false alarms and increased mortality of premature hatchlings due to aquatic predation.

Our discriminant function analyses based on standardized spectra suggest that a more promising strategy would be to combine information from multiple frequency ranges. Although all frequencies characteristic of predators are also strongly represented in most benign disturbances, rain excites frequencies above the range of predator vibrations, and wind excites only very low frequencies (Fig. 2). Discriminant functions positively weighted predator attack frequencies and negatively weighted extremely low frequencies characteristic of wind and high frequencies characteristic of rain (Table 1). A potentially effective rule to distinguish predators from rain could be to hatch in response to frequencies characteristic of attack unless frequencies excited only by rain are also present. A more complex rule in which predator frequencies are stimulatory and higher rain frequencies are inhibitory could also be effective. A similar rule might aid discrimination between predators and wind. Embryos could hatch in response to broad bands of low frequencies characteristic of predators but avoid hatching in response to narrower bands of very low frequencies excited by wind.

How effectively red-eyed treefrog embryos might employ any potential decision rule depends on their sensory physiology and information-processing abilities as well as on the information available. How these embryos receive and process vibrational information is unknown. Nonetheless, the property distributions of stimulus types affect their discriminability by even an ideal receiver (Macmillan and Creelman, 2005). Our most effective discriminant function separated predator attacks and rainstorms, with over 90%

correct classifications, very few missed cues and a moderate level of false alarms. Embryos using a similar decision rule would hatch in almost every predator attack but would occasionally hatch prematurely during rain. Classifications in our DFA were based on 5-min samples of rain vibrations and several minutes of predator attack. In snake attacks, however, on average, embryos begin hatching in 16 s and all embryos in the clutch hatch in under 5 min (Warkentin et al., 2007). Thus, embryos tend to base their hatching decisions on shorter samples of vibration than we used in our analyses. Given the variation in frequency distributions among individual vibrations within attacks and storms (Warkentin, 2005), shorter samples should result in poorer estimates of cue frequency distributions. Therefore, embryos discriminating between predator and rain vibrations on the basis of frequency properties alone would be expected to make more classification errors than our analyses did. Classification results erring towards either missed cues or false alarms could be achieved by changing the weightings of frequencies characteristic of predator attacks or rain. Similarly, selection by arboreal and aquatic predators should refine the hatching response to both reduce and balance missed cues and false alarms, in proportion to the risks posed by both classes of predators.

Vibration frequency properties and escape hatching behavior

Frequency properties clearly affect *A. callidryas*' vibration-cued hatching. Embryos hatch only in response to a specific range of frequencies and are unresponsive to frequencies outside of this range. This reinforces the conclusion from work on temporal properties (Warkentin et al., 2006b) that escape hatching is not a general response to any vibrational disturbance. Frequencies that induce hatching are largely consistent with the frequency range excited during predator attacks. Furthermore, variation in the hatching response within this range is largely consistent with the relative distribution of energies in predator attacks (Figs 2 and 5). We observed the highest levels of hatching in response to very low vibrational frequencies strongly excited by predators, moderate levels of hatching to somewhat higher frequencies, and almost no hatching to frequencies absent in predator attack.

Although high-amplitude vibrations at extremely low frequencies produce large displacements of MCI tines, potentially confounding vibrational and tactile cues, embryos also hatched strongly in response to the 0–10 Hz stimulus delivered via a vibrating substrate. The hatching response of indirectly stimulated clutches indicates that tactile cues from movement of an object through the clutch are

not required for a strong response to low frequencies and that very low frequency vibrations themselves function as strong hatching stimuli. The stronger response of embryos to direct stimulation, rather than vibrations transferred through the substrate, could reflect either improved mechanical coupling between the shaker and clutch or the contribution of an additional tactile component to the stimulus.

The experiments presented here do not fully elucidate the frequency-based decision rules that *A. callidryas* embryos use but they begin to narrow the possibilities. The fine-scale frequency–response pattern shows no sharp discontinuities but rather a gradual modulation, with hatching increasingly less likely as frequencies rise to ranges that are not strongly excited by predator attacks. Thus, the perception of frequency does not appear to be categorical (Wyttenbach et al., 1996), and embryos do not simply hatch in response to frequencies within some range. Our results do, however, suggest the converse: if they perceive them, embryos may classify frequencies above the range of predator vibrations as benign (Fig. 4).

