

A Study on the Release Calls of Four Anauran Frogs from the Upper Peninsula of  
Michigan

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## Abstract

The Anuran release call has not had been heavily studied within the scientific community, but is thought of as the cornerstone of anuran auditory interaction and has displayed evidence of little evolution (Tada et al. 2001). In this study, I hypothesize that a universal factor is present in the Ranidae release calls that facilitates all species to effectively communicate their desire to be released from the amplexus clasp. Amplexus was stimulated on *Rana clamitans* (green frog), *R. pipiens* (Leopard frog), *R. septentrionalis* (mink frog), and *R. sylvatica* (wood frog) by clasping the frog behind its forelimbs. The calls were then transformed into sonograms and analyzed by way of frequency, amplitude, call length, pulses per call, calls per bout, calls per minute, and total calls. After statistical analysis, call length and pulses per call showed statistical evidence that it can be a possible factor in the universality of the Ranidae release call. Further research is needed to determine if other factors are present in the release calls that make them a universal auditory signal.

## Introduction

Auditory signals from anurans are typically divided into four distinct categories: advertisement calls, distress calls, aggression calls, and release calls (Bevier et al. 2004). Advertisement calls are auditory calls that are produced by male frogs to attract a female mate and to ward off other males that are in their

territory (Bevier, et al. 2004). These calls are composed of differing variations of call notes between species, therefore advertisement calls have been heavily relied on for species identification (Bevier et al. 2004, Tada et al. 2001). Despite recent interest in advertisement calls, anuran release, aggression, and distress call have received significantly less attention in the scientific literature (Tada et al. 2001).

Anuran advertisement calls are believed to have evolved from less complex release calls (Gerhardt 1994; Schmidt 1976) leading to the argument that release call are the evolutionary being of anuran auditory interaction (Tada et al. 2001). A release call is a signal that is produced by the frog when it is inappropriately clasped by another frog (Boyd 1992; Gerhardt 1994; Tada et al. 2001). Release calls are heavily utilized during the mating season when males mistakenly clasp other male or unreceptive females to commence amplexus (Boyd 1992; Gerhardt 1994; Tada et al. 2001). In 1947, Blair (1947) demonstrated that toad species will appropriately release the unreceptive female/male when the victim emits a release call. Frogs produce release calls by forcefully ejecting air through the larynx; an inspiration phase follows the call in which air is forced back into the frog's lungs (Schmidt 1976). Release calls tend to be highly variable in call structure, call duration, and call periodicity (Gerhardt 1994). Schmidt (1976) found that female release calls were too variable at low amplitudes to compare to male release calls. This variability in female release calls is attributed to the

female frogs' underdeveloped laryngeal apparatus compared to the male counterpart.

Few studies have examined the universality of the frog release call. In most instances, it has been accepted that this call has a universal feature that enables frogs to effectively communicate their desire to be released to frogs of other species and sex. Previous studies on Ranidae advertisement calls have shown that the calls of each species have their own unique characteristics (Bevier et al. 2004, Tada et al. 2001, Bee et al. 2001). From their advertisement calls, scientist are able to be identified the frogs to their species (Bee et al. 2001). Release calls, however, should not be species specific because the release call exceeds the species boundary of communication.

In this study, I hypothesize that a universal factor is present within Ranidae release calls that facilitates all species to effectively communicate their desire to be released from the amplexus clasp.

## Materials and Methods

### Study Site

The study was conducted at the University of Notre Dame Environmental Science Research Center (UNDERC). The center is located in the Western portion of Michigan's Upper Peninsula in Southern Gogebic County. The property is composed of approximately 7,750 acres hardwood forest, bogs, lakes,

and early successional fields that have remained relatively undisturbed for 60 years (Allan 1973). For this study, Ranidea specimens were collected at various locations on property, but most specimen collections was conducted at bogs and vernal ponds.

