Acoustic variation and species discrimination in southeastern sibling species, the cricket frogs *Acris crepitans* and *Acris gryllus*

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ABSTRACT

Jonathan P. Micancin: Acoustic variation and species discrimination in cricket frogs, genus *Acris*

(Under the direction of R. Haven Wiley)

Mate recognition systems, both the signals produced and the sensory mechanisms to receive them, often diverge to result in pre-mating reproductive isolation between closely related species. Comparative and experimental studies of anurans have contributed to our understanding of this process. By comparing populations of related species in allopatry, sympatry, and syntopy, it is possible to identify the environmental factors associated with divergence in mate recognition systems, including interactions with related species.

Many pairs of species meet along the Fall Zone in the Southeast, but few have been well researched. My study compared the two species of cricket frogs, *Acris crepitans* and *A. gryllus,* at 36 sites in North Carolina. I assessed the acoustic and morphological traits used to identify the species, determined the extent of their ranges and sympatry, and identified 4 syntopic sites in the upper Coastal Plain. The dominant frequency and call rate of male vocalizations varied widely and overlapped between the species. Body mass had the largest effect on these features. In contrast, the effects of seasonality and temperature were minor. Additional variation between sites could not be attributed to sympatry or syntopy, so there was no evidence of reproductive character displacement in dominant frequency or call rate.

In playback experiments at a syntopic site, females of both species discriminated between conspecific and heterospecific signals on the basis of click structure, a fine-scale temporal feature, and had no preference for dominant frequency among conspecific signals. Reproductive isolation in *Acris* is promoted by divergence in the temporal structure of male signals. Only studies like this one, conducted on geographic scales appropriate for comparisons of local populations, can identify patterns of geographic variation in signals that contribute to reproductive isolation.

For the frogs and the people who appreciate them

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PREFACE

Early in my graduate training, I heard a zoologist at a state agency express disappointment in the majority of biological research occurring at public universities. He stated that the enormous intellectual, physical, and financial resources consumed by these institutions ought to be used for research on basic wildlife ecology and conservation rather than to address esoteric questions that few people understood and even fewer people could apply toward practical ends. After my initially defensive feelings subsided, I recognized that the lack of even cursory knowledge of many species that are native to the United States justified his opinion.

Like many behavioral ecologists, childhood experiences as an amateur naturalist informed my interest in this academic field. My experiences emphasized field study for the sake of knowledge and stewardship of nature. I was drawn to behavioral ecology because of its basic premise that field research is crucial to understanding behavior. Field work usually depends on identifying an organism, knowing where it ranges, and having some information about its relationships with closely related taxa. Such information is lacking for many species in the Southeast. Although I addressed some important theoretical issues in my study, I also aimed to correct this oversight for the cricket frogs *Acris crepitans* and *A. gryllus* in North Carolina.

I believe that the most demanding and rewarding scientific research addresses theory but has practical applications outside of academia. From the outset, the research recounted in this dissertation has been conducted with that approach. My goal was to develop a system for the study of evolution, in particular speciation and communication, while simultaneously developing basic tools, like field marks and range maps, that could be used for conservation and human appreciation of natural history.

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Acoustic and morphological identification and range overlap of the cricket frogs *Acris crepitans crepitans* and *Acris gryllus gryllus*

Abstract

Studies of speciation, including studies of reinforcement and reproductive character displacement, require the accurate identification of sibling taxa and the assessment of the degree of contact between them. In order to use two cricket frogs, *Acris crepitans crepitans* and *Acris gryllus gryllus,* as subjects for the investigation of communication and mate choice in the context of speciation, I determined an efficient means to identify live and preserved cricket frogs, described their ranges in North Carolina, and determined the degree of co-occurrence at breeding sites (syntopy). I was able to identify preserved specimens by the extent of hind-foot webbing and the diameter of anal tubercles, but the small size and high variability of these features made it difficult to identify live frogs morphologically. I determined that acoustic identification of breeding *Acris* was a practical approach in the field and used it to establish that *A. c. crepitans* and *A. g. gryllus* had ranges that overlapped (i.e. were sympatric) in the upper Coastal Plain of North Carolina. The actual range of *A. c. crepitans* was more extensive than expected from published report because I found the species along rivers deep into the lower Coastal Plain. The range of *A. g. gryllus* was more restricted than expected because I could not find it in much of its published range in the

northern half of the state. Therefore, the conservation status of *A. g. gryllus* should be monitored carefully. *A. c. crepitans* and *A. g. gryllus* were syntopic at a few breeding sites in the upper Coastal Plain.

Introduction

Much effort has recently been made by evolutionary biologists to address still-unresolved issues to explain the origin and maintenance of species (Coyne and Orr, 2004). Species concepts are still debated and there is limited understanding of how reproductive isolation arises in nature. One way to address these open questions is to study previously non-utilized groups of organisms that are believed to be sibling species. Comparative and experimental studies of closely-related amphibians have contributed to our understanding of evolution and ecology including mechanisms of speciation (Gerhardt and Huber, 2002). Cricket frogs (*Acris*, Hylidae) are a good example. *Acris* are small, primarily terrestrial treefrogs of eastern North America. Two species, *A. crepitans* (Northern Cricket Frog) and *A. gryllus* (Southern Cricket Frog), and five subspecies are currently recognized (Crother et al., 2001). Two subspecies of *A. crepitans* have been extensively studied in the Midwest. *A. crepitans blanchardi* (Blanchard's Cricket Frog) occupies open, xeric habitat in the Great Plains, while *A. crepitans crepitans* (Eastern Cricket Frog) occupies denser, mesic mixed hardwood forests of the eastern United States (Nevo, 1973a, 1973b).

Where the subspecies are parapatric (have adjoining but non-overlapping ranges) along the eastern edge of the Great Plains, differences in advertisement calls occur among populations and subspecies (Ryan et al., 1990). Female *A. c. blanchardi* and *A. c. crepitans*

prefer vocalizations representing local males of their own subspecies to vocalizations of the other subspecies or of *A. gryllus gryllus* (Coastal Plain Cricket Frog) (Nevo and Capranica, 1985). Divergence in *Acris* calls might be the result of selection for increased transmission of vocalizations in different habitats, rather than indirect selection on body size or reinforcement of reproductive isolation in sympatry (Nevo and Capranica, 1985; Ryan and Wilczynski, 1988; Ryan et al., 1990; Sun et al., 2000; Witte et al., 2005). Such behavioral evidence as well as recent molecular work (Beauclerc et al., 2007) suggest that the two subspecies of *A. crepitans* have recently become reproductively isolated. Because the two subspecies are apparently never found at the same breeding sites, they are not a candidate for a study of secondary contact after divergence in allopatry. By Rivas' (1964) definitions, the two subspecies are sympatric (their ranges overlap) but not syntopic (so close that they could interbreed). Variation in male vocalizations and female preferences along the border between grasslands and forests occupied by *A. c. blanchardi* and *A. c. crepitans* and the possibility of reinforcement of differences in vocalizations remain open topics.

In the southeastern United States, *A. c. crepitans* and *A. g. gryllus* provide an opportunity to investigate relationships between closely related populations because of their occurrence in allopatry and sympatry, high abundance, and long breeding season (Nevo and Capranica, 1985). The previously-published southeastern ranges of *A. c. crepitans* and *A. g. gryllus* correspond approximately to the Piedmont and Coastal Plain, respectively (Conant and Collins, 1991). In allopatry, *A. c. crepitans* and *A. g. gryllus* occupy different habitats (Bayless, 1969; Nevo and Capranica, 1985). The mixed hardwood and pine community of the Piedmont is drier and cooler than the extensive pine savannas or bottomland cypress-gum

(*Taxodium*, *Nyssa*) swamps of the Coastal Plain. Precipitation and temperature differences between allopatric habitats of *A. c. crepitans* and *A. g. gryllus* in the Southeast resemble those between *A. c. blanchardi* and *A. c. crepitans* in the Midwest (Bayless, 1969; Nevo and Capranica, 1985).

The two species occupy an extensive area of sympatry on or near the Fall Zone (Mecham, 1964), the region where the Piedmont and Coastal Plain meet (Stuckey, 1965). This sympatric zone, which extends from southeastern Virginia to the Mississippi River (Nevo and Capranica, 1985; Conant and Collins, 1991), is apparently narrowest at its northeastern limit in Virginia and gradually widens to encompass most of Alabama and Mississippi (Nevo and Capranica, 1985). In Alabama, Mecham (1964) reported no hybrids of *A. crepitans* and *A. gryllus* despite the absence of hybrid inferiority in a laboratory experiment. In contrast, Mount (1975) found cricket frogs with intermediate morphological features in Alabama's Coastal Plain and suggested that these could be hybrids.

These contradictory findings may reflect the perpetual confusion about the morphological identification of species and subspecies in *Acris*. This difficulty suggests that some of the morphological features traditionally used to identify cricket frogs to species are not distinctive or geographically concordant, even though the basis for naming different *Acris* taxa is almost entirely morphological. The descriptions of *Acris crepitans* from "the northern states" by Baird in 1854 and *Rana gryllus*, presumably from Georgia, by LeConte in 1825, did not include type specimens (Dunn, 1938). Viosca (1923) argued that in Louisiana, there were two *Acris* occurring in close proximity without interbreeding. Dunn (1938) reviewed the original descriptions of *Acris* and later specimens in the collection of the Academy of

Natural Sciences of Philadelphia and agreed that there were two species. In allopatry, *A. crepitans* and *A. gryllus* were concluded to have distinct morphologies (Dunn, 1938; Brimley, 1944; Wright and Wright, 1949; Neill, 1950; Mecham, 1964; Martof et al., 1980). *A. crepitans* is larger overall, has a thicker build, broader snout, and shorter appendages relative to its body size than does *A. gryllus*. *A. crepitans* has more rugose skin and more extensive webbing on the hind feet than does *A. gryllus*, and the anal tubercles (whitish glands on either side of the vent) are larger. Stripes of dark pigment on the back of the thigh are wider, but with more irregular margins, in *A. crepitans* than in *A. gryllus*. Subsequent morphological and ecological studies (Brimley, 1944; Neill, 1950; Boyd, 1964; Mecham, 1964; Mount, 1975) confirmed the existence of two species in the Southeast which occasionally occurred in syntopy, but did not reach a consensus on whether they were reproductively isolated.

Brimley (1944) loosely described the ranges of *A. crepitans* and *A. gryllus* in North Carolina and suggested that the ranges overlapped and that there were no morphologically intermediate individuals. Subsequently, no publications have investigated the identification, ranges, or status of *A. c. crepitans* or *A. g. gryllus* in North Carolina. *Acris* in or near the Fall Zone are still difficult to identify (Alvin Braswell, Curator of Herpetology at the North Carolina Museum of Natural Sciences, personal communication). The status of *A. g. gryllus* in North Carolina and southeastern Virginia, at the northern extent of its range, is presumed to be stable, but the ability to monitor *A. g. gryllus* is probably obscured by its morphological and ecological similarity to *A. c. crepitans.* A new study of *Acris* is needed not only to reexamine the traits used to identify the species in proximity to each other but also to promote their conservation.

My goals in this study were to determine an efficient means to identify *Acris crepitans crepitans* and *Acris gryllus gryllus* in the field and museum, to describe their ranges in North Carolina, and to investigate the possibility of syntopy at breeding sites. *Acris* in North Carolina exhibited morphological and acoustic distinctions that permitted identification. They had overlapping ranges, were syntopic at a few sites, and appeared to be reproductively isolated. These results confirmed that *A. c. crepitans* and *A. g. gryllus* are attractive subjects for an investigation of communication and mate choice in the context of speciation.

Methods

Field survey

On visits to potential breeding wetlands for *Acris* in the Piedmont and Coastal Plain of North Carolina in 2004, 2005, and 2007, I recorded frogs in 36 sites (Figure 1.1; 17 sites from 4 May to 21 July 2004, 17 sites from 8 May to 24 July 2005, including 2 sites from 2004, and 4 sites in 2007). These sites included public and private properties in the western and eastern Piedmont (15, including 4 sites just west of the Fall Zone), and the upper and lower Coastal Plain (21, including 2 sites in the Sandhills). At each site, there were one or more permanent freshwater bodies with choruses of cricket frogs. At each chorus visited (up to 5 at each site), I recorded and collected a representative sample of the calling frogs (6 to 10 frogs for 10 bouts each. When the chorus was too small to remain active if I removed more males, I reduced the number of frogs collected (3 sites) or refrained from collecting altogether (3 sites). Recording began at or after 2100 hours and ended when the chorus

waned (between 0100 and 0300 hours) or I had a complete sample. Recordings were made with a Marantz PMD-221 or PMD-421 portable tape recorder (2004) or PMD-670 digital recorder (2005 and 2007), and Audio-Technica 815a microphones. I digitized tape recordings from 2004 at a sampling rate of 22.05 kHz (WildSpectra version 060118, Wiley, 2007). Recordings in 2005 and 2007 produced WAV files at the same sampling rate. I also photographed each recorded male, as well as any females and satellite males in obvious association with it, with a Canon Powershot A80 4.1 MP digital camera. After capturing the male, I measured the surface temperature at its calling site with a Miller and Weber T-6000 fast-read cloacal thermometer. Within 12 to 36 hours after collection, I weighed each frog, euthanized it in a chlorotone solution, preserved a forefoot in a dimethyl sulfoxide and salt solution for genetic analysis, and fixed the frog for morphological study and deposition in the collection of the North Carolina Museum of Natural Sciences, raleigh, North Carolina.

Acoustic analysis

Based on studies of acoustic communication in *A. crepitans* in the Midwest (especially Wagner, 1989c) I developed terminology to describe the temporally complex vocalizations of cricket frogs (Chapter 2 and 3). Two studies (Blair, 1958, Nevo and Capranica, 1985) that compared the vocalizations of *A. crepitans* and *A. gryllus* at a continental scale have suggested that a temporal component of their vocalizations could be used for identification. Wagner (1989c) interchangeably refers to this component as a "call" or "click". I use "click" exclusively for this component and avoid "call" when referring to components of cricket frog vocalizations. Blair's (1958) description of the differences within

clicks was primarily qualitative, but Nevo and Capranica (1985) showed that *A. gryllus* clicks contain more pulses, produced at a faster and more consistent rate, than do *A. crepitans* clicks. Consecutive pulses decrease in amplitude in *A. gryllus* clicks but remain at about the same amplitude in *A. crepitans* clicks. Figure 1.2 shows representative clicks of the two species in North Carolina.

To verify that these differences occur in North Carolina, I used WildSpectra (version 061025, Wiley, 2007) to analyze the structure within each click of a bout from six frogs from each of four sites in or near the putative zone of sympatry (Figure 1.1). I chose two sites near the Fall Zone where clicks resembled published descriptions of one of the species (*A. c. crepitans* at Mason Farm Biological Reserve (MF), NAD83 Lat. 35.89005°, Long. -79.00866°; *A. g. gryllus* at the Pineberry Bay Tract of the Sampson County Gamelands (PB), NAD83 Lat. 34.97567°, Long. -78.48394°). These sites were outside but near the edge of the presumed zone of overlap according to distribution maps (Conant and Collins, 1991; Martof et al., 1980). I also included two sites in the upper Coastal Plain (Merchants Millpond State Park (MM), NAD83 Lat. 36.43179°, Long. -76.69666°; and Cliffs of the Neuse State Park (CN), NAD83 Lat. 35.22819°, Long. -77.88223°) where both types of clicks occurred. The SongSignatures function in WildSpectra was used for all acoustic measurements. SongSignatures recognizes each note with user-defined starting and ending amplitude thresholds in a selected portion of a spectrogram. It outlines each note identified and produces a file with spectral and temporal information for each. I measured all the clicks in each bout at a sampling rate of 44.1 kHz and transform size of 16 to emphasize temporal resolution (0.18 milliseconds) at the expense of frequency resolution (5.512 kHz). The data

produced by SongSignatures allowed convenient measurement of the mean duration, mean number of pulses, and mean interpulse interval of each bout. JMP 6.0.3 (SAS Institute, 2006) was used for all statistical analyses.

Morphometric analysis

To compare the morphological traits of the acoustically-identified species in North Carolina, I randomly selected (Haahr, 2007) preserved males from among frogs that had previously been identified by click type and used in additional acoustic analyses (Chapters 3 and 4). Vernier calipers (SPI Dial Max 31-415-3) were used to measure snout-vent length, length of the upper hind limb from the vent to the distal surface of the second leg joint (knee), length of the lower hind limb from the skin fold distal to the knee to the distal surface of the ankle (heel), lengths of the upper and lower thigh stripes from the their proximal origins at or near the vent to their distal ends on the upper hind limb (thigh), widths of the upper and lower thigh stripes at their widest and narrowest points, and diameter of the anal tubercle, all to the nearest 0.1 millimeter. Thigh stripes are often discontinuous, and in such cases the narrowest point of the stripe was recorded as 0.0 millimeters. To measure the extent of webbing on the hind foot, I used a Wild M5 dissecting stereomicroscope at 6x magnification. I recorded the number of phalanges on the first (shortest) toe that were free of webbing, not including the small ridge of tissue that runs the length of each toe. Because the extent of webbing on either side of the fourth (longest) toe often differed, I recorded the number of half-phalanges of the fourth toe that were free of webbing on the both the third and fifth toe sides. All these measurements were taken on the right side of the frog. To

control for influence of body size on hind limb length and thigh stripe length, I calculated the ratios of total hind limb length to snout-vent length and thigh stripe length to upper hind limb (thigh) length for each individual. Regularity in the width of each thigh stripe was assessed by dividing the minimal width of each stripe by the maximal width.

JMP 6.0.3 (SAS Institute, 2006) was used for all statistical analyses. I used logistic regressions to investigate the associations of six morphological features with each species: the three measurements of toe webbing, anal tubercle diameter, snout-vent length, the ratio of total leg length to snout-vent length, the ratio of upper and lower thigh stripe lengths to upper hind limb (thigh) length, and the regularity of width of the upper and lower thigh stripes.

Results

Acoustic analysis

The audible differences in the clicks of cricket frogs from Mason Farm Biological Reserve and the Pineberry Bay Tract (Table 1.1 and 1.2a to 1.2d, Figure 1.3a to 1.3d) matched published differences between *A. crepitans* and *A. gryllus*. The clicks of *Acris* at Mason Farm were similar to the clicks of *A. crepitans* in the Midwest: the click duration (Figure 1.3a), number of pulses (Figure 1.3b), and interpulse interval (Figure 1.3c) varied substantially between successive clicks. Some Mason Farm clicks at or near the ends of bouts resembled the "rattle" clicks identified as a distinctive feature of *A. crepitans* in the Midwest. The interpulse interval in the rattle was more regular than for other *A. crepitans* clicks, but still much longer than in *A. gryllus.* Several rattles produced in succession have a throbbing quality. At the Pineberry Bay Tract, clicks of *Acris* were different, with regular,

repeated pulses that decreased in amplitude like the clicks of *A. gryllus* studied by Nevo and Capranica (1985). The distinctions between clicks at these two sites also separated the two types of clicks at the remaining two sites. The lower number of pulses and duration of clicks for *A. g. gryllus* at the Pineberry Bay Tract compared to the syntopic sites is probably an artifact of higher background noise in the Pineberry Bay Tract recordings. The fading of *A. g. gryllus* pulses over the course of every click made it difficult to count the final pulses in the background noise. As a consequence, these clicks appeared to have fewer pulses and shorter durations in comparison to those at the other sites.

Once the distinct differences between clicks of *A. c. crepitans* and *A. g. gryllus* described by other authors were confirmed in North Carolina, I was able to identify frogs from recordings as well as calling individuals in the field. Based on these criteria, *A. c. crepitans* occurred throughout the Piedmont and near large rivers in the upper Coastal Plain, and *A. g. gryllus* occupied the lower Coastal Plain and upland sites of the upper Coastal Plain (Figure 1.1). In the Sandhills region in south-central North Carolina, with the highest elevations in the Coastal Plain (Stuckey, 1965), *A. g. gryllus* occupied the two sites with cricket frogs. *A. c. crepitans* and *A. g. gryllus* occurred in syntopy at four sites: the Crumpler Pond at Cliffs of the Neuse State Park, throughout the wetlands of Merchants Millpond State Park, a borrow pit downstream from Merchants Millpond, and Hare's Millpond. The three latter sites were within 25 kilometers of one another in the Chowan River basin of northeastern North Carolina. Nowhere in the survey were there clicks that combined attributes of both species, such as short, regularly-spaced pulse intervals with no reduction in

amplitude or by combining diminishing pulse amplitude with long, irregular interpulse intervals.

Morphometric analysis

The two species, as identified by their distinct acoustic features, differed significantly in each morphological measurement except in the ratio of lower thigh stripe to upper hind limb (thigh) length (Table 1.3 to 1.5). Nested analyses of variance (ANOVA) and the assessment of variance components by the restricted maximum likelihood method (REML) showed that species accounted for little variance in snout-vent length, leg length relative to snout-vent length, and thigh stripe characteristics. However, species did explain a substantial proportion of the variation in anal tubercle diameter and the extent of toe webbing. The first principal component calculated from the last two features (Figure 1.4 and Table 1.6) also differs markedly between species. A discriminant function analysis of anal tubercle diameter and the three measurements of webbing was used to predict species. The discriminant function was calculated from 43 *A. c. crepitans* and 27 *A. g. gryllus* specimens (43% of total sample) selected randomly from each site and tested on the remaining 58 *A. c. crepitans* and 35 *A. g. gryllus*. It misidentified 3 *A. c. crepitans* and no *A. g. gryllus* (3.2% of the specimens).

To identify cricket frogs morphologically, the anal tubercle diameter and extent of webbing on the first and fourth toe of the hind foot are sufficient. The webbing on the third-toe side of the fourth toe of *A. c. crepitans* includes all but the last two phalanges while in *A. g. gryllus* the webbing ends along the third phalange from the end. In both species, the

webbing is usually less extensive on the outside of the fourth toe but the difference between species is maintained. The webbing on the first toe typically ends at the base of the last phalange in *A. c. crepitans* and along the second to last phalange in *A. g. gryllus*. The anal tubercles of *A. c. crepitans* are typically ≥ 0.5 mm in diameter, but are much smaller, even invisible at 6x magnification, in *A. g. gryllus*.

Discussion

Acoustic identification of cricket frogs

The consistent differences between clicks of *A. c. crepitans* and *A. g. gryllus* can identify *Acris* to species. With experience, these differences can be used to identify *Acris* at breeding sites by ear. While *A. c. crepitans* and *A. g. gryllus* vocalizations can have similar patterns of repetition over the course of seconds or longer, they differ substantially in the structure of the individual clicks. Because of the rapid and regular production of pulses, a single click of *A. g. gryllus* sounds continuous, multiple clicks of one individual sound nearly identical, and the clicks of different individuals or different populations are often indistinguishable. In *A. c. crepitans*, the few pulses produced at a slow and irregular rate make the clicks sound discontinuous and highly variable. The clicks of neighboring *A. c. crepitans* individuals at a single wetland often sound different and the populations at neighboring *A. c. crepitans* breeding sites can often sound recognizably different. The high variation between individuals and populations of *A. c. crepitans* is consistent with previous findings that the temporal and spectral structure of *A. crepitans* vocalizations varies according to the intensity of competition among males (Wagner, 1989a, 1989b, 1989c, 1992;

Burmeister et al., 1999). The rattle click contributes to acoustic variation in both areas and is perhaps the most distinctive feature of *A. c. crepitans* vocalizations in North Carolina. I found no individuals with intermediate vocalizations while conducting this study.

Morphological identification of cricket frogs

Anuran specimens in museums rarely have associated audio recordings, so even recent taxonomic studies of *Acris* (McCallum and Trauth, 2006; Rose et al., 2006) have usually been restricted to the use of morphological features. The inclusion of acoustic features in this study makes it possible to categorize *Acris* and then to assess morphological features for identification. This analysis indicates that diameter of the anal tubercles and the extent of webbing on the fourth (longest) toe on the hind foot are the best morphological traits for distinguishing preserved *A. c. crepitans* and *A. g. gryllus*. Nevertheless, occasional misidentification of *A. c. crepitans* as *A. g. gryllus* can occur when using morphological traits. Given the absence in this study of any frogs with intermediate vocalizations, these misidentifications probably reflect the difficulty of identification by morphology rather than hybridization between *A. c. crepitans* and *A. g. gryllus*.

Additional morphological features of *A. c. crepitans* and *A. g. gryllus* might be useful for identification of live specimens in the field. Formalin and alcohol dramatically alter the dermal pigmentation, texture, and turgor of *Acris* specimens. Preservation probably impaired the assessment of thigh stripes and eliminated the use of dermal texture and pigmentation as identifying features. During two seasons of field work at a syntopic site, I became proficient in identifying frogs by the texture of the skin and the appearance of thigh stripes before

confirming this identification using the anal tubercles. The skin of *A. c. crepitans* was distinctly rugose, which probably contributed to the waxy quality of its dorsal coloration. The skin of *A. g. gryllus* was much smoother and looked translucent, as though the pigmentation was deposited under a layer of unpigmented tissue. The thigh stripes of *A. c. crepitans* were broader and had a more mottled appearance than *A. g. gryllus*, as if they had been drawn with a charcoal pencil. The thigh stripes of *A. g. gryllus* appeared to have been painted with a fine watercolor brush.

Ranges of cricket frogs in North Carolina

The range of *A. c. crepitans* appears to extend farther east than indicated by maps in many field guides such as Conant and Collins (1991), Martof et al. (1980), Bartlett and Bartlett (2006), and Dorcas et al. (2007). The suggestion that *A. c. crepitans* might penetrate the Coastal Plain along rivers (Martof et al., 1980) appears to be valid. There is concern that Coastal Plain *A. c. crepitans* populations in the Carolinas are isolated and, as a result, at greater risk for extirpation (Gray and Brown, 2005, Gray et al., 2005). In the three coastal river systems that I was able to survey thoroughly (Roanoke, Neuse, and Cape Fear), *A. c. crepitans* choruses were easy to find and more or less evenly distributed on or near the major rivers. The few breeding sites more than 2 km from a major river in the upper Coastal Plain were all occupied by *A. g. gryllus*.

The range of *A. g. gryllus* in North Carolina appears to be less extensive than field guides show, at least in the northern part of the upper (western) Coastal Plain. This area of North Carolina has relatively little public or private protected land, which made surveys

difficult. It is possible that *A. g. gryllus* is common on remote private properties in that area, but because the known *A. g. gryllus* populations in this study usually occurred in either sandy pine savannah or bottomland cypress swamp habitats, neither of which is common in the northwestern section of North Carolina's Coastal Plain, it seems unlikely that *A. g. gryllus* currently has any significant presence there.

Extensive surveying from 2002 onward produced no evidence that *Acris* of either species occur in the extreme northeastern part of the state, east of the Chowan river basin and north of the Albemarle Sound. This area, which includes the Great Dismal Swamp and land that was formally a part of it, has been extensively ditched and drained for agriculture. Little appropriate habitat was found for *Acris* in this area.

Implications for conservation

Species can be more sensitive to environmental change at the edges of their ranges than elsewhere (Mehlman, 1997). The two subspecies of *A. crepitans* are in severe decline at the northern edges of their ranges in the upper Midwest and Northeast and though the precise causes of this decline are not established, several anthropogenic changes may be responsible (see Lannoo, 2005, for several discussions of decline in *A. crepitans*). *Acris gryllus* appears to be in local decline in several southern areas of its range, possibly because of increased pine silviculture (Jensen, 2005). The Coastal Plain of North Carolina contains vast pine plantations and edges of the ranges for both *A. crepitans* and *A. gryllus.*

A. crepitans populations in North Carolina, in the middle of its latitudinal distribution, may not be as sensitive to environmental disturbance as elsewhere, and concern for isolation

of *A. c. crepitans* populations in the Coastal Plain appears to be unwarranted. The results of this survey indicate that *A. c. crepitans* is fairly common in the Coastal Plain.

In contrast, *A. g. gryllus* is less common than initially expected. The status of *A. gryllus* in North Carolina, near the northern limit of its range, is probably obscured by difficulties in separating it from *A. crepitans*. A reevaluation of Coastal Plain specimens based on webbing and anal tubercles could establish the historical range of *A. g. gryllus* in North Carolina. A recently introduced statewide anuran monitoring program (the North Carolina Calling Amphibian Survey Project) might define the current range of *Acris*, provided volunteers can accurately identify the two species acoustically. In combination, these efforts could determine the recent stability of *A. gryllus* in North Carolina. Although *A. g. gryllus*, like *A. c. crepitans* has an extensive range, regional concern for the species is warranted.

The two species of *Acris* in North Carolina can be identified by acoustic and morphological features and have overlapping ranges that include shared breeding sites. These conditions make these sibling species a suitable subject for studies of behavioral ecology in sympatry and allopatry. Preserved specimens can be accurately identified by the extent of hind-foot webbing and the diameter of anal tubercles, but the size and variability of these features makes field identification of live frogs difficult. Other morphological features that do not preserve well might be more useful for identification of silent frogs in the field, but acoustic identification of breeding frogs is the most accurate way to separate these frogs.

The ranges of the two species in the Coastal Plain of North Carolina are different from published reports and the conservation status of *A. g. gryllus* should be monitored carefully.

Table 1.1. Click measurements by site and click type.

		CN (1)	MM (1)	MF (1)	total (1)	CN (2)	MM (2)	PB (2)	total (2)
Click Duration (ms)	Mean	0.1479	0.1552	0.1658	0.1587	0.0429	0.0449	0.027	0.0355
	SD	0.0807	0.0104	0.0484	0.0483	0.0135	0.0027	0.0035	0.0109
	Min	0.0922	0.1436	0.1126	0.0922	0.0348	0.0419	0.0203	0.0203
	Max	0.2405	0.1637	0.2339	0.2405	0.0585	0.0472	0.0296	0.0585
Pulses (N)	Mean	5.28	4.79	5.9	5.47	11.16	12.38	8.79	10.28
	SD	1.38	1.06	0.79	1.03	1.18	2.89	1.58	2.35
	Min	4.33	3.86	5.11	3.86	9.95	10.09	6.17	6.17
	Max	6.86	5.94	7.06	7.06	12.3	15.63	10.43	15.63
Interpulse Interval (ms)	Mean	0.0147	0.0181	0.0153	0.0159	0.0021	0.002	0.0017	0.0019
	SD	0.0041	0.0027	0.0039	0.0036	0.0005	0.0004	0.0002	0.0003
	Min	0.0116	0.0151	0.0111	0.0111	0.0017	0.0016	0.0015	0.0015
	Max	0.0193	0.0202	0.0209	0.0209	0.0026	0.0022	0.0019	0.0026
N Bouts		3	3	6	12	3	3	6	12

Table 1.2a. Means and standard deviations of click duration by site and click type

Site (click type)	N	Mean	Standard Deviation	Standard Error Mean	Lower 95%	Upper 95%
CN (1)	3	0.14794	0.080702	0.04659	-0.0525	0.34842
MM (1)	3	0.155151	0.010394	0.006	0.1293	0.18097
MF (1)	6	0.16583	0.048353	0.01974	0.1151	0.21657
CN (2)	3	0.042934	0.013495	0.00779	0.0094	0.07646
MM (2)	3	0.044936	0.002713	0.00157	0.0382	0.05168
PB (2)	6	0.027006	0.003463	0.00141	0.0234	0.03064

Table 1.2b. Means and standard deviations of total pulses per click by site and click type

Site (click type)	N	Mean	Standard Deviation	Standard Error Mean	Lower 95%	Upper 95%
CN (1)	3	5.2798	1.37611	0.7945	1.8613	8.698
MM (1)	3	4.7906	1.05634	0.6099	2.1665	7.415
MF (1)	6	5.8972	0.78854	0.3219	5.0697	6.725
CN (2)	3	11.1648	1.17656	0.6793	8.242	14.087
MM (2)	3	12.3782	2.88862	1.6677	5.2025	19.554
PB (2)	6	8.7894	1.57693	0.6438	7.1345	10.444

Table 1.2c. Means and standard deviations of inter-pulse interval by site and click type

Site (click type)	N	Mean	Standard Deviation	Standard Error Mean	Lower 95%	Upper 95%
CN (1)	3	0.014657	0.004062	0.00235	0.00457	0.02475
MM (1)	3	0.018114	0.002665	0.00154	0.01149	0.02473
MF (1)	6	0.015321	0.003926	0.0016	0.0112	0.01944
CN (2)	3	0.002064	0.000475	0.00027	0.00088	0.00325
MM (2)	3	0.002029	0.000373	0.00022	0.0011	0.00296
PB (2)	6	0.00168	0.000154	0	0.00152	0.00184

Table 1.2d. Means and standard deviations of the first principal component of click features by site and click type

Site (click type)	N	Mean	Standard Deviation	Standard Error Mean	Lower 95%	Upper 95%
CN (1)	3	1.338	0.747265	0.43143	-0.518	3.194
MM (1)	3	1.7635	0.328772	0.18982	0.947	2.58
MF (1)	6	1.43	0.659745	0.26934	0.738	2.122
CN (2)	3	-1.5674	0.127317	0.07351	-1.884	-1.251
MM (2)	3	-1.7655	0.523273	0.30211	-3.065	-0.466
PB (2)	6	-1.3144	0.260549	0.10637	-1.588	-1.041

Table 1.3. Logistic regressions of morphological features. Asterisks after *P* values indicate models with a significant difference between the species in the feature.

