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Substrate-Borne Communication in Chameleons: Do Vibrations Induce Behavioral Changes?

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SUBSTRATE-BORNE COMMUNICATION IN CHAMELEONS:

DO VIBRATIONS INDUCE BEHAVIORAL CHANGES?

A Capstone Project Presented in Partial Fulfillment

of the Requirements for the Degree Bachelor of Biology

with Honors College Graduate Distinction at

Western Kentucky University

By

Emily J. Hamilton

August 2017

*****

CE/T Committee:

Dr. Michael Smith, Chair

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I dedicate this thesis to my sister, Elise Hamilton, who inspires and motivates me to be

the best I can be.

ACKNOWLEDGEMENTS

ABSTRACT

Understanding the modes of communication used by a species is essential to understanding their ecology, behavior, and evolution. Substrate-borne vibrations have been reported to be produced by the veiled chameleon (*Chamaeleo calyptratus*), possibly implemented by use of a gular pouch. We found that veiled chameleons produced vibrations under dominance and mating behavioral contexts. We tested the sensitivity of veiled chameleons to vibrations by placing chameleons, one at a time, on a wooden dowel attached to a permanent magnetic shaker and recording each chameleon's behavior before, during, and after a three-pulse vibrational stimulus of 25, 50, 150, 300, or 600 Hz. Vibrations were measured via an accelerometer attached to the dowel. The chameleons exhibited a stop-behavioral response (i.e., lack of movement) when exposed to stimulus of 50 and 150 Hz. Further experiments testing behavioral responses at lower (25 Hz) and higher (300 and 600 Hz) frequencies showed little to no reduction in movement. For induced sounds produced by chameleons, there was no significant correlation between size of the chameleon and average dominant vibrational frequency or duration of a vibrational pulse and dominant frequency. Chameleon vibrational response was also studied under various behavioral contexts by pairing chameleons on a dowel and recording the natural vibrational responses of chameleons under these conditions via an accelerometer. The pairing of chameleons in various behavioral contexts, including male-male, male-female, and interspecific interactions resulted in natural vibrational responses that were much shorter in duration and more pulse-like than the induced low-frequency tonal vibrations previously studied in *C. calyptratus*, suggesting the possibility of different types of vibrational responses. These findings improve the understanding of

behavioral responses between chameleons, and can be utilized as a basis for further

research into the morphology and physiology of chameleons.

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EDUCATION

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Hamilton, E., Smith, M., & Huskey, S. (2017). *Substrate-borne communication in chameleons: Do vibrations induce behavioral changes?* Poster presented at the WKU Student Research Conference. Bowling Green, KY.

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SECTION ONE

INTRODUCTION

Knowledge concerning the modes of communication used by a species is essential to the

understanding of how members of the same species relay information, as well as understanding

how this information may be relayed to members of other species. Communication via vibrations

through a substrate, such as through the soil or plant matter, is a mode of transmission utilized

across a number of animal taxa (Hill, 2009). The study of vibrational communication is a

relatively new area of inquiry, as this modality of communication has not yet been investigated

in the majority of animal species, but has been studied extensively in various species of insects

and arachnids (Fabre et al., 2012; Shamble et al., 2016; Hill, 2009). It has been estimated that

nearly 200,000 species of insects either exclusively rely on substrate-level communication, or

use it collectively with other communication styles (Cocroft & Rodríguez, 2005). The versatility

of substrate communication is apparent in the observations of various insect species, with certain

species using different substrate media for communication, including dirt, plant matter, water, or

other constructs that are unique to a particular species (Hill, 2009). While the study of vibrational

communication is a fairly new discipline, the exclusive use of substrate signaling, or the use of

substrate signaling in conjunction with other methods of communication has proven to be a large

constituent of communication in some taxa.

Although the use of vibrational communication has primarily been reported for insects

and arachnids, it is also been reported in some species of other taxa including Mammalia, Aves,

Reptilia, Amphibia, Osteichthyes, Crustacea, and Nemotoda (reviewed in Hill, 2009). In terms of

using vibrations as a source of information, current findings report that 16 species from the class

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Amphibia (with 2 from the order Urodela, and 14 from the order Anura), and 12 species from the

class Reptilia utilize substrate-borne communication (Hill, 2009). The 12 species of reptile

reported to implement substrate-level communication are all from the order Squamata, which is

composed of lizards and snakes (Hill, 2009). While this represents a broad categorization,

current publications on substrate-borne communication are limited, likely due to the presence of

more observable communication responses, such as reactions to acoustic or visual signals (Hill,

2009).

In a study conducted by Barnett et al. (1999), the veiled chameleon, *C. calyptratus*, was

observed producing low-frequency vibrations that traveled through the plant-based substrate as a

form of communication (Barnett et al., 1999). These vibrations typically ranged from 50-150 Hz,

were not generally audible to human hearing, and were elicited in response to different

behavioral contexts, including male-male dominance, male-female mating, and in the presence of

a perceived threat (Barnett et al., 1999). The researchers used an accelerometer placed on a

branch that the chameleon was perched on to record the rapid, low-frequency vibrational

"pulses" (Barnett et al., 1999). In corroboration of these findings, veiled chameleons, as well as

C. gracilis and *C. dilepis*, produced vibrational responses when handled in our laboratory,

particularly when the chameleon's casque was gently squeezed or a limb gently poked (personal

observation). Some of these responses were barely audible as a low-frequency hoot-like sound,

while most of them were inaudible.

We postulate that chameleons are able to produce branch-borne vibrations through the

use of a gular pouch (Huskey, pers. comm.), a specialized vocal structure commonly found in

birds and, to a lesser extent, some species of amphibians (Dooling et al., 2000; Rosenthal et al.,

2004). The gular pouch has also been described in a few chameleon species, as an inflatable sac

that is connected to the ventral wall of the trachea just behind the larynx (Germershausen, 1913; Klaver, 1981; Klaver and Böhme, 1986). A recent comparative morphological study of chameleons observed considerable variability in the gular pouch between species, with some being large and extensive, and others being vestigial or non-existant (Boka, 2012), but the specific relationship between gular pouch morphology and specific functions have not yet been studied. Boka (2012) referred to the gular pouch of chameleons as "resonators", proposing that these structures are used to amplify vibrations for signal production and communication in a similar way that the swim bladder acts as a resonator to amplify some fish sound production signals (reviewed in Parmentier & Fine, 2016). Further, Boka (2012) hypothesized that chameleons with vestigial or non-existant resonators would not be able to communicate via vibrations.

Gular pouches in species of birds and amphibians that utilize substrate-level communication use the gular pouch in order to produce vibrational signals used for communication (Bartlett & Bartlett, 2001; Starnberger, 2014). For example, gular pouches are typically utilized in bird species such as the Brandt's cormorant, which inflates and vibrates the gular pouch during mating interactions (Williams, 1942). Gular pouches in birds can have other, non-communicative functions as well. For example, the Namaqua dove (*Oena capensis*) vibrates the gular pouch in order to maintain heat balance (Gerson et al., 2014). Gular pouches are also utilized in amphibian species, such as the white-lipped frog (*Leptodactylus albilabris*), that can strike the substrate to produce seismic signals (Narins, 1990; Hedges & Sibley, 1994). The presence of a gular pouch in several species of chameleons in conjunction with the close evolutionary ties between reptiles and birds supports the hypothesis that chameleons utilize the

gular pouch for vibration production and communication in a similar fashion to birds (Dooling et al., 2000).

Barnett et al. (1999) is the only published research describing vibration production and potential communication in chameleons, and they only presented data from one species (*Chamaeleo calyptratus*). While the few chameleons tested demonstrated the ability to elicit vibrations via the substrate, it is unclear whether or not this was strictly a communication pattern, or exactly what these signals mean.

