Tree Calls Of Three Treefrogs (hyla Femoralis, H. Gratiosa, And H. Squirella): Analysis Of Environmental, Behavioral, And Acoustic Characteristics

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TREE CALLS OF THREE TREEFROGS
(*HYLA FEMORALIS*, *H. GRATIOSA*, AND *H. SQUIRELLA*):
ANALYSIS OF ENVIRONMENTAL, BEHAVIORAL, AND ACOUSTIC CHARACTERISTICS

by

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A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science
in the Department of Biology
in the College of Sciences
at the University of Central Florida
Orlando, Florida

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Male frogs typically call near water at dusk to attract females for breeding. During the breeding season, male treefrogs also emit diurnal “tree calls” or “rain calls” from the tops of trees. Very little is known about tree calls, although many treefrogs use them. Tree calls may be used to attract females, deter males or be triggered by weather conditions favorable for breeding: high temperature and relative humidity, and a drop in barometric pressure. As dusk approaches, male treefrogs continue tree calls from lower in the trees, and if conditions are favorable, jump to the ground and travel to a nearby breeding pond where they begin their repetitive nocturnal mating calls. The scant published information is mostly descriptive and does not address the fitness benefit of calling from treetops far from breeding ponds. My goal was to determine the function of tree calls based on their environmental, behavioral, and acoustic characteristics.

My data indicate tree calls are not rain calls. Each treefrog species that I studied (Hyla femoralis, H. gratiosa, and H. squirella) called most frequently at different combinations of mean environmental characteristics (temperature, relative humidity, and barometric pressure). Hyla femoralis and H. gratiosa gave tree calls at ambient air temperatures that differed significantly from the distributions recorded when no treefrogs called. Temperature, relative humidity, and barometric pressure distributions of calling activity differed significantly among all three species and from the distributions recorded when no treefrogs called. Hyla squirella called most often at a significantly different mean relative humidity of 1015 mbar; whereas H. gratiosa and H. femoralis called at a median1017 mbar. Means and fluctuations (summarized as SD) of the three weather parameters explained significant variation in tree calling activity (32-60%). Tree calling activity for all three treefrog species were also not significantly affected by
These results indicate that tree calls were not given at random with respect to environmental conditions. My data suggest tree calls are advertisement calls that deter males from an area, as evidenced by partitioning of tree calls among species during the day. In a playback experiment conducted at Chuluota Wilderness Area, Florida (28°38.31’N 81°07.24’W) no significant effect on mating behavior was found for either call indicating that neither tree calls alone or in conjunction with mating calls are necessary for mating. However, due to habitat differences between treatments and a limited number of experimental replicates, further research is needed. Preliminary results indicate an additional four natural ponds should be sampled to determine a possible effect for tree calls. Acoustic analysis showed that tree calls had fewer pulses per call, more time between pulses within a single call, and a higher minimum call frequency than mating calls. Call duration and maximum call frequency of tree and mating calls did not differ significantly.

My research has greatly increased the information known about tree calls. My results indicate tree calls are not only “rain calls,” a common misperception about daytime tree calls. However, more research is needed to fully understand the function of tree calls.
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CHAPTER ONE:
INVESTIGATING THE ENIGMATIC TREE CALLS OF HYLID FROGS —
ENVIRONMENTAL TRIGGERS OF CALLING FREQUENCY

Introduction

Understanding the function of animal communication signals is challenging because a response may be subtle, delayed, or not occur at all (Krebs and Davies 1993). For example, it took years for researchers to discover the presence and function of ultrasonic bat calls and infrasonic elephant calls (Payne et al. 1986, Neuweiler 1990). Because their mating calls are audible to the human ear, readily manipulated, and evoke rapid behavioral responses, anurans (frogs and toads) provide ideal model systems for studying the role of acoustic communication in courtship and its evolutionary significance (Blair 1958, Littlejohn 1977, Wells 1977a, Gerhardt 1994, Ryan and Rand 2003). Typical anuran courtship consists of males gathering near water, usually at night, and giving species-specific calls to attract mates. Females locate groups of calling males and then search for a potential mate within them (Wells 1977a, 1977b, Gerhardt 1982, Arak 1983) using acoustic cues to assess the fitness of prospective mates. Fitness of male frogs increases with the number of mates, female body size (and hence clutch size), tenure in the chorus, and many aspects of their nocturnal mating call (Fellers 1979a, Klump and Gerhardt 1987, Gerhardt 1991, Welch et al. 1998, Doty and Welch 2001, Hobel and Gerhardt, Ryan and Rand 2003).

While the function of mating calls is well understood, many Holarctic hylid frogs also give tree calls (Table 1), which males broadcast diurnally from the tops of trees during the breeding season (Blair 1958). Tree calls often are given far from breeding sites and usually are
shorter and have fewer pulses than mating calls (see Chapter 3). The function of tree calls is unknown but many authors suggest that they are rain calls that indicate impending rain, which triggers breeding (Neill 1952, Bogert 1960, Fellers 1979b, Honda and Matsui 1996). Tree calls also may be a cooperative call to help orient other frogs to the nearest breeding pond (Neill 1958). Specific triggers of tree calls are unknown, but being sprinkled with water, sounds of splashing water, feeding, turning lab lights on and off, and airplanes flying overhead induced them in *Hyla cinerea* (Bogert 1960, Capranica 1965).

I examined the relationship between environmental variables (temperature, relative humidity, and barometric pressure) that change before and during summer rainstorms, and tree call activity (number of calls x h\(^{-1}\)) in three species of hylid frogs common throughout the southeastern U.S. coastal plain. My goal was to determine if daytime tree calls were associated with changes in these environmental variables that indicate conditions favorable for breeding. In the southeastern U.S., summer rains that trigger anuran breeding are usually preceded by increasing relative humidity and decreasing temperature and barometric pressure (Ruffner 1985, Wood 2001). Therefore, I tested the null hypothesis that tree calling frequency (number of tree calls x h\(^{-1}\)) is not affected by environmental variables vs. the alternate hypothesis that tree calling frequency (number of tree calls x h\(^{-1}\)) is affected by environmental variables.
Methods

I studied three species of small arboreal treefrogs: *Hyla femoralis* (herinafter, HFEM: 25-38 mm snout-vent length (SVL)), *H. gratiosa* (HGRA: 51-67 mm SVL) and *H. squirella* (HSQU: 22 to 41 mm SVL). All three species typically breed from May to September (Martof 1975, Caldwell 1982, Hoffman 1988) and give tree calls throughout this period. I monitored their tree calls within the Econ River Wilderness Area, Seminole County, Florida (28°39.15’N 81°10.13’W; Figure 3) during a portion of the 2004 breeding season before the impacts of Hurricanes Charlie, Frances, and Ivan made my study site inaccessible. The area was dominated by pine flatwoods comprised of longleaf pine (*Pinus palustris*), slash pine (*Pinus elliottii*) and pond pine (*P. serotina*). The understory contained live oak (*Quercus virginiana*), saw palmetto (*Serenoa repens*) and wiregrass (*Aristida stricta*), which is a typical native plant association in central Florida (Abrahamson and Hartnett 1990).

Eight times per month from 7 June - 11 August 2004, I recorded time, temperature, relative humidity and barometric pressure using an automated data logger (HOBO® Micro Station; Onset Computer Corporation, Massachusetts, part # H21-002) each time I heard a tree call. As daily rain fronts approach in Florida, temperature and barometric pressure typically drop, while relative humidity rises. For example, temperature can decrease as much as 5-10°C during rain events (Ruffner 1985, Wood 2001). These changing environmental variables could indicate impending rain and thereby potential breeding conditions for treefrogs. Each recording session was 4 h long: 0600-1000 h, 1000-1400 h, 1400-1800 h, and 1800 h-dusk. Sessions were repeated twice per month with the time sequence determined at random.
I used an automated data logger to record environmental variables at the time tree calls were made versus intervals without tree calls. The data logger consisted of a HOBO® Micro Station with a barometric pressure sensor (part # S-BPA-CM10 [accuracy: \(\pm 4\) mbar]) and temperature/relative humidity sensor (part # S-THA-M002 [accuracy: \(\pm 0.7^\circ C\) and \(\pm 3\%\) RH]) that recorded temperature, relative humidity and barometric pressure every second. The Micro Station also included a pulse input adapter (part # S-UCA-M006) connected to a Radio Shack SPST push on-push off switch (part # 275-011), which I operated manually when tree calls were heard. The Micro Station and data loggers were enclosed by an Onset Solar Radiation Shield (part # M-RSA) to protect them from rain and sun and the entire unit was mounted atop a 2 m high polyvinyl chloride (PVC) pipe.