Morphological feature	Model type	*A. c. crepitans* mean (SD)	*A. g. gryllus* mean (SD)	R^2	*P* value
snout-vent length (SVL)	overall	21.62 (1.09)	21.05 (1.1)	0.05	0.0009*
	allotopy	21.84 (1.04)	21.01 (1.2)	0.1	0.0001*
	syntopy	21.02 (0.94)	21.15 (0.91)	0	0.60
leg length/SVL	overall	0.98 (0.04)	1.01 (0.04)	0.06	0.0002*
	allotopy	0.98 (0.04)	1.01 (0.05)	0.05	0.0053*
	syntopy	0.97 (0.04)	1.01 (0.04)	0.13	0.0023*
upper thigh stripe/thigh length	overall	0.81 (0.06)	0.86 (0.05)	0.13	<0.0001*
	allotopy	0.8 (0.06)	0.86 (0.05)	0.16	<0.0001*
	syntopy	0.81 (0.06)	0.85 (0.05)	0.08	0.0213*
lower thigh stripe/thigh length	overall	0.70 (0.13)	0.69 (0.14)	0	0.81
	allotopy	0.66 (0.12)	0.70 (0.16)	0.01	0.173
	syntopy	0.80 (0.11)	0.68 (0.11)	0.19	0.0003*
lower/upper thigh stripe length	overall	0.87 (0.16)	0.8 (0.16)	0.03	0.0115*
	allotopy	0.83 (0.14)	0.81 (0.18)	0	0.47
	syntopy	0.98 (0.13)	0.80 (0.12)	0.33	<0.0001*
upper thigh stripe difference	overall	0.95 (0.37)	0.66 (0.29)	0.13	<0.0001*
	allotopy	0.96 (0.39)	0.63 (0.29)	0.18	<0.0001*
	syntopy	0.84 (0.29)	0.70 (0.3)	0.04	0.0945
lower thigh stripe difference	overall	1.12 (0.36)	0.82 (0.24)	0.14	<0.0001*
	allotopy	1.13 (0.35)	0.79 (0.22)	0.2	<0.0001*
	syntopy	1.06 (0.38)	0.87 (0.28)	0.06	0.0437*
anal tubercle size	overall	0.59 (0.22)	0.12 (0.17)	0.54	<0.0001*
	allotopy	0.60 (0.23)	0.15 (0.18)	0.5	<0.0001*
	syntopy	0.53 (0.17)	0.08 (0.16)	0.61	<0.0001*
4th toe webbing (3rd toe side)	overall	2.02 (0.14)	2.7 (0.28)	0.72	<0.0001*
	allotopy	2.02 (0.16)	2.72 (0.32)	0.64	<0.0001*
	syntopy	2.00 (0)	2.65 (0.23)	1	<0.0001*
4th toe webbing (5th toe side)	overall	2.39 (0.33)	2.99 (0.06)	0.57	<0.0001*
	allotopy	2.42 (0.34)	2.96 (0.18)	0.47	<0.0001*
	syntopy	2.31 (0.29)	3.00 (0)	0.89	<0.0001*
1st toe webbing	overall	1.01 (0.09)	1.39 (0.3)	0.42	<0.0001*
	allotopy	0.96 (0.23)	1.39 (0.33)	0.42	<0.0001*
	syntopy	1.02 (0.1)	1.38 (0.27)	0.41	<0.0001*

Table 1.4. Analyses of variance of morphological features. Asterisks after *P* values indicate levels of analysis with a significant difference between the species in the feature.

Morphological feature	Analysis level	R^2	adjusted R^2	DF	F ratio	*P* value
snout-vent length (SVL)	whole model	0.42	0.31	171	3.82	<0.0001*
	Species			1	18.13	<0.0001*
	Site [Species]			26	3.37	<0.0001*
leg length/SVL	whole model	0.45	0.34	171	4.33	<0.0001*
	Species			1	18.67	<0.0001*
	Site [Species]			26	3.70	<0.0001*
upper thigh stripe/thigh length	whole model	0.37	0.25	171	3.15	<0.0001*
	Species			1	37.27	<0.0001*
	Site [Species]			26	1.87	0.0111*
lower thigh stripe/thigh length	whole model	0.34	0.21	171	2.71	<0.0001*
	Species			1	0.44	0.5091
	Site [Species]			26	2.81	<0.0001*
lower/upper thigh stripe length	whole model	0.37	0.26	171	3.19	<0.0001*
	Species			1	3.82	0.0527
	Site [Species]			26	2.98	<0.0001*
upper thigh stripe difference	whole model	0.31	0.18	171	2.35	0.0007*
	Species			1	31.51	<0.0001*
	Site [Species]			26	1.27	0.1866
lower thigh stripe difference	whole model	0.3	0.16	171	2.24	0.0013*
	Species			1	29.94	0.0001*
	Site [Species]			26	1.05	0.42
anal tubercle size	whole model	0.73	0.68	171	14.41	<0.0001*
	Species			1	239.44	<0.0001*
	Site [Species]			26	3.89	<0.0001*
4th toe webbing (3rd toe side)	whole model	0.8	0.76	170	21.46	<0.0001*
	Species			1	438.30	<0.0001*
	Site [Species]			26	2.43	0.0005*
4th toe webbing (5th toe side)	whole model	0.69	0.63	165	11.87	<0.0001*
	Species			1	193.17	<0.0001*
	Site [Species]			25	2.93	<0.0001*
1st toe webbing	whole model	0.69	0.63	169	11.76	<0.0001*
	Species			1	160.99	<0.0001*
	Site [Species]			26	5.33	<0.0001*

Table 1.5. Assessment of total variance (REML method) in morphological features. Variations in anal tubercle size and toe webbing were strongly associated with species.

Morphological feature	Analysis level	R^2	Variance component	% of total variance
snout-vent length (SVL)	Species	0.38	0.19092	13.68
	Site[Species]		0.34837	24.97
	Residual		0.85592	61.35
leg length/SVL	Species	0.42	0.00028	12.44
	Site[Species]		0.00068	29.65
	Residual		0.00132	57.90
upper thigh stripe/thigh length	Species	0.31	0.00161	30.45
	Site[Species]		0.00049	9.16
	Residual		0.0032	60.39
lower thigh stripe/thigh length	Species	0.29	-0.00036	0.00
	Site[Species]		0.00386	21.22
	Residual		0.01432	78.78
lower/upper thigh stripe length	Species	0.33	0.00044	1.78
	Site[Species]		0.00595	24.04
	Residual		0.01836	74.18
upper thigh stripe difference	Species	0.21	0.04571	27.83
	Site[Species]		0.00548	3.34
	Residual		0.11305	68.83
lower thigh stripe difference	Species	0.18	0.04239	28.95
	Site[Species]		0.00095	0.65
	Residual		0.10308	70.40
anal tubercle size	Species	0.72	0.10216	69.87
	Site[Species]		0.01521	10.40
	Residual		0.02883	19.72
4th toe webbing (3rd toe side)	Species	0.79	0.22775	83.70
	Site[Species]		0.0094	3.45
	Residual		0.03495	12.84
4th toe webbing (5th toe side)	Species	0.67	0.16973	68.39
	Site[Species]		0.02095	8.44
	Residual		0.05752	23.17
1st toe webbing	Species	0.68	0.08895	56.43
	Site[Species]		0.03156	20.02
	Residual		0.03711	23.54

Table 1.6. Analysis of variance of the first principal component of morphological features by species.

R^2	0.82
DF (Species)	1
DF (Error)	161
F ratio	742.45
Prob > F	<.0001

	N	Mean	Std Error
A. c. crepitans	101	-1.1827	0.07038
A. g. gryllus	62	1.9267	0.08982

Figure 1.1. *Acris* survey sites in North Carolina

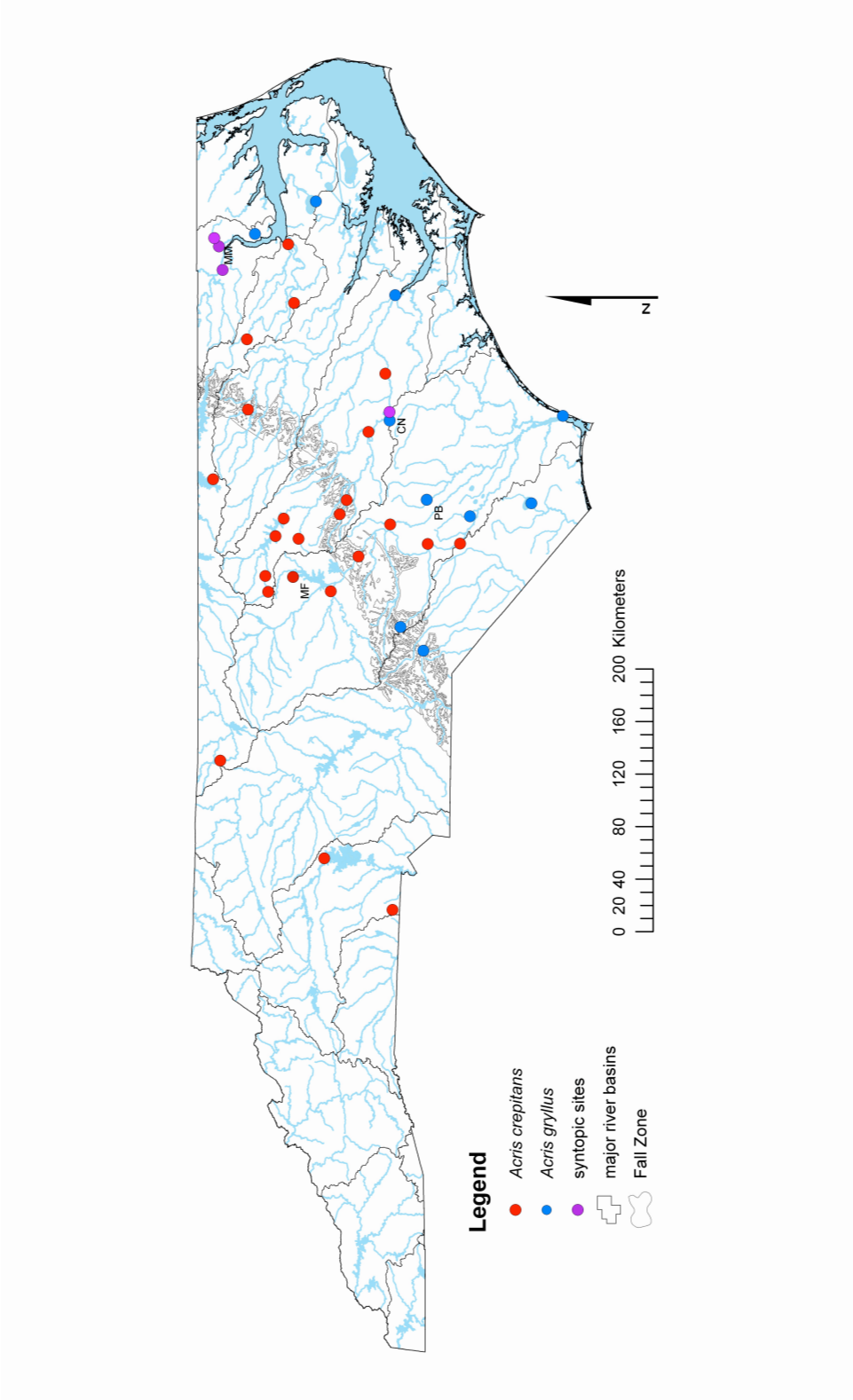


Figure 1.2. Representative clicks of *Acris c. crepitans* and *A. g. gryllus* in North Carolina

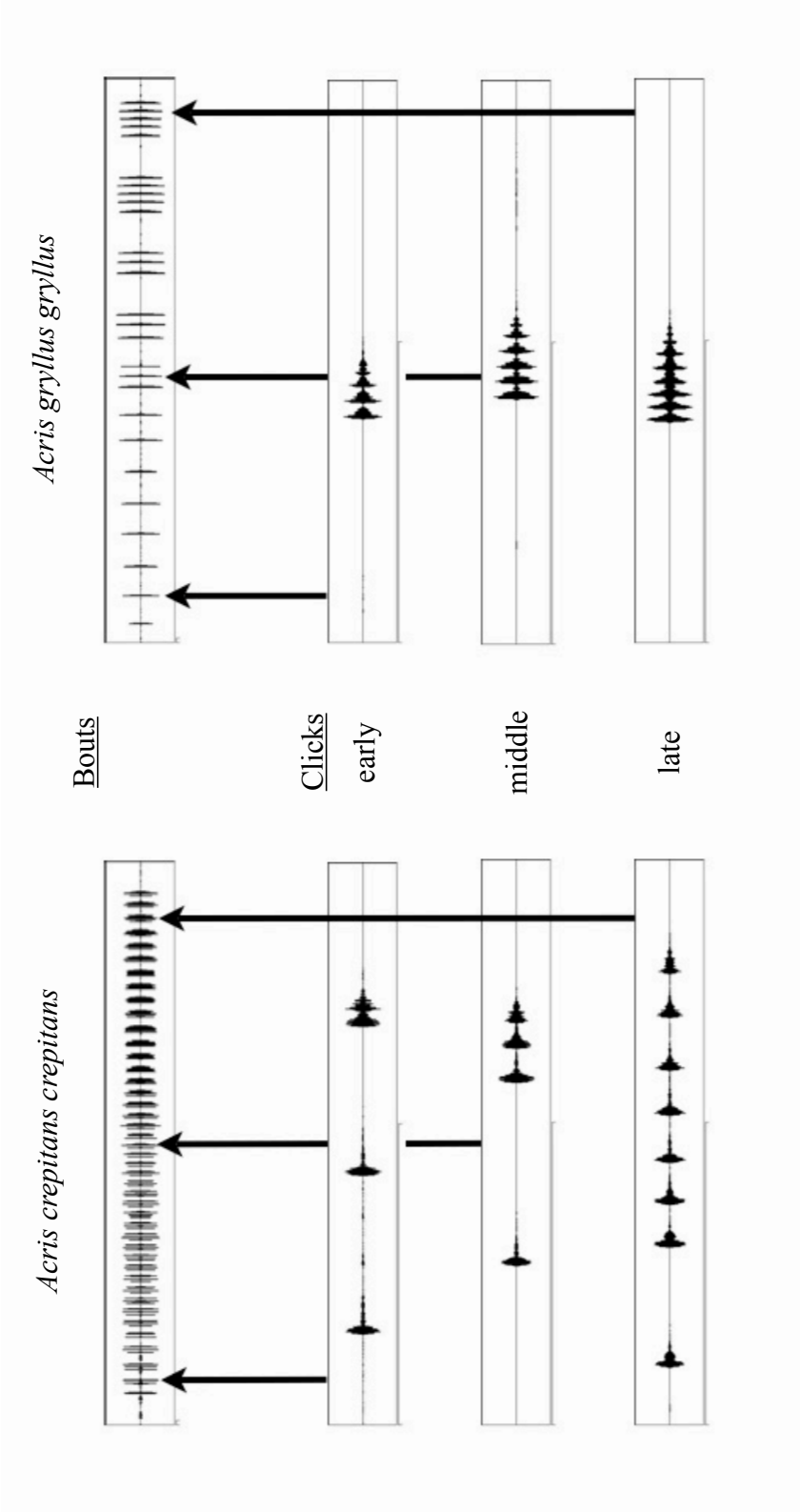


Figure 1.3a. Click duration by site and click type. Each point represents a bout. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations.

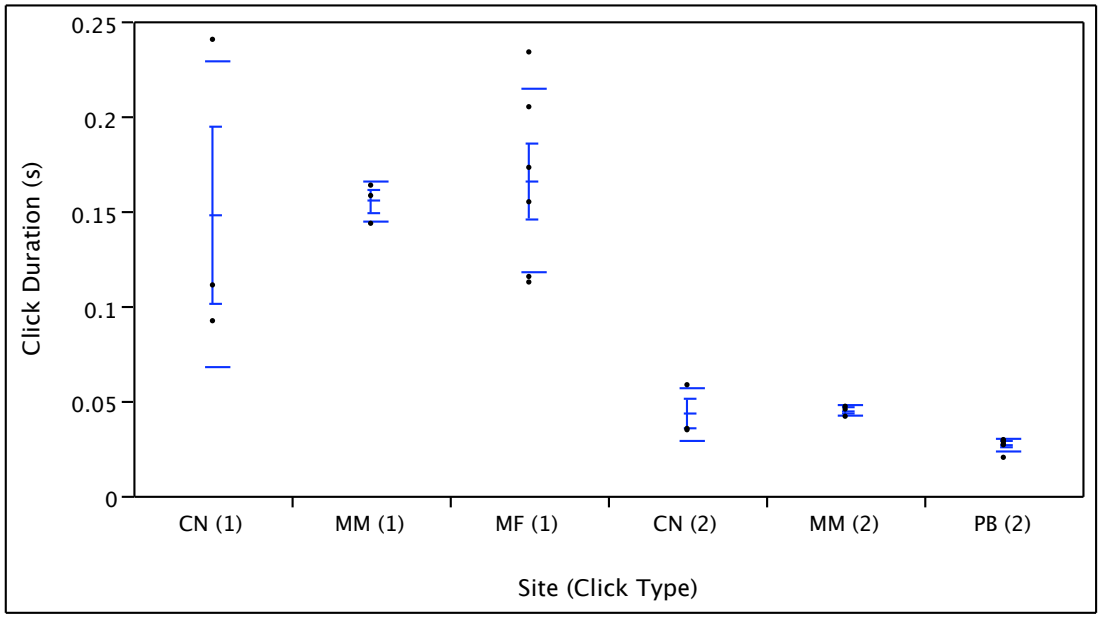


Figure 1.3b. Total pulses per click by site and click type. Each point represents a bout. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations.

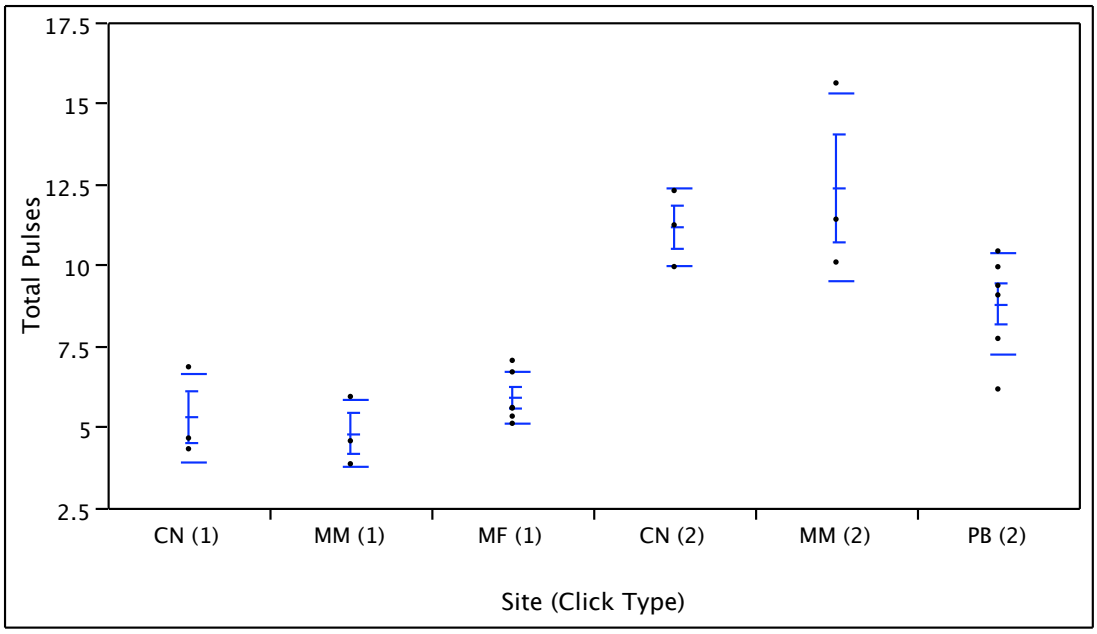


Figure 1.3c. Inter-pulse interval by site and click type. Each point represents a bout. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations.

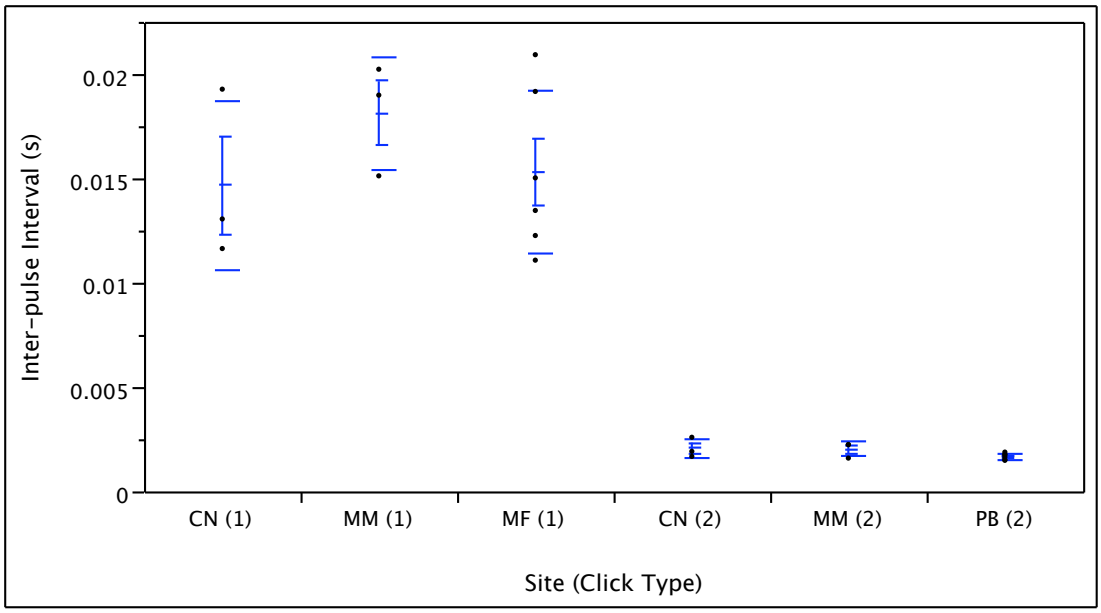


Figure 1.3d. First principal component of click features by site and click type. Each point represents a bout. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations.

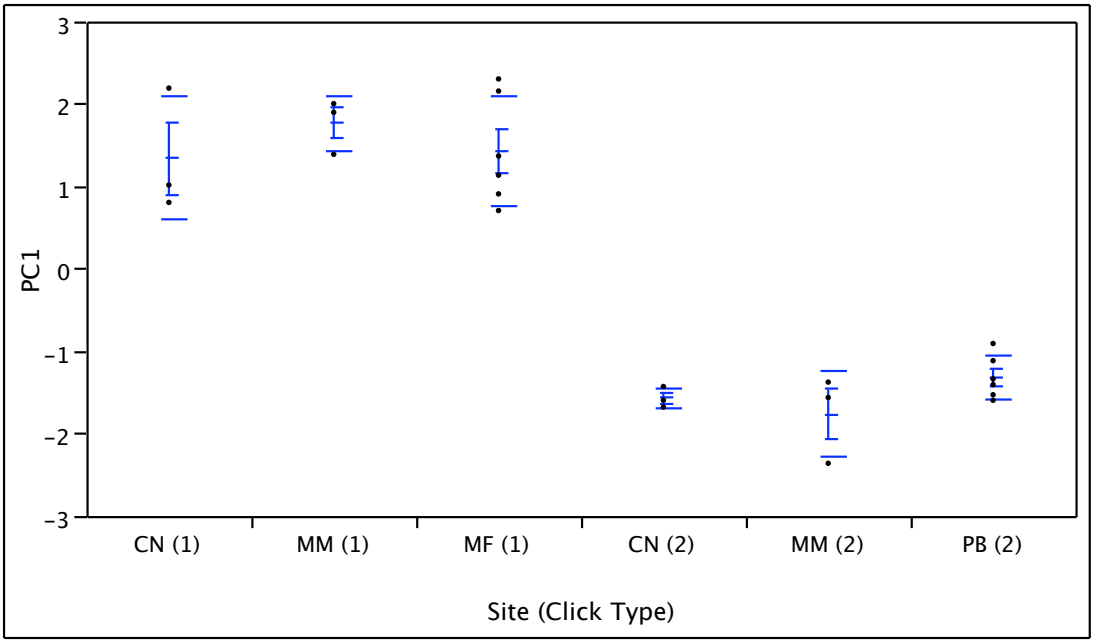
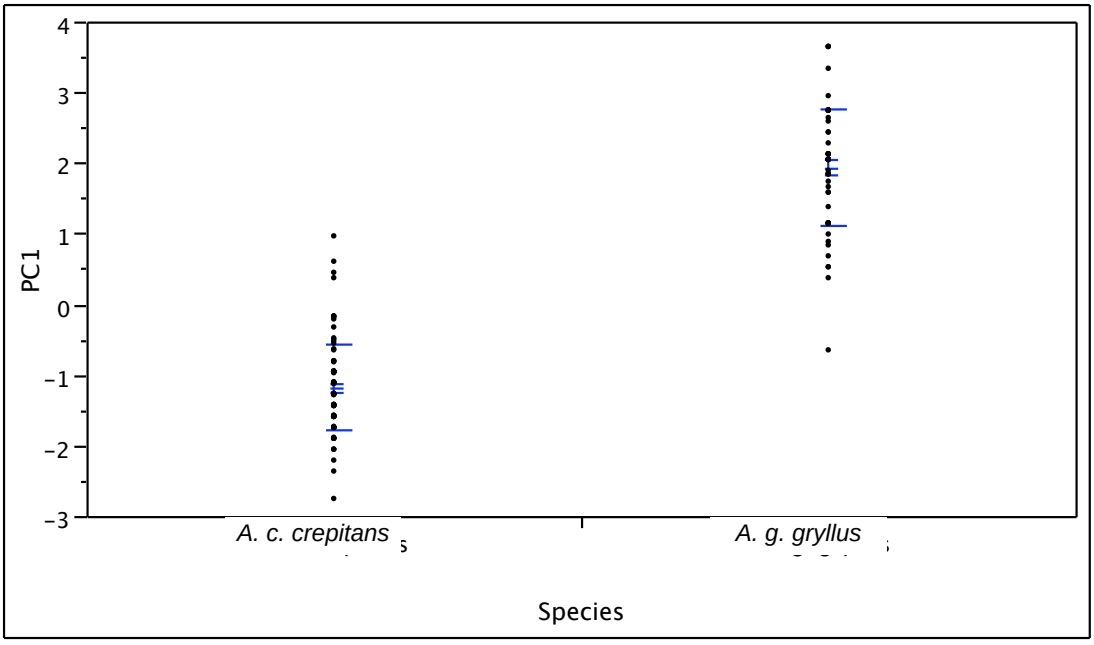


Figure 1.4. First principal component of morphological features by species. Each point represents a frog. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations.



Chapter 2

Acoustic variation among breeding sites and the influences of temperature and mass in the Eastern Cricket Frog, *Acris crepitans crepitans*

Abstract

Reproductive character displacement occurs when selection for recognition of conspecifics produces greater differences in systems for mate recognition in an area where two species are in contact than in areas where they are not. Detection of reproductive character displacement is complicated by the effects of temperature and body size on mating signals and the geographic scale at which populations are studied. I assessed variation in vocalizations of *A. c. crepitans,* the Eastern Cricket Frog, at a geographic scale appropriate for reproductive character displacement in order to determine the effects of temperature and body size. Among 27 sites in the Coastal Plain and Piedmont of North Carolina, the dominant frequency and click rate of vocalizations of *A. c. crepitans* varied substantially. Most of this variation was correlated with differences in mass, not temperature. Additional differences that were not explained by mass or temperature existed among sites. Possible explanations for this additional variation include differences among sites in the level of male competition or reproductive character displacement in response to sympatry or syntopy with *A. g. gryllus*.

Introduction

Mate recognition systems, both the signals produced and the sensory mechanisms that receive them, often diverge and result in pre-mating reproductive isolation between related species (Coyne and Orr, 2004). Because selection for accurate mate recognition is greatest where two species occur together, mate recognition systems can differ more in sympatry than in allopatry, a situation called reproductive character displacement (Brown and Wilson, 1956).

Divergence of mating signals or sensory reception in sympatry can have other explanations besides selection for accurate mate recognition. Allopatric environmental selection on mate recognition systems or on morphological traits that influence signaling or reception, as well as genetic drift, can also cause divergence. Therefore, identifying character displacement in natural populations is difficult, and it is particularly important to assess spatial variation precisely (Goldberg and Lande, 2006). Of particular concern are environmental clines that can produce differences in sympatry that resemble differences resulting from character displacement, or alternatively, produce great differences in allopatry that mask slight differences in sympatry that result from character displacement (Goldberg and Lande, 2006). Signals can diverge as a result of geographic gradients in environmental conditions that directly affect signals, such as temperature (Gerhardt, 1978), or that indirectly affect signals by influencing morphological traits like body size (Ryan et al., 1990; Hobel and Gerhardt, 2003). Detection of reproductive character displacement is thus dependent on concurrent assessment of other biotic and abiotic factors that may influence mate recognition systems. Another important consideration that receives less attention in character

displacement studies is the scale of geographic assessment of sympatry between species with continuous (non-disjunct) ranges. Rivas (1964) distinguished between overlap between species in geographic distribution (sympatry) and in macrohabitat (syntopy); the latter occurs at a smaller scale at which the species could possibly interbreed. Many studies assess reproductive character displacement on large scales, familiar from range maps, which are often too coarse to indicate the actual chances of individual interactions. Detection of reproductive character displacement would be improved by comparing mate recognition systems in syntopy and allotopy.

The acoustic communication system of cricket frogs, *Acris*, has been thoroughly studied in the midwestern United States, where two subspecies, *A. c. crepitans* and *A. c. blanchardi*, have parapatric (adjoining but non-overlapping) ranges. Advertisement calls differ among populations and subspecies (Ryan et al., 1990). The dominant frequency of male vocalizations (Ryan and Wilczynski, 1991) and the frequency response of the ear (Wilczynski, et al., 1992) change gradually in a cline across the line of contact. Divergence in mate recognition systems in these populations could result from allopatric selection on vocalizations for increased transmission in different habitats, rather than indirectly from selection on body size or directly from reinforcement in sympatry (Nevo and Capranica, 1985; Ryan and Wilczynski, 1988; Ryan et al., 1990; Sun et al., 2000; Witte et al., 2005). Studies of reproductive character displacement in these western *Acris* are limited because the subspecies do not overlap sufficiently to allow comparisons of populations in sympatry and allopatry. In their study of acoustic variation in *A. crepitans* on a continental scale, Nevo and Capranica (1985) compared this species with the other species of cricket frog, *A. gryllus*,

which overlaps *A. c. crepitans* in the southeastern United States (Conant and Collins, 1991). In *A. crepitans,* body size, environmental factors including temperature, and geographic distance all correlated with temporal and spectral features of vocalizations. They concluded that divergence in the mate recognition systems of different populations of the two species resulted from allopatric selection rather than selection in sympatry against heterospecific mating. However, the study compared geographic variation on a scale far too coarse to assess reproductive character displacement. Indeed, their study included only one syntopic site. Reproductive character displacement between *A. crepitans* and *A. gryllus* might have been obscured by large ecological differences among sites. In North Carolina, allotopic and syntopic breeding choruses of *A. crepitans* and *A. gryllus* are separated only a few kilometers (Chapter 1).

Chapter 1 describes the extent of sympatry between *Acris crepitans crepitans,* the Eastern Cricket Frog, and *Acris gryllus gryllus*, the Coastal Plain Cricket Frog, in North Carolina and identifies syntopy at breeding sites in the upper Coastal Plain. In this study, I assess geographic variation in the dominant frequency and rate of vocalizations of *A. c. crepitans* in relationship to temperature and body size. Chapter 3 discusses variation in these features in *A. g. gryllus* and compares populations of the two species in allopatry, sympatry, and syntopy to determine whether reproductive character displacement contributes to acoustic divergence between species of *Acris*.