My project focused on understanding the behavioral contexts in which chameleons both produce and respond to low-frequency vibrations, with the ultimate goals of the project being to: 1) test the vibrational sensitivity of *C. calyptratus* and *C. gracilis* across different frequencies, 2) examine the relationship between chameleon size and frequency of vibration production, and 3) to document the behavioral contexts under which *C. calyptratus*, *C. gracilis*, and *C. dilepis* produces vibrations. *C. calyptratus* was my focal species for these behavioral experiments since it has been shown that this species is the only chameleon documented to utilize vibrations as a possible means of communication (Barnett et al., 1999), although preliminary data for a few *C. gracilis* and *C. dilepis* were collected as well.

I hypothesized that the chameleons' sensitivity to vibrational communication would be observable with a stop-behavioral response, that larger chameleons would produce vibrations at lower frequencies, and that the chameleons would utilize substrate-borne communication to relay information to other chameleons under certain behavioral contexts. In order to test these hypotheses, I developed a protocol for the detection of behavioral responses to the presence of a vibrational stimulus, and detecting and recording the vibrations of chameleons under different behavioral contexts. In general, each experiment involved placing a chameleon or pair of

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chameleons on a wooden dowel, and simulataneously recording both video and accelerometer

vibration data with a specially-designed data analysis software. Then both the animals' behavior

and vibrations were able to be synchronized in terms of their response to vibrations as well as

their own vibration production.

Figure 1. Dissection of *C. calyptratus* revealing the gular pouch, inflated using compressed air. The gular pouch is thought to be responsible for the chameleon's ability to communicate via vibrations through the substrate. Photo courtesy of Dr. Steve Huskey.

SECTION TWO

MATERIALS AND METHODS

Animal Care

      C. calyptratus, ranging in snout-vent lengths between 12.6 and 20.1 cm, *C. dilepis*, with a

snout-vent length of 8.9 cm, and *C. gracilis*, ranging in snout-vent lengths between 11.4 and 11.8

cm (Table 1), were kept in a Zoo Med Reptibreeze open air screen cage. Larger chameleons were

kept in 24 x 24 x 48 inch cages, while smaller chameleons were kept in 18 x 18 x 36 inch cages.

In order to mimic the humidity and dense foliage found in a chameleon's natural habitat, a

mixture of real and artificial plants were used as well as Zoo Med Reptirain automatic misting

machines and water drippers. A total sample size of seven chameleons (five *C. calyptratus* and

two *C. gracilis*) were observed when testing for vibrational sensitivity. For experiments testing

the relationship between chameleon size and vibration frequency produced, six *C. calyptratus*

were used. For experiments examining vibration production under different behavioral contexts,

four *C. calyptratus*, one *C. gracilis*, and one *C. dilepis* were used. Subjects used for these trials

received no training prior to, or throughout the duration of this research, as the objective was to

observe and record the natural behaviors and vibrational responses of each animal. These

experiments were done under the approval of the Institutional Animal Care and Use Committee

of Western Kentucky University (Animal Welfare Assurrance #A3448-01).

Overview of Experiments & Video Analysis

      The objective of these experiments was to better understand the behavioral contexts in

which *C. calyptratus* and *C. gracilis* both produce and respond to low-frequency vibrations.

Three experiments were designed in order to meet this objective: 1) testing the vibrational sensitivity of the chameleons across different frequencies, 2) examining the potential relationship between chameleon size and frequency of vibration produced, and 3) observing the behavioral contexts under which chameleons naturally produce vibrations. The sensitivity of veiled chameleons was first tested by placing a chameleon on a dowel that was connected to a permanent magnetic shaker and presented a vibrational stimulus in order to gauge behavioral responses at certain frequencies. Once behavioral data for a specific stimulus frequency was established, preparation for the next set of experiments was conducted. For these experiments, a chameleon was placed upon the dowel and induced to produce a vibrational response, which was recorded by an accelerometer attached to the chameleon's casque and recorded by software uniquely designed for this project to record video and vibration signals simultaneously. This software was developed in LabVIEW by Dr. Doug Harper of the WKU Physics Department. Vibrational responses from each trial were then exported as .wav files and subsequently processed using Audacity software in order to determine the dominant frequency of each chameleon's response. Lastly, multiple chameleons were placed upon the dowel and recorded eliciting vibrations in natural behavioral contexts. Using data aquisition software, the simultaneous recording of vibrational and video data allowed the observation of possible physical indicators of vibration production. In detail, the experiments were:

Experiment 1: Behavioral Response to Vibrations

For each trial, an individual chameleon (N=7) was placed upon a wooden dowel 77 cm in length and 0.5 cm in diameter, balanced 15 cm above the table surface using support rods and clamps covered in acoustical foam. The vibrational stimulus used during these experiments was a series of three, rapid, low-frequency pulses which were produced by a Kistler 10 MHz Function

Generator connected to a 0.4 amp LDS Power Amplifier and an LDS V203 permanent magnet shaker, with the stimulus being presented after the chameleon began to walk across the dowel. The shaker was mechanically connected to the dowel via a metal rod connected with screws. The short duration of the pulses (three, approximately 0.5 s pulses within approximately 2.5 s), along with the frequency range of 25-600 Hz was used in order to mimic previous observations of chameleon vibrations (Barnett et al., 1999). These vibrations were detected by a PCB Piezotronics, Inc. accelerometer (Model 394C06), which was mounted via beeswax underneath the dowel 5 cm from the end distal to the shaker, and recorded using National Instruments Sound and Vibration Assistant software.

In each trial, only one frequency was used for the three pulses. In subsequent trials, the same individuals were tested with different frequencies, at least an hour after the previous frequency, but usually on a different day. In total, five frequencies were used in order to gauge a chameleon's sensitivity to pulse vibrations: 25, 50, 150, 300, and 600 Hz. Each trial was video recorded in order to observe the chameleon's behavioral response before and after the vibrational stimulus. In order to minimize background vibrations from affecting the outcome of each trial, the experiments were conducted in a WhisperRoom, Inc. Sound Isolation Enclosure (SE 2000 series). Video analysis recorded the velocity of the chameleon before and after stimulus, with velocity being reported in body lengths/second. The dowel was marked in 1 cm increments to facilitate the measurement of distances. An LED light connected to the function generator served as a visual indicator for when the vibrational stimulus was presented during each trial. The average pre-stimulus velocity was calculated from the time the chameleon started moving on the dowel until the first vibrational pulse of the stimulus, while the post-stimulus velocity of the chameleon was recorded for the first 5 seconds after the first vibrational "pulse" was produced

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and recorded. This was used to calculate the chameleon's percent change in velocity due to the stimulus.

An overall analysis of variance (ANOVA) was performed to examine the effect of stimulus frequency on the percent change in chameleon velocity for both *C. calyptratus* and *C. gracilis*. When an overall frequency effect was found, post-hoc Tukey's Honestly Significant difference (HSD) test was performed to test for differences in percent change in velocity between different stimulus-frequency treatments. The Wilcoxon signed-rank test was then used to test for differences in chameleon velocity before and after the vibrational pulse stimulus for each frequency tested.

Experiment 2: Size-Frequency Relationship

For each trial an individual chameleon was placed upon a wooden dowel 122 cm in length and 1.75 cm in diameter. Separate experiments recorded vibrations made by individual chameleons, either by gently squeezing the casque, or poking the upper arm of a chameleon to produce a vibration. The PCB accelerometer was attached to the casque of each chameleon in order to record vibrational responses from the chameleon while minimizing unwanted vibrational recordings caused by the chameleon's movement. The accelerometer was connected to a DAQ board and vibrational responses were recorded in conjunction with video streaming using the data aquisition software. Video recording occurred at a frame rate of 30 frames/second at 10 kHz. The vibrations for each trial were analyzed using Audacity software in order to describe the frequency and duration of each pulse. Five vibrational responses from each chameleon were analyzed in order to calculate the mean dominant frequency and duration for each chameleon. These values would then be compared to the size of each chameleon in order to understand the effect that size has on frequency of the vibrational productions. Linear regression analysis was

used to test the relationships between chameleon mass and dominant frequency of vibration production and between vibration pulse duration and dominant frequency of vibrations.