I downloaded data using BoxCar® Pro 4 software (Onset Computer Corporation, Massachusetts) and summarized it within the two hour observational periods. I used a G-test with Bonferroni adjustment (Fry 1993) to test the null hypothesis that tree call activity (number of calls per hour) by each species was independent of time of day, grouped into one-hour intervals. I used Kolmogorov-Smirnov (K-S) tests to evaluate the null hypothesis that tree calling activity was independent of prevailing temperature, relative humidity and barometric pressure. To do this, I compared the distribution of environmental conditions at which tree calls were heard with a null distribution comprised of the environmental conditions that did not elicit tree calls. Environmental data were collected every second, but were grouped into two-hour time periods for analysis. If tree call activity were random, these two distributions would be identical, while if tree calls were given under specific environmental conditions, the two distributions would differ significantly. To determine whether means and fluctuations (summarized as SD) of these environmental variables predicted tree call activity, I used backward stepwise regression of data
grouped into the two-hour observational periods. This analysis tested whether calling activity varied with changes in these variables (or a combination thereof) instead of to average conditions. I also analyzed the seasonality of tree call use by comparing the observed and expected number of tree calls per day throughout the breeding season.

If tree calls were truly rain calls, tree calling frequency (number of calls per hour) would increase prior to rain events. To test this, I compared the number of tree calls broadcast one half hour before rain to the same time of day when it did not rain. Rain fell twice while I collected these environmental data: started at 1109h on 9 Jun 2004 and 1731h on 7 July 2004. This allowed for a comparison of three additional days of no rain for each time period with rain. I used a two-way ANOVA to investigate the interactions between the two time periods and rain/non-rain events. All statistical analyses were performed using JMP (v. 6.0.2, SAS Institute, Inc., 2006).
Results

Time of Day

Daily tree call activity differed significantly from a null distribution for *H. femoralis* (G = 922.58, 12 d.f., p < 0.01) and *H. gratiosa* (G = 38.00, 12 d.f., p < 0.01) but not *H. squirella* (G = -154.62, 12 d.f., p > 0.05). *Hyla femoralis* called almost twice as often as expected from 0600-0759 h and much less than expected from 1400-1559 h; its major calling period was dawn and it was almost silent in mid-afternoon (Figure 1a). In contrast, *H. gratiosa* called more than twice as often as expected from 0800-0959 h and less than expected from 0600-0759 h and 1400-1559; its major calling period was mid-morning (Figure 1b). Tree call activity by *Hyla squirella* peaked in the middle to late afternoon, and was virtually absent near dusk. However, tree call use during these time periods was not significantly different from the rest of the day.

Seasonality

Tree call activity throughout the breeding season differed significantly from a null distribution for *H. femoralis* (G = 2504.01, 16 d.f., p < 0.01) and *H. gratiosa* (G = 117.09, 16 d.f., p < 0.01) and *H. squirella* (G = 56.097, 16 d.f., p > 0.01). *Hyla femoralis* and *H. gratiosa* called more often early in the season (middle of June). In contrast, *H. squirella* called more often from June to mid-July (Figure 2).

Temperature

*Hyla femoralis* and *H. gratiosa* gave tree calls at ambient air temperatures that differed significantly (*H. femoralis* (HFEM): D=0.032, 15 d.f., p<0.01; *Hyla gratiosa* (HGRA): D=0.168,
15 d.f., p<0.01) from the distributions recorded when no treefrogs called. *Hyla femoralis* called more often than expected at lower temperatures (21-29 °C), although these temperatures comprised < 72% of temperature observations. Air temperatures usually were in the 29-33 °C range but *H. femoralis* gave few tree calls under such conditions (Figure 2a). *Hyla gratiosa* called more often than expected at temperatures of 27-31 °C, just below and at the lower end of the most common temperatures recorded (Figure 2b). In contrast, *H. squirella* called often at 24 °C and 29-33 °C, although tree call activity did not vary significantly with temperature in this species (Figure 2c).

Temperature distributions of calling activity differed significantly among all three species (K-S tests: HFEM vs. HGRA, D=0.171, 15 d.f., p<0.01; HFEM vs. HSQU, D=0.058, 15 d.f., p<0.01; HGRA vs. HSQU, D=0.058, 15 d.f., p<0.01). *Hyla femoralis* tended to give more tree calls in the lower range of ambient temperatures than the other two species; whereas *H. gratiosa* tended to broadcast more tree calls at the middle range of ambient temperature and *H. squirella* tended to broadcast tree calls consistently across observed temperatures.

Relative Humidity

All three species gave tree calls at relative humidities that differed significantly from the distributions recorded when no treefrogs called (HFEM: D=0.032, 15 d.f., p<0.01; HGRA: D=0.051, 15 d.f., p<0.01; HSQU: D=0.049, 15 d.f., p<0.01). *Hyla femoralis* rarely called at relative humidities of 75-93% which were the most commonly recorded values. Instead, *H. femoralis* called more often than expected at 72% and 96-98 % relative humidity. *Hyla gratiosa* called more often than expected at middle relative humidities (54 - 84% RH, Figure 2e). *Hyla squirella* called more often than expected through out the range of relative humidities recorded
Relative humidity distributions of calling activity differed significantly among all three species (K-S tests: HFEM vs. HGRA, D=0.171, 15 d.f., p<0.01; HFEM vs. HSQU, D=0.058, 15 d.f., p<0.01; HGRA vs. HSQU, D=0.175, 15 d.f., p<0.01). *Hyla femoralis* tended to give more tree calls at higher relative humidities than the other two species (72% and 96 – 99% RH); whereas *H. gratiosa* tended to broadcast more tree calls than the other two species at the middle range (54 – 84% RH) and *H. squirella* tended to broadcast more tree calls at a lower range (45 - 69% RH) and higher range (90 – 96% RH) of humidities recorded when compared to the other two species.

**Barometric Pressure**

All three species gave tree calls at barometric pressures that differed significantly from distributions recorded when no treefrogs called (Figure 2g-i). *Hyla femoralis* (K-S test: D=0.032, 10 d.f., p<0.01) called more often than expected at 1016-1019 mbar, while *H. gratiosa* (K-S test: (D=0.168, 10 d.f., p<0.01) called more often than expected at 1015-1018 mbar and never called at the lowest pressures (1010-1013 mbar, Figure 2g,h). *Hyla squirella* used tree calls at all barometric pressures recorded, but called more often at middle pressures recorded (1014 – 1015 mbar) (D=0.049, 10 d.f., p<0.01; Figure 2i).

*Hyla squirella* called at significantly different relative humidities compared to both *H. femoralis* and *H. gratiosa* (HSQU vs. HFEM: D=0.058, 10 d.f., p<0.01; HSQU vs. HGRA: D=0.175, 10 d.f., p<0.01). There was no significant difference between calling activity of *H. femoralis* and *H. gratiosa*. *Hyla squirella* called most often at a significantly different mean relative humidity of 1015 mbar; whereas *H. gratiosa* and *H. femoralis* called at a median
mbar.

Variation in Environmental Variables

Means and fluctuations (summarized as SD) of the three weather parameters explained significant variation in tree calling activity (Table 3). In *H. femoralis*, tree calling activity increased with increasing barometric pressure and decreased during the 1400 – 1600h time period: together, these regressors explained 32% of the variation in tree call activity. Tree calling activity of *H. gratiosa* increased with increasing mean temperature, temperature standard deviation, and mean relative humidity: together, these three regressors explained 53% of the variation in tree call activity. Tree calling activity of *H. squirella* increased with increasing mean barometric pressure, and Sds of relative humidity and barometric pressure, and decreased with increasing temperature SD and during the time period 1400 – 2000h. Together, these five regressors explained 60% of the variation in tree call activity by *H. squirella*.

Responses to impending rain

Tree calling activity for all three treefrog species were not significantly affected by subsequent rain (HFEM: F=0.2601, 7 d.f., p>0.05; HGRA: F=0.9792, 7 d.f., p>0.05; HSQU: F=0.7545, 7 d.f., p>0.05). *Hyla femoralis* and *H. squirella* called on all days and time periods sampled; whereas *H. gratiosa* only called one day during these time periods (Table 3).
Discussion

Tree calls must be more than simple indicators of approaching rain or good breeding conditions for two reasons: 1) tree call activity by the three treefrog species peaked at different times and under different conditions and 2) tree calls were not given more often under environmental conditions associated with impending rainfall, and therefore opportunities to breed. Tree calls were not evenly distributed throughout the day, suggesting temporal partitioning among the three species. *Hyla femoralis* called most often at dawn, *H. gratiosa* at mid-morning, and *H. squirella* throughout the day. This separation of calling times may reduce interspecies calling interference in a manner similar to that reported for temporal partitioning of mating calls (e.g. Littlejohn and Martin 1969, Littlejohn 1977, Ryan 1985, Gerhardt 1994, Brenowitz and Rose 1999). *Hyla femoralis* and *H. gratiosa* both give mating calls through the evening, but each species peaks chorusing at significantly different times (Bridges and Dorcas 2000) indicating an avoidance of interspecies calling interference.