Methods

Field survey

I recorded *A. c. crepitans* at 27 sites in the Piedmont and Coastal Plain of North Carolina in 2004, 2005, and 2007 (Figure 2.1). At all but two sites, I collected and preserved frogs after recording them. Recording took place from 4 May to 21 July 2004 (13 sites), 8 May to 24 July 2005 (14 sites including 2 from 2004), and 3-4 June and 15 July 2007 (2 sites). Three sites were in the western Piedmont, 12 sites were in the eastern Piedmont (including 4 sites just west of the Fall Zone), and 12 sites were in the upper and lower Coastal Plain. At each site were 1-5 permanent bodies of freshwater with choruses of cricket frogs. At each chorus, I recorded 6-10 frogs for 10 bouts each. I reduced the number of frogs collected when the chorus was too small to remain active otherwise. Recording began at or after 2100 hours and ended when a chorus waned (between 0100 and 0300 hours) or I had a complete sample. I used a Marantz PMD-221 or PMD-421 portable tape recorder (2004) or PMD-670 digital recorder (2005 and 2007) and an Audio-Technica 815a microphone to record each male. Immediately after recording, I photographed the male and any females and satellite males in obvious association with it with a Canon Powershot A80 4.1 MP digital camera. After capturing the male, I measured the surface temperature at its calling site with a Miller and Weber T-6000 fast-read cloacal thermometer. Within 12-36 hours after collection, I weighed each frog, euthanized it in a chlorotone solution, preserved a forefoot in a dimethyl sulfoxide and salt solution for genetic analysis, and fixed the frog for morphological study and deposition in the collection of the North Carolina Museum of Natural Sciences, Raleigh, North Carolina.

Acoustic analysis

Cricket frog sounds have complex temporal structure in which smaller components are repeated to produce larger patterns at several levels (see Figure 1.2 of the previous chapter). Wagner's (1989c) terminology for these components was based on terms from Nevo and Capranica (1985). I have modified Wagner's terminology to refer unambiguously to the structure of cricket frog vocalizations in North Carolina. The terms are pulse, pulse group, click, click group, bout, and episode, and they refer to temporal patterns of increasing duration. Pulses are typically less than 10 ms long. One or more pulses in succession constitute a pulse group. One or several pulse groups are a click 25-125 ms long (Ryan et al., 1995), the shortest component of easily resolvable by the human ear. A bout is one or more click groups lasting a few seconds to over a minute. An episode is the production of bouts over several minutes, preceded and succeeded by several minutes of silence. A male has many episodes in a night of calling. The vocalizations of *A. crepitans* and *A. gryllus* have different pulse patterns (Nevo and Capranica, 1985) that I used for species identification (Chapter 1). This study focuses on clicks and bouts, the two most prominent temporal patterns in *Acris* vocalizations.

I used WildSpectra (version 060125; Wiley, 2007) to digitize cassette recordings at a sampling rate of 22.05 kHz (2004) and to import WAV files recorded at 22.05 kHz (2005 and 2007). For this study, I included 275 male *A. c. crepitans*, identified by pulse pattern, from the 27 sites. I selected 5 bouts from each male with a random number generator (Haahr, 2007) unless < 5 bouts were available for analysis (1365 total bouts, 4.96 bouts per individual). Previous studies of *A. crepitans* sounds (Nevo and Capranica, 1985; Wagner,

1989a, 1989b, 1989c; Burmeister et al., 1999; Burmeister et al., 2002) have measured a sample of clicks from the beginning, middle, and end of each bout in order to assess variation within bouts. By using the SongSignatures procedure in WildSpectra, I could measure every click in a bout, use the data to calculate means for each bout overall, and retain data for future analysis of within-bout variation. The SongSignatures procedure visually indicates each note identified and produces a text file with spectral and temporal information for each note and for the entire selection. With this procedure, I measured the duration of each bout, the dominant frequency of each click in the bout, and the interval between each click. I then calculated the click rate, mean dominant frequency and mean interval between clicks for each bout. To confirm the accuracy of the measurements made by SongSignatures, I manually measured the duration and overall dominant frequency of 19 randomly-selected bouts on spectrograms with the same resolutions. In addition, I manually counted the number of clicks in each bout before using the SongSignatures procedure.

I emphasized frequency resolution (21 Hz) at the expense of temporal resolution (46.43 milliseconds) by using a sampling rate of 11.025 Khz and a transform size of 1024. This temporal resolution was sufficient for detecting temporal variation in bouts. SongSignatures recognizes each "note" in a selected portion of a spectrogram with user-defined starting and ending thresholds of the power spectrum. For each bout, I adjusted the amplitude thresholds to optimize detection of clicks. Perfect detection of clicks occurred when all clicks in the bout were recognized as distinct notes. This was difficult for some *A. crepitans* bouts because the high click rates and multiple pulse groups within each click caused Song Signatures to mistakenly identify and assess multiple clicks as one note. For

these bouts, I lowered the starting amplitude threshold and used WildSpectra's Exclude Selection procedure to remove background noise between some clicks, particularly in the last third of the bout. With these adjustments, I could obtain perfect detection of clicks.

Previous studies of *Acris* (Nevo and Capranica, 1985; Wagner, 1989c; Ryan and Wilczynski, 1991) have used linear regression to adjust acoustic features for the effects of temperature. For studies with large samples and wide ranges of temperature such as Wagner (1989c), linear regression for each site can accurately describe temperature-dependent variation. For studies with small sample sizes and narrow temperature ranges at each site, such as Nevo and Capranica (1985) or the present study, it is less satisfactory. Furthermore, regressions on temperature can obscure the influence of other factors on properties of vocalizations. For instance, body size has an important influence on acoustic features in *A. crepitans* (Ryan and Wilczynski, 1988; McClelland, et al., 1996), and calling site temperature and body size of *Acris* can change over the long breeding season. Therefore, rather than control for relationships that are more complex than a linear regression can describe, I used a statistical approach to assess the relative influences of multiple factors, including temperature and mass, without a priori adjustment for any of them.

Analyses of variance (ANOVA) of 5 measurements of bouts (mean dominant frequency, total duration, total clicks, click rate, and mean interval between clicks) and linear regressions of dominant frequency and click rate with temperature and mass were computed with JMP 6.03 (SAS Institute, 2006). To include differences in temperature, body size, and time in the breeding season, I relied on nested ANOVA to assess the importance of all of these factors on click rate and dominant frequency. These analyses of variance were

computed in two ways: either with individual frogs as the most nested factor or with individual's mass and temperature as continuous covariates. Because conditions changed over the breeding season and I visited most sites only once, I nested sites within months and then individual (or temperature and mass covariates) within sites to compare sites within months. I calculated the variance components for random effects with the restricted maximum likelihood method (REML, Quinn and Keough, 2002; SAS Institute, 2006). From the results of these tests, I could determine the statistical significance of the overall model, as well as the contributions of each variance component to the total variance in each acoustic feature.

To illustrate the effects of mass and temperature on vocalizations, I calculated linear regressions of dominant frequency and mean click rate by temperature and mass using pooled data from the entire survey. I used the regression equations for temperature to adjust dominant frequency and click rate to a mean temperature of 22.5° to compare site differences with the effect of temperature removed.

Results

There was significant variation within months as well as among months, sites, and individuals for all acoustic features measured (Table 2.1). Mass and temperature at calling sites also varied significantly among months and sites. Intervals between clicks, numbers of clicks, and durations of bouts are interrelated temporal features contributing to click rate. Nested ANOVAs for these variables had only slightly lower coefficients of determination (R^2) than click rate itself, so I do not present further analyses of these features. Differences

among sites in click rate and dominant frequency (Table 2.2a and 2.2b; Figure 2.2a and 2.2b) are likely to have been partly determined by differences in mass and temperature (Table 2.2c and 2.2d; Figure 2.2c and 2.2d), which in turn might have resulted from recording in different months. Temperature at calling sites increased significantly (nested ANOVA, d.f. = 2, $p < 0.0001$) from May (20.3°) to June (23.0°) to July (25.9°), was significantly different among sites in each month (nested ANOVA, d.f. = 28, $p < 0.0001$), and was particularly variable among sites in May (12.2-24.3°). Mean body mass also changed significantly (nested ANOVA, d.f. = 2, $p = 0.0004$) from May (1.06) to June (1.13) to July (1.01) and varied among sites in each month (nested ANOVA, d.f. = 26, $p < 0.0001$; May, 0.85-1.26 g; June, 0.92-1.28 g; July, 0.80-1.22 g)

To estimate variance components, I used nested analyses of variance with random effects for dominant frequency and click rate (Table 2.3a and 2.3b). For both acoustic features, higher R^2 occurred in analyses with individual as the terminal effect (Table 2.3a), rather than with temperature and mass as covariates (Table 2.3b). Because each individual has a single measurement of mass and temperature, the greater R^2 presumably results from additional variation among males not related to differences in mass and temperature. The largest percentage of the total variance was attributed to mass in analyses of dominant frequency (85.2%) and click rate (55.0%). Temperature and month contributed little to the total variance. Differences among sites contributed more to total variation (dominant frequency, 8.4%; click rate, 15.0%) than did temperature differences (dominant frequency, 0.7%; click rate, 2.3%). Residual differences contributed more to variation in click rate

(27.7%) than any factors other than mass. Month had inconsequential effects on either acoustic feature (dominant frequency, 1.9%; click rate, 0.0%).

In the linear regressions (Table 2.4 and Figure 2.3a to 2.3d), there were substantial positive relationships between the temperature at a calling site and click rate (Figure 2.3a; adjusted $R^2 = 0.32$, d.f. = 1365, $p < 0.0001$) and dominant frequency (Figure 2.3b; ANOVA: adjusted $R^2 = 0.32$, d.f. = 1365, $p < 0.0001$) and and significant negative relationships between mass and click rate (Figure 2.3c; adjusted $R^2 = 0.05$, d.f. = 1282, $p < 0.0001$) and dominant frequency (Figure 2.3d; adjusted $R^2 = 0.34$, d.f. = 1282, $p < 0.0001$). Mean click rate and dominant frequency at each site are shown adjusted to the study's mean temperature of 22.5° C in Table 2.5a and 2.5b and Figure 2.4a and 2.4b.

Discussion

Acoustic variation in North Carolina

Statistically significant differences occurred in the dominant frequency and click rate of *A. c. crepitans* vocalizations at 27 sites separated by a maximum of 530 km in the Coastal Plain and Piedmont of North Carolina (Table 2.1 to 2.2b; Figure 2.2a and 2.2b). The range of mean dominant frequency among sites in North Carolina (3428-4132 Hz; 3542-4031 Hz after temperature correction to 22.5°) was not as large as Nevo and Capranica (1985) noted throughout the range of *A. crepitans* in eastern North America (2995-4484 Hz, 3164-4254 Hz after correction to 22.79°) but was equivalent to the range among their 5 southeastern sites for *A. c. crepitans* from eastern Texas to eastern Georgia (3972-4485 Hz; 3749-4254 Hz after correction to 22.79°). The range of click rates within North Carolina (1.9-5.1 clicks/s;

2.9-4.9 clicks/s after temperature correction to 22.5°) was almost as large as they found throughout North America (2.3-5.7 clicks/s; 2.8-5.4 clicks/s after temperature correction to 22.79°) and larger than they observed among their southeastern sites for *A. c. crepitans* (3.5-5.4 clicks/s; 3.8-4.6 clicks/s after temperature correction to 22.79°). Ryan and and Wilczynski (1991) conducted a study of acoustic variation in *A. c. crepitans* and *A. c. blanchardi* in which 16 of 17 sites were located along a 500 km longitudinal transect in the eastern half of Texas. Among these sites, temperature-adjusted mean dominant frequency ranged from 3520 to 3990 Hz and varied in a longitudinal cline with lower dominant frequencies in the *A. c. blanchardi* populations to the west and higher dominant frequencies in the *A. c. crepitans* populations to the east. Among the 6 *A. c. crepitans* populations, mean dominant frequency again ranged from 3520 to 3990 Hz and click rate ranged from 4.03 to 6.25 clicks/s. Because of the variance in each population in Texas and North Carolina, *A. crepitans* in the two states have equivalent highest and lowest mean dominant frequencies and click rates across a similar geographic scale of 500 km. Therefore, dominant frequency and click rate among *A. c. crepitans* populations vary as extensively in North Carolina as throughout the Southeast or in Texas.

Differences in mass (Table 2.2c and Figure 2.2c) accounted for most of the acoustic variation in *A. c. crepitans* within breeding choruses (Table 2.3b) in North Carolina. Temperature at calling site varied widely (Table 2.2d and Figure 2.2d), undoubtedly resulting from the long breeding season and variety of habitats (from small rivers to large lakes) of *A. c. crepitans*. Temperature was significantly correlated with dominant frequency and click rate, but had only a slight effect in comparison with mass (Table 2.3b). After adjusting

dominant frequency and click rate to a mean temperature (Table 2.5a and 2.5b; Figure 2.4a and 2.4b), differences between adjacent sites within river systems usually remained. Therefore, differences in temperature between individuals and sites in a study of this scale did not greatly affect variation in vocalizations.

The seasonal changes in mass reflected the mean age or condition of males. In May, calling males were likely to be individuals which had over-wintered from the previous year. The slight increase in mass by June could have resulted from smaller males with lower energetic reserves abandoning calling activity after a few weeks or, because of a seasonal increase in prey availability, an increase in the mass of most males. The large decrease in mean mass in July probably resulted from the sudden addition of small, newly-metamorphosed males to the chorus. Despite the seasonal shift in body size, differences in mean mass were greater among sites in the same month than within months. Consequently, seasonality accounts for little of the differences in vocalizations among sites while non-seasonal differences in mass contribute substantially to vocal variation among sites. Variation among sites was determined mostly by differences in mass.

A. c. blanchardi facultatively decrease dominant frequency (Wagner, 1989a, 1992; Burmeister et al., 1999) and click rate of calls (Wagner, 1989b, 1989c) in response to a perceived increase in proximity of neighboring males (and increased competition as a result). Such facultative shifts in behavior might explain the differences between individuals and sites not explained by mass and temperature in the present study. The number and density of males and the intensity of calling varied substantially among sites. Many sites, most notably Merchants Millpond State Park (MeMi), contained large, high-density choruses in which

competition was undoubtedly high for physical position, acoustic space, and access to females. Facultative shifts in calling behavior would be expected in these circumstances. At a few sites, particularly Hanging Rock State Park (HaRo) and Hares Millpond (HARE), the few frogs in a chorus were widely separated so competition was probably low. The remaining choruses, though not large in extent or numbers, had males in close proximity to one another. If competition at the majority of sites was similar, interactions among males would not explain the additional variation among sites in dominant frequency and click rate. Among other possible differences between populations that might produce differences in vocalizations is overlap with a closely related species.

Geographic scale in studies of acoustic variation

Reproductive character displacement is a possible explanation for acoustic divergence only in areas where the species or subspecies in question could have interacted reproductively with a related species. At larger scales, the effects of reproductive character displacement could be masked by environmental gradients (Goldberg and Lande, 2006). Because of these limitations, studies of reproductive character displacement must assess variation at a small geographic scale. Comparison should be made among populations with enough spatial separation that they differ in their interaction with a related species but are not so spatially removed that large environmental differences also occur among sites. Syntopy, in which individuals of two species co-occur at a locality and could possibly interbreed (Rivas, 1964), rather than sympatry, as indicated by a general overlap in the ranges of two species, is more biologically relevant for reproductive character displacement.

Nevo and Capranica (1985) conducted a continental study of widely separated populations and included only one syntopic site (between *A. crepitans* and *A. gryllus* in Alabama). They concluded that environmental selection in allopatry, not reproductive character displacement, accounted for acoustic divergence in *Acris.* However, their scale of assessment was probably too large to test reproductive character displacement as an influence on divergence between *A. crepitans* and *A. gryllus*. Not only could environmental selection have masked reproductive character displacement, but reproductive character displacement would probably have occurred at much smaller scale than they examined. In North Carolina, *A. c. crepitans* and *A. g. gryllus* occur in syntopy at few sites within a large area of sympatry (Chapter 1). Allotopic sites of one or the other species were only a few kilometers away. Reproductive character displacement in *Acris* could plausibly occur at syntopic sites relative to allotopic sites at this scale.

Ryan and Wilczynski (1991) intended to address acoustic divergence between *A. c. crepitans* and *A. c. blanchardi* at a geographic scale that included populations that could interact reproductively. Reproductive character displacement and selection on body size were rejected as possible explanations for acoustic variation. While confirming that environmental selection in allopatry is an important influence in the divergence of *Acris* vocalizations, the study took place at a scale that, again, was too large to address reproductive character displacement as a possible influence. In their study, populations of *A. c. crepitans* and *A. c. blanchardi* were parapatric, not sympatric, were separated by at least 50 km, and occupied different habitats (forest and grassland, respectively), any of which could have precluded interactions between individuals of the two taxa. With no reproductive

interaction, reproductive character displacement cannot plausibly occur between subspecies of *A. c. crepitans* and *A. c. blanchardi* and testing the relative influences of allopatric and sympatric selection is impossible. Sympatry and syntopy between *A. crepitans* and *A. gryllus*, however, invites assessment of these influences. Ryan and Wilczynski (1991) avoided *A. c. crepitans* populations that potentially overlapped with *A. gryllus* because of potential reproductive character displacement between species of *Acris*.

This study assessed variation in dominant frequency and click rate in *A. c. crepitans* among many breeding populations, occurring in allopatry, sympatry, allotopy, and syntopy with *A. g. gryllus*, at a scale much smaller than Nevo and Capranica (1985) and equivalent to Ryan and Wilczynski (1991). The variation among populations in dominant frequency and click rate in North Carolina was at least as large the variation described by Nevo and Capranica (1985) among fewer populations over the entire Southeast and was equivalent to variation in an area of Texas where no sympatry occurs with other *Acris* taxa. Variation in mass accounted for most of the acoustic variation in North Carolina, but some size-independent variation between sites also occurred (Table 2.3b).

The dominant frequency and click rate of vocalizations of *A. c. crepitans,* the Eastern Cricket Frog, vary substantially in a small part of its geographic range. Most of this variation can be attributed to differences in mass. In contrast, the effect of temperature is slight. There are additional differences among sites that are not explained by mass or temperature. The level of competition between males might differ among populations, but it is unlikely to account for all of the differences. There is sufficient size-independent variation in

vocalizations of *A. c. crepitans* in North Carolina to investigate whether sympatry or syntopy with *A. g. gryllus* accounts for any of the variation within *A. c crepitans*. Reproductive character displacement might account for some of this additional variation, a possibility examined in Chapter 3.

Table 2.1 Nested analyses of variance of acoustic features, temperature, and mass

Feature	Summary of fit		Source	DF	F ratio	*P* value
mean dominant frequency (Hz)	R^2	0.93	Model	274	49.68	0.000
	adjusted R^2	0.91	Month	2	807.07	<.0001
	Root mean square error	65.90	Site[Month]	28	218.61	0.000
	Mean of response	3860.92	Indiv.[Site, Month]	244	23.76	<.0001
	Observations	1365	Error	1090		
click rate (N/s)	R^2	0.75	Model	274	11.69	<.0001
	adjusted R^2	0.68	Month	2	207.19	<.0001
	Root mean square error	0.65	Site[Month]	28	44.04	<.0001
	Mean of Response	4.02	Indiv.[Site, Month]	200	5.10	<.0001
	Observations	1365	Error	1090		
mean interclick interval (s)	R^2	0.52	Model	274	4.37	<.0001
	adjusted R^2	0.40	Month	2	45.26	<.0001
	Root mean square error	0.09	Site[Month]	28	13.57	<.0001
	Mean of Response	0.27	Indiv.[Site, Month]	244	2.99	<.0001
	Observations	1365	Error	1090		
clicks (N)	R^2	0.63	Model	274	6.86	<.0001
	adjusted R^2	0.54	Month	2	25.75	<.0001
	Root mean square error	11.86	Site[Month]	28	20.08	<.0001
	Mean of Response	35.00	Indiv.[Site, Month]	244	5.11	<.0001
	Observations	1365	Error	1090		
duration (s)	R^2	0.62	Model	274	6.62	<.0001
	adjusted R^2	0.53	Month	2	19.16	<.0001
	Root mean square error	3.68	Site[Month]	28	20.01	<.0001
	Mean of Response	9.32	Indiv.[Site, Month]	244	5.13	<.0001
	Observations	1365	Error	1090		
temperature at calling site (°C)	R^2	0.85	Model	30	47.70	<.0001
	adjusted R^2	0.84	Month	2	353.60	<.0001
	Root mean square error	1.48	Site[Month]	28	24.79	<.0001
	Mean of Response	22.48	Error	244		
	Observations	275				
mass (g)	R^2	0.44	Model	28	6.37	<.0001
	adjusted R^2	0.37	Month	2	8.16	0.0004
	Root mean square error	0.13	Site[Month]	26	6.06	<.0001
	Mean of Response	1.06	Error	229		
	Observations	258				

Table 2.2a. Mean click rate of *A. c. crepitans* at each site. Sites are grouped by river system. Upstream sites precede downstream sites in each system.

Site	N (bouts)	Mean	Standard Deviation	Standard Error Mean	Lower 95%	Upper 95%
HARE	13	3.99063	0.5819	0.16139	3.639	4.3423
MeMi	105	3.42242	1.00851	0.09842	3.2272	3.6176
GATE	50	3.90575	0.67228	0.09507	3.7147	4.0968
HaRo	10	2.85223	0.52512	0.16606	2.4766	3.2279
Kerr	45	4.57894	0.7181	0.10705	4.3632	4.7947
ROAN2	30	4.26342	0.78461	0.14325	3.9704	4.5564
ROAN1	45	5.09503	1.02237	0.15241	4.7879	5.4022
CASH	25	4.65047	1.0707	0.21414	4.2085	5.0924
HAL	40	4.49782	0.65753	0.10396	4.2875	4.7081
OcMo	30	4.57753	0.89854	0.16405	4.242	4.9131
EnRi	29	4.34694	0.80949	0.15032	4.039	4.6549
FaLa-RV	40	4.12938	1.02087	0.16141	3.8029	4.4559
FaLa-CC	44	4.47786	0.83063	0.12522	4.2253	4.7304
WBUm	165	4.25805	1.21332	0.09446	4.0715	4.4446
MCB1	50	3.22871	0.71534	0.10116	3.0254	3.432
MCB2	30	1.90413	0.60386	0.11025	1.6786	2.1296
WHP	45	3.50713	0.47837	0.07131	3.3634	3.6508
ClNe-syn	47	3.60478	1.17745	0.17175	3.2591	3.9505
NRFK	50	3.48765	0.9464	0.13384	3.2187	3.7566
MFBR	45	2.65282	0.89389	0.13325	2.3843	2.9214
TLCJ	75	3.50557	0.91826	0.10603	3.2943	3.7168
RaRo	105	4.80112	0.9604	0.09373	4.6153	4.987
GAIN	27	4.97919	1.08381	0.20858	4.5505	5.4079
TAHE	50	4.86466	1.06607	0.15076	4.5617	5.1676
RHOD	30	4.48626	0.82269	0.1502	4.1791	4.7935
LaNo	95	3.96749	1.06624	0.10939	3.7503	4.1847
CrMo	45	3.63942	0.99453	0.14826	3.3406	3.9382
Total	1365	4.01623	1.15947	0.03138	3.9547	4.0778

Table 2.2b. Mean dominant frequency of *A. c. crepitans* at each site. Sites are grouped by river system. Upstream sites precede downstream sites in each system.

Site	N (bouts)	Mean	Standard Deviation	Standard Error Mean	Lower 95%	Upper 95%
HARE	13	3681.46	54.522	15.122	3648.5	3714.4
MeMi	105	3948.26	214.223	20.906	3906.8	3989.7
GATE	50	3857.59	161.606	22.855	3811.7	3903.5
HaRo	10	3738.03	68.162	21.555	3689.3	3786.8
Kerr	45	3980.64	108.061	16.109	3948.2	4013.1
ROAN2	30	3752.84	112.333	20.509	3710.9	3794.8
ROAN1	45	4062.62	157.731	23.513	4015.2	4110
CASH	25	3960.19	108.327	21.665	3915.5	4004.9
HAL	40	4011.61	111.683	17.659	3975.9	4047.3
OcMo	30	3845.44	145.247	26.518	3791.2	3899.7
EnRi	29	3734.75	105.843	19.655	3694.5	3775
FaLa-RV	40	3761.58	94.534	14.947	3731.3	3791.8
FaLa-CC	44	3942.16	192.573	29.031	3883.6	4000.7
WBUm	165	3894.83	171.211	13.329	3868.5	3921.1
MCB1	50	3598.67	142.152	20.103	3558.3	3639.1
MCB2	30	3579.27	157.689	28.79	3520.4	3638.2
WHP	45	3644.91	79.527	11.855	3621	3668.8
ClNe-syn	47	3927.27	111.899	16.322	3894.4	3960.1
NRFK	50	3759.73	111.143	15.718	3728.1	3791.3
MFBR	45	3427.5	176.972	26.381	3374.3	3480.7
TLCJ	75	3837.5	140.327	16.204	3805.2	3869.8
RaRo	105	4091.55	132.056	25.414	4080	4184.5
GAIN	27	4132.23	132.056	25.414	4080	4184.5
TAHE	50	3975.67	200.97	28.421	3918.6	4032.8
RHOD	30	3949.68	111.386	20.336	3908.1	3991.3
LaNo	95	3800.96	125.529	12.879	3775.4	3826.5
CrMo	45	3727.38	112.157	16.719	3693.7	3761.1
Total	1365	3860.92	216.335	5.855	3849.4	3872.4

Table 2.2c. Mean mass of calling *A. c. crepitans* males at each site. Sites are grouped by river system. Upstream sites precede downstream sites in each system.

Site	N (males)	Mean	Standard Deviation	Standard Error Mean	Lower 95%	Upper 95%
MeMi	21	0.988	0.130	0.028	0.928	1.047
HaRo	2	0.980	0.141	0.100	-0.291	2.251
Kerr	9	1.066	0.136	0.045	0.961	1.170
ROAN2	6	1.222	0.089	0.036	1.128	1.315
ROAN1	9	1.084	0.095	0.032	1.011	1.158
CASH	5	0.924	0.124	0.056	0.770	1.078
HAL	8	0.978	0.161	0.057	0.843	1.112
OcMo	6	0.998	0.079	0.032	0.916	1.081
EnRi	6	1.280	0.115	0.047	1.160	1.400
FaLa-RV	7	1.264	0.142	0.054	1.133	1.396
FaLa-CC	9	1.121	0.186	0.062	0.978	1.264
WBUm	32	1.091	0.180	0.032	1.026	1.156
MCB1	10	1.185	0.084	0.027	1.125	1.245
MCB2	6	1.108	0.119	0.048	0.984	1.233
WHP	9	1.169	0.086	0.029	1.103	1.235
ClNe-syn	10	0.965	0.065	0.021	0.918	1.012
NRFK	10	1.146	0.082	0.026	1.087	1.205
MFBR	9	1.186	0.116	0.039	1.097	1.275
TLCJ	15	1.039	0.142	0.037	0.960	1.117
RaRo	19	0.849	0.152	0.035	0.776	0.922
GAIN	6	0.800	0.074	0.030	0.723	0.877
TAHE	10	0.991	0.147	0.047	0.886	1.096
RHOD	6	0.947	0.085	0.035	0.858	1.035
LaNo	19	1.049	0.132	0.030	0.985	1.113
CrMo	9	1.140	0.110	0.037	1.055	1.225
Total	258	1.056	0.169	0.010	1.035	1.077

Table 2.2d. Mean calling site temperature of *A. c. crepitans* at each site. Sites are grouped by river system. Upstream sites precede downstream sites in each system.

Site	N (males)	Mean	Standard Deviation	Standard Error Mean	Lower 95%	Upper 95%
HARE	3	26.50	0.95	0.55	24.13	28.87
MeMi	21	22.53	2.63	0.57	21.34	23.73
GATE	10	22.79	1.24	0.39	21.90	23.68
HaRo	2	20.80	1.13	0.80	10.64	30.97
Kerr	9	25.27	0.59	0.20	24.81	25.72
ROAN2	6	24.67	0.45	0.18	24.19	25.14
ROAN1	9	29.58	1.91	0.64	28.11	31.05
CASH	5	26.52	0.89	0.40	25.42	27.63
HAL	8	23.98	0.99	0.35	23.15	24.80
OcMo	6	20.77	0.50	0.20	20.25	21.29
EnRi	6	25.27	0.39	0.16	24.85	25.68
FaLa-RV	8	22.55	0.63	0.22	22.02	23.08
FaLa-CC	9	25.11	0.82	0.27	24.48	25.74
WBUm	33	23.21	1.54	0.27	22.67	23.75
MCB1	10	18.90	1.52	0.48	17.82	19.98
MCB2	6	16.90	3.06	1.25	13.69	20.12
WHP	9	19.47	0.57	0.19	19.03	19.91
ClNe-syn	10	20.12	3.18	1.00	17.85	22.39
NRFK	10	22.66	1.07	0.34	21.90	23.42
MFBR	9	12.20	3.50	1.17	9.51	14.89
TLCJ	15	19.09	1.59	0.41	18.21	19.97
RaRo	21	24.30	1.17	0.26	23.77	24.84
GAIN	6	26.50	0.53	0.22	25.94	27.06
TAHE	10	25.30	1.01	0.32	24.58	26.02
RHOD	6	27.93	1.53	0.63	26.33	29.54
LaNo	19	20.04	1.02	0.23	19.55	20.53
CrMo	9	20.42	0.72	0.24	19.87	20.98
Total	275	22.48	3.66	0.22	22.04	22.91

Table 2.3a: Variance components for dominant frequency and click rate (individual model)

	Summary of Fit		Random Effect	Variance Ratio	Variance Component	Standard Error	95% Confidence intervals lower	upper	% of Total Variance
Click Rate (clicks/s)	R^2	0.74	Month	0.47	0.20	0.24	-0.27	0.67	13.47
	Root MSE	0.65	Site[Month]	0.89	0.38	0.12	0.14	0.62	25.69
	Mean	4.02	Indiv.[Site,Month]	1.11	0.47	0.05	0.37	0.57	31.97
	Observations	1365	Residual		0.43	0.02	0.39	0.47	28.87
			Total		1.48				100.00
Dominant Frequency (Hz)	R^2	0.93	Month	1.92	8349.85	10206.22	-11654.34	28354.04	16.56
	Root MSE	65.89	Site[Month]	4.14	17966.41	5531.14	7125.37	28807.44	35.64
	Mean	3860.92	Indiv.[Site,Month]	4.55	19750.01	1862.43	16099.65	23400.38	39.18
	Observations	1365	Residual		4341.77	185.96	3999.15	4730.61	8.61
			Total		50408.04				100.00

Table 2.3b. Variance components for dominant frequency and click rate (mass and temperature model)

	Summary of Fit		Random Effect	Variance Ratio	Variance Component	Standard Error	95% Confidence intervals lower	upper	% of Total Variance
Click Rate (clicks/s)	R^2	0.51	Month	-0.05	-0.04				0.00
	Root MSE	0.85	Site[Month]	0.54	0.39	0.16	0.07	0.71	14.99
	Mean	4.02	Temp.[Site,Month]	0.08	0.06	0.02	0.02	0.10	2.27
	Observations	1282	Mass[Site,Month]	1.99	1.42	0.91	-0.37	3.21	55.03
			Residual		0.71	0.03	0.66	0.78	27.72
			Total		2.54				100.00
Dominant Frequency (Hz)	R^2								
	Root MSE	0.72	Month	0.50	6948.97	10236.32	-13114.21	27012.15	1.91
	Mean	118.25	Site[Month]	2.18	30492.34	9934.44	11020.83	49963.85	8.38
	Observations	3861.5	Temp.[Site,Month]	0.17	2405.49	908.60	624.63	4186.34	0.66
		1282	Mass[Site,Month]	22.16	309904.09	94844.73	124008.42	495799.76	85.20
			Residual		13983.26	575.00	12921.32	15182.39	3.84
			Total		363734.15				100.00

Table 2.4. Linear regression formulas for click rate and dominant frequency on temperature and mass, including summaries of fit and analyses of variance.

Regression	Summary of Fit		Analysis of variance	
Click Rate =	R^2	0.32	d.f.	1363
0.05 + (0.181 x temperature)	adjusted R^2	0.32	F ratio	652.5
	Root MSE	0.95	*P* value	0.0001
	Mean	4.02		
	Observations	1365		
Dominant Frequency =	R^2	0.32	d.f.	1363
3104.48 + (33.67 x temperature)	adjusted R^2	0.32	F ratio	646.45
	Root MSE	3861	*P* value	<0.0001
	Mean	3862		
	Observations	1365		
Click Rate =	R^2	0.05	d.f.	1281
5.325 - (1.519 x mass)	adjusted R^2	0.05	F ratio	62.45
	Root MSE	1.15	*P* value	< 0.0001
	Mean	4.02		
	Observations	1282		
Dominant Frequency =	R^2	0.34	d.f.	1281
4663.72 - (759.48 x mass)	adjusted R^2	0.34	F ratio	666.25
	Root MSE	177	*P* value	< 0.0001
	Mean	3862		
	Observations	1282		

Table 2.5a. Mean click rate (adjusted to 22.5° C) of *A. c. crepitans* at each site. Sites are grouped by river system. Upstream sites precede downstream sites in each system.