Experiment 3: Behavioral Contexts

For each trial, two chameleons were placed on a wooden dowel 122 cm in length and 1.75 cm in diameter. Coupling of chameleons was determined based on behavioral contexts that would most likely produce a natural vibrational response from one or both chameleons (i.e., a dominant or mating behavioral context). For example, two males of different sizes were considered a dominance context, a male and a female *C. calyptratus* was considered a courtship context, and a *C. gracilis* with a *C. dilepis* was an interspecific context. A PCB accelerometer was placed upon the dowel and vibrational responses were connected to a DAQ board and recorded using the data aquisition software. Video recordings for each trial were recorded using the same parameters as those outlined in the size-frequency relationship trials. No additional stimulation was provided outside of the natural stimulation provided by the presence of another chameleon, as these experimental trials were focused on recording responses within a natural behavioral context. Vibrational responses were analyzed using Audacity software in order to quantify low-frequency vibrations characteristic to veiled chameleons, as well as to observe other possible vibrational responses not previously studied. For each behavioral context, five pulses were analyzed to calculate the mean (±S.E.) duration.

Experiment	Mass (g)	SVL (cm)	Chameleon	Species
1	37.6	11.4	GM1	*gracilis*
1	41.1	11.8	GF1	*gracilis*
1	135	17.8	CM6	*calyptratus*
1	169	19.6	CM3	*calyptratus*
1	185	20.1	CM7	*calyptratus*
1	94.6	13.2	CF3	*calyptratus*
1	41	12.6	CM5	*calyptratus*
2	190	21.2	CM1	*calyptratus*
2	183.4	20.8	CM2	*calyptratus*
2	169	19.6	CM3	*calyptratus*
2	80.8	15.3	CM4	*calyptratus*
2	54.3	12.9	CM5	*calyptratus*
2	88.8	14.6	CF1	*calyptratus*
2	47.3	11.9	GF1	*gracilis*
3	183.4	20.8	CM2	*calyptratus*
3	80.8	15.3	CM4	*calyptratus*
3	47.3	11.9	GF1	*gracilis*
3	26.8	8.9	DM1	*dilepis*
3	121.3	17.9	CF2	*calyptratus*
3	54.3	12.8	CM5	*calyptratus*

Table 1. Chameleons used throughout the course of the three experiments. Listed are the species of chameleon used for each experiment, along with the mass in grams, the snout-vent length (SVL) in cm, and their identifying labels.
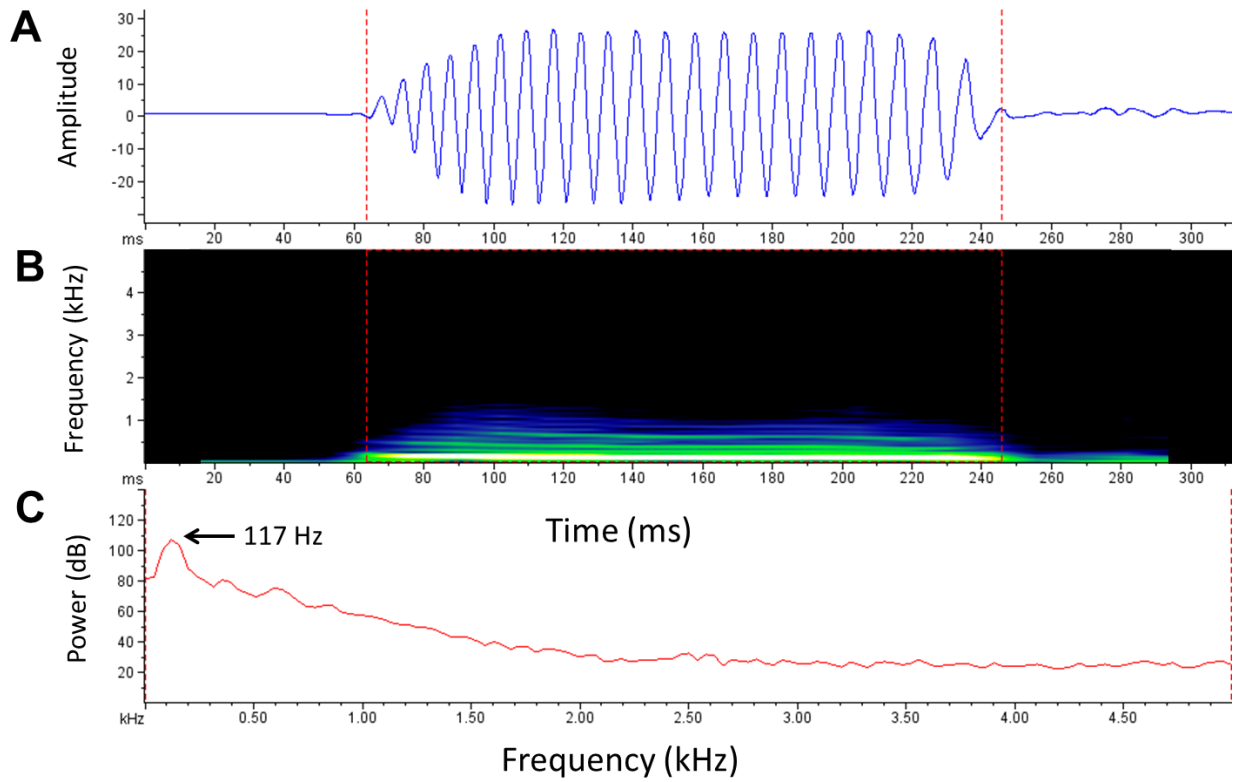
Figure 2. A) An enlarged view of a typical sinusoidal vibrational "hoot" from a male *C. calyptratus* (CM1). B) Spectrogram and C) power spectrum of the hoot from Figure 2A, showing that the vibration is low frequency (dominant frequency at 117 Hz) and tonal.
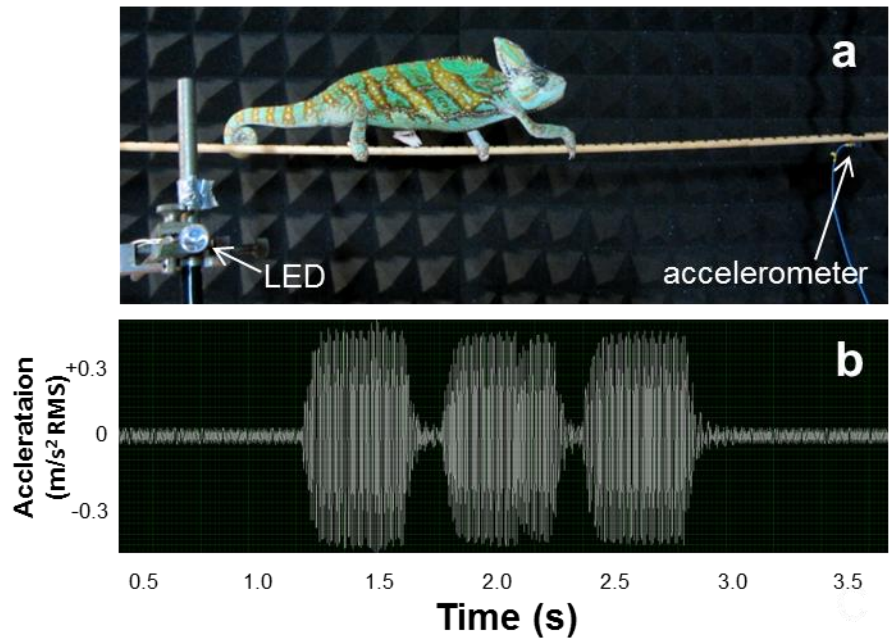
Figure 3. A) Experimental setup for the vibration sensitivity behavior trials with a veiled chameleon (*C. calyptratus*) walking across the dowel, towards the accelerometer. The LED light was turned on when the pulse vibrations were produced by a function generator and shaker. B) Three 150 Hz vibration pulses produced by the function generator as recorded by the accelerometer.