In many anurans, repetition rate of mating calls is strongly and positively correlated with ambient air or water temperature (e.g. Zweifel 1959, Gayou 1984). In contrast, tree call activity did not increase monotonically with increasing air temperature in any of the treefrogs I studied. Instead, tree call activity peaked at species-specific temperatures. *Hyla femoralis* called more often than expected at lower temperatures (<29 °C), although these temperatures comprised less than 58% of observations. *Hyla gratiosa* called more often than expected at temperatures of 27-31 °C, just below and at the lower end of the most common temperatures recorded. In contrast, *H. squirella* called about equally at all temperatures recorded. If tree calls were rain calls, all
three species were expected to call at the lowest temperatures recorded (20 °C), but this did not occur (all three species together only called 12% of the time at this temperature).

Tree calls are colloquially known as “rain calls” (Neill 1952, Bogert 1960, Fellers 1979b, Honda and Matsui 1996), and if this description is accurate, calling should increase at higher relative humidities which indicate impending rain. Tree call activity did vary significantly with relative humidity in all three treefrog species and peaked at relative humidities > 90%. This supports the prevailing idea that tree calls are “rain calls.” However, in all three species tree call activity was also more frequent than expected at lower relative humidities (<72% RH), which can be physiologically stressful for active anurans (Lee 1968). Instead of remaining in water conserving postures (Johnson 1969, Pough et al. 1983), male treefrogs of all three species gave numerous tree calls, suggesting that tree calls may function as territorial advertisements. I tested this hypothesis using playback experiments (see Chapter 2).

If tree calls were solely “rain calls”, their frequency should increase with decreasing barometric pressure because advancing low pressure systems generate numerous thunderstorms during the May to October wet season in central Florida (Ruffner 1985, Wood 2001). I recorded a range of barometric pressures from 1010 to 1020 mbar; if the “rain call” hypothesis were correct, tree call activity should peak near 1010 mbar. Instead, calling activity of all three treefrog species peaked at intermediate barometric pressures: 1017, 1016, and 1015 mbar for \textit{H. femoralis}, \textit{H. gratiosa}, and \textit{H. squirella} respectively.

Tree call activity immediately preceding rains was not significantly different from similar time periods without rain. It tree calls were only rain calls, I should have found a significantly higher number of tree calls broadcast before rain events and this did not occur. However, this is an exploratory data set and more data are needed to make more informed predictions. A power
analysis of these data indicate a range of 36 to 72 data points are necessary to show significance with \( p<0.05 \), depending on the species.

Tree calling activity differed significantly with a change in mean or standard deviation in some environmental variables. I examined variations within these environmental variables to determine if these treefrogs responded to the environmental changes that precede a storm front. Tree calling activity of *H. femoralis* increased with increasing barometric pressure and decreased during the 1400 – 1600h time period. This does not coincide with the idea that tree calls are rain calls because tree calls in *H. femoralis* should increase, not decrease when barometric pressure drops before a storm. In contrast, tree calling activity by *H. gratiosa* increased with increasing mean relative humidity, mean temperature, and the standard deviation of temperature. Again, these results indicate that these calls are not true rain calls because *H. gratiosa* calls more often with the increase of all temperature-related variables, when a rain call would be expected with a decrease in temperature. Tree calling activity by *H. squirella* increased with increasing mean barometric pressure, and standard deviations of relative humidity and barometric pressure. If these were rain calls, calling activity would increase with decreasing barometric pressure and during less variation within relative humidity and barometric pressure. Results from analyzing these changes further indicate that tree calls are not rain calls in that tree calling activity does not increase with the expected environmental changes preceding a storm.

The results of my study indicate that tree calls probably are more than just rain calls. Calls were not broadcast more often when conditions forecast approaching rain, such as high temperature and relative humidity and low barometric pressure. Instead, I hypothesize that tree calls are advertisement or territorial calls. Vocalizations made high in trees carry farther than those made from the ground (Kime et al. 2000) and males may use tree calls to advertise their
presence near breeding ponds. A mathematical model of spring peeper (*Pseudacris crucifer*) behavior indicated that a higher vertical calling position significantly increased the area in which a call was heard (Parris 2002). While males of most Holarctic treefrog species do not defend oviposition sites, many do defend sites favored for giving nocturnal mating calls (e.g., Resetarits and Wilbur 1991). It is plausible that males use tree calls to deter other males from particular trees or even from a general area (see Chapter 2).

More research is needed to better understand tree calls, which represent another energetically-costly component of breeding for male treefrogs. For example, male spring peepers (*Pseudacris crucifer*) expend more energy on nocturnal mating calls than on forced exercise in a lab (Taigen et al. 1985). If males also give tree calls before moving to breeding ponds - which seems likely as I heard males calling as they descended from trees before an advancing storm - the total energetic cost of anuran calling will be much higher than previously estimated. By calling from the tops of trees during the day, male treefrogs subject themselves to environmental conditions that otherwise could be avoided by remaining in desiccation-resistant postures, and also risk predation by diurnal predators such as raptors and arboreal snakes. Tree calls likely are a costly behavior, and evolutionary theory predicts that they must confer a larger relative fitness benefit if they are maintained by selection (Krebs and Davies 1993).

Better understanding of tree calls will aid amphibian conservation efforts because daytime calls are easy to identify and count, and can be incorporated easily into monitoring protocols for the fourteen species of hylid frogs in North America known to give tree calls (Table 1; e.g. North American Amphibian Monitoring Program (NAAMP), Amphibian Research and Monitoring Initiative (ARMI), Frogwatch USA). Because tree calls may be monitored at any time of day, they also may be useful for evaluating the quality of terrestrial habitat; an aspect of
References


Table 1. North American treefrogs that emit tree calls.

<table>
<thead>
<tr>
<th>Species</th>
<th>Region</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hyla andersonii</em></td>
<td>Sandhills region of eastern U.S.</td>
<td>P. Morin <em>pers. comm.</em></td>
</tr>
<tr>
<td>Pine Barrens Treefrog</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. avivoca</em></td>
<td>U.S. Gulf Coast and Atlantic Coast</td>
<td>J. Fauth <em>pers. obs.</em></td>
</tr>
<tr>
<td>Bird-Voiced Treefrog</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. chrysoscelis</em></td>
<td>Eastern North America</td>
<td>J. Fauth <em>pers. obs.</em></td>
</tr>
<tr>
<td>Cope’s Gray Treefrog</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. cinerea</em></td>
<td>Southeastern U.S.</td>
<td>Bogert 1960</td>
</tr>
<tr>
<td>Green Treefrog</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. eximia</em></td>
<td>Mountains of Arizona and New Mexico</td>
<td>Maslin 1957</td>
</tr>
<tr>
<td>Mountain Treefrog</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. femoralis</em></td>
<td>Southeastern U.S. Coastal Plain</td>
<td>Hoffman 1988</td>
</tr>
<tr>
<td>Pine Woods Treefrog</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. gratiosa</em></td>
<td>U.S. Atlantic and Gulf Coastal Plains</td>
<td>Wright 1932</td>
</tr>
<tr>
<td>Barking Treefrog</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. regilla</em></td>
<td>Western U.S.</td>
<td>L. Kats <em>pers. comm.</em></td>
</tr>
<tr>
<td>Pacific Treefrog</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. squirella</em></td>
<td>Southeastern U.S. Coastal Plain</td>
<td>Blair 1958</td>
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<tr>
<td>Squirrel Treefrog</td>
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<td></td>
</tr>
<tr>
<td><em>H. versicolor</em></td>
<td>Eastern U.S.</td>
<td>Pettus 1955</td>
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<tr>
<td>Gray Treefrog</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudacris cadaverina</em></td>
<td>California</td>
<td>L. Kats <em>pers. comm.</em></td>
</tr>
<tr>
<td>California Treefrog</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. crucifer</em></td>
<td>Eastern U.S.</td>
<td>L. Kats <em>pers. comm.</em></td>
</tr>
<tr>
<td>Spring Peeper</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Osteopilus septentrionalis</em></td>
<td>Cuba and Florida</td>
<td>Meshaka 2001</td>
</tr>
<tr>
<td>Cuban Treefrog</td>
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<td></td>
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<tr>
<td><em>Calyptahyla crucialis</em></td>
<td>Jamaica</td>
<td>Garrick et al. 1985</td>
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<td>Jamaican Treefrog</td>
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Table 2. Number of tree calls observed preceding rain and compared to similar time periods on non-rain days.