Site	N (bouts)	Mean	Standard Deviation	Standard Error Mean	Lower 95%	Upper 95%
HARE	13	3.23878	0.46776	0.12973	2.9561	3.5214
MeMi	105	3.41639	0.77405	0.07554	3.2666	3.5662
GATE	50	3.85326	0.57698	0.0816	3.6893	4.0172
HaRo	10	3.15993	0.64238	0.20314	2.7004	3.6195
Kerr	45	4.07817	0.69444	0.10352	3.8695	4.2868
ROAN2	30	3.87125	0.81287	0.14841	3.5677	4.1748
ROAN1	45	3.81395	1.09349	0.16301	3.4854	4.1425
CASH	25	3.92285	0.99772	0.19954	3.511	4.3347
HAL	40	4.23084	0.65146	0.10301	4.0225	4.4392
OcMo	30	4.89127	0.87908	0.1605	4.563	5.2195
EnRi	29	3.84825	0.80549	0.14958	3.5419	4.1546
FaLa-RV	40	4.12033	1.03790	0.16411	3.7884	4.4523
FaLa-CC	44	4.0048	0.85576	0.12901	3.7446	4.265
WBUm	165	4.1297	1.12712	0.08775	3.9564	4.303
MCB1	50	3.88031	0.69404	0.09815	3.6831	4.0775
MCB2	30	2.91773	0.49459	0.0903	2.7331	3.1024
WHP	45	4.05616	0.48772	0.0727	3.9096	4.2027
ClNe-syn	47	3.99721	0.75224	0.10973	3.7763	4.2181
NRFK	50	3.45869	0.85855	0.12142	3.2147	3.7027
MFBR	45	4.51712	0.45156	0.06731	4.3815	4.6528
TLCJ	75	4.12218	0.83759	0.09672	3.9295	4.3149
RaRo	105	4.47446	0.9436	0.09209	4.2918	4.6571
GAIN	27	4.26927	1.0716	0.20623	3.8454	4.6932
TAHE	50	4.35786	0.97413	0.13776	4.081	4.6347
RHOD	30	3.50282	0.87592	0.15992	3.1757	3.8299
LaNo	95	4.41237	1.04309	0.10702	4.1999	4.6249
CrMo	45	4.01550	0.94070	0.14023	3.7329	4.2981
Total	1365	4.11596	0.95383	0.02582	4.06532	4.16661

Table 2.5b. Mean dominant frequency (adjusted to 22.5° C) of *A. c. crepitans* at each site. Sites are grouped by river system. Upstream sites precede downstream sites in each system.

Site	N (bouts)	Mean	Standard Deviation	Standard Error Mean	Lower 95%	Upper 95%
HARE	13	3541.6	60.98	16.913	3504.8	3578.5
MeMi	105	3947.14	187.329	18.281	3910.9	3983.4
GATE	50	3847.83	162.821	23.026	3801.6	3894.1
HaRo	10	3795.27	92.105	29.126	3729.4	3861.2
Kerr	45	3887.48	102.124	15.224	3856.8	3918.2
ROAN2	30	3679.89	106.837	19.506	3640.0	3719.8
ROAN1	45	3824.31	175.139	26.108	3771.7	3876.9
CASH	25	3824.83	98.613	19.723	3784.1	3865.5
HAL	40	3961.95	130.553	20.642	3920.2	4003.7
OcMo	30	3903.8	152.566	27.855	3846.8	3960.8
EnRi	29	3641.98	111.259	20.66	3599.7	3684.3
FaLa-RV	40	3759.9	84.506	13.362	3732.9	3786.9
FaLa-CC	44	3854.16	192.73	29.055	3795.6	3912.8
WBUm	165	3870.95	163.922	12.761	3845.8	3896.2
MCB1	50	3719.88	124.514	17.609	3684.5	3755.3
MCB2	30	3767.82	213.947	39.061	3687.9	3847.7
WHP	45	3747.04	79.38	11.833	3723.2	3770.9
ClNe-syn	47	4000.27	111.444	16.256	3967.5	4033.0
NRFK	50	3754.34	93.886	13.277	3727.7	3781.0
MFBR	45	3774.3	244.253	36.411	3700.9	3847.7
TLCJ	75	3952.2	129.53	14.957	3922.4	3982.0
RaRo	105	4030.79	154.93	15.12	4000.8	4060.8
GAIN	27	4000.17	131.594	25.325	3948.1	4052.2
TAHE	50	3881.39	178.53	25.248	3830.7	3932.1
RHOD	30	3766.74	94.884	17.323	3731.3	3802.2
LaNo	95	3883.71	126.57	12.986	3857.9	3909.5
CrMo	45	3797.33	104.627	15.597	3765.9	3828.8
Total	1365	3877.90	178.273	4.825	3868.4	3887.4

Figure 2.1. Survey sites for *Acris c. crepitans* in North Carolina

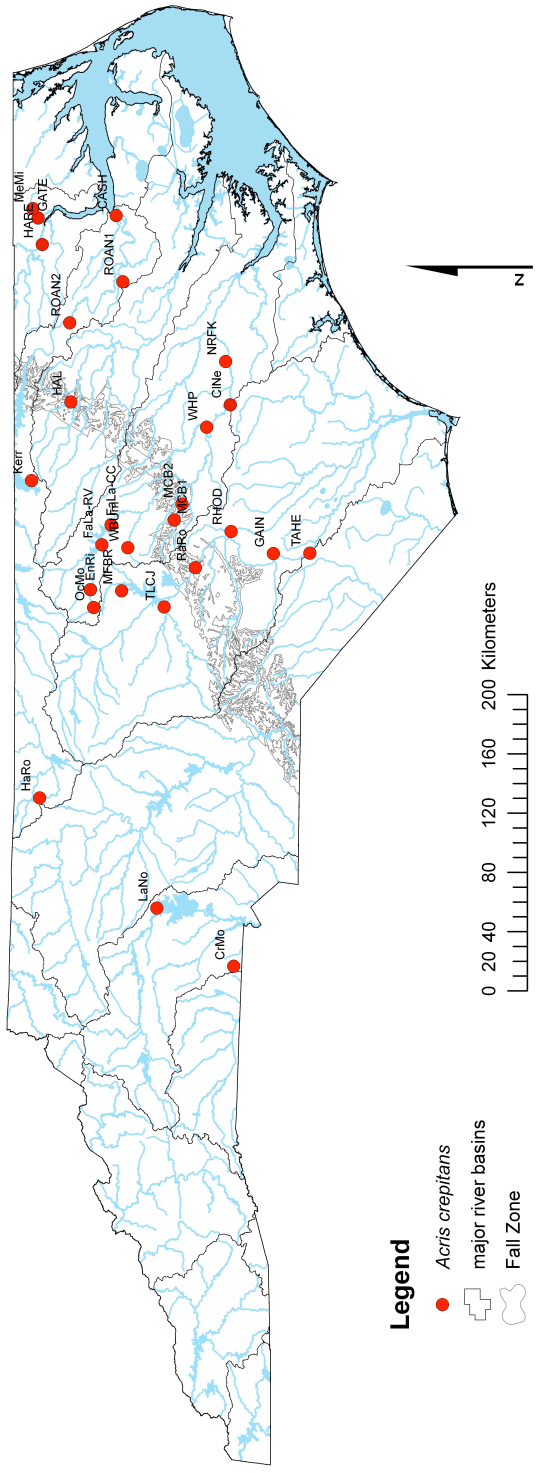


Figure 2.2a. Mean click rate of *A. c. crepitans* at each site. Each point represents a bout. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations. Sites are grouped geographically by river system. Within each system, the site furthest upstream is on the left and the site furthest downstream is on the right.

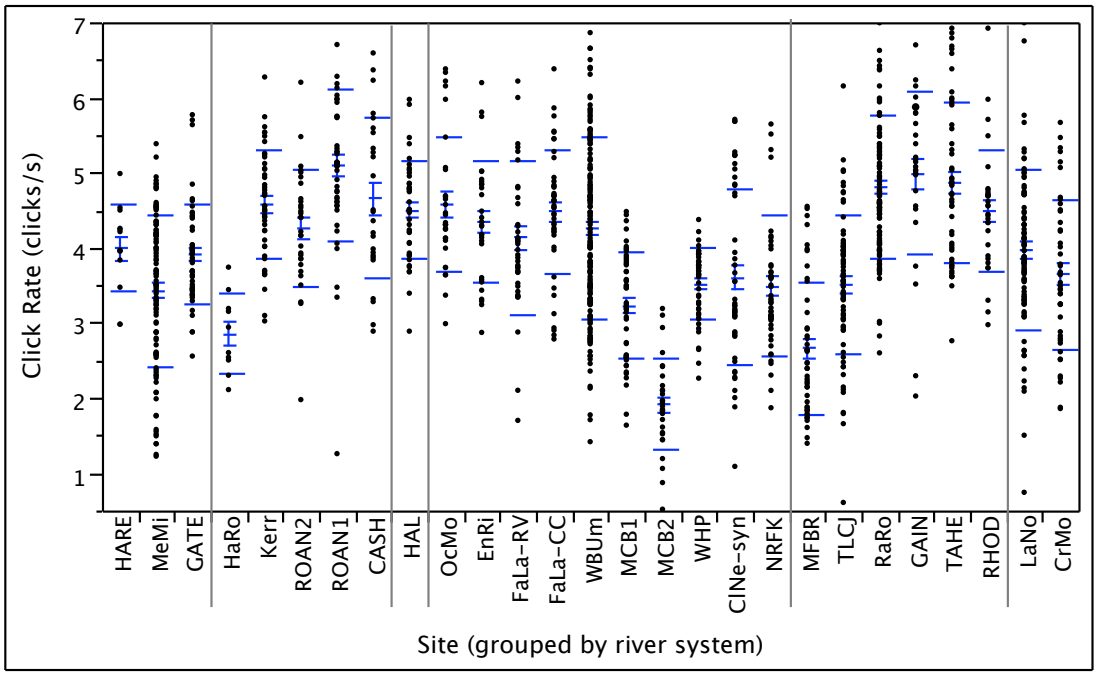


Figure 2.2b. Mean dominant frequency of *A. c. crepitans* at each site. Each point represents a bout. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations. Sites are grouped geographically by river system. Within each system, the site furthest upstream is on the left and the site furthest downstream is on the right.

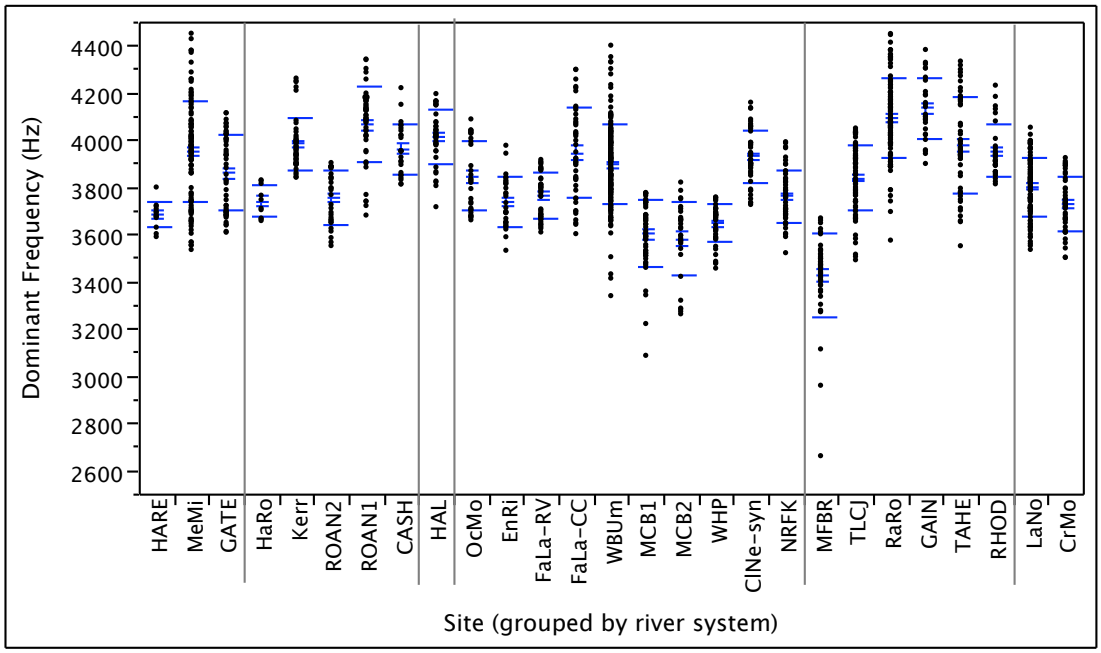


Figure 2.2c. Mean mass of *A. c. crepitans* at each site. Each point represents a frog. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations. Sites are grouped geographically by river system. Within each system, the site furthest upstream is on the left and the site furthest downstream is on the right.

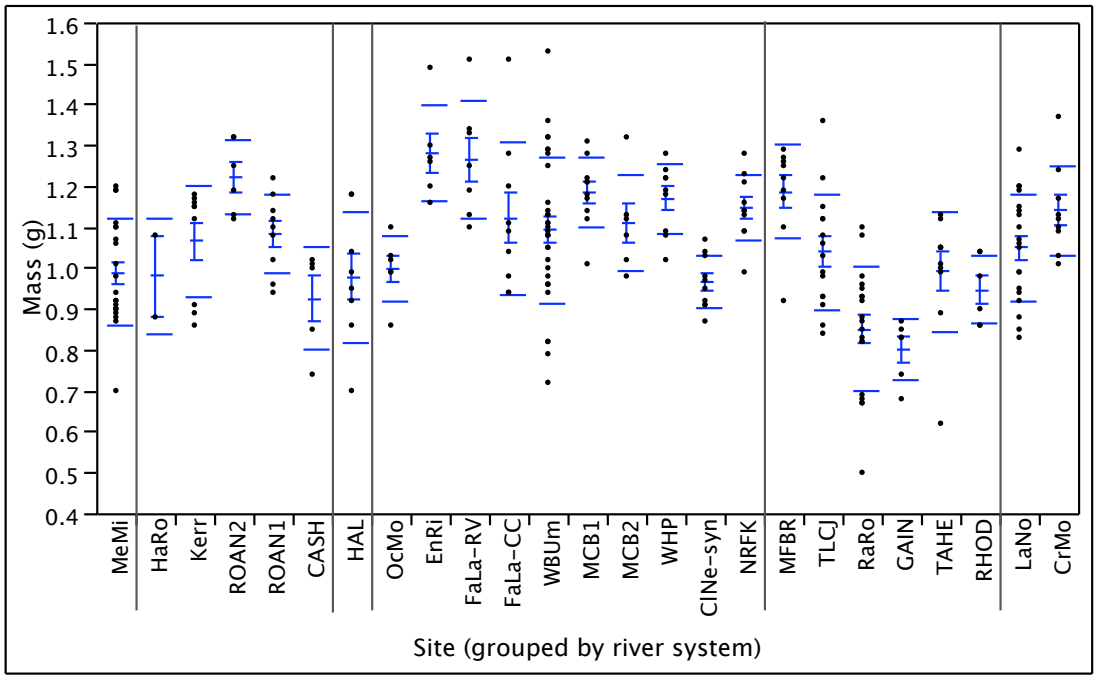


Figure 2.2d. Mean calling temperature of *A. c. crepitans* at each site. Each point represents a bout. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations. Sites are grouped geographically by river system. Within each system, the site furthest upstream is on the left and the site furthest downstream is on the right.

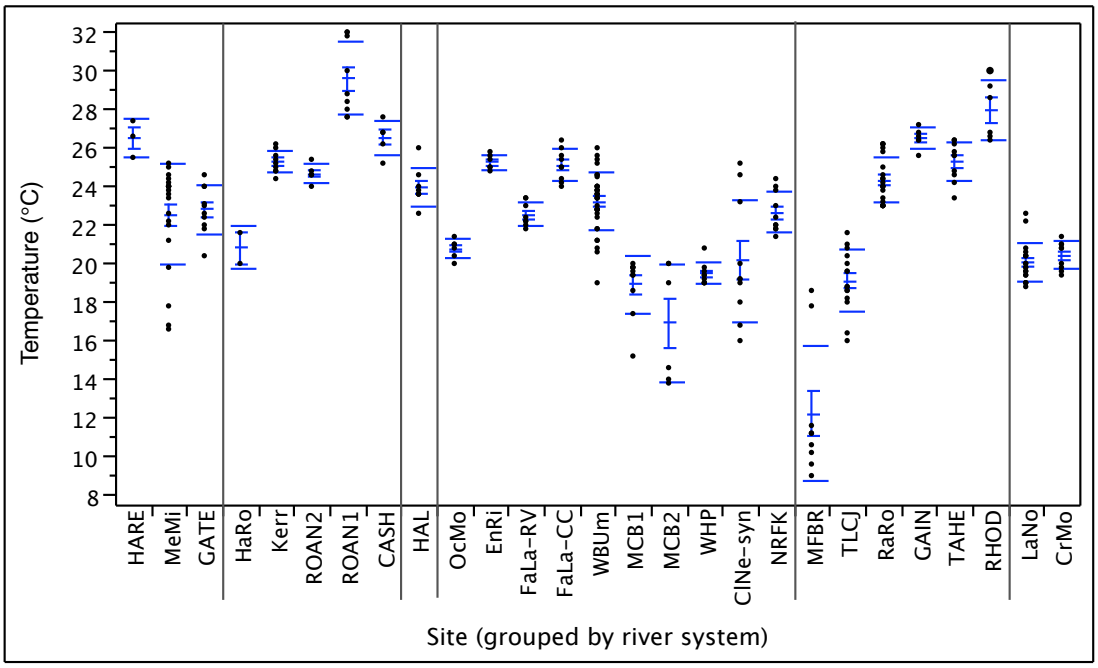
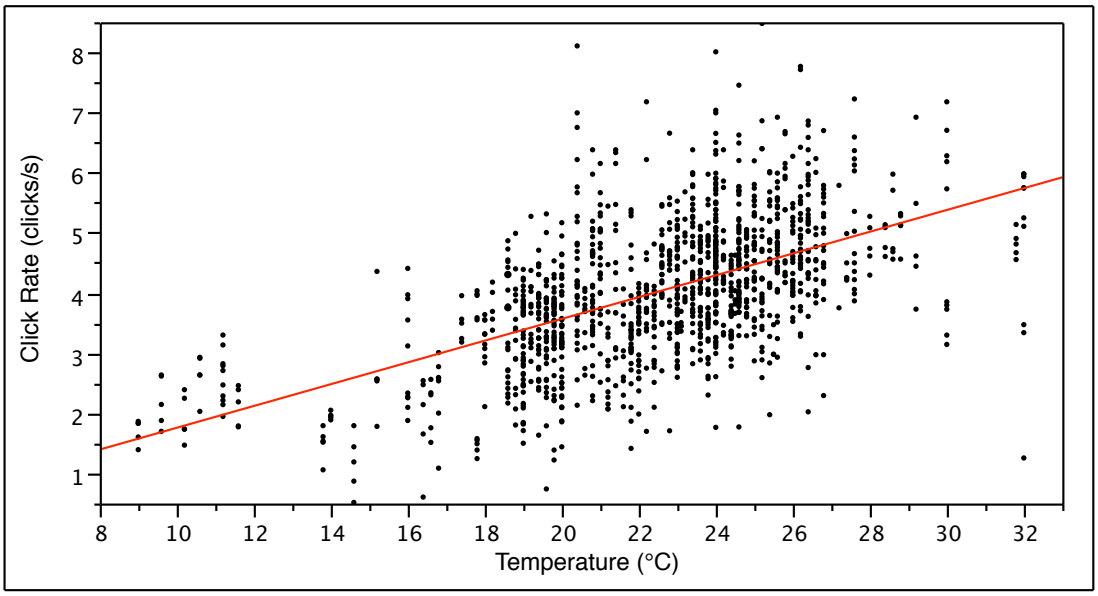
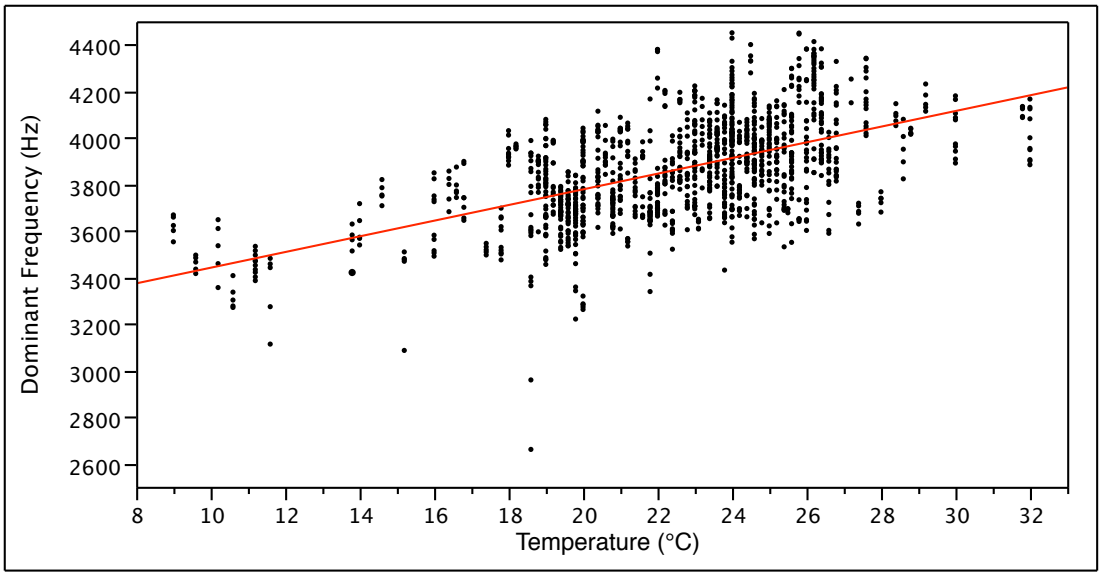


Figure 2.3a. Linear regression of click rate on temperature. Each point represents a bout.



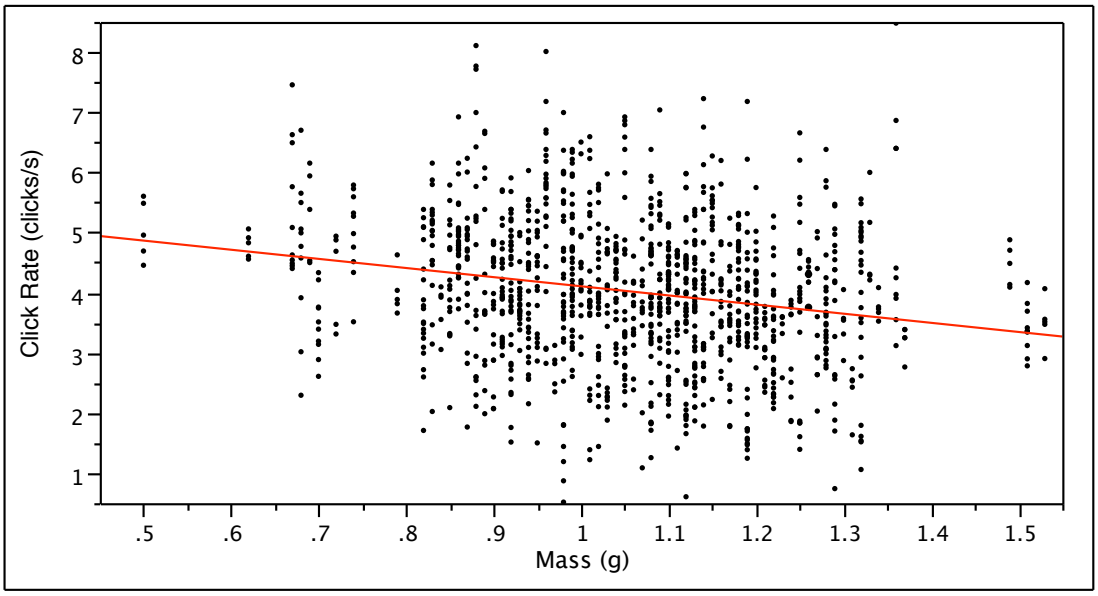
Click Rate = -0.05 + (0.181 x temperature)

Figure 2.3b. Linear regression of dominant frequency on temperature. Each point represents a bout.



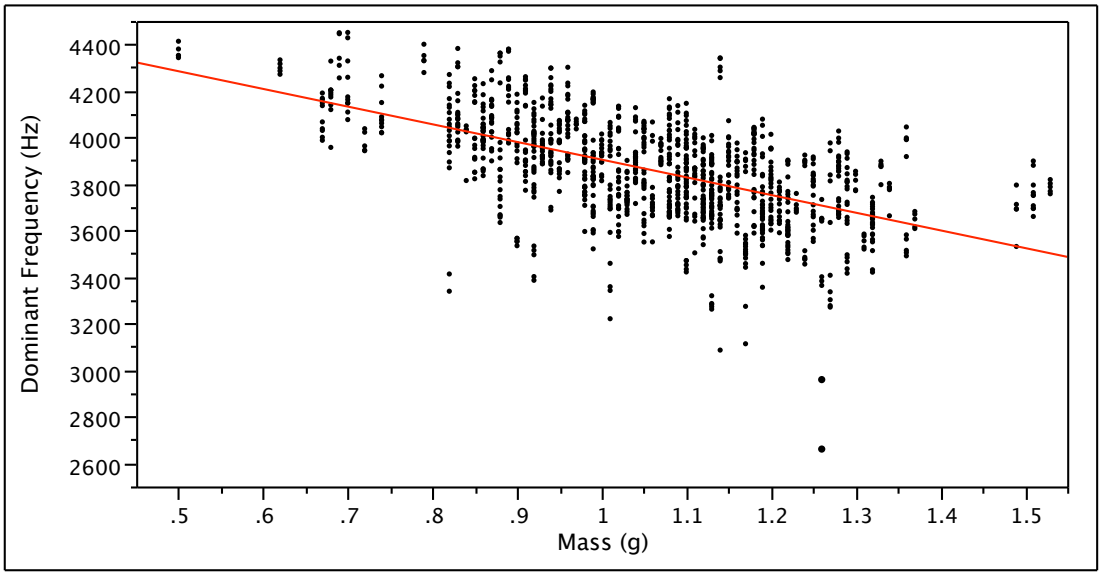
Dominant Frequency = 3104.48 + (33.67 x temperature)

Figure 2.3c. Linear regression of click rate on mass. Each point represents a bout.



Click Rate = 5.325 - (1.519 x mass)

Figure 2.3d. Linear regression of dominant frequency on mass. Each point represents a bout.



Dominant Frequency = 4663.72 - (759.48 x mass)

Figure 2.4a. Mean click rate at 22.5° of *A. c. crepitans* at each site. Each point represents a bout. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations. Sites are grouped geographically by river system. Within each system, the site furthest upstream is on the left and the site furthest downstream is on the right.

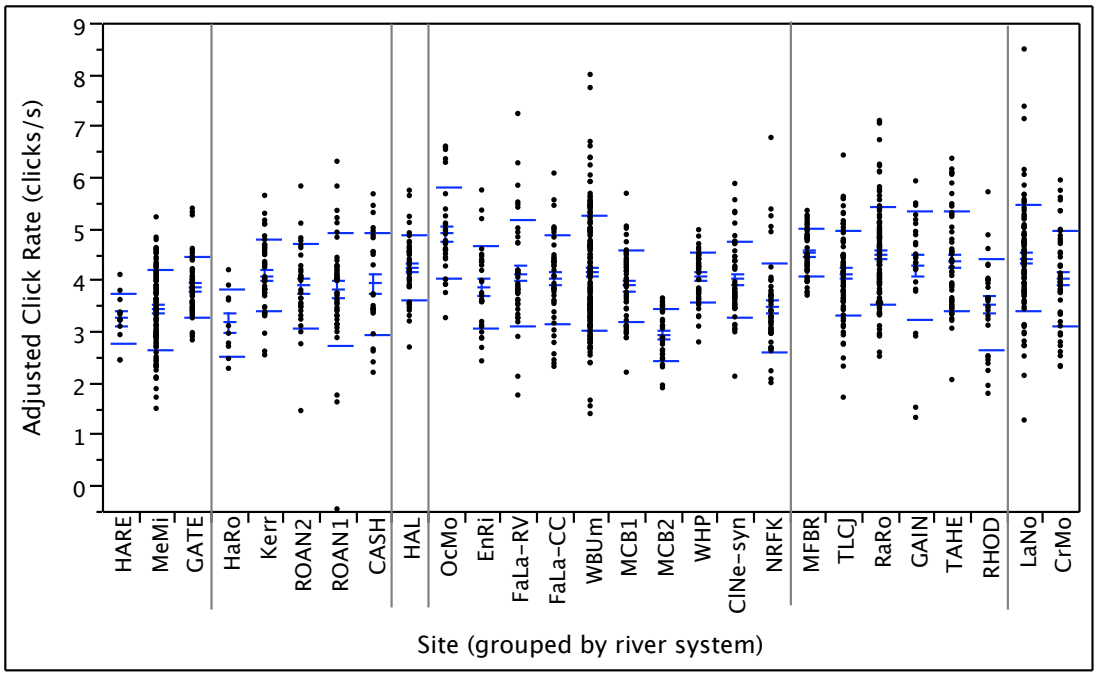
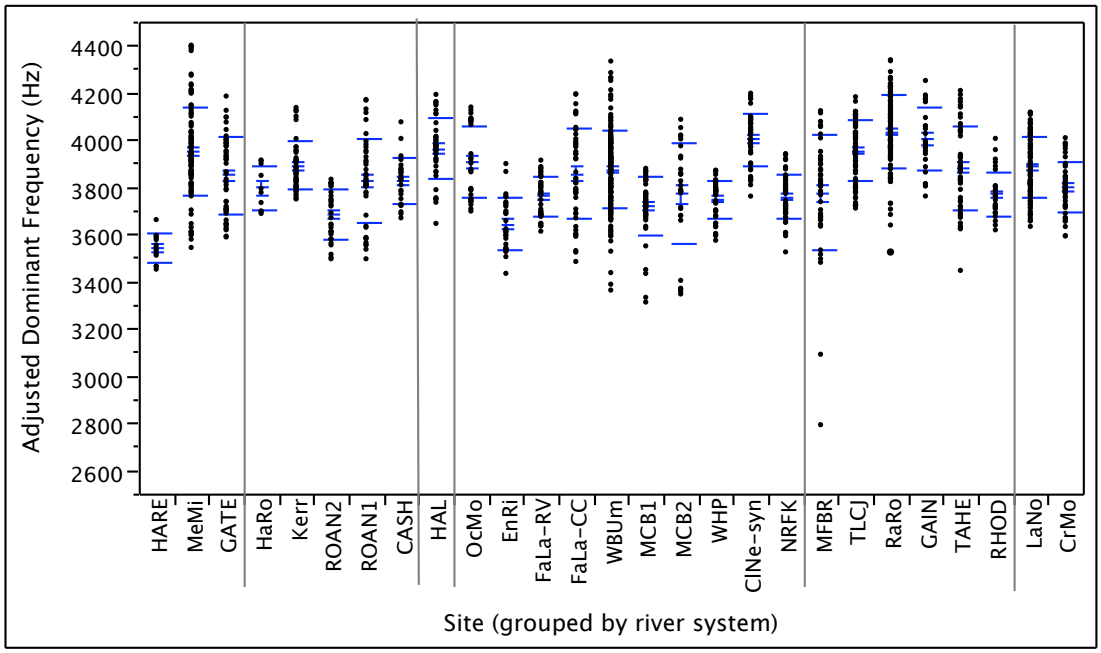


Figure 2.4b. Dominant frequency at 22.5° of *A. c. crepitans* at each site. Each point represents a bout. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations. Sites are grouped geographically by river system. Within each system, the site furthest upstream is on the left and the site furthest downstream is on the right.



Chapter 3

Acoustic variation in the Coastal Plain Cricket Frog, *Acris gryllus gryllus*, and the absence of reproductive character displacement in syntopic and sympatric *Acris*

Abstract

Detection of reproductive character displacement depends on assessing variation in mate recognition systems at a biologically-relevant geographic scale and describing the influences of other factors such as temperature and body size. I assessed variation in the click rate and dominant frequency of vocalizations of *Acris gryllus gryllus,* the Coastal Plain Cricket Frog, in order to determine the effects of temperature and body size and the degree of geographic variation among 14 sites in the Coastal Plain of North Carolina. Click rate and dominant frequency varied substantially and overlapped with a sympatric and syntopic congener, *A. crepitans crepitans*. As in *A. c. crepitans*, most acoustic variation was attributed to differences in mass, but unlike in *A. c. crepitans* and many other anurans, temperature had no influence on dominant frequency. In both species, differences among sites not explained by mass were unlikely to result from reproductive character displacement in sympatry or syntopy. The vocalizations of the two species were most similar at syntopic sites, where both had lowest mass.

