ECOLOGY AND DISTRIBUTION OF THE FLORIDA BOG FROG AND FLATWOODS SALAMANDER ON EGLIN AIR FORCE BASE

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ABSTRACT

I studied the ecology and distribution of the Florida bog frog (*Rana okaloosae*) and flatwoods salamander (*Ambystoma cingulatum*) on Eglin Air Force Base in northwest Florida. I report data on the breeding ecology, population dynamics, home ranges, microhabitat, and distribution of the endemic bog frog and make comparisons to its closest relative, the bronze frog (*Rana clamitans clamitans*). Bog and bronze frogs occur in the same habitats and are suspected to hybridize.

I investigated the potential for auditory and visual mate recognition errors between *R. okaloosae* and *R. clamitans*. I also described the vocal repertoire of the bog frog and observed the response of resident males to the playback of unfamiliar conspecific and heterospecific (*R. clamitans*) calls. The advertisement calls of bog frogs vary among individuals, and individual voice recognition is possible.

I exposed tadpoles of bog frogs, bronze frogs, and leopard frogs (*R. sphenocephala*) to chemical cues from 2 predators, the banded water snake (Colubridae: *Nerodia fasciata*) and the red fin pickerel (Esocidae: *Esox americanus*) to evaluate whether swimming behavior or habitat selection differed among tadpole species. The time spent swimming differed among tadpole species and predator treatments, differences which potentially could affect growth rates, survivorship rates, and distribution patterns.

Lastly, I discuss the relationship between fire and the federally–threatened flatwoods salamander (*Ambystoma cingulatum*). I compared 13 breeding wetlands with different fire histories in addition to burned and unburned halves of a partially–burned wetland. In general, areas that burned more recently had more open canopies, higher dissolved oxygen concentrations, higher water temperatures, more understory vegetation, and were shallower than unburned areas. Rangewide surveys indicate that prescribed fires typically are applied in winter and early spring in areas inhabited by flatwoods salamanders. Based on what is known about the natural history of the species, the historical burning regime of the longleaf ecosystem, and the effects of fires on ephemeral wetlands, I suggest that land mangers diversify their fire–management strategy to increase the likelihood of burning the breeding wetlands of flatwoods salamanders.

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This research was supported by a grant from the natural resources branch (Jackson Guard) at Eglin Air Force Base and administered through the USGS Biological Resources Division, Virginia Cooperative Fish and Wildlife Research Unit of Virginia Tech. Throughout this document, I use the singular pronoun to maintain consistency; however, I had significant help from others.

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FOREWORD

This dissertation summarizes my research on the ecology and distribution of the Florida bog frog (Ranidae: *Rana okaloosae*) and flatwoods salamander (Ambystomatidae: *Ambystoma cingulatum*) on Eglin Air Force Base (AFB). Eglin AFB is the largest forested military base in North America, encompassing 187,630 hectares (464,000 acres) in Okaloosa, Santa Rosa, and Walton counties in northwest Florida. Principal communities include sandhill, upland pine forest, upland mixed forest, mesic and wet flatwoods, wet prairie, baygall, seepage slope, floodplain swamp, depression marsh, and blackwater stream (Printiss and Hipes 2000). Eglin AFB contains much of the remaining old–growth (150–300 years old) longleaf pine (*Pinus palustris*) forests in the southeastern coastal plain and is managed actively for rare species, such as the endangered red–cockaded woodpecker (*Picoides borealis*).

The distribution component of my research was published in a separate report (Bishop 2004) to protect the locations of species and limit the size of my dissertation. Bog frogs have been verified at 58 locations on Eglin, plus 3 north of Eglin (as of December 2004). However, there has been little effort to survey potential sites outside of the base. There are gaps in the distribution of bog frogs within and among drainages on Eglin for unknown reasons. Flatwoods salamanders have been captured at 18 wetlands on Eglin. I captured salamanders at 7 of these between 2002 and 2004, but there likely was little population recruitment because the wetlands dried prematurely. The number of occupied breeding wetlands and the number of salamanders appears to have declined severely since the original surveys were conducted in the early 1990s. Individuals wishing to view this report can request a copy from natural resources personnel at Eglin

AFB (Jackson Guard, Wildlife Section, 107 Highway 85 N, Niceville FL 32580). Survey data for both species are available now in 2 monitoring databases, maintained by Jackson Guard.

Besides the survey component, the final product of my research deviated significantly from my original research plan. Weather issues (i.e., drought or too much rain) prevented burning experimental areas as scheduled. Weather problems, coupled with the rarity of flatwoods salamanders on Eglin AFB, limited the salamander component of my research primarily to surveying historic and potential wetlands for larvae and establishing a monitoring protocol (Bishop 2004). However, I did collect limited data on the effects of fires on the characteristics of breeding wetlands (e.g., vegetation, dissolved oxygen concentrations and water temperatures) and discuss the potential negative effects of fire suppression on larval flatwoods salamanders (Ch. 6).

This document is organized into 6 chapters plus a summary section and appendix. Each chapter has its own literature cited, tables, and figures. If you are reading a hard copy of this dissertation, some figures may be lower quality (and not in color) than in the pdf version. A pdf version is available through the Virginia Tech library. This research was conducted under Florida FWCC permits WV01232 and WV04037, USFWS permit TE049502, and Virginia Tech Animal Care Committee protocol numbers 04-012-F&W and 02-084-F&W.

LITERATURE CITED

Bishop, D. C. 2004. Summary of surveys for Florida bog frogs and flatwoods salamanders on Eglin Air Force Base through 2004. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Printiss, D. J., and D. L. Hipes. 2000. Flatwoods salamander survey and habitat evaluation of Eglin Air Force Base, Hurlburt Field, and Tyndall Air Force Base. Final Report to U.S. Department of Defense, U.S. Air Force. Florida. Natural Areas Inventory, Tallahassee, Florida.

CHAPTER 1. BREEDING BIOLOGY, POPULATION DYNAMICS, BODY SIZE, AND MICROHABITAT OF FLORIDA BOG FROGS

ABSTRACT.—I monitored the breeding ecology, population dynamics, and body size of Florida bog frogs (*Rana okaloosae*) at one location in Okaloosa County, Florida, for 3 breeding seasons. The number and size of individuals at my study site varied among years. Mark–recapture estimates were similar to the number of actual males captured, suggesting I effectively censused the study site. Males selected calling positions in low–flow, shallow areas, typically near the stream bank and cover. Calling individuals occasionally had satellite males; satellites were not always smaller than callers. Egg masses were found March–August and had a mean clutch size of 234.7 eggs (range: 152–345, N = 10). Eggs were deposited close to male calling locations, suggesting that males choose calling sites that also are suitable for oviposition. Mating success was correlated with the number of nights a male spent calling, but not with body size.

INTRODUCTION

The Florida bog frog (*Rana okaloosae*) is one of the least studied amphibians in North America and has one of the most limited distributions (Moler 1993, Bishop 2004). Since its discovery in 1982 (Moler 1985), no natural history study has been conducted on *R. okaloosae*. Current knowledge of the species is limited to the original description (Moler 1985), a few surveys (Moler 1986; Printiss and Hipes 1999; Enge 2002) and a recent phylogenetic analysis of the *Rana catesbeiana* group (Austin et al. 2003).

The distribution of the bog frog is restricted to a few drainages in Okaloosa, Santa Rosa, and Walton counties in northwestern Florida (Moler 1993, Bishop 2004). It is designated a ‘species of special concern’ in the state of Florida, and because the majority of its known distribution occurs within the boundary of Eglin Air Force Base, it recently was enrolled in the ‘species at risk’ program, a partnership between the U. S. Fish and Wildlife Service and the Department of Defense. The goal of this program is to protect rare species on military lands to reduce the likelihood that federal listing will be needed in the future.

A primary objective of the species at risk program is to establish a management plan for each enrolled species (e.g., Jackson 2004). Before a management plan can succeed, however, we must first understand the basic ecology of the species of interest. Data on distribution, body size, sex discrimination, population dynamics, breeding biology, and microhabitat preferences provide the foundation on which management plans and monitoring techniques are based. I monitored Florida bog frogs for 3 seasons at one location in Okaloosa County, Florida, and report data on each of these topics for the first time.

METHODS

Study site.—My study site (30°30’N, 86°43’W) is a 120–m first order tributary of Live Oak Creek (LOC) on Eglin Air Force Base in Okaloosa County, Florida (Site ID 125, Appendix 1, Bishop 2004). The creek is located in a longleaf pine (*Pinus palustris*) and turkey oak (*Quercus laevis*) sandhill community that is managed for the endangered red cockaded woodpecker (*Picoides borealis*). The creek varies from 7–22 m in width in the section I monitored. The upper 60 m of the creek is divided into two channels, separated by a strip of land approximately 5 m in width. The substrate in the creek consists of bare sand in the flowing sections and organic matter in the low–flow sections. The water level remained fairly stable throughout the study (2002–2004) and ranged from < 1 to 20 cm in depth across the site. The creek becomes progressively deeper farther downstream. Dominant canopy species along the creek are sweetbay (*Magnolia virginiana*) and black titi (*Cliftonia monophylla*).

Sex differentiation.—I determined the sex of each captured frog by applying slight pressure to the abdomen with my thumb, a technique that caused males to inflate their paired vocal sacs (Appendix). Vocal sacs are not internal, as previously reported (Moler 1985, 1993; Bartlett and Bartlett 1999). Females lack external vocals sacs and typically have smaller tympanums than males (see Results). Gravid females can be identified by focusing a concentrated light source (e.g., head–lamp) through their abdomen and observing eggs.

Mark–recapture.—I thoroughly searched the study site for bog frogs on 30 nights (27 May–10 September) in 2002, 84 nights (27 March–3 August) in 2003, and 32 nights (1 April–8 July) in 2004. I toe–clipped each individual for permanent identification and marked each capture location with a wire flag that identified the capture date and frog identification number. Each observation period typically lasted 2–3 h per night and was terminated after all calling males were captured and the entire study site was searched.

I estimated the number of calling males present at the study site for each week in June and July in 2002 and 2003 using the robust design mark–recapture method (Pollock 1982) in program Mark 4.0 (White and Burnham 1999). My objective was to evaluate whether the numbers of males I captured were similar to mark–recapture estimates. The robust design technique combines the power of closed population models for estimating population size and the ability to estimate survivorship as in an open population model. I considered each week a closed sampling period for which I estimated the number of calling males present. If there were more than 2 nights sampled within a week (2003 data), I selected the 2 nights with the fewest number of days between them. I considered the periods between weeks as open, between which animals could move into or out of the study area. Hence, there were 8 primary sampling intervals (weeks 1–4 in June and July) and 16 secondary sampling intervals each year. No data, however, were collected the last week of June in 2002 because of inclement weather. I selected June and July because I had the best data for these months, and they are times when bog frogs call actively.

Body size.—For each newly captured frog, I measured its snout–vent length (SVL) and the diameters of its right tympanum and right eye with dial calipers. I weighed each individual to the nearest 0.5 g using a Pesola™ spring scale after attempting to void the bladder. In addition to comparing the size distribution within the LOC study site, I combined measurement data with frogs captured at other locations on Eglin to provide more information on body size and sex differences within the species.

Breeding biology.—I searched for recently deposited *R. okaloosae* egg masses on each observation night in 2003 and typically the following day. I calculated the average number of calling males per observation night and the total number of egg masses deposited for 2–week intervals for 2003. I measured the length (long axis) and width of each egg mass, the depth of the water in which it was deposited, and the distance deposited from the bank. I took digital photographs of 10 egg masses (April–July), from which I counted clutch size.

I identified the father of each egg mass by comparing the known spatial distribution of calling males each night (Ch. 2) relative to oviposition locations. Because of the limited movements of bog frogs (Ch. 2) and frequent site visits, I believe I was able to identify the father for most egg masses using this method. I tested whether the number of clutches fathered by each male in the 2003 breeding season was correlated with SVL or the number of nights a male was actively calling. To evaluate whether male calling locations were the same as oviposition locations (i.e., do males call from suitable oviposition sites?), I measured the distance between each egg mass and the calling position of the father (i.e., closest male) the night the mating occurred.

Bog frogs adopt a satellite strategy on rare occasions, where non–calling males position themselves near calling individuals (Wells 1977a). I identified satellite males by their lack of calling and low posture. I never observed satellites on evenings when oviposition occurred, thus simplifying the identification of the father of each egg mass. In 2003 and 2004, I measured the SVLs of observed satellite–caller pairs to evaluate whether body size was related to whether an individual was a satellite or caller.

Male calling sites.—Lastly, to characterize the microhabitat of male calling sites, I compared 50 known calling positions to 50 random locations within the study area. To select random locations, I established 10 transects at 10–m intervals in the study site and selected 5 random positions along each transect. At each of the 100 locations, I measured water depth, water temperature, distance to the bank, whether water was flowing (yes or no), whether emergent structure was present within 10 cm of the calling location (yes or no), and the distance to cover. I defined emergent structure as any living or nonliving item (e.g., woody debris) that extended > 2 cm above the water level and cover as vegetation or structure < 20 cm above the water level that a frog could hide under. All measurements were made between 0800 h and 1100 h on the same day. I used logistic regression analysis to identify significant variables associated with known (1) and random (0) locations, using backward elimination with Wald's statistics to select significant variables (P to enter and remove variables = 0.05). I used SPSS 11.0 for all statistical analyses in this chapter and subsequent chapters.

RESULTS

Mark–recapture.—The number of males and females captured at the LOC site varied among years. I captured 39 frogs in each of the second and third years. Of these, 8 (20.5%) each year were recaptures from the previous season(s) (Table 1). I only captured 1 individual in all 3 years. The sex ratio (N males to each female) of captured frogs was 1.7 in 2002, 1.1 in 2003, and 2.3 in 2004. Mark–recapture estimates for the number of calling males each week in June and July were similar to the number of captured individuals except for the last 2 weeks of July 2002 (Table 2). Estimated survivorship during June and July was 96.5% in 2002 and 90.0% in 2003. The activity patterns varied among individuals across the breeding season in 2003 (Figure 1).

Body size.—I measured 107 bog frogs (males = 63, females = 44) from the LOC study site, plus 22 males from other locations (total N = 129). When comparing body measurements between males and females captured at the LOC site, there was no significant statistical difference in SVL or mass, but males had larger tympanums (Table 3). When I combined the body sizes of males captured from other sites (N = 22) along with the LOC site males (N = 63), mean SVL (± SD) was 42.7 ± 5.1 mm (range: 34.4–

53.9 mm) and mass was 7.1 ± 2.5 g (range: 3.7–14.0 g). When data were combined for all measured frogs (N = 129), the sexes overlapped in tympanum diameter (Figure 2). Therefore, the presence (males) or absence (females) of vocals sacs is more consistent discriminator of sex than relative tympanum size. There was a significant linear relationship between SVL and mass in both sexes (Figure 3). The size distribution of individuals at the LOC study site varied among years (Figure 4).

Breeding biology.—I located 37 *R. okaloosae* egg masses in 2003 and identified the father for 35. These 35 egg masses were fathered by 15 different individuals (out of 20 males captured in 2003). The mean number of clutches fertilized by each male present in 2003 was 1.8 ± 21.2 (range: 0–9, N = 20). The number of clutches a male fertilized was correlated with the number of nights spent calling (r = 0.630, P = 0.003) but not his SVL (r = 0.135, P = 0.571). The smallest mated male was 37.0 mm SVL, but males as small as 34.4 mm SVL gave advertisement calls (Type I, Ch. 3). The smallest gravid female captured was 37.6 mm SVL. The average number of calling males per night and the number of egg masses deposited varied over the course of the breeding season (Figure 5).

The mean distance between male calling sites and oviposition locations was 13.6 ± 18.4 cm (range: 0–85 cm, N = 35). Twenty–seven (77%) egg masses were deposited ≤ 5 cm of male calling locations. Egg masses had a mean length of 12.5 ± 3.2 cm (range: 7.0–21.0 cm, N = 30) and width of 9.1 ± 2.0 cm (range: 6.0–13.0 cm, N = 30). Mean water depth at oviposition locations was 3.5 ± 1.0 cm (range: 1.9–7.0 cm, N = 31). Eggs were deposited an average of 111.3 ± 260.7 cm (range: 0 – 1300 cm, N = 32) from the bank. Egg masses typically were deposited as a free–floating single surface layer but occasionally were folded or attached to the bank or other vegetation. Eggs always were located in non–flowing sections of the stream (Table 4). Mean clutch size was 234.7 ± 58.9 eggs (range: 152–345, N = 10).

I observed 15 pairs of satellites and callers in 2002 and 2003. Average SVL was 42.3 ± 1.3 mm (range: 40.1–44.2 mm) for callers and 40.8 ± 0.8 mm (37.2–46.9 mm) for satellites. Calling males were larger than satellites in 10 (66.7%) of the pairs. Mean size difference (caller SVL – satellite SVL) was 1.5 ± 3.3 mm (range: –5.3–6.0 mm), but this difference was not significant statistically ($t = 1.713$, $df = 14$, $P = 0.109$).

Male calling sites.—The microhabitat differed between calling sites and random locations (Table 4). The best fit logistic regression model for differentiating between calling and random locations was significant ($\chi^2_4 = 8.890$, $P = 0.003$), with 92% of the calling sites classified correctly. Significant variables in the final model included distance to the bank, distance to cover, whether water was flowing, and water temperature (calling locations = –8.56flow – 0.59temp + 0.07cover + 0.028bank + 21.067).

DISCUSSION

Population turnover at the LOC site was fairly high (80%) among years. It is unknown if this is the result of winter mortality, toe–clip related mortality (McCarthy and Parris 2004) or migration into and out of the study area. I recaptured 3 individuals in 2004 that originally were marked in 2002. Two of these were not captured in 2003, suggesting that bog frogs may move into and out of the study area. Although bog frogs appear to move very little during the breeding season (Ch. 2), movements during the post–breeding season have never been studied. The network of streams likely provides a corridor system through which dispersal can occur (Bishop 2004). In addition, frogs may move over land during rain events (Ch. 2). *Rana clamitans*, the closest relative of *R. okaloosae* (Austin et al. 2003), may move to different habitats during the breeding season in some locations (Lamoureux and Madison 1999).

Because my capture methods were biased towards calling males (Ch. 2), and I did not use radiotelemetry, survivorship estimates are limited. For example, if I was unable to locate a previously marked individual, it is impossible to determine if his absence was the result of migration out of the study area, decreased calling activity (i.e., present but missed), or death. Snakes likely are a major predator of larvae (Bishop 2003, Ch. 4) and adults. Winter mortality may result from poor selection of overwintering sites, but death from cold weather probably is minimal in north Florida. Moler found a single individual under *Sphagnum* moss during the winter (P. Moler, personal communication), but the winter ecology and habitat of *R. okaloosae* has never been studied. Recent investigations (McCarthy and Parris 2004) suggest that toe–clipping in anurans may be a greater source of mortality than previously thought; hence, I recommend that future studies consider alternative individual marking techniques (e.g., elastomer pigments or PIT tags).

Differences in the total number of males captured among years at the LOC site appears to be independent of the amount of time I spent searching for frogs. For example, there were almost 3 times as many observation nights in 2003 compared to other years, but the total number of males captured was lowest in 2003. The observed sex ratio each season, however, likely was related to search effort. Females are more difficult to find than males because they rarely vocalize (Ch. 3). My greater search effort in 2003 allowed me to capture more females at the study site than in the other years, thus the sex ratio was closer to 1. There is no evidence to suggest that sex ratios are skewed in bog frogs. Unlike some species of anurans, where sexes differ in the timing of migration to breeding sites (e.g., Martof 1953, Guttman et al. 1991, Hardy and Raymond 1991), both male and female bog frogs were captured throughout the season in the breeding area.

My data suggest that simple population mark–recapture methods may provide a reasonable estimate of the number of calling males present at a location during a limited period of time. The estimated number of males was similar to actual number of males I captured for most weeks in June and July; however, there was significant variation during the last 2 weeks of July in 2002. During these weeks, I captured several unmarked males for the first time, suggesting that immigration into the study area may occur. Mark–recapture estimates conducted over a short period (e.g., 1 week) did not account adequately for all males that used the site throughout the season.

In most ranids, females are larger than males (Wood et al. 1998). This may be an advantage in species where female SVL is correlated with clutch size (Shine 1979). Because the mother of each egg mass rarely was identified in this study, it is unknown if this is true for bog frogs. I found no significant difference in SVL or mass between male and female bog frogs at the LOC study site. Large male body size may be an advantage in species that engage in physical combat (Shine 1979, Howard 1980) or if large individuals are more successful at attracting and securing mates than smaller males (Howard 1978a, Berven 1981, Morris 1989). The high site fidelity and limited movements of male bog frogs during the breeding season suggest they may be territorial

(Ch. 2), but I never observed physical combat (e.g., Brode 1959, Schroeder 1968, Howard 1978a) between males during the 3 years of my study. Male bog frogs, however, do react vocally to the playback of unfamiliar conspecifics (Ch. 3). Body size was not related to mate success for males, although mating success was correlated positively with the number of nights a male spent calling, a pattern documented in other anurans (Gerhardt et al. 1987, Morrison et al. 2001). Because calling is expensive energetically (Wells 2001), calling activity may provide a better measure of fitness than body size for females to select mates in species with prolonged breeding seasons.

All females measured in this study came from the LOC study site because females are more difficult to find than males. The largest males I captured were from other locations. The discrepancy in size among locations for males may be the result of differences in growth or survivorship rates due to habitat quality, reflect natural fluctuations in population composition, or result from differences in intraspecific or interspecific competition (likely with *Rana clamitans*, Ch's 2, 3, 5) among sites. At one location containing large bog frogs, the density of bronze frogs was much lower than at the LOC study site.

Bog frogs have a breeding season that extends 5–6 months. I found egg masses from 27 March to 26 August. The number of calling males and deposited egg masses varied over the course of the breeding season. Males called March–September (Bishop 2004). Bog frogs do not clasp approaching females readily, as explosive breeding species do (e.g., Howard 1988). On a few occasions, females were observed sitting next to calling males. In addition, I positioned a couple of females next to calling males but detected no noticeable reaction, suggesting that females may initiate the mating sequence.

The breeding season of bronze frogs, *Rana clamitans*, overlaps that of the bog frog. The two species also overlap spatially (Ch. 2). The egg masses of the two species, however, can be differentiated easily. Bog frog clutches typically contain a few hundred eggs, whereas *R. clamitans* typically deposits several thousand eggs per clutch (Wells 1976, personal observations). Because bronze frogs typically occur in the same locations as bog frogs and produce an order of magnitude more eggs per clutch, there is potential for strong larval competition that may affect population size and distribution of *R. okaloosae*. Because bog frogs have a limited distribution (Bishop 2004) and their reproductive output is limited, protecting all known breeding areas should be a high conservation priority.

There is potential for conflict if male calling sites differ from preferred female oviposition locations (Resetarits and Wilbur 1991). Similar to other anuran species with prolonged breeding seasons, *R. okaloosae* males space themselves out and call to attract females (Wells 1977b). Based on the distance between calling sites and oviposition locations, males appear to choose calling locations that are acceptable to ovipositing females. The ability to select suitable oviposition sites may affect fitness in amphibians (Howard 1978b; Resetarits and Wilbur 1989). The distance between calling and oviposition locations was small and may have been smaller if individuals were never disturbed. For example, although I always flagged the original calling location and released each captured male at the same position, an individual who moved to a new calling location and mated after he was released would increase the measured distance to the egg mass.

Oviposition sites may be chosen based on physical criteria such as water temperature (Caldwell 1986; Waldman 1982) or to minimize predation (Petranka and Fakhoury 1991) and competition (Crump 1991; Dillon and Fiaño 2000). Males selected shallow, non–flowing calling locations that typically were located near the bank or other structural items, such as woody debris or vegetation. Although water depth was not included in the final model, it undoubtedly is a major microhabitat factor that influences whether a site is suitable for *R. okaloosae*. Because random locations also were selected in the headwaters area of the LOC study area, depth likely was omitted from the model because the entire site was shallow. If microhabitat selection is viewed at a larger scale, such as the distribution of frogs within the entire stream, *R. okaloosae* avoided downstream locations where water was deeper and flowing. Bog frogs may avoid calling from high–flow locations because they are unsuitable for egg deposition. Deeper sections also contain large predatory fish, such as pickerel (*Esox* sp.) which readily eat tadpoles (Ch. 5).