<table>
<thead>
<tr>
<th>Species</th>
<th>1039-1108h with Rain</th>
<th>1039-1108h without Rain</th>
<th>1701-1730h with Rain</th>
<th>1701-1730h without Rain</th>
</tr>
</thead>
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<tr>
<td>Hyla femoralis</td>
<td>48</td>
<td>2</td>
<td>72</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>57</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Hyla gratiosa</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hyla squirella</td>
<td>9</td>
<td>1</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>3</td>
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</tbody>
</table>
Figure 1. Mean number of tree calls broadcast within two hour blocks during the day by a) *Hyla femoralis*, b) *H. gratiosa*, and c) *H. squirella*.

Black bars give the mean number of tree calls observed and gray bars are the number of tree calls expected, under the null hypothesis that calling activity was evenly distributed throughout the sampling periods. Expected bars are not equal across all times because there was an extra 30 minutes of data collection from 2000 – 2030h.
Figure 2. Seasonal variation of tree calling behavior.

Circles indicate observed number of tree calls broadcast by treefrogs and triangles represent the expected number of tree calls each day if they were broadcast equally throughout the breeding season.
Figure 3. Number of tree calls broadcast per hour as a function of air temperature, relative humidity and barometric pressure.

Black bars indicate fraction of time treefrogs gave tree calls and gray bars indicate the fraction when the same environmental variable was recorded but no tree calls were heard, a-c) temperature, d-f) relative humidity, g-i) barometric pressure. Tree calling activity is the proportion of time intervals tree calls were heard or not heard.
Table 3. Results of backward multiple regression of tree calling activity (proportion of time intervals tree calls were heard/not heard) as a function of mean and standard deviations (SD) of environmental variables. All regressors have 1 d.f.

<table>
<thead>
<tr>
<th></th>
<th>Hyla femoralis</th>
<th>Hyla gratiosa</th>
<th>Hyla squirella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean temperature (°C)</td>
<td>β = 0.0899</td>
<td>p = 0.0157</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>β = 0.2624</td>
<td>p = 0.0002</td>
<td>β = -16.2105</td>
</tr>
<tr>
<td>SD temperature (°C)</td>
<td>NS</td>
<td>p = 0.0122</td>
<td>β = -16.2105</td>
</tr>
<tr>
<td>Mean relative humidity (%)</td>
<td>β = 0.0193</td>
<td>p = 0.0226</td>
<td>NS</td>
</tr>
<tr>
<td>SD relative humidity (%)</td>
<td>NS</td>
<td>NS</td>
<td>β = 3.7872</td>
</tr>
<tr>
<td>Mean barometric pressure (mbar)</td>
<td>β = 0.0014</td>
<td>p = 0.0214</td>
<td>β = 0.0241</td>
</tr>
<tr>
<td>SD barometric pressure (mbar)</td>
<td>NS</td>
<td>NS</td>
<td>β = 0.5330</td>
</tr>
<tr>
<td>Time of day (two-hour periods)</td>
<td>β = -0.3538 at 1400-1600h</td>
<td>p = 0.0256</td>
<td>β = -10.5406 at 1400-2000h</td>
</tr>
<tr>
<td>y-intercept (when significant)</td>
<td>0.0224</td>
<td>0.0189</td>
<td>0.0421</td>
</tr>
<tr>
<td>R²</td>
<td>0.3226</td>
<td>0.5300</td>
<td>0.5960</td>
</tr>
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</table>
Figure 4. Econ River Wilderness Area, Seminole County, Florida.

The author collecting data on tree call use at Econ River Wilderness Area, Seminole County, Florida, June 2004.
CHAPTER TWO:
BENEFITS OF TREE CALLS IN THE PINE WOODS TREEFROG
(HYLA FEMORALIS)

Introduction

During their breeding season, males of several treefrog species (Table 1) give a
diurnal tree call from the tops of trees. The function of this tree call is unknown, but may
be used to attract females (Neill 1958), deter males (Whitney and Krebs 1975a, 1975b) or
be triggered by changing weather conditions (see Chapter 1). By initiating daytime tree
calls when fewer frogs are calling, males also may reduce competition within the
nocturnal breeding chorus. Amphibian calls broadcast from trees can be heard at greater
distances than those broadcast from the ground (Kime et al. 2000) and mathematical
models indicate that a higher vertical calling position significantly increased the area in
which spring peeper (Pseudacris crucifer) calls were audible (Parris 2002).

I used the pinewoods treefrog (Hyla femoralis) as a model anuran species to test
the benefits of tree calls. Hyla femoralis is a small arboreal frog about 25-38 mm snout-
vent length (SVL) that inhabits the southeastern U.S. coastal plain (Hoffman 1988). It
typically occurs in pine flatwoods and in Florida breeds from May through September.
Male mating calls vary from 0-5000 Hz and have been described as the sound of Morse
code being tapped out. Their tree call is similar, but shorter and trails off at the end
(Figure 4). The tree call is used during the day, whereas the mating call primarily is used
after dusk. Breeding typically begins at dusk after heavy rainfall in ponds lacking fish
and females lay 500-800 eggs per mating, which typically occurs two to three times each
breeding season (Livezey and Wright 1947).

I used a field experiment to test the potential mating benefit of tree calls. My goal was to determine whether the tree call alone increased the number of female frogs attracted to a pond, if only a mating call was needed, or if a combination of the two calls was necessary. Due to a prolonged summer drought in central Florida during 2006, I completed only a single replicate of this experiment. However, results obtained during a trial in 2005 permit evaluation of my main questions and can be used in power analyses to plan future experiments (Sokal and Rohlf 1995, Zar 1999).
Methods

To determine whether male and female behaviors were altered by tree calls, I manipulated tree and mating calls using a playback experiment based on published playback methodologies (Sullivan 1985, Kroodsma 1989, McGregor 1992, Schwartz 1993, Kroodsma et al. 2001). I recorded calls of *H. femoralis* at Econ River Wildlife Area, Seminole County, Florida (28°39.15’N 81°10.13’W) using a Digital Audio Tape Recorder, Sennheiser microphone with windshield, and DAT tapes (Table 4, Figure 5). I digitized calls at a rate of 44.1 kHz using Signal 4.0 and isolated tree calls and mating calls within sonograms using CoolEdit Pro (Table 3). Playback treatment pools consisted of: 1) control with no calls, 2) tree calls played from 0600 - 2030 h, 3) mating calls played from 2030 - 0600 h, and 4) both tree calls and dusk mating calls played at their corresponding times. Treatments were applied to a set of four artificial pools (one treatment per pool) similar to those described by Binckley and Resetarits (2003), which were deployed at Chuluota Wilderness Area, Seminole County, Florida (28°38.31’N 81°07.24’W; Figure 7). I used 416 L Tuff Tub containers (86 cm x 130 cm x 51cm) and filled them with dechlorinated city water pumped from a large water bladder transported by truck (Figure 5). To each pool, I added all leaf litter found beneath it to serve as a source of nutrients for the aquatic food chain. Tight-fitting fiberglass window screen kept animals out until pools were opened for breeding.

I placed four of these experimental pools around one natural pond, equally spaced around it and 100 meters from its edge (Figure 7). Next to each pool (except the control)
four automated speakers played pre-recorded tree or mating calls of *H. femoralis* (Figures 5, 6). Speakers broadcasting tree calls were secured to trees approximately 4 m above ground and directed away from the pool (Figure 6). Only posts, used to hold speakers at the other treatments, were placed around the control pool.

I used marine CD players and speakers to broadcast the recorded tree and mating calls. The pool with only tree calls had four CD players equipped with a waterproof marine speaker to broadcast four individual tree calls. Marine speakers were mounted in plastic containers to protect them from moisture (Figure 5). The pool with only mating calls had one CD player with four speakers to broadcast a chorus of mating calls. The pool with both tree and mating calls broadcast four individual tree calls during the day and a chorus of mating calls at night using the systems described above. Four different tree calls and four unique mating call choruses were previously recorded and randomly ordered for playback. Volume of all calls was adjusted to mimic natural groups of calling frogs, as perceived by the investigator.