Introduction

Reproductive character displacement occurs when differences in mating signals or the sensory systems to receive them are greater where two species are in close proximity (Brown and Wilson, 1956). This differentiation contributes to pre-mating reproductive isolation (Coyne and Orr, 2004). Mate recognition systems can diverge as a result of selection against mistakes in species detection but are also directly affected by external conditions such as temperature (Gerhardt, 1978) or selection on traits like body size (Ryan et al., 1990; Hobel and Gerhardt, 2003). Environmental clines can produce differences like those produced by reproductive character displacement (Goldberg and Lande, 2006). These factors create challenges in determining whether differences in mate recognition systems arose in sympatry (where ranges overlap) or through direct or indirect selection on mating signals or reception in allopatry (where ranges do not overlap).

The acoustic communication of cricket frogs, *Acris*, has been extensively studied. Nevo and Capranica (1985) proposed four hypotheses for divergence in mating signals within *Acris*: reproductive character displacement in sympatry, selection on calls in allopatry, selection on other traits in allopatry that incidentally affected calls, and genetic drift. They found that across the range of *Acris* in eastern North America, acoustic variation was correlated with body length, environmental factors, and geographic distance associated with eastern woodland and western grassland habitats in *A. c. crepitans*, *A. c. blanchardi*, and two other subspecies, *A. g. gryllus* and *A. g. dorsalis*. Ryan et al. (1990) provided evidence that selection on *A. c. crepitans* for efficient transmission of calls in forested habitat contributed to acoustic divergence between *A. c. crepitans* and *A. c. blanchardi*. Ryan and Wilczynski

(1991) found that among parapatric (adjacent but non-overlapping) *A. c. crepitans* and *A. c. blanchardi* populations in Texas, dominant frequency was correlated with longitude and body length. Because body length did not vary longitudinally, it did not account for the cline in dominant frequency. In these studies, size-independent clinal variation indicated that allopatric selection in different habitats accounted for most of the acoustic divergence within *A. crepitans* and selection to avoid interbreeding (reproductive character displacement) and allopatric selection on body size were rejected as possible explanations for acoustic divergence.

Despite this body of information, reproductive character displacement in vocalizations remains untested in *Acris* (Chapter 2). Ryan and Wilczynski (1991) assessed potential determinants of acoustic variation between *A. c. crepitans* and *A. c. blanchardi* in an area where the two subspecies have never been found in syntopy (at the same locality so they could possibly interbreed, Rivas, 1964). Since the subspecies have apparently never had the opportunity to interbreed, no selection for reproductive character displacement has had the opportunity to occur. They also avoided populations of *A. c. crepitans* occurring in sympatry with *A. gryllus* because of the potential for reproductive character displacement between the species of *Acris*. Nevo and Capranica (1985) assessed variation on a large geographic scale. They demonstrated the influence of environmental conditions on *Acris* vocalizations, but the scale of analysis was too large to detect acoustic differences directly resulting from overlap between *A. crepitans* and *A. gryllus*. Therefore, it is an unresolved question whether or not reproductive character displacement exists where *A. crepitans* overlaps with *A. gryllus*.

Chapter 2 describes the variation in click rate and dominant frequency of *Acris crepitans crepitans*, the Eastern Cricket Frog, in North Carolina, and the large influence of mass on these features compared with month, site, and temperature differences. In this study, I discuss analogous variation in these features in *A. g. gryllus*. I also determine whether or no there is evidence for reproductive character displacement in click rate or dominant frequency of either species. In Chapter 4, I discuss species recognition by females of both species at a syntopic site.

Methods

Field survey

The data for this study came from the same fieldwork described in Chapter 2 and is described similarly. I recorded and collected *Acris* at 33 sites and recorded without collecting frogs at three additional sites in the Piedmont and Coastal Plain of North Carolina in 2004, 2005, and 2007 (Figure 3.1). Recording took place from 4 May to 21 July 2004 (17 sites), 8 May to 24 July 2005 (17 sites including 2 from 2004), and 3-4 June and 15-16 July 2007 (4 sites). Three sites were in the western Piedmont, 12 sites were in the eastern Piedmont (including 4 sites just west of the Fall Zone), and 21 sites were in the upper and lower Coastal Plain. At each site were 1-5 permanent bodies of freshwater with choruses of cricket frogs. At each chorus, I recorded 6-10 frogs for 10 bouts each. I reduced the number of frogs collected at a few locations where a chorus was too small to remain active otherwise. Recording began at or after 2100 hours and ended when a chorus waned (between 0100 and 0300 hours) or I had a complete sample. I used a Marantz PMD-221 or PMD-421 portable

tape recorder (2004) or PMD-670 digital recorder (2005 and 2007) and an Audio-Technica 815a microphone to record each male. Immediately after recording, I photographed the male and any females and satellite males in obvious association with it with a Canon Powershot A80 4.1 MP digital camera. After capturing the male, I measured the surface temperature at its calling site with a Miller and Weber T-6000 fast-read cloacal thermometer. Within 12-36 hours after collection, I weighed each frog, euthanized it in a chlorotone solution, preserved a forefoot in a dimethyl sulfoxide and salt solution for eventual genetic analysis, and fixed the frog for morphological study and deposit in the collection of the North Carolina Museum of Natural Sciences, Raleigh, North Carolina.

Acoustic analysis

Chapter 2 describes the terminology modified from Wagner (1989) to describe cricket frog vocalizations in North Carolina. I used WildSpectra (version 060125; Wiley, 2007) to digitize cassette recordings at a sampling rate of 22.05 kHz (2004) or to import WAV recordings with a sampling rate of 22.05 kHz (2005 and 2007). Recorded frogs were identified as *A. c. crepitans* or *A. g. gryllus* by pulse patterns (Chapter 1). The study included 275 male *A. c. crepitans* from 27 sites and 140 male *A. g. gryllus* from 14 sites (4 sites had both species). Five bouts were selected from each male with a random number generator (Haahr, 2007) unless < 5 bouts were available for analysis (2055 total bouts, 4.95 bouts per individual). I measured every click in a bout using the SongSignatures procedure in WildSpectra. SongSignatures visually indicates each identified note and produces a text file with spectral and temporal information. I used this process to measure the duration of each

bout, the dominant frequency of each click in the bout, and the interval between each click. I then calculated the click rate, mean dominant frequency and mean interval between clicks for each bout. In addition, I manually counted the number of clicks in each bout before using SongSignatures. I optimized frequency resolution (21 Hz) at the expense of temporal resolution (46.43 milliseconds) by using a sampling rate of 11.025 Khz and a transform size of 1024; this temporal resolution was sufficient for detecting temporal variation in bouts. SongSignatures recognizes each note in a selected portion of a spectrogram with user-defined starting and ending thresholds of amplitude of the power spectrum. For each bout, I adjusted the amplitude thresholds to maximize detection of clicks and used WildSpectra's Exclude Selection procedure to remove background noise between clicks when noise interfered with detection of clicks.

Statistical analyses

Statistics were calculated with JMP 6.03 (SAS Institute, 2006). Analyses of variance (ANOVA) of 5 measurements of bouts (mean dominant frequency, total duration, total clicks, click rate, and mean interval between clicks) were computed to assess variation within and among populations of *A. g. gryllus*. To include differences in temperature, body size, and time in the breeding season, I relied on nested ANOVA to assess the importance of all of these factors on click rate and dominant frequency. These analyses of variance were computed in two ways: either with individual frogs as the most nested factor or with individual's mass and temperature as continuous covariates. Because conditions changed over the breeding season and I visited most sites only once, I nested sites within months and

then individual (or temperature and mass covariates) within sites to compare sites within months. I calculated the variance components for random effects with the restricted maximum likelihood method (REML, Quinn and Keough, 2002; SAS Institute, 2006). From the results of these tests, I could determine the statistical significance of the overall model, as well as the contributions of each variance component to the total variance in each acoustic feature.

To illustrate the effects of mass and temperature on vocalizations, I calculated linear regressions of dominant frequency and mean click rate by temperature and mass using pooled data for each species from the entire survey. I used the regression equations for temperature to adjust dominant frequency and click rate to a mean temperature of 22.5° before using nested ANOVAs to determine the specific effects of syntopy and sympatry on click rate, dominant frequency, and mass. I nested sites within biogeographic status (syntopy, allopatric sympatry, or allopatry).

Results

Variation in A. g. gryllus

There was significant variation in all acoustic features among months, sites, and individuals, and significant variation in mass and temperature among sites and individuals in *A. g. gryllus* (Table 3.1). As with *A. c. crepitans* (Chapter 2), intervals between clicks, number of clicks, and duration of the bout all contributed to click rate and all had slightly lower coefficients of determination (R^2) than did click rate in the nested ANOVAs. Differences among sites in click rate and dominant frequency (Table 3.2a and 3.2b; Figure

3.2a and 3.2b) were expected to be related to differences in mass and temperature (Table 3.2c and 3.2d; Figure 3.2c and 3.2d). Mean temperature at calling sites for *A. g. gryllus* increased significantly (nested ANOVA, d.f. = 2, $p < 0.0001$) from May (21.9°) to June (24.1°) to July (25.8°) and was significantly different among sites in each month (nested ANOVA, d.f. = 14, $p < 0.0001$). The monthly means and range of temperatures among sites (19.9-26.9°) used by *A. g. gryllus* were both warmer than the corresponding values for *A. c. crepitans*. Mean body mass of *A. g. gryllus* differed significantly among months (nested ANOVA, d.f. = 2, $p = 0.031$). There was a decrease from May (1.06) to June (1.02) to July (0.98), and mass differed among sites in each month (nested ANOVA, d.f. = 11, $p = 0.028$). The variation in mean mass among *A. g. gryllus* sites was substantial (May, 1.03-1.18 g; June, 0.75-1.13 g; July, 0.84-1.05 g).

As in *A. c. crepitans*, higher R^2 values for individual models (Table 3.3a) compared with the corresponding temperature and mass models (Table 3.3b) indicate that there was additional variation in dominant frequency and click rate among individuals that was unrelated to mass or temperature. As in *A. c. crepitans*, the largest percentage of total variance was attributed to mass in the analysis of dominant frequency (89.2%), but for click rate, mass (36.5%) was less important than the residual (47.8%). Month and temperature had no effect on the dominant frequency of *A. g. gryllus* (month, 0.0%; temperature; 0.1%), compared with the small contributions of month and temperature to dominant frequency in *A. c. crepitans*. The response of click rate to month and temperature in each species was nearly identical (*A. g. gryllus*: month, 0.0%; temperature, 14.8%).

The differences in the effects of mass and temperature on vocalizations in *A. c. crepitans* and *A. g. gryllus* are best illustrated by linear regressions (Table 3.4 and Figure 3.3a to 3.3d). The positive relationship between temperature and click rate in *A. g. gryllus* (Figure 3.3a; adjusted $R^2 = 0.12$, d.f. = 689, p = <0.0001) had a much lower adjusted R^2 and slope than in *A. c. crepitans,* an indication of a weaker relationship in *A. g. gryllus*. The positive effect of temperature on dominant frequency was significant in *A. c. crepitans*, but there was no effect of temperature on dominant frequency in *A. g. gryllus* (Figure 3.3b; adjusted $R^2 = 0.003$, d.f. = 689, p = 0.064). Unlike *A. c. crepitans*, there was no significant relationship between click rate and mass in *A. g. gryllus* (Table 3.3c: adjusted $R^2 = 0.00$, d.f. = 521, p < 0.86). As with *A. c. crepitans*, there was a significant negative relationship between mass and dominant frequency for *A. g. gryllus* (Table 3.3d: adjusted $R^2 = 0.19$, d.f. = 521, p < 0.0001),

The range of mean dominant frequency among sites in North Carolina (3314-3658 Hz) is as great as Nevo and Capranica (1985) found among five sites throughout most of the range of *A. g. gryllus* (3426-3587 Hz, 3174-3597 Hz after adjustment to 22.79°). The range of click rates within North Carolina (1.9-3.1 clicks/s; 1.4-2.7 clicks/s after temperature correction to 22.5°) was narrower than they found throughout the Southeast (1.5-3.7 clicks/s; 1.5-3.5 clicks/s after temperature correction to 22.79°). Substantial overlap occurred between *A. g. gryllus* and *A. c. crepitans* in click rate, dominant frequency, and mass (Table 3.5a and Figure 3.4a to 3.4c).

Acoustic and mass differences in syntopy and sympatry

Variance components showed that biogeographic relationships contributed no more than 4.9% of the differences in click rate, dominant frequency, or mass in *A. c. crepitans* and *A. g. gryllus* (Table 3.5c). Individual and residual variation, in combination, accounted for most differences in click rate for both species (*A. c. crepitans*, 87.1%; *A. g. gryllus*, 84.9%) while biogeographic status contributed only 3.1% to *A. c. crepitans* and 0.1% to *A. g. gryllus*. Click rate was the only response with substantial differences among the three biogeographic zones for each species (ANOVA; d.f. = 2, $p < 0.001$ for both species). Because *A. c. crepitans* had a slower click rate and *A. g. gryllus* had a faster click rate in syntopy and sympatry than in allopatry, their click rates were more similar the closer they occurred to each other. For dominant frequency, variation among individuals accounted for most differences (*A. c. crepitans*, 57.3%; *A. g. gryllus*, 64.8%) and biogeographic status made no significant contribution to dominant frequency in *A. c. crepitans* (0.0%; ANOVA, $p = 0.529$) despite a slightly higher mean in syntopy. Biogeographic status made almost no contribution to dominant frequency (0.5%; ANOVA, $p < 0.001$) in *A. g. gryllus*. In *A. g. gryllus*, dominant frequency was lower in non-syntopic sympatry than in syntopy or allopatry. Mass was almost entirely determined by site (40.7%) and residual variation (58.5%) in *A. c. crepitans* and by residual variation (87.6%) in *A. g. gryllus*. Both species had lower masses in syntopy than at other sympatric sites.

Differences in the Neuse River basin

Because the distribution of *A. c. crepitans* suggests that it colonized the Coastal Plain by moving down rivers from the Piedmont (Martof et al., 1980), adjacent populations within a river basin are likely to be more closely related to each other than to populations in adjacent basins. Of the eight distinct river systems in the study, the Neuse River basin is the only one in which allopatric, sympatric, and syntopic wetlands were located for both species. In sympatry and syntopy, the two species overlapped in click rate, dominant frequency, and mass (Table 3.7a to 3.7c; Figure 3.6a to 3.6c). The syntopic wetland was one of the two ponds at Cliffs of the Neuse State Park (only *A. g. gryllus* bred at the other pond), less than 2 km from the Neuse River. At allopatric and sympatric sites upstream of the syntopic site, the temperature-corrected mean dominant frequency of *A. c. crepitans* varied from 3642 to 3904 Hz (Table 3.7b and Figure 3.6b). At the two *A. c. crepitans* sites adjacent to the syntopic pond (both within 25 m of the Neuse River), one 25 km upstream at the Waynesborough Historic Park (WHP) and the other 30 km downstream near Kinston (NRFK), the mean dominant frequencies were statistically indistinguishable (3747 and 3754 Hz; ANOVA d.f. = 95, $p = 0.1683$). At the syntopic pond, the mean dominant frequency of *A. c. crepitans* was 4000 Hz, 96 Hz higher than anywhere else in the basin and 250 Hz higher than the two non-syntopic sites nearby. Click rates were similar at the three sites (Table 3.7a and Figure 3.6a). Because mass has a larger influence than site on dominant frequency in *A. c. crepitans* (Chapter 2), the high dominant frequency of *A. c. crepitans* at the syntopic site is likely to be related to the lower masses of frogs at that location (Table 3.7c and Figure 3.6c), rather than the presence of *A. g. gryllus*.

Discussion

A. g. gryllus varied extensively in click rate, dominant frequency, and mass within and among 14 breeding sites in the Coastal Plain of North Carolina (Table 3.1 to 3.2c; Figure 3.2a to 3.2c). On average, *A. g. gryllus* called at a lower dominant frequency (despite a lower mean mass) and slower rate than *A. c. crepitans*, but the two species overlapped in these features, even at syntopic sites (Table 3.5a; Figure 3.4a to 3.4c and 3.6a to 3.6c). Mass had no effect, and temperature had only a slight effect, on click rate in *A. g. gryllus* (Table 3.3b). Individual variation unrelated to mass or temperature differences was the most important influence on click rate in *A. g. gryllus*. As in *A. c. crepitans*, the most important influence on dominant frequency was body mass. Unexpectedly and mysteriously, the dominant frequency of *A. g. gryllus* was not significantly affected by temperature (Table 3.4 and Figure 3.3b), unlike in *A. c. crepitans* (Chapter 2) and many other anurans (Gerhardt and Huber, 2002).

There is little evidence that reproductive character displacement has occurred in the click rate or dominant frequency of *A. c. crepitans* or *A. g. gryllus* vocalizations in North Carolina. There was no significant influence of syntopy and sympatry on the dominant frequency of *A. c. crepitans*, and their influence on the dominant frequency of *A. g. gryllus* and the click rates of both species was small in comparison with other effects (Table 3.5a and 3.5b). Rather than an increased difference in the click rates of *A. c. crepitans* and *A. g. gryllus* in sympatry, the difference was smaller, and the species were most similar in syntopy (Table 3.5a). The difference between the species in dominant frequency was smaller in syntopy as well, because in syntopy compared with allotopy, the dominant frequency of *A. g.*

gryllus was higher and the dominant frequency of *A. c. crepitans* was not significantly different (despite a higher mean). The masses of both species were lower in syntopy than allotopy within the sympatric area, which accounts for the higher dominant frequencies in syntopy. Within the Neuse River basin, the dominant frequency of *A. c. crepitans* was highest at a syntopic site, but its reduced body size at that location was more of a factor than the presence of *A. g. gryllus*. The linear regressions of dominant frequency on mass for *A. c. crepitans* (Chapter 2) and *A. g. gryllus* (Table 3.4 and Figure 3.3d) have non-overlapping slopes and the two species overlap in mass (Figured 3.4c), so the differences in dominant frequency in the two species are the result of differences in mass.

The lower mass of both species in syntopy was probably not a result of decreased survival or reduced prey availability that might favor reproduction at a younger age and smaller size. The choruses at syntopic sites were dense, spatially extensive, and long-lasting. The high number of calling males at syntopic sites did not appear to wane during drought conditions that attenuated breeding activity at other permanent wetlands. The insects that *Acris* were observed to eat seemed limitless at syntopic sites. High habitat quality and prey availability at the syntopic breeding site might instead reduce pressure to reach a larger body size before commencing a long and energy-intensive breeding period. The ecological constraints that dictate the range limits of *A. crepitans* and *A. gryllus* are unknown, but appear to be relaxed in syntopy. The same factors that cause the species to occur in proximity to each other may also cause them to breed at a smaller body size than in other areas.

The extensive overlap between *A. c. crepitans* and *A. g. gryllus* in click rate and dominant frequency and the absence of important differences within each species among syntopic and allotopic populations suggest that some other acoustic feature, not dominant frequency or click rate, is used for species recognition by *A. c. crepitans* and *A. g. gryllus.* The distinct differences between these species in click structure (Chapter 1) are a good candidate. *A. c. crepitans* produces fewer pulses per click, at a slower and more variable rate, than *A. g. gryllus*, and no overlap occurs in click structure in North Carolina. Acoustic divergence between species of *Acris* appears to most pronounced in this small-scale temporal feature, not in dominant frequency or in a large-scale temporal feature such as click rate. The four hypotheses that Nevo and Capranica (1985) proposed to explain divergence in the acoustic signals of *Acris* are still applicable for explaining differences in click structure and could be tested in future studies

The dominant frequency and click rate of vocalizations of *A. g. gryllus,* the Coastal Plain Cricket Frog, vary substantially in a small part of its geographic range. Like its sympatric congener *A. c. crepitans*, most of this variation is correlated with differences in mass, but unlike *A. c. crepitans*, temperature had no influence on dominant frequency. In both species, differences among sites that are not explained by mass are unlikely to result from reproductive character displacement in sympatry or syntopy.

Table 3.1 Nested analyses of variance of *A. g. gryllus* acoustic features, temperature, and mass

Feature	Summary of fit		Source	DF	F ratio	*P* value
mean dominant frequency (Hz)	R^2	0.82	Model	139	18.11	<.0001
	adjusted R^2	0.78	Month	2	35.14	<.0001
	Root mean square error	94.06	Site[Month]	14	50.73	<.0001
	Mean of response	3487.44	Indiv.[Site, Month]	123	14.56	<.0001
	Observations	690	Error	550		
click rate (N/s)	R^2	0.54	Model	139	4.63	<.0001
	adjusted R^2	0.42	Month	2	34.61	<.0001
	Root mean square error	0.65	Site[Month]	14	15.08	<.0001
	Mean of Response	2.2	Indiv.[Site, Month]	123	2.67	<.0001
	Observations	690	Error	550		
mean interclick interval (s)	R^2	0.48	Model	139	3.59	<.0001
	adjusted R^2	0.34	Month	2	18.80	<.0001
	Root mean square error	0.20	Site[Month]	14	8.90	<.0001
	Mean of Response	0.55	Indiv.[Site, Month]	123	2.54	<.0001
	Observations	690	Error	550		
clicks (N)	R^2	0.46	Model	139	3.33	0.009
	adjusted R^2	0.32	Month	2	4.75	<.0001
	Root mean square error	12.29	Site[Month]	14	5.39	<.0001
	Mean of Response	29.36	Indiv.[Site, Month]	123	3.09	<.0001
	Observations	690	Error	550		
duration (s)	R^2	0.51	Model	139	4.12	<.0001
	adjusted R^2	0.39	Month	2	17.15	<.0001
	Root mean square error	8.65	Site[Month]	14	8.54	<.0001
	Mean of Response	15.70	Indiv.[Site, Month]	123	3.36	<.0001
	Observations	690	Error	550		
temperature at calling site (°C)	R^2	0.66	Model	16	14.74	<.0001
	adjusted R^2	0.61	Month	2	48.13	<.0001
	Root mean square error	1.40	Site[Month]	14	7.99	<.0001
	Mean of Response	24.48	Error	123		
	Observations	140				
mass (g)	R^2	0.23	Model	13	2.08	0.022
	adjusted R^2	0.12	Month	2	3.62	0.031
	Root mean square error	0.16	Site[Month]	11	2.09	0.028
	Mean of Response	1.01	Error	92		
	Observations	106				

Table 3.2a. Mean click rate of *A. g. gryllus* at each site. Sites are grouped by river system. Upstream sites precede downstream sites in each system.

Site	N (bouts)	Mean	Standard Deviation	Standard Error Mean	Lower 95%	Upper 95%
HARE	20	3.14	0.96	0.21	2.69	3.59
MeMi	93	1.98	0.81	0.08	1.81	2.14
GATE	28	2.29	0.77	0.15	1.99	2.59
BENN	50	1.91	0.58	0.08	1.74	2.07
Pett	40	1.97	0.70	0.11	1.75	2.19
ClNe-allo	65	3.21	1.18	0.15	2.92	3.51
ClNe-syn	76	2.13	0.73	0.08	1.96	2.30
COOL	40	1.70	0.58	0.09	1.51	1.89
WeWo	20	2.44	0.57	0.13	2.17	2.70
Jone	54	2.09	0.62	0.08	1.92	2.25
CaBe	59	2.22	0.80	0.10	2.01	2.43
PBBT	35	1.86	0.72	0.12	1.62	2.11
SGL	45	2.31	0.59	0.09	2.14	2.49
LaWa	65	2.03	0.59	0.07	1.88	2.18
Total	690	2.20	0.86	0.03	2.13	2.26

Table 3.2b. Mean dominant frequency of *A. g. gryllus* at each site. Sites are grouped by river system. Upstream sites precede downstream sites in each system.

Site	N (bouts)	Mean	Standard Deviation	Standard Error Mean	Lower 95%	Upper 95%
HARE	20	3406.16	96.92	21.67	3360.80	3451.50
MeMi	93	3502.55	148.86	15.44	3471.90	3533.20
GATE	28	3657.97	190.23	35.95	3584.20	3731.70
BENN	50	3384.39	110.42	15.62	3353.00	3415.80
Pett	40	3613.34	226.29	35.78	3541.00	3685.70
ClNe-allo	65	3473.00	189.44	23.50	3426.10	3519.90
ClNe-syn	76	3560.72	163.04	18.70	3523.50	3598.00
COOL	40	3642.56	121.30	19.18	3603.80	3681.40
WeWo	20	3313.90	88.86	19.87	3272.30	3355.50
Jone	54	3473.85	135.27	18.41	3436.90	3510.80
CaBe	59	3352.28	186.34	24.26	3303.70	3400.80
PBBT	35	3508.08	153.45	25.94	3455.40	3560.80
SGL	45	3466.01	354.22	52.80	3359.60	3572.40
LaWa	65	3443.60	161.16	19.99	3403.70	3483.50
Total	690	3487.44	198.46	7.56	3472.61	3502.28

Table 3.2c. Mean mass of *A. g. gryllus* at each site. Sites are grouped by river system. Upstream sites precede downstream sites in each system.

Site	N (males)	Mean	Standard Deviation	Standard Error Mean	Lower 95%	Upper 95%
MeMi	19	1.033	0.136	0.031	0.967	1.098
BENN	2	1.125	0.007	0.005	1.062	1.189
Pett	7	0.837	0.158	0.060	0.691	0.983
ClNe-allo	13	1.048	0.145	0.040	0.960	1.135
ClNe-syn	13	0.936	0.175	0.048	0.831	1.042
COOL	8	0.975	0.116	0.041	0.878	1.072
WeWo	4	1.180	0.218	0.109	0.833	1.527
Jone	11	1.081	0.188	0.057	0.955	1.207
CaBe	9	0.994	0.122	0.041	0.901	1.088
PBBT	7	0.961	0.178	0.067	0.797	1.126
LaWa	13	1.036	0.182	0.050	0.926	1.146
Total	106	1.010	0.167	0.016	0.978	1.042

Table 3.2d. Mean calling temperature of *A. g. gryllus* at each site. Sites are grouped by river system. Upstream sites precede downstream sites in each system.

Site	N (males)	Mean	Standard Deviation	Standard Error Mean	Lower 95%	Upper 95%
HARE	4	26.85	0.66	0.33	25.80	27.90
MeMi	19	22.86	2.57	0.59	21.62	24.10
GATE	6	23.03	1.20	0.49	21.77	24.30
BENN	10	23.52	2.17	0.69	21.97	25.07
Pett	8	25.30	1.48	0.52	24.07	26.54
ClNe-allo	13	26.17	1.35	0.37	25.35	26.99
ClNe-syn	16	23.41	3.24	0.81	21.69	25.14
COOL	8	22.05	0.81	0.28	21.38	22.72
WeWo	4	25.90	0.50	0.25	25.10	26.70
Jone	11	25.82	1.74	0.53	24.65	26.99
CaBe	12	25.52	0.66	0.19	25.10	25.94
PBBT	7	26.20	0.49	0.19	25.75	26.65
SGL	9	25.76	0.53	0.18	25.35	26.16
LaWa	13	23.82	0.86	0.24	23.30	24.33
Total	140	24.48	2.25	0.19	24.11	24.86

Table 3.3a: Variance components for dominant frequency and click rate (individual model) in *A. g. gryllus*

	Summary of Fit		Random Effect	Variance Ratio	Variance Component	Standard Error	95% Confidence intervals lower	upper	% of Total Variance
Click Rate (clicks/s)	R^2	0.50	Month	0.12	0.05	0.09	-0.11	0.22	7.04
	Root MSE	0.65	Site[Month]	0.31	0.13	0.06	0.01	0.25	17.54
	Mean	2.20	Indiv.[Site,Month]	0.34	0.14	0.03	0.09	0.20	19.09
	Observations	690	Residual		0.42	0.03	0.38	0.48	56.33
			Total		0.75				100.00
Dominant Frequency (Hz)	R^2	0.82	Month	-0.15	-1348.93	-	-	-	0.00
	Root MSE	94.06	Site[Month]	0.68	6048.09	-	-	-	15.02
	Mean	3487.44	Indiv.[Site,Month]	2.87	25384.93	-	-	-	63.02
	Observations	690	Residual		8847.01	476.65	7982.36	9860.93	21.96
			Total		38931.09				100.00

Table 3.3b. Variance components for dominant frequency and click rate (mass and temperature model) in *A. g. gryllus*

	Summary of Fit		Random Effect	Variance Ratio	Variance Component	Standard Error	95% Confidence intervals lower	upper	% of Total Variance
Click Rate (clicks/s)	R^2	0.33	Month	-0.03	-0.02	0.03	-0.08	0.05	0.00
	Root MSE	0.74	Site[Month]	0.32	0.17	0.09	0.00	0.35	14.78
	Mean	2.20	Temp.[Site,Month]	0.02	0.01	0.01	0.00	0.02	0.87
	Observations	522	Mass[Site,Month]	0.77	0.42	0.46	-0.47	1.32	36.52
			Residual		0.55	0.04	0.48	0.62	47.83
			Total		1.14				100.00
Dominant Frequency (Hz)	R^2								
	Root MSE	0.41	Month	-0.05	-1086.84	447.40	-1963.75	-209.94	0.00
	Mean	141.09	Site[Month]	0.24	4754.98	2221.30	401.23	9108.72	2.07
	Observations	3490.45	Temp.[Site,Month]	0.01	148.01	-	-	-	0.06
		522	Mass[Site,Month]	10.29	204776.67	86919.49	34414.47	375138.87	89.19
			Residual		19906.37	1267.49	17638.26	22644.35	8.67
			Total		228499.18				100.00

Table 3.4. Linear regression formulas for click rate and dominant frequency on temperature and mass for *A. g. gryllus*, including summaries of fit and analyses of variance.

Regression	Summary of Fit		Analysis of variance	
Click Rate =	R^2	0.12	d.f.	689
-1.12 - (0.135 x temperature)	adjusted R^2	0.12	F ratio	93.45
	Root MSE	0.80	*P* value	< 0.0001
	Mean	2.12		
	Observations	690		
Dominant Frequency =	R^2	0.00	d.f.	689
3644.41 - (6.40 x temperature)	adjusted R^2	0.00	F ratio	3.44
	Root MSE	198.11	*P* value	0.0637
	Mean	3487.44		
	Observations	690		
Click Rate =	R^2	0.00	d.f.	521
2.238 + (0.041 x mass)	adjusted R^2	0.00	F ratio	0.03
	Root MSE	0.88	*P* value	0.8598
	Mean	2.20		
	Observations	522		
Dominant Frequency =	R^2	0.19	d.f.	521
3960.99 - (465.06 x mass)	adjusted R^2	0.19	F ratio	123.58
	Root MSE	159.71	*P* value	< 0.0001
	Mean	3490.45		
	Observations	522		

Table 3.5a. Mean click rate, dominant freqency, and mass of *A. c. crepitans* and *A. gryllus* in allopatry, sympatry, syntopy, and throughout North Carolina.

A. c. crepitans

	Status	N	Mean	SD
Click Rate (clicks/s)	Allopatry	878	4.15	0.96
	Sympatry	272	3.91	0.96
	Syntopy	215	3.63	0.76
	Total	1365	4.02	0.95
Dominant Frequency (Hz)	Allopatry	878	3862.80	178.96
	Sympatry	272	3820.31	150.91
	Syntopy	215	3911.13	193.95
	Total	1365	3861.94	178.17
Mass (g)	Allopatry	170	1.07	0.18
	Sympatry	61	1.05	0.16
	Syntopy	44	0.98	0.11
	Total	275	1.06	0.17

A. g. gryllus

	Status	N	Mean	SD
Click Rate (clicks/s)	Allopatry	204	1.77	0.69
	Sympatry	269	1.95	0.91
	Syntopy	217	2.04	0.74
	Total	690	1.93	0.80
Dominant Frequency (Hz)	Allopatry	204	3500.70	209.59
	Sympatry	269	3467.55	204.72
	Syntopy	217	3540.78	169.55
	Total	690	3500.38	197.97
Mass (g)	Allopatry	41	0.98	0.16
	Sympatry	54	1.06	0.17
	Syntopy	45	0.99	0.16
	Total	140	1.01	0.17

Table 3.5b. Nested analyses of variance of click rate, dominant freqency, and mass of *A. c. crepitans* and *A. g. gryllus.* Status refers to classification of each site (allopatry, sympatry, or syntopy with the other species). Asterisks after *P* values indicate levels of analysis with a significant difference within the species.

