POPULATION-LEVEL VARIATION IN VOCALIZATIONS OF *RANA BOYLII*, THE FOOTHILL YELLOW-LEGGED FROG

A Thesis

Presented

to the Faculty of

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In Partial Fulfillment

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Master of Science

in

Biology

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by

Courtney S. Silver
Summer 2017

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DEDICATION

I would like to dedicate this thesis to my amazing, strong, and intelligent mother, Janice Silver. Thank you for encouraging me to be different.

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ABSTRACT

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Vocalizations are the main form of communication between anurans, and each species can have an array of calls that can range from simple to complex. Local dialects from different populations of the same species can inhibit intraspecific communication, especially when females prefer a specific range of spectral and temporal properties in their mates' calls. Vocal sacs are anatomical features used for communication in anurans and their structure and size are variable among anuran species. *Rana boylii*, a candidate species under the California Endangered Species Act and the focus of this study, primarily call underwater using small, paired subgular vocal sacs. Most studies of frog vocalizations have focused on aerial calls, partly due to the ease of recording vocalizations above water. Consequently, characterization of *R. boylii's* call spectrum, as with other taxa that vocalize under water, has proven challenging. One previous study distinguished five *R. boylii* calls, but did not characterize variation among populations. This study examined variation in spectral (dominant frequency and high frequency) and temporal properties (call duration, pulse number, pulse rate, pulse duration, note number, and note duration) of call types among three disjunct populations of *R. boylii*. *Rana boylii* had a substantial amount of variation among populations not only in the spectral and temporal properties of calls, but also in the call types they emit. Two novel call types were described: the chuckle and the warble. Two locations had call types with frequency ranges that peaked above 20 kHz (ultrasonic), which could be a direct effect of the distinct environmental conditions at these sites. *Rana boylii's* diverse array of calls showed previously undocumented significant differences between populations. The results of this study highlight that different populations of the same species might not possess the same communication strategies.

CHAPTER I

INTRODUCTION

Vocalizations are the main form of communication between frogs. Each species of frog has unique vocalizations or calls. Anurans' (frogs') ability to detect, recognize, and isolate sounds is key for their reproductive success and ability to effectively interpret interspecific competition between signals. Recent studies have shown that each male has an individual call signature and can distinguish species and distance of advertisement calls (Gerhardt and Huber, 2002; Narins et al., 2007). Some species of frogs have a very complex array of calls. These elaborate frog calls can be equivalent in complexity to the vocalizations of higher vertebrates, for example human phonetic segments, where others may only produce simple tones or trills (Gerhardt and Huber, 2002; Narins et al., 2007). Within a species of frog, there can be variation in vocal signals, graded or otherwise (Narins et al., 2007). Variations in calls among and within populations can have a dramatic effect on intraspecific interactions. In a study conducted by Ryan et al. (1992), *Acris crepitan* females did not show any preference for local or foreign males, but instead for males with the lowest of frequencies. Yet, many other studies show homotypic preferences in females for local males (Ryan and Wilczvnski, 1988).

Among anurans, spectral properties (dominant frequency and high frequency) are considered to be static and to have less variation between males than temporal properties (call duration, pulse number, pulse rate, pulse duration, note number, and note duration), which are more dynamic (Gerhardt, 1994). Female anurans have been found to prefer calls with static properties that are closer to the average values found in natural populations and dynamic properties that are equal to or greater than the average value in a natural population (Gerhardt, 1994). This therefore suggests stabilizing selection for static spectral properties and positive directional selection for the dynamic temporal properties within populations (Gerhardt, 1994). There have been local dialects ("modifications of spectral and/or temporal attributes of vocalizations") found among some anuran populations due to selection pressures from opposing sounds found within an environment (Narins et al., 2007).

Most anurans use vocal sacs for communication, but some species lack vocal sacs, like *Rana muscosa*. The reduction in size of vocal sacs in ranids has led to the evolution of underwater vocalizations (Stebbins et al., 2012). Vocal sacs are correlated with above-water (aerial) calls, whereas reduced (or lack of) vocal sacs correspond to underwater vocalizations. Vocal sacs amplify calls made in the air, but not those made underwater (Hayes and Krempels, 1986). Studies of above water vocalizations are more common because they are easier to record and more commonly heard. Reduction in vocal sacs, however, does not diminish the importance of communication. Members of the "*R. boylii* group" (Case, 1978) all have limited development of their vocal sac apparatus; *R. aurora, R. cascadae, R. muscosa* and *R. pretiosa* lack vocal sacs entirely and the remaining two (*R. draytonii and R. boylii*) have small, paired subgular sacs (Hayes and Krempels, 1986). *Rana boylii* (Fig. 1) and the rest of the *R. boylii* group, all have the ability to vocalize underwater (Davidson, 1995; Hayes and Krempels, 1986, Stebbins and McGinnis, 2012). Northen (1993) stated that the underwater vocalizations presented a challenge in fully capturing *Rana boylli's* call spectrum and in identifying behaviors associated with their calls (Northen, 1993). While researching for this current study, information on underwater vocalizations on these above species was scarce. The exclusively aquatic pipid frogs also vocalize underwater, but have had little attention paid towards their vocalizations except for one species, *Xenopus leavis* (Yager, 1992).

Figure 1: *Rana boylii* in Little Yosemite, Sunol Regional Park, Alameda County, California

A previous study of vocalization in *Rana boylii* identified five distinct calls based on temporal and spectral properties (Northen, 1994). However, that study involved only one location. The current study characterized the spectral and temporal properties of calls of *Rana boylii* in three disjunct populations, focusing on three questions. First, will calls vary among populations in their spectral and temporal properties? Second, are more than five calls used in the repertoire of *R. boylii?* Third, do all *R. boylii* populations use the same calls? Addressing these questions will provide a better understanding of variation in *R. boylii* communication across its geographic range.

CHAPTER II

METHODOLOGY

Study System:

Rana boylii, commonly known as the foothill yellow-legged frog, can be found in the rivers and streams of foothills from Northwest Oregon down through Southern California (Nafis, 2014). For 23 years, the California Department of Fish and Wildlife has designated *R. boylii* as a California Species of Special Concern, and the USDA Forest Service lists it as a Sensitive Species due to range-wide declines (Jennings et al., 1994, Hayes et al. 2016). More recently (2017), *R. boylii* was listed as a candidate species under the California Endangered Species Act, permanent listing as a California Endangered Species is pending final vote. It is also under consideration for listing under the and Federal Endangered Species Act, but it is still under review.

Historically, the foothill yellow-legged frog was found commonly throughout its geographic range. However, it appears to be near extirpation in at least two-thirds of its range. One area in which populations are decreasing at an alarming rate is the Sierra Nevada, which makes up roughly 25% of their historic range. In areas of the Sierra Nevada where the species persists, populations are sparse (Lind, 2005). Lind (2005) showed that *R. boylii* no longer inhabits 51% of their historical range within the Sierra Nevada. These findings indicate that *R. boylii* is at risk not only of local extinction, but range wide extinction as well (Hayes et al., 2016). Further analysis of its calling behavior can inform a conservation plan for this species.

Understanding their vocalizations will help us to form a clearer picture of their social system, which may then give us insight into their rapid decline.

Past studies on the foothill yellow-legged frog (family: Ranidae) considered it to be a single species, yet Zweifel (1955) found there to be color and morphology differences between populations found in the southern Sierra Nevada and north coast populations. More recent studies (Lind 2005; Lind et al. 2011) have shown genetic variation within the species. Mitochondrial DNA analysis identified “significant genetic partitioning” (Hayes et al., 2016) between populations that inhabit the coast and the populations in the Sierra Nevada range. As well as two distinct groupings found in *Rana boylii* peripheral ranges, the northern limit into central Oregon and to the south in the Sierrian and coast range of the Central Valley (Lind et al. 2011)

Sites:

Three geographically isolated locations were chosen for this study (Table 1). Isolated locations were selected to minimize the chance of gene flow between populations. Based on observations and yearly egg mass surveys in Angelo and Sunol (Dr. Sarah Kupferberg, unpublished data) and surveys conducted in the Big Chico Creek Ecological Reserve (BCCER) (BCCER staff, unpublished data), BCCER and Angelo Coast Range Reserve have larger populations than Sunol. Sunol has seen a steep decrease in population over the past decade (Kupferberg, 2015).

Table 1: Locations in which vocalizations of *Rana boylii* were recorded during the mating seasons (March-May) of 2015 and 2016. Locations include the Big Chico Creek Ecological Reserve, Angelo Coast Range Reserve, and the Sunol Reginal Wilderness Area.

Location	Latitude & Longitude
Big Chico Creek Ecological Reserve	39.825818 N, -121.720907 W
Angelo Coast Range Reserve	39.729167 N, -123.63887 W
Sunol Reginal Wilderness Area	37.5109275 N, -121.823626 W

Big Chico Creek Ecological Reserve (BCCER) is 1,598.5 hectares of canyon and ridge habitat along Big Chico Creek in Butte County. Breeding habitat for *Rana boylii* in BCCER is found in secluded areas along Big Chico Creek in pools or runs. Unlike in many other breeding sites, at BCCER *R. boylii* lay their clutches attached to boulders or larger rocks located within the creek. Cobble bars, where clutches are more commonly found outside of BCCER, are scarce within the creek at BCCER. Observations indicated *R. boylii* use pooled areas along the side of the creek that are in small alcoves. Due to the water depth, individual males and females were extremely hard to see and males that were found at shallower depths were not making vocalizations.