Data were collected on two nights in late summer 2005 at the same study area in Chuluota Wildlife Area, Seminole County, Florida (Figure 6). Between 2200 - 0200 h, I visually identified: 1) number of male and female *H. femoralis* in and near each pool, and 2) size (snout-vent length: SVL) of adult frogs. The next morning, I visited ponds and photographed all eggs laid in them. I placed eggs in shallow plastic dishpans and digitally photographed them for later counting (Resetarits and Wilbur 1991). *Hyla femoralis* lays 500-800 eggs per clutch (Livezy and Wright 1947) and I used this observation to determine the number of clutches laid in each pool. After data were collected, I returned all eggs to the nearby natural pond. I used two-way analysis of
variance (ANOVA) for an unreplicated design to test the null hypothesis that tree and mating calls did not affect mating success of *H. femoralis*. The unreplicated design precluded testing the mating call x tree call interaction, which was the error term in the ANOVA. I therefore used a G-test of heterogeneity to test this hypothesis. While this experiment had limited statistical power, my data are still helpful as preliminary results and can be used to plan future experiments.
Results

On 31 August, I found five male *H. femoralis* calling at the treatment playing only mating calls and two males at the treatment with both mating and tree calls. No males were at the treatment with only tree calls or the control. No females were seen at any of these tubs and I found no egg masses the next morning. On 2 September, I observed calling males at all treatments (Table 4). Eleven *H. femoralis* were calling near the treatment with mating calls, compared to a total of five males in the other three treatments combined. I also found one pair of *H. femoralis* in amplexus at the mating call only treatment, and the next morning, one clutch of eggs was in this pool. Additional eggs were in the pool with both mating and tree calls (Table 4).

Experimental treatments had no significant effect on the mean number of male treefrogs attracted to pools (ANOVA; Table 5). Power analyses indicate a sample size of 10 was needed to detect a statistically significant effect (p < 0.05 and η² = 0.50) for mating calls and 14 for tree calls, respectively. This indicates the experiment should be replicated around at least four more natural ponds. Implementing a more statistically powerful experiment would require 10 natural ponds (β = 0.90). The mean number of *H. femoralis* males attracted to experimental pools differed significantly from that expected if mating calls and tree calls were independent (G = 32.75, 1 d.f., p < 0.05). Males were attracted to pools with synthesized mating calls alone over twice as often as expected, but less often than expected at pools with only tree calls or no calls at all.
Discussion

In 1955, Frank Blair published the first sonogram of the tree call of a treefrog: the squirrel treefrog (*Hyla squirella*; Blair 1955). Since that time, the function of tree calls has remained enigmatic (Bogert 1960, Fellers 1979, Honda and Matsui 1996). My playback experiment is the first attempt to determine the function of tree calls in any frog. Calls broadcast through speakers were in addition to any given by males residing in the area. If tree calls advertise occupied territory and cause other males to stay away from nearby breeding sites, one would predict that increasing the number of tree calls near artificial pools would decrease the number of males compared to pools with no calls or only mating calls. However, no significant effect was found for either call (Table 5). On average, pools where tree calls were broadcast attracted one male, compared to four males at pools without tree calls.

If the energetically-costly male tree calls attract females, one would predict that experimentally increasing the number of tree calls near experimental pools would increase the number of females attracted and eggs laid compared to pools without tree calls. However, on both sampling nights combined, I saw only one female, which laid approx. 480 eggs in the mating call treatment. I also found three clumps of eggs (approx. 250, 298, 237 eggs) in the experimental pool with both calls. Because females typically lay 500-800 eggs per mating event (Livezey and Wright 1947), I presumed that one female bred at each of these two pools. This is a much lower response than I expected, and likely reflects the time of this experiment, which was conducted at the very end of
the breeding season.

In contrast, if both tree and mating calls are necessary to attract mates, or if one call negated or overwhelmed the effects of the other, one would predict that tree and mating calls would have a synergistic effect on the number of females and males at a pool or the number of eggs laid. I tested this hypothesis using contingency table analysis and found a significant difference between the number of males at the mating-call only treatment and at all other treatments combined (23 total males; \( G = 26.51, 1 \text{ d.f.}, p < 0.05 \)). This supports the interpretation that tree calls are not necessary to attract males to breeding ponds.

This interpretation should be considered preliminary because each experimental pool was in a somewhat different habitat type. The control and tree call-only treatments had fewer pine trees, more oak trees, and a more dense understory than the other two treatments. The tree call-only treatment also was in an area disturbed by hurricane damage the previous year. Lack of replication left these habitat differences confounded with treatment, which is a limitation of this experiment. Nevertheless, results of my experiment still provide useful information on the life history and mating behavior of \( H. \text{femoralis} \), which is an indicator species used by the U.S. Forest Service to detect the consequences of wetlands management (USDA 2002). Currently, there is little published data on the ecology and behavior of \( H. \text{femoralis} \) (Wilbur 1982, Pechmann et al. 1989, Resetarits and Wilbur 1989, Warner et al. 1993, LaFiandra and Babbitt 2004). My data show that tree call playbacks elicited responses by \( H. \text{femoralis} \) under field conditions and can be used to design more powerful experiments to further test the fitness benefits of tree calls. A power analysis indicates a sample size of 10 pools is needed to detect an
effect for mating calls and 14 pools for tree calls. Thus, an experiment with a total of 16 pools distributed around four different natural ponds will yield a powerful test of the null hypothesis that tree and mating calls do not affect reproductive success of *H. femoralis*. 
References


acoustic conditions. 32: 401-414.


Figure 5. Sonogram of a tree call of *Hyla femoralis*.

I recorded this call at Econ Wilderness Area, Oviedo, FL on 25 July 2004 at 0833h, at an air temperature of 25.95 °C. The x-axis indicates duration of call, while the y-axis shows call frequency. Each pink band is a pulse and this group of pulses over time comprises one tree call.
Table 4. Materials used to record calls and conduct field experiments.

<table>
<thead>
<tr>
<th>Item</th>
<th>Part Number</th>
<th>Company</th>
<th>City, State</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digital Audio Tape Recorder</td>
<td>DA-P1</td>
<td>Tascam</td>
<td>Montebello, CA</td>
</tr>
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<td>Professional Dynamic Stereo Headphones</td>
<td>MDR-7506</td>
<td>Sony Electronics, Inc.</td>
<td>Los Angeles, CA</td>
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<td>MKH 20 – P48</td>
<td>Sennheiser</td>
<td>Wedemark, Germany</td>
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<td>Wind shield for microphone</td>
<td>MZW20-1</td>
<td>Sennheiser</td>
<td>Wedemark, Germany</td>
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<td>Audio Pro DAT 65 tapes</td>
<td>R-65DA</td>
<td>Maxell</td>
<td>Iidabashi, Tokyo</td>
</tr>
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<td>Tubs</td>
<td>KMT 100</td>
<td>Tuff Tubs</td>
<td>Downy, CA</td>
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<td>Signal computer program</td>
<td>v. 4.0</td>
<td>Engineering Design</td>
<td>Belmont, MA</td>
</tr>
<tr>
<td>CoolEdit Pro computer program</td>
<td>v. 1.2</td>
<td>Syntrillium Software</td>
<td>San Jose, CA</td>
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<td>CD player</td>
<td>MCD5112</td>
<td>Jensen</td>
<td>Hauppauge, NY</td>
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<td>Waterproof coaxial marine speakers</td>
<td>WM-6500B</td>
<td>West Marine®</td>
<td>Watsonville, CA</td>
</tr>
<tr>
<td>Plastic containers for speakers</td>
<td>1952</td>
<td>Sterilite</td>
<td>Townsend, MA</td>
</tr>
</tbody>
</table>
Figure 6. Experimental pool at Chuluota Wildlife Area on 1 September 2005.

Solid red arrows indicate four speakers placed on posts around the pool to broadcast mating calls, the spotted arrow marks the toolbox housing the CD player and the striped arrow points to the marine battery that powers it. When the photo was taken, a blue rectangular plastic container and ruler used to count eggs were floating in the pool.
Figure 7. Alex Feliciano ’07 attaching a speaker about 4 m high on a tree 26 August 2006.
Figure 8. Aerial photo of the experiment at Chuluota Wilderness Area, Seminole County, Florida.

Each experimental pool (blue circles) contained a different treatment and was placed 100 m from the edge of a natural pond: control with no recorded calls, only tree calls broadcast during the day, only mating calls broadcast at night, and both tree and mating calls broadcast at their usual times. Yellow lines indicate hiking trails, red line indicates park boundary.
Table 5. Results of two-way ANOVA comparing mean numbers of male *H. femoralis* among treatments.

Table entries are d.f. = degrees of freedom, SS = sum of squares, MS = mean square, F = F-ratio, P = P value.

<table>
<thead>
<tr>
<th>Source of Variance</th>
<th>d.f.</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mating Calls</td>
<td>1</td>
<td>72.250</td>
<td>57.250</td>
<td>2.3884</td>
<td>0.3656</td>
</tr>
<tr>
<td>Tree Calls</td>
<td>1</td>
<td>42.250</td>
<td>57.250</td>
<td>1.3967</td>
<td>0.4471</td>
</tr>
<tr>
<td>Error</td>
<td>1</td>
<td>30.250</td>
<td>30.250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>144.750</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6. Responses of *Hyla femoralis* to playback experiment.