A. c. crepitans

		R^2	adjusted R^2	DF	F Ratio	*P* value
Click Rate (clicks/s)	Model	0.62	0.53	274	6.62	
	Status			2	25.31	<0.001*
	Site[Status]			24	14.51	<0.001*
	Individual[Site,Status]			248	5.40	<0.001*
	Error			1090	0.00	
Dominant Frequency (Hz)	Model	0.89	0.86	274	32.42	
	Status			2	0.64	0.529
	Site[Status]			24	121.72	<0.001*
	Individual[Site,Status]			248	22.91	<0.001*
	Error			1090	0.00	
Mass (g)	Model	0.43	0.37	24	7.36	
	Status			2	8.95	<0.001
	Individual[Site,Status]			22	7.46	<0.001
	Error			233	0.00	

A. g. gryllus

		R^2	adjusted R^2	DF	F Ratio	*P* value
Click Rate (clicks/s)	Model	0.48	0.34	139	3.60	
	Status			2	16.21	<0.001*
	Site[Status]			11	14.45	<0.001*
	Individual[Site,Status]			126	2.57	<0.001*
	Error			550	0.00	
Dominant Frequency (Hz)	Model	0.82	0.77	139	18.00	
	Status			2	46.00	<0.001*
	Site[Status]			11	42.83	<0.001*
	Individual[Site,Status]			126	15.43	<0.001*
	Error			550	0.00	
Mass (g)	Model	0.18	0.10	10	2.16	
	Status			2	4.15	0.019*
	Individual[Site,Status]			8	1.97	0.058
	Error			95	0.03	

Table 3.5c. Variance components (REML) of click rate, dominant freqency, and mass of *A. c. crepitans* and *A. gryllus*. Status refers to classification of each site (allopatry, sympatry, or syntopy with the other species).

A. c. crepitans

		R^2	Variance Component	% Variance
Click Rate (clicks/s)	Model	0.61	0.93	100.0
	Status		0.03	3.1
	Site[Status]		0.09	9.8
	Individual[Site,Status]		0.38	41.2
	Residual		0.43	45.9
Dominant Frequency (Hz)	Model	0.89	32619.83	100.0
	Status		-767.46	0.0
	Site[Status]		9930.42	29.7
	Individual[Site,Status]		19115.08	57.3
	Residual		4341.79	13.0
Mass (g)	Model	0.42	0.03	100.0
	Status		0.00	0.7
	Site[Status]		0.01	40.7
	Residual		0.02	58.5

A. g. gryllus

		R^2	Variance Component	% Variance
Click Rate (clicks/s)	Model	0.43	0.66	100.0
	Status		0.00	0.1
	Site[Status]		0.10	15.0
	Individual[Site,Status]		0.14	20.6
	Residual		0.42	64.3
Dominant Frequency (Hz)	Model	0.82	40363.61	100.0
	Status		212.14	0.5
	Site[Status]		5145.21	12.7
	Individual[Site,Status]		26158.61	64.8
	Residual		8847.64	21.9
Mass (g)	Model	0.13	0.03	100.0
	Status		0.00	4.9
	Site[Status]		0.00	7.5
	Residual		0.03	87.6

Table 3.6a. Mean click rate (adjusted to 22.5° C) of *A. g. gryllus* at each site. Sites are grouped by river system. Upstream sites precede downstream sites in each system.

Site	N (bouts)	Mean	Standard Deviation	Standard Error Mean	Lower 95%	Upper 95%
HARE	20	2.55	0.95	0.21	2.11	3.00
MeMi	93	1.91	0.69	0.07	1.77	2.05
GATE	28	2.21	0.72	0.14	1.93	2.49
BENN	50	1.77	0.52	0.07	1.62	1.91
Pett	40	1.59	0.76	0.12	1.35	1.84
ClNe-allo	65	2.72	1.21	0.15	2.42	3.02
ClNe-syn	76	1.99	0.69	0.08	1.83	2.15
COOL	40	1.76	0.59	0.09	1.57	1.95
WeWo	20	1.98	0.58	0.13	1.70	2.25
Jone	54	1.64	0.62	0.08	1.47	1.81
CaBe	59	1.81	0.80	0.10	1.60	2.02
PBBT	35	1.36	0.70	0.12	1.12	1.61
SGL	45	1.87	0.57	0.09	1.70	2.05
LaWa	65	1.85	0.57	0.07	1.71	1.99
Total	690	1.94	0.81	0.03	1.88	2.00

Table 3.6b. Mean dominant frequency (adjusted to 22.5° C) of *A. g. gryllus* at each site. Sites are grouped by river system. Upstream sites precede downstream sites in each system.

Site	N (bouts)	Mean	Standard Deviation	Standard Error Mean	Lower 95%	Upper 95%
HARE	20	3434.00	95.78	21.42	3389.20	3478.80
MeMi	93	3505.66	147.98	15.35	3475.20	3536.10
GATE	28	3661.58	191.64	36.22	3587.30	3735.90
BENN	50	3390.91	107.00	15.13	3360.50	3421.30
Pett	40	3631.26	231.25	36.56	3557.30	3705.20
ClNe-allo	65	3496.48	192.24	23.84	3448.80	3544.10
ClNe-syn	76	3567.34	172.19	19.75	3528.00	3606.70
COOL	40	3639.68	122.23	19.33	3600.60	3678.80
WeWo	20	3335.66	89.59	20.03	3293.70	3377.60
Jone	54	3494.83	132.98	18.10	3458.50	3531.10
CaBe	59	3371.60	186.21	24.24	3323.10	3420.10
PBBT	35	3531.76	155.21	26.23	3478.40	3585.10
SGL	45	3486.85	353.54	52.70	3380.60	3593.10
LaWa	65	3452.02	160.39	19.89	3412.30	3491.80
Total	690	3487.44	198.46	7.56	3472.61	3502.28

Table 3.7a. Click rates (adjusted to 22.5° C) of *A. c. crepitans* and *A. g. gryllus* in the Neuse River basin. Upstream sites precede downstream sites.

Species	Site	N (bouts)	Mean	Standard Deviation	Standard Error Mean	Lower 95%	Upper 95%
A. c. crepitans	OcMo	30	4.89	0.88	0.16	4.56	5.22
A. c. crepitans	EnRi	29	3.85	0.81	0.15	3.54	4.15
A. c. crepitans	FaLa-RV	40	4.12	1.04	0.16	3.79	4.45
A. c. crepitans	WBUm	165	4.13	1.13	0.09	3.96	4.30
A. c. crepitans	FaLa-CC	44	4.00	0.86	0.13	3.74	4.27
A. c. crepitans	MCB1	50	3.88	0.69	0.10	3.68	4.08
A. c. crepitans	MCB2	30	2.92	0.49	0.09	2.73	3.10
A. c. crepitans	WHP	45	4.06	0.49	0.07	3.91	4.20
A. g. gryllus	ClNe-allo	65	2.72	1.21	0.15	2.42	3.02
A. c. crepitans	ClNe-syn	47	4.00	0.75	0.11	3.78	4.22
A. g. gryllus	ClNe-syn	76	1.99	0.69	0.08	1.83	2.15
A. c. crepitans	NRFK	50	3.46	0.86	0.12	3.21	3.70
A. g. gryllus	COOL	40	1.76	0.59	0.09	1.57	1.95

Table 3.7b. Dominant frequencies (adjusted to 22.5° C) of *A. c. crepitans* and *A. g. gryllus* in the Neuse River basin. Upstream sites precede downstream sites.

Species	Site	N (bouts)	Mean	Standard Deviation	Standard Error Mean	Lower 95%	Upper 95%
A. c. crepitans	OcMo	30	3903.80	152.57	27.86	3846.80	3960.80
A. c. crepitans	EnRi	29	3641.98111.	20.66	3599.70	3684.30	4.15
A. c. crepitans	FaLa-RV	40	3759.90	84.51	13.36	3732.90	3786.90
A. c. crepitans	WBUm	165	3870.95	163.92	12.76	3845.80	3896.20
A. c. crepitans	FaLa-CC	44	3854.16	192.73	29.06	3795.60	3912.80
A. c. crepitans	MCB1	50	3719.88	124.51	17.61	3684.50	3755.30
A. c. crepitans	MCB2	30	3767.82	213.95	39.06	3687.90	3847.70
A. c. crepitans	WHP	45	3747.04	79.38	11.83	3723.20	3770.90
A. g. gryllus	ClNe-allo	65	3496.48	192.24	23.84	3448.80	3544.10
A. c. crepitans	ClNe-syn	47	4000.27	111.44	16.26	3967.50	4033.00
A. g. gryllus	ClNe-syn	76	3567.34	172.19	19.75	3528.00	3606.70
A. c. crepitans	NRFK	50	3754.34	93.89	13.28	3727.70	3781.00
A. g. gryllus	COOL	40	3639.68	122.23	19.33	3600.60	3678.80

Table 3.7c. Masses of *A. c. crepitans* and *A. g. gryllus* in the Neuse River basin. Upstream sites precede downstream sites.

Species	Site	N (frogs)	Mean	Standard Deviation	Standard Error Mean	Lower 95%	Upper 95%
A. c. crepitans	OcMo	6	1.00	0.07	0.01	0.97	1.03
A. c. crepitans	EnRi	6	1.28	0.11	0.02	1.24	1.32
A. c. crepitans	FaLa-RV	8	1.26	0.13	0.02	1.22	1.31
A. c. crepitans	WBUm	9	1.09	0.18	0.01	1.06	1.12
A. c. crepitans	FaLa-CC	9	1.12	0.18	0.03	1.07	1.18
A. c. crepitans	MCB1	10	1.19	0.08	0.01	1.16	1.21
A. c. crepitans	MCB2	6	1.11	0.11	0.02	1.07	1.15
A. c. crepitans	WHP	9	1.17	0.08	0.01	1.14	1.19
A. g. gryllus	ClNe-allo	13	1.05	0.14	0.02	1.01	1.08
A. c. crepitans	ClNe-syn	10	0.96	0.06	0.01	0.94	0.98
A. g. gryllus	ClNe-syn	16	0.93	0.17	0.02	0.89	0.97
A. c. crepitans	NRFK	10	1.15	0.08	0.01	1.12	1.17
A. g. gryllus	COOL	8	0.98	0.11	0.02	0.94	1.01

Figure 3.1. Survey sites for *A. c. crepitans* and *A. g. gryllus* in North Carolina

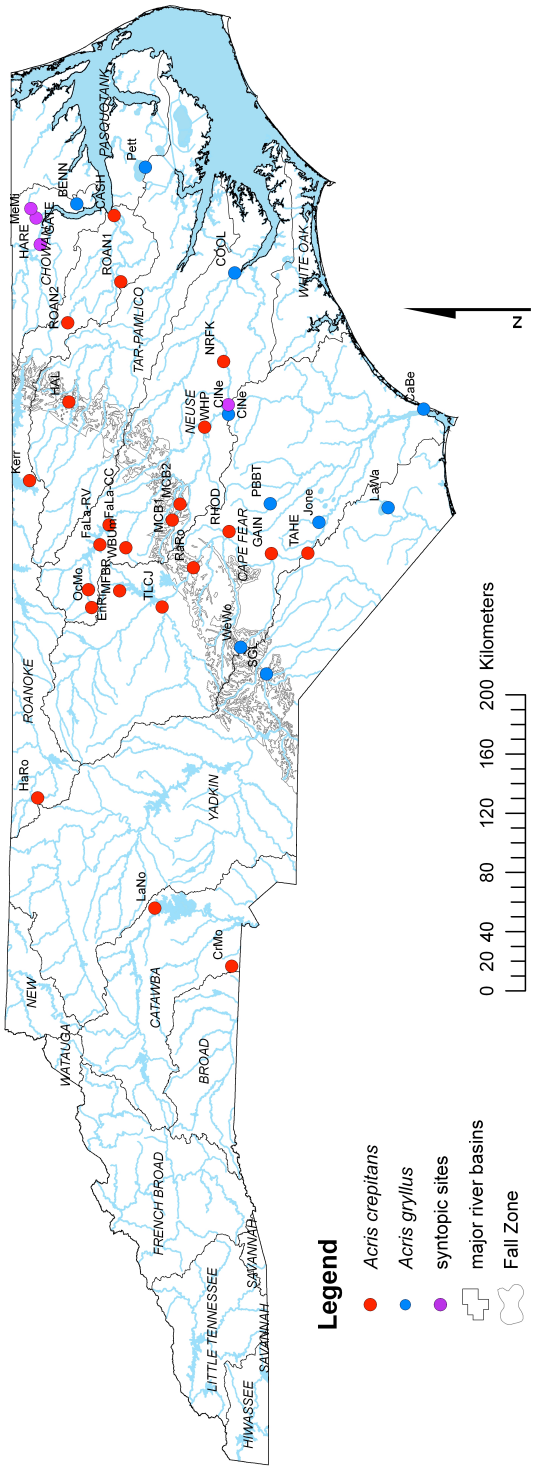


Figure 3.2a. Mean click rate of *A. g. gryllus* at each site. Each point represents a bout. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations. Sites are grouped geographically by river system. Within each system, the site furthest upstream is on the left and the site furthest downstream is on the right.

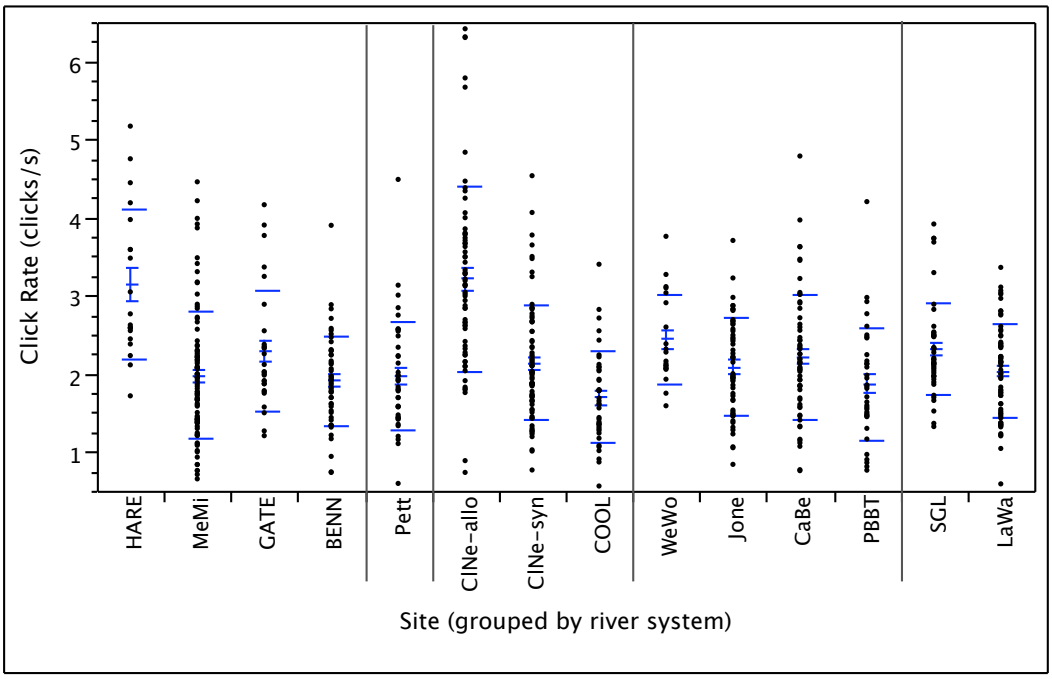


Figure 3.2b. Mean dominant frequency of *A. g. gryllus* at each site. Each point represents a bout. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations. Sites are grouped geographically by river system. Within each system, the site furthest upstream is on the left and the site furthest downstream is on the right.

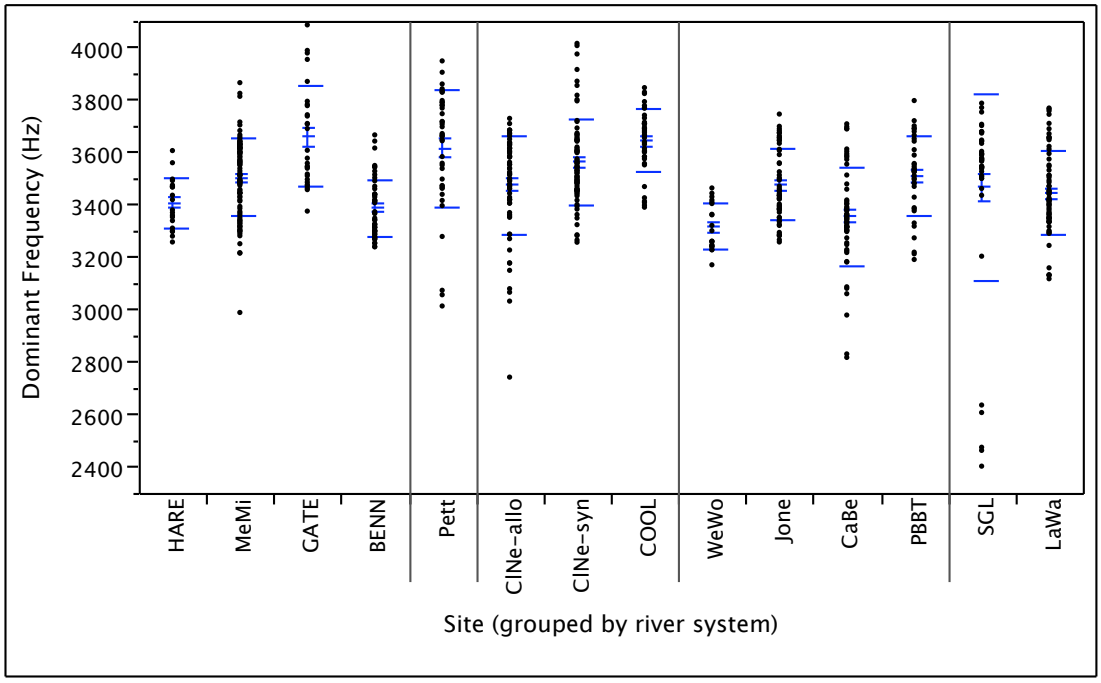


Figure 3.2c. Mean mass of *A. g. gryllus* at each site. Each point represents a frog. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations. Sites are grouped geographically by river system. Within each system, the site furthest upstream is on the left and the site furthest downstream is on the right.

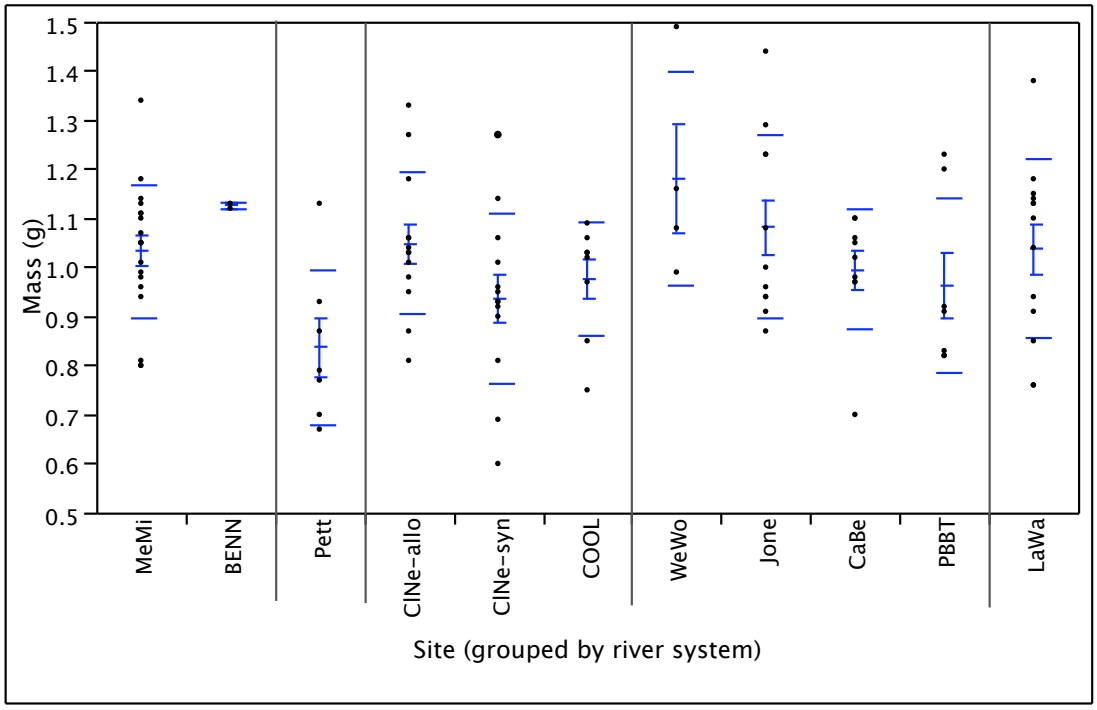


Figure 3.2d. Mean calling temperature of *A. g. gryllus* at each site. Each point represents a bout. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations. Sites are grouped geographically by river system. Within each system, the site furthest upstream is on the left and the site furthest downstream is on the right.

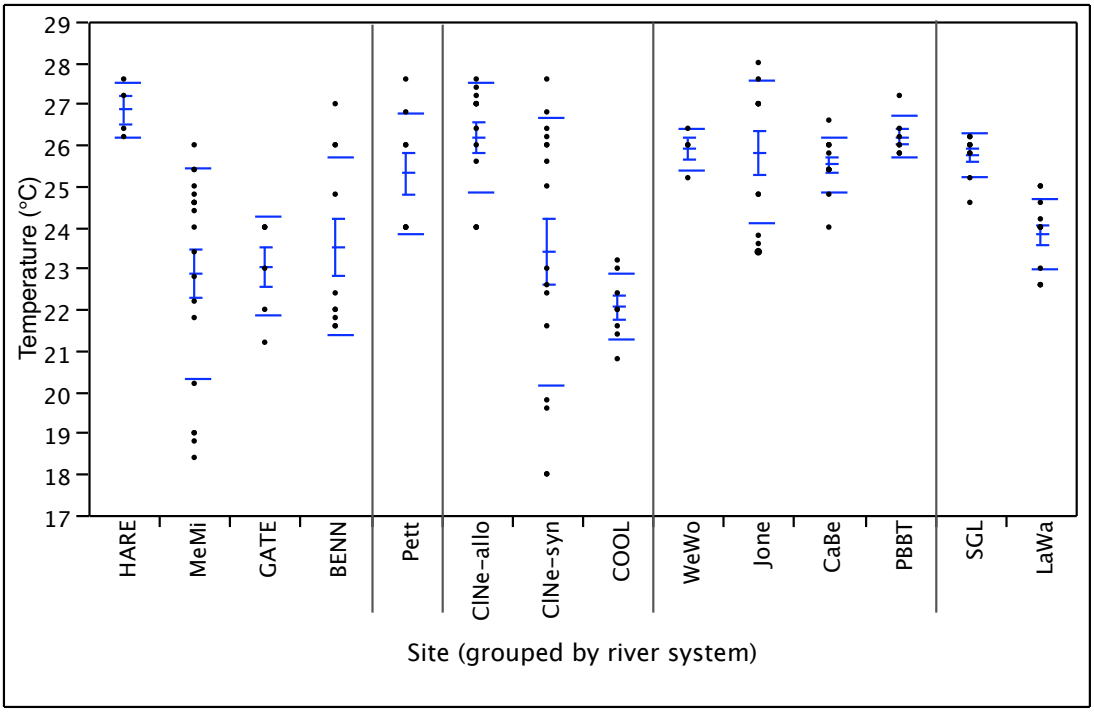
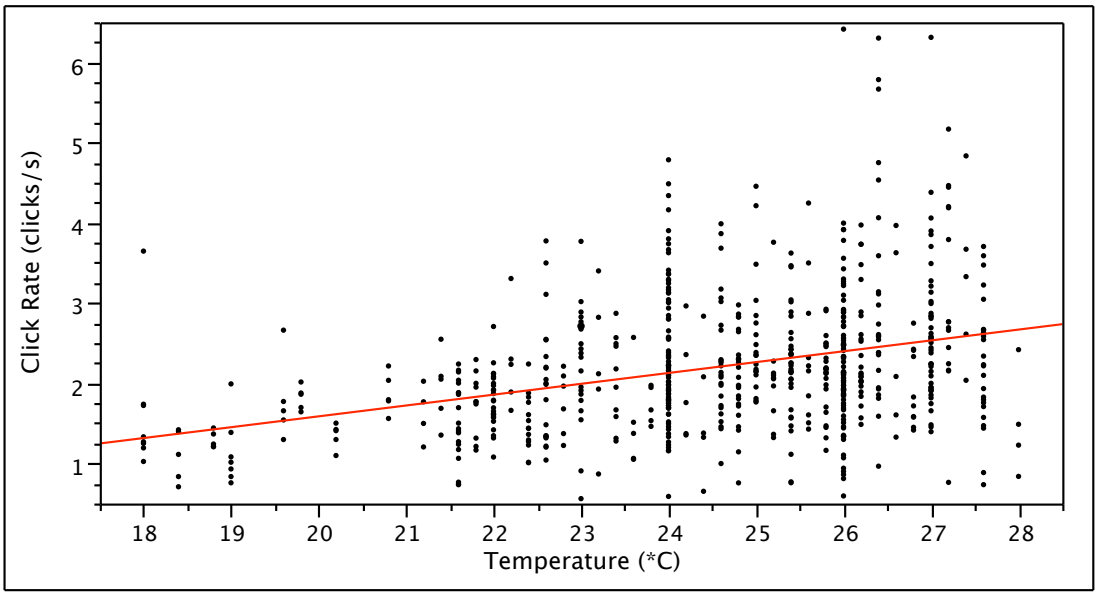
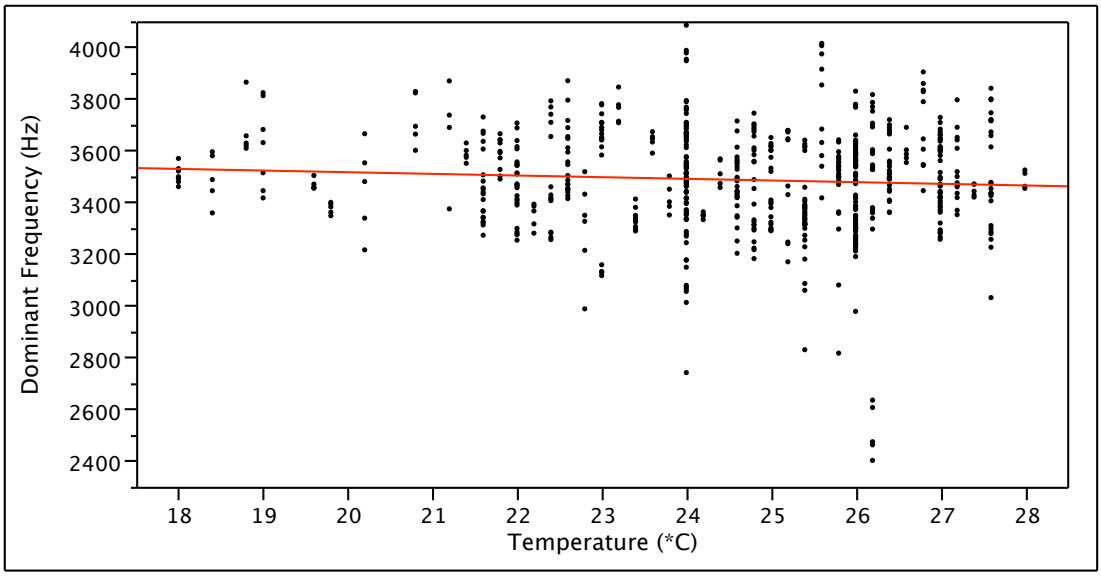


Figure 3.3a. Linear regression of click rate on temperature for *A. g. gryllus*. Each point represents a bout.



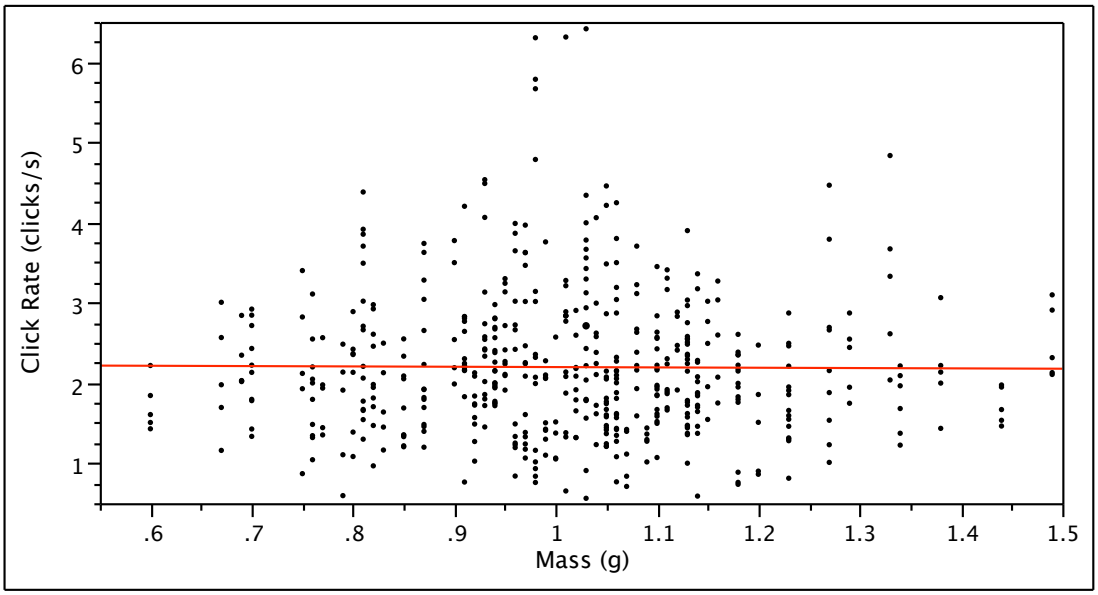
Click Rate = -1.12 - (0.135 x temperature)

Figure 3.3b. Linear regression of dominant frequency on temperature for *A. g. gryllus.* Each point represents a bout.



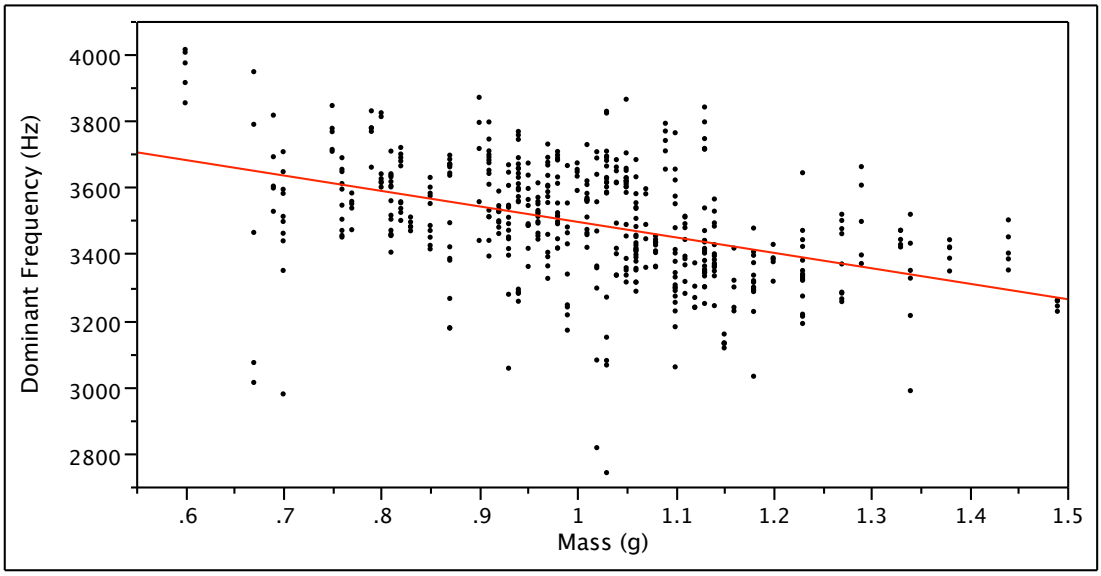
Dominant Frequency = 3644.41 - (6.40 x temperature)

Figure 3.3c. Linear regression of click rate on mass for *A. g. gryllus*. Each point represents a bout.



Click Rate = 2.238 + (0.041 x mass)

Figure 3.3d. Linear regression of dominant frequency on mass for *A. g. gryllus*. Each point represents a bout.



Dominant Frequency = 3960.99 - (465.06 x mass)

Figure 3.4a. Overlap in click rate between *A. c. crepitans* and *A. g. gryllus*. Each point represents a bout. Points are jittered (spread on x-axis) to show distribution on y-axis.