### Specimen Collection and Storage

Random sites on the UNDERC property were chosen for frog collection from June 11, 2007 to June 17, 2007, June 20, 2007, and June 25, 2007 to June 27, 2007. Ranidae Anuran species that were sexually mature were captured by hand and placed in large plastic storage bags for temporary purposes. Once back at the lab, specimens were placed in individual 591 mL container with air ventilation and a sufficient amount of water. Specimens were left in the climate controlled lab for at least an hour before testing. After testing, specimens received fresh water and were placed in the lab refrigerator for no longer than 30 hours. The specimens were release a day after their capture at their capture site.

### Recording

Prior to recording, the specimens' water was drained from their container. The frogs were weighted, sexed, and identified to species. The female frogs were inspected to determine gravidity. The frogs were transported to a dark room for recording. Release calls were stimulated on the frogs by clasping frogs in the

pectoral region behind the forearms and applying pressure for 1 to 3 minutes (Tada et al. 2001; Boyd 1992; and Schmidt 1976). The release calls produced by the frogs were recorded on a condenser Lo-Z microphone (Audio-Technica, Stow, Ohio, Type Line and Gradient AT8156) and a portable recorder (M-Audio, Irwindale, California, Type USD 499.95 MSRP). After recording, the frogs' snout-vent length and cloacal temperature was recorded. Toe clipping was performed to prevent recapture and ensure that duplicates were not present within the data. The frogs were then return to their original individual container with new water and stored in the refrigerator over night to induce a hibernative state.

#### Release Call Analysis

The release calls produced from the frogs were transformed into sonograms through Raven 1.2.1 software (Cornell Lab of Ornithology Bioacoustics Research Program 2003-2004). Call frequency (kHz) , peak amplitude, call length, call period, pulses per call, calls per bout, calls per minute, and total calls were calculated for each individual frog in Raven 1.2.1. Since few frogs displayed a consistent call period, this variable was not used for further analysis. During call analysis, an un-pulsed call with random call periods and no bouts (uniform call) and a pulsed call that occurred in bouts with normally distributed intervals (pulsed call) were discovered using Raven 1.2.1 software. Tada et al. (2001) reported similar multi-release calls in neotropical toads. The

three classified calls found in the study were a uniform release call, a release trill with a high pulse rate, and a release trill with a low pulse rate (Tada et al. 2001

### Statistical Analysis

Statistical analysis of the data was completed using SYSTAT 12 (Wilkinson, 2007) and R (R DEVELOPMENT CORE TEAM 2005). Frog weight, call length, and call amplitude were log transformed to improve normality. Gender, gravidity, length, weight, and cloacal temperature were include as covariates for ANCOVA test to test interspecific differences in frequency and amplitude for both uniform and pulsed calls. A Tukey's post hoc analysis was conducted to determine interspecific pairwise differences. Proportion testing using a bonferroni adjustment of 0.9917 was used to compare species versus, calls per bout, pulses per call, calls per minute, and total call for both uniform and pulsed calls. Hypothesis testing of the equality of two proportions was used to compare species versus calls per bout, pulses per call, calls per minute, and total calls for both uniform and pulsed calls. A Bonferroni adjustment of 0.9917 was used in the Hypothesis testing to account for multiple tests being performed. To test if amplitude and frequency was significantly different between uniform and pulsed calls an ANOVA nested by species was preformed using the program R (R DEVELOPMENT CORE TEAM 2005). Two sample T-tests were conducted in SYSTAT (Wilkinson 2007) to evaluate if a

significance difference was present between the uniform and the pulsed calls within a species

## Results

### Sonogram Analysis for the Uniform Call

The uniform call (Figure 1) showed no significant difference between species for call length and amplitude. Frequency of the uniform call was significantly affected by the snout-vent length (p-value= 0.03, df=1) and the weight (p-value= 0.02, df=1) of the frog. The frequency of the uniform call was also significantly different between *R. clamitans* and *R. pipiens* (p-value= 0.01, 95% confidence: 0.41, 3.84) and between *R. pipiens* and *R. septentrionalis* (p-value=0.01, 95% confidence: -5.43, -0.68) (Figure 5). For calls per bout in the uniform call, there was no significant difference between any. For pulses per call, there was no significant difference between species. For calls per minute in the uniform call, a significant difference was detected between *R. clamitans* and *R. pipiens* (99.17% confidence: -0.76, -0.1). For proportion of total uniform calls produced, no significance was detected.