Males selected calling sites near emergent structure. This often was the bank itself, but it also could be living vegetation or woody debris. There are two potential benefits to calling near structural items. First, this structure may create the shallow water and non–flowing microhabitat they seem to prefer. Second, the presence of structure and cover provides protection from predators. Bog frogs rarely fled when approached by observers, and structural items helped conceal frogs, at least from me. On a few occasions, individuals retreated under the nearby bank or cover item when I approached. Males occasionally called from dry land or from vegetation, such as *Sphagnum*, but this behavior was rare. Although I did not quantify the microhabitat of females, they were found in similar locations as males.

Male bog frogs occasionally adopt a satellite strategy, where noncalling males position themselves near calling individuals. Satellite behavior may increase the likelihood that small males secure mates while minimizing energetic expenditure (Wells 1977b, Halliday and Tajedo 1995, Ritke and Semlitsch 1991) or enable nonterritorial males to quickly occupy vacant territories (Wells 1977b). In *R. okaloosae*, callers often were slightly larger than satellites, but this was not consistent or statistically significant. The roles of individuals often varied over the course of a few nights; i.e., those that were callers on some nights were satellites on others. As previously mentioned, no physical aggression was observed toward other nearby males. However, satellite males did adopt a low–crouching posture, a behavior that suggests that males may react to visual cues in close encounters.

LITERATURE CITED

Austin, J. D., S. C. Lougheed, P. E. Moler, and P. T. Boag. 2003. Phylogenetics, zoogeography, and the role of dispersal and vicariance in the evolution of the *Rana catesbeiana* (Anura: Ranidae) species group. Biological Journal of the Linnean Society 80:601–624.

Bartlett, R. D., and P.P. Bartlett. 1999. A field guide to Florida reptiles and amphibians. Gulf Publishing Company, Houston, Texas, USA.

Berven, K. A. 1981. Mate choice in the wood frog, *Rana sylvatica*. Evolution 35:707–722.

Bishop, D. C. 2003. Florida Bog Frog (*Rana okaloosae)*. Predation. Herpetological Review 34:235.

Bishop, D. C. 2004. Summary of surveys for Florida bog frogs and flatwoods salamanders on Eglin Air Force Base through 2004. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Brode, W. E. 1959. Territoriality in *Rana clamitans*. Herpetologica 15:140.

Caldwell, J. P. 1986. Selection of egg deposition sites: a seasonal shift in the southern leopard frog, *Rana sphenocephala*. Copeia 1986:249–253.

Crump, M. L. 1991. Choice of oviposition site and egg load assessment by a treefrog. Herpetologica 47:308–315.

Dillon, M. E., and J. Fiaño. 2000. Oviposition site selection by the Túngara frog (*Physaleaemus pustulosus*). Copeia 2000:883–885.

Enge, K. M. 2002. Herpetofaunal drift–fence survey of steephead ravines and seepage bogs in the western Florida Panhandle. Florida Fish and Wildlife Conservation Commission Final Performance Report, Tallahassee, Florida, USA.

Gerhardt, H. C., R. E. Daniel, S. A. Perrill, and S. Schramm. 1987. Mating behaviour and male mating success in the green treefrog. Animal Behaviour 35:1490–1503.

Guttman, D., J. E. Bramble, and D. J. Sexton. 1991. Observations on the breeding immigration of wood frogs *Rana sylvatica* reintroduced in east–central Missouri. American Midland Naturalist 125:269–274.

Halliday, T., and M. Tejedo. 1995. Intrasexual selection and alternative mating behavior. Pages 419–468 *in* Harold Heatwole, editor. Amphibian Biology Vol 2: Social Behavior. Surrey Beatty and Sons, Chipping Norton, NSW, Australia.

Hardy, L. M., and L. R. Raymond. 1991. Observations on the activity of the pickerel frog, *Rana palustris* (Anura: Ranidae), in northern Louisiana. Journal of Herpetology 25:220–222.

Howard, R. D. 1978a. The evolution of mating strategies in bullfrogs, *Rana catesbeiana*. Evolution 32:850–871.

Howard, R. D. 1978b. The influence of male–defended oviposition sites of early embryo mortality in bullfrogs. Ecology 59:789–798.

Howard, R. D. 1980. Mating behaviour and mating success in woodfrogs, *Rana sylvatica*. Animal Behaviour 28:705–716.

Howard, R. D. 1988. Sexual selection on male body size and mating behaviour in American toads, *Bufo americanus*. Animal Behaviour 36:1796–1808.

Jackson, D. R. 2004. Management guidance document for species at risk on Eglin Air Force Base, Niceville, Florida: Florida bog frog (*Rana okaloosae*). Final report to U.S. Fish and Wildlife Service, Panama City, Florida. Florida Natural Areas Inventory, Tallahassee, USA.

Lamoureux, V. S., and D. M. Madison. 1999. Overwintering habitats of radio–implanted green frogs, *Rana clamitans*. Journal of Herpetology 33:430–435.

Martof, B. S. 1953. Territoriality in the green frog, *Rana clamitans*. Ecology 34:165–174.

McCarthy, M. A., and K. M. Parris. 2004. Clarifying the effects of toe clipping on frogs with Bayesian statistics. Journal of Applied Ecology 41:780–786.

Moler, P. E. 1985. A new species of frog (Ranidae: *Rana*) from northwestern Florida. Copeia 1985:379–383.

Moler, P. E. 1986. Distribution of the Florida bog frog. Florida Game and Fresh Water Fish Commission, Bureau Wildlife Research Final Report, Tallahassee, Florida, USA.

Moler, P. E. 1993. *Rana okaloosae* Moler Florida bog frog. Catalogue of American amphibians and reptiles.561.1–3.

Morris, M. R. 1989. Female choice of large males in the treefrog *Hyla chrysocelis*: the importance of identifying the scale of choice. Behavioral Ecology and Sociobiology 25:275–281.

Morrison, C., J. M. Hero, and W. P. Smith. 2001. Mate selection in *Litoria chloris* and *Litoria xanthomera*: females prefer smaller males. Austral Ecology 26:223–232.

Petranka, J. W., and K. Fakhoury. 1991. Evidence of a chemically–mediated avoidance response of ovipositing insects to blue–gills and green frog tadpoles. Copeia 1991:234–239.

Pollock, K. H. 1982. A capture–recapture design robust to unequal probability of capture. Journal of Wildlife Management 46:757–760.

Printiss, D., and D. Hipes. 1999. Rare amphibian and reptile survey of Eglin Air Force Base, Florida. Final Report, Florida Natural Areas Inventory, Tallahassee, Florida, USA.

Resetarits, W. J., and H. M. Wilbur. 1989. Choice of oviposition site by *Hyla chrysocelis*: role of predators and competitors. Ecology 70:220–228.

Resetarits, W. J., and H. M. Wilbur. 1991. Calling site choice by *Hyla chrysocelis*: effects of predators, competitors, and oviposition sites. Ecology 72:778–786.

Ritke, M. E., and R. D. Semlitsch. 1991. Mating behavior and determinants of male mating success in the gray treefrog, *Hyla chrysocelis*. Canadian Journal of Zoology 69:246–250.

Schroeder, E. E. 1968. Aggressive behavior in *Rana clamitans*. Journal of Herpetology 1:95–96.

Shine, R. 1979. Sexual selection and sexual dimorphism in the Amphibia. Copeia 1979:297–306.

Waldman, B. 1982. Adaptive significance of communal oviposition in wood frogs (*Rana sylvatica*). Behavioral Ecology and Sociobiology 10:169–174.

Wells, K. D. 1976. Multiple egg clutches in the green frog (*Rana clamitans*). Herpetologica 32:85–87.

Wells, K. D. 1977a. Territoriality and male mating success in the green frog (*Rana clamitans*). Ecology 58:750–762.

Wells, K. D. 1977b. The social behavior of anuran amphibians. Animal Behaviour 25:666–693.

Wells, K. D. 2001. The energetics of calling frogs. Pages 45–60 *in* M. J. Ryan, editor. Anuran Communication, Smithsonian Institution Press, Washington, USA.

White, G.C., and K. P. Burnham. 1999. Program MARK: Survival estimation from populations of marked animals. Bird Study 46 Supplement, 120–138.

Wood, K. V., J. D. Nichols, H. F. Percival, and J. E. Hines. 1998. Size–sex variation in survival rates and abundance of pig frogs, *Rana grylio*, in northern Florida wetlands. Journal of Herpetology 32:527–535.

Table 1. Number of bog frogs (*Rana okaloosae)* captured each year at the Live Oak Creek study site in Okaloosa County, FL, from 2002–2004. The number of recaptures is the number of frogs that were first captured and marked in an earlier season.

Year	Total Frogs	N Recaptures	N Males	N Females
2002	46	NA	29	17
2003	39	8	20	19
2004	39	8	27	12

Table 2. Comparison of observed and estimated number of *Rana okaloosae* males each week in June and July at the Live Oak Creek study site in Okaloosa County, FL. Mark–recapture estimates were made using the robust design method. The number of sample nights is the number of observation nights during that week. If there were ≥ 2 sample nights during a week, I selected 2 nights with the fewest number of days between them. The numbers (1–4) below each month correspond to the weeks of the month.

		June				July			
		1	2	3	4	1	2	3	4
2002	Estimated N	16	17	15	NA	17	18	17	26
	Observed N	16	17	16	NA	17	18	9	12
	Sample nights	2	2	2	0	2	2	2	2
2003	Estimated N	8	10	8	9	11	9	8	7
	Observed N	10	11	11	10	9	11	8	10
	Sample nights	5	5	4	7	3	7	4	7

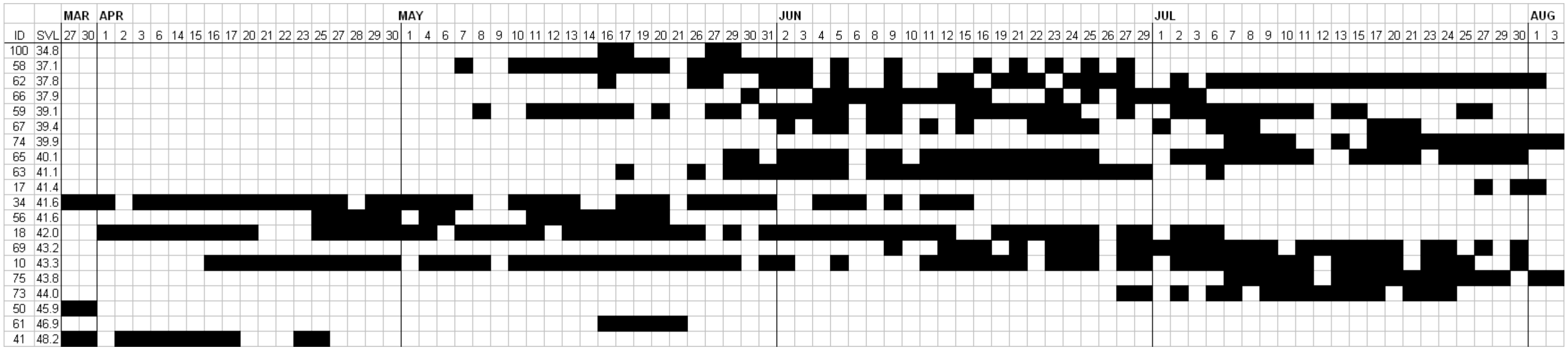
Table 3. Comparison of body measurements of male (N = 63) and female (N = 44) *Rana okaloosae* captured at the Live Oak Creek study site in Okaloosa County, FL from 2002–2004. A Satterthwaite t–test was used when variances were unequal. SVL = snout–vent length.

	Males		Females		t–test		
	Mean ± SD	Range	Mean ± SD	Range	t	df	P
SVL (mm)	40.2 ± 3.0	34.4–56.9	41.5 ± 4.3	33.5–48.8	–1.429	74.775	0.157
Mass (g)	5.9 ± 1.2	3.7–8.3	6.2 ± 1.9	2.8–9.8	–0.632	69.463	0.530
Eye (mm)	4.9 ± 0.8	2.9–6.5	4.9 ± 0.7	2.7–6.3	0.328	104	0.743
Tympanum (mm)	4.6 ± 0.9	2.7–6.8	3.5 ± 0.7	1.7–5.2	5.963	104	< 0.001

Table 4. Summary of microhabitat variables for 50 known calling locations for male *Rana okaloosae* and 50 random locations at Live Oak Creek study site in Okaloosa County, FL. For quantitative variables, the means, standard deviations, and ranges are given. For categorical data, the percentage is given for each category.

Variable	Known	Random
Water depth (cm)	2.2 ± 0.9 (0.6–4.9)	2.8 ± 1.8 (0.4–8.0)
Temperature (°C)	26.0 ± 1.6 (23.0–50.0)	23.9 ± 1.7 (22.0–31.0)
Distance to cover (cm)	7.0 ± 11.0 (0–45.6)	30.3 ± 24.9 (0–84.8)
Distance to bank (cm)	20.7 ± 24.1 (1.5–121.6)	56.6 ± 37.0 (11.5–175.8)
Water flow (yes or no)	0% Yes	56% Yes
Emergent structure (yes or no)	96% Yes	50% Yes

Figure 1. Activity patterns for 20 male Florida bog frogs (*Rana okaloosae*) over 84 observation nights in 2003 at the Live Oak Creek study site in Okaloosa County, FL. Black boxes indicate nights when individuals were captured. SVL = snout–vent length (mm).



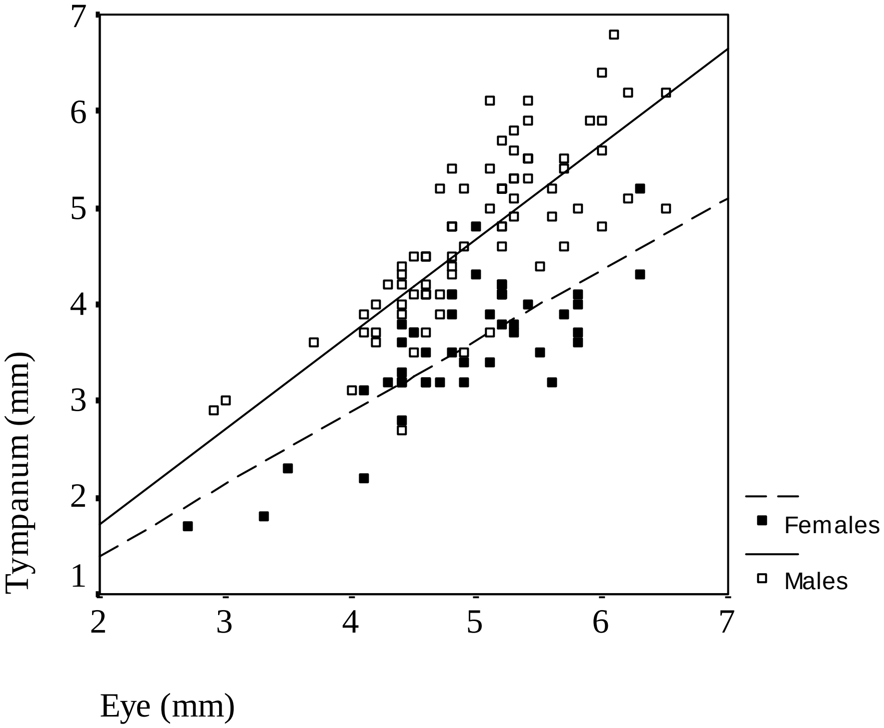


Figure 2. Relationship between tympanum and eye diameter in male and female bog frogs (*Rana okaloosae*). Female Tymp. = 0.741Eye – 0.008 ($R^2 = 0.631$, $P < 0.001$, $N = 44$), Male Tymp. = 0.984Eye – 0.236 ($R^2 = 0.605$, $P < 0.001$, $N = 83$). The regression was done to differentiate between the sexes; it does not imply a cause and effect relationship between the two variables.

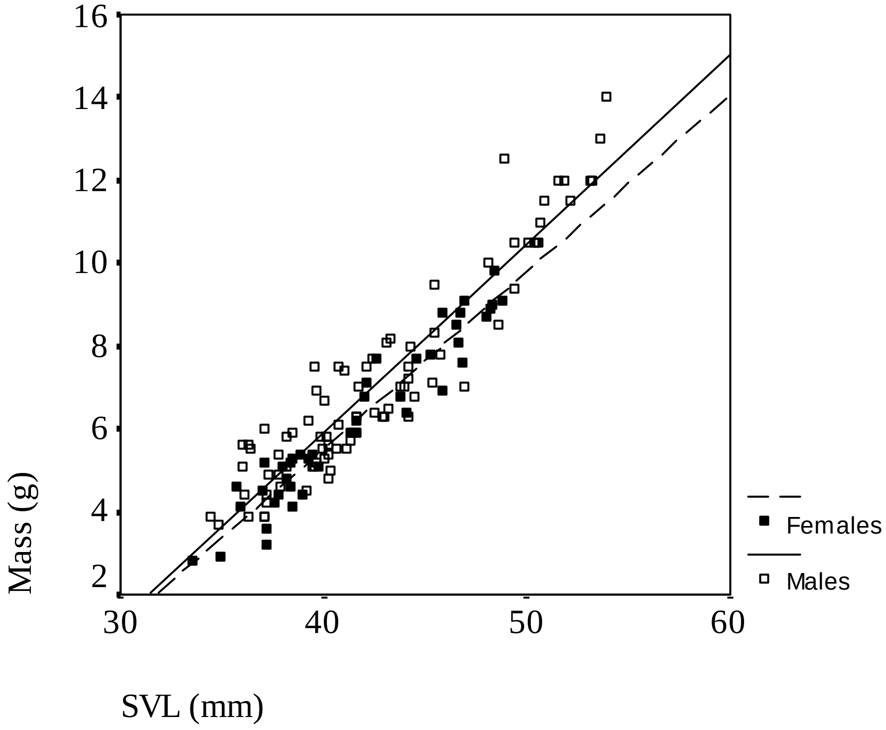


Figure. 3. Relationship between mass and snout–vent length (SVL) in male and female bog frogs (*Rana okaloosae*). Female mass = 0.427SVL – 11.457 (R^2 = 0.922, $P < 0.001$, N = 44), Male mass = 0.456SVL – 12.314 (R^2 = 0.882, $P < 0.001$, N = 83).

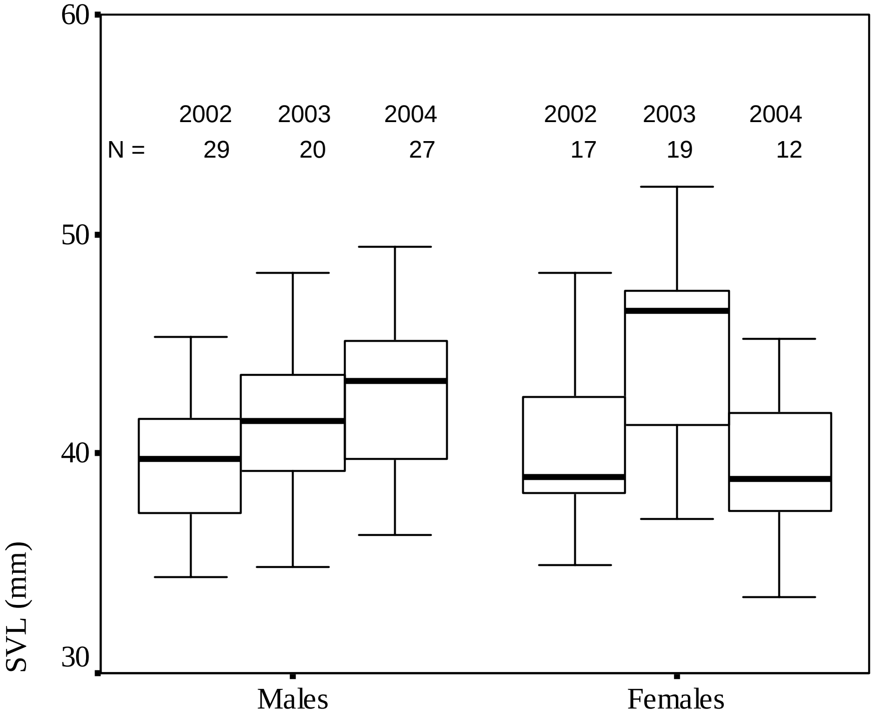


Figure 4. Size distribution (snout–vent length, SVL) of male and female bog frogs (*Rana okaloosae*) at Live Oak Creek study site in Okaloosa County, FL by year. Boxes display interquartile range. The dark line is the median.

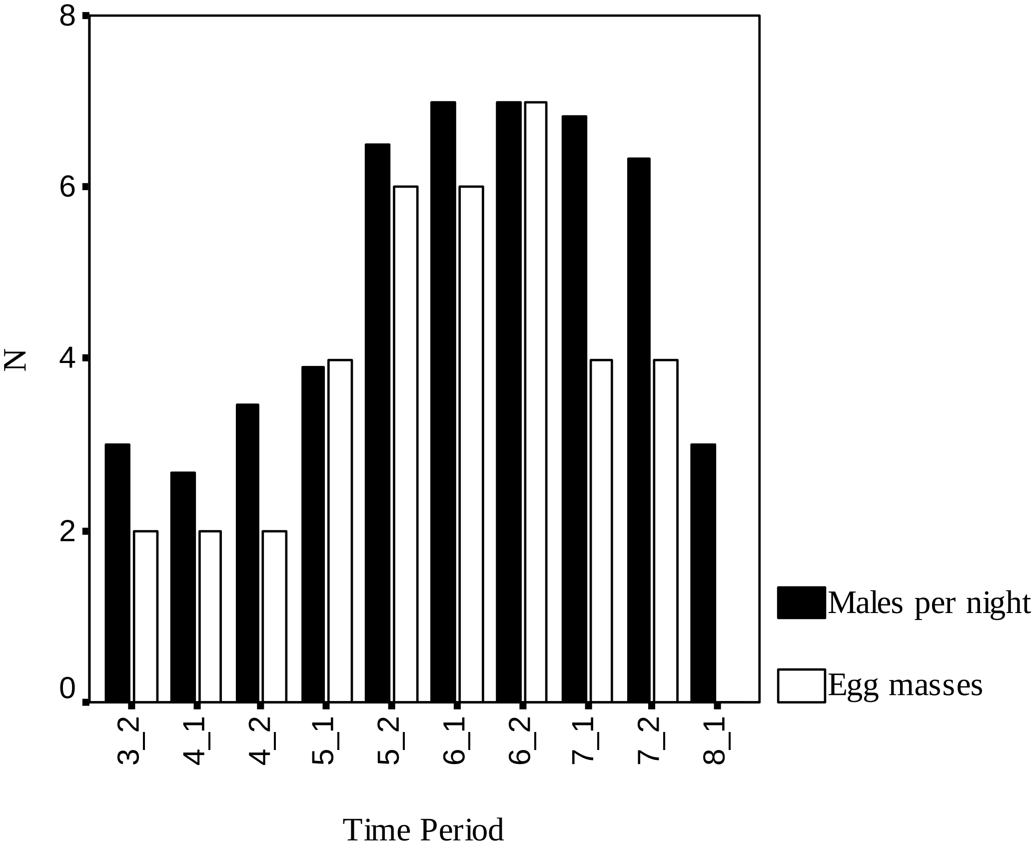


Figure 5. Average number of calling male bog frogs (*Rana okaloosae*) per night and total number of egg masses deposited during 2–week intervals (27 March–3 August) in 2003 at Live Oak Creek study site in Okaloosa County, FL. Each month was broken into 2 time periods (period 1 = days 1–15, period 2 = days 16–31). For the x–axis, the first number indicates the month and second number indicates the half.

CHAPTER 2. MOVEMENTS, HOME RANGES, AND SPATIAL INTERACTIONS OF FLORIDA BOG FROGS AND BRONZE FROGS

ABSTRACT.—I monitored the movements and spatial distribution of Florida bog frogs (*Rana okaloosae*) and bronze frogs (*Rana clamitans clamitans*) at one location in Okaloosa County, Florida. In linear habitats, such as the streams where bog frogs often occur, unconstrained kernel home range estimators have limited application because they include surrounding upland (atypical) habitat. In these habitats, the maximum distances moved may be more appropriate for determining core protection areas. Both species used similar calling locations, although *R. clamitans* also called from microhabitats that were unused by *R. okaloosae*. *Rana clamitans* had larger home ranges than *R. okaloosae*. When comparing the spatial distribution of males on a given night, bog frogs had calling positions closer to bronze frogs than to other bog frogs. The spatial overlap between these two species suggests there is potential for competition.