There are numerous predators of *R. boylii* found in BCCER. Garter snakes (*Thamnophis* sp.) are a primary predator of *R. boylii* (Lind and Welsh, 1994) and can be found within the reserve. Newts (*Taricha sp.*) also prey on egg masses of *R. boylii (*Jones et al., 2005). Non-native crayfish are found at the reserve and have become an alien predator to many species of amphibians in the United States (Lodge et al., 2000).

The Angelo Coast Range Reserve is on 3,100 hectares of the upper watershed of the South Fork of the Eel River in Mendocino County. Five kilometers of the upper South Fork Eel River are located within the boundaries of the reserve. The *R. boylii* breeding habitat at the reserve is typical for the species: partial shade, shallow riffles, and cobble sized substrate or bigger. Each spring, *R. boylii* assemble in the same sites in the main stream for breeding (Kupferberg, 1996). Unlike at BCCER, the water depth at breeding sites was not deep. Receptive males and females were easy to spot and were often calling from shallow water. Breeding and egg-laying sites in Angelo are located near tributary confluences. *Rana boylii* stay close to the shore on the side of the creek with shallow water with a low velocity. Most breeding sites within the reserve were also found at gravel bars, unlike at BCCER (Kupferberg, 1996).

Angelo, like BCCER, has many *R. boylii* predators. Aquatic garter snakes, *Thamnophis atratus,* are plentiful, as are rough skinned newts, *Taricha granulosa.* Signal cray fish*, Pacifastacus leniusculus,* are also common. Other vertebrate predators include mergansers, river otters, and occasional great blue heron.

The Sunol Regional Wilderness is in Alameda County. The site consists of 2,772 hectares of former ranch land, with chaparral, oak woodland, grassland, and riparian habitat. Alameda Creek runs through the park, which is where *Rana boylii* breed. Unlike the other two locations, the breeding habitat of *R. boylii* is very diverse (substrate size and water depth) from site to site within Alameda creek. There were two hydrologically distinct reaches that make up the sampling site (Adams et al., 2017). The lowest elevation breeding site for this survey, located near the visitor center of the park, is much like Angelo with shallow slow-moving water with cobble-sized substrate. *Rana boylii* attach clutches to the substrate along cobble bars. The second location, Little Yosemite, is a scenic gorge about two miles upstream from the lower site. Little Yosemite is made up of large pools located between large boulders with little vegetation. There are waterfalls ranging in size that connect the pools. Here *R. boylii* attach egg masses to boulders, like at BCCER. The main recording site within little Yosemite was a large pool with a steady waterfall flowing into it. Sunol has the most altered and urbanized landscape of the three locations for this study. It is located not far from a large city (Fremont, Ca), experiences the highest volume of human visitors, has been impacted and altered by damns, and has paved (and often driven) roads within the park.

There are many predators to *R. boylii* within the park. Rough skinned newts, *Taricha granulosa,* and the common garter snake, *Thamnophis sirtalis,* are common predators within the park. However, the predator with the most impact is the invasive American bullfrog, *Lithobates catesbeianus*. Bullfrogs not only prey on egg masses, they also eat adult and juvenile frogs. In addition to direct predation, bullfrog larvae compete with *R. boylii* for resources and adult bullfrogs carry the deadly fungus chytrid (Schloegel et al., 2012). *Batrachochytrium dendrobatidis* (chytid) has had a detrimental effect on the native population of *R. boylii,* in recent years (Adams et al., 2017).

The introduction of bullfrogs, and the subsequent chytrid fungus outbreak, has had a cascade of negative effects on *R. boylii* in Sunol. where bullfrogs were present, for example, male *R. boylii* were not observed to be calling. This is likely related to predator avoidance, since bullfrogs will eat almost anything they come across including other frogs. In Little Yosemite where bullfrogs are not found, calling was far more common. Adams et al. (2017) found a high pathogen load of the chytrid fungus in dead and dying juveniles along the creek in the fall of 2013. The upstream site locations historically had a larger population size of *R. boylii* prior to the recent drought (2012-2015) (Kupferberg et al., 2012). The creek practically dried up in the upper locations, forcing *R. boylii* to move downstream into the area that bullfrogs have invaded and potentially exposing them to the chytrid fungus (Adams et al., 2017). The drought also made it easier for bullfrogs to expand upstream to habitats that usually have faster flowing waters. Besides bullfrogs being vectors of the chytrid fungus, bullfrogs had other negative effects on the fitness of native *Rana boylii*. Kupferberg (1997) found that *R. boylii* tadpoles took longer to develop and had decreased mass when housed with bullfrog tadpoles and/or adults. This is thought to be due to resources availability, changes in behavior, and habitat use. These stressors combined can increase *R. boylii* susceptibility to chytrid (Adams et al., 2017).

Recordings:

Underwater and aerial vocalizations were recorded March through May (2015 and 2016), during the breeding season. During each census, recordings were collected midday; between 12:00 pm to 5:00 pm, in all locations (Table 2). Aerial calls were recorded with a Zoom H4n Recorder. Underwater calls were collected with the Zoom H4n Recorder (Zoom North America, Hauppauge, NY) and H2a-XLR Hydrophone (Aquarian Audio & Scientific, AFAB Enterprises, LLC, Anacortes, WA USA). Aerial (release) calls were recorded while holding frogs around the midsection and any sounds made were recorded. Underwater calls were recorded by placing the H2a-XLR Hydrophone in the water near the banks of the creek, and the recorder was turned on during calling (Fig. 2, 3). In some cases, multiple locations were sampled within each site. Locations were chosen non-randomly to try to record calls from multiple individual animals. In some cases, frogs that were being recorded were not observed visually. Calls recorded from unobserved and observed animals were marked as such and noted in data tables. Water temperature was also measured at each location (Table 3).

Figure 2: Hydrophone placed underwater, to the right is to a male *Rana boylii.* Photo taken in Little Yosemite in the Sunol Reginal Wilderness Area.

Figure 3: Recording underwater vocalizations of *Rana boylii* in the Big Chico Creek Ecological Reserve. The hydrophone is underwater and the Zoom H4n recorder is being held.

Table 2: Sampling effort for recording vocalizations of *Rana boylii* at three northern California locations; Big Chico Creek Ecological Reserve, Angelo Coast Range Reserve, and Sunol Reginal Wilderness Area.

Location	Number of Days	Total Hours
Big Chico Creek Ecological Reserve	10	30
Angelo Coast Range Reserve	3	15
Sunol Reginal Wilderness Area	4	20

Table 3: Mean (SD) temperature (°C) at three northern California locations in which vocalizations of *Rana boylii* were recorded. Temperature was measured once each day of recording. Means were not significantly different across sites by a one-way ANOVA *(p* = 0.48*).*

Locations	Mean (SD) Temperature
Big Chico Creek Ecological Reserve	12 (1.73)
Angelo Coast Range Reserve	11.9 (2.12)
Sunol Reginal Wilderness Area	13.5 (.481)

Sound Analysis:

Recordings were processed using Raven Pro (Cornell Laboratories, Ithaca NY). Call types within each recording were classified based on visual characteristics of the spectrograph. Duration was found by measuring the length of the call visually on the spectrograph while simultaneously measuring the length of the audio sound of the call in Raven Pro. Pulse length was found by measuring from peak to peak (highest point of amplitude on a waveform) of each pulse in the waveform of each call in Raven Pro. Note length was found the same way as pulse length, but was measured from the starting peak of each note to the following note's starting peak. Note and pulse number were found by counting each note and pulse visually. Initial and ending pulse rate was found by measuring the length of the starting and ending pulse within a call. Pulses per second was found by dividing the number of pulses by the length of the call. Dominant frequency was measured automatically by Raven Pro by finding the highest concentration of frequency emitted in each call. High frequency was measured by finding the highest point of the call on the spectrograph.

Statistical Analysis:

Each call type’s measurements were compared among locations using a one-way ANOVA or t-test and Tukey’s post hoc test (when applicable). Calls that did not clearly fall into a category, had too much back-ground noise, or were recorded at too far of a distance were omitted from analysis. Measurements done by hand (pulse/note length and initial/ending pulse rate), i.e., not automatically by Raven Pro, were carried out by only one individual to reduce human error.

CHAPTER III

FINDINGS AND RESULTS

A total of 902 calls were recorded and of those, 299 were chosen for analysis of spectral (dominant frequency (Hz) and high frequency (Hz)) and temporal (duration, note number, note duration, number of pulses, pulse/sec, pulse duration average, initial pulse rate, and ending pulse rate) properties (Table 4).

Table 4: Number of calls in each of the three location (recorded and analyzed) made by *Rana boylii*, separated in to call groups. Locations include Angelo Coast Range Reserve, Sunol Reginal Wilderness Area, and Big Chico Creek Ecological Reserve. Parentheses indicate the percentage of each call type out of all calls in each designated location.