### Sonogram Analysis for the Pulsed Call

Like the uniform call, the pulsed call (Figure 2) only displayed a significant difference in the call length between species. Amplitude of the pulsed



call is significantly affected by snout-vent length of the frog (p-value=0.035, df=1). Amplitude of pulsed calls are only significantly different between the species of *R. clamitans* and *R. septentrionalis* (p-value=0.019, 95% confidence: -0.96, -0.11) and between *R. clamitans* and *R. sylvatica* (p-value=0.037, 95% confidence: -0.94, -0.10). Frequency of the pulsed call was significantly affected by weight (p-value=0.013, df=1) and was marginally affected by the cloacal temperature of the frog (p-value=0.081, df=1). Frequency of the pulsed calls were only significantly different between *R. clamitans* and *R. pipiens* (p-value=0.028, 95% confidence: 0.14, 1.54) (Figure 5). For the proportion of bouts per call, a significant difference was present between *R. clamitans* and *R. pipiens* (99.17% confidence: -0.96, -0.2 (Table 1). For the proportion of frogs that had 2 or more pulses per call, a significant difference was present between *R. clamitans* and *R. pipiens* (99.17% confidence: 0.18, 1.05), and *R. clamitans* and *R. septentrionalis* (99.17% confidence: 0.32, 10.8) (Table 1). For proportion of calls per minute in pulsed calls, a significant difference was detected between *R. clamitans* and *R. pipiens* (99.17% confidence: -1.13, -0.47) (Table 1). For proportion of total pulsed calls produced by each frog, a significant difference was displayed between all *R. pipiens* and *R. septentrionalis* (99.17% confidence: 0.46, 10.8), *R. pipiens* and *R. sylvatica* (99.17% confidence: 0.16, 1.17), and *R. septentrionalis* and *R. sylvatica* (99.17% confidence: 0.16, 1.17) (Table 1).

## Sonogram comparison of the Uniform and Pulsed Call

An analysis comparing the pulsed and uniform calls revealed that there is no significant difference in frequency between the calls. Although frequency was not significant, a significant difference does exist in the peak amplitude of the pulsed and uniform calls (p-value= 0.005). The difference in peak amplitude was only significantly different between pulsed and uniform calls for *R. pipiens* (p-value=0.02, df=18.94, 98.75% confidence: -1.23, 0.05) and in *R. septentrionalis* (p-value=0.01, df=8, 98.75% confidence: -4.55, 2.32). Besides the statistical physics aspect of the calls, the pulsed and uniform calls display different physical structures on the sonogram (Figure 1, Figure 2).

## Discussion

At first glance, it appears that a universal factor does not exist between species for release calls. Upon further examination of the data, other significantly different hypotheses can be made.

I originally suspected that frequency and amplitude would not be significantly different in uniform and pulsed release calls and therefore frequency and amplitude would be a universal factor that enables intra species communication through the release call. This, though, did not hold true. Frequency, as seen in the results, is dependant on factors of snout-vent length and weight for uniform calls, and weight for pulsed calls. Even though there is not a

significant difference in peak amplitude in uniform calls, there is for pulsed calls (Figure 5). Therefore, frequency and amplitude cannot be considered the universal factor for both pulsed and uniform calls.

From the data, the only tested variables that did not show a significant difference between species and between pulsed and uniform calls was call length and sex. I believe call length could play a factor in the universality of the release call, but I also believe that call length is not the only factor that makes the release call universal between species. Since the release call is considered the first developed call of all frogs and the call in which advertisement, aggression, and distress calls stem from (Gerhardt 1994; Schmidt 1976), it makes sense that this primitive call will display a common short call length period throughout the Ranidae species. Tada et al. 2001 found that in the uniform call of neotropical toads, “a complete overlap with respect to signal features among all taxa suggest[s] the same function within the specific communication system” (Tada et al. 2001). Despite the fact that the data did not prove any similarity in frequency and amplitude in the uniform and pulsed call, there could be other features of the call that I did not examine that are key factors which make the release call universal.