INTRODUCTION

Studies of movement behavior and home ranges of rare species are helpful for determining adequate protection areas and appropriate management guidelines. In addition, understanding the spatial interactions between sympatric species contributes to understanding distribution patterns and the potential for competition and territoriality. Most studies investigating movements and spatial interactions of anurans focus on a single species within a community (e.g., Currie and Bellis 1969; Wells 1977a; Shepard 2002); few document spatial interactions between sympatric species (however, see MacNally 1979; Given 1990). I monitored the movements of two sympatric ranids, the

Florida bog frog (*Rana okaloosae*) and bronze frog (*Rana clamitans clamitans*), at a single site in northwestern Florida.

As mentioned previously, the bronze frog is the closest relative of the bog frog (Austin et al. 2003). In addition to comparing the movement behavior between these two species, I monitored their spatial distribution to evaluate whether the two species partition calling locations or whether there is potential for competition of shared resources. My research provides a foundation to which future home range telemetry studies can be compared, a biological basis for establishing management guidelines, and new information on the social system of *R. okaloosae*.

METHODS

Study site and captures.—I conducted all research at the Live Oak Creek (LOC) study site on Eglin Air Force Base, previously described in chapter 1. I located frogs by thoroughly searching the LOC site. Most searches were at night, but I also conducted day searches, especially in 2003. Although most captures were of calling males, I routinely located non–calling individuals throughout the breeding season. Individuals captured during the day rarely vocalized. I omitted females from analyses because their recapture rates were lower than males. I toe–clipped each individual for identification, measured snout–vent length (SVL) to the nearest 0.1 mm using dial calipers, and mass to the nearest 0.5 g with a spring scale (Pesola™ 10 g or Avinet™ 50 g). I marked the capture location of each individual with a wire flag and later recorded flag locations with a GPS unit (Trimble™ Asset Surveyor TDC2, resolution < 1 m). I captured frogs on 30 days between 27 May and 10 September in 2002 (*R. okaloosae* only) and on 84 days between 27 March and 3 August in 2003 (*R. okaloosae* and *R. clamitans*).

Movements.— I calculated home ranges (50% and 95% fixed kernel (FK) and 100% minimum convex polygon (MCP)) and maximum linear distances (MLD) moved (i.e., distance between the two farthest capture locations) for individuals captured ≥ 10 times within a year. I selected ≥10 as the cutoff value for several reasons. First, when I plotted home range size against the number of locations for each frog, there was no natural break or asymptotic value where home range size leveled off (Figure 1). This may be the result of the high variability between individuals or the limited number of individuals with a large number (> 50) of recaptures. Second, the number of captures was not correlated significantly (P > 0.05) with fixed kernel home ranges once I attained ≥ 10 locations per individual, although there was a positive relationship. When the outliers were removed from the dataset, this positive relationship was less pronounced. Lastly, kernel estimators tend to overestimate home range sizes for individuals with too few locations, rather than underestimate like minimum convex polygons (Seaman and Powell 1996); therefore, it may be better to err on the cautious side when estimating the amount of space required by a rare species, such as the bog frog. I estimated home ranges with 50% and 95% fixed kernels (FK) with least squares cross validation and with 100% minimum convex polygons (MCP) using the Animal Movement Program extension (Hooge et al. 1999) in ArcView GIS 3.2.

Spatial distribution.—I created figures to show the relative spatial positions of *R. okaloosae* males during two week periods (May–July) at the LOC study site, times corresponding to peak calling activity (see Figure 4 in Ch. 1). To compare the spatial distribution and overlap between *R. okaloosae* and *R. clamitans* males at the LOC site, I combined individual home range polygons (95% FK and MCP) and created a composite polygon representing the space use of each species. I then calculated the percent overlap of the 2 composite polygons. I chose to combine individual polygons, rather than look at the overlap of individual home ranges, because there were temporal differences among the activity times of individuals. By combining the home range polygons of all individuals for each species, I hoped to get a general measurement of the spatial distribution of each species within the study site.

For another estimate of spatial overlap and to evaluate whether both species select the same calling locations within the study site, I compared capture locations of males of both species over the entire 2003 season, ignoring temporal differences. At each location where I captured a calling male, I measured the distance (± 1 m) to the closest heterospecific and conspecific male capture locations. For example, if I captured *R. okaloosae* No. 19 at point A on any given night, I measured the distance from point A to the closest *R. clamitans* and *R. okaloosae* (different individual) capture locations. If I also captured a *R. clamitans* at point A on the same or different date, the heterospecific distance was 0 m, indicating that the two species used the same location, although there may have been temporal differences in when location A was used.

Lastly, for the nights (N = 34) on which I captured ≥ five individuals of each species, I measured the distance to the nearest calling *R. okaloosae* and *R. clamitans* from each calling male. This is similar to the calculations described previously; however, for this measurement, I did not ignore temporal differences. Therefore, this analysis measures the potential for direct interactions between individuals, not just similarities in microhabitat selection. I calculated average distances between calling individuals for each night and compared whether individuals selected calling sites that were closer to conspecifics or heterospecifics. I used the ratio of the number of calling males of each species present each night as a covariate. I tested for correlations between the numbers of active callers each night and the average neighbor distance and between body size (SVL) and average neighbor distance. I omitted satellite males (Ch. 1) from analyses.

RESULTS

I captured 76 (44 males, 32 females) different bog frogs during the two breeding seasons and 55 (45 males, 10 females) bronze frogs in the second season at the LOC study site. I estimated maximum linear movement distances and home ranges for 28 *R. okaloosae* males and 18 *R. clamitans* males with ≥ 10 locations (Table 1). There were no significant correlations ($P > 0.05$) between the number of capture locations and kernel home range estimates within each species, although there was a positive linear relationship that may be biologically significant (Figure 1). There were no correlations between SVL and movement estimates for either species (Figure 2). There were significant positive correlations between all home range and movement estimates for both species (Table 2). Bronze frogs had larger home ranges (Table 1) than bog frogs for 50% FK ($t = –1.804$, $df = 18.448$, $P = 0.088$), 95% FK ($t = –2.174$, $df =18.565$, $P = 0.043$), and MCP ($t = –2.239$, $df = 44$, $P = 0.030$) estimates. Bronze frogs also moved farther maximum linear distances than bog frogs ($t = –2.520$, $df = 44$, $P = 0.015$).

When I combined the 2003 individual home ranges (MCP and 95% FK) for each species (*R. okaloosae*, $N = 18$; *R. clamitans*, $N = 18$), the combined MCP was 1592 m^2

for *R. okaloosae* and 1562 m^2 for *R. clamitans*. Combined fixed kernel (95%) polygons were 2000 m^2 for *R. okaloosae* and 3298 m^2 for *R. clamitans*. Bronze frog polygons overlapped 87.1% (MCP) and 90.8% (FK) of bog frog polygons. Polygons for bog frogs overlapped 88.7% (MCP) and 55.1% (FK) of bronze frog polygons.

In 2003, I captured 20 *R. okaloosae* males at 496 locations. The mean distance (± SD) from each *R. okaloosae* capture location to the closest conspecific capture location (ignoring temporal differences) was 1.2 ± 1.2 m (range: 0–11 m). The mean distance to the closest *R. clamitans* capture location was 0.97 ± 1.0 m (range: 0–11 m). Intraspecific distances between *R. okaloosae* males (i.e., bog frog to bog frog) were greater than interspecific distances (i.e., bog to bronze) ($t = 3.658$, $df = 926.497$, $P = < 0.001$). I made the same measurements for 43 *R. clamitans* males captured at 565 locations. The mean distance from each *R. clamitans* location to the closest conspecific capture location was 1.9 ± 2.1 m (range: 0–13 m) and 0.93 ± 0.8 m (range: 0–6 m) to the closest *R. okaloosae* capture location, a difference that also was significant ($t = 9.875$, $df = 722.355$, $P < 0.001$).

Bog frog males typically remained in the same general locations for several nights before moving to a new location (Figures 3–7, 8). The distances between callers and their nearest neighbors were measured on 34 nights for 18 different *R. okaloosae* and 41 *R. clamitans* (Table 3). The average distance between calling bog frogs was greater than the distance between bog and bronze frogs (ANCOVA, $F = 7.189$, $df = 2$, $P = 0.002$), suggesting that bog frogs avoid call sites near each other. There was no significant difference between intraspecific and interspecific distances from calling bronze frogs (ANCOVA, $F = 0.035$, $df = 2$, $P = 0.966$). There was a general trend for spacing between individuals to be reduced when more callers were present, with *R. clamitans* affected more than *R. okaloosae* (Table 4). There were no significant correlations between the size (SVL) of the caller and the mean distance to the closest calling conspecific for *R. okaloosae* (r = 0.131, P = 0.604, N = 18) or *R. clamitans* (r = –0.144, P = 0.367, N = 41).

DISCUSSION

The most difficult aspect in conducting a movement study is attaining adequate capture locations per individual and determining the minimum number of independent locations necessary to estimate movements accurately. This is especially difficult when telemetry is not used. The small size of bog frogs ($\bar{x}$ = 7.1 g for males, range: 3.7–14.0 g, Ch. 1) limits the longevity of currently available transmitters; thus, I had to rely on active searching. One common method for determining the minimum number of locations necessary is to plot the number of capture locations against the home range estimator and look for the asymptote (Rose 1982), a technique often used for MCPs. Minimum convex polygons have been criticized for their dependence on a large number of captures (Kernohan et al. 2001). Fixed kernel estimators generally are recommended over MCPs (reviewed in Powell 2000 and Kernohan et al. 2001) and are starting to be applied to frog studies, even when sample sizes are small (e.g., Matthews and Pope 1999, Watson et al. 2003). As a general recommendation, Seaman et al. (1999) suggested that a minimum of 30 locations per individual (preferably > 50) be obtained for fixed kernel estimators.

I calculated home ranges for individuals with ≥ 10 capture locations. I do not believe this is an adequate number to fully characterize the movements and home ranges of either bog or bronze frogs. However, these data may allow a comparison of relative movements between the two species. In addition, I report these estimates because they are the only data available for *R. okaloosae*. Bronze frogs had home ranges more than double the size of bog frogs and also moved greater maximum linear distances. This may reflect the size difference between the two species; *R. clamitans* is considerably larger than *R. okaloosae* (Ch. 3). Martof (1953a) studied *R. clamitans melanota* in Michigan and calculated a mean home range (MCP) size of 61 m^2 (range: 20–200 m^2) for individuals (N = 43) captured ≥ 5 times, a result much smaller than my estimate (301 m^2). Possible explanations of this difference may include my higher number of recaptures or variation in habitat between sites.

My results suggest that kernel estimates may be of limited use in narrow habitats such as streams. The 95% FK home range estimates for both species often included upland habitat surrounding the study site. Blundell et al. (2001) encountered the same problem with home range estimates for river otters. Although bronze frogs often called from terrestrial locations along the banks of the creek, bog frogs rarely were found out of the water (Ch. 1). One easy solution to this problem is to constrain the kernel estimate to the boundaries of the creek. I did not do this because both species also occupy nonlinear habitats at other sites where estimates may be applicable.

For frogs found in linear habitats, such as streams, the maximum linear distance moved may be a better measure for determining conservation areas than home range estimators. Assuming the movements of individuals at the LOC study site are similar to other sites, a minimum of 75.0 m of stream length (upstream and downstream, total 150.0 m, Table 1) at each known location would be needed to protect the habitat used by 100%

of adult *R. okaloosae* during the breeding season (March–August, Ch. 1). A minimum of 83.8 m (total 167.6 m, Table 1) would be needed to protect the area used by *R. clamitans*. This also would protect oviposition areas for both species, which are in the same location (Ch. 1). Juveniles and tadpoles routinely are found in the same habitat as adults (Ch. 5, Bishop 2004), but I do not know the extent of their movements. Obviously, upstream impacts (e.g., damming, siltation) will affect the lower drainage; hence, my recommendations cannot be used to determine the total area in need of protection. They can, however, be used as a guideline to help minimize point disturbances, such as erosion, feral pig damage, vehicle traffic, and military maneuvers.

Because I only monitored movements in a limited section of a stream at one study site, maximum linear movement distances may be underestimated for some individuals. In general, individuals that move greater distances are more likely to escape detection in movement studies, thus biasing estimates (Koenig et al. 1996). Frogs that dispersed farther downstream may have been undetected, although the number of calling frogs heard downstream was minimal. Most bronze frogs dispersed away from the LOC site in mid–July. My movement estimates, therefore, likely capture only a portion of their total utilized area. Lamoureux and Madison (1999) followed radio–implanted *R. clamitans* in New York distances up to 560 m in late summer to their overwintering habitats.

Bog frogs also are capable of moving relatively large distances, even through unsuitable habitats. For example, I found one individual (male, 42.0 mm SVL) in an isolated cypress wetland (Site ID 226, Appendix 1, Bishop 2004), approximately 130 m from the LOC site, where it previously was marked. Separating the two sites was dry sandhill habitat dominated by saw palmetto (*Serenoa repens*), longleaf pine, and turkey oak. Several days of rain occurred between the previous capture date at the main site (11 July 2003) and the date (15 July) it was found in the isolated wetland. Others have reported long–distance movements in ranids during rain events (Martof 1953a; Dole 1965). No other *R. okaloosae* males were heard calling in the cypress wetland, but several *R. clamitans* males were active there throughout the summer.

It was not uncommon for males, especially *R. okaloosae*, to remain in the same location during an entire sampling event on one night (2–3 h) and be in the same location the following night. In addition, those captured during the day often were in the same calling location used the previous night. Both species typically remained in the same general location for several days or weeks at a time. These stationary periods were separated by larger movements to other locations. Often a male returned to a location he used previously in the season. Martof (1953a) also reported that *R. clamitans* moved very little between captures. Site fidelity has been interpreted as evidence for territoriality in frogs (e.g., Given 1988).

There was substantial spatial overlap in combined home ranges between the two species. Because home ranges were calculated only for individuals with sufficient recaptures, my calculations likely underestimate the amount of spatial overlap. The low estimate of home range overlap of bog frogs with bronze frogs (FK = 55.1%) may be the result, in part, of a few *R. clamitans* inhabiting deeper sections of the stream, areas bog frogs typically avoided.

When I ignored temporal differences and compared the distances to the closest conspecific and heterospecific calling locations from each captured *R. okaloosae* male, the distances between bog frog capture locations were greater than interspecific distances.

The opposite pattern was found for *R. clamitans*; intraspecific distances were less than interspecific distances. These results agree with the home range overlap results. Bronze frogs used the same sites as bog frogs but also used deeper sections of the stream that bog frogs typically avoided.

Males of species with prolonged breeding seasons often space themselves out to minimize competition (Littlejohn 1977; Wells 1977b). Males may use the calls of neighbors to determine minimum spacing distances (Dyson and Passmore 1992; Halliday and Tejedo 1995). Other studies also have reported nonrandom spacing of frogs (Whitney and Krebs 1975; Given 1988; Wilczynski and Brenowitz 1988). Because of similar resource requirements and competition for mates, intraspecific competition is often assumed to be greater than interspecific competition, resulting in higher aggression levels between conspecifics than between heterospecifics (Gerhardt and Schwartz 1995). Presumably, this may lead to greater distances between calling conspecifics than between heterospecifics. Conversely, males may prefer to call in conspecific aggregations (Martof 1953b; Given 1988), possibly because of similar habitat preferences or an increased chance of attracting mates. Spacing differences also may be related to the distribution of preferred calling sites or the density of frogs (Shepard 2002).

When I compared the spatial distribution of calling males within a single night, I got results similar to the other analyses where I ignored temporal differences. Bog frogs selected calling positions that were closer to calling bronze frogs than to other *R. okaloosae*. Although multiple individuals may use a single calling location throughout the breeding season, they avoid overlapping spatially during the same time. Male bog frogs react more strongly to the playback of conspecific vocalizations than those from *R.*

clamitans (Ch. 3). Both of these factors, coupled with the limited movements of bog frogs, suggest that *R. okaloosae* may be territorial. There was no difference in inter– or intraspecific distances from *R. clamitans* calling positions. This result is surprising because bronze frogs also are believed to be territorial (Wells 1977a). As suggested earlier, bronze frogs may have been space–limited at the LOC site because of their greater numbers, an explanation supported by the observed decrease in inter–male distances on evenings when more bronze frogs were calling.

If frogs use call properties of neighbors to help determine inter–individual distances, and call properties are related to body size (Ch. 3, Given 1988, Bee et al. 1999), then inter–male spacing may be affected by the size of the calling individual. Further, large males also are more likely to win aggressive encounters (Given 1988, Dyson and Passmore 1992) and may have larger home ranges (Howard 1978) than small males. However, I found no significant correlations between male body size (SVL) and neighbor distances over 34 nights for either species or between SVL and home ranges.

In summary, *R. okaloosae* and *R. clamitans* differ in home range size and maximum linear movement distances. Because I did not use radiotelemetry, the number of capture locations per individual was low; hence, I recommend using these data cautiously. Currently, this is the best available data set on bog frog movements and may provide a biological basis for establishing core protection areas. It also serves as a foundation with which future home range studies can be compared. Bog frogs and bronze frogs overlap spatially. Other ranids typically are absent from sites containing *R. okaloosae*; cricket frogs (*Acris* sp.) are the only other terrestrial anuran consistently active during the breeding season at these sites. Adults of the two ranids used similar microhabitats within a location, an observation that suggests the two species may compete for calling sites, oviposition locations, and food resources. Overlap in breeding season microhabitat also may contribute to mate recognition errors; apparent hybrids between the two species are fairly common (Ch. 3).

LITERATURE CITED

Austin, J. D., S. C. Lougheed, P. E. Moler, and P. T. Boag. 2003. Phylogenetics, zoogeography, and the role of dispersal and vicariance in the evolution of the *Rana catesbeiana* (Anura: Ranidae) species group. Biological Journal of the Linnean Society 80: 601–624.

Bee, M. A., S. A. Perrill, and P. C. Owen. 1999. Size assessment in simulated territorial encounters between male green frogs (*Rana clamitans*). Behavioral Ecology and Sociobiology 45:177–184.

Bishop, D. C. 2004. Summary of surveys for Florida bog frogs and flatwoods salamanders on Eglin Air Force Base through 2004. Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA.

Blundell, G. M., J. A. K. Maier, and E. M. Debevec. 2001. Linear home ranges: effects of smoothing, sample size, and autocorrelation on kernel estimates. Ecological Monographs 71:469–489.

Currie, W., and E. D. Bellis. 1969. Home range and movements of the bullfrog, *Rana catesbeiana* Shaw, in an Ontario pond. Copeia 1969:688–692.

Dole, J. W. 1965. Summer movements of adult leopard frogs, *Rana pipiens* Schreber, in northern Michigan. Ecology 46:236–255.

Dyson, M. L., and N. I. Passmore. 1992. Inter–male spacing and aggression in African painted reed frogs, *Hyperolius marmoratus*. Ethology 91:237–247.

Gerhardt, H. C., and J. J. Schwartz. 1995. Interspecific interactions in anuran courtship. Pages 601–632 *in* Harold Heatwole, editor. Amphibian Biology Vol 2: Social Behavior. Surrey Beatty and Sons, Chipping Norton, UK.

Given, M. F. 1988. Territoriality and aggressive interactions of male carpenter frogs, *Rana virgatipes*. Copeia 1988:411–421.

Given, M. F. 1990. Spatial distribution and vocal interaction in *Rana clamitans* and *R. virgatipes*. Journal of Herpetology 24:377–382.

Halliday, T., and M. Tejedo. 1995. Intrasexual selection and alternative mating behavior. Pages 419–468 *in* H. Heatwole, editor. Amphibian Biology Vol 2: Social Behavior. Surrey Beatty and Sons, Chipping Norton, UK.

Hooge, P. N., W. Eichenlaub, and E. Solomon. 1999. The animal movement program. United States Geological Survey, Alaska Biological Science Center, Anchorage, Alaska, USA.

Howard, R. D. 1978. The evolution of mating strategies in bullfrogs, *Rana catesbeiana*. Evolution 32:850–871.

Kernohan, B. J., R. A. Gitzen, and J. J. Millspaugh. 2001. Analysis of animal space use and movements. Pages 125–126 *in* J. J. Millspaugh and J. M. Marzluff, editors. Radio Tracking and Animal Populations, Academic Press, San Diego, California USA.

Koenig, W. D., D. Van Vuren, and P. N. Hooge. 1996. Detectability, philopatry, and the distribution of dispersal distances in vertebrates. Trends in Ecology and Evolution 11:514–517.

Lamoureux, V. S., and D. M. Madison. 1999. Overwintering habitats of radio–implanted green frogs, *Rana clamitans*. Journal of Herpetology 33:430–435.

Littlejohn, M. J. 1977. Long–range acoustic communication in anurans: an integrated and evolutionary approach. Pages 263–294 *in* D. H. Taylor and S. I. Guttman, editors. The Reproductive Biology of Amphibians, Plenum Press, New York, USA.

MacNally, R. C. 1979. Social organization and interspecific interactions in two sympatric species of *Ranidella* (Anura). Oecologia 42:293–306.

Martof, B. 1953a. Home range and movements of the green frog, *Rana clamitans*. Ecology 34:529–543.

Martof, B.. 1953b. Territoriality in the green frog, *Rana clamitans*. Ecology 34:165–174.

Matthews, K. R., and K. L. Pope. 1999. A telemetric study of the movement patterns and habitat use of *Rana muscosa*, the mountain yellow–legged frog, in a high–elevation basin in Kings Canyon National Park, California. Journal of Herpetology 33:615–624.

Powell, R. A. 2000. Animal home ranges and territories and home range estimators. Pages 65–110 *in* L. Boitani and T. K. Fuller, editors. Research Techniques in Animal Ecology, Columbia University Press, New York, USA.

Rose, B. 1982. Lizard home ranges: methodology and functions. Journal of Herpetology 16:253–269.

Seaman, D. E., and R. A. Powell. 1996. An evaluation of the accuracy of kernel density estimators for home range analysis. Ecology 77:2075–2085.

Seaman, D. E., J. J. Millspaugh, B. J. Kernohan, G. C. Brundige, K. J. Raedeke, and R. A. Gitzen. 1999. Effects of sample size on kernel home range estimates. Journal of Wildlife Management 63:739–747.

Shepard, D. B. 2002. Spatial relationships of male green frogs (*Rana clamitans*) throughout the activity season. American Midland Naturalist 148:394–400.

Watson, J. W., K. R. McAllister, and D. J. Pierce. 2003. Home ranges, movements, and habitat selection of Oregon spotted frogs (*Rana pretiosa*). Journal of Herpetology 37:292–300.

Wells, K. D. 1977a. Territoriality and male mating success in the green frog (*Rana clamitans*). Ecology 58:750–762.

Wells, K. D. 1977b. The social behavior of anuran amphibians. Animal Behaviour 25:666–693.

Whitney, C. L., and J. R. Krebs. 1975. Spacing and calling in Pacific tree frogs, *Hyla regilla*. Canadian Journal of Zoology 53:1519–1527.

Wilczynski, W., and E. A. Brenowitz. 1988. Acoustic cues mediate inter–male spacing in a neotropical frog. Animal Behaviour 36:1054–1063**.**

Table 1. Mean home range size and maximum linear movement distances (MLD) for male bog frogs (*Rana okaloosae*, *N* = 28) and bronze frogs (*R. clamitans*, *N* = 18) at the Live Oak Creek study site in Okaloosa County, FL (2002, 2003). Home ranges were estimated as 50% and 95% fixed kernels (FK) and 100% minimum convex polygons (MCP). The number of days monitored is the total days elapsed between first and last captures. Maximum linear movement distance is the distance between the two farthest capture locations. SVL = snout–vent length.