Location	Treatment	Short Call (%)	Long Call (%)	Rattle (%)	Squeak (%)	Chuckle (%)	Release (%)	Warble (%)	Total
Angelo	Recorded	268 (53)	49 (9.7)	122 (24.1)	38 (7.5)	16 (3.1)	13 (2.6)	0(0)	506
	Analyzed	33	21	30	14	16	10	0	124
Sunol	Recorded	93 (84.5)	9(8.2)	2 (1.8)	0 (0)	6(5.5)	0 (0)	0(0)	110
	Analyzed	36	9	30	0	6	0	0	81
BCCER	Recorded	101 (35.3)	22 (7.7)	151 (52.8)	4 (1.4)	2(.7)	0 (0)	6(2.1)	286
	Analyzed	31	22	30	3	2	0	6	94
Total	Recorded	462	80	275	42	24	13	6	902
	Analyzed	100	52	90	17	24	10	6	299

The criteria distinguishing calls is in Table 5. Short croaks, long croaks, rattles, releases, and chuckles all had a repeating uniform pattern in their spectrographs. There was variation between calls, even within call type, but their calls all had a uniform pattern. Squeaks and warbles however, were irregular in their pattern.

Table 5: General characteristics of the seven types of calls by *Rana boylii* recorded at three northern California locations. Pulse refers to a short repeating beat whereas note refers to a set of multiple repeating pulses separated by a period of no pulses. Pattern refers to overall appearance of the wave form. Repeated means that the pulses/notes are repetitive throughout the call. Irregular means that there was no identifiable pattern and in these cases, there were no pulses or notes.

Call Type	Pulsed	Noted	Length (Seconds)	Pattern
Short Croak	Yes	No	0.13 - 0.49	Repeated
Long Croak	Yes	No	0.48 - 0.77	Repeated
Rattle	Yes	Yes	0.3 – 3	Repeated
Squeak	No	No	0.01- 0.08	Irregular
Release	Yes	No	0.03- 0.15	Repeated
Chuckle	Yes	No	0.03- 0.2	Repeated
Warble	No	No	0.2- 0.35	Irregular

Short Calls:

Short call duration in Angelo was 25.5% longer than short calls in BCCER (Fig. 4A, F = 4.52, ndf = 2, ddf = 95, p = 0.01). Mean number of pulses in short calls from Angelo was 45.2% greater than in BCCER and 47.6% greater than in Sunol (Fig. 4B, F = 9.13, ndf = 2, ddf = 88, p < 0.001). The mean dominant frequency of short calls was 50.4% higher in Angelo than in BCCER and 42.8% higher than in Sunol (Fig. 4D, F = 12.6, ndf = 2, ddf = 95, p < 0.001). The highest overall frequency of short calls in Sunol was 286.4% higher than in BCCER and 144.3% higher than in Angelo (Fig. 4D, F = 24.2, ndf = 2, ddf = 94, p < 0.001).

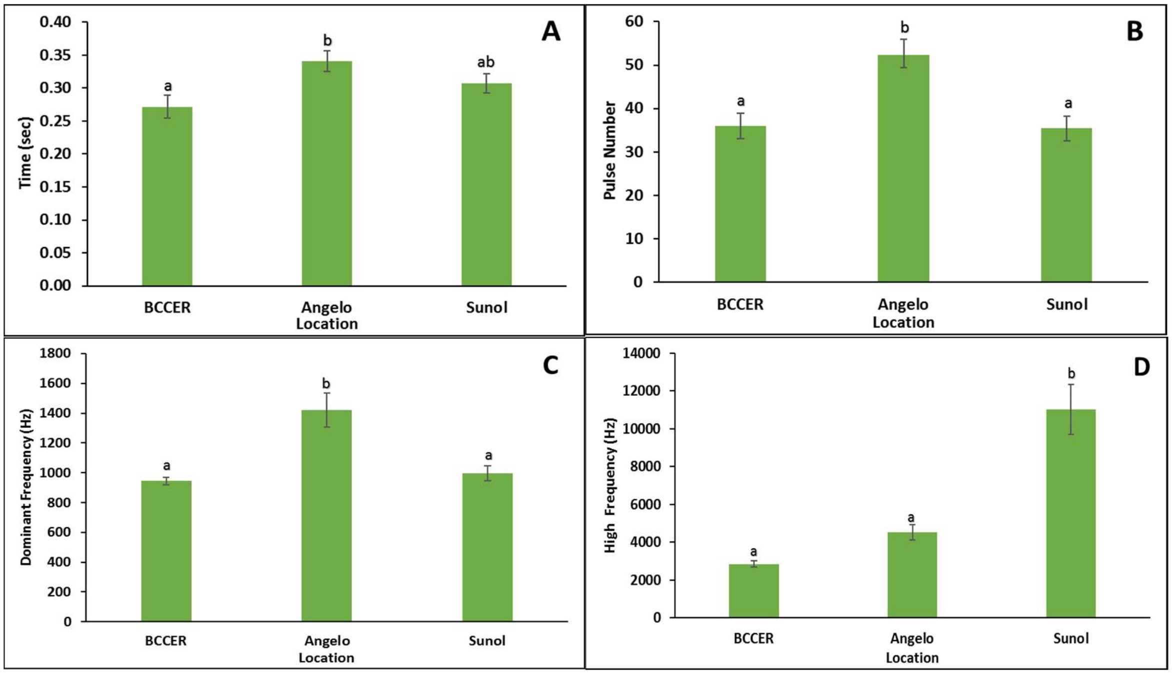

Figure 4: Characteristics of short call vocalizations of *Rana boylii* recorded in three northern California locations. Mean (±SE) (A) duration. (B) number of pulses (A series of amplified wavelengths) (C) dominant frequency and (D) highest overall frequency recorded.

Sunol had a higher overall frequency (Fig. 5A). On the other hand, Angelo had a higher mean dominant frequency (Fig. 5C). Short calls ranged from 0.130-0.049 seconds, with a wide range of number of pulses per call, from 11-103.

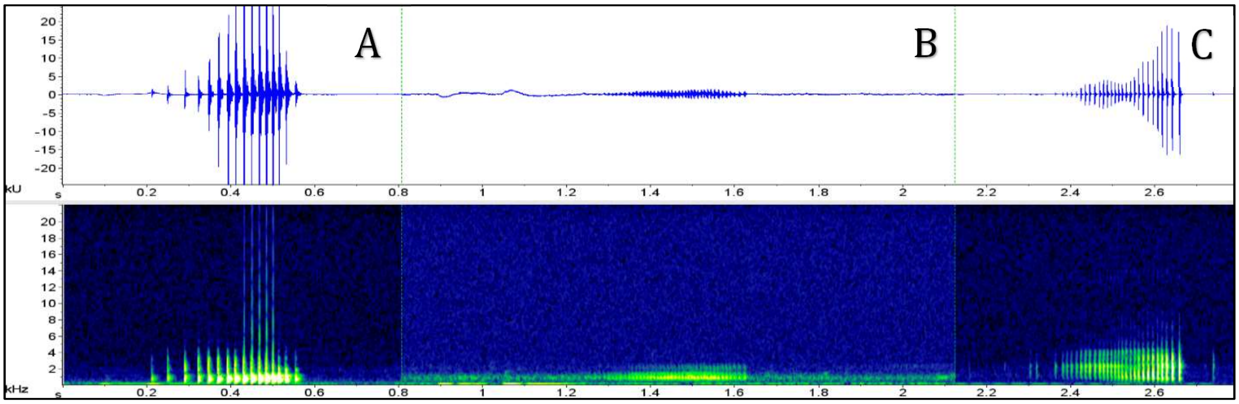

Figure 5: Examples of waveform (top) and spectrograph (bottom) of short calls in three northern California locations: (A) Sunol, (B) BCCER, (C) Angelo.

Long Calls:

Long calls ranged from 0.480- 0.770 seconds and were made up of repeating pulses (Fig. 9). The mean dominant frequency of long calls in Angelo was 64.1% higher than in BCCER, and 41.9% higher in Sunol than in BCCER (Fig. 6 , *F* = 12.75, *ndf* = 2 , *ddf* = 45, *p* <0.0001). The mean long call duration in Sunol was 26.9% longer than in BCCER and 23.4% longer than in Angelo (Fig. 7, *F* = 13.81, *ndf* = 2 , *ddf* = 45 , *p* <0.0001). The mean number of pulses per long call in Angelo was 48.8% more than in BCCER and 80.2% more in Sunol than in BCCER (Fig. 8A, *F* = 18.90, *ndf* = 2, *ddf* = 45 , *p* <0.0001). The mean pulse duration in BCCER was 43.1% longer than in Angelo and 43.7% longer than in Sunol (Fig. 8B, *F* =12.49, *ndf* = 2 , *ddf* = 45, *p* <0.0001). The mean number of pulses per second found in long calls was 45.3% more in Angelo than in BCCER and 40.7% more in Sunol than in BCCER (Fig. 8C, *F*= 16.04, *ndf* = 2, *ddf* = 45, *p* <0.0001). The mean of the highest frequency in long calls in Sunol is 234.6% higher than in BCCER (Fig. 9C, *F* = 7.523, *ndf* = 2, *ddf* = 45, *p* = 0.0015).