The data concluded that there was not a significant difference between male, female, and gravid female release calls. This contradicted Schmidt (1976) study in which he concluded that female release calls could not be compared to

males because of their variability and low amplitude. My data suggests otherwise. The female release calls are not different the males' in frequency, call length, or amplitude. Again, this points to the theory that the release call is the evolutionary beginning of all anuran calling. If the release call did evolve first in frog vocalization, it is a reasonable hypothesis that female's larynx are developed enough to produce these calls, but did not advance in development because they did not have to need to produce more advanced calls such as advertisement calls.

During analysis of the Ranidae frog calls, I discovered 2 different calls. These called differed in their physical appearance on the sonogram and the sound they produce. As seen in Fig 1 and Fig 2, the sonogram of the uniform and pulsed call display visually different characteristics. Because of these calls differing in sound and physical appearance, I analyzed them separately considering them to be 2 different types of release calls. A study done by Tada et al. (2001) discovered 3 different release calls present in neotropical toads. The neotropical toads produced a uniform call, a trill call with a high pulse rate, and a trill call with a low pulse rate (Tada et al. 2001). The uniform call in this study is similar the uniform call produced in Tada et al. (2001) neotropical toads. Tada et al. 2001 found that the uniform call was the only call that was given in all taxa of toads which were examined. When examining the trill calls, it was found that these calls were significantly different between taxa. Tada et al. (2001) theorized that these trill might not be release calls. Tada et al. (2001) noted that these trill calls

are, “remarkably similar to the advertisement call”. When looking at the release of uniform calls vs. pulsed calls (Figure 3) in this study, similar to Tada et al (2001) results, the uniform call was produced over twice as much as the pulsed call in the examined Ranidae species. In Fig. 4, it is apparent all species produce the uniform call more than the pulsed call except *R. pipiens*. Since such a small sample size of *R. sylvatica* was examined, it is possible that *R. sylvatica* will also produce the pulse call more than the uniform call. I hypothesize that it is possible that the pulsed call is not the release call of the frogs, but instead a distress or modified advertisement call. In the data, pulsed calls did not display any similarities across species except for call length. Also, pulsed calls are a more complex call than uniform calls, which suggests that it is not the part of the original, simple release call that is the evolutionary beginning of frog vocalization (Gerhardt 1994; Schmidt 1976). It is possible that the pulsed call evolved separately in each species from the uniform call, hence its variability across Ranidea species.

During the stimulated clasping of amplexus, a consider amount of frogs in all species released chest vibrations, but might failed to produce a release call. Although this data was not recorded, it is possible that these vibrations are a vital part to the release call mechanism and universality. For further studies, I suggest that the vibrations produced by the frog during clasping be quantified and

determined whether they are link to release calls. Also, whether the vibrations are produced in all Ranidae species

The pulsation of calls, bouts per call, and calls per minute were also examined within this study. Pulsed calls all displayed a significant difference for the previous categories. Uniform calls displayed no significance in the area of calls per bouts, pulses per call, and proportion of total calls. When referring to the proportion of calls per bout, no frog who produced a uniform call called in a bout. Therefore, when the proportions test was preformed, the system crashed and was unable to correctly analyze the data. As a result, proportion of bouts per call is discarded from the data. Total call is also discarded from the data due to the fact that the amount of calls produce could have been directly connect to the amount of pressure place on the frog's abdomen during stimulated amplexus clasp. Even though I attempted to apply the same amount of pressure for every specimen, I cannot guarantee that the pressure was equal throughout all 93 tested frogs. There was no significant difference in the proportion of pulses per call in the uniform call. I believe that this could be a key component of the universality of the release call. As I previously stated, the pulsed call could possibly not even be a release call. Going off of that hypothesis, most of the species who produced a uniform call only had one pulse to the call. Since the release call is thought of as the first auditory signal of the frog (Gerhardt 1994; Schmidt 1976), it makes sense that this call is simple and lacks any ornate pulses. Lastly, even though the stimulated

clasping produced calls, I cannot be sure that the same pressure was applied to all frogs. Therefore, greater pressure could have induced release calls which would produce error in the calls per minute for the frog. Therefore, calls per minute in pulsed and uniform calls was not considered within the study.