		SVL (mm)	No. locations	No. days monitored	Home Range (m^2)			MLD (m)
					95% FK	50% FK	MCP	
R. okaloosae	Mean	40.5	22.1	55.6	187.7	36.6	140.8	22.3
	S.D.	2.8	14.9	23.0	183.2	41.0	204.6	16.1
	Range	36.0–48.2	10–64	25–105	6.7–820.1	0.6–164.8	4.5–964.8	3.8–75.0
R. clamitans	Mean	64.8	26.3	66.9	547.6	106.0	301.5	37.1
	S.D.	4.7	13.7	31.7	687.0	159.8	282.3	24.0
	Range	56.8–71.1	10–54	18–124	25.8–2251.5	4.5–598.9	13.4–1031.6	5.9–83.8

Table 2. Correlations among movement estimates for male Florida bog frogs (*Rana okaloosae*, N=28, above diagonal line) and bronze frogs (*R. clamitans*, N=18, below line) at the Live Oak Creek study site in Okaloosa County, FL. Home ranges were estimated as 50% and 95% fixed kernels (FK) and 100% minimum convex polygons (MCP). Movements were monitored between 27 May–10 September in 2002 (*R. okaloosae* only) and 27 March–3 Aug 2003 (*R. okaloosae* and *R. clamitans*).

		50% FK	95% FK	MCP	MLD
50% FK	r		0.923	0.582	0.674
	P		< 0.001*	< 0.001*	< 0.001*
95% FK	r	0.972		0.726	0.777
	P	< 0.001*		< 0.001*	< 0.001*
MCP	r	0.914	0.949		0.941
	P	< 0.001*	< 0.001*		< 0.001*
MLD	r	0.867	0.914	0.898	
	P	< 0.001*	< 0.001*	< 0.001*	

* $P < 0.05$

Table 3. Mean neighbor distances for *Rana okaloosae* (ROK) and *R. clamitans* (RCL) at the Live Oak Creek study site in Okaloosa County, FL in 2003. On each observation night (N = 34), the distance to the nearest calling conspecific and heterospecific individual was measured for each calling male.

	Mean	SD	Range	95% CI
Calling Males Present (N):				
R. okaloosae	7.3	1.5	5–10	6.8–7.8
R. clamitans	9.6	3.2	5–16	8.5–10.7
Total frogs	16.9	3.5	11–24	15.7–18.2
Distances (m):				
ROK to ROK	9.17	3.6	4.41–18.25	7.92–10.43
ROK to RCL	6.50	2.2	2.34–12.11	5.74–7.26
RCL to RCL	7.75	2.7	3.23–16.24	6.82–8.68
RCL to ROK	7.73	3.4	3.76–19.08	6.54–8.92

Table 4. Correlations between the number of active calling frogs and inter–male distances at the Live Oak Creek study site in Okaloosa County, FL in 2003. Distances were averaged for each observation night (N = 34). A negative relationship indicates that average distances between calling males were reduced when more individuals were present.

		Distances			
Increasing number of:		ROK to ROK	ROK to RCL	RCL to RCL	RCL to ROK
Total Frogs	r	0.155	–0.374*	–0.444*	–0.276
	P	0.383	0.029	0.009	0.115
R. okaloosae (ROK)	r	–0.235	–0.018	–0.072	–0.582*
	P	0.181	0.921	0.686	0.000
R. clamitans (RCL)	r	0.282	–0.410*	–0.463*	–0.037
	P	0.106	0.016	0.006	0.834

* P< 0.05

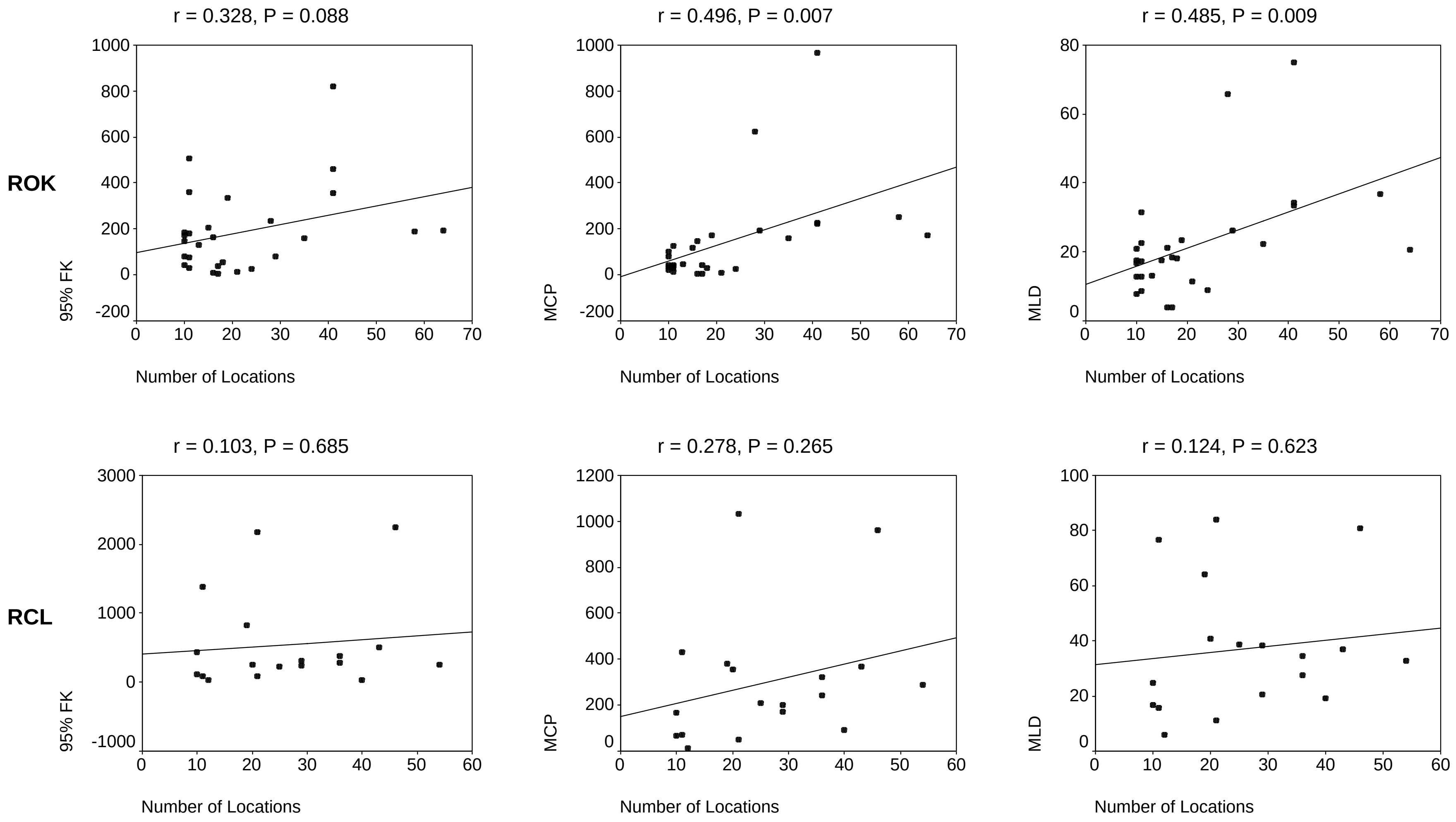


Figure 1. Correlations between the number of capture locations and home range size (m^2) or maximum linear movement distance (m) for Florida bog frogs (*Rana okaloosae*, ROK, *N* = 28) and bronze frogs (*R. clamitans*, RCL, *N* = 18) at the Live Oak Creek study site in Okaloosa County, FL. Home ranges were estimated as 50% and 95% fixed kernels (FK) and 100% minimum convex polygons (MCP). Maximum linear movement distance (MLD) is the distance between the two farthest capture locations. Movements were monitored 27 May – 10 September in 2002 (ROK only) and 27 March – 3 Aug in 2003 (ROK and RCL).

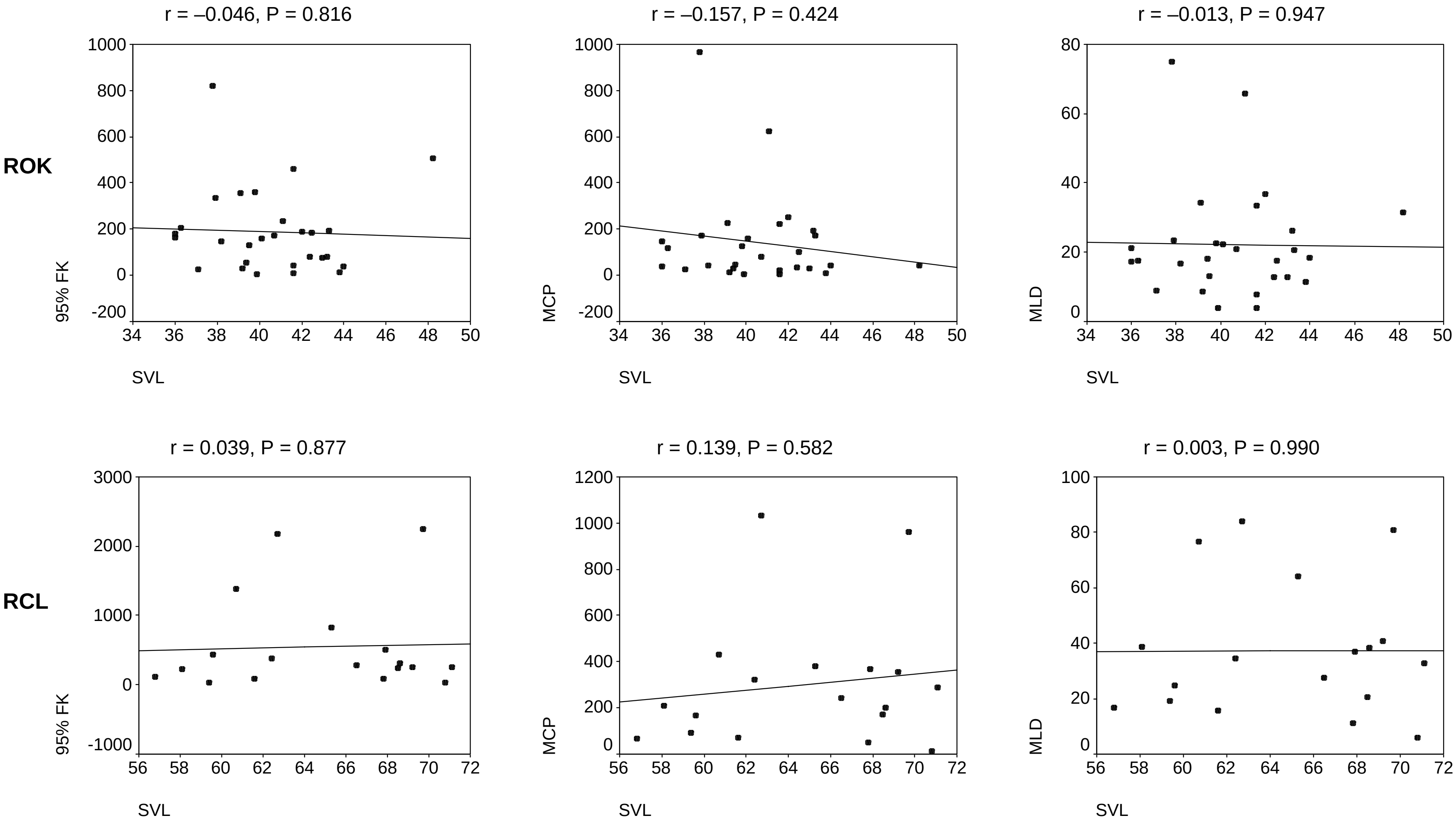


Figure 2. Correlations between snout–vent length (SVL, mm) and home range size (m^2) or maximum linear movement distance (m) for Florida bog frogs (*Rana okaloosae*, ROK, *N* = 28) and bronze frogs (*R. clamitans*, RCL, *N* = 18) at the Live Oak Creek study site in Okaloosa County, FL. Home ranges were estimated as 50% and 95% fixed kernels (FK) and 100% minimum convex polygons (MCP). Maximum linear movement distance (MLD) is the distance between the two farthest capture locations. Movements were monitored 27 May – 10 September in 2002 (ROK only) and 27 March – 3 Aug in 2003 (ROK and RCL).

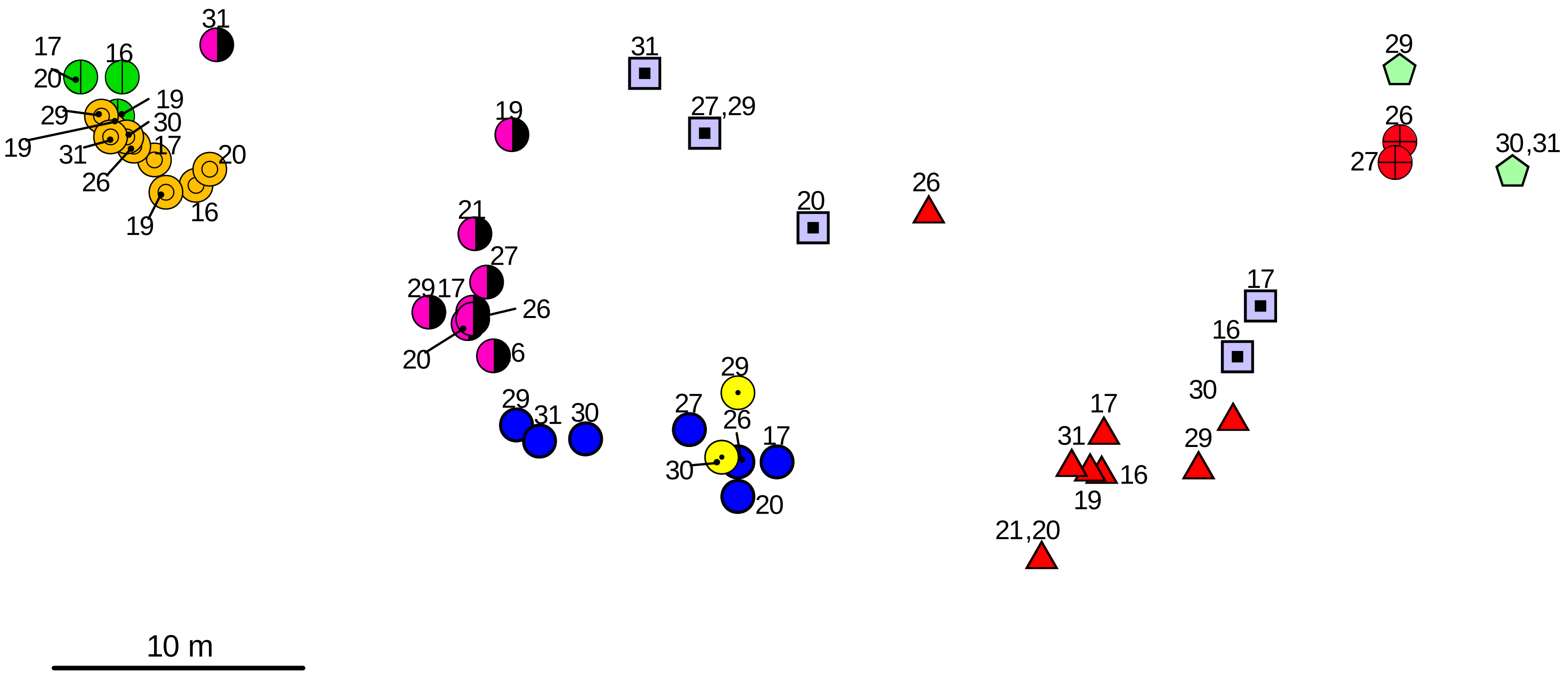


Figure 3. Relative spatial distribution of *R. okaloosae* males during the last 2 weeks of May at Live Oak Creek study site in Okaloosa County, FL. Males are identified by unique symbols. The numbers indicate the capture date(s). Symbols with multiple dates indicate an individual was captured in the same location on multiple dates.

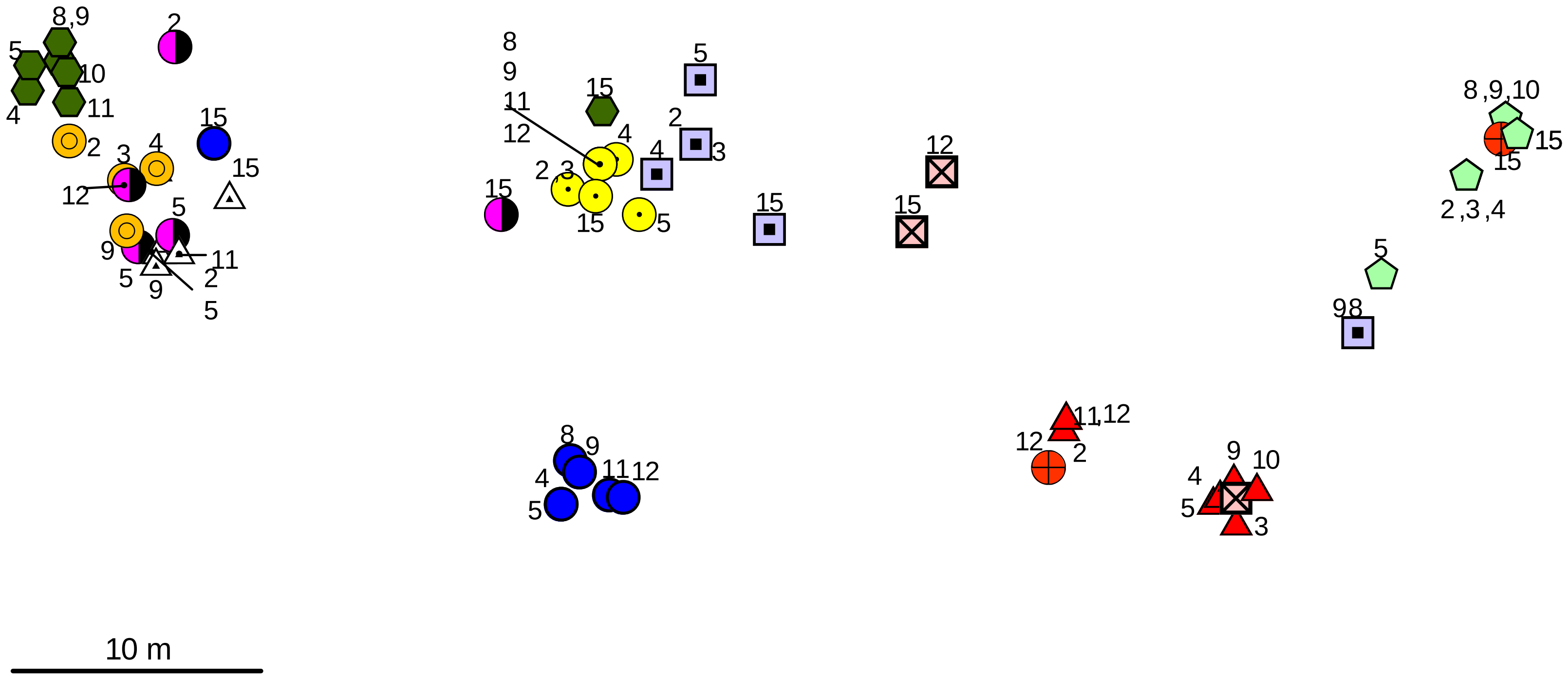


Figure 4. Relative spatial distribution of *R. okaloosae* males during the first 2 weeks of June at Live Oak Creek study site in Okaloosa County, FL. Males are identified by unique symbols. The numbers indicate the capture date(s). Symbols with multiple dates indicate an individual was captured in the same location on multiple dates.

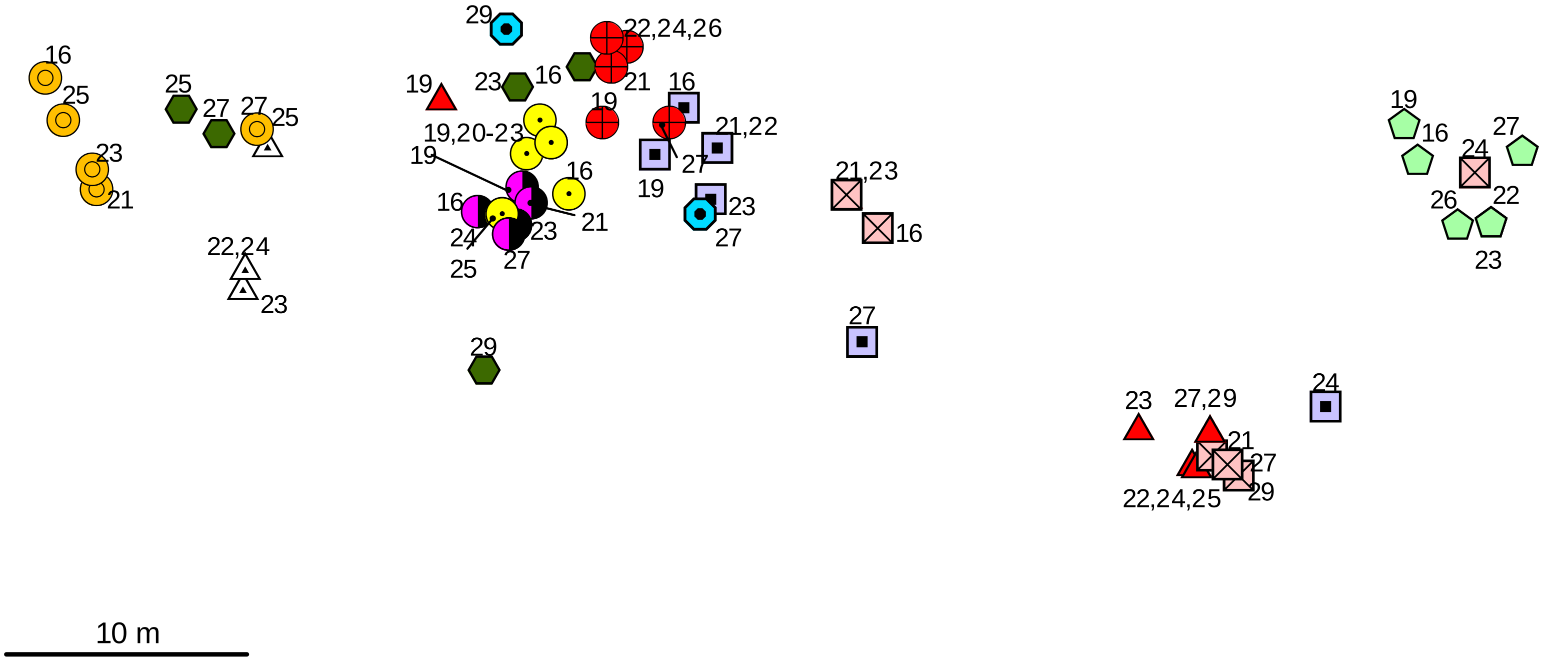


Figure 5. Relative spatial distribution of *R. okaloosae* males during the last 2 weeks of June at Live Oak Creek study site in Okaloosa County, FL. Males are identified by unique symbols. The numbers indicate the capture date(s). Symbols with multiple dates indicate an individual was captured in the same location on multiple dates.