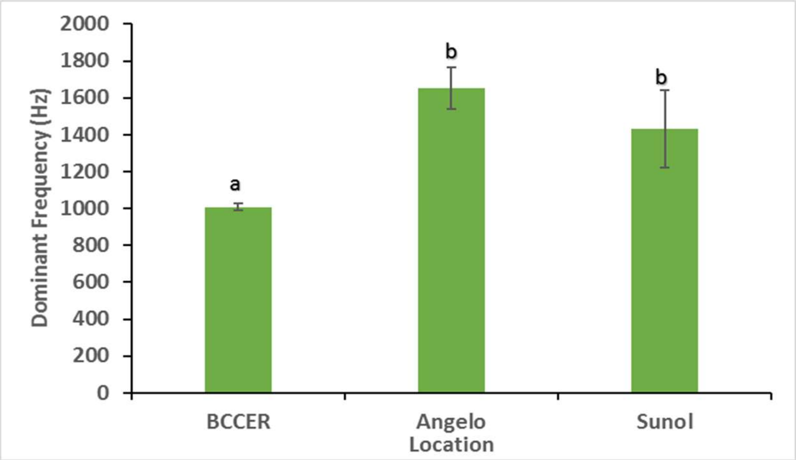

Figure 6: Mean (±SE) of long call dominant frequency emitted by *Rana boylii* found at BCCER, Angelo, and Sunol. Means with the same letters were not significantly different at α = 0.05 by a Tukey's HSD test.

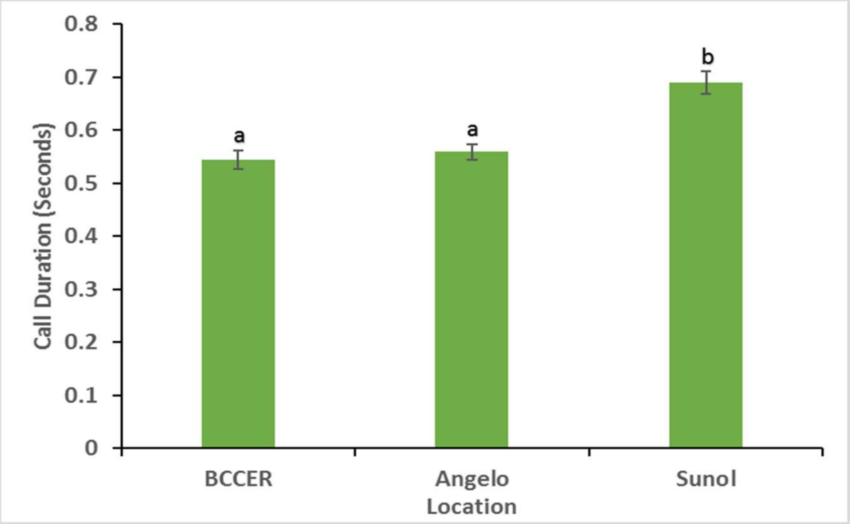

Figure 7: Mean (±SE) of long call duration emitted by *Rana boylii* found at BCCER, Angelo, and Sunol. Means with the same letters were not significantly different at α = 0.05 by a Tukey's HSD test.

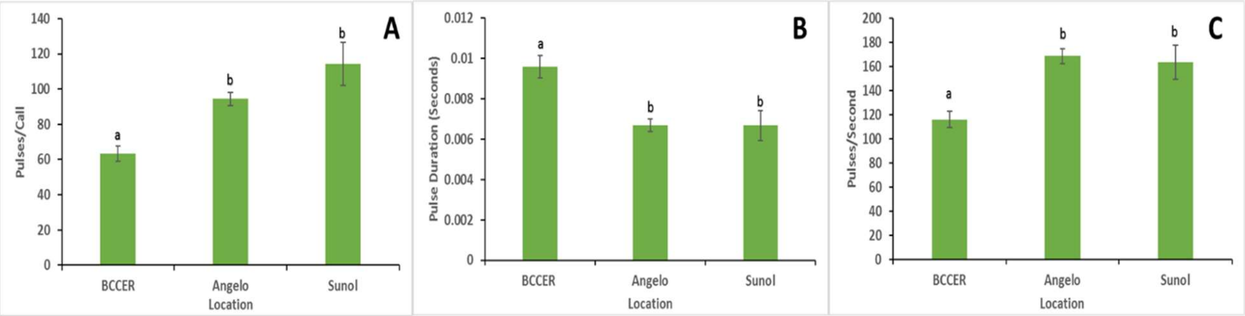

Figure 8: Pulse characteristics of long calls. (A) Mean (±SE) number of pulses per long call at BCCER, Angelo, and Sunol. (B) Mean (±SE) of long call pulse duration at BCCER, Angelo, and Sunol. (C) Mean (±SE) of number of pulses per second in long calls at BCCER, Angelo, and Sunol. Means with the same letters were not significantly different at α = 0.05 by a Tukey's HSD test.

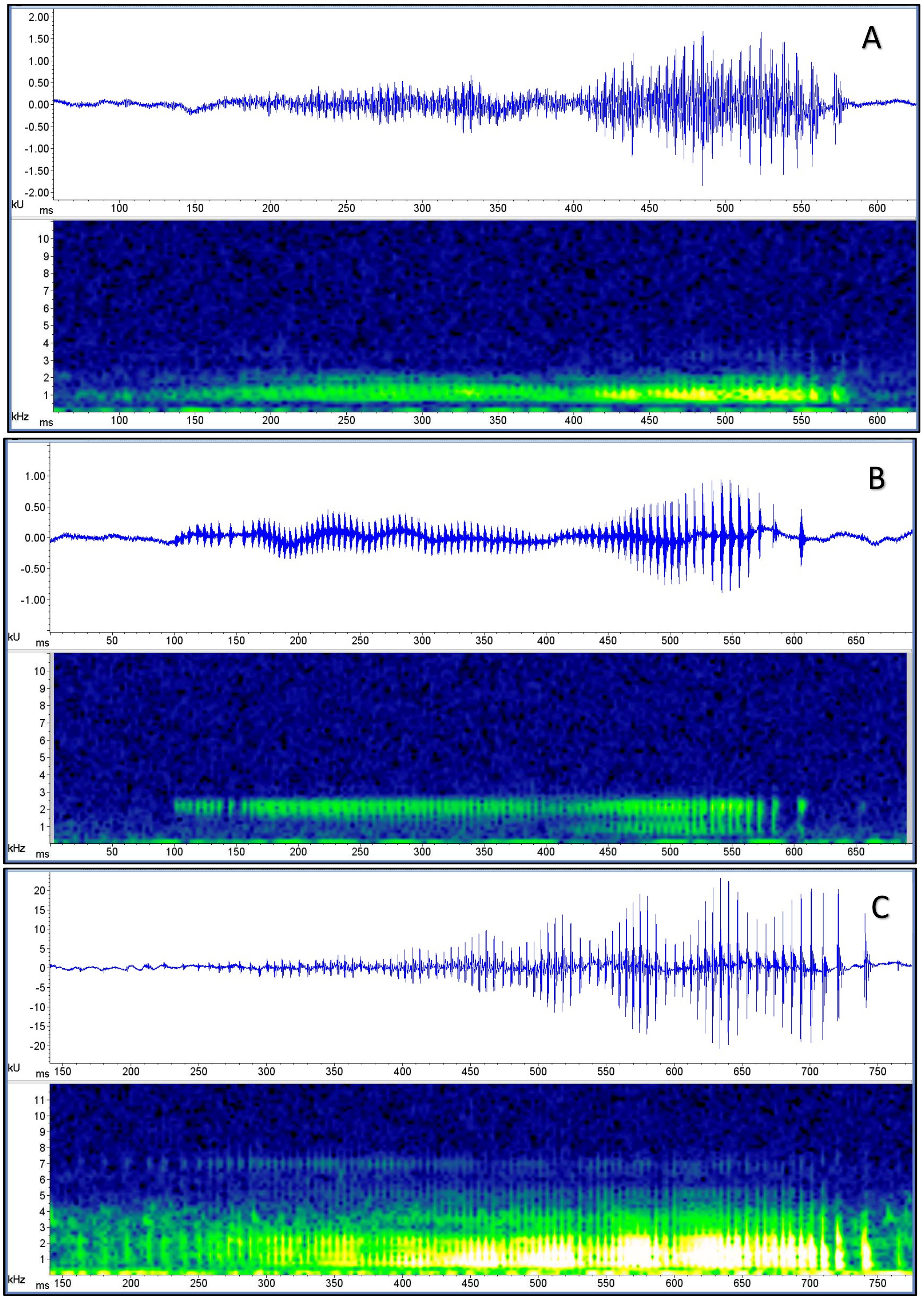

Figure 9: Waveform (top) and Spectrograph (bottom) of long calls: (A) BCCER, (B) Angelo, and (C) Sunol. This figure shows the variation that is found in all three locations. Waveform is measured in kU (y-axis) and Spectrograph is measured in kHz (y-axis), time (milliseconds) is on the x-axis for all calls and in the spectrograph and waveform. Note the kHz difference between Sunol (C) and the other two locations.