In further studies, I suggest the use of an even sampling field. For this study, 59 *R. clamitans*, 13 *R. septentrionalis*, 12 *R. pipiens*, and 6 *R. sylvatica* were used. It is possible that our sample size was not large enough for the *R. septentrionalis*, *R. pipiens*, and *R. sylvatica* to produce results that are representative of these species. I also recommend more complex analysis of the release calls. In this study, I only examined frequency and peak amplitude of calls, it is possible that by narrowing the study to just frequency and peak amplitude, I missed key factors in the release calls.

In conclusion, call length and pulses per call might be one of the universal factors present in the Ranidae release call that enables all intra species communication of the desire to be released from the mistaken amplexus clasp. Others factors were unable to be determined based off the studies, therefore I reject my hypothesis that a universal factor is present in the Ranidae release call.

Table

Table 1. P-values and confidence for calls/bout, pulses/call, calls/minute, and total calls for pulsed calls. 99.17% confidence. Significant values are bolded.

Species 1	Species 2	Proportion of bouts per call		Proportion of pulses per call		Proportion of pulses per minute		Proportion of total calls	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
R. pipiens	R. septentrionalis	-0.56	1.06	-0.13	0.29	-0.39	1.05	0.46	1.08
R. pipiens	R. sylvatica	-0.89	0.73	-1.33	0.17	-0.39	1.05	0.16	1.17
R. septentrionalis	R. sylvatica	-1.35	0.68	-1.39	0.05	-1.02	1.02	0.16	1.17
R. clamitans	R. septentrionalis	-1.05	0.39	0.32	1.08	-1.26	0.33	-0.51	0.12
R. clamitans	R. pipiens	<b>-0.96</b>	<b>-0.21</b>	<b>0.18</b>	<b>1.05</b>	<b>-1.13</b>	<b>-0.47</b>	<b>-1.03</b>	<b>-0.9</b>
R. clamitans	R. sylvatica	-1.39	0.05	-0.78	0.85	-1.26	0.33	-0.81	0.21



Figures

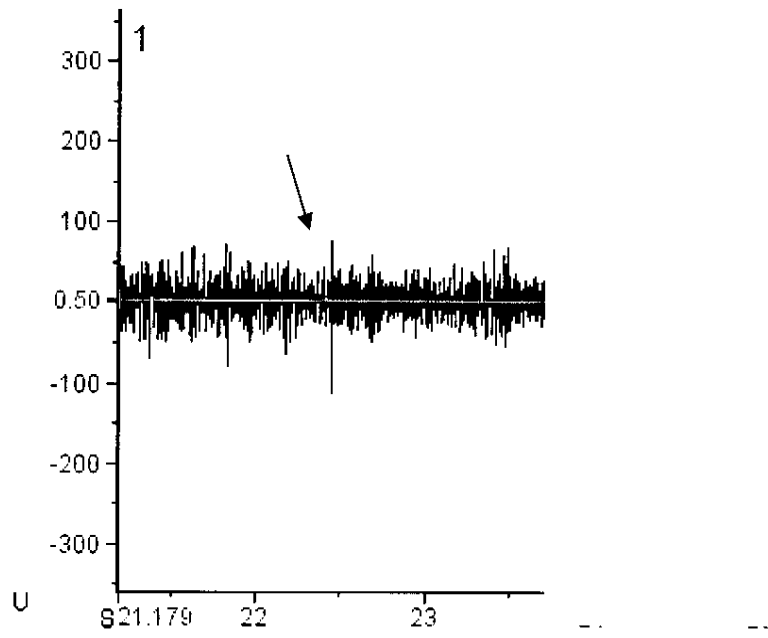


Fig. 1. The arrow indicates the sonogram peak of a uniform call from *Rana Clamitans*