The dominant frequencies of vibrations excited by wind, rain and routine embryo movements all fall within the range that stimulates high hatching. Moreover, all of the energy in wind vibrations, most of the energy in embryo movements and much of the energy in rain vibrations falls within a range that stimulates hatching. Therefore, the relative (rain) or apparently complete (wind, embryos) lack of hatching in response to these vibrations in nature cannot be attributed to a lack of stimulatory frequencies.

Discriminant function analysis suggests that some frequencies may stimulate hatching while other frequencies inhibit it. The playbacks presented here do not directly test the inhibition hypothesis for vibrations above the range that stimulates hatching, but they are consistent with it. In other playback experiments (Warkentin and Caldwell, in press) (M.S.C., J.G.McD. and K.M.W., unpublished), we have found that high-frequency energy reduces the hatching response to low-frequency vibrations presented concurrently. Such inhibition by high frequencies may contribute to the lack of hatching in rainstorms, which strongly excite frequencies above those that induce hatching.

Both DFA and examination of wind spectra (Fig. 2) suggest that extremely low frequencies (<16 Hz) could also inhibit hatching, at least in the absence of broader-band low-frequency vibrations. Our playback results clearly reject this hypothesis. The 0–10 Hz stimulus elicited a hatching response as strong as any we have recorded in playback, even though its acceleration amplitude was one-third that of the other playbacks. Thus neither frequency-dependant hatching inhibition nor a lack of stimulatory frequencies can explain the absence of hatching in wind. Temporal properties may account for this, or wind may not excite vibrations with sufficient amplitude to elicit hatching. Insufficient amplitude may also account for the lack of hatching in response to routine embryo movements, which consistently excite vibrations of low amplitudes (Fig. 3) (M.S.C., J.G.McD. and K.M.W., unpublished).

To construct our frequency–response curves for escape hatching, we chose a temporal pattern of 1 s vibration:1 s silence that elicits strong hatching when the stimulus includes broadband low-frequency energy (Warkentin et al., 2006b). The hatching response to this ‘scary’ temporal pattern was dependent upon vibration frequency. Low frequencies again elicited hatching, but frequencies outside the range characteristic of predator attacks ablated the response to this temporal pattern. Embryo response to variation in temporal properties shows a similar pattern. Even at ‘scary’ frequencies, embryos do not hatch unless both vibration durations

and intervibration intervals are within narrow ranges (Warkentin et al., 2006b). These three properties are therefore non-redundant (Bradbury and Vehrencamp, 1998; Partan and Marler, 2005). None alone is sufficient to elicit hatching; if any of them is out of range, embryos will not hatch. By combining information from the frequency, duration and spacing properties of vibrations, *A. callidryas* embryos substantially limit the range of stimuli that elicit hatching and thereby refine their hatching response.

Information from incidental cues

We know a great deal about how animals communicate using stereotyped signals (Bradbury and Vehrencamp, 1998; Endler, 1992; Guilford and Dawkins, 1991). While foraging or attempting to avoid predators, however, animals often extract information from cues that have not evolved for efficacy in communication. These cues can be highly variable in form, with property distributions that overlap those of other environmental stimuli, creating an information-processing challenge. Our studies of *A. callidryas*’ use of vibrational cues begin to elucidate how one animal solves this challenge (Warkentin and Caldwell, in press).

The detection of incidental cues is complicated by their variability. Because communication signals are stereotyped, short samples are highly informative estimates of mean values. By contrast, brief samples of cue properties may be less indicative of their overall distribution. Individual vibrations in *A. callidryas* clutches, such as from different raindrops or snake movements, vary substantially in frequency spectra and other properties (Warkentin, 2005). Thus, information sampled from a small number of vibrations does not reliably identify disturbance types. It appears that embryos rely on longer sampling periods to extract useful information from the statistical regularities of cues. It is not clear how embryos integrate frequency or other information over time. Nonetheless, they typically sample cues for some period before hatching, and this delay varies with the rate at which information accrues (Warkentin et al., 2007).