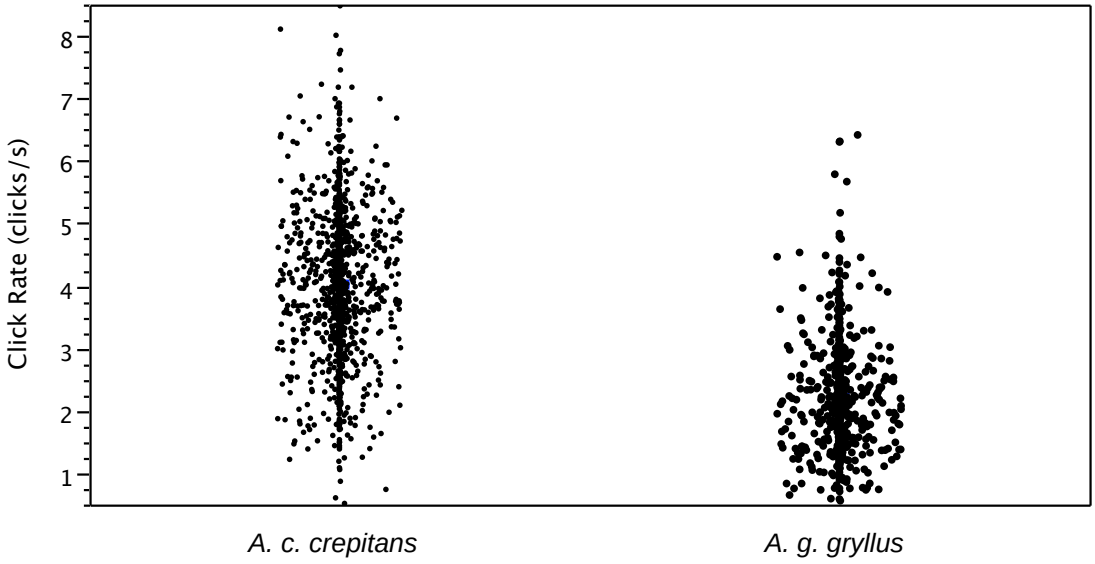


Figure 3.4b. Overlap in dominant frequency between *A. c. crepitans* and *A. g. gryllus*. Each point represents a bout. Points are jittered (spread on x-axis) to show distribution on y-axis.

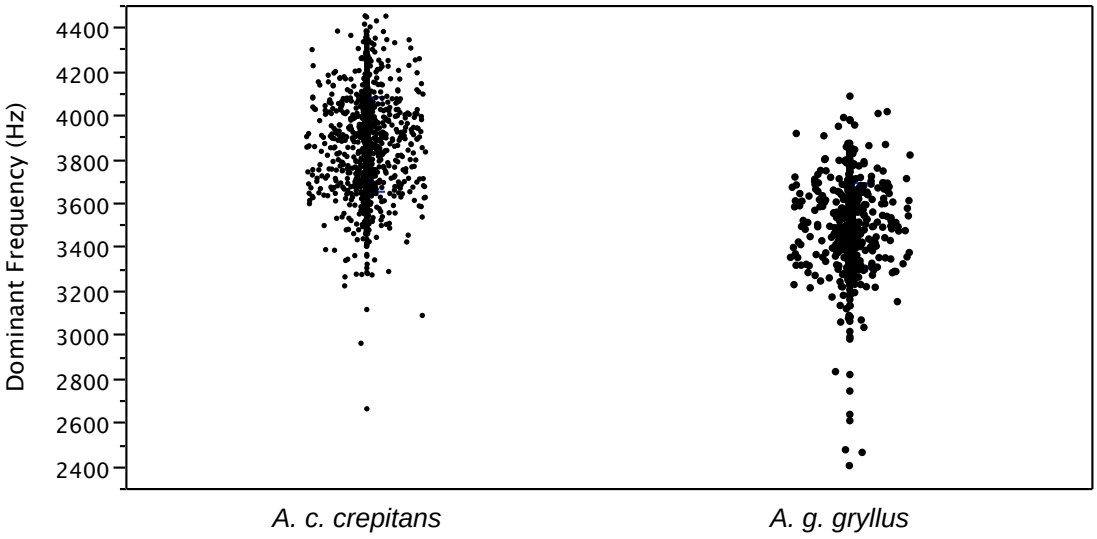


Figure 3.4c. Overlap in mass between *A. c. crepitans* and *A. g. gryllus*. Each point represents a frog. Points are jittered (spread on x-axis) to show distribution on y-axis.

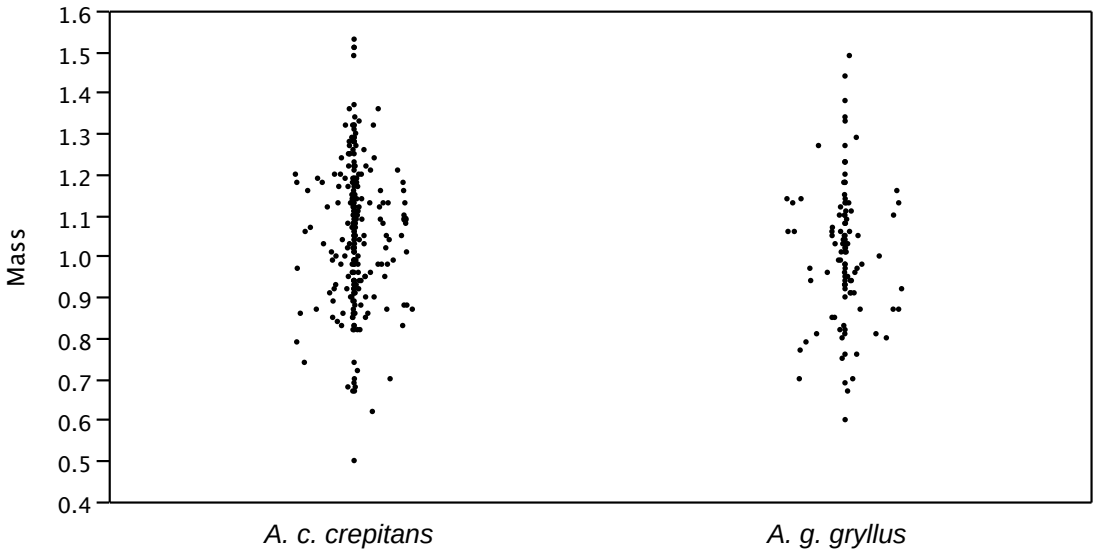


Figure 3.5a. Mean click rate at 22.5° of *A. g. gryllus* at each site. Each point represents a bout. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations.. Sites are grouped geographically by river system. Within each system, the site furthest upstream is on the left and the site furthest downstream is on the right.

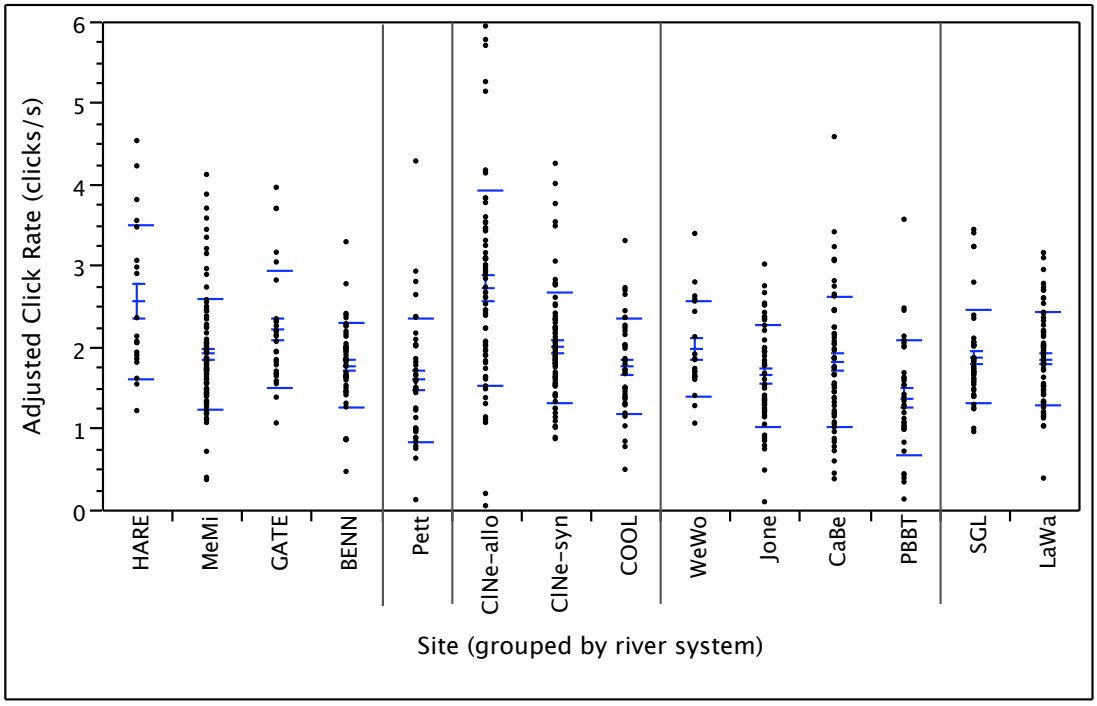


Figure 3.5b. Dominant frequency at 22.5° of *A. g. gryllus* at each site. Each point represents a bout. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations.. Sites are grouped geographically by river system. Within each system, the site furthest upstream is on the left and the site furthest downstream is on the right.

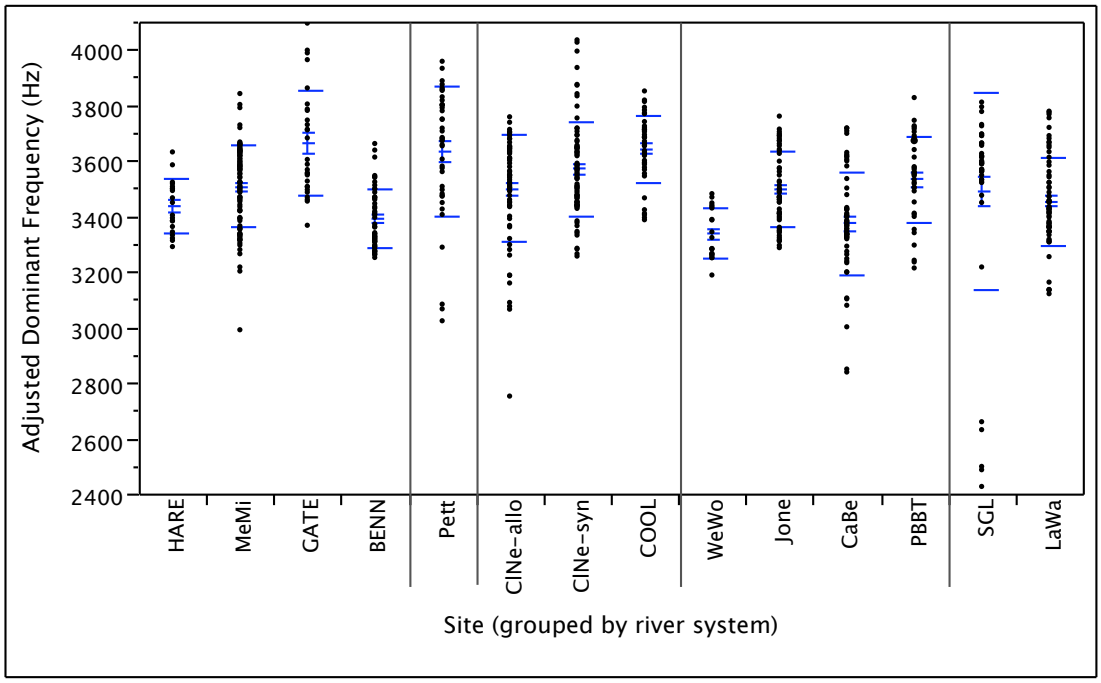


Figure 3.6a. Click rates of *A. c. crepitans* and *A. g. gryllus* in the Neuse River basin. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations.

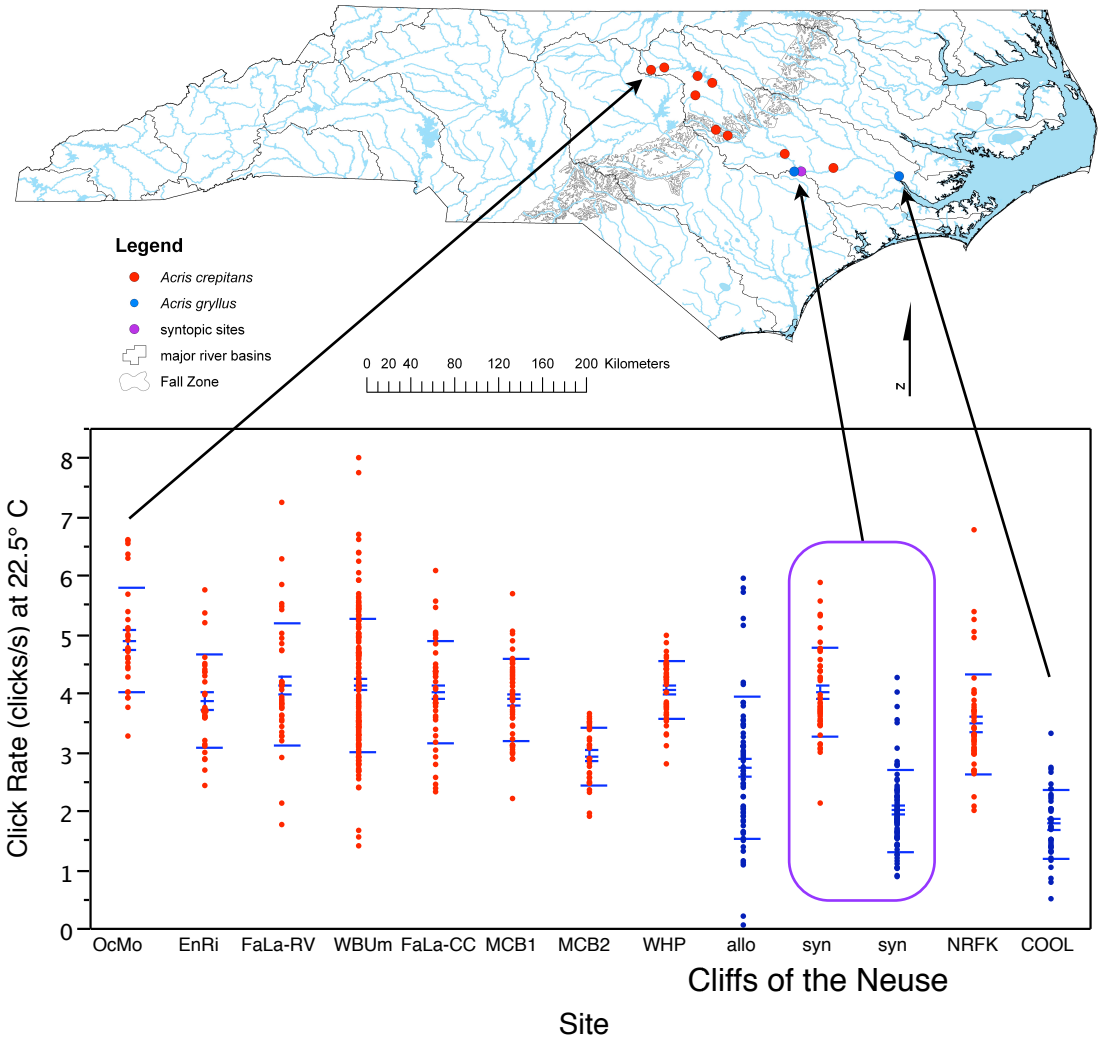


Figure 3.6b. Dominant frequencies of *A. c. crepitans* and *A. g. gryllus* in the Neuse River basin. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations.

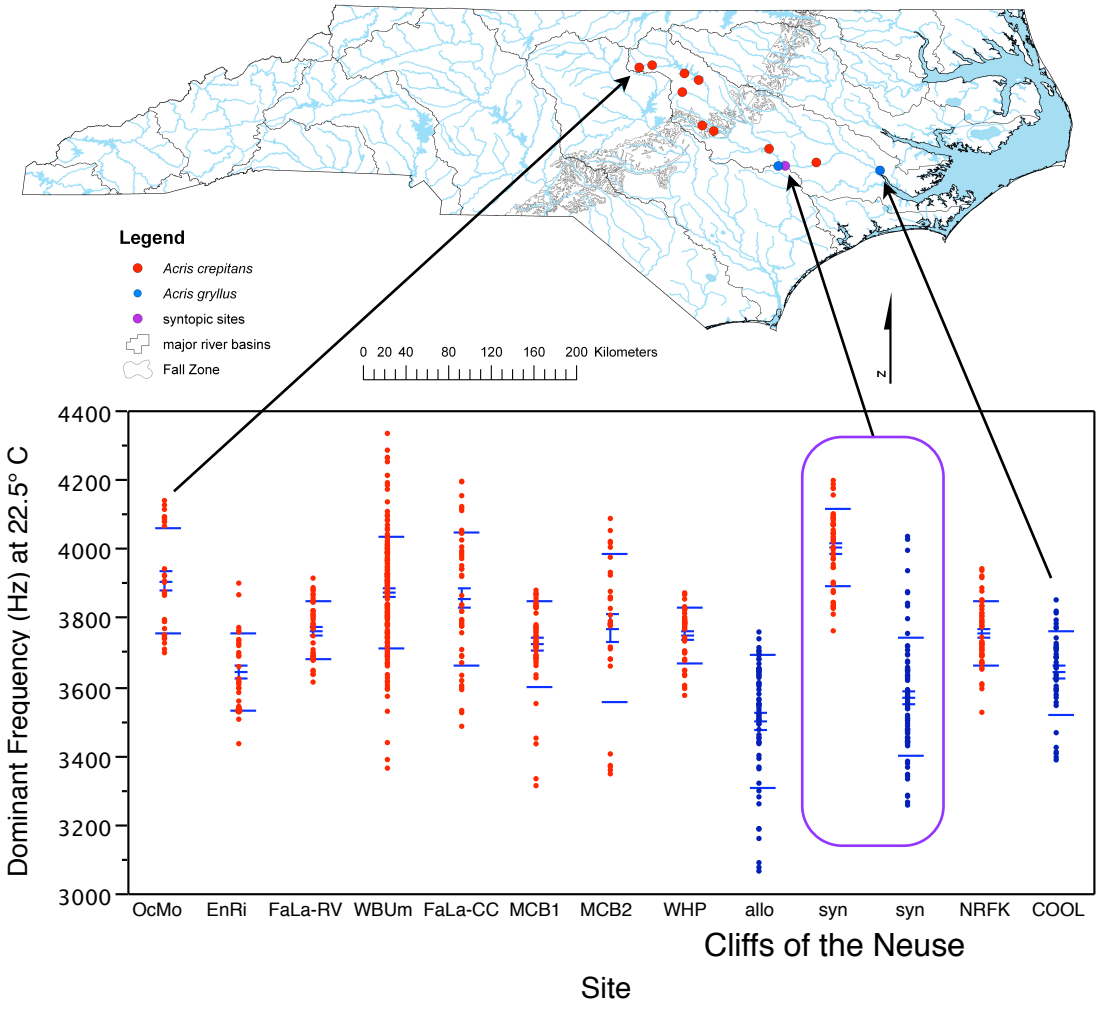
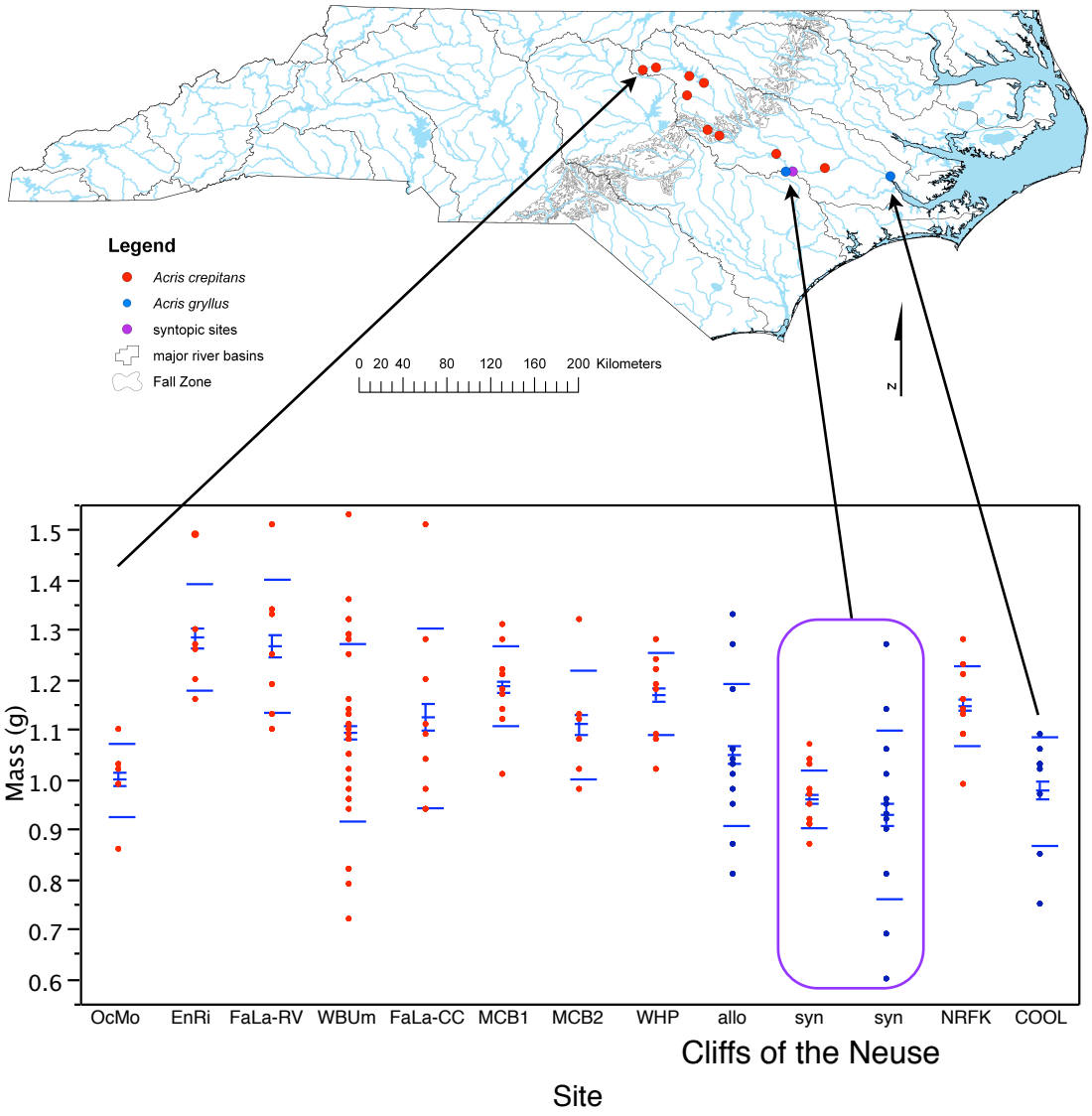


Figure 3.6c. Mass of *A. c. crepitans* and *A. g. gryllus* in the Neuse River basin. Central bars indicate the mean and standard errors. Outer bars indicate standard deviations.



Chapter 4

Acoustic species discrimination in syntopy by female cricket frogs, *Acris crepitans crepitans* and *Acris gryllus gryllus*

Abstract

The cricket frogs *Acris crepitans crepitans* and *A. gryllus gryllus* occur in syntopy in North Carolina and appear to be reproductively isolated, but the isolation mechanism has not previously been known. I conducted antiphonal playback experiments on females of both species with natural and synthesized vocalizations of males to determine whether differences in vocalizations contributed to pre-mating isolation. Both species discriminated between conspecific and heterospecific vocalizations differing in fine-scale temporal components and showed no preferences between conspecific vocalizations differing in dominant frequency. Although mate-quality discrimination can be compromised when high-quality conspecifics resemble heterospecifics, the results of the present study suggest that selection for species recognition is stronger than selection for mate-quality discrimination in syntopic *Acris*.

Introduction

Divergence in mate recognition systems often results in pre-mating reproductive isolation between related species (Coyne and Orr, 2004). The evolution of mate recognition systems can be complicated by the conflict between species recognition and mate-quality

discrimination when high-quality conspecifics resemble heterospecifics (Pfennig, 1998), as in the spadefoot toad, *Spea multiplicata* (Pfennig, 2000). Signal detection theory (Wiley and Richards, 1982; Wiley, 1994, 2006) predicts that organisms optimize transmission and reception of species-identifying signals and the identification of conspecific mates.

The cricket frogs *Acris crepitans crepitans* and *A. gryllus gryllus* have broadly overlapping ranges and are syntopic in at least four wetlands in North Carolina (Chapter 1). Morphological identification of *Acris* has previously been difficult and whether reproductive isolation exists between species and subspecies has been uncertain. *A. crepitans* females from an allopatric population in New Jersey and a sympatric population in Georgia successfully discriminated against the calls of *A. gryllus* but females from a Texas population did not (Nevo and Capranica, 1985). Mount (1975) identified frogs with intermediate morphological traits in Alabama as hybrids of *A. crepitans* and *A. gryllus*, but Mecham (1964) reported finding no frogs with intermediate morphologies in wild populations despite successfully hybridizing the species in the laboratory. Limited genetic divergence (Kaela Beauclerc, personal communication) has prevented development of mitochondrial (Rose et al., 2006) and microsatellite (Beauclerc et al., 2007) markers suitable for differentiating *Acris* species until recently. The mating vocalizations of both species of *Acris* are highly variable in dominant frequency and large-scale temporal structure (Nevo and Capranica, 1985). These factors all suggest that divergence within *Acris* is minor, and perhaps has only occurred recently. Characterizing the distinct differences between *A. c. crepitans* and *A. g. gryllus* in a small-scale temporal feature (the pulses within clicks) led to identifying morphological distinctions between the species at syntopic sites (Chapter 1). No hybrids

have been found in North Carolina (Chapter 1; Brimley, 1944). Thus, despite only slight morphological, genetic, and behavioral divergence, reproductive isolation appears to be strong in *Acris*. Pre-mating isolation, whether or not there is post-mating isolation, is probable.

The previous analyses of variation in click structure (Chapter 1) and click rate and dominant frequency in *A. c. crepitans* (Chapter 2) and *A. g. gryllus* (Chapter 3) indicated that the most consistent acoustic differences between the species were the patterns of pulses within clicks. Furthermore, reproductive character displacement had not occurred in dominant frequency or click rate even where the species are syntopic. These results suggested that dominant frequency or large-scale temporal features were less likely than the fine-scale structure of vocalizations to contribute to species recognition by females of either species. This study tests the hypotheses that female *A. c. crepitans* and *A. g. gryllus* use the distinct differences in click structure rather than the slight differences in dominant frequency for species discrimination in syntopy.

Methods

Natural playback experiments on A. g. gryllus

I conducted a test of the ability of female *A. g. gryllus* to discriminate species based on natural male vocalizations recorded for studies of variation between populations (Chapters 2 and 3) and species (Chapter 3) in *Acris*. I used recordings from five sites (Figure 4.1): the syntopic populations at Merchants Millpond ("MM"; NAD83 Lat. 36.43179°, Long. -76.69666°); two sites that were near Merchants Millpond but had only one of the species

("proximate allotopic"; *A. c. crepitans* from the Cashie River in Martin County, "CA", NAD83 Lat. 35.92478°, Long. -76.73445°; *A. g. gryllus* from Lake Phelps in Washington County, "PT", NAD83 Lat. 35.73400°, Long. -76.44083°); and two sites far from Merchants Millpond with only one species ("distant allotopic"; *A. c. crepitans* near Middle Creek in Johnston County, "MC", NAD83 Lat. 35.57138°, Long. -78.58368°; *A. g. gryllus* near Swift Creek in Cool Springs, Craven County, "CS", NAD83 Lat. 35.19123°, Long. 77.08344°). Within these geographic groups, I paired bouts of each species that differed by no more than 4 clicks and 2 s in total duration, but differed in the structure of pulses within clicks, dominant frequency, and bout-scale temporal structure. From these pairs, I randomly selected four pairs of Merchants Millpond bouts, two pairs of Cashie River and Lake Phelps bouts, and two pairs of Middle Creek and Neuse River bouts (Table 4.1). I used Sound Studio 2.2.4 (Felt Tip Software, 2005) to concatenate the two bouts of each pair into a two-channel AIFF file with 1 s of silence separating the antiphonal bouts. Half of the exemplar pairs began with an *A. c. crepitans* bout. Each file contained an equal number of bouts of the two species and ended after approximately 15 min (once the bout nearest the 15 min point was complete). I removed background vocalizations with the Silence function in Sound Studio and equalized the amplitudes of the two channels.

Amplexed *A. g. gryllus* females were collected from a chorus at Merchants Millpond containing both species from 10 June to 22 June 2006 between 2100 and 2400 hours. I photographed each female with its mate, captured the pair and confirmed the species of each female and male based on anal tubercle size, thigh stripe characteristics, and rugosity of the dorsum (Chapter 1), and released the male at the site of capture. I tested females within 5

hours of capture and released them on Merchants Millpond at another chorus at least 1500 meters from the collection site. The playback arena (240 x 120 x 71 cm high) had walls covered with acoustic tile. An incandescent red bulb was suspended over the center of the arena. An amplified speaker (Radioshack 277-1008) located 20 cm from each end of the arena was connected to the playback device (third-generation iPod, Apple Inc.). I alternated the speaker presenting each species from one night to the next. Before each trial, I measured the amplitude of a test vocalization emitted by each speaker with a sound pressure level meter (Realistic 33-2050, C weighting, fast response) and adjusted the speaker until each click peaked at 80 dB. For each trial, I randomly selected a female, placed her at the center of the arena under a perforated plastic cone, and released her remotely after one round of antiphonal bouts of a randomly selected playback file. Each playback continued for fifteen minutes or until a female left the arena or made a choice by demonstrating a phonotactic response to one of the signals and moving to within 20 cm of the speaker producing that signal.

Natural models for synthetic playback experiments

The temporal structure of synthesized clicks was modeled on recordings of five *A. c. crepitans* and four *A.g. gryllus* males recorded on 12 May 2005 at a mixed-species chorus at Merchants Millpond. I focused on recordings from one night and chorus to address click differences between the species from a single mixed chorus (Figure 4.2) rather than mean click differences between the two species. This decision also excluded any changes in click features by either species in choruses with different numbers of heterospecific males or

differences between males calling at substantially different temperatures. The resultant synthesized clicks therefore represented the vocalizations of males as a female would perceive them in a mixed chorus rather than the average vocalizations for each species or population. I used the SongSignatures function in WildSpectra (version 061025, Wiley, 2007a) to assess the structure of pulses within each click for 5 bouts of each of the 5 *A. c. crepitans* males and 2 *A. g. gryllus* males, 4 bouts for one *A. g. gryllus* male, and 2 bouts for 1 *A. g. gryllus* male. SongSignatures recognizes each note in a selected portion of a spectrogram based on a user-defined starting and ending amplitude thresholds and produces a text file with spectral and temporal information for each note and the selection overall. I used a sampling rate of 44.1 kHz and transform size of 16 for these measurements, which optimized temporal resolution (under 1 ms) at the expense of spectral resolution. SongSignatures indicated the number of pulses in each click, the duration of each pulse, and the interval from one pulse to the next. JMP 6.03 (SAS Institute, 2006) was used to calculate the median number of pulses, mean pulse duration, and mean interval between pulses in each quartile of a bout for each bout, individual, and species. This procedure generated means for the click structure in each quartile of a bout for each species. I modeled the dominant frequency of synthesized vocalizations on measurements from the previous study of dominant frequency in both species (Chapters 2 and 3).

Synthesis of files for playback

SoundSynth2 version 070516b (Wiley, 2007b) was used to synthesize the clicks as uncompressed AIFF files (Figure 4.3a and 5.3b). I created 4 *A. c. crepitans* clicks, one for

each quartile of a bout. For each quartile, I selected the 12 May 2005 natural click that most closely reflected the median pulse number and mean click duration and used it as the model for the intervals between pulses and the relative amplitude of each pulse in the synthesized click. I used the calculated median pulse numbers, mean intervals between pulses, and mean click durations to model the *A. g. gryllus* clicks directly. Because *A. g. gryllus* clicks varied so little in the latter 3 quartiles of a bout, I only synthesized 2 clicks: one for the first quartile and one to represent the 3 other quartiles. The latter synthesized click consisted of the click for the first quartile with two additional pulses and an amplitude profile adjusted to account for the additional pulses. I synthesized each click with each of three dominant frequencies. I used the population mean temperature-adjusted dominant frequency for each species (*A. c. crepitans*, 3962 Hz; *A. g. gryllus*, 3502 Hz) to synthesize clicks for both species, and the dominant frequency one standard deviation above the mean for *A. c. crepitans* (4150 Hz) and one standard deviation below the mean for *A. g. gryllus* (3353 Hz). To model the large-scale temporal structure of bouts for each species, I categorized natural bouts from Merchants Millpond *A. c. crepitans* (N=102) and *A. g. gryllus* (N=96) according to the number of clicks in each bout and randomly selected a bout from each quartile. This resulted in models representing short bouts (those in the first quartile for click number), intermediate bouts (in the second and third quartiles for click number), and long bouts (in the last quartile for click number) for each species. To assemble synthesized bouts, I concatenated the SoundSynth2 text files for each click into larger files. Finally, I used the natural bout as a template for the start time of each click in the synthesized bout and selected the synthesized click that most

reflected the structure of the natural click at that point in the bout. Figure 4.3c shows the two shortest bouts synthesized for Experiment 1.

I created two-channel AIFF files for each playback experiment with Sound Studio 2.2.4 (Felt Tip Studio, 2005). Each file began with 3 min of silence as an acclimation period and then consisted of antiphonal bouts separated by 1 s of silence for approximately 15 min (once the bout nearest the 15 min point was complete). For each of the 4 exemplar pairs in an experiment, I created files differing in the initial exemplar and the channel of each exemplar. Altogether I produced 16 files for each experiment.