Table entries indicate the number of male and female treefrogs and eggs observed at each of the four experimental treatments: pools with only mating calls, only tree calls, both mating and tree calls, and the control with no calls broadcast. I counted eggs on 1 and 3 September 2005 that had been laid in the experimental pools the night before.

<table>
<thead>
<tr>
<th>Date</th>
<th>Only Mating Calls Broadcast</th>
<th>Only Tree Calls Broadcast</th>
<th>Both Calls Broadcast</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Clutches</td>
<td>Males</td>
</tr>
<tr>
<td>31-Aug-05</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2-Sep-05</td>
<td>11</td>
<td>1</td>
<td>1 (~480 eggs)</td>
<td>1</td>
</tr>
</tbody>
</table>
CHAPTER THREE:
ACOUSTIC DIFFERENCES BETWEEN TREE AND MATING CALLS OF THE PINE WOODS TREEFROG (HYLA FEMORALIS)

Introduction

Anurans use four basic calls: advertisement, reciprocation, release, and distress calls. Advertisement calls, including mating calls, can both deter males and attract females; reciprocation calls are emitted by females in response to males; release calls are broadcast by males when amplexed by another male; and distress calls are used in response to potential trouble (Wells 1977a, Arak 1983, Duellman and Trueb 1986, see Chapter 2). Typical anuran courtship consists of males gathering near water and calling to advertise to conspecific females, which search for a potential mate within the nocturnal chorus (Wells 1977a, 1977b, Gerhardt 1982, Arak 1983). Once a mated pair forms, they proceed with amplexus and deposit eggs into nearby oviposition sites, which generally are not defended by males (Arak 1983). Mating calls are an essential part of anuran breeding behavior and are the topic of extensive research (e.g. Blair 1958, Littlejohn 1977a, Wells 1977a, b, Gerhardt 1994, Murphy 1994, Ryan and Rand 2003).

In addition to a mating call, males of several treefrog species (Table 1) also emit a diurnal “tree call” or “rain call” from the tops of trees during the breeding season (Deckert 1915, Blair 1955). As dusk approaches, these treefrogs continue tree calls from lower in the tree, and if conditions are favorable jump to the ground and travel to a nearby breeding pond where they begin their repetitive mating calls. Males continue calling until they attract a mate. By calling from the treetops several hours before mating typically begins, males may attract either more females or more distant females. It also is possible that tree calls deter
other males from the area (Chapter 2). By initiating daytime tree calls when fewer frogs are
calling, males may reduce competition within the night-time breeding chorus. For example,
*Hyla cinerea* living near ponds with other treefrog species called from significantly higher
perches than individuals in a monospecific chorus (Hobel and Gerhardt 2003). Amphibian
calls broadcast from elevated sites undergo less sound degradation than those broadcast from
ground (Kime et al. 2000), and a mathematical model of spring peeper (*Pseudacris crucifer*)
behavior predicted that a higher vertical calling position significantly increased the area in
which a call was heard (Parris 2002).

However, calling from high in trees probably increases energy expenditure and risk of
predation on males. Additional energy is needed to climb up and down trees and deal with
extreme environmental conditions. In Chapter 1, I reported *H. femoralis* giving repetitive
tree calls at 20-35 °C and 40-100% relative humidity; a challenging range of conditions for
any amphibian. I heard individual frogs calling at a temperature of 35.7 °C and only 39.75%
relative humidity. While the energetics of tree calls is unexplored, anuran mating calls are
very expensive energetically. In *H. versicolor*, mating call rate and duration explained over
80% of the variance in metabolism (Andersson 1994), illustrating the high cost of sustained
calling. Rate of glycogen depletion in the trunk muscles of hylids significantly increased in
species with higher mating call rates (Bevier 1997). In *Hyla cinerea*, *H. gratiosa* and *H.
squirella*, the energy cost of broadcasting mating calls is equal to or greater than the energy
needed for locomotion (Prestwich et al. 1989). Time spent broadcasting mating calls in a
chorus is determined by energy limitations and mortality risk (Murphy 1994). Energetic
costs of tree calls presumably would be even higher (per call) because of stressful daytime
temperatures and increased movement.
Treefrogs giving calls from the tops of trees also may be more easily detected by predators than individuals that remain motionless in water-conserving postures (illustrated in Duellman and Trueb 1986, p. 199). In southern Florida, frogs comprised over three-fourths of all prey that swallow-tailed kites (*Elanoides forficatus*) delivered to their nests (Meyer et al. 2004). *Hyla femoralis* also is consumed by black rat snakes (*Elaphe obsoleta, sensu lato*; Wright, 1932), which are exceptional climbers, and by eastern ribbon snakes (*T. sauritus sauritus*; Brown 1979). Other hylids were consumed by eastern garter snakes (*Thamnophis sirtalis sirtalis*) and northern black racers (*Coluber constrictor constrictor*; Brown 1979).

Here, I compare acoustics of mating and tree calls of the pine woods treefrog (*Hyla femoralis*), which is a small arboreal frog about 25-38 mm snout-vent length (SVL). *Hyla femoralis* inhabits the southeastern U.S. coastal plain from southeastern Virginia to southern Florida (except the Everglades) and west through southern Louisiana (Hoffman 1988). *Hyla femoralis* typically occurs in pine woods habitat and in Florida breeds from May-September.

Male mating calls vary from about 0-5000 Hz and have been described as the sound of Morse code being tapped out (Blair 1958; Figures 4, 8a). Their tree call is similar, but is shorter and trails off at the end (Figure 8b). The tree call is used during the day, whereas the mating call is given after dusk (pers. obs.).

I predicted that because tree and mating calls are broadcast at different times of the day and may have different functions, they will differ acoustically. Different energy requirements and environmental conditions often affect acoustic characteristics of anuran calls; for example, there is a positive correlation between pulse repetition rate and temperature and a negative correlation between mean number of pulses within a call and temperature in *Hyla versicolor* (Gayou 1984), and a negative correlation between call
duration and body temperature in *H. versicolor* (Blair 1958). Based on this information and likely differences in function (Chapter 2), I predicted that tree calls would be of shorter duration with fewer pulses and a longer duration between pulses, and have a higher frequency than mating calls of *H. femoralis*. 
Methods

I recorded thirty tree and ten mating calls of *H. femoralis* at Econ River Wilderness Area, Seminole County, Florida (28°39.15’N 81°10.13’W) from 7 June 2004 - 27 July, 2005 (Figure 9; Appendix C). I recorded calls with a Digital Audio Tape Recorder, microphone, wind shield and DAT tapes (Table 3). Tree calls were recorded from 0600 - 1900 h with a mean air temperature at ground level of 25.2 ± 2.7 °C (mean ±1 SE). Mating calls were recorded from 2200 - 2400 h, with a mean air temperature at ground level of 24.3 °C ± 0.2 °C (Global Ecosystems Database Project 2000).

Calls were digitized at a rate of 44.1 kHz using Signal 4.0 (Engineering Design, Belmont, MA). I used CoolEdit Pro (Syntrillium Software, San Jose, CA) to isolate tree calls and mating calls within sonograms. I then measured minimum and maximum frequency, duration of call, number of pulses per call and mean time between pulses for each call (Figure 10). I used these data to calculate the pulse repetition rate for each call type. Pulse repetition rate was calculated as \((N-1)/(\text{Duration of Call} \times 100)\); where \(N = \) the number of pulses per call and \(\text{Duration of Call} = \) length of call in seconds from first pulse to last pulse (Gayou 1984). I compared characteristics of individual tree and mating calls using t-tests for minimum frequency, duration of call and number of pulses per call. Due to non-normal distributions, I used rank-sum tests for mean duration between pulses within each call, pulse repetition rate and maximum frequency. All statistical analyses were done using JMP (v. 6.0.2, 2006, Cary, NC).
Results