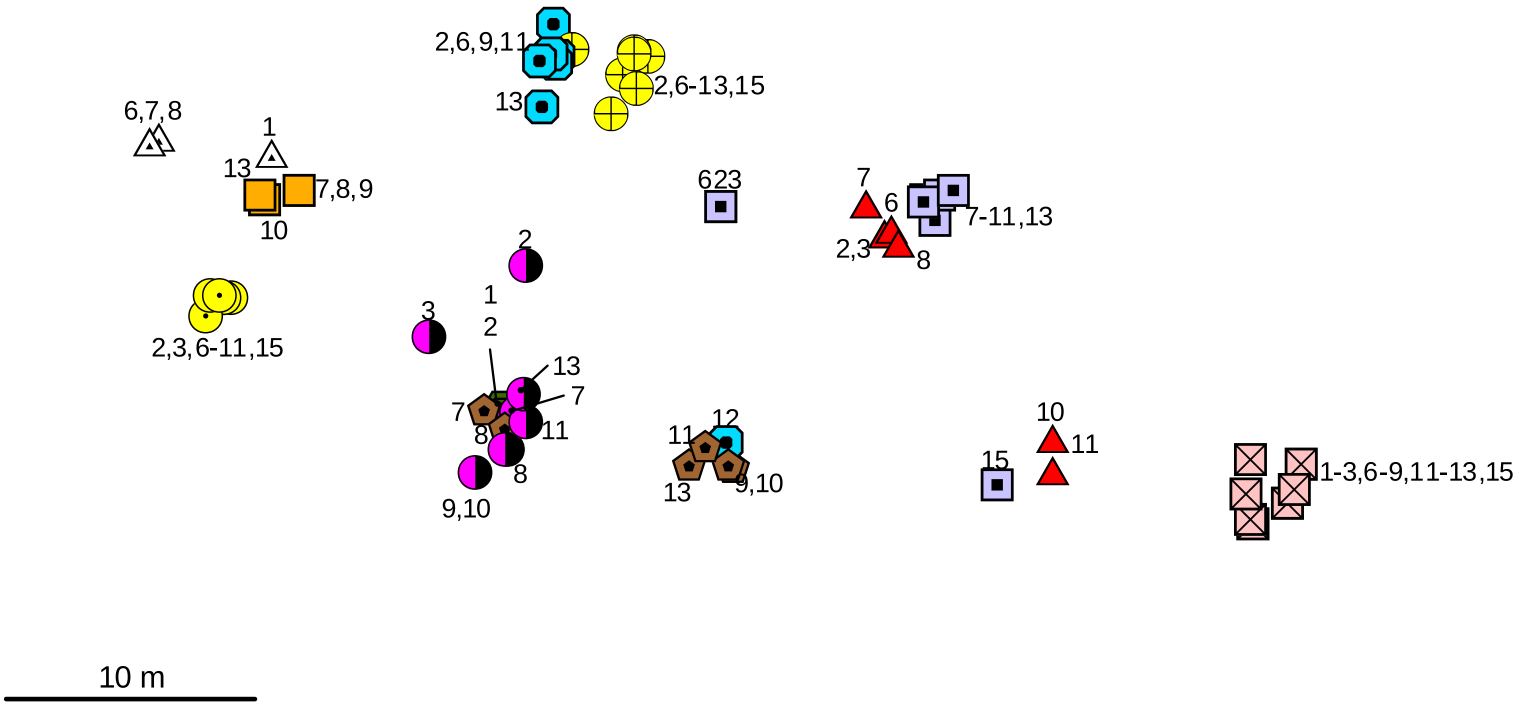


Figure 6. Relative spatial distribution of *R. okaloosae* males during the first 2 weeks of July at Live Oak Creek study site in Okaloosa County, FL. Males are identified by unique symbols. The numbers indicate the capture date(s). Symbols with multiple dates indicate an individual was captured in the same location on multiple dates.

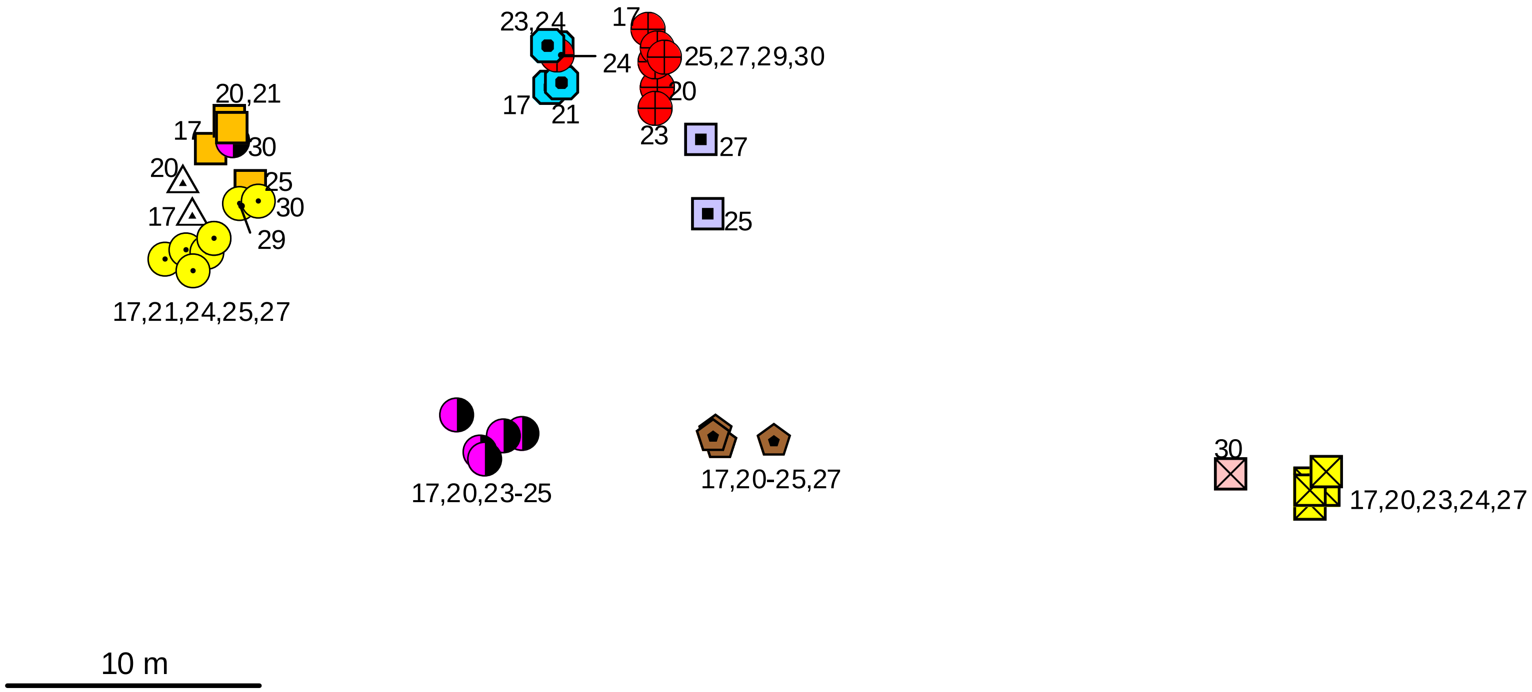


Figure 7. Relative spatial distribution of *R. okaloosae* males during the last 2 weeks of July at Live Oak Creek study site in Okaloosa County, FL. Males are identified by unique symbols. The numbers indicate the capture date(s). Symbols with multiple dates indicate an individual was captured in the same location on multiple dates.

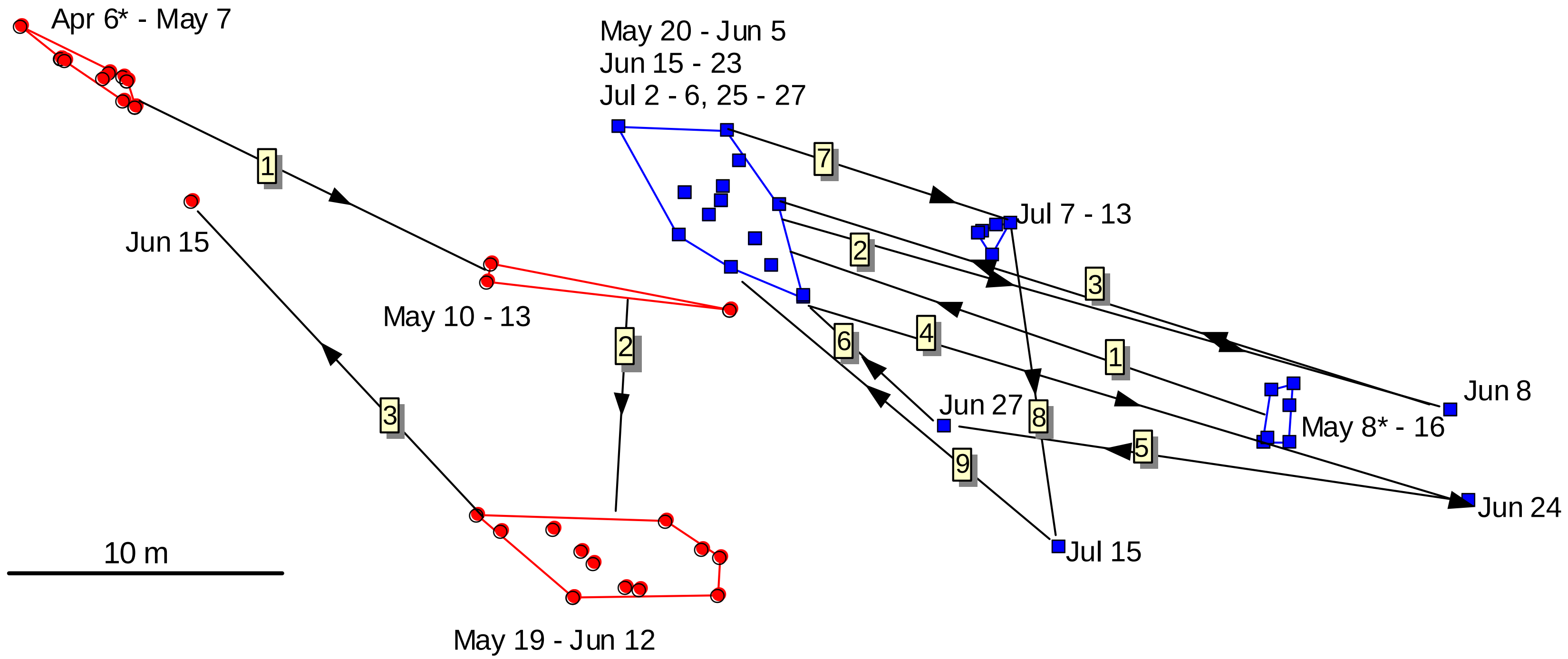


Figure 8. Movements and relative spatial distribution of 2 male Florida bog frogs (*Rana okaloosae*) in 2003 at Live Oak Creek study site in Okaloosa County, FL. A minimum convex polygon was used to cluster capture locations in the same vicinity. Arrows indicate movement paths to a new area. The numbers in boxes on arrows indicate the order of movements. * indicates starting location.

CHAPTER 3. VOCAL REPERTOIRE OF THE FLORIDA BOG FROG AND RESPONSE TO CONSPECIFIC AND HETEROSPECIFIC CALLS

ABSTRACT.—I recorded the calls of Florida bog frogs (*Rana okaloosae*) and describe the vocal repertoire. In addition, I broadcast conspecific and heterospecific (bronze frog, *R. clamitans clamitans*) advertisement calls to resident bog frog males to evaluate the vocal response to potential territorial intruders. The bog and the bronze frog are close relatives, occur in the same habitat, and are believed to hybridize. Bog frogs decreased type I advertisement calls and increased type II chuck calls during playback of conspecific male calls. In addition, resident *R. okaloosae* males produced type III response calls that overlapped the calls of other males. The vocal reaction to playback of *R. clamitans* calls was not as great as in conspecific trials, although some males had similar vocal responses. Lastly, to evaluate if hybridization between bog and bronze frogs could result from visual or auditory recognition errors, I investigated whether the species of an individual could be identified using only body size or the dominant frequency of the call. There is a large size difference between the two species, and it is unlikely that visual mate recognition errors occur. Most individuals also were classified to the correct species by the dominant frequency of their calls, although there were a few errors as a result of frequency overlap.

INTRODUCTION

Investigations of the acoustic signals may provide insight into the ecology, behavior, and evolutionary history of a species. Vocal interactions may be the primary form of competition for species with a prolonged breeding season (Wells 1977a).

Acoustic properties of frog calls may be related to fitness–related traits such as body size, fighting ability, experience, age, and resource-holding potential (Ryan 1980, Telford et al. 1989, Lode and Le Jacques 2003); hence, females may prefer to mate with some males over others because of properties of their vocalizations (Wagner and Sullivan 1995, Howard and Young 1998). Further, because calling is expensive energetically (Wells 2001), actively calling males may be more attractive to females and have greater mating success than individuals who do not have the energetic resources to sustain long calling bouts (Ch. 1).

In addition to communicating to potential mates, male advertisement calls provide information to other males on spatial position, territorial ownership, fighting ability, and body size (Wells 1977a, Gerhardt 1994, Littlejohn 2001). Males also may have encounter or aggressive calls, which are used as warning signals to other males in close proximity interactions, often as a precursor to physical aggression (McDiarmid and Adler 1975, Wells 1977a). Although body size constrains the frequency range of vocalizations an individual can produce (Ryan 1986, Gerhardt 1994), interacting males may change the dominant or fundamental frequencies of their calls in an attempt to bluff potential rivals into thinking they are larger than they are (Howard and Young 1998, Given 1999, Bee et al. 2000).

Calls also function as species isolating mechanisms (Blair 1964), reducing the frequency of aggressive interactions and matings between heterospecifics. Although sympatric species often partition acoustic (Chek et al. 2003) and geographic (Given 1990) space, hybridizations still may occur (Gerhardt 1974, Oldham and Gerhardt 1975). Playback experiments that compare the response of male and female anurans to calls from heterospecifics, conspecifics, and hybrids have been conducted (Littlejohn and Martin 1969, Oldham and Gerhardt 1975, Littlejohn and Watson 1976, Schwartz and Wells 1984, Given 1990, Ryan and Rand 1993) and may be useful in investigating the evolutionary history of a species. For example, Ryan and Rand (1995, 1999) observed that túngara frogs responded to hypothesized ancestral calls in addition to the calls of conspecifics.

I studied the vocal repertoire of the Florida bog frog (*Rana okaloosae*). Besides a brief description and sonogram of the type I call (Moler 1985, 1993), limited information is available on the calling behavior and diversity of vocalizations of Florida bog frogs. In addition to recording and describing the vocal repertoire of *R. okaloosae* males, I conducted playback experiments, in which I broadcast the advertisement calls of unfamiliar conspecifics to resident males to investigate the functions of different calls. I performed the same playback experiment to evaluate the response to heterospecific males in which I broadcast the calls of bronze frogs (*R. clamitans clamitans*) to bog frog males. As previously mentioned, *R. clamitans* is the closest relative of *R. okaloosae* (Austin et al. 2003), occurs in the same habitats (Bishop 2004), uses similar calling and oviposition sites (Chs. 1,2), and is suspected of hybridizing with *R. okaloosae* (Austin et al. 2003, Bishop 2004). To investigate potential sources of mate recognition errors between these two species, I look at the ability to identify the species of an unknown individual using the dominant frequency of his call or body size.

METHODS

Vocal repertoire of R. okaloosae.—Male bog frogs have 3 common call types. The type I call is a series of pulses strung together in a rattling train (Figure 1). Each pulse is divided further into subpulses. The type I call is a broadband signal, with most of the acoustic energy located in 2 frequency bands under 2500 Hz. The type II call is a single note ‘chuck’ (Figure 2). The type III call (Figure 3) is produced in response to the calls of other males and consists of a single pulse, similar to those in the type I call. If multiple type III calls are given in a series, they always are delivered at a slower rate than in a type I call. There is a fourth type of call, a release call (Wells 1978), that rarely is given by captured individuals and has not been recorded. Because I have heard it on only a few occasions, I will not describe it here.

Recording procedures.—I recorded all frogs on Eglin Air Force Base in Okaloosa and Santa Rosa counties, Florida, between April and July in 2003 and 2004. After I located a calling male, I positioned a tripod–mounted Sennheiser™ shotgun microphone (ME66) 1 m from the frog and recorded vocalizations using a Marantz™ recorder (PMD222) and type II audio cassettes. While recording, I sat quietly 3–4 m from the male under ambient light conditions. Individuals typically resumed calling soon after the microphone was in position and did not seem to be influenced by my presence. After I completed recording, I measured, and toe–clipped each frog for permanent identification and recorded the water temperature at the calling location.

Analysis of bog frog calls.—I digitized audio tapes at a sampling rate of 44 kHz using Goldwave™ 5.09 software (www.goldwave.com). I made temporal and spectral measurements of calls using Raven™ 1.2 software (Cornell Lab of Ornithology, Ithaca, NY). For type I calls, I measured the duration of each call and the number of pulses on the waveform (i.e., oscillogram), which I used to calculate a pulse rate (pulses/s). I created spectrograms and power spectra for each call (spectrogram and power spectra parameters: Hann window = 100 ms / 4410 samples, DFT size = 8192, 3 dB bandwidth = 14.4 Hz) and recorded the dominant frequency; i.e., the harmonic with the greatest power. Because type I calls typically have two frequency peaks that are close in amplitude (Figure 1), I also measured the frequency of the first peak in the power spectrum. The dominant frequency of type I calls may be either of these two peaks and may shift among calls for the same individual. I also calculated an average calling interval (i.e., time between calls) for males that gave ≥ 1 type I call. Finally, I measured the dominant frequencies for type II and III calls.

Playback experiment.—I conducted playback experiments in 2004 to evaluate how resident males respond to the calls of unfamiliar conspecific and heterospecific males. I broadcast previously recorded bog frog type I calls to 20 actively calling *R. okaloosae* males using a single speaker (Radioshack™ model 40–4000) mounted on a stake and connected to a portable CD player. I positioned the speaker 1 m from a calling male at water–level and broadcast calls at approximately 75 dB, a level within the natural range for *R. okaloosae* at this distance (unpublished data). I also broadcast type I calls (Figure 4) from previously recorded *R. clamitans* to 11 *R. okaloosae* males to evaluate the response to heterospecific calls. The vocal repertoire of *R. clamitans* is described elsewhere (Wells 1978, Bee and Perrill 1996). I broadcast calls for both species at 30-s intervals. Each playback session consisted of recording a male 10 min prior to playback and 10 min during playback. For different playback trials, I alternated among calls from

5 different individuals for each species to reduce the possibility of accidentally selecting an atypical call (Kroodsma 1986). I created all playback tracks using Goldwave™ software. For individuals that had ≥ 1 type II call before and during playback, I compared the average dominant frequency of type II calls to evaluate whether bog frogs alter the frequency of their calls during vocal interactions with other males

Interspecific comparisons.—To compare the similarities between calls of adult *R. okaloosae* and *R. clamitans* and the potential for species recognition errors, I compared the dominant frequencies of type I calls between the two species. I randomly selected a single call from each individual for comparisons. The temporal properties of advertisement calls differ greatly between the two species and can be distinguished easily in the field. The bronze frog type I call is a single pulse (Figure 4), whereas the bog frog type I call consists of a series of pulses (Figure 1). Because the type II chuck call of *R. okaloosae* is temporally similar to the advertisement call of *R. clamitans*, I also compared the dominant frequencies between these 2 calls (i.e., type II for *R. okaloosae* and type I for *R. clamitans*). I also evaluated the potential for visual recognition errors by comparing body sizes between the two species using data collected from all individuals measured between 2002 and 2004. Using logistic regression, I evaluated my ability to differentiate between species using only the dominant frequency of the call or snout–vent length (SVL).

RESULTS

Description of bog frog calls.—I recorded type I calls from 40 *R. okaloosae* males ($\bar{x}$ SVL = 45.7 ± 5.5 mm, range: 37.0–53.7 mm). Type I calls averaged 1.38 ± 0.79 s in length (range: 0.28–4.25 s, CV = 57.2%) and 7.5 ± 3.8 pulses (range: 2–18 pulses, CV = 50.7%), with a mean pulse rate of 0.18 ± 0.02 pulses/s (range: 0.14–0.23 pulses/s, CV = 11.1%). The average dominant frequency (DF) was 1505.3 ± 635.8 Hz (range: 651.4–2465.6 Hz, CV = 42.2%), and the frequency of the first peak of the power spectrum was 771.8 ± 88.6 Hz (range: 640.6–942.1 Hz, CV = 11.5%). The average calling interval (i.e., time between calls) for type I advertisement calls was 136.3 ± 123.4 s (range: 23.4–600.0 s, CV = 90.5%, N = 33).

Body size (SVL) was correlated negatively with the frequency of the first spectral peak in type I calls, with larger frogs producing deeper calls (Table 1, Figure 5). There was no significant correlation between SVL and DF for type II calls when all individuals were included in the dataset. However, when the data were separated into 2 groups, those whose DF was the first spectral peak (N = 17, $\bar{x}$ = 651.4 ± 84.2 Hz, range: 651.4–920.5 Hz, CV = 12.9%) and those whose DF occurred at a higher frequency (N = 23, $\bar{x}$ = 2027.6 ± 200.9 Hz, range: 1588.1–2465.6 Hz, CV = 8.1%), the relationship was significant within each group (low: DF = –10.551SVL +1270.268, R^2 = 0.662, P < 0.001; high: DF = –25.724SVL + 3234.212, R^2 = 0.316, P < 0.006). There was no significant difference in SVL between frogs whose DF was the first spectral peak ($\bar{x}$ = 44.2 ± 6.5 mm, range: 37.0–53.7 mm) and those that had a DF at a higher frequency ($\bar{x}$ = 46.8 ± 4.5 mm, range: 37.1–52.1 mm) (Satterthwaite t-test: t = –1.361, df = 25.018, P = 0.186).

I recorded type II calls from 36 *R. okaloosae* males ($\bar{x}$ SVL = 45.4 ± 5.1 mm, range: 37.2–53.7 mm). Type II calls had a mean DF of 1041.1 ± 340.7 Hz (range: 640.6–2072.6 Hz, CV = 32.7%). Dominant frequency was not correlated with SVL for type II calls (Figure 5). I recorded type III calls from 38 *R. okaloosae* males ($\bar{x}$ SVL = 44.4 ±

5.6 mm, range: 37.0–53.7 mm). The mean DF was 742.2 ± 80.5 Hz (range: 613.3–904.4 Hz, CV = 10.8%), which was correlated negatively with SVL (Figure 5).

Playback experiment.—Bog frog males decreased the number of type I calls and increased type II and III calls in response to calls of unfamiliar conspecific males (Figure 6). Males averaged 4.0 ± 3.1 (range: 0–9, N = 20) type I calls (per 10 min) prior to playback but only 0.8 ± 1.3 (range: 0–4) during playback of conspecific calls, a statistically significant decline (Wilcoxon signed ranks test: z = –2.922, P = 0.003, N = 20). Males averaged 4.5 ± 3.2 (range: 0–13, N = 11) type II calls prior to playback and 10.3 ± 8.4 (range: 1–33) during playback, also a significant increase (z = –3.499, P = < 0.001). Males averaged 9.2 ± 1.6 (range: 0–20) type III response calls during playback (compared to 0 type III calls before playback), a significant increase (z = –3.519, P < 0.001). Seventeen out of 20 (85%) males gave ≥ 1 type III call in response to playback of conspecific calls.

When bog frogs were broadcast calls from *R. clamitans*, there was less change in calling behavior than in conspecific playback trials (Figure 6). Males averaged 5.7 ± 4.0 (range: 0–13) type I advertisement calls prior to playback and 4.3 ± 3.1 (range: 0–11) during playback of heterospecific calls, a decline that was not significant statistically (z = –1.474, P = 0.141). Males averaged 3.6 ± 1.4 type II chuck calls (range: 1–6) prior to playback and 3.3 ± 1.7 (range: 0–6) during playback, a change that was not significant (z = –0.423, P = 0.672). Males averaged 0.6 ± 1.4 type III response calls (range: 0–4) during playback of *R. clamitans* calls (z = –1.342, P < 0.180). Two out of 11 (18%) males gave response calls during heterospecific playback trials.

Interspecific comparisons.—The average DF of type I calls for *R. okaloosae* males was 1505.3 ± 635.8 Hz (range: 651.4–2465.6 Hz, N = 40) and 500.7 ± 21.7 Hz (range: 393.3–753.7 Hz, N = 22) for *R. clamitans* (Figure 7). The logistic regression model for differentiating species using only the DF of type I calls was highly significant (Y = –0.024DF + 65.57, $\chi^2_1 = 57.050$, $P < 0.001$), with 97.5% (39 of 40) of *R. okaloosae* calls and 90.9% (20 of 22) of *R. clamitans* calls correctly classified (grand $\bar{x} = 95.2\%$). The type II calls of *R. okaloosae* had a mean DF of 1041.1 ± 340.7 Hz (range: 640.6–2072.6 Hz, N = 36). The logistic regression model for differentiating species using the DF of bronze frog type I calls and bog frog type II chuck calls also was significant (Y = – –0.024DF + 15.948, $\chi^2_1 = 60.908$, $P < 0.001$), with 97.2% (35 of 36) of *R. okaloosae* and 90.9% (20 of 22) of *R. clamitans* classified correctly ($\bar{x} = 94.8\%$).