A. callidryas embryos also improve their ability to detect predators by combining information from multiple, independently variable cue properties. Each added property effectively reduces the overlap between cues from dangerous and benign sources and the consequent unavoidable decision errors (Macmillan and Creelman, 2005). The strategy of combining information from multiple sources is likely widespread in animals attempting to exploit incidental cues in any context. These sources of information may be multiple properties of a single cue (e.g. size, shape or angular velocity of a visual cue) (Cooper et al., 2003; Helfman, 1989; Layne, 1998; Lorenz, 1939; Tinbergen, 1948) or may come from cues in different sensory modalities, such as visual and acoustic cues (Amo et al., 2004; Bouwma and Hazlett, 2001; Chivers et al., 2001; Hartman and Abrahams, 2000; Maier et al., 2004; Mathis and Vincent, 2000).

Detection strategies that rely on statistical regularities of cues and combine information from multiple sources are likely to be common wherever animals interpret environmental information outside of a signaling context. The lack of established methodologies for studying incidental, non-stereotyped cues may, however, contribute to the relative scarcity of research in this area. When investigating properties of cues, and the ways animals use them, it may be important to rely less on point measures, such as dominant frequency, and instead focus on complete distributions. It may also be productive to shift focus from attempts to identify outstanding characteristics of the cues of interest to broader characterizations of both the cues and their background (Barth et al., 1988; Casas et al., 1998), considering multiple cue properties and different sensory modalities. This should improve our understanding of both

information-processing tasks in nature and the ways in which animals may solve them.