Figure 4. Image from a video of a dominant interaction between two male veiled chameleons (*C. calyptratus*). The accelerometer is attached to the right end of the dowel (with blue wire) in order to record possible vibrational responses during the interaction.

SECTION THREE

RESULTS

Behavioral Response to Vibrations

Percent change in chameleon velocity significantly differed by stimulus frequency in

both *C. calyptratus* (F=10.33, P<0.001) and *C. gracilis* (F=4.43, P=0.018). Both species

responded to some of the frequencies of pulsed vibrational stimuli similarly (i.e., they stopped or

slowed down their locomotion). Velocity change significantly differed between 50 and 300 Hz,

150 and 300 Hz, and 150 and 600 Hz in *C. calyptratus* (P<0.001 for all comparisons), and

between 150 and 300 Hz in *C. gracilis* (P=0.014). A significant negative percent change in

velocity, indicative of a stop-behavioral response occurring after presenting the stimulus, was

observed at 50 and 150 Hz in *C. calyptratus* (Fig. 5). *C. calyptratus* behavioral responses at 25,

300, and 600 Hz did not show a significant decrease in voleocity, however there was a trend

toward an increase in velocity at 300 Hz (Fig. 5). The behavioral responses to the vibrational

stimuli for *C. gracilis* (N=2) were similar to that of *C. calyptratus*, with a decrease in velocity at

150 Hz, and an increase at 300 Hz (Fig. 6).

Size-Frequency Relationship

There was no significant linear regression relationship between duration of vibration

pulses and the pulse dominant frequency in *C.* calyptratus, although there was a trend of

increasing frequency with longer pulse durations (Fig. 7A). Similarly, there was no significant

linear regression relationship between chameleon mass and dominant frequency of its vibration

pulses (Fig. 7B). For *C. calyptratus*, dominant frequency of vibration production ranged from

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106-157 Hz (N=6; Fig. 7). C. *gracilis* (N=1) was observed to have a mean vibrational frequency of 688 Hz, averaged over five pulses. A veiled chameleon with 15% more mass than the *C. gracilis* subject had an average vibrational frequency of 142 Hz. Comparison between *C. gracilis* and *C. calyptratus* of similar mass suggests that differences in vibrational frequency are also species-dependent. Further comparison between the vibrations produced by *C. calpytratus* and *C. gracilis* demonstrate that the induced vibrational responses of *C. calyptratus* are typically low-frequency, sinusoidal hoots characteristic to the species, while *C. gracilis* typically elicits higher-frequency, pulse-like vibrations (Fig. 8).

Behavioral Context

During these trials, chameleons were paired in three different contexts: dominance, courtship, and interspecific. Data from these different behavioral contexts show that vibration pulse durations from unprovoked chameleons differed compared to the vibrational responses observed during the size-frequency trials in which vibrations were induced by the experimenter (Table 2). For a dominance behavioral context, two calyptratus males, CM2 and CM4, were placed on the dowel and recorded for a total time of 11 minutes, spanning over two separate recordings. Behavioral responses were evident in this recording, as CM4 appeared to take on a submissive stance, while CM2 displayed dominance by advancing towards CM2. These behavioral responses are likely due to the noticeable size difference between the two chameleons, as CM4 has a mass of 80.8 g and CM2 has a mass of 183.4 g. Vibrational responses within this context were elicited as short-duration pulses, instead of the typical "hooting" sound known to be produced by *C. calyptratus*. Five pulses from this trial had a mean duration of 0.010 seconds (Table 2). Previous vibrational responses from *C. calyptratus* during the size-frequency experiments had a mean duration of 0.155 seconds, suggesting that the duration of a pulse

vibration from this behavioral trial only lasted approximately 6.5% of the pulse duration recorded during the size-frequency trials (Fig. 9).

For the courtship context, a male and female *C. calyptratus* (CM5 and CF2, respectively) were placed on the dowel and recorded for a total time of 9 minutes. Mating did not occur during this trial, as CF2's coloration did not indicate receptiveness to mating, while CM5 displayed aggressive behavior by gaping his mouth and advancing towards CF2. Vibrational responses were recorded during this interaction, with mean pulse duration calculated over five pulses to be 0.016 seconds (Table 2). Along with their very short duration, these reponses were also pulse-like instead of the characteristic "hoot" response (Fig. 10). Although frequency values were unable to be calculated due to the short duration of these responses, the consistency in duration of response across the three behavioral contexts suggests that *C. calyptratus* have the capability of producing different types of vibrational responses, with the type of response possibly dependent upon factors such as environmental surrounding and the presence of a perceived threat.

The interspecific trial involved a female *C. gracilis* (GF1) paired with a male *C. dilepis* (DM1). Interactions between GF1 and DM1 were recorded over a period of 6 minutes, resulting in multiple pulse-like vibrational responses (Fig. 11). DM1 displayed visual behavioral responses during the interaction with GF1, such as gaping his mouth and flaring his neck flaps. Out of five pulses, the mean pulse duration was 0.028 seconds (Table 2), although it is unknown which chameleon was eliciting these vibrations. Dominant frequency was unable to be accurately calculated via bioacoustical software due to the short durations of these pulses.