There was little overlap in body size between adult *R. okaloosae* and *R. clamitans* males and females (Figure 8). As reported in Ch. 1, average SVL was 42.7 ± 5.1 mm (range: 34.4–53.9 mm) for the 85 bog frog males and 41.5 ± 4.3 mm (range: 33.5–44.8 mm) for the 44 females I measured. Bronze frog averaged 64.9 ± 6.3 mm (range: 56.2–81.1 mm, N = 39) for males and 67.3 ± 6.1 mm (range: 58.6–77.0 mm, N = 12) for females. The logistic regression model classified 100% of the males (Y = –12.089SVL + 665.090, $\chi^2_1 = 153.662$, $P < 0.001$) and females (Y = –3.405SVL + 182.902, $\chi^2_1 = 58.193$, $P < 0.001$) correctly, using only SVL.

DISCUSSION

The vocal repertoire of *Rana okaloosae* is similar to that of other members of the genus, consisting of a few call types that presumably serve numerous functions including, species recognition, mate attraction, and inter–male competition (e.g., Wiewandt 1969, Wells 1978, Given 1987). The type I call of the bog frog can be classified as the *advertisement* call (Wells 1977a), likely used to attract females and convey information to other males. Because the number of type II chuck calls increased during playback, it may be labeled an *encounter* call (McDiarmid and Adler 1974), used in vocal interactions between nearby males. However, type II calls also were given regularly when males were not in close proximity. Females also have a call similar to the type II chuck of males, which may be a *reciprocation* call given during courtship (Littlejohn 1977). This call, however, is rare and unrecorded. Given (1987) reported that female carpenter frogs (*Rana virgatipes*) also give a low intensity call during courtship similar to the single note aggressive calls of males.

Perhaps the most interesting aspect of the calling behavior of male Florida bog frogs is the presence of a type III *response* call, a call given intentionally during the vocalizations of other males. Most frogs avoid acoustic interference (Shwartz and Wells 1983, 1984; Grafe 1996) because overlapping calls limit the orientation and recognition ability of females (Wollerman 1999, Gerhardt 1982). Response calls typically occur in reaction to type I advertisement calls of neighboring conspecific males but also may be given in response to type II chuck calls. Occasionally, bog frogs respond to the advertisement calls of *R. clamitans*, especially when other bog frogs are not calling actively in the area. I even heard type III calls given in response to machine gun fire and other military ordinance. It is unknown why bog frogs give response calls. Because response calls are similar to the pulses that make up the type I advertisement pulse train, type III calls may provide similar information about body size, territorial ownership, and identity of an individual (Ch. 4) as type I calls, possibly with less energetic costs. Response calls also may interfere with the ability of nearby males to attract mates.

In general, anurans react to the calls of nearby males by either decreasing their overall calling activity (Whitney and Krebs 1975, Perrill and Shepherd 1989) or increasing calling intensity and the occurrence of certain call types (Wells 1978, Given 1987, Bee and Perrill 1996). Bog frogs decreased the number of type I advertisement calls and increased the number of type II and III calls in response to playbacks of advertisement calls of unfamiliar conspecific males. There was no statistical difference in the number of calls given during heterospecific playback trials; however, sample size (N = 11) was limited.

Only on a single occasion did a resident male approach a speaker during conspecific playback, which supports my previous field observations that bog frogs are not as aggressive toward intruding males as other ranids (Ch. 1). However, bog frogs do space themselves throughout a calling area (Ch. 2), and if two males are next to each other, one adopts a satellite strategy (Ch. 1), behaviors that suggest they may be territorial. Because body size is correlated negatively with call frequency, bog frogs may convey fighting ability, territorial ownership, and body size vocally and avoid physical combat (Bee et al. 2000, Bee 2002).

Others studies reported that males lowered the dominant or fundamental frequency of their advertisement call in response to territorial intruders (Howard and Young 1998, Bee et al. 2000, Bee and Bowling 2002). I found no statistical difference in average DF between type II chuck calls of bog frogs given before and during playback of conspecific calls. I looked for frequency differences in chuck calls because they increased during playback, whereas advertisement calls decreased. Because some individuals stopped giving advertisement calls during playback, I did not have adequate data to compare frequency changes in advertisement calls. I also did not have adequate data to address whether the call (i.e., from big or small male) used during playback experiments affected the vocal response of resident males (see Bee et al. 2000).

Temperature often affects the calling behavior or call properties of frogs (Littlejohn 1977, Given 1999, Friedel and Klump 2002). Although I measured water temperature each time I recorded a frog, I did not include these data because of confounding variables. There were significant correlations between water temperature and some acoustic properties. However, water temperature also was correlated with SVL; i.e., the largest frogs I captured and recorded generally were located in other sites that had higher water temperatures. Therefore, I was unable to separate the effects of water temperature from SVL. This pattern raises questions of whether warm water locations have frogs that grow faster or live longer than cooler locations or whether large frogs select warm locations (see also Ch. 1). Other environmental variables also may affect calling behavior. For example, casual observations suggest that *R. okaloosae* may call less during and after rain and possibly on bright nights associated with full moons (Bishop 2004).

The current overlap in the distribution of *R. okaloosae* and *R. clamitans* likely is the result of range expansion of the bog frog after the initial speciation event (likely in the Pleistocene, Austin et al. 2003). Because of the low–elevation of the streams in the area, a slight change in sea–level may have caused populations of *R. clamitans* to become isolated and possibly provided the vicariance event needed for speciation. Because of similarities in their resource requirements and breeding phenology, I do not think sympatric speciation occurred. There is little mitochondrial genetic differentiation between bog frogs and bronze frogs, likely the result of incomplete lineage sorting (Austin et al. 2003). Pre–mating isolating mechanisms may evolve before complete genetic separation (Blair 1964). Littlejohn (1977) listed 4 potential methods used by frogs to reduce acoustic interference, which may act as isolating mechanisms: 1) spectral stratification, 2) spatial separation of individuals, 3) temporal partitioning, or 4) different coding patterns if similar frequency bands are used and both species are active at the same time.

Because bog frogs and bronze frogs overlap in distribution (Bishop 2004), breeding seasons (Ch. 1), oviposition and calling locations (Chs. 1, 2), and appear to have similar calling patterns within a night (personal observations), methods 2 and 3 in the Littlejohn model may be eliminated as potential species isolating mechanisms. The spectral properties of the advertisement calls of both species are similar, consisting of multiple harmonics with sound energy concentrated in 2 frequency peaks under 2500 Hz. I successfully differentiated between most *R. okaloosae* and *R. clamitans* using only the DF of their calls. There were a few misidentifications, however, a result that may explain hybridizations if DF is an important mate–recognition feature. The temporal coding patterns (e.g., duration, number of pulses) of advertisement calls differ greatly between the two species, and it is unlikely that females would confuse these two calls if temporal properties also are used to identify appropriate mates. Although the type II calls of *R. okaloosae* are more similar temporally than type I calls to the advertisement calls of *R. clamitans*, species were still identified successfully by DF. Because of the large size difference between the two species, it also is unlikely that females would make visual identification errors.

Although no single isolating mechanism may keep species from hybridizing, their combined effects may be sufficient (Blair 1958). However, when suitable mates are unavailable, the frequency of hybridizations may increase. Male bog and bronze frogs do not clasp females indiscriminately, a characteristic observed in explosive breeding species (Wells 1977b); females must initiate the mating process (Ch. 1, Wells 1977c). Although I do not report these results in detail, I conducted an exploratory 2–speaker mate preference playback experiment (e.g., Howard and Young 1998, Gerhardt 1991) where a female *R. okaloosae* or *R. clamitans* was given a choice between a conspecific and heterospecific call. Neither species responded after 30 min of audio playback. Mate–choice experiments like this have been successful in explosive breeding species but, to my knowledge, not in a species with a prolonged breeding season.

It is unknown if the hybridization between *R. okaloosae* and *R. clamitans* is directional. It also is unknown if hybrids are sterile or if introgression does occur. Austin et al. (2003) found that a single *R. okaloosae* male shared the same mitochondrial haplotype as *R. clamitans* and suggested that hybridization may have occurred between a male bog frog and a female bronze frog. If hybridization between these species is directional and I ignored the limited genetic evidence, I think hybridization would be more likely between a female *R. okaloosae* and male *R. clamitans* for the following reasons. First, as mentioned earlier, Ryan and Rand (1995, 1999) found that female túngara frogs responded to hypothesized ancestral calls as well as conspecific calls. If bog frogs recently diverged from its ancestor, *R. clamitans* (Austin et al. 2003), female bog frogs may still respond to the vocalizations of bronze frog males. Second, the size difference between the two species is striking, and it is unlikely the arms of a male *R. okaloosae* could fully clasp a mature female *R. clamitans* during mating. Because mitochondrial DNA is inherited maternally, future genetic analysis may be able to reveal the direction of hybridization (e.g., Lamb and Avise 1986).

LITERATURE CITED

Austin, J. D., S. C. Lougheed, P. E. Moler, and P. T. Boag. 2003. Phylogenetics, zoogeography, and the role of dispersal and vicariance in the evolution of the *Rana catesbeiana* (Anura: Ranidae) species group. Biological Journal of the Linnean Society 80: 601–624.

Bee, M. A. 2002. Territorial male bullfrogs (*Rana catesbeiana*) do not assess fighting ability based on size–related variation in acoustic signals. Behavioral Ecology 13:109–124.

Bee, M. A., and A. C. Bowling. 2002. Socially mediated pitch alteration by territorial male bullfrogs, *Rana catesbeiana*. Journal of Herpetology 36:140–143.

Bee, M. A., and S. A. Perrill. 1996. Responses to conspecific advertisement calls in the green frog (*Rana clamitans*) and their role in male–male communication. Behaviour 133:283–301.

Bee, M. A., S. A. Perrill, and P. C. Owen. 2000. Male green frogs lower the pitch of acoustic signals in defense of territories: a possible dishonest signal of size? Behavioral Ecology 11:169–177.

Bishop, D. C. 2004. Summary of surveys for Florida bog frogs and flatwoods salamanders on Eglin Air Force Base through 2004. Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA.

Blair, W. F. 1958. Mating call in the speciation of anuran amphibians. American Naturalist 92:27–51.

Blair, W. F. 1964. Isolating mechanisms and interspecies interactions in anuran amphibians. The Quarterly Review of Biology 39:334–344.

Chek, A. A., J. P. Bogart, and S. C. Lougheed. 2003. Mating signal partitioning in multi–species assemblages: a null model test using frogs. Ecology Letters 6:235–247.

Gerhardt, H. C. 1974. Vocalization of some hybrid treefrogs: acoustic and behavioral analyses. Behaviour 49:130–151.

Gerhardt, H. C. 1982. Sound pattern recognition in some North American treefrogs (Anura: Hylidae): implications for mate choice. American Zoologist 22:581–595.

Gerhardt, H. C. 1991. Female mate choice in treefrogs: static and dynamic acoustic criteria. Animal Behaviour 42:615–635.

Gerhardt, H. C. 1994. The evolution of vocalization in frogs and toads. Annual Review of Ecology and Systematics 25:293–324.

Given, M. F. 1987. Vocalizations and acoustic interactions of the carpenter frog, *Rana virgatipes*. Herpetologica 43:467–481.

Given, M. F. 1990. Spatial distribution and vocal interaction in *Rana clamitans* and *R. virgatipes*. Journal of Herpetology 24:377–382.

Given, M. F. 1999. Frequency alteration of the advertisement call in the carpenter frog, *Rana virgatipes*. Herpetologica 55:304–317.

Grafe, T. U. 1996. The function of call alternation in the African reed frog (*Hyperolius marmoratus*): precise call timing prevents auditory masking. Behavioral Ecology and Sociobiology 38:149–158.

Friedl, T. W. P., and G. M. Klump. 2002. The vocal behaviour of male European treefrogs (*Hyla arborea*): implications of inter– and intrasexual selection. Behaviour 139:113–136.

Howard, R. D., and J. R. Young. 1998. Individual variation in male vocal traits and female mating preferences in *Bufo americanus*. Animal Behaviour 55:1165–1179.

Kroodsma, D. E. 1986. Design of playback experiments. Auk 103:640–642.

Lamb, T., and J. C. Avise. 1986. Directional introgression of mitochondrial DNA in a hybrid population of tree frogs: the influence of mating behavior. Proceedings of the National Academy of Sciences, USA 83:2526–2530.

Littlejohn, M. J. 1977. Long–range acoustic communication in anurans: an integrated and evolutionary approach. Pages 263–294 i*n* D. H. Taylor and S. I. Guttman, editors. The Reproductive Biology of Amphibians. Plenum Press, New York, New York, USA.

Littlejohn, M. J. 2001. Patterns of differentiation in temporal properties of acoustic signals of anurans. Pages 102–120 *in* M. J. Ryan, editor. Anuran Communication., Smithsonian Institution Press, Washington, D.C., USA.

Littlejohn, M. J., and A. A. Martin. 1969. Acoustic interaction between two species of leptodactylid frogs. Animal Behaviour 17:785–791.

Littlejohn, M. J., and G. F. Watson. 1976. Effectiveness of a hybrid mating call in eliciting phonotaxis by females of the *Geocrinia laevis* complex (Anura: Leptodactylidae). Copeia 1976:76–79.

Lode, T., and D. Le Jacques. 2003. Influence of advertisement calls on reproductive success in the male midwife toad *Alytes obstetricians*. Behaviour 140:885–898.

McDiarmid, R. W., and K. Adler. 1974. Notes on territorial and vocal behavior of neotropical frogs of the genus *Centrolenella*. Herpetologica 30:75–78.

Moler, P. E. 1985. A new species of frog (Ranidae: *Rana*) from northwestern Florida. Copeia 1985:379–383.

Moler, P. E. 1993. *Rana okaloosae* Moler Florida bog frog. Catalogue of American amphibians and reptiles.561.1–3.

Oldham, R. S. and H. C. Gerhardt. 1975. Behavioral isolating mechanisms of the treefrogs *Hyla cinerea* and *H. gratiosa*. Copeia 1975:223–231.

Perrill, S. A., and W. J. Shepherd. 1989. Spatial distribution and male–male communication in the northern cricket frog, *Acris crepitans blanchardi*. Journal of Herpetology 23:237–243.

Ryan, M. J. 1980. Female mate choice in a neotropical frog. Science 219:1087–1089.

Ryan, M. J. 1986. Factors influencing the evolution of acoustic communication: biological constraints. Brain, Behavior, and Evolution 28:70–82.

Ryan, M. J., and A. S. Rand. 1993. Species recognition and sexual selection as a unitary problem in animal communication. Evolution 47:647–657.

Ryan, M. J., and A. S. Rand. 1995. Female responses to ancestral advertisement calls in Túngara frogs. Science 269:390–392.

Ryan, M. J., and A. S. Rand. 1999. Phylogenetic influence on mating call preferences in female túngara frogs, *Physaleaemus pustulosus*. Animal Behaviour 57:945–956.

Schwartz, J. L., and K. D. Wells. 1983. An experimental study of acoustic interference between two species of neotropical treefrogs. Animal Behaviour 31:181–190.

Schwartz, J. L., and K. D. Wells. 1984. Interspecific acoustic interactions of the neotropical treefrog *Hyla ebraccata*. Behavioral Ecology and Sociobiology 14:211–224.

Telford, S. R., M. L. Dyson, and N. I. Passmore. 1989. Mate choice occurs only in small choruses of painted reed frogs *Hyperolius marmoratus*. Bioacoustics 2:47–53.

Wagner, W. E., Jr. and B. K. Sullivan. 1995. Sexual selection in the Gulf Coast toad, *Bufo valliceps*: female choice based on variable characters. Animal Behaviour 49:305–319.

Wells, K. D. 1977a. The social behavior of anuran amphibians. Animal Behaviour 25:666–693.

Wells, K. D. 1977b. The courtship of frogs. Pages 233–262 *in* D. H. Taylor and S. I. Guttman, editors. The Reproductive Biology of Amphibians. Plenum Press, New York, New York, USA.

Wells, K. D. 1977c. Territoriality and male mating success in the green frog (*Rana clamitans*). Ecology 58:750–762.

Wells, K. D. 1978. Territoriality in the green frog (*Rana clamitans*): vocalizations and agonistic behaviour. Animal Behaviour 26:1051–1063.

Wells, K. D. 2001. The energetics of calling frogs. Pages 45–60 *in* M. J. Ryan, editor. Anuran Communication, Smithsonian Institution Press, Washington, D.C. USA.

Whitney, C. L., and J. R. Krebs. 1975. Spacing and calling in Pacific tree frogs, *Hyla regilla*. Canadian Journal of Zoology 53:1519–1527.

Wiewandt, T. A. 1969. Vocalization, aggressive behavior, and territoriality in the bullfrog, *Rana catesbeiana*. Copeia1969:276–285.

Wollerman, L. 1999. Acoustic interference limits call detection in a Neotropical frog *Hyla ebraccata*. Animal Behaviour 57:529–536.

Table 1. Correlations between call properties of type I calls and snout–vent length (SVL) for 40 *Rana okaloosae* males.

		SVL	Dominant frequency	1st peak frequency	Pulse rate	N pulses	Call length
Call length	r	0.183	0.049	0.027	0.291	0.972*	1
	P	0.273	0.763	0.869	0.068	0.000	.
N pulses	r	0.203	0.099	0.026	0.470*	1	
	P	0.221	0.542	0.873	0.002	.	
Pulse rate	r	–0.074	–0.091	0.080	1		
	P	0.657	0.578	0.624	.		
1st peak frequency	r	–0.848*	–0.083	1			
	P	0.000	0.613	.			
Dominant frequency	r	0.090	1				
	P	0.592	.				
SVL	r	1					
	P	.					

* $P < 0.05$

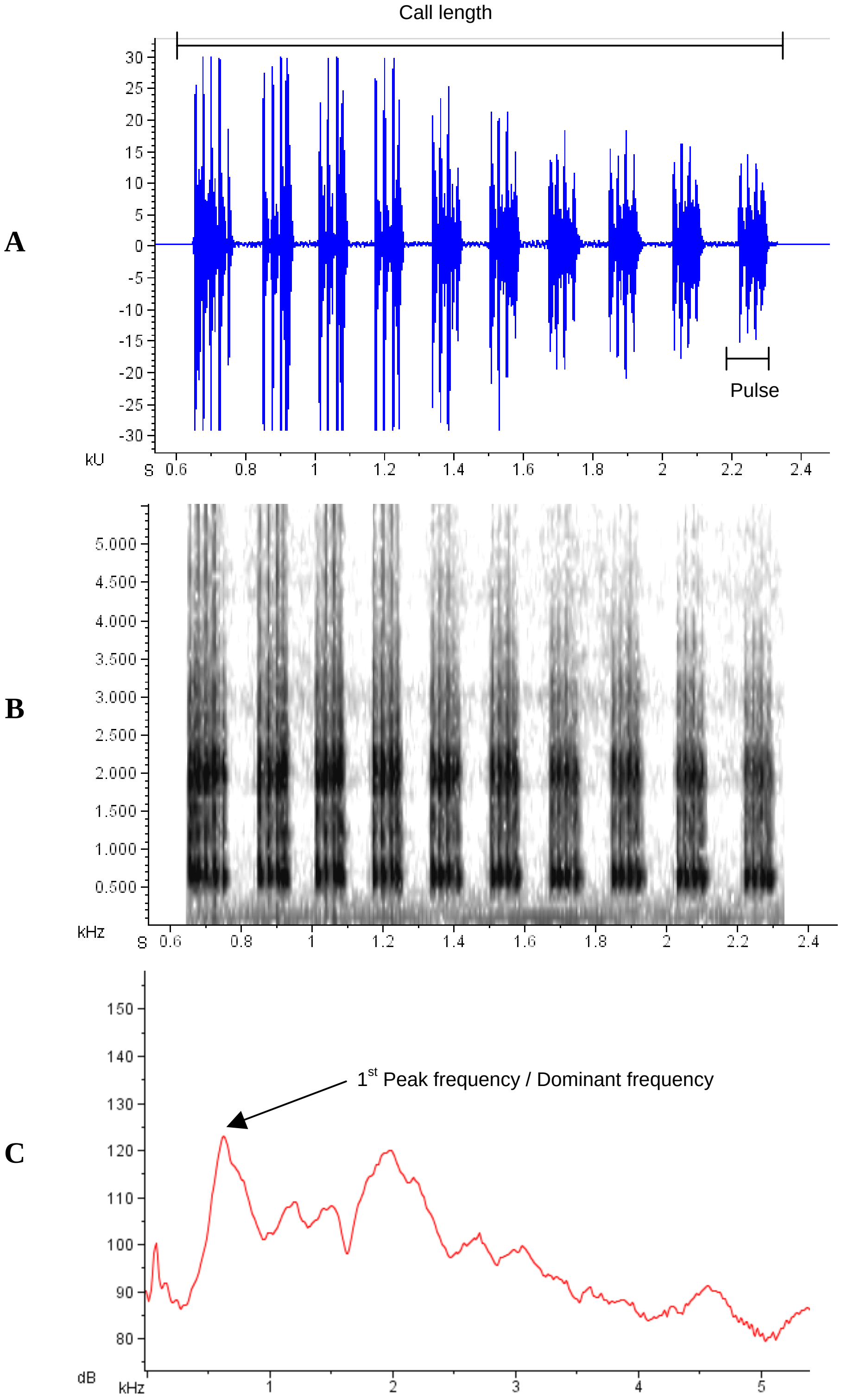


Figure 1. Waveform (A), spectrogram (B), and power spectrum (C) of a type I call from a male *Rana okaloosae* in Okaloosa County, Florida.

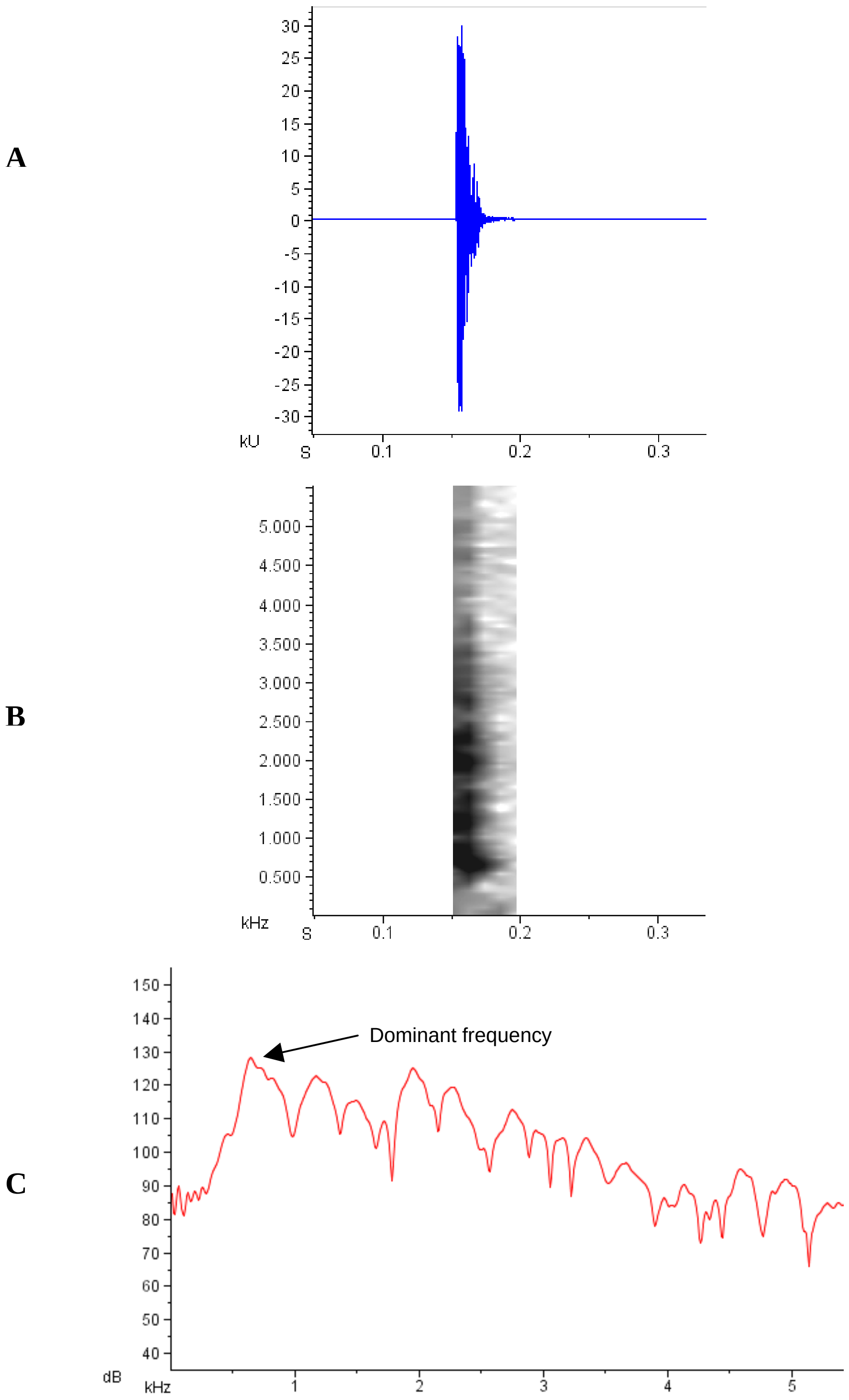


Figure 2. Waveform (A), spectrogram (B), and power spectrum (C) of a type II call from a male *Rana okaloosae* in Okaloosa County, Florida.