Tree and mating calls differed significantly in four characteristics: number of pulses per call, duration between pulses within a single call, pulse repetition rate, and minimum call frequency. Tree calls had significantly fewer pulses per call than mating calls (t-test: \( t = 3.35 \), 34 d.f., \( p < 0.01 \)); on average, tree calls had \( 30 \pm 15 \) pulses per call compared to \( 48 \pm 18 \) for mating calls. Tree calls had significantly longer duration between pulses than mating calls (Rank-sum test: \( X^2 = 83.73 \), 1 d.f., \( p < 0.01 \)); medians and ranges were \( 0.118 \) s (\( 0.010 – 2.325 \) s) in tree calls versus \( 0.068 \) s (\( 0.027 – 1.399 \)) in mating calls. Tree calls had significantly lower pulse repetition rate than mating calls (Rank-sum test: \( X^2 = 10.57 \), 1 d.f., \( p < 0.01 \)); medians and ranges were \( 0.061 \) pulses/s (\( 0.014 – 0.086 \)) in tree calls versus \( 0.086 \) pulses/s (\( 0.047 – 0.111 \)) in mating calls. Tree calls also were broadcast at a significantly higher average minimum frequency than mating calls (t-test: \( t = -5.74 \), 38 d.f., \( p < 0.01 \)). Tree calls had a mean minimum frequency of \( 1726 \pm 88 \) Hz, while that of mating calls was \( 1545 \pm 98 \) Hz. Tree and mating calls did not differ significantly in duration (t-test: \( t = 0.38 \), 38 d.f., \( p < 0.704 \)) or maximum frequency (Rank-sum test: \( X^2 = 3.34 \), 1 d.f., \( p < 0.068 \)), which averaged \( 5.145 \) s and \( 4546 \) Hz and \( 5.411 \) s and \( 4845 \) Hz, respectively.
Discussion

Very little is known about the function and possible benefits of tree calls, which were first described by Deckert (1915) using *Hyla femoralis* and Blair (1958) using *Hyla squirella*. Since then, little new information has been published about this enigmatic type of anuran call (Bogert 1960, Fellers 1979, Honda and Matsui 1996). This chapter is the first acoustic description of the tree call used by *Hyla femoralis* and the first quantitative comparison of the tree and mating calls of any anuran.

My results indicate that tree and mating calls of *Hyla femoralis* were of similar duration and maximum frequencies, but differed in the number of pulses per call, time between pulses, pulse repetition rate and minimum call frequency. On average, tree calls had 37.5% fewer pulses, 42.4% longer duration between pulses, and a 10.5% higher minimum calling frequency than mating calls. Tree calls of *H. femoralis* thus required less energy than mating calls. In male frogs, energy use during calling can be ten to twenty times higher than at rest (Taigen and Wells 1985, Wells and Taigen 1986, 1989, Prestwich et al. 1989), so tree calls represent a substantial energetic investment, particularly under stressful daytime conditions. In Chapter 1, I recorded tree calls of *H. femoralis* at a maximum temperature of 35°C and a minimum relative humidity of 40%; challenging conditions for any amphibian (Duellman and Trueb 1986). Although *Hyla femoralis* has cryptic coloration, calling from treetops in daylight also may attract predators, specifically birds and arboreal snakes (Duellman 1978, Arak 1983).

Absorption of sound energy varies with physical habitat structure. Sound frequency was lower in dense forest than in open habitats (Marten and Marler 1977) and
energy of higher frequency (> 2500 Hz) passerine bird calls decreased faster with increasing distance because sound reflected from nearby trees (Morton 1975). These dynamics suggest that more energy is needed to broadcast high-frequency calls from trees than from the more open conditions typical of breeding ponds. Females frequently favor mating calls with more energy, in the form of call intensity, complexity and repetition rate (Ryan 1988, Gerhardt 1991, Gerhardt 1994). However, my results indicate tree calls contain less energy than mating calls, which may indicate that males need to conserve energy for mating calls later in the day or that past research signifying female preference for high energy calls can only be attributed for mating calls and not tree calls. Alternatively, a high density of females in the area may require tree calls of only minimal energy (Whitney and Krebs 1975).

Compared to mating calls, tree calls had significantly fewer pulses per call, more time between pulses within a call, and a lower pulse repetition rate. This pattern is opposite that expected due to differences between nocturnal and daytime temperatures alone; in hylid frogs, number of pulses, time between pulses, and pulse repetition rate generally increases with increasing temperature (Gerhardt 1978, Gayou 1984) and therefore should be higher in tree compared to mating calls. Similarly, call duration is negatively correlated with body temperature in mating calls of diverse anurans (e.g. *H. veriscolor*, Blair 1958; *Bombina variegata*, Zwiefel 1959, 1968). However, call duration of tree and mating calls did not differ significantly; both calls lasted a mean of 5.211 s. This past research on mating calls can only be effectively compared to other mating calls and not necessarily tree call characteristics. Perhaps daytime with higher temperatures include other more stressful environmental conditions that treefrogs must cope with and
does not allow for the necessary energy needed to increase number of pulses, time between pulses, and pulse repetition rate.

Maximum tree call frequency (Hz) for *H. femoralis* was similar to mating calls. However, minimum tree call frequency (Hz) was significantly higher than mating calls. Mating call frequency increases with increasing temperature (Blair 1955, Zweifel 1968) suggesting that higher daytime temperatures should induce higher maximum tree calling frequency. However, this was not the case. Four call characteristics were significantly different (number of pulses per call, time between pulses within a call (s), pulse repetition rate (number of pulses per second), and minimum tree call frequency (Hz)) between tree and mating calls. Two call characteristics were not significantly different (maximum tree call frequency (Hz) and call duration (s)) between tree and mating calls. These differences between the two types of calls may be due to individual or daily variation in calling. However, each call used for analysis was broadcast by a different individual, so these consistent differences in calling characteristics must be due to other factors, such as body size, seasonality, and environmental variation (Runkle et al. 1994, Bridges and Dorcas 2000).

By calling from the tops of trees during the day, male *H. femoralis* expend considerable energy and expose themselves to stressful environmental elements (Chapter 1), which could be avoided by staying in water-conserving postures. Relative to mating calls, tree calls may equal or surpass energy requirements needed for mating calls. Individual frogs may give tree calls every few minutes throughout the day for possibly weeks at a time (pers. obs.). To be maintained by natural selection, tree calls must confer a fitness benefit that offsets these costs. Better understanding of the fitness benefits of
tree calls can help conservation efforts because daytime calls are easy to identify and count, and can be easily incorporated into monitoring protocols for the 16 species of hylid frogs in North America that have tree calls. In addition, playbacks of tree and mating calls could be used to lure treefrogs to new or preferred breeding habitats instead of ecological traps (Schlaepfer et al. 2002) such as small pools of water found on roads and residential areas. As urban sprawl and development increase, treefrog habitat will probably decrease and urban acoustic interference will increase (Rabin et al. 2003). Using a simple playback technique may aid in maintaining healthy treefrog populations in protected and restored natural areas.
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Figure 9. Sonograms of a) mating call and b) tree call of Hyla femoralis.

Y-axis indicates call duration (seconds) and x-axis is frequency (Hertz) of calls. I recorded both calls at Econ River Wildlife Area: the mating call on 2 June 2005 at 2047h and the tree call on 25 July 2004 at 0827h with an air temperature of 25.9 °C.
Figure 10. Kristine Schad recording tree calls at Econ River Wilderness Area summer 2004.
Figure 11. Tree call sonogram.

Sonogram showing: a) minimum frequency, b) maximum frequency, c) call duration, d) number of pulses per call (arrows point to four individual pulses) and e) duration between pulses within a single call. I recorded this call at Econ River Wilderness Area on 25 July 2004 at 0834h with an air temperature of 25.95 °C.
APPENDIX A:
DIRECTION FOR FUTURE RESEARCH
Possible hypotheses and ideas for future research concerning tree call use by treefrogs.

1. **Tree calls may be broadcast as a means of practicing for mating calls.** Birds have been shown to practice their calls, so frogs may be doing the same thing. Perhaps these frogs are preparing their vocal sacs for the mating season to broadcast mating calls.

2. **Try to trigger treefrogs to broadcast tree calls in the lab.** If this could be accomplished, much more could be learned about tree call use by eliminating many other variables.

3. **Determine the number of frogs that broadcast tree calls compared to the number of those same frogs that also broadcast mating calls at night.** It may be possible that some ‘sneaker males’ are benefiting by not broadcasting tree calls during the day.

4. **Determine the number of frogs that broadcast tree calls compared to the number of those same frogs that also mate that night.** It may be possible that some ‘sneaker males’ are gaining a mating benefit by staying nearby other treefrogs that did broadcast tree calls throughout the day, while they did not incur the costs of broadcasting tree calls.

5. **Tree calls may be an attempt at predator avoidance.** Calling from the tops of trees during the day should be an obvious prey. Perhaps tree calls are actually a way to warn others about predators or the frogs are able to project their voice to trick predators into going elsewhere.

6. **Determine if the treefrogs are able to broadcast tree calls on consecutive days.** It is probably quite energetically costly for treefrogs to broadcast tree calls every day. However, depending on the benefit to calling, this may be worth this cost.