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Figure 5. Percent change in *C. calyptratus* velocity walking across a dowel 5 sec following the vibratory stimulus. Velocity change differed significantly by frequency of the vibrational stimulus (ANOVA; $F=10.33$, $P<0.001$). A negative percent change in velocity (i.e., a stop behavioral response) is observed for the 50 and 150 Hz trials, suggesting 50-150 Hz to be the frequency range at which *C. calyptratus* display the greatest behavioral sensitivity to vibrational stimuli. **$P<0.01$,*$P<0.05$; Wilcoxon Signed Rank Test, N=6.
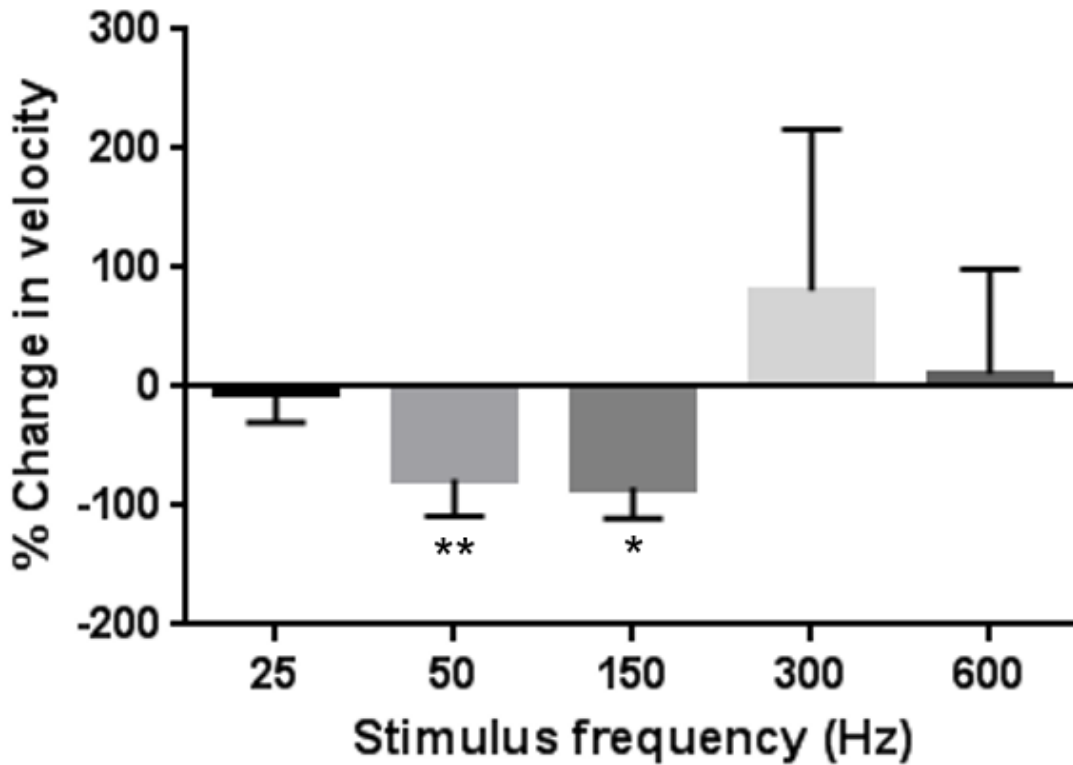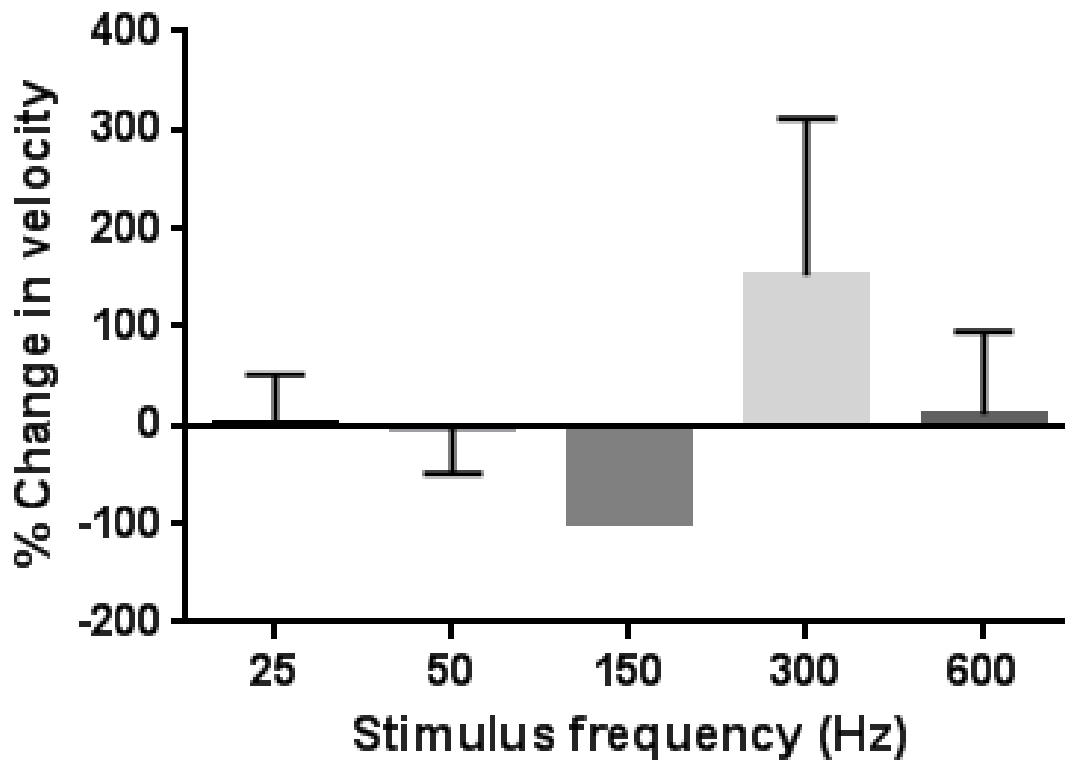
Figure 6. Percent change in *C. gracilis* velocity walking across a dowel 5 sec following the vibratory stimulus. Velocity change differed significantly by frequency of the vibrational stimulus (ANOVA; F=4.43, P=0.018). A negative percent change in velocity (i.e., a stop behavioral response) is observed for the 150 Hz trial, suggesting 150 Hz may be the frequency at which *C. gracilis* display the greatest behavioral sensitivity to vibrational stimuli. N=2.
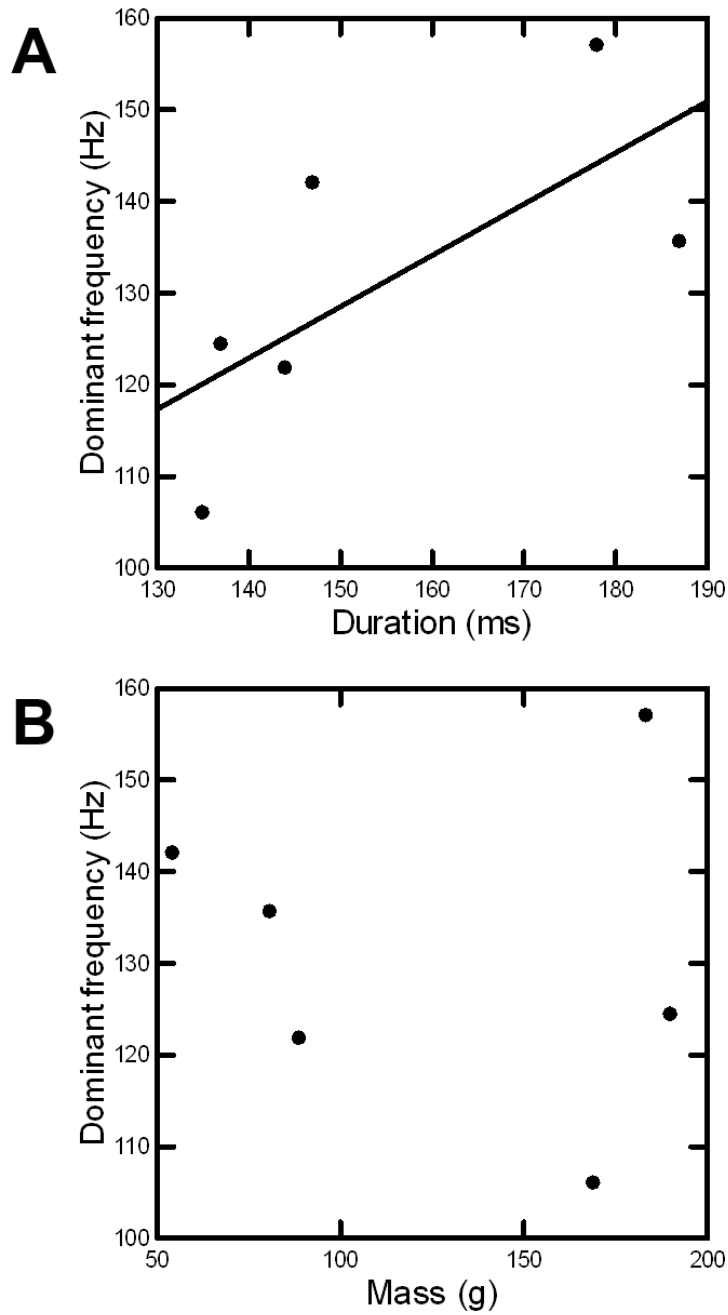
Figure 7. Mean dominant frequency of *C. calyptratus* (N=6 chameleons, 5 pulses each)

vibrations in relation to pulse duration (A) and chameleon mass (B). The trendline in A

is not a significant regression line.