Rattles:

Rattles are long pulsed and noted calls (Fig. 13), and each note is made up of repeating pulses. Rattles are the only *R. boylii* call that contain notes. Calls from Sunol had 54.15% more pulses per call than calls from Angelo, and 91.51% more pulses than calls from BCCER (Fig. 10A, $F = 9.358$, $ndf = 2$, $ddf = 86$, $p = 0.0002$). The number of pulses between BCCER and Angelo were not significantly different (Fig. 10A). The pulse duration at BCCER was 42.12% longer than at Sunol, while at Angelo it was 26.82% longer than at Sunol (Fig. 10B, $F = 11.52$, $ndf = 2$, $ddf = 86$, $p < 0.0001$). The pulse durations at BCCER and Angelo were not significantly different (Fig. 10B). Calls from Sunol consequently had 24.29% more pulses per second than calls from Angelo, and 44.17% more than calls from BCCER (Fig. 10C, $F = 9.629$, $ndf = 2$, $ddf = 86$, $p = 0.0001$). There was no significant difference in notes per call among locations ($F = 1.102$, $ndf = 2$, $ddf = 86$, $p = 0.3366$). Similarly, there was no significant difference in note duration among locations ($F = 0.4398$, $ndf = 2$, $ddf = 86$, $p = 0.6456$). Furthermore, there was no significant difference in the duration of rattles among locations ($F = 1.772$, $ndf = 2$, $ddf = 86$, $p = 0.1761$).

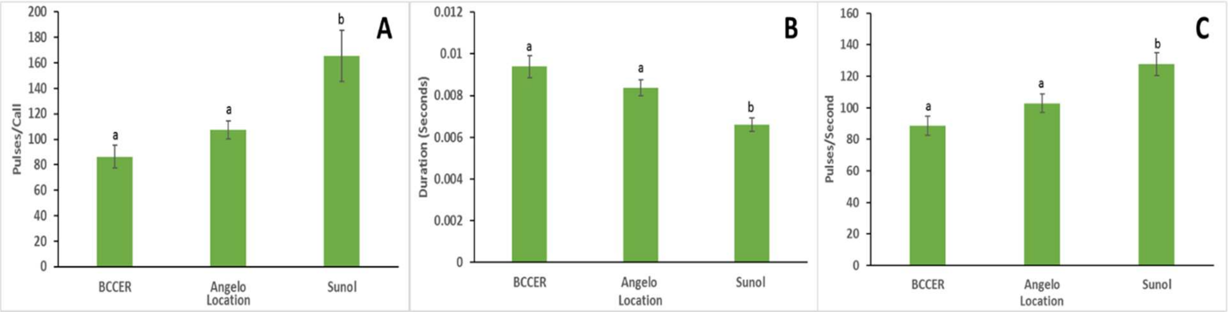

Figure 10: Pulse characteristics of rattles at three northern California locations. (A) Mean (±SE) pulses per call, (B) Mean (±SE) pulse duration, (C) Mean (±SE) pulses per second. Means with the same letters were not significantly different at $\alpha = 0.05$ by a Tukey's HSD test.

The dominant frequency found in rattles in Angelo was 120.5% higher than those in BCCER, and 61.96% higher than those in Sunol. Dominant frequency in Sunol was 36.14% higher than in BCCER (Fig. 11A, $F = 51.83$, $ndf = 2$, $ddf = 87$, $p < 0.0001$). The highest overall frequency was 144.32% higher in Sunol than in BCCER and 52.62% higher than in Angelo. Angelo's Highest overall frequency was 60.08% higher than in BCCER's (Fig. 11B, $F = 95.24$, $ndf = 2$, $ddf = 86$, $p < 0.0001$).

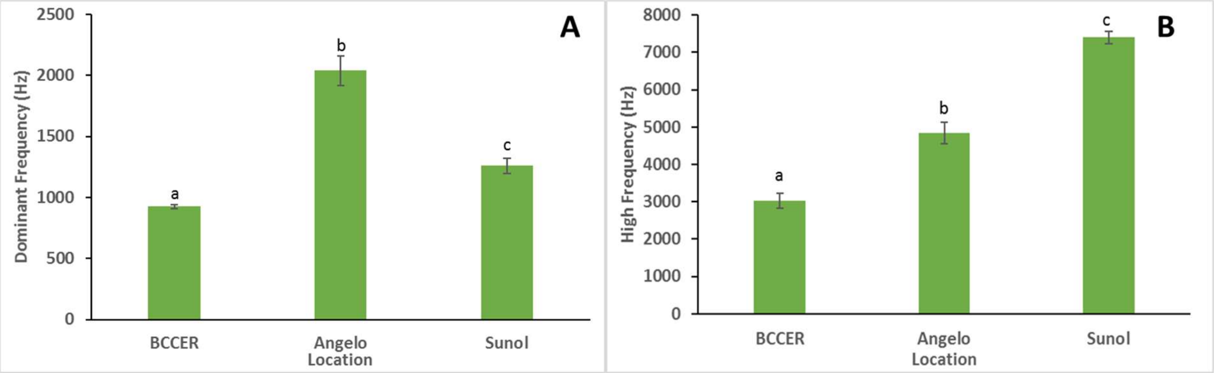

Figure 11: Frequency characteristics of rattles at three northern California locations. (A) dominant frequency and (B) overall high frequency. Means with the same letters were not significantly different at $\alpha = 0.05$ by a Tukey's HSD test.

The spectrographs of rattles (Fig. 12) shows that, despite the higher mean dominant frequency of Angelo (B), Sunol had a higher overall frequency than BCCER (A) and Angelo (B). The different shapes of each rattle waveform and spectrograph (Fig. 12), were unique to each area.

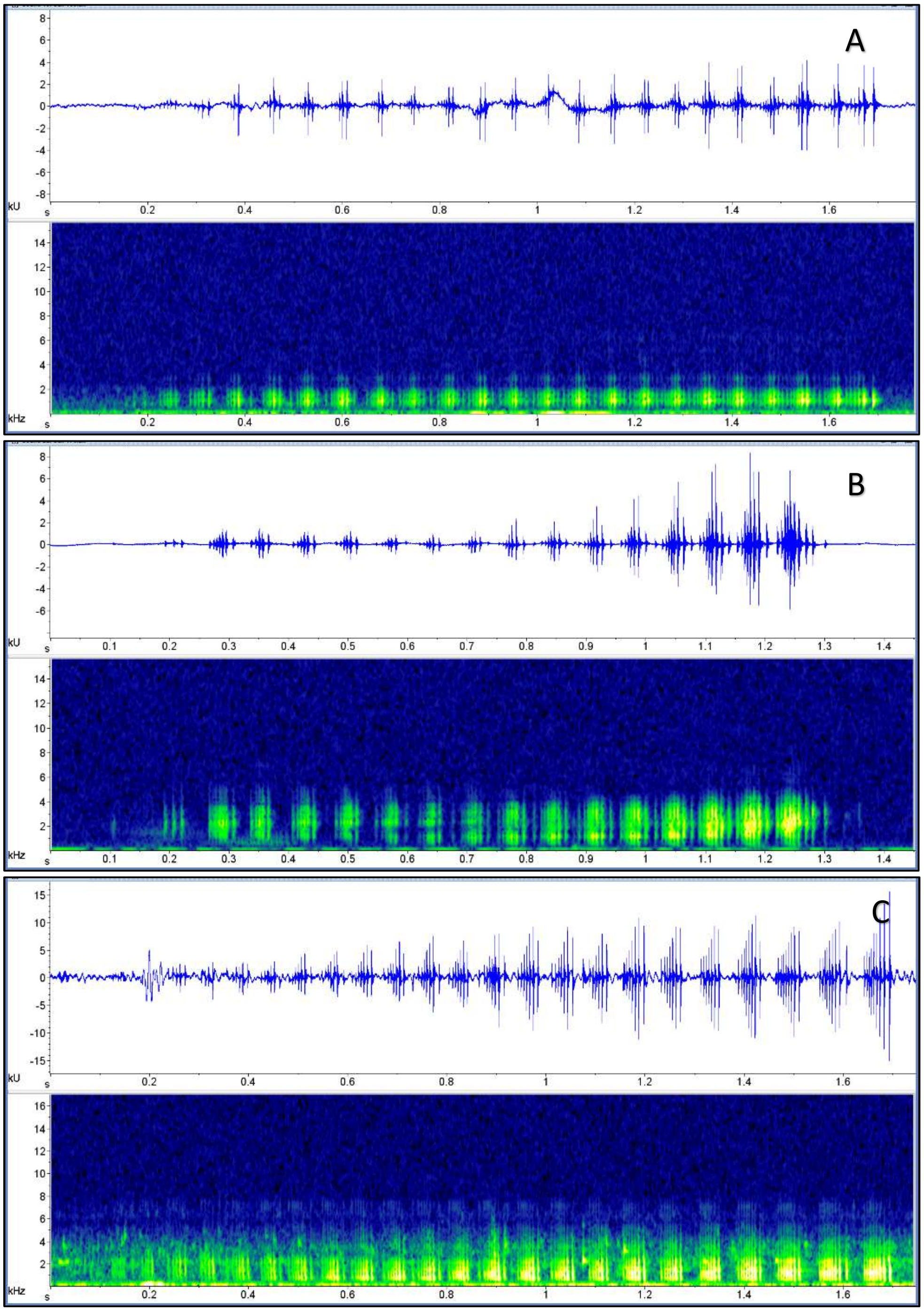

Figure 12: Spectrographs (bottom) and waveforms (top) of rattles made by *Rana boylii* from three northern California locations. (A) BCCER, (B) Angelo, (C) Sunol. Waveform is measured in kU (y-axis) and Spectrograph is measured in kHz (y-axis), time (milliseconds) is on the x-axis for all calls and in the spectrograph and waveform.

Chuckles:

The structure of these calls, termed chuckles in this study (Fig. 13), is different from that of a short call. Each pulse is separated by an average of 18 ms (Fig. 14). Also, the duration of chuckles, ranging from 0.030-0.200 seconds, is shorter than that of short calls. The duration range and number of pulses per second was consistent throughout all locations.