7. **Use treefrogs species, other than those used here, to determine which environmental variables trigger tree calling behavior (repeat Chapter 1 observations).** If all species that
use tree calls were to be sampled, it is possible that trends for environmental triggers may become more obvious.

8. **Determine the number of individual treefrogs broadcast tree calls compared to the total number of treefrogs in the entire population.** Counting the number of treefrogs that broadcast tree calls would help determine the population size needed for only a few treefrogs to continue tree calls. This may help in applying counts of tree calls to amphibian monitoring programs.

9. **Repeat the behavioral study explained in Chapter 2.** A power analysis indicated four more replicates are needed. The implementation of this experiment could further determine whether tree calls have a mating benefit.

10. **Compare the acoustic characteristics between tree and mating calls for other species (similar to Chapter 3).** Tree call sonograms of very few species have been published. And before my research, no acoustic comparisons between tree and mating calls have been published. The more species that are studied, the better we will be able to understand tree calls.
## Table 7. Tree calling activity at Econ River Wilderness Area.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time Period</th>
<th>Number of tree calls by <em>Hyla femoralis</em></th>
<th>Number of tree calls by <em>Hyla gratiosa</em></th>
<th>Number of tree calls by <em>Hyla squirella</em></th>
<th>Mean Temperature (°C)</th>
<th>Variance in Temperature</th>
<th>Mean Relative Humidity (%)</th>
<th>Variance in Relative Humidity</th>
<th>Mean Barometric Pressure (Pascals)</th>
<th>Variance in Barometric Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-Jun-04</td>
<td>1400-1559</td>
<td>32</td>
<td>2</td>
<td>24</td>
<td>35.0</td>
<td>0.2</td>
<td>44</td>
<td>5</td>
<td>101716</td>
<td>434</td>
</tr>
<tr>
<td>7-Jun-04</td>
<td>1600-1800</td>
<td>143</td>
<td>2</td>
<td>51</td>
<td>32.9</td>
<td>0.3</td>
<td>48</td>
<td>4</td>
<td>101629</td>
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<td>28</td>
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<td>25</td>
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<td>59</td>
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APPENDIX C:
SONOGRAMS OF TREEFROG CALLS
Table 8. Acoustic summary of tree call characteristics.

n/a refers to instances when acoustic interference (such as birds calling) did not allow for accurate counting.

<table>
<thead>
<tr>
<th>Tree Call</th>
<th>Duration (s)</th>
<th>Number of Pulses</th>
<th>Minimum Frequency</th>
<th>Maximum Frequency</th>
<th>Mean Time between Pulses (s)</th>
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Table 9. Acoustic summary of mating call characteristics.

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<th>Tree Call</th>
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<th>Number of Pulses</th>
<th>Minimum Frequency</th>
<th>Maximum Frequency</th>
<th>Mean Time between Pulses (s)</th>
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Calls of *Hyla femoralis* recorded for acoustic analysis in Chapter 3: thirty tree calls and ten mating calls.

**Figure 12.** Tree call 1 recorded at Econ River Wildlife Area on 25 July 2004 at 0800h with an air temperature of 22.48 °C.

**Figure 13.** Tree call 2 recorded at Econ River Wildlife Area on 25 July 2004 at 0802h with an air temperature of 22.48 °C.

**Figure 14.** Tree call 3 recorded at Econ River Wildlife Area on 25 July 2004 at 0803h with an air temperature of 22.48 °C.
Figure 15. Tree call 4 recorded at Econ River Wildlife Area on 25 July 2004 at 0803h with an air temperature of 22.48 °C.

Figure 16. Tree call 5 recorded at Econ River Wildlife Area on 25 July 2004 at 0813h with an air temperature of 23.24 °C.

Figure 17. Tree call 6 recorded at Econ River Wildlife Area on 25 July 2004 at 0814h with an air temperature of 23.63 °C.

Figure 18. Tree call 7 recorded at Econ River Wildlife Area on 25 July 2004 at 0816h with an air temperature of 23.63 °C.
Figure 19. Tree call 8 recorded at Econ River Wildlife Area on 25 July 2004 at 0818h with an air temperature of 24.01 °C.

Figure 20. Tree call 9 recorded at Econ River Wildlife Area on 25 July 2004 at 0819h with an air temperature of 24.01 °C.

Figure 21. Tree call 10 recorded at Econ River Wildlife Area on 25 July 2004 at 0825h with an air temperature of 24.79 °C.

Figure 22. Tree call 11 recorded at Econ River Wildlife Area on 25 July 2004 at 0826h with an air temperature of 24.79 °C.
Figure 23. Tree call 12 recorded at Econ River Wildlife Area on 25 July 2004 at 0826h with an air temperature of 24.79 °C.

Figure 24. Tree call 13 recorded at Econ River Wildlife Area on 25 July 2004 at 0827h with an air temperature of 25.17 °C.

Figure 25. Tree call 14 recorded at Econ River Wildlife Area on 25 July 2004 at 0827h with an air temperature of 25.17 °C.

Figure 26. Tree call 15 recorded at Econ River Wildlife Area on 25 July 2004 at 0833h with an air temperature of 25.95 °C.
Figure 27. Tree call 16 recorded at Econ River Wildlife Area on 25 July 2004 at 0833h with an air temperature of 25.95 °C.

Figure 28. Tree call 17 recorded at Econ River Wildlife Area on 25 July 2004 at 0834h with an air temperature of 25.95 °C.

Figure 29. Tree call 18 recorded at Econ River Wildlife Area on 25 July 2004 at 0833h with an air temperature of 25.95 °C.

Figure 30. Tree call 19 recorded at Econ River Wildlife Area on 25 July 2004 at 0834h with an air temperature of 25.95 °C.
Figure 31. Tree call 20 recorded at Econ River Wildlife Area on 25 July 2004 at 0834h with an air temperature of 25.95 °C.

Figure 32. Tree call 21 recorded at Econ River Wildlife Area on 25 July 2004 at 0836h with an air temperature of 25.95 °C.

Figure 33. Tree call 22 recorded at Econ River Wildlife Area on 25 July 2004 at 0836h with an air temperature of 25.95 °C.

Figure 34. Tree call 23 recorded at Econ River Wildlife Area on 25 July 2004 at 0836h with an air temperature of 25.95 °C.
Figure 35. Tree call 24 recorded at Econ River Wildlife Area on 25 July 2004 at 0836h with an air temperature of 25.95 °C.

Figure 36. Tree call 25 recorded at Econ River Wildlife Area on 25 July 2004 at 0844h with an air temperature of 26.73 °C.

Figure 37. Tree call 26 recorded at Econ River Wildlife Area on 25 July 2004 at 0844h with an air temperature of 26.73 °C.

Figure 38. Tree call 27 recorded at Econ River Wildlife Area on 25 July 2004 at 0859h with an air temperature of 27.52 °C.
Figure 39. Tree call 28 recorded at Econ River Wildlife Area on 25 July 2004 at 0859h with an air temperature of 27.52 °C.

Figure 40. Tree call 29 recorded at Econ River Wildlife Area on 25 July 2004 at 0859h with an air temperature of 27.52 °C.

Figure 41. Tree call 30 recorded at Econ River Wildlife Area on 25 July 2004 at 0900h with an air temperature of 27.91 °C.
Figure 42. Mating call 1 recorded at Econ River Wildlife Area on 2 June 2005 at 2047h with an air temperature of 24.3 °C.

Figure 43. Mating call 2 recorded at Econ River Wildlife Area on 2 June 2005 at 2047h with an air temperature of 24.3 °C.

Figure 44. Mating call 3 recorded at Econ River Wildlife Area on 2 June 2005 at 2047h with an air temperature of 24.3 °C.
Figure 45. Mating call 4 recorded at Econ River Wildlife Area on 2 June 2005 at 2047h with an air temperature of 24.3 °C.

Figure 46. Mating call 5 recorded at Econ River Wildlife Area on 2 June 2005 at 2047h with an air temperature of 24.3 °C.

Figure 47. Mating call 6 recorded at Econ River Wildlife Area on 2 June 2005 at 2047h with an air temperature of 24.3 °C.
Figure 48. Mating call 7 recorded at Econ River Wildlife Area on 2 June 2005 at 2049h with an air temperature of 24.4 °C.

Figure 49. Mating call 8 recorded at Econ River Wildlife Area on 2 June 2005 at 2049h with an air temperature of 24.4 °C.

Figure 50. Mating call 9 recorded at Econ River Wildlife Area on 2 June 2005 at 2049h with an air temperature of 24.4 °C.

Figure 51. Mating call 10 recorded at Econ River Wildlife Area on 2 June 2005 at 2049h with an air temperature of 24.4 °C.