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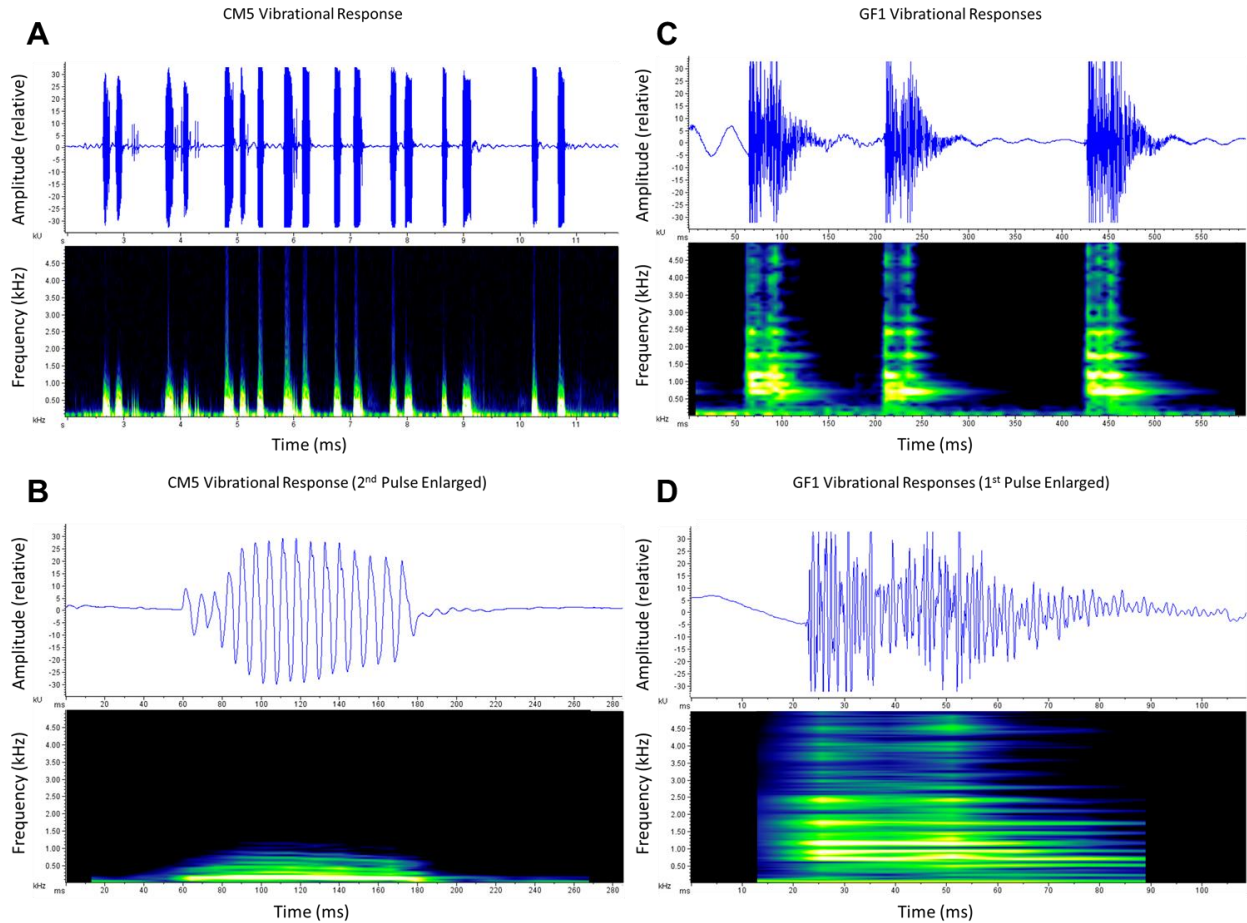
Figure 8. A) A series of induced vibrational responses from a male *C. calyptratus* (CM5) with an average dominant frequency of 134 Hz. Vibrational responses are typically elicited in pairs of two or three pulses, with a spectrogram showing the pulses to be of low frequency. B) An expanded view of the second vibrational pulse from A), displaying sinusoidal activity. C) Vibrational recordings of a female *C. gracilis* (GF1) showing three pulses with an average dominant frequency of 689 Hz. The spectrogram shows that these pulses are occurring at a much higher frequency compared to *C. calyptratus.* D) An expanded view of the first vibrational pulse from C), showing a more broadband signal compared to vibrations produced by *C. calyptratus*.

CM2 vs. CM4
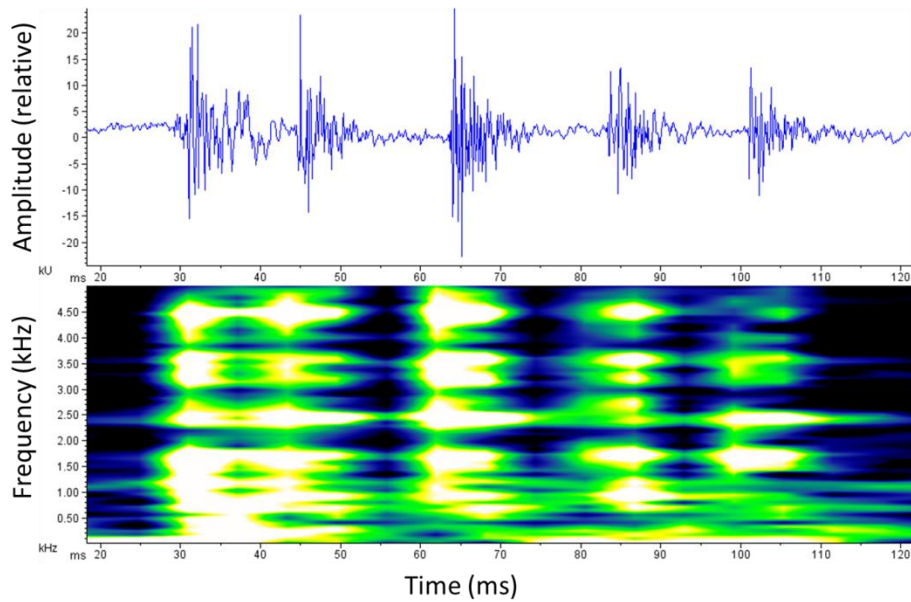
Figure 9. Vibrational recordings between subjects CM2 and CM4, indicating a dominance behavioral context. These responses are more pulse-like and are not characteristic of the typical sinusoidal hoot elicited by *C. calyptratus*. Average duration of these five pulses was calculated to be 0.01 s. While frequency cannot be accurately determined, the spectrogram shows that these pulses are produced at a much higher frequency than the "hooting" pulses, which are typically produced in the 100-150 Hz range.