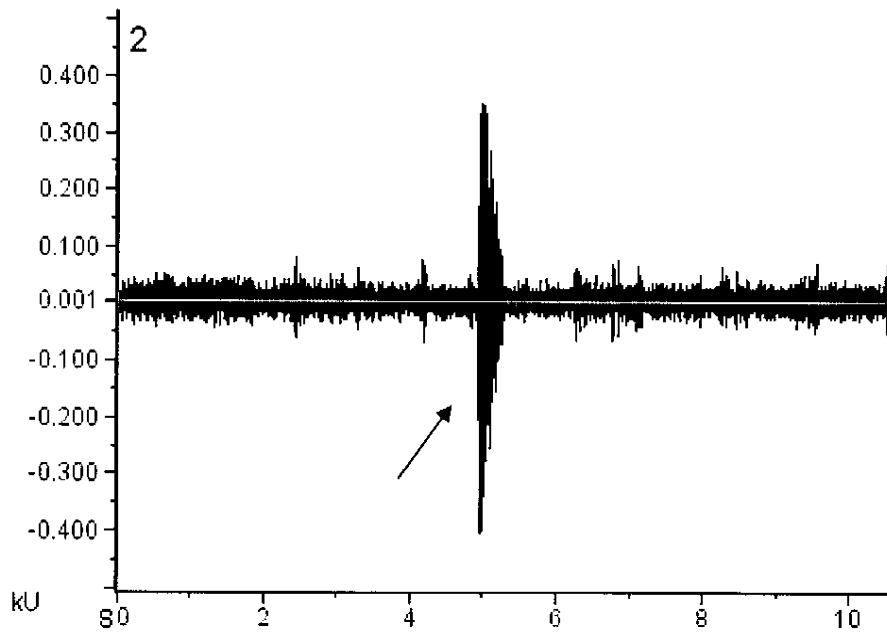


Fig. 2 The arrow indicates a sonogram peak of a pulsed call for *Rana Clamitans*

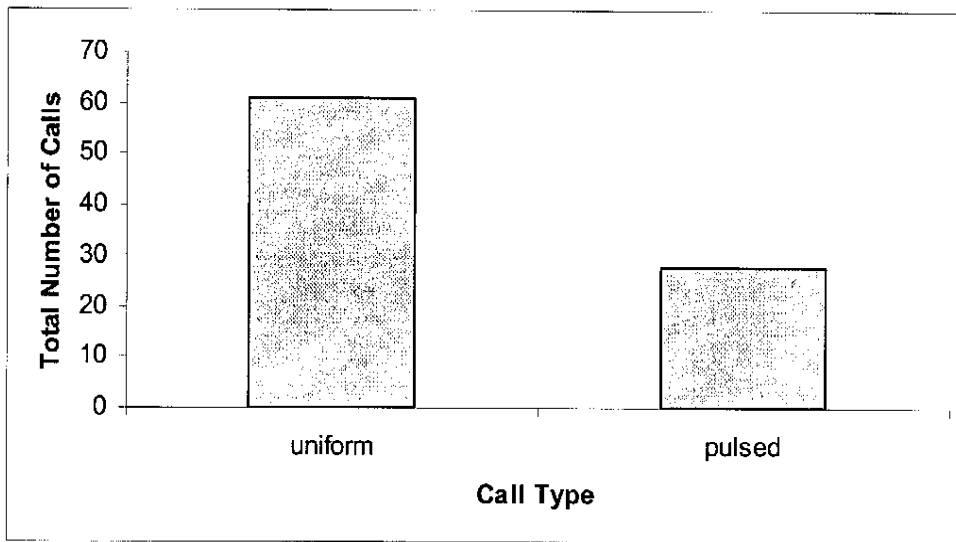


Fig. 3 Bar graph displaying the raw number of uniform calls and pulsed calls produced by *Rana clamitans*, *Rana pipiens*, *Rana septentrionalis*, and *Rana sylvatica*