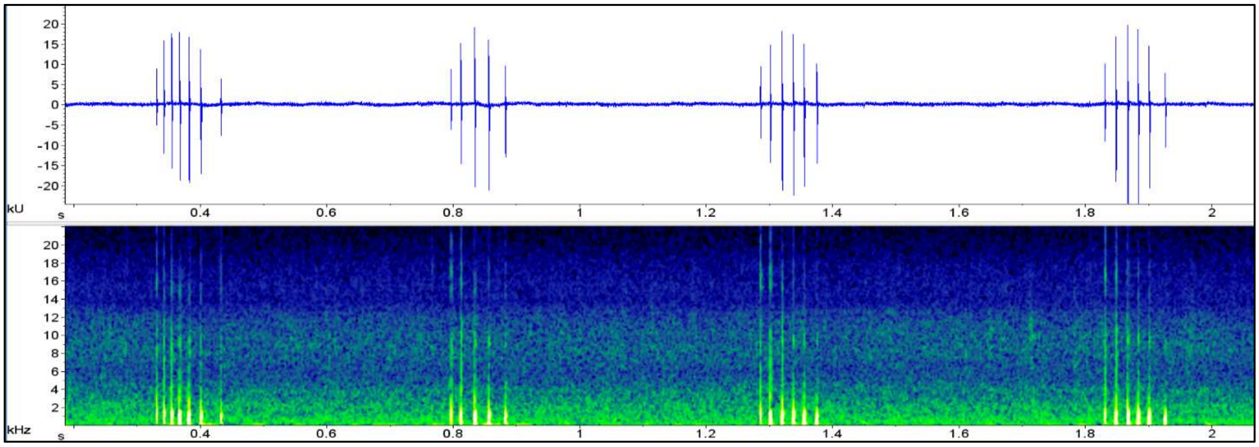

Figure 13: Waveform and spectrograph of repeating chuckles found in the Angelo Coast Range Reserve. Recording was made in April of 2015. This image was created with Raven Pro and depicts four repeating chuckle calls. Spectrograph (bottom) is measured in kHz, the waveform (top) is measured in kU.

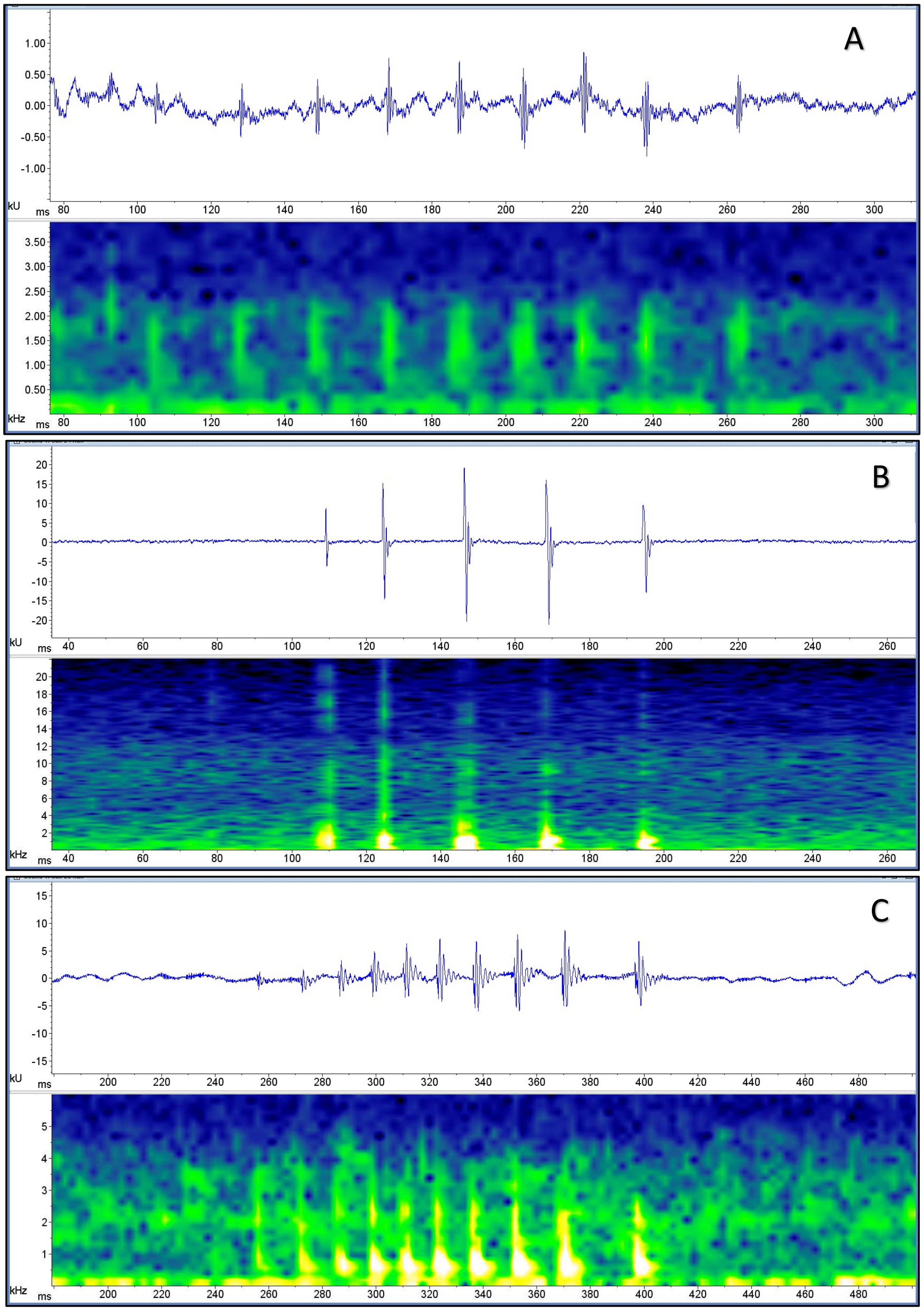

Figure 14: Waveform and Spectrograph of chuckles: (A) BCCER, (B) Angelo, (C) Sunol. This figure shows the variation that is found across all three locations: The Big Chico Creek Ecological Reserve (BCCER), Angelo Coast Range Reserve, and Sunol Reginal Park. Waveform (top) is measured in kU and Spectrograph (bottom) is measured in kHz.

Chuckle duration in BCCER was 54.6% longer than in Angelo and chuckle duration in Sunol was 44.1% longer than in Angelo (Fig. 15, *F*= 9.45, *ndf* = 2*, ddf* = 18, *p* = 0.001). The average number of pulses in BCCER was 42.6% larger than in Angelo and Sunol was 58.4% larger than in Angelo (Fig. 15, *F* = 17.7, *ndf* = 2*, ddf* = 16, *p* < 0.001). However, pulses were not significantly different. There was no significant difference in the duration of the pulses (*F*= 1.82, *ndf* = 2*, ddf* = 19, *p* = 0.188) or the number of pulses per second in chuckles (*F* = 0.644, *ndf* = 2*, ddf* = 17, *p* = 0.537) at any of the three locations. There was overall a significant difference among sites in dominant frequency (*F*= 3.65, *ndf* = 2*, ddf* = 19, *p* = 0.046), but the Tukey's post hoc test did not identify any significant pairwise differences. Lastly, the highest overall frequency of chuckles was in 779.5% higher in Angelo than in BCCER and 212.9% higher than in Sunol (Fig. 17, *F* = 251.4, *ndf* = 2, *ddf* = 21, *p* < 0.001). A total of 24 chuckles were recorded during field seasons, 16 from Angelo, 6 from Sunol, and 2 from BCCER.

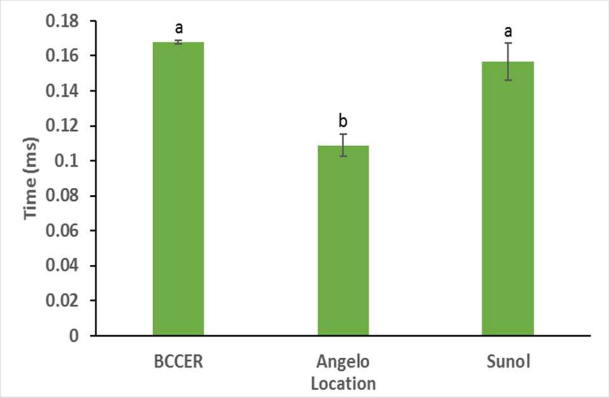

Figure 15: Chuckle Duration in three northern California locations. Means with the same letters were not significantly different at α = 0.05 by a Tukey's HSD test.

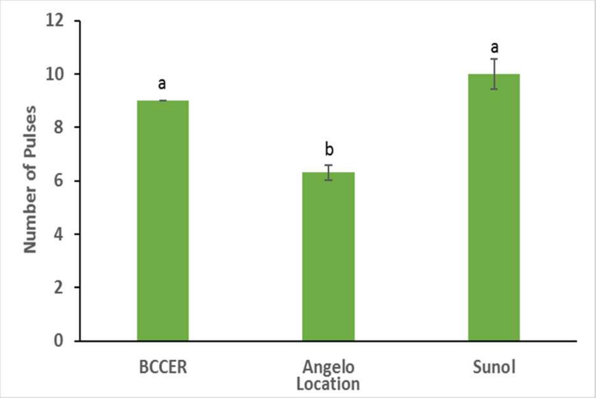

Figure 16: Number of pulses in chuckles in three northern California locations. Means with the same letters were not significantly different at α = 0.05 by a Tukey's HSD test.