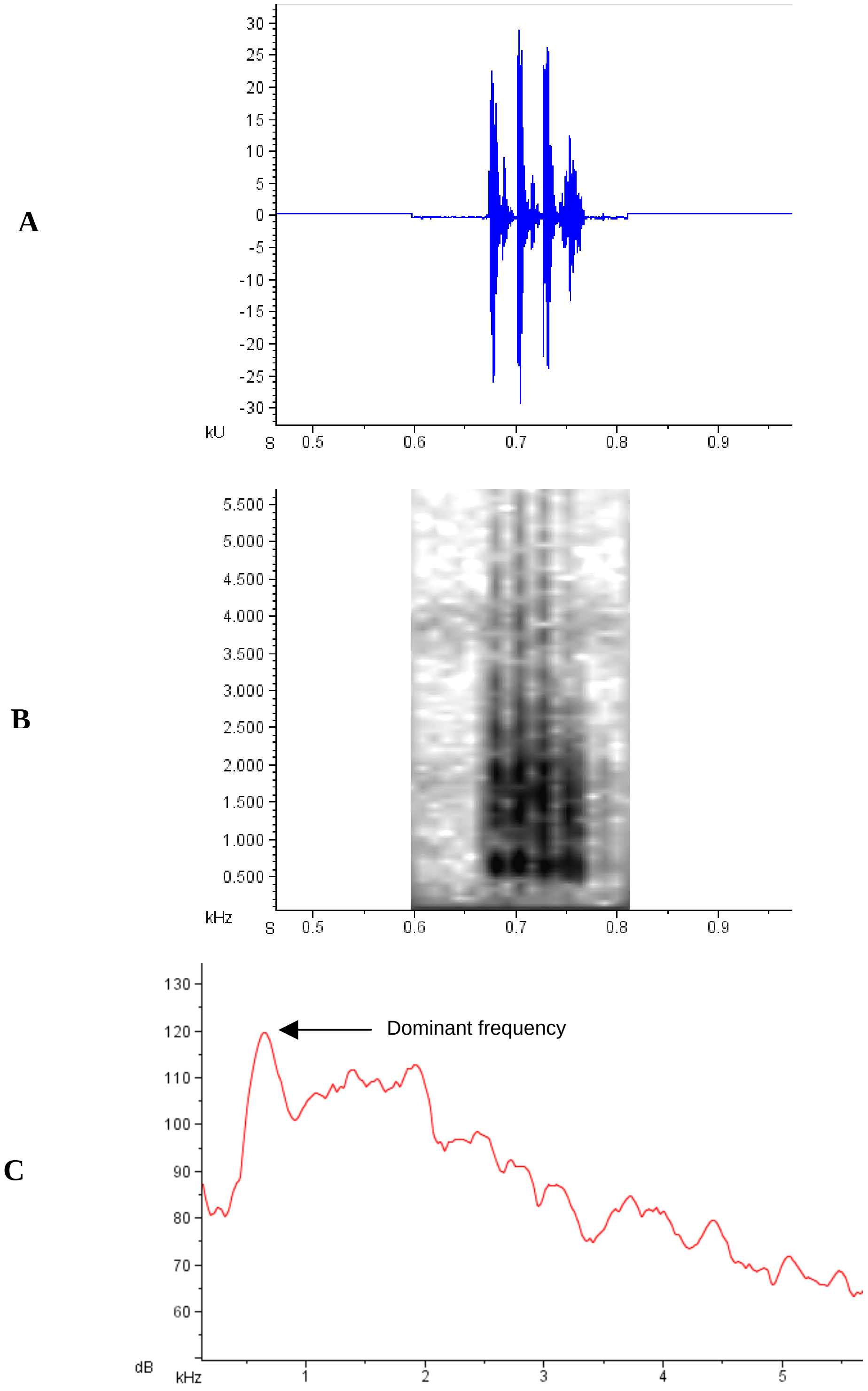


Figure 3. Waveform (A), spectrogram (B), and power spectrum (C) of a type III call from a male *Rana okaloosae* in Okaloosa County, Florida.

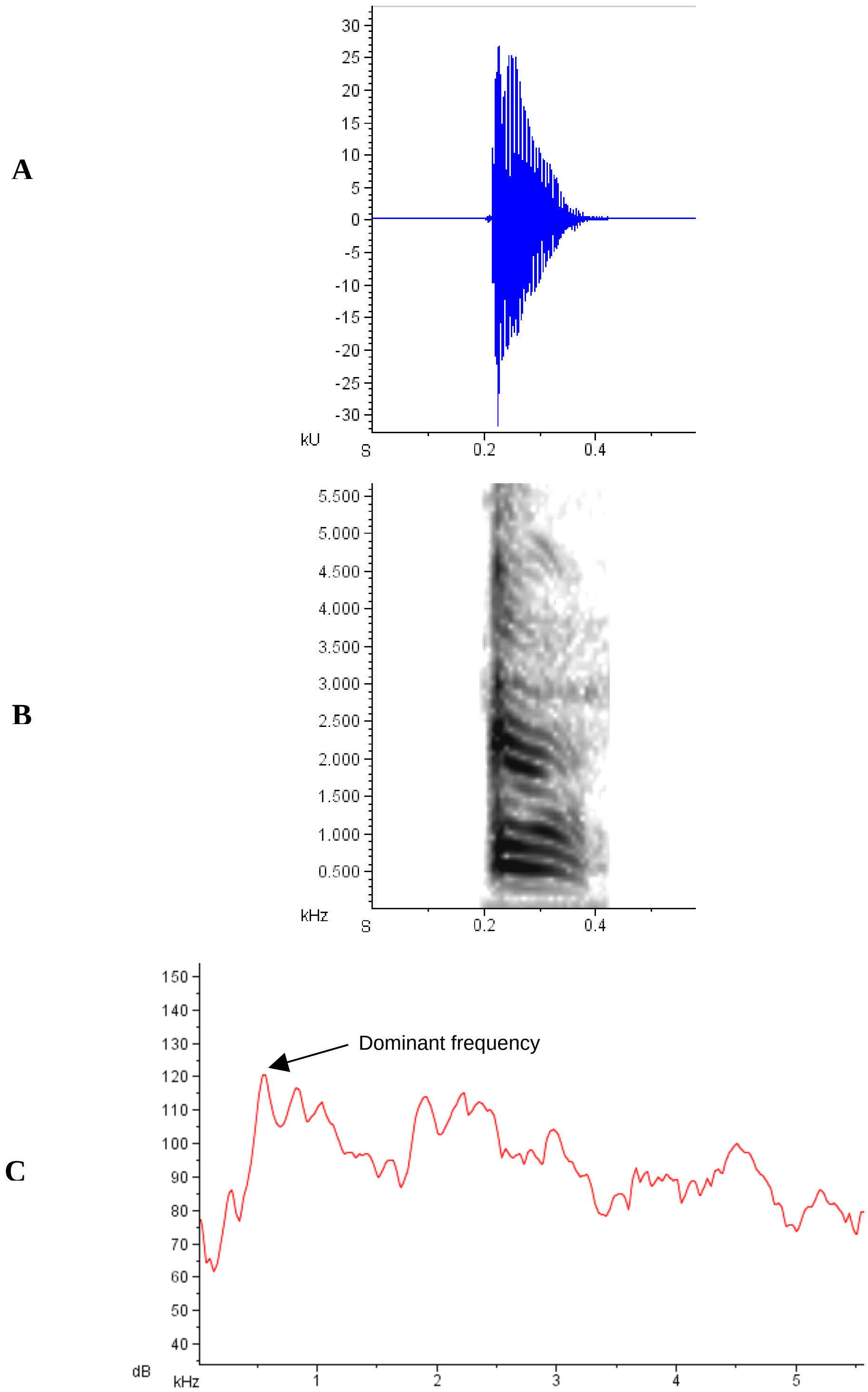


Figure 4. Waveform (A), spectrogram (B), and power spectrum (C) of a single type I advertisement call from a male *Rana clamitans* in Okaloosa County, Florida.

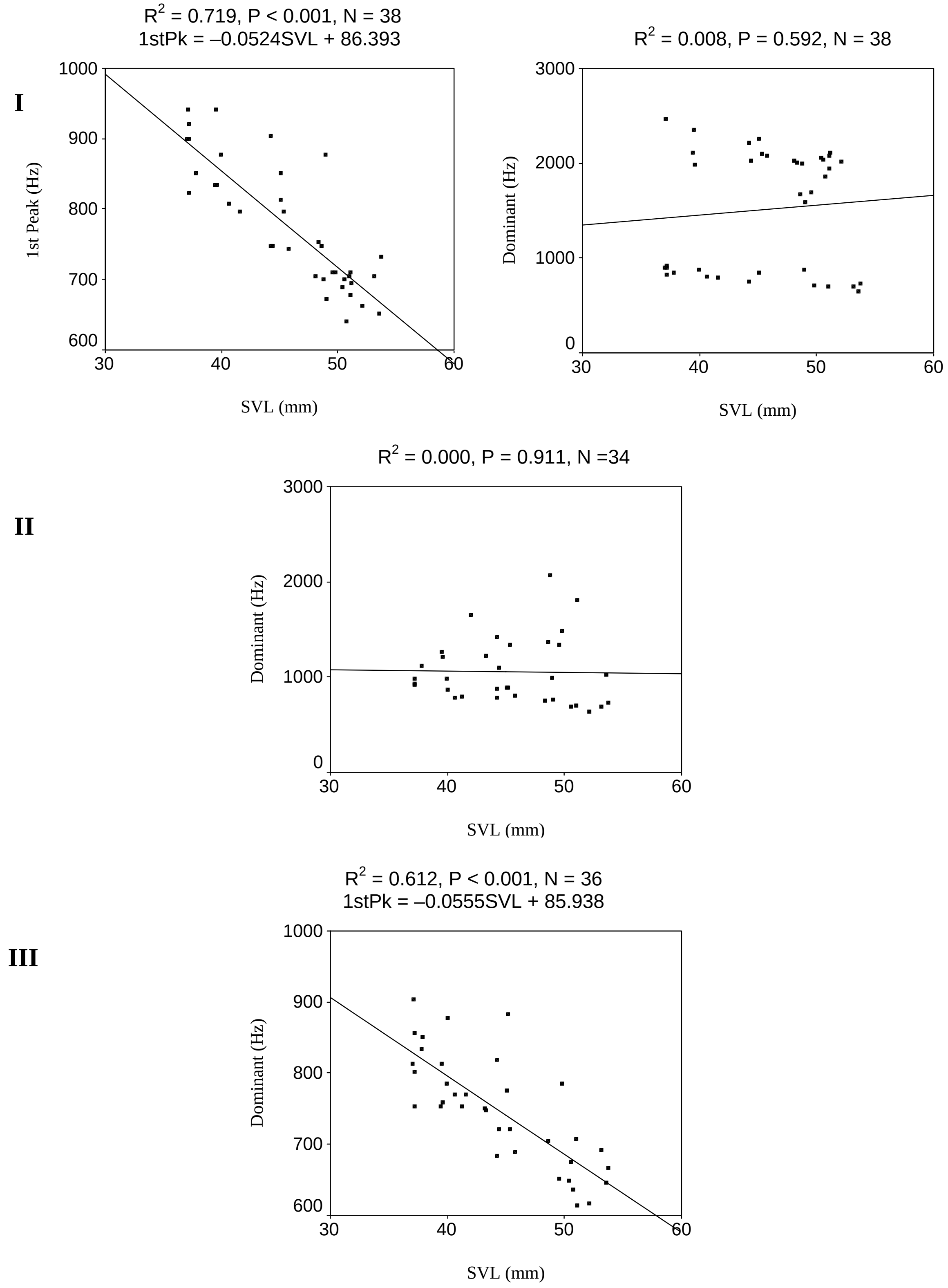


Figure 5. Relationship between snout–vent length (SVL) and dominant frequency or the frequency of the first spectral peak for type I, type II, and type III calls of *Rana okaloosae*.

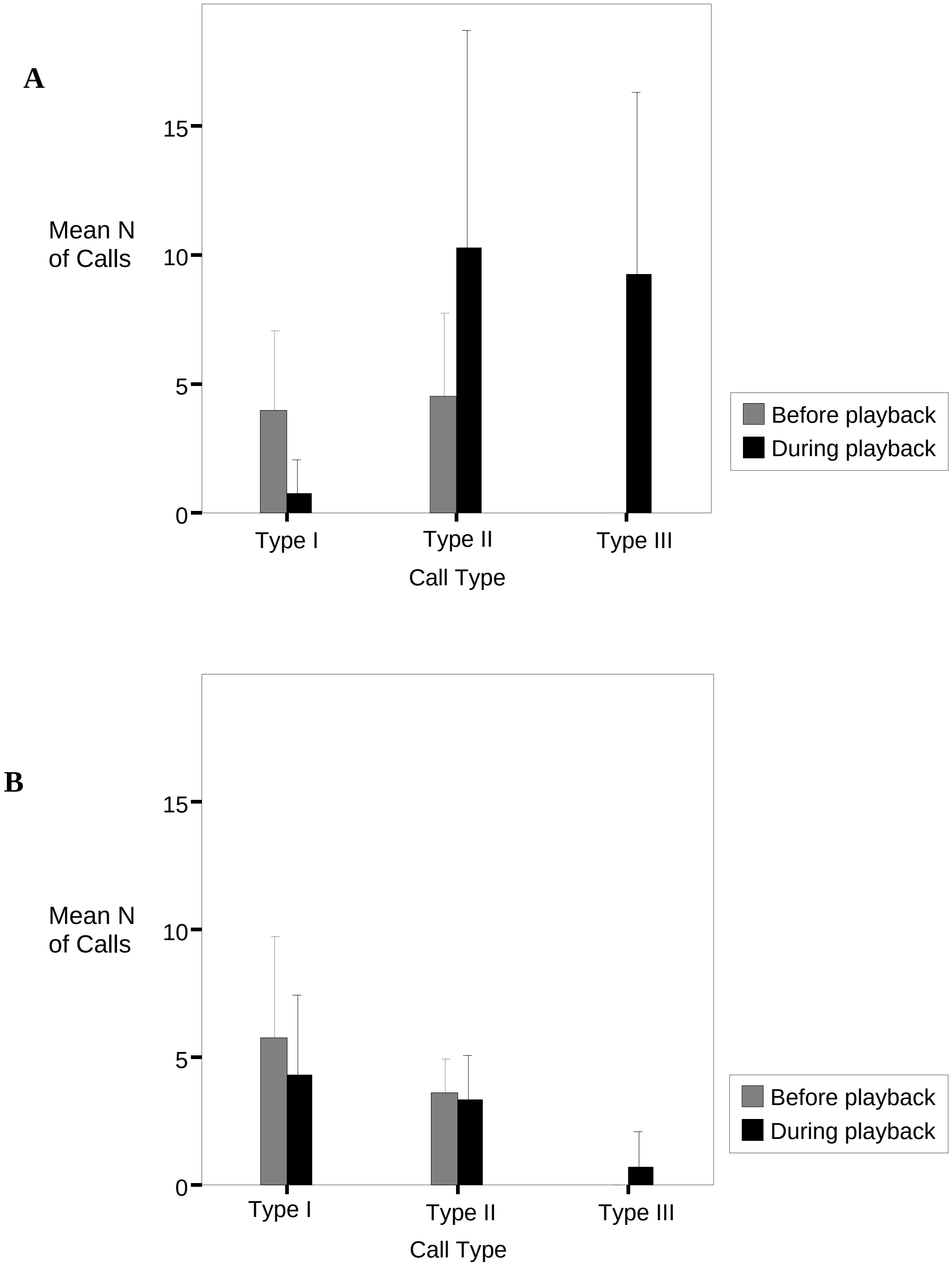


Figure 6. Mean number (+SD bars) of type I advertisement and type II chuck calls by *Rana okaloosae* males before and during playback of conspecific (A) and heterospecific (B) calls. Bog frog males were recorded for 10 min prior to playback and 10 min during playback. During playback, males were presented with calls from a conspecific (N = 20) or heterospecific (*R. clamitans*, N = 11) male.

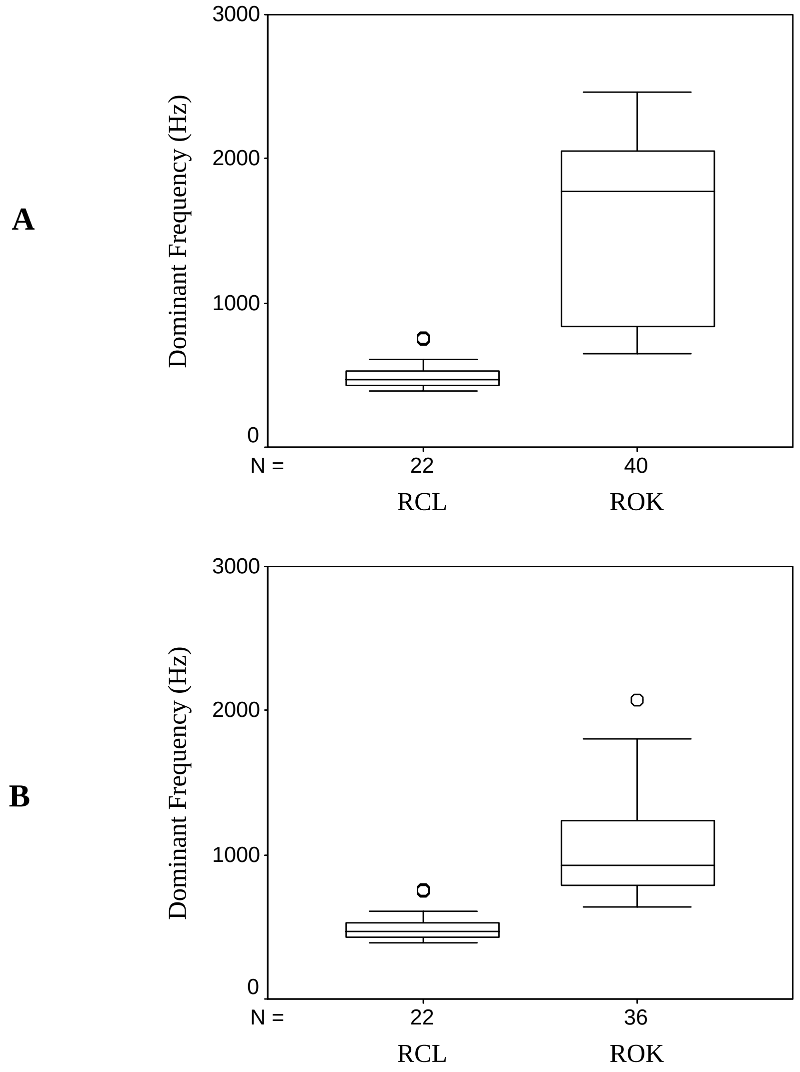


Figure 7. Comparison of dominant frequencies between calls from *Rana clamitans* (RCL) and *R. okaloosae* (ROK) males. In A, type I calls from both species are compared. In B, type II calls from ROK are compared to type I calls for RCL. Box plots display interquartile range. The middle line is the median. Outliers are identified by circles.

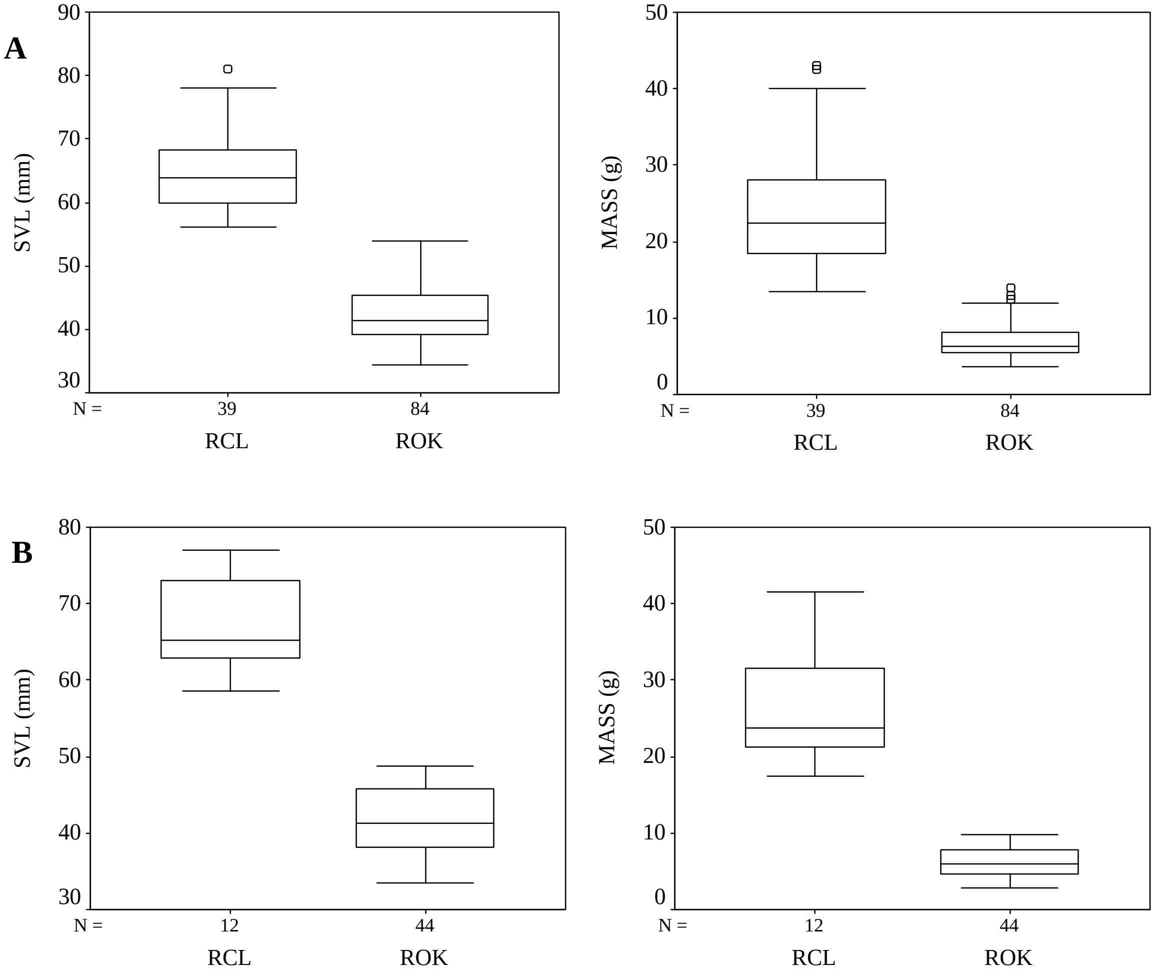


Figure 8. Comparison of body size between adult *Rana clamitans* (RCL) and *R. okaloosae* (ROK) males (A) and females (B). Box plots display interquartile range. The middle line is the median. Outliers are identified by circles.

CHAPTER 4. INDIVIDUAL VOICE RECOGNITON IN FLORIDA BOG FROGS

ABSTRACT.—I analyzed the spectral and temporal variation of advertisement calls of Florida bog frogs (*Rana okaloosae*). I used discriminant analysis with cross–validation to evaluate whether individual males could be recognized by the temporal and spectral properties of their vocalizations. Discriminant analysis classified 76% of all calls to the correct individual when all 20 individuals were included in the dataset. Classification success increased when fewer individuals were included. I evaluated whether individual voice recognition could be used to estimate the number of actively calling males in previously unrecorded populations using cluster analysis. There was a high misclassification rate for calls using cluster analysis; therefore, it is unlikely that individual voice recognition can be used to differentiate among individuals or estimate population size if prior classification criteria are unavailable.