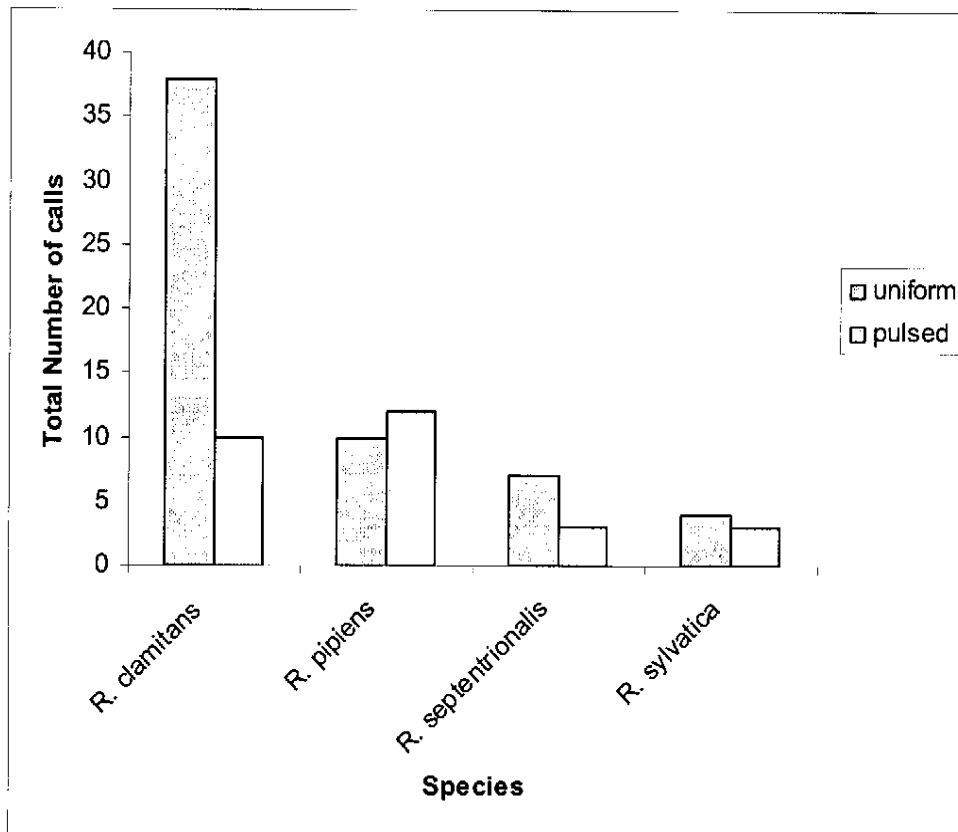


Fig. 4 Bar graph of raw number of uniform and pulsed calls produced broken down by species