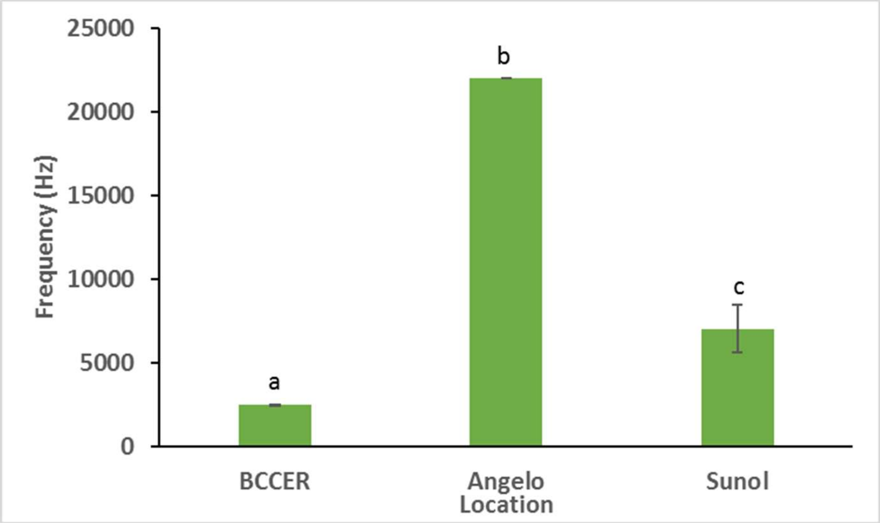

Figure 17: Mean (±SE) of highest overall frequency of chuckles emitted by *Rana boylii* found at three northern California locations. Means with the same letters were not significantly different at α = 0.05 by a Tukey's HSD test.

Release Calls and Warbles:

Release calls were only recorded at the Angelo Coast Range Reserve, despite attempts to record them at all locations. A total of 10 release calls were recorded and analyzed (Table 5). They were pulsed calls, but unlike most other call types, they were not as uniform in their look and pattern. Figure 18 shows the variety of release calls that were recorded in Angelo

The warble is a previously undescribed call, and was only recorded at the Big Chico Creek Ecological Reserve from one mating pair. Only 6 "warbles" were recorded; they were emitted while a mating pair was in amplexus. This call was unlike other calls recorded in this study. The call is unpulsed and unnoted and consists of multiple parallel wavy lines in the spectrograph (Fig. 19) They ranged from 0.200 - 0.350 seconds in length with a high pitched dominant frequency (Table 6).

Table 6: Mean & SE for release call and warble call characteristics of *Rana boylii*. Both of these call types were found only at one location, the release call was recorded at Angelo Coast Range Reserve and the warble was recorded at the Big Chico Creek Ecological Reserve (BCCER). Warbles do not possess any pulses, hence the Not Applicable (NA) in the tables.

	Duration (Seconds)	Dominant Frequency (Hz)	High Frequency (Hz)	Pulse Number	Pulse Duration (Seconds)	Pulses/ Second
Release Call	0.0703	757.97	19075.71	5.6	0.016	86.74
Standard Error	0.0085	145.72	1420.8	1.4	0.004	20.5
Warble Call	0.2661	1492.9	2560.56	NA	NA	NA
Standard Error	0.0208	36.324	84.73	NA	NA	NA

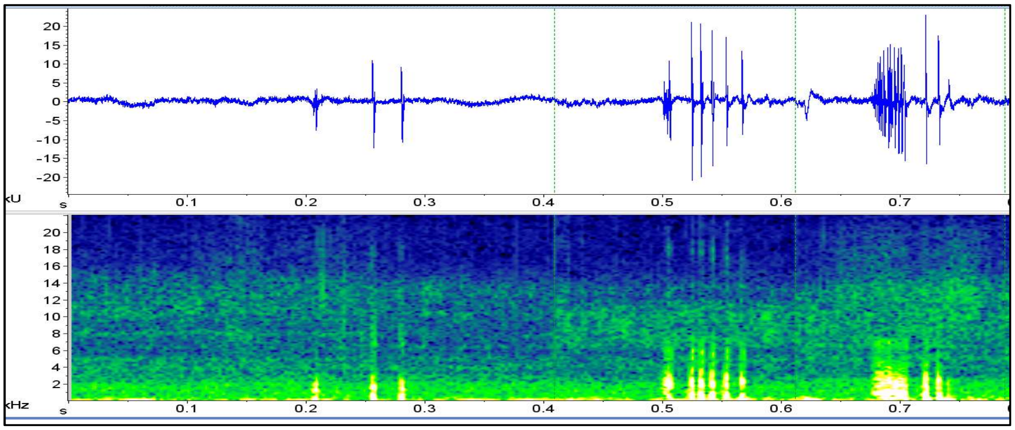

Figure 18: Multiple repeating release calls (cropped) made by the same individual *Rana boylii* at the Angelo Coast Range Reserve. Time (ms) is on the x-axis, kU is on the y-axis for the waveform and kHz is on the y-axis for the spectrograph.

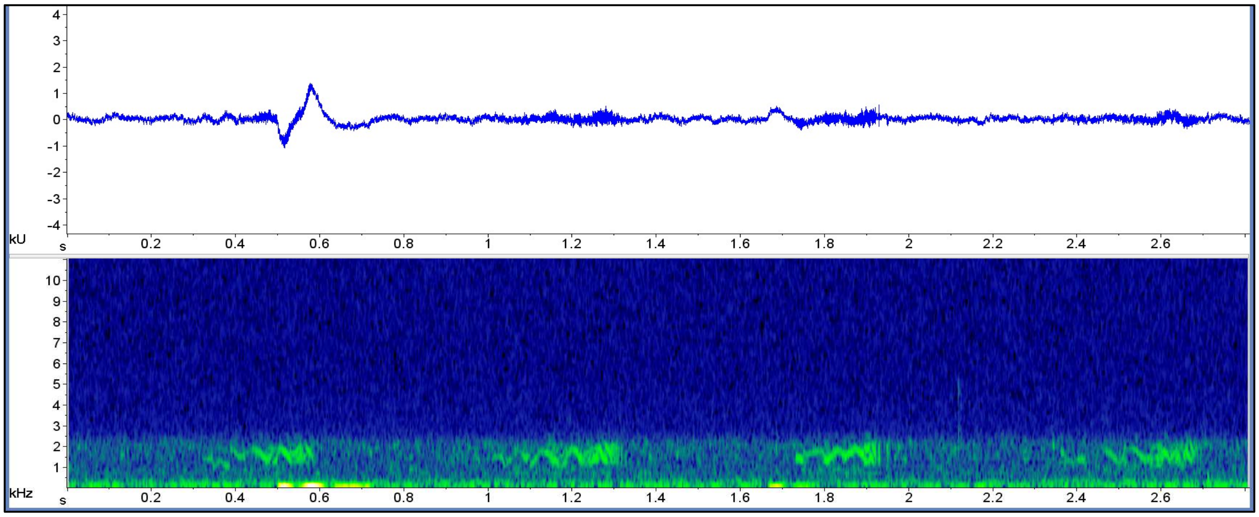

Figure 19: Repeating warbles made by the same *Rana boylii* individual at the Big Chico Creek Ecological Reserve. These warbles were recorded from a pair in amplexus. Warbles were only found at BCCER. Time (ms) is on the x-axis, kU is on the y-axis for the waveform and kHz is on the y-axis for the spectrograph.

Squeaks:

Squeaks recorded from Angelo and BCCER looked different from one another (Fig. 20), but no significant differences were found. Squeak duration ranged from 0.010 to 0.080 seconds. BCCER's duration was on average longer with a mean (SE) of 0.054 (0.012) seconds and Angelo with a mean (SE) of 0.022 (0.001) seconds, but not significantly so ($t = 2.54$, $df = 2$, $p = 0.13$). Dominant frequency ($p = 0.42$) and overall high frequency ($p = 0.12$) also did not differ.