In Experiment 1, I tested the effectiveness of the synthesized vocalizations in eliciting species recognition by females. I created 4 *A. c. crepitans* bouts, at 3962 Hz, and 4 *A. g. gryllus* bouts, at 3502 Hz, each with the number and intervals of clicks and total duration patterned on a natural bout. I randomly paired a bout from each species to create the 4 exemplar pairs for the experiment, so in each exemplar pair, the two signals differed not only in temporal and spectral structure of clicks but also in bout duration and the number and pattern of clicks in the bout. Each exemplar pair emphasized differences between the species and included both the dominant frequency and click differences that might be used by females for species recognition.

In Experiment 2, I tested the ability of females to discriminate between vocalizations differing only in specific click structure. I paired each bout used in Experiment 1 with a bout in which I replaced the clicks of one species with the clicks of the other species, so within each pair, the exemplars had exactly the same dominant frequency and large-scale structure. This produced 4 exemplar pairs for use on each species.

In Experiment 3, I tested the ability of females to discriminate between vocalizations with the mean conspecific dominant frequency (*A. c. crepitans*, 3962 Hz; *A. g. gryllus*, 3502 Hz) and with dominant frequencies one standard deviation above the mean for *A. c. crepitans* (4150 Hz) and below the mean for *A. g. gryllus* (3353 Hz). I paired each bout used in Experiment 1 with a bout resynthesized at the other dominant frequency, again producing 4 exemplars for use with females of each species.

Synthetic playback experiments

I collected amplexed females from a mixed-species chorus at Merchants Millpond State Park between 2100 and 0030 hours from 16 May to 10 July 2007. I photographed each female with its mate, captured the pair and determined the species of each female and male based on thigh stripe characteristics and rugosity of the dorsum (Chapter 1), and released the male at the site of capture. I tested each female within 5 h of capture before releasing it the following night on Merchants Millpond at another chorus at least 700 meters from the site of capture. I conducted experiments on both species concurrently. Experiment 1 (synthesis) took place from 16 May to 30 May and Experiment 2 (click structure) and 3 (dominant frequency) concurrently from 30 May to 4 July. I used the same arena as in 2006 but replaced the single incandescent bulb with a pair of red button-cell LEDs for more uniform lighting and connected the speakers to a compact two-channel mixer (Radioshack 32-2056) with input from a third-generation iPod (Apple Inc.). Before each trial, I measured the amplitude of a test vocalization emitted by each speaker with a sound pressure meter

(Radioshack 33-2055, C weighting, fast response) and adjusted the speaker and mixer until each click peaked at 80 dB.

For each trial, a female was selected randomly, placed at the center of the arena under a perforated plastic cone, and released remotely after one round of antiphonal bouts of a randomly selected playback file. I recorded the time and direction of the female's first hop and each hop thereafter. Each playback continued for 15 min (no choice) or until a female demonstrated a phonotactic response to one of the signals and moved to within 20 cm of the speaker producing that signal (choice). In Experiment 1, I tested each female once. I continued testing females until I recorded one choice for each of the 16 playback files for both species. Because each exemplar was played an equal number of times from either side of the arena, any side bias in the arena could be detected and corrected before progressing to the other experiments. In Experiment 2 and 3, I randomly assigned an experiment and playback file to each female and continued testing females until I recorded 4 choices for each exemplar pair. Because no bias was detected in the first experiment, I randomized the presentation of each exemplar pair instead of seeking a response to each of the 16 playback files. If a female made a choice in the initial trial, it was placed in a sample of pond water for 60 seconds and then returned to the center of the arena to begin a trial for the other experiment.

A Sign Test was used to assess preference and JMP 6.0.3 (SAS Institute, 2006) was used to conduct Wilcoxon tests on the latency to first hop and latency to choice for each experiment.

Results

Table 4.2 summarizes the results. In 2006, female *A. g. gryllus* preferred the vocalizations of conspecific males (Sign Test: $N = 15$, $p < 0.05$). In the one trial in which a female chose an *A. c. crepitans* recording, the stimulus was the first syntopic exemplar pair. The latency to choice for this female did not differ significantly from the 15 choices for *A. g. gryllus* (Wilcoxon Test: $\chi^2 = 0.29$, d.f. = 1, $p = 0.59$).

In the first experiment of 2007, both species preferred conspecific vocalizations (Sign Test: *A. c. crepitans*, $N = 15$, $p < 0.05$; *A. g. gryllus*, $N = 13$, $p < 0.05$), an indication that the synthesized signals were sufficient for species discrimination. Because the stimulus pairs differed to varying degrees in duration and other large-scale temporal features, these were unlikely to have influenced species discrimination. In both species, there were no significant differences in the latency to first hop between females that chose conspecific signals and those that chose heterospecific signals (Wilcoxon Test: *A. c. crepitans*, $\chi^2 = 1.42$, d.f. = 1, $p = 0.23$; *A. g. gryllus*, $\chi^2 = 0.11$, d.f. = 1, $p = 0.74$) or latency to choice (Wilcoxon Test: *A. c. crepitans*, $\chi^2 = 1.42$, d.f. = 1, $p = 0.23$; *A. g. gryllus*, $\chi^2 = 0.11$, d.f. = 1, $p = 0.74$)

While the second and third experiments were taking place, *A. c. crepitans* stopped amplexing at the collection site before 16 choices were made in each experiment. Nonetheless, enough choices were available for statistical analysis. In Experiment 2, which tested preference for pulse structure of clicks, 8 of 8 *A. c. crepitans* females preferred the conspecific signal (Sign Test, $p < 0.05$). In Experiment 3,there was no preference for conspecific signals at the mean dominant frequency or one standard deviation above the mean (Sign Test; 8 for mean, 6 for SD, $p > 0.05$) and no difference in latency to choice

(Wilcoxon Test; $\chi^2 = 0.27$, d.f. = 1, $p = 0.60$). There was a difference in latency to first hop (Wilcoxon Test; $\chi^2 = 9.75$, d.f. = 1, $p = 0.0018$) between females that chose the high-frequency bout (mean = 44 s) and the low-frequency bout (mean = 204 s).

Every female *A. g. gryllus* preferred bouts with conspecific clicks in Experiment 2 (Sign Test; N = 16, $p < 0.05$). In Experiment 3, *A. g. gryllus* females had no preference for conspecific signals at the mean dominant frequency or one standard deviation below the mean, as half the females chose each signal (Sign Test; N = 16, $p > 0.05$). There was no difference in the latency to first hop (Wilcoxon Test; mean = 100 s., $\chi^2 = 0.07$, d.f. = 1, $p = 0.79$) or latency to choice (Wilcoxon Test; mean = 319 s., $\chi^2 = 0.28$, d.f. = 1, $p = 0.60$).

Discussion

With pairs of natural *A. g. gryllus* and *A. c. crepitans* bouts, with varying differences in dominant frequency and large-scale temporal structure, female *A. g. gryllus* were able to recognize the conspecific signal (Table 4.1). The experiments with synthesized vocalizations showed that the temporal structure within clicks was sufficient for species discrimination by females of both species (Table 4.2). Females had no preference between synthesized conspecific vocalizations differing in dominant frequency, except that *A. c. crepitans* females that chose the signal with the higher dominant frequency hopped sooner than those that chose the lower dominant frequency. No preference occurred in either species for the dominant frequency that was most removed from the dominant frequency of the heterospecific males.

Several differences separate the clicks of the two species, including duration, pulse number, variance in the interpulse interval (Chapter 1), pulse shape, and pulse rate. One or a

combination of these differences could have been used by females for identification of conspecific males. Furthermore, because the clicks of *A. c. crepitans* vary more than those of *A. g. gryllus* and because the difference in variation was reflected in the synthesized clicks, the extent of variation among the clicks, rather than any specific component of the clicks themselves, might have been used for discrimination. In grey treefrogs, *Hyla chrysoscelis* and *H. versicolor*, differences in pulse rate were sufficient for species discrimination but changes in pulse duration and interpulse interval also affected female preference in *H. versicolor* (Gerhardt and Huber, 2002). Among field crickets (Orthoptera: Gryllidae), pulse rate or period is the most important feature used by females for mate selection, including species discrimination (Gerhardt and Huber, 2002). The taxonomic and geographic relatedness of grey treefrogs to *Acris* makes an informative comparison. The acoustic similarity of *Acris* to the Gryllidae and two other families of orthopteran insects is not just a inspiration for nomenclature, but invites comparison of mate recognition systems.

Experiments on the spectral and temporal preferences of female *A. crepitans* in the Midwest have found inconsistent results. The basilar papilla of the female ear was tuned below the mean dominant frequency of three *A. crepitans* populations in which females also demonstrated preferences for lower dominant frequency (Ryan and Wilczynski, 1988; Ryan et al., 1992). In another study, female *A. crepitans blanchardi* did not have a preference among vocalizations with different dominant frequencies, but they preferred vocalizations that included facultative temporal changes made by males in proximity to other males (Kime et al., 2004). Temporal complexity might have affected the discriminability of vocalizations differing in dominant frequency (Witte et al., 2001; Kime et al., 2004). Pulse-scale temporal

features did not affect the preferences of female *A. crepitans* in Illinois, but dominant frequency did (Perrill and Lower, 1994). Smaller females prefer dominant frequencies that are higher than those preferred by larger females because basilar papilla tuning is size-dependent (Ryan et al., 1992), so even within populations, selection by females is not purely directional. It is probable that differences in female preferences in the Midwest are population-specific (Perrill and Lower, 1994). Selection on female choice could differ among populations, but genetic drift may also produce such differences.

The results of this study indicate that there is strong selection in syntopy for avoidance of heterospecific mating. At the edge of the range of *A. crepitans* and in syntopy with *A. gryllus*, the geographic, ecological, and morphological overlap between the species, together with the low incidence of hybridization in syntopy, indicates a reliable reproductive isolating mechanism. When choosing conspecific males, females of both species used the most distinct difference between species, temporal structure within clicks (Chapter 1), for species discrimination. Females disregarded a feature, dominant frequency, that correlates with body size (Chapters 2 and 3) and might thus indicate male quality but that overlaps with the dominant frequency of heterospecifics. In *A. c. crepitans* in the Midwest, selection on temporal components of vocalizations for decreased signal degradation during propagation has been more important than selection on spectral features (Wilczynski and Ryan, 1999). The importance of temporal features for *A. c. crepitans* and *A. g. gryllus* and the consistency with previous experiments on *A. crepitans* indicates that similar interpretations might be apply to the Southeast.

Important aspects of the reproductive ecology of *Acris* in syntopy were not addressed in this study. Although post-metamorphic *Acris* with hybrid phenotypes have never been detected in North Carolina, cross-fertilization and rearing experiments would determine whether or not post-mating isolation mechanisms, such as hybrid inviability or failure of hybrids to reach the adult stage, might result in selection for pre-mating isolation. Because of their disregard for dominant frequency, it is not clear how females of either species assess conspecific mate quality in the syntopic chorus. The lower mass of males in syntopy (Chapter 3) and, in this study and others, the lack of female preference for lower dominant frequency associated with body size, should be explored further. *A. c. crepitans* breeding activity appeared to peak earlier in the summer than that of *A. g. gryllus*, perhaps because the species is better adapted for cooler temperatures, but it is still unclear what factors determine the occurrence of each species at a particular site within the zone of sympatry.

Female *Acris crepitans crepitans* and *Acris gryllus gryllus* from a syntopic site discriminated the vocalizations of conspecific and heterospecific males based on the structure of clicks and not on dominant frequency. Discrimination of large-scale temporal structure was not directly assessed, but such differences did not appear to affect female responses.

Table 4.1 Natural vocalizations used for playback to female *A. g. gryllus* in 2006

Category		Site	Species	N (clicks)	duration (s)	mean dominant frequency (Hz)
Syntopic	1	CASH	A. c. crepitans	23	4.36	4076
		Pett	A. g. gryllus	20	4.46	3470
			difference	3	-0.1	606
	2	CASH	A. c. crepitans	35	12.12	3811
		Pett	A. g. gryllus	35	13.65	3537
			difference	0	-1.53	274
	3	MCB1	A. c. crepitans	46	17.92	3480
		COOL	A. g. gryllus	45	18.52	3582
			difference	1	-0.6	-102
	4	MCB1	A. c. crepitans	21	8.63	3747
			A. g. gryllus	18	8.49	3777
			difference	3	0.14	-30
Non-syntopic	1	MeMi	A. c. crepitans	36	10.63	3700
		MeMi	A. g. gryllus	32	11.28	3563
			difference	4	-0.65	137
	2	MeMi	A. c. crepitans	20	13.18	3796
		MeMi	A. g. gryllus	17	13.09	3480
			difference	3	0.09	316
	3	MeMi	A. c. crepitans	36	14.99	3703
		MeMi	A. g. gryllus	36	15.69	3301
			difference	0	-0.7	402
	4	MeMi	A. c. crepitans	63	24.14	4173
		MeMi	A. g. gryllus	66	24.25	3549
			difference	-3	-0.11	624

Table 4.2. Results of playback experiments in 2006 and 2007. Asterisks indicate significant preferences by females.

	Experiment	Subject	Stimulus	N (choices)	mean latency to first hop (s)	mean latency to choice (s)
2006:	Natural vocalizations	*A. g. gryllus*	*A. c. crepitans*	1	-	393
			A. g. gryllus	15*	-	302
			total	16		308
2007:	Synthesis	*A. c. crepitans*	*A. c. crepitans*	15*	147	487
			A. g. gryllus	1	335	497
			total	16	187	488
		A. g. gryllus	*A. c. crepitans*	3	189	491
			A. g. gryllus	13*	194	370
			total	16	193	393
	Click type	*A. c. crepitans*	*A. c. crepitans*	8*	120	446
			A. g. gryllus	0	-	-
			total	8	-	-
		A. g. gryllus	*A. c. crepitans*	0	-	-
			A. g. gryllus	16*	82	346
			total	16	-	-
	Dominant frequency	*A. c. crepitans*	4150	8	44	381
			3962	6	204	418
			total	14	182	397
		A. g. gryllus	3502	8	101	338
			3353	8	81	300
			total	16	100	319

Figure 4.1. Recording sites for 2006 female choice experiments

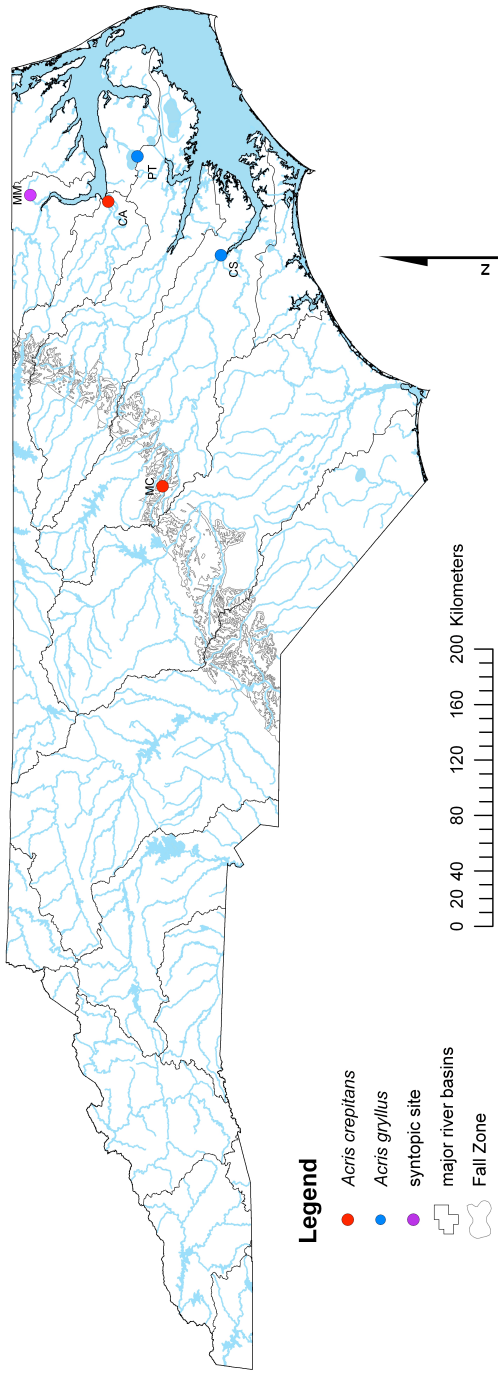


Figure 4.2. Oscillograms of typical clicks of *Acris c. crepitans* and *A. g. gryllus*

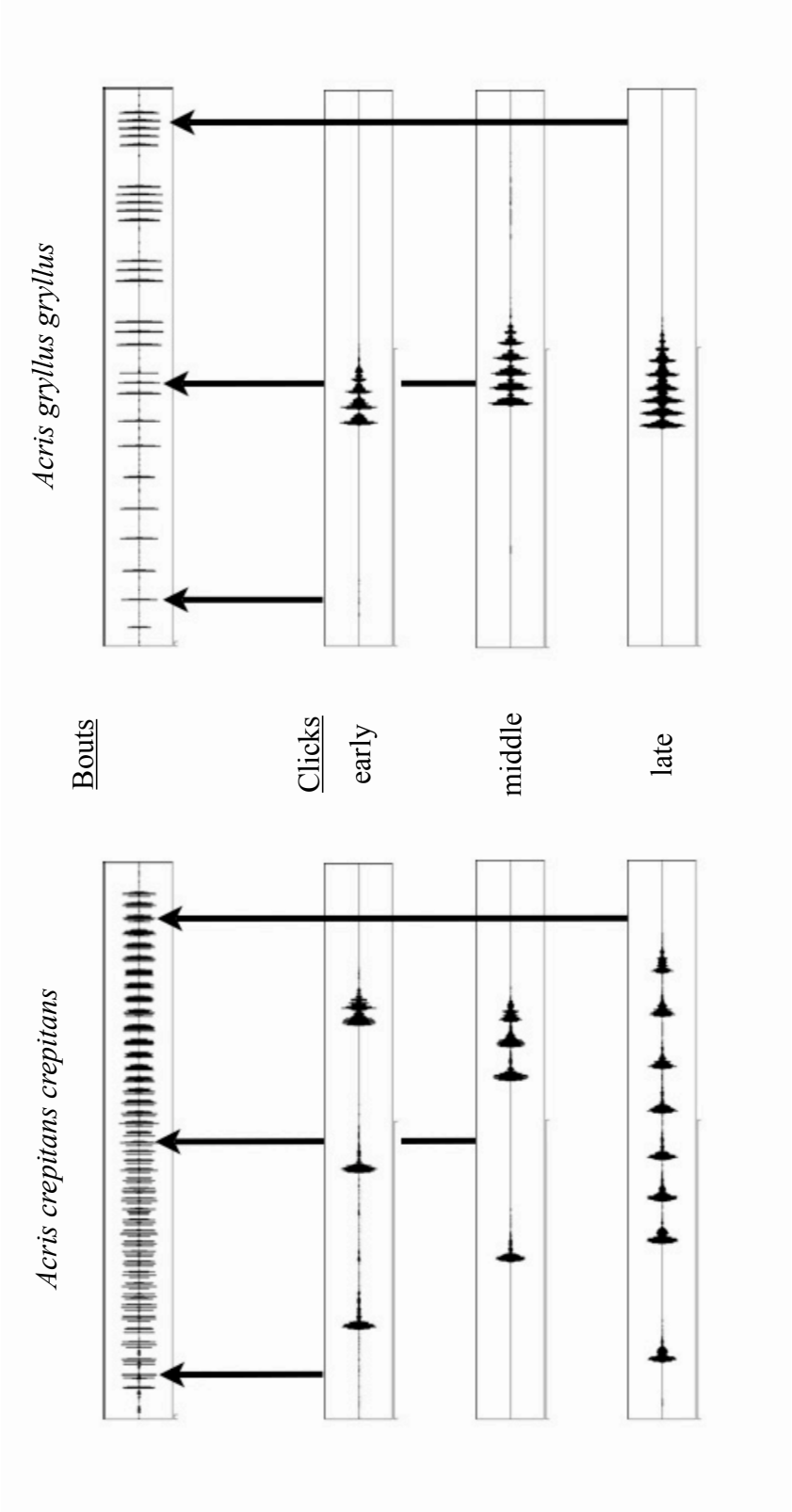


Figure 4.3a. Oscillograms of 4 synthesized clicks of *Acris c. crepitans* used in playback experiments in 2007.

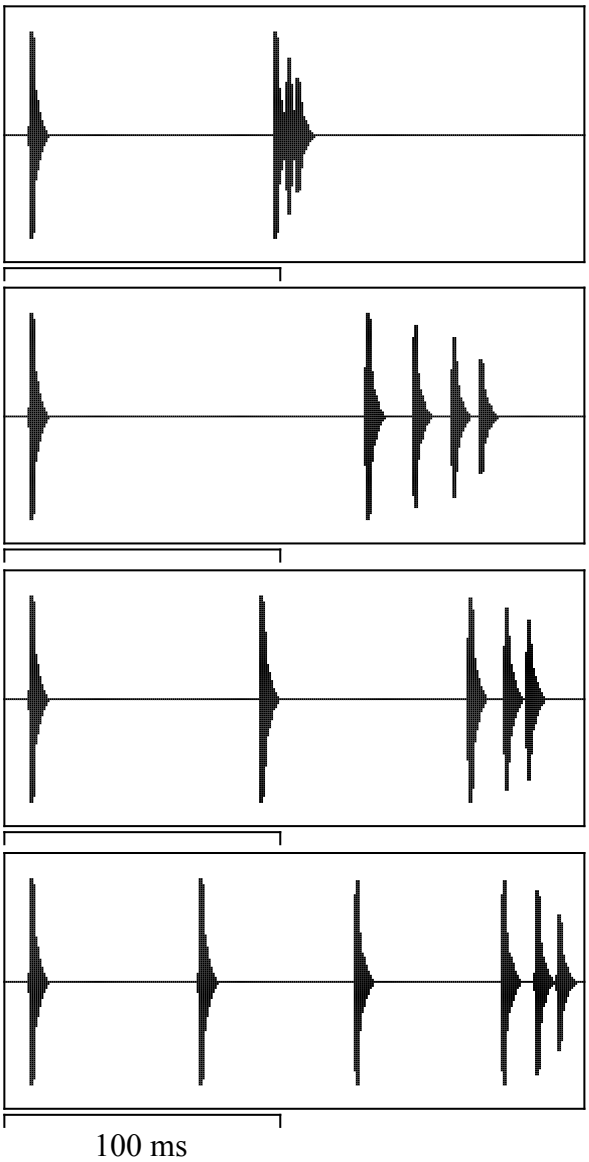


Figure 4.3b. Oscillograms of 2 synthesized clicks of *Acris g. gryllus* used in playback experiments in 2007.

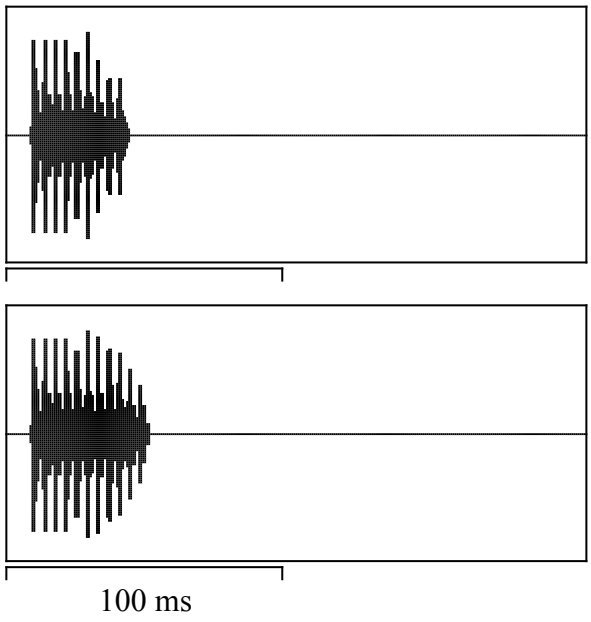
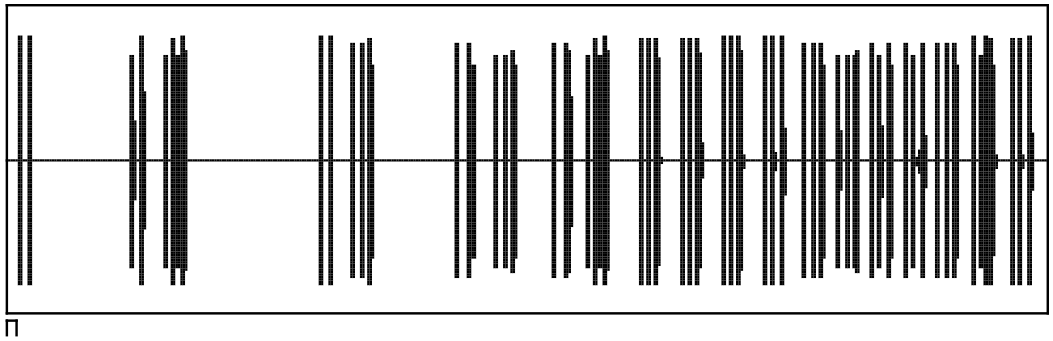
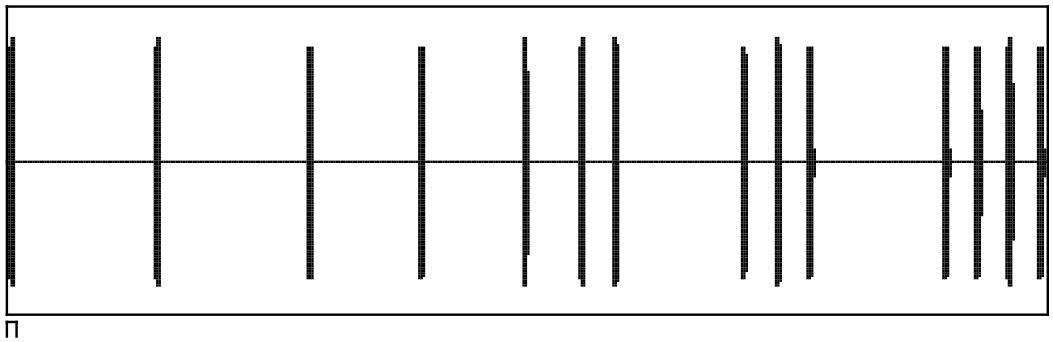


Figure 4.3c. Oscillograms of short (10 s) *Acris* bouts synthesized for Experiment 1 in 2007. These are modeled on bouts in the first quartile for click number at Merchants Millpond.

A. c. crepitans



A. g. gryllus



Appendix. Field sites, management agencies, and geographic coordinates (NAD83 datum). NCDPR: North Carolina Department of Parks and Recreation. NCWRC: North Carolina Wildlife Resources Commission. RCWC: Roanoke-Chowan Wildlife Club. TLC: Triangle Land Conservancy. UNC: University of North Carolina. WHC: Waynseborough Historic Commission.

Site	Location	Management	Species	Latitude	Longitude
BENN	Bennett's Millpond	Chowan Co.	*A. g. gryllus*	36.15287	-76.66357
CaBe	Carolina Beach State Park	NCDPR	*A. g. gryllus*	34.04309	-77.90969
CASH (CA)	Cashie River	none	*A. c. crepitans*	35.92418	-76.73445
ClNe-allo	Cliffs of the Neuse State Park	NCDPR	*A. g. gryllus*	35.23613	-77.88506
ClNe-syn (CN)	Cliffs of the Neuse State Park	NCDPR	both	35.22819	-77.88223
COOL (CS)	Cool Springs boat launch	NCWRC	*A. g. gryllus*	35.19123	-77.08344
CrMo	Crowder's Mountain State Park	NCDPR	*A. c. crepitans*	35.21016	-81.29326
EnRi	Eno River State Park	NCDPR	*A. c. crepitans*	36.08026	-79.00616
FaLa-CC	Falls Lake State Rec. Area	NCDPR	*A. c. crepitans*	35.95424	-78.61367
FaLa-RV	Falls Lake State Rec. Area	NCDPR	*A. c. crepitans*	36.01103	-78.73368
GAIN	Gainie Road Gravel Pit	private	*A. c. crepitans*	34.96911	-78.78715
GATE	Gatesville borrow pit	private	both	36.39785	-76.74918
HAL	Halifax beaver pond	private	*A. c. crepitans*	36.19955	-77.86637
HARE	Hare's Millpond	RCWC	both	36.37260	-76.91055
HaRo	Hanging Rock State Park	NCDPR	*A. c. crepitans*	36.38864	-80.27112
Jone	Jones Lake State Park	NCDPR	*A. g. gryllus*	34.68122	-78.59874
Kerr	Kerr Lake State Rec. Area	NCDPR	*A. c. crepitans*	36.43823	-78.34382
LaNo	Lake Norman State Park	NCDPR	*A. c. crepitans*	35.67728	-80.94046
LaNo	Lake Norman State Park	NCDPR	*A. c. crepitans*	35.67551	-80.93837
LaNo	Lake Norman State Park	NCDPR	*A. c. crepitans*	35.67762	-80.94091
LaWa	Lake Waccamaw State Park	NCDPR	*A. g. gryllus*	34.26094	-78.52350
LaWa	Lake Waccamaw State Park	NCDPR	*A. g. gryllus*	34.25921	-78.48366
LaWa	Lake Waccamaw State Park	NCDPR	*A. g. gryllus*	34.26000	-78.51872
MCB1 (MC)	Middle Creek Bottomlands	TLC	*A. c. crepitans*	35.57138	-78.58368
MCB2	Middle Creek Bottomlands	TLC	*A. c. crepitans*	35.52441	-78.48677
MeMi (MM)	Merchants Millpond State Park	NCDPR	both	36.43179	-76.69667

Site	Location	Management	Species	Latitude	Longitude
MeMi	Merchants Millpond State Park	NCDPR	both	36.43122	-76.69647
MeMi	Merchants Millpond State Park	NCDPR	both	36.43150	-76.69663
MeMi	Merchants Millpond State Park	NCDPR	both	36.42441	-76.67717
MFBR (MF)	Mason Farm Biological Reserve	UNC	*A. c. crepitans*	35.89005	-79.00866
MFBR	Mason Farm Biological Reserve	UNC	*A. c. crepitans*	35.89215	-79.02037
MFBR	Mason Farm Biological Reserve	UNC	*A. c. crepitans*	35.89018	-79.00935
NRFK	Neuse River in Kinston, NC	none	*A. c. crepitans*	35.25923	-77.62067
OcMo	Occoneechee Mountain SNA	NCDPR	*A. c. crepitans*	36.06011	-79.11407
PBBT (PB)	Pineberry Bay	NCWRC	*A. g. gryllus*	34.97568	-78.48395
Pett (PT)	Pettigrew State Park	NCDPR	*A. g. gryllus*	35.73400	-76.44083
RaRo	Raven Rock State Park	NCDPR	*A. c. crepitans*	35.44016	-78.87911
RaRo	Raven Rock State Park	NCDPR	*A. c. crepitans*	35.44158	-78.87788
RaRo	Raven Rock State Park	NCDPR	*A. c. crepitans*	35.44576	-78.87048
RaRo	Raven Rock State Park	NCDPR	*A. c. crepitans*	35.44359	-78.87098
RaRo	Raven Rock State Park	NCDPR	*A. c. crepitans*	35.44142	-78.87304
RaRo	Raven Rock State Park	NCDPR	*A. c. crepitans*	35.43929	-78.87323
RaRo	Raven Rock State Park	NCDPR	*A. c. crepitans*	35.44673	-78.86413
RHOD	Rhodes Millpond	NCWRC	*A. c. crepitans*	35.22589	-78.65241
ROAN1	Roanoke River Wetlands	NCWRC	*A. c. crepitans*	35.88325	-77.13712
ROAN2	Roanoke River boat launch	NCWRC	*A. c. crepitans*	36.20569	-77.38601
SGL	Sandhills Gameland	NCWRC	*A. g. gryllus*	34.99828	-79.51797
TAHE	Tar Heel boat launch	NCWRC	*A. c. crepitans*	34.74723	-78.78435
TLCJ	Justice Tract	TLC	*A. c. crepitans*	35.62966	-79.11966
TLCJ	Justice Tract	TLC	*A. c. crepitans*	35.63414	-79.10582
WBUm	WB Umstead State Park	NCDPR	*A. c. crepitans*	35.86411	-78.75387
WBUm	WB Umstead State Park	NCDPR	*A. c. crepitans*	35.87117	-78.76282
WBUm	WB Umstead State Park	NCDPR	*A. c. crepitans*	35.84007	-78.74436
WBUm	WB Umstead State Park	NCDPR	*A. c. crepitans*	35.83947	-78.74673
WeWo	Weymouth Woods	NCDPR	*A. g. gryllus*	35.15509	-79.35639
WHP	Waynesborough Historic Village	WHC	*A. c. crepitans*	35.37415	-78.01898

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