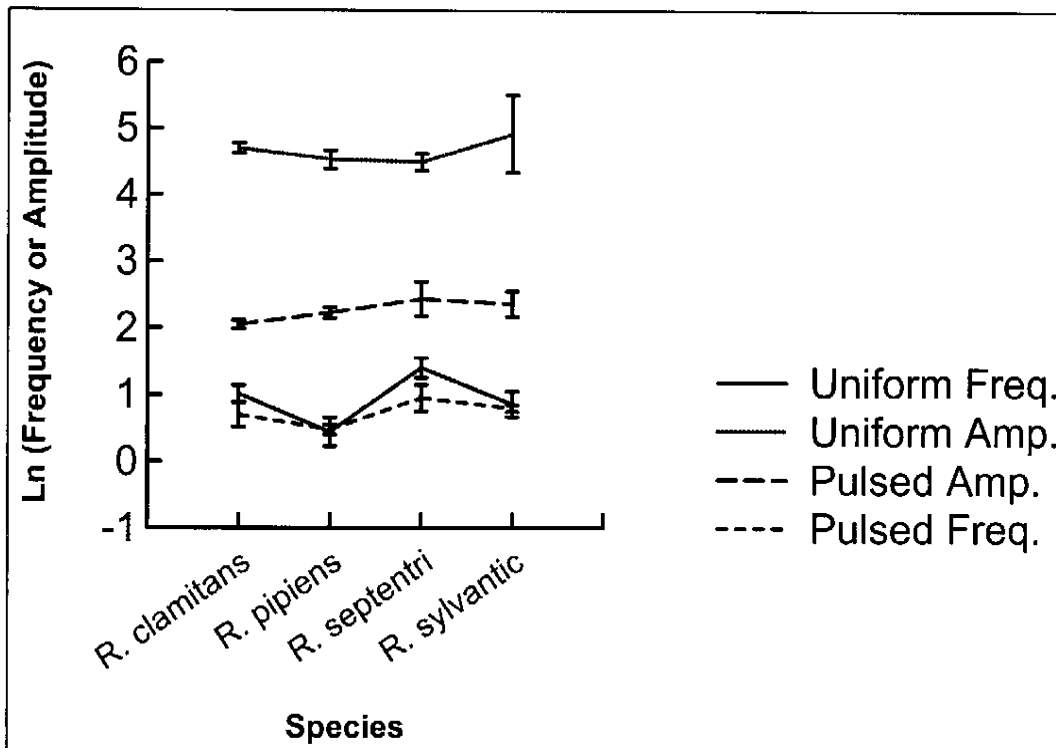


Fig. 5 Mean values ( $\pm$  standard error) of log transformed frequency or amplitude of uniform and pulsed calls across species. Species differed for Uniform Freq., Pulsed Freq., and Pulsed Amp. (ANCOVAs:  $F_{3,52}=4.97, p<0.01$ ;  $F_{3,19}=4.06, p=0.02$ ;  $F_{3,23}=4.60, p=0.01$ ). See text for specific post hoc test analysis.

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### Reference Cited

- Allan, J.D. 1973. Competition and the relative abundances of two cladocerans. *Ecology* 54: 484-498.
- Bee, M.A., Kozich, C.E., Blackwell, K.J., Gerhardt, H.C. 2001. Individual variation in advertisement calls of territorial male green frogs, *Rana clamitans*: implications for individual discrimination. *Ethology* 107: 65-84.
- Bevier, C.R., Larson, K., Reilly, K., Tat, S. 2004. Vocal repertoire and calling activity of the mink frog, *Rana septentrionalis*. *Amphibia-Reptilia* 25:255-264.

- Blair, A.P. 1947. The male warning vibration in *Bufo*. American Museum Novitates 1344: 1-7.
- Bosch, J., Riva, I. De la. 2004. Are frog calls modulated by the environment? An analysis with anuran species from Bolivia. *Can. J. Zool.* 82: 880-888.
- Boyd, S. K. 1992. Sexual differences in hormonal control of release calls in bullfrogs. *Hormones and Behavior* 26: 522-535.
- Cornell Lab of Ornithology Bioacoustics Research Program. 2003-2004. Raven Version 1.2.1 for Windows®. Ithaca, NY.
- Gerhardt, H.C. 1994. The evolution of vocalization in frogs and toads. *Annu. Rev. Ecol. Syst.* 25: 293-324.
- \_\_\_\_\_. 2005. Advertisement-call preferences in diploid-tetraploid treefrogs (*Hyla chrysoscelis* and *Hyla versicolor*): implications for mate choice and the evolution of communication systems. *Evolution* 59: 395-408.
- Given, M.F. 1990. Spatial distribution of vocal interaction in *Rana clamitans* and *R. virgatipes*. *Journal of Herpetology* 24: 377-382.
- R DEVELOPMENT CORE TEAM. 2005. R: A language and environment for statistical computing. *in*. R Foundation for Statistical Computing, Vienna, Austria.
- Ratnam R., Feng, A.S. 1998. Detection of auditory signals by frog interior collicular neurons in the presence of spatially separated noise. *J Neurophysiol* 80: 2848-2859.

- Schmidt, R.S. 1976. Release-call pulse differences between two members of *Rana pipiens* complex. *Copeia* 1976: 721-727.
- Tada, I.E. Di, Martino, A., Sinsch, U. 2001. Release vocalizations in neotropical toads (*Bufo*): ecological constraints and phylogenetic implications. *J. Zool. Syst. Evol.* 39: 13-23.
- Wilkinson, L. 2007. SYSTAT: The System for Statistics. Version 12. Evanston, Illinois, U.S.A.