CM5 vs. CF2
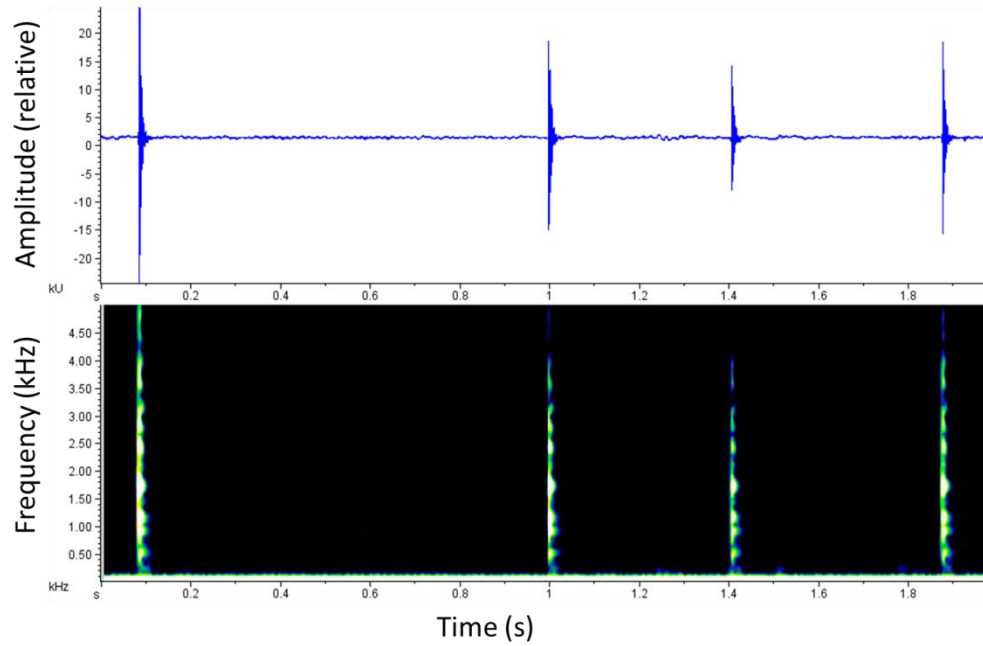
Figure 10. A group of four vibrational responses between a male (CM5) and female (CF2) *C. calyptratus*, suggesting a courtship behavioral context. Average duration of these responses is 0.016 s. The spectrogram shows that these pulses are broadband signals.
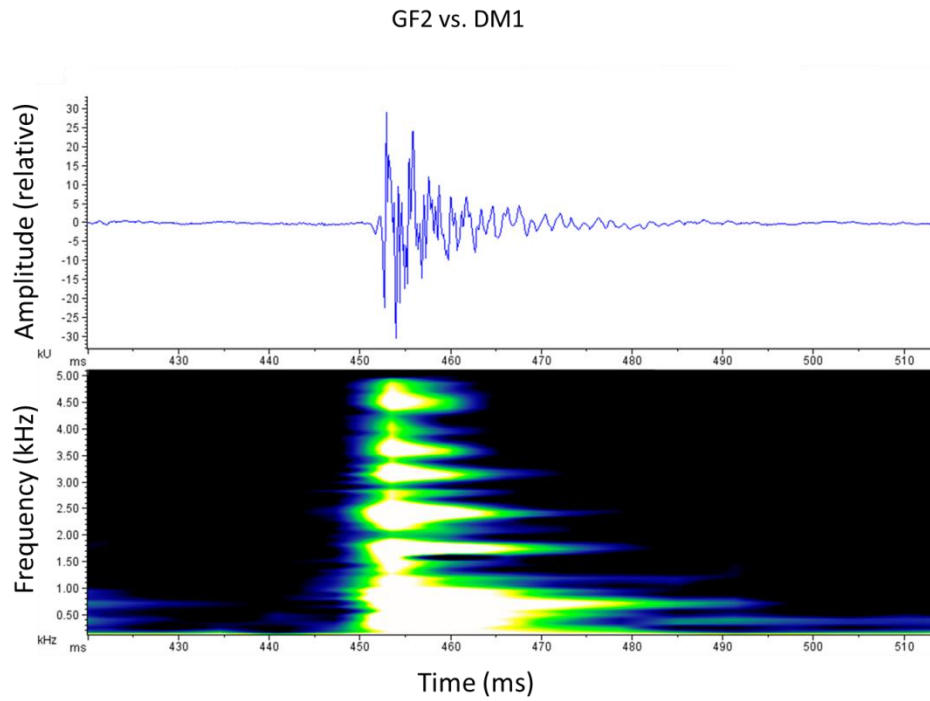
Figure 11. Vibrational recording of an interspecific interaction between *C. gracilis* (GF1) and *C. dilepis* (DM1). The duration of this pulse was recorded at 0.019 seconds. The waveform of this response is similar to those recorded during an individual recording of *C. gracilis* (Fig. 7D), indicating that this species may characteristically produce higher-frequency "pulses" instead of low frequency "hoots."

Experiment	Chameleons	Context	# Pulses	Mean Pulse Duration	SE
3	CM2 vs. CM4	Dominant	5	0.010	0.0003
3	CM5 vs. CF2	Mating	5	0.016	0.0007
3	GF1 vs. DM1	Interspecific	5	0.021	0.0034
2	CM1	Individual	5	0.1372	0.0159
2	CM2	Individual	5	0.1776	0.0413
2	CM3	Individual	5	0.1350	0.0108
2	CM4	Individual	5	0.1868	0.0739
2	CM5	Individual	5	0.1472	0.0109
2	CF1	Individual	5	0.1442	0.0114

Table 2. Mean (±S.E.) vibration pulse duration of the three behavioral context trials (Experiment 3) and the size-frequency trials (Experiment 2). Each mean was calculated from five pulses for each behavioral context (Experiment 3) or individual (Experiment 2).

SECTION FOUR

DISCUSSION

This study shows that *C. calyptratus* communicates via substrate vibrations in various

behavioral contexts, and that vibrations from this species are typically produced in two general

types- a low frequency hoot and a broadband pulse. Additionally, I found that other chameleon

species, i.e., *C. gracilis* and *C. dilepis*, also produce substrate-borne vibrations as a potential

mode of communication. During the vibrational sensitivity trails, *C. calyptratus* was observed to

react to vibrational stimuli using a stop-behavioral response at 50 and 150 Hz, and *C. gracilis*

was observed to react to vibrational stimuli at 150 Hz. For both *C. calyptratus* and *C. gracilis*,

the most notable stop-behavioral response was observed at 150 Hz. Sensitivity to vibrational

stimuli outside of the 50-150 Hz range was diminished, as stop-behavioral responses were not

readily observed at the 25, 300, and 600 Hz range (Fig. 6). While a decrease in chameleon

velocity was evident at 150 Hz, the response at 300 Hz was the opposite- an overall increase in

velocity for *C. calyptratus* and *C. gracilis* (Fig. 5 and 6, respectively), suggesting that 300 Hz

vibrations may induce greater activity in these species. This could have been due to the

chameleons becoming more comfortable with their surroundings, and therefore gained

momentum while moving across the dowel, in conjunction with being presented with vibrational

stimuli that may have been outside of their detectable frequency range. Evidence against this

interpretation is that at 600 Hz, there is almost no change in velocity in both *C. calyptratus* and

C. gracilis, and the pattern is similar between both species- decreased velocity at 150 Hz,

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increased velocity at 300 Hz, and minimal change in velocity at 600 Hz. This suggests that both species may perceive 150 Hz and 300 Hz vibrations as two different classes of signals.

The results of the vibrational response trials demonstrate that veiled chameleons are sensitive to vibrations at 50 and 150 Hz. This overlaps with the range of dominant frequencies of the low-frequency hoots induced in Experiment 2. It may be that the stop-behavioral response to these signals suggests that this frequency range is used by veiled chameleons to communicate with conspecifics. Stopping locomotion may enhance the chameleon's ability to localize the vibration source and discriminate features in the vibration that may be informative. Both *C. calyptratus* as well as *C. gracilis* demonstrated a significant stop-behavioral response during 150 Hz trials. While a previous study conducted by Barnett et al. (1999) supports the hypothesis that *C. calyptratus* produce low frequency vibrations, there was no previous evidence that *C. gracilis* would respond to the same frequency range. The reasoning as to why *C. gracilis* would exhibit a stop-behavioral response at 150 Hz is further complicated by the data obtained during the size-frequency correlation trials, as the average dominant frequency of vibrations produced by this species was observed to be 688 Hz. A possible explanation as to why *C. gracilis* responds to vibrational frequencies of 150 Hz could be that the intended audience for vibrations elicited by veiled chameleons in the 50-150 Hz range are not only utilized as a mode of intraspecific communication, but for interspecific communication with other species of chameleons as well, although these two species do not have geographical distributions that overlap (Stahl, 1997; Gonwouo, 2006).