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REFERENCES

- Amo, L., Lopez, P. and Martin, J. (2004). Wall lizards combine chemical and visual cues of ambush snake predators to avoid overestimating risk inside refuges. *Anim. Behav.* **67**, 647-653.
- Bacher, S., Casas, J. and Dorn, S. (1996). Parasitoid vibrations as potential releasing stimulus of evasive behaviour in a leafminer. *Physiol. Entomol.* **21**, 33-43.
- Bacher, S., Casas, J., Wackers, F. and Dorn, S. (1997). Substrate vibrations elicit defensive behaviour in leafminer pupae. *J. Insect Physiol.* **43**, 945-952.
- Barth, F. G., Bleckmann, H., Bohnenberger, J. and Seyfarth, E. A. (1988). Spiders of the genus *Cupiennius* Simon 1891 (Araneae, Tenuidae). II. On the vibratory environment of a wandering spider. *Oecologia* **77**, 194-201.
- Bell, P. D. (1980). Transmission of vibrations along plant stems: implications for insect communication. *J. NY Entomol. Soc.* **88**, 210-216.
- Blumstein, D., Cooley, L., Winternitz, J. and Daniel, J. (2008). Do yellow-bellied marmosets respond to predator vocalizations? *Behav. Ecol. Sociobiol.* **62**, 457-468.
- Bouwma, P. and Hazlett, B. A. (2001). Integration of multiple predator cues by the crayfish *Orconectes propinquus*. *Anim. Behav.* **61**, 771-776.
- Bradbury, J. W. and Vehrencamp, S. L. (1998). *Principles of Animal Communication*. Sunderland: Sinauer.
- Casas, J., Bacher, S., Tautz, J., Meyhofer, R. and Pierre, D. (1998). Leaf vibrations and air movements in a leafminer-parasitoid system. *Biol. Control* **11**, 147-153.
- Casas, J., Magal, C. and Sureau, J. (2007). Dispersive and non-dispersive waves through plants: implications for arthropod vibratory communication. *Proc. Biol. Sci.* **274**, 1087-1092.
- Catania, K. C. (2008). Worm grunting, fiddling and charming: humans unknowingly mimic a predator to harvest bait. *PLoS ONE* **3**, e3472.
- Chivers, D. P., Mirza, R. S., Bryer, P. J. and Kiesecker, J. M. (2001). Threat-sensitive predator avoidance by slimy sculpins: understanding the importance of visual versus chemical information. *Can. J. Zool.* **79**, 867-873.
- Clegg, J. M. and Barlow, C. A. (1982). Escape behaviour of the pea aphid *Acyrtosiphon pisum* (Harris) in response to alarm pheromone and vibration. *Can. J. Zool.* **60**, 2245-2252.
- Cocroft, R. B. and Rodriguez, R. L. (2005). The behavioral ecology of insect vibrational communication. *Bioscience* **55**, 323-334.
- Cocroft, R. B., Shugart, H. J., Konrad, K. T. and Tibbs, K. (2006). Variation in plant substrates and its consequences for insect vibrational communication. *Ethology* **112**, 779-789.
- Cooper, W. E., Martin, J. and Lopez, P. (2003). Simultaneous risks and differences among individual predators affect refuge use by a lizard, *Lacerta monticola*. *Behaviour* **140**, 27-41.
- Dillon, W. R. and Goldstein, M. (1984). *Multivariate Analysis, Methods and Applications*. New York: Wiley.
- Djemai, I., Casas, J. and Magal, C. (2001). Matching host reactions to parasitoid wasp vibrations. *Proc. Biol. Sci.* **268**, 2403-2408.
- Duellman, W. E. (2001). *The Hylid Frogs of Middle America*. St Louis, MO: Society for the Study of Amphibians and Reptiles.
- Endler, J. A. (1992). Signals, signal conditions, and the direction of evolution. *Am. Nat.* **139**, S125-S153.
- Evans, T. A., Lai, J. C. S., Toledano, E., McDowall, L., Rakotonarivo, S. and Lenz, M. (2005). Termites assess wood size by using vibration signals. *Proc. Natl. Acad. Sci. USA* **102**, 3732-3737.
- Gerhardt, H. C. and Huber, F. (2002). *Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions*. Chicago: University of Chicago Press.
- Ginsberg, J. H. (2001). *Mechanical and Structural Vibrations: Theory and Applications*. New York: Wiley.
- Gnatzy, W. and Kämper, G. (1990). Digger wasp against crickets. II. An airborne signal produced by a running predator. *J. Comp. Physiol. A* **167**, 551-556.
- Guilford, T. and Dawkins, M. S. (1991). Receiver psychology and the evolution of animal signals. *Anim. Behav.* **42**, 1-14.
- Hartman, E. J. and Abrahams, M. V. (2000). Sensory compensation and the detection of predators: the interaction between chemical and visual information. *Proc. Biol. Sci.* **267**, 571-575.
- Helfman, G. S. (1989). Threat-sensitive predator avoidance in damselfish-trumpetfish interactions. *Behav. Ecol. Sociobiol.* **24**, 47-58.
- Hergenröder, R. and Barth, F. G. (1983). The release of attack and escape behavior by vibratory stimuli in a wandering spider (*Cupiennius salei* Keys.). *J. Comp. Physiol. A* **152**, 347-359.
- Hill, P. S. M. (2008). *Vibrational Communication in Animals*. Boston, MA: Harvard University Press.
- Kats, L. B. and Dill, L. M. (1998). The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* **5**, 361-394.
- Lawrence, P. O. (1981). Host vibration: a cue to host location by the parasite, *Biosteres longicaudatus*. *Oecologia* **48**, 249-251.