INTRODUCTION

Acoustic signals convey information to competitors and potential mates. They are used among males to convey body size, fighting ability, and territorial ownership and provide a fitness–related measure of mate quality to females (see Ch. 3). Not surprisingly, many species can identify individuals by their unique acoustic signals (Brooks and Falls 1975, Myrberg and Riggio 1985, Davis 1987, Bee and Gerhardt 2002), an ability that serves numerous functions including parent–offspring recognition in colonial breeding species (Charrier et al. 2003) and differentiation between territorial neighbors and intruding conspecifics (i.e., ‘dear enemy’ effect; reviewed in Temeles 1994).

By analyzing the temporal and spectral properties of vocalizations or other acoustic signals, investigators have succeeded in identifying individuals in species of birds (Weary et al. 1990, Jones and Smith 1997, Galeotti and Sacchi 2001, Christie et al. 2004), mammals (Burnett et al. 2001, Frommolt et al. 2003, Sousa–Lima et al. 2003, Ceugniet and Izumi 2004), and frogs (Bee and Gerhardt 2001a, Bee et al. 2001, Bee 2004). Most of these studies focused on the development, function, and evolution of unique acoustic signals. However, a few researchers suggest that unique individual acoustic signals could be used to monitor populations of rare or hard to capture species (Saunders and Wooler 1988, Jones and Smith 1997, Burnett et al. 2001, Peake and McGregor 2001).

I investigated variation in advertisement calls among male Florida bog frogs (*Rana okaloosae*) and evaluated whether individuals could be identified by their vocalizations. Because *R. okaloosae* is a prolonged breeder that may be territorial (Ch. 2), calls throughout the breeding season to attract females (Ch. 1), responds vocally to the calls of unfamiliar conspecifics (Ch. 3), and is related to other ranids that have unique vocal signatures (Bee and Gerhardt 2001a, Bee et al. 2001), I hypothesized that advertisement calls would be unique to individuals. The purpose of this study was to test whether individual differences in advertisement calls exist in bog frogs that might provide an acoustic foundation on which future behavioral experiments could be based. In addition, I investigated the potential for using individual voice recognition to estimate population size.

METHODS

Call analyses.—I recorded advertisement calls (type I, Ch. 3) from 20 *Rana okaloosae* males between April and July in 2003 and 2004 at multiple locations in Okaloosa and Santa Rosa County, Florida (see chapter 3 for a description of recording techniques). I selected the first 6 advertisement calls from each male that were recorded during a single calling bout. I measured the duration of each call and the number of pulses on the waveform (i.e., oscillogram), from which I calculated a pulse rate (pulses/s) (Figure 1). I created spectrograms and power spectra for each call (Hann window = 100 ms / 4410 samples, DFT size = 8192, 3 dB bandwidth = 14.4 Hz) and recorded the dominant frequency (DF); i.e., the harmonic with the greatest power. I also measured the frequency of the first peak in the power spectrum. I made all temporal and spectral measurements using Raven™ 1.2 software (Cornell Lab of Ornithology, Ithaca, NY). Because all recordings were made with frogs in similar water temperatures (range: 19.9–22.9 °C) and data are limited on how water temperature affects call properties (Ch. 3), no adjustments were made to call variables to standardize for temperature differences.

Statistical analyses.—I calculated a coefficient of variation (CV = [SD/Mean] x 100) within each individual and between individuals for the 5 call variables measured. For within–individual coefficients of variation (CV_w), I calculated a grand mean and standard deviation using the average of 6 calls for each individual. For between–male coefficient of variations (CV_b), I randomly selected a single call from each individual and calculated a mean and standard deviation for all 20 males. If $CV_b/CV_w > 1.0$ for any call variable, there is potential for individual discrimination because greater variation exists among individuals than within each individual (Robisson et al. 1993, Bee et al. 2001). I also plotted the mean and 95% confidence intervals of each call variable to evaluate whether individuals could be identified qualitatively using a single measurement; i.e., do call variables overlap among individuals?

I used discriminant analysis (DA) to evaluate whether individuals could be differentiated using the 5 call variables I measured. Discriminant analysis is a classification technique that attempts to extract dominant gradients of variation by condensing multivariate data into a smaller set of new variables (i.e., canonical functions) that are linear combinations of the original data (McGarigal et al. 2000). I used stepwise DA with the Wilk’s Lambda statistic to enter and remove variables and cross–validation to calculate classification accuracy. In cross–validation, each call of an individual is classified using the discriminant functions created by the n–1 remaining calls. In addition to conducting a DA with all 20 males, I randomly selected 5, 10, and 15 males from the dataset and calculated an average classification success of 10 trials for each subset.

Lastly, to evaluate the potential for using individual vocal recognition to estimate population size, I performed a cluster analysis (CA) using the same data and variables included in the discriminant analysis. Cluster analysis is a classification technique, like DA, that attempts to group similar cases (i.e., individuals) by similar properties. However, unlike DA, group membership is unknown beforehand in CA and there is no prior training data set used to develop classification criteria (McGarigal et al. 2000). I performed a hierarchical CA on the complete dataset (i.e., 6 calls for 20 males) and on smaller subsets consisting of 5, 10, or 15 randomly selected males. I tried several cluster methods, including average linkage, furthest neighbor, nearest neighbor, and Ward’s minimum variance linkage and constructed dendrograms to look at classification success.

I qualitatively assessed classification success by looking at clusters in dendrograms. I also quantified the success of CA at identifying individuals by setting the number of clusters to be formed equal to the number of different frogs included in the dataset. I then calculated the percentage of clusters that were correct (i.e., a cluster contained all 6 calls from the same individual and no calls from other individuals). For example, for the subsets of 15 males, I set the number of clusters to be formed at 15. If 3 correct clusters were formed, the classification success was 20%.

RESULTS

There were few significant correlations between the 5 selected call measurements (Table 1). Variation within males (CV_w) was less than variation between males (CV_b) for all call variables except DF (Table 2). Variation in DF was high because bog frog advertisement calls typically have two frequency peaks that are close in amplitude (Figure 1). As mentioned in Ch. 3, the peak that is dominant differs among individuals and among calls for the same individual. Qualitative differentiation of all 20 frogs was not possible using a single call variable because there was substantial overlap among individuals (Figure 2).

Five canonical functions were extracted in the DA (Table 3). The first 4 functions had eigenvalues > 1 and accounted for 98.5% of the variance. Discriminant analysis classified 75.8% of the calls to the correct individual when all 20 males were analyzed, a rate 15.2 times greater than expected by chance (5%). Mean classification success increased to 78.1% (range: 73.3–85.65%, N = 10) for subsets of 15 males, 81.1% (range: 71.7–90.0%, N = 10) for 10 males, and 87% (range: 80.0–93.3%, N = 10) for 5 males

(Figure 3). Despite the high variation in DF within individuals, it still was included in the stepwise DA for 10, 15, and 20 males but was not always included in the final canonical functions for groups of 5 males.

Classification success using cluster analysis was highly variable among individuals (Figure 4). The different clustering methods varied slightly, but no method was superior at classifying individuals correctly. No correct clusters formed when all 20 individuals were included in the dataset. The number of correct clusters averaged 2.0 ± 3.2% (range: 0–6.2%, N = 10) for 15 individuals, 6.0 ± 7.0% (range: 0–20.0%, N = 10) for 10 individuals, and 16.0 ± 18.4% (range: 0–40.0%, N = 10) for 5 individuals. Clusters regularly grouped calls from multiple individuals, regardless of the number of males in the dataset, a result suggesting cluster analysis has limited applications for identifying individual *Rana okaloosae* using the call variables measured in this study.

DISCUSSION

If an acoustic signal is to be useful to identify individuals, variability among individuals should be high and individual variation should be low (Falls 1982). Discriminant analysis of bog frog advertisement calls included both temporal and spectral measurements, with the frequency of the first peak in the power spectrum being the most useful characteristic for differentiating individuals. To my knowledge, only a few studies have verified that frogs can be identified statistically through vocal analysis (Bee et al. 2001, Bee and Gerhardt 2001a, Bee 2004); however, others have reported greater variation among males than within males (e.g., Shy 1985, Howard and Young 1998), a result that suggests individual discrimination likely is possible in many other species.

It is unknown whether *Rana okaloosae* males will demonstrate a ‘dear enemy’ response to the calls of conspecifics, responding more aggressively to the calls of unfamiliar males than established neighboring males (Temeles 1994). This behavior pattern has been observed in a few anurans (Davis 1987, Bourne et al. 2001, Bee and Gerhardt 2001b, 2002). Because male bog frogs typically use the same calling location for several sequential nights during the breeding season and remain in the breeding area for several months (Chs. 1, 2), they likely become familiar with the spatial positions and vocalizations of neighbors, two factors that may be important in neighbor recognition (Davis 1987, Bee and Gerhardt 2001b, 2002).

The results of my study provide an acoustic foundation on which future behavioral studies investigating the social system of *R. okaloosae* could be based. Bog frogs react to the playback of calls from unfamiliar conspecifics by increasing the frequency of chuck (type II) calls and reducing the frequency of advertisement (type I) calls (Ch. 3). Their response to calls from familiar males (i.e., neighbors) has never been tested. Because similar playback experiments on individual recognition have been done already using *R. clamitans* (Bee et al. 1999, 2000, 2001), the closest relative of the Florida bog frog (Austin et al. 2003), and *R. catesbeiana* (Davis 1987, Bee and Gerhardt 2001a,b, 2002, Bee 2004), evolutionary comparisons could be made among other members of the genus that differ in breeding systems and habitats. For example, do explosive breeding species, such as the wood frog (*Rana sylvatica*) have calls that are unique to individuals?

As expected, classification success rate increased when fewer individuals were included in the DA. The number of males needed to be identified by call classification varies depending on the situation or application. I captured an average of 12 males per night in 2002 (range: 5–15) and 7 males (range: 2–10) in 2003 in June and July at the Live Oak Creek (LOC) study site (Chs. 1, 2). This site had a greater density of bog frogs than most locations; thus, it is unlikely that bog frogs would have to identify more than 15 active males on any given night. However, there were 29, 20, and 27 different males captured at the same location in 2002, 2003, and 2004, respectively. If the application is to evaluate the ability to use vocal identification to estimate population size, more frogs would need to be included and classification accuracy would decrease.

I used calls recorded from a single calling bout during the same night. Because acoustic signals may change temporally (Weary et al. 1990, Puglisi and Adamo 2004) and depend on social context (Howard and Young 1998, Given 1999, Bee et al. 1999, 2000), recordings over several nights should be compared to see how acoustic properties change temporally or in different social contexts. Although my study did not investigate temporal variation in calls, the ability to distinguish individuals vocally within a single night still may have biological significance. Bee and Gerhardt (2001b, 2002) found that bull frogs (*R. catesbeiana*) habituated to calls within a single night, after which males could differentiate between calls of familiar and unfamiliar individuals. Because bog frogs occasionally switch calling sites throughout the breeding season (Ch. 2), males may have to re–learn the spatial distribution and vocalizations of different neighbors on each night.

My ability to accurately estimate the number of different bog frogs in a sample using voice recognition was limited. Cluster analysis did not reliably group calls from the same individuals, although it was successful for some. Thus, it is unlikely that population size can be estimated using vocal mark–recapture methods, at least using the call variables I measured. Although DA did fairly well at identifying individuals by call, the applications of using this classification technique are limited to previously recorded populations. Even if a location that contains bog frogs has been recorded, any new individuals appearing on subsequent nights would be misclassified by DA because they would not be included in the original training dataset. Burnett et al. (2001) found similar problems in their study of bat acoustic signals. A better and easier approach to monitor populations of bog frogs may be to look at the relationship between relative calling activity (e.g., calls per min) at a location with reproduction, population size, and environmental conditions (e.g., Nelson and Graves 2004, Stevens and Paszkowski 2004).

LITERATURE CITED

Austin, J. D., S. C. Lougheed, P. E. Moler, and P. T. Boag. 2003. Phylogenetics, zoogeography, and the role of dispersal and vicariance in the evolution of the *Rana catesbeiana* (Anura: Ranidae) species group. Biological Journal of the Linnean Society 80: 601–624.

Bee, M. A. 2004. Within–individual variation in bullfrog vocalizations: implications for a vocally mediated social recognition system. Journal of the Acoustical Society of America 116:3770–3781.

Bee, M. A., and H. C. Gerhardt. 2001a. Neighbor–stranger discrimination by territorial male bullfrogs (*Rana catesbeiana*): I. Acoustic basis. Animal Behaviour 62:1129–1140.

Bee, M. A., and H. C. Gerhardt. 2001b. Neighbor–stranger discrimination by territorial male bullfrogs (*Rana catesbeiana*): II. perceptual basis. Animal Behaviour 62:1129–1140.

Bee, M. A., and H. C. Gerhardt. 2002. Individual voice recognition in a territorial frog (*Rana catesbeiana*). Proceedings of the Royal Society of London, Series B 269:1443–1448.

Bee, M. A., S. A. Perrill, and P. C. Owen. 1999. Size assessment in simulated territorial encounters between male green frogs (*Rana clamitans*). Behavioral Ecology and Sociobiology 45:177–184.

Bee, M. A., S. A. Perrill, and P. C. Owen. 2000. Male green frogs lower the pitch of acoustic signals in defense of territories: a possible dishonest signal of size? Behavioral Ecology 11:169–177.

Bee, M. A., C. E. Kozich, K. J. Blackwell, and H. C. Gerhardt. 2001. Individual variation in advertisement calls of territorial male green frogs, *Rana clamitans*: implications for individual discrimination. Ethology 107:65–84.

Bourne, G. R., A. C. Collins, A. M. Holder, and C. L. McCarthy. 2001. Vocal communication and reproductive behavior of the frog *Colostethus beebei* in Guyana. Journal of Herpetology 35:272–281

Brooks, R. J., and J. B. Falls. 1975. Individual recognition by song in white–throated sparrows. III. Song features used in individual recognition. Canadian Journal of Zoology 53:1749–1761.

Burnett, S. C., K. A. Kazial, and W. M. Masters. 2001. Discriminating individual big brown bat (*Eptesicus fuscus*) sonar vocalizations in different recording situations. Bioacoustics 11:189–210.

Ceugniet, M., and A. Izumi. 2004. Vocal individual discrimination in Japanese monkeys. Primates 45:119–128.

Charrier, I., N. Mathevon, and P. Jouventin. 2003. Vocal signature recognition of mothers by fur seal pups. Animal Behaviour 65:543–550.

Christie, P. J., D. J. Mennill, and L. M. Ratcliffe. 2004. Chickadee song structure is individually distinctive over long broadcast distances. Behaviour 141:101–124.

Davis, M. S. 1987. Acoustically mediated neighbor recognition in the North American bullfrog, *Rana catesbeiana*. Behavioral Ecology and Sociobiology 21:185–190.

Falls, J. B. 1982. Individual recognition by sound in birds. Pages 237–273 *in* D. E. Kroodsma and E. H. Miller, editors. Acoustic Communication in Birds, Vol. 2. Academic Press, New York, USA.

Frommolt, K. H., M. E. Goltsman, and D. W. Macdonald. 2003. Barking foxes, *Alopex lagopus*: field experiments in individual recognition in a territorial mammal. Animal Behaviour 65:509–518.

Galeotti, P., and R. Sacchi. 2001. Turnover of territorial Scops owls *Otus scops* as estimated by spectrographic analyses of male hoots. Journal of Avian Biology 32:256–262.

Given, M. F. 1999. Frequency alteration of the advertisement call in the carpenter frog, *Rana virgatipes*. Herpetologica 55:304–317.

Howard, R. D., and J. R. Young. 1998. Individual variation in male vocal traits and female mating preferences in *Bufo americanus*. Animal Behaviour 55:1165–1179.

Jones, D. N., and G. C. Smith. 1997. Vocalisations of the marbled frogmouth II: an assessment of vocal individuality as a potential census technique. Emu 97:296–304.

McGarigal, K., S. Cushman, and S. Stafford. 2000. Multivariate Statistics for Wildlife and Ecology Research. Springer–Verlag. New York, New York, USA.

Myrberg, A. A., and R. J. Riggio. 1985. Acoustically mediated individual recognition by a coral reef fish (*Pomacentrus partitus*). Animal Behaviour 33:411–416.

Nelson, G. L., and B. M. Graves. 2004. Anuran population monitoring: comparison of the North American Amphibian Monitoring Program’s calling index with mark–recapture estimates for *Rana clamitans*. Journal of Herpetology 38:355–359

Peake, T. M., and P. K. McGregor. 2001. Corncrake *Crex crex* census estimates: a conservation application of vocal individuality. Animal Biodiversity and Conservation 24:81–90.

Puglisi, L., and C. Adamo. 2004. Discrimination of individual voices in male great bitterns (*Botaurus stellaris*) in Italy. The Auk 121:541–547.

Robisson, P., T. Aubin, and J. C. Bremond. 1993. Individuality in the voice of the emperor penguin *Aptenodytes fosteria*: adaptation to a noisy environment. Ethololgy 94:279–290.

Saunders, D. A., and R. D. Wooller. 1988. Consistent individuality of voice in birds as a management tool. Emu 88:25–32.

Shy, E. 1985. Individuality in the mating calls of the treefrog, *Hyla arborea savignyi*. Amphibia–Reptilia 6:343–353.

Sousa–Lima, R. S., A. P. Paglia, and G. A. B. Da Fonseca. 2002. Signature information and individual recognition in the isolation calls of Amazonian manatees, *Trichechus inunguis* (Mammalia: Sirenia). Animal Behaviour 63:301–310.

Stevens, C. E., and C. A. Paszkowski. 2004. Using chorus–size ranks from call surveys to estimate reproductive activity of the wood frog (*Rana sylvatica*). Journal of Herpetology 8:404–410.

Temeles, E. J. 1994. The role of neighbours in territorial systems: when are they ‘dear enemies’? Animal Behaviour 47:339–350.

Weary, D. M., K. J. Norris, and J. B. Falls. 1990. Song features birds use to identify individuals. The Auk 107:623–625.

Table 1. Correlations among call variables and snout–vent length (SVL). Spectral and temporal measurements were averaged for 6 advertisement (type I) calls from 20 *Rana okaloosae* males.

		SVL	Dominant frequency	1st peak frequency	Pulse rate	N pulses	Call length
Call length	r	0.226	–0.081	0.010	0.253	0.944*	1
	P	0.367	0.735	0.967	0.283	0.000	.
N pulses	r	0.299	–0.015	–0.004	–0.063	1	
	P	0.228	0.948	0.988	0.792	.	
Pulse rate	r	–0.139	–0.044	–0.064	1		
	P	0.581	0.854	0.788	.		
1st peak frequency	r	–0.874*	–0.094	1			
	P	0.000	0.694	.			
Dominant frequency	r	0.100	1				
	P	0.692	.				
SVL	r	1					
	P	.					

*P < 0.01

Table 2. Mean, range, and variability within (CV_w) and between (CV_b) individuals for call variables. Spectral and temporal measurements were made for 6 advertisement calls from 20 *Rana okaloosae* males.

Call Property	Grand Mean ± SD	Range	Mean CV_w	CV_w Range	Mean CV_b	CV_b/CV_w
Call length (s)	1.48 ± 0.62	0.49–2.69	39.67%	5.34–80.00%	53.46%	1.35
N pulses	7.7 ± 3.3	2.8–13.5	35.90%	5.56–72.86%	53.22%	1.48
Pulse rate (pulses/sec)	0.19 ± 0.02	0.15–0.23	4.50%	0.00–11.11%	10.55%	2.34
1st peak frequency (Hz)	788.9 ± 90.3	658.9–944.8	2.98%	1.01–5.12%	11.86%	3.98
Dominant frequency (Hz)	1461.7 ± 584.6	112.5–2485.3	48.37%	1.01–479.45%	44.65%	0.92

Table 3. Factor loading of each call variable on the canonical functions for 20 *Rana okaloosae* males. A discriminant analysis was performed classifying 6 advertisement calls for each male.

	Function				
	1	2	3	4	5
Call length	0.413*	0.863*	0.204	0.001	–0.207
N pulses	–0.118	0.066	0.129	0.964*	0.190
Pulse rate	–0.038	0.025	0.100	0.954*	0.278
1st peak frequency	0.395	–0.274	0.408	0.759*	0.164
Dominant frequency	–0.050	0.016	0.678	–0.246	0.689*

*Largest correlation between variable and any discriminant function

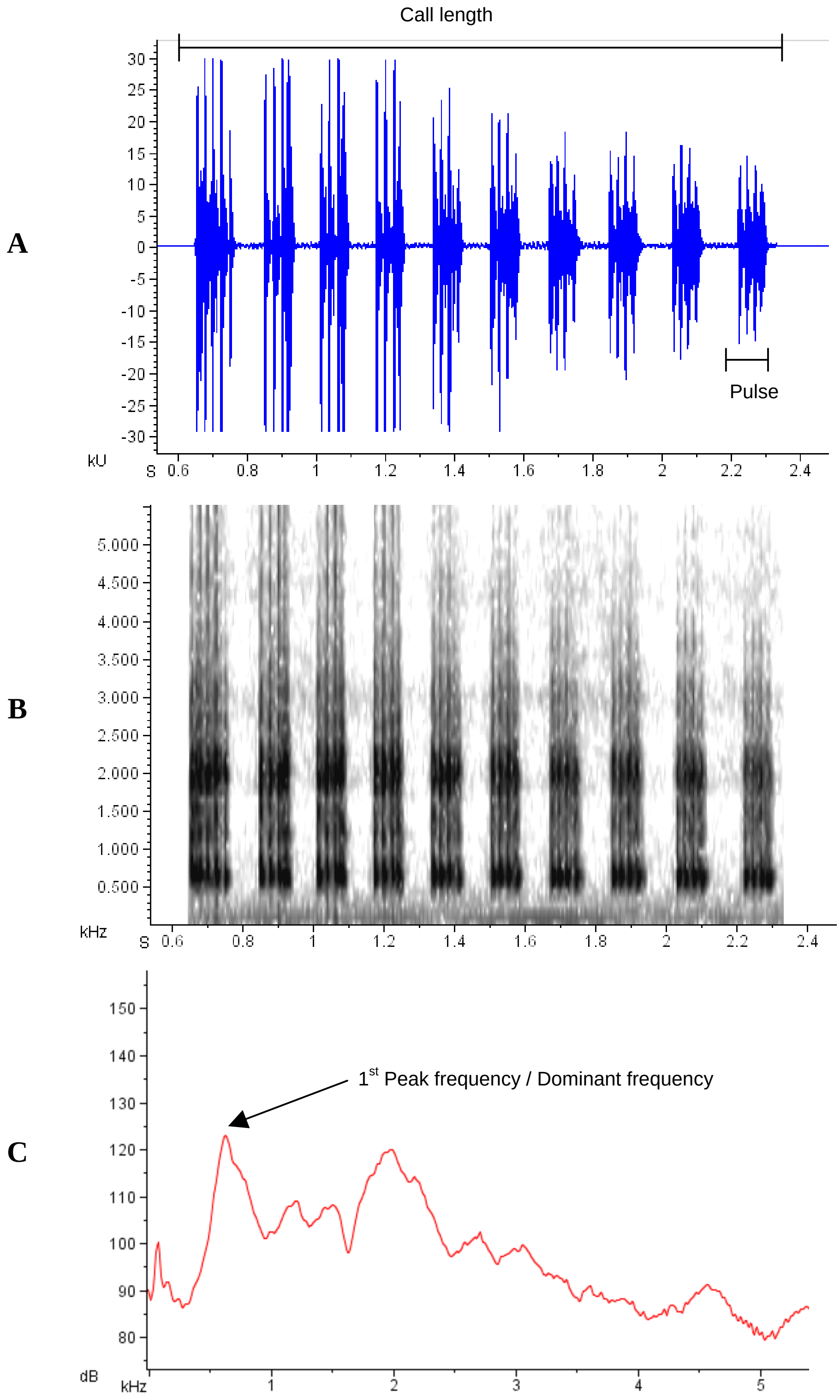