For the vibrational sensitivity trials, the stimulus elicited during the trials had a vibration acceleration of 6 mm/s^2. This vibration acceleration value is much greater than the sensitivity of reptiles and amphibians that utilize substrate-borne vibrations. For example, in a study published

by Koyama et al. (1982), the American bullfrog (*Lithobates catesbeianus*) had an observable response to vibration acceleration values of 0.05 mm/s^2 (Koyama et al., 1982). Other animals have also been reported to demonstrate an ability to detect vibrational stimuli at intensities similar to that of the American bullfrog. Most notably, a study conducted by Hartline (1971) concluded that several species of snakes displayed a high-degree of sensitivity to substrate-borne vibrations (Hartline, 1971). When presented with a vibrational stimulus, the snakes in Hartline's study were found to exhibit a response to stimuli at intensities of 0.2 mm/s^2 (Hartline, 1971). The intensity used for this project (6 mm/s^2) is clearly above the threshold that chameleons are able to detect. In order to find a threshold for the chameleon's sensitivity to vibrations, this study would need to be replicated at lower vibration acceleration values. While the behavioral trials showed a significant response at 50 and 150 Hz in *C. calyptratus*, there is still more to learn concerning threshold sensitivity to vibration in *C. calyptratus* and *C. gracilis*.

Results from Experiment 1 corroborated with the original hypothesis that *C. calyptratus* will display a stop-behavioral response at 50 and 150 Hz, but results from the size-frequency trials did not support the hypothesis that larger veiled chameleons produced lower frequencies. When comparing dominant frequency to the mass of each chameleon, no significant correlation between frequency and mass was observed, suggesting that the frequency is not dependent upon size. Comparisons between dominant frequency and duration of the response were also plotted, but no significant trend was found.

Our reasoning behind why the original hypothesis that larger chameleons would, on average, have a lower dominant frequency is due to the inverse relationship between frequency and wavelength in bioacoustics (e.g., sound produced by a larger animal with longer resonating surfaces would have a longer wavelength and therefore be of a lower frequency compared to a

smaller animal). Results from the size-frequency trials now suggest the possibility that vibrations elicited by *C. calyptratus* do not depend on size, but instead may depend on the contraction of specific muscles in order to elicit the vibration at a certain frequency. If this were the case in *C. calyptratus*, frequency would be dependent on the contraction rate of the muscles used by the chameleon to produce the vibration. Muscle contraction rate controlling frequency has been observed in other animals. For example, sound producing fish such as *Parablennius parvicornis*, *Cynoscion regalis*, *Myripristis kuntee*, *Myripristis violacea*, *Eutrigla gurnardus, Pygocentrus nattereri*, had dominant frequencies that were controlled by sonic muscle contraction rate (reviewed in Parmentier & Fine, 2016). The goal of research currently being conducted at WKU (Samuel Tegge, unpublished data), is to measure the electrical activity of specific muscles surrounding the gular pouch used by *C. calyptratus* during vibration production, and how this may affect dominant frequency.

Responses of *C. calyptratus* during the frequency-size correlation trials also exhibited some differences in frequency and duration compared to a previous study of frequency response range and duration conducted by Barnett et al. (1999). The Barnett study analyzed 128 vibrational recordings of a male *C. calyptratus* (N=1) and found a broader range of frequencies produced by the chameleon, as well as a difference in duration depending upon the frequency of response (Barnett et al., 1999). For the frequency range produced by the chameleon, they reported that 65% of responses were at a frequency of 105 Hz or greater, with an average duration of 142 ms and a dominant frequency of 156 Hz. 24% of responses elicited by the male *C. calyptratus* were below 90 Hz, and exhibited a dominant frequency of 49 Hz and an average duration of 948 ms; 12% of responses were found by Barnett to be a mixture between the first two types of responses reported (Barnett et al., 1999).

Results from the size-frequency trials of this project showed differing results from those published by Barnett et al. (1999), as none of the *C. calyptratus* (N=6) used during the size-frequency trials were observed to elicit a response below 105 Hz, with results ranging in averaged frequencies of 106-157 Hz. Furthermore, average duration for the vibrational responses analyzed across the six samples was found to be 155 ms. These results are similar to those that Barnett observed at responses above 105 Hz, but the discrepancy between the range and frequency between the two studies will need to be examined further. Particularly, the relationship between dominant frequency and duration requires further study, as both studies see a positive trend between dominant frequency and duration of response. While the relationship between dominant frequency and duration of response were not found to be significant in this project, it is not specified whether this relationship is significant within the one individual recorded in the Barnett study.

While the Barnett study examined the frequency range of vibrational responses exhibited by *C. calyptratus* during a male-female mating context, the possibility of differences in vibration signals depending on behavioral context was not explored, as only one context (male-female courtship) was observed during this study and was not the focus of the research (Barnett et al., 1999). When recording the behavioral and vibrational responses of chameleons under male-male dominance, male-female mating, and interspecific contexts, results displayed pulse-like vibrational responses that were very short in duration, more broadband, and higher in frequency. When compared to the characteristic low-frequency, sinusoidal hoots elicited by *C. calyptratus* during the size-frequency trials, vibrational responses observed during the behavioral contexts trials may suggest a different kind of vibrational response. What is most surprising about these results is the difference in duration between the induced vibrations of the size-frequency

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experiments and the natural vibrations of the behavioral contexts experiment, as the induced

vibrations had an average duration of 155 ms, while the natural vibrations had an average

duration 18 ms across the three behavioral contexts. The most compelling evidence that these

rapid pulse-like vibrations are indeed being produced by a chameleon is that these responses

were produced in groups of two or three similar signals (Figs. 7, 9 & 10), which is characteristic

to previously-recorded vibrational responses of *C. calyptratus* (Barnett et al., 1999). In addition,

vibrations were recorded when the animals were not moving on the dowel, to rule out any

background pulse-like vibrations produced by their movement as they walk along the dowel.

While quantifiable data for the behavioral context trials only focused on the duration of

the responses throughout the trial, additional behavioral cues were observed which may add to

the complexity of chameleon communication. Tremulation is a mode of communication that

utilizes movement of the entire body as a vibrational response is produced, and is widely

represented in orders of insects, including Diptera, Hemiptera, Heteroptera, Trichoptera,

Plecotpera, and Neuroptera (Ponce, 2014). Tremulation is being utilized as a communication cue

by the chameleon, with tremulation occurring as the chameleon produces the vibration and the

vibrational signal travels down the chameleon's limbs and through the substrate. Although head

twitching has been characterized as a behavioral response of *C. calyptratus* (Smith & Huskey,

pers. obs.), the presence of a tremulatory response during interactions with other conspecifics has

not been reported (Barnett et al., 1999). Tremulatory responses during chameleon interactions

further justify the results that chameleons use substrate-borne vibrations as a mode of

communication.

Utilizing substrate-borne vibrations for communication purposes may be advantageous

for *C. calyptratus* and other species of chameleon relative to other signaling modalities. While

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chameleons are well-known for their cryptic abilities, the typical environment in which chameleons live is dense foliage, which would greatly hinder the ability of a chameleon to perceive visual communications and responses. In these environments, the capability to communicate via the substrate would be quite beneficial. Additionally, acoustical modes of communication would not be advantageous to *C. calyptratus*, as they do not possess a tympanum (ear drum), a membrane that is paramount to the processing of sound waves, as the tympanic membrane vibrates in response to sound waves and sends this signal to the inner ear (Nečas, 1999). Thus, their hearing is quite poor (Wever, 1968, 1969).

Results from this project have provided stronger support for the use of vibrational signals through the substrate as a mode of communication for *C. calyptratus*, and that the use of vibrational signals may vary in duration and dominant frequency depending on the behavioral context. Understanding how chameleons communicate and under which context this communication occurs can lead to further knowledge of the underlying mechanisms of chameleon communication, which will prove invaluable to the disciplines of functional morphology and neuroethology.

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