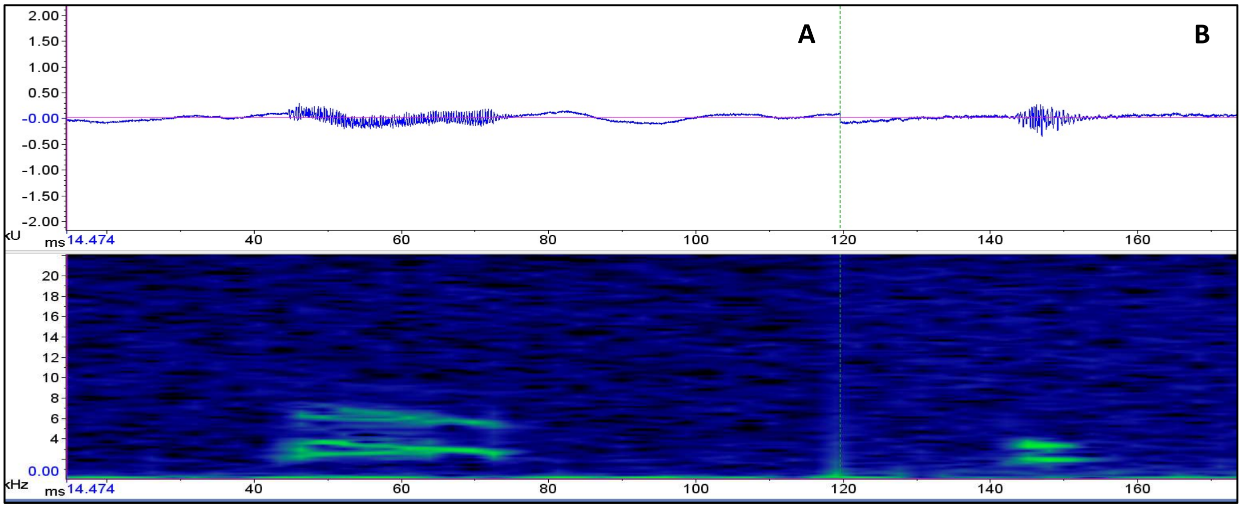

Figure 20: Squeaks emitted by *Rana boylii* from BCCER (A) and Angelo (B). No significant differences were found between the two locations. Time is on the x-axis for both the spectrograph (bottom) and the waveform (top). kHz is on the y-axis for the spectrograph (bottom) and kU is on the y-axis for the waveform (top).

CHAPTER IV

DISCUSSION

Marked variations within vocalizations made by *Rana boylii* were observed among the three populations of this study. These distinct dialects associated with each location could be a direct effect of the different environmental pressures found in each location. Not all frogs have a diverse array of call types (Gerhardt and Huber, 2002). This study, however, has shown *Rana boylii* to be a species with a wide range of call types (a total of seven). In addition, calls types emitted varied among locations. Within the call types emitted, the temporal and spectral properties that makeup each type of call varied significantly among locations. The consequences of these variations are not yet understood, but intraspecific communication could be impeded due to lack of recognition of call types due to the variations found. If the variations in calls between sites inhibit communication this could directly affect breeding success if populations were ever to mix.

Will these calls differ among populations in their spectral and temporal properties?

Several calls varied among populations. Within short calls, Sunol had a higher overall frequency (fig 4D) with a mean of 11.03 kHz. Many of the short calls from Sunol even reached higher than 22 kHz (Ultrasonic vocal signals are signals that are over 20 kHz (Vitt et al, 2013)). Within chuckles, Angelo had the highest overall frequency with a mean that was also over 22 kHz. Like the short calls from Sunol, the ultrasonic high frequency found in chuckles in Angelo was restricted to one call type. There are only two species of frogs that are known to produce and hear ultrasonic vocal signals, hole-in-the-head frog (*Huia cavitympanum*) and concave-eared torrent frog (*Odorrana tormota*). Both species live in noisy habitats, use different mechanisms to produce these sounds, and are not closely related (Vitt et al, 2013). Other species of frogs also have high-frequency calling in noisy areas, whether natural or man-made. Masking, or acoustic interference, occurs when background noise reduces how far a call can travel (Brumm et al, 2005). Littlejohn (1965) showed that natural acoustical interference can cause a species to develop more effective communication. Warren et al. (2006) and Parris et al. (2009) found that anthropogenically generated noise, like traffic and other urban noises, tend to shift frogs' and birds' dominant frequency higher because urban noises tends to be emitted at a lower frequency. Urban noise frequency tends to fall below 2 kHz, higher pitched signals have less acoustic interference than lower pitched signals (Warren et al, 2006).

Boeckle et al. (2009) showed that *Staurois latopalmatus,* the rock skipper frog, calls at higher frequency in the presence of a waterfall due to the low frequency interference. This suggests that characteristics of microhabitats put strong selective pressures on acoustic signals of frogs. Multiple ultrasonic short calls were recorded in Little Yosemite within Sunol. Not only is there noise from constant human visitors to the area, but there is also a natural acoustical interference: a waterfall. This waterfall has a dominant frequency of 1.7 kHz (personal observation). Thus, the ultrasonic frequency found in Sunol may compensate for the masking that occurs as a result of the waterfall. The ultrasonic frequency found in chuckles in Angelo are harder to explain. There is no waterfall present at this site and few human visitors or other potential sources of masking. However, the Eel River and the surrounding area is filled with communicating wildlife as well as noise from a fast-moving river. Feng et al. (2006) proposed that the background noise from the fast-moving stream in the Huangshan Hot Springs in China caused the concave-eared torrent frog to extend its call frequencies into the ultrasonic range to prevent the frog's vocal signals from being masked. Other acoustical interference that was not measured may also occur. Alternatively, high frequency calls may not be an anomaly; perhaps *Rana boylii* populations routinely call at ultrasonic frequencies. More research is needed to characterize the extent and cause of these intriguing ultrasonic signals.

There were few consistent trends in call characteristics. Sunol, in general, had a higher overall frequency in several vocalizations whereas calls from Angelo often had a higher dominant frequency. Otherwise, there was substantial variation in call characteristics across locations that did not seem to follow any sort of trend or pattern.

Short calls from Angelo had a higher dominant frequency, a faster pulse rate, and a longer duration than the other locations. However, long call duration was longer in Sunol with more pulses per call at shorter pulse durations, leading to a faster pulse rate. Angelo again had a higher dominant frequency of long calls, but Sunol had a higher overall frequency with some calls reaching above 22 kHz. Chuckle duration was longer and had more pulses in BCCER and Sunol compared to Angelo. There was no difference in temporal properties of the chuckle pulses (pulse duration and pulse rate) or dominant frequency between locations for chuckles, but Angelo had the higher overall frequency reaching above 22 kHz. Squeaks were only recorded at Angelo and BCCER with no significant difference in any of the characteristics between the two locations. Even though there was no significant difference in the duration of rattles among the three locations, other spectral and temporal properties seem to mirror what was observed for the long calls. Rattles recorded at Sunol, for example, had more pulses per call with shorter pulse durations, leading to a faster pulse rate. Also, rattles at Angelo had a higher dominant frequency and rattles from Sunol had a higher overall frequency. There was no difference in the note characteristics between the locations.

Temperature is known to alter anurans' call properties within and between individuals that breed over a wide range (Blair, 1958; Gerhardt, 1978; Narins et al., 2007). Temporal call properties are the most affected by temperature (Narins et al., 2007). However, it is unlikely that the temperature difference between the three locations of this study were significant enough to have caused the differences reported herein. As temperature increases, pulse rate increases and duration decreases (Narins et al., 2007). In Sunol (highest temperature 13.5 C), there were longer durations in long call and chuckles, which goes against the known standard. Furthermore, in Angelo (lowest temperature 11.9 C), there was a faster pulse rate in short calls and long calls.

Are more than five calls used in their repertoire?

Two call types not previously reported were recorded: the chuckle and the warble. The chuckle was recorded from all three locations. The spectral and temporal properties of chuckles were consistent among all locations. The only difference found was in duration, with BCCER and Sunol being longer than Angelo. The second additional call, the warble, was recorded only at BCCER. It is suspected that this call is only made during amplexus, possibly by females. All of the warbles were recorded from a single mating pair in amplexus in which the male and female appeared to be calling in a duet. The calls coming from the male were classifiable, but those from the female were not. Recordings of mating pairs were not conducted in other locations. More data are needed to better understand the complexities (spectral and temporal properties) of these calls. Although rare, male-female mating duets have been reported elsewhere (Tobias et al., 1998). During mating, male and female African clawed frogs (*Xenopus* *laevis*) vocalize in what Tobias et al. (1998) calls a duet. Female African clawed frogs make calls out of amplexus in contrast to female *R. boylii*, but both *Xenopus laevis* and *R. boylii* call underwater.

Do all *Rana boylii* populations use the same calls?

Release calls and squeaks were not present in all three locations. Release calls are emitted when an individual frog is grasped, often in amplexus, and is uninterested in mating. It is a signal for the individual grasping to let go. Release calls were only recorded in Angelo and squeaks only in Angelo and BCCER. Short calls, long calls, rattles, and chuckles were found in all three locations. To record a release call, individuals were grasped around their midsection. Despite efforts, only one individual was caught and used in Angelo to signal for a release. After 20 hours of recording from Sunol, no squeaks were recorded. Squeaks and releases might not be present in all three populations, or they may have been missed.

In conclusion, this study underscores the importance of understanding variation among geographically isolated populations. It cannot be assumed that all populations are identical in communication strategies. Earlier studies (Zweifel, 1955; Lind, 2005; Lind et al., 2011) identified genetic and morphological variation among *R. boylii* populations. The current study reveals additional population-level variation not previously anticipated in key characteristics of call properties and call types, including previously undescribed calls and ultrasonic calls. What is not known, however, is in the consequences of this variation for future conservation efforts. Reintroductions, head-starting, and translocation efforts appear to be methods most likely to be adopted in order to successfully protect this declining species (Lind, 2005; Gascon, 2007; Griffiths and Pavajeau, 2008). First, however, an understanding patterns of variation in communication and behavior among sites is needed. An important next step would be to identify the function of each call type and use play-back studies to examine whether females distinguish calls from non-local males (Ryan and Wilczvnski, 1988; Ryan et al., 1992). Such experiments would clarify whether mixing frogs from different populations would be able to communicate and therefor breed successfully.

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