- Layne, J. E. (1998). Retinal location is the key to identifying predators in fiddler crabs (*Uca pugilator*). *J. Exp. Biol.* **201**, 2253-2261.
- Lima, S. L. and Dill, L. M. (1989). Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* **68**, 619-640.
- Lorenz, K. (1939). Vergleichende Verhaltensforschung. *Zool. Anz. Suppl.* **12**, 69-102.
- Macedonia, J. M. and Yount, P. L. (1991). Auditory assessment of avian predator threat in semi-captive ringtailed lemurs (*Lemur catta*). *Primates* **32**, 169-182.
- Macmillan, N. A. and Creelman, C. D. (2005). *Detection Theory: A User's Guide*. Mahwah, NJ: Lawrence Erlbaum Associates.
- Maier, J. X., Neuhoff, J. G., Logothetis, N. K. and Ghazanfar, A. A. (2004). Multisensory integration of looming signals by rhesus monkeys. *Neuron* **43**, 177-181.
- Mann, D. A., Lu, Z. and Popper, A. N. (1997). A clupeid fish can detect ultrasound. *Nature* **389**, 341-341.
- Mathis, A. and Vincent, F. (2000). Differential use of visual and chemical cues in predator recognition and threat-sensitive predator-avoidance responses by larval newts (*Notophthalmus viridescens*). *Can. J. Zool.* **78**, 1646-1652.
- Michelsen, A., Fink, F., Gogala, M. and Traue, D. (1982). Plants as transmission channels for insect vibrational songs. *Behav. Ecol. Sociobiol.* **11**, 269-281.
- Miller, L. A. and Surlykke, A. (2001). How some insects detect and avoid being eaten by bats: tactics and counter-tactics of prey and predator. *Bioscience* **51**, 570-581.
- Moiseff, A., Pollack, G. S. and Hoy, R. R. (1978). Steering responses of flying crickets to sound and ultrasound: mate attraction and predator avoidance. *Proc. Natl. Acad. Sci. USA* **75**, 4052-4056.
- Narins, P. M., Lewis, E. R., Jarvis, J. J. U. M. and O'Riain, J. (1997). The use of seismic signals by fossorial southern African mammals: a neuroethological gold mine. *Brain Res. Bull.* **44**, 641-646.
- Partan, S. R. and Marler, P. (2005). Issues in the classification of multimodal communication signals. *Am. Nat.* **166**, 231-245.
- Pfannenstiel, R. S., Hunt, R. E. and Yeargan, K. V. (1995). Orientation of a hemipteran predator to vibrations produced by feeding caterpillars. *J. Insect Behav.* **8**, 1-9.
- Schüch, W. and Barth, F. G. (1985). Temporal patterns in the vibratory courtship signals of the wandering spider *Cupiennius salei* Keys. *Behav. Ecol. Sociobiol.* **16**, 263-271.
- Swaigood, R. R., Rowe, M. P. and Owings, D. H. (1999). Assessment of rattlesnake dangerousness by California ground squirrels: exploitation of cues from rattling sounds. *Anim. Behav.* **57**, 1301-1310.
- Tautz, J. and Markl, H. (1978). Caterpillars detect flying wasps by hairs sensitive to airborne vibration. *Behav. Ecol. Sociobiol.* **4**, 101-110.
- Tinbergen, N. (1948). Social releasers and the experimental method required for their study. *Wilson Bull.* **60**, 6-51.
- Virant-Doberlet, M. and Cokl, A. (2004). Vibrational communication in insects. *Neotrop. Entomol.* **32**, 121-134.
- Warkentin, K. M. (1995). Adaptive plasticity in hatching age: a response to predation risk trade-offs. *Proc. Natl. Acad. Sci. USA* **92**, 3507-3510.
- Warkentin, K. M. (1999). The development of behavioral defenses: a mechanistic analysis of vulnerability in red-eyed tree frog hatchlings. *Behav. Ecol.* **10**, 251-262.
- Warkentin, K. M. (2000). Wasp predation and wasp-induced hatching of red-eyed treefrog eggs. *Anim. Behav.* **60**, 503-510.
- Warkentin, K. M. (2005). How do embryos assess risk? Vibrational cues in predator-induced hatching of red-eyed treefrogs. *Anim. Behav.* **70**, 59-71.
- Warkentin, K. M. and Caldwell, M. S. (In press). Assessing risk: embryos, information, and escape hatching. In *Cognitive Ecology II: The Evolutionary Ecology of Information Processing and Decision-Making* (ed. R. Dukas and J. M. Ratcliffe). Chicago: University of Chicago Press.
- Warkentin, K. M., Buckley, C. R. and Metcalf, K. A. (2006a). Development of red-eyed treefrog eggs affects efficiency and choices of egg-foraging wasps. *Anim. Behav.* **71**, 417-425.
- Warkentin, K. M., Caldwell, M. S. and McDaniel, J. G. (2006b). Temporal pattern cues in vibrational risk assessment by red-eyed treefrog embryos, *Agalychnis callidryas*. *J. Exp. Biol.* **209**, 1376-1384.
- Warkentin, K. M., Caldwell, M. S., Siok, T. D., D'Amato, A. T. and McDaniel, J. G. (2007). Flexible information sampling in vibrational assessment of predation risk by red-eyed treefrog embryos. *J. Exp. Biol.* **210**, 614-619.
- Wilcox, R. S., Jackson, R. R. and Gentile, R. (1996). Spiderweb smokescreens: spider trickster uses background noise to mask stalking movements. *Anim. Behav.* **51**, 313-326.
- Wytenbach, R. A., May, M. L. and Hoy, R. R. (1996). Categorical perception of sound frequency by crickets. *Science* **273**, 1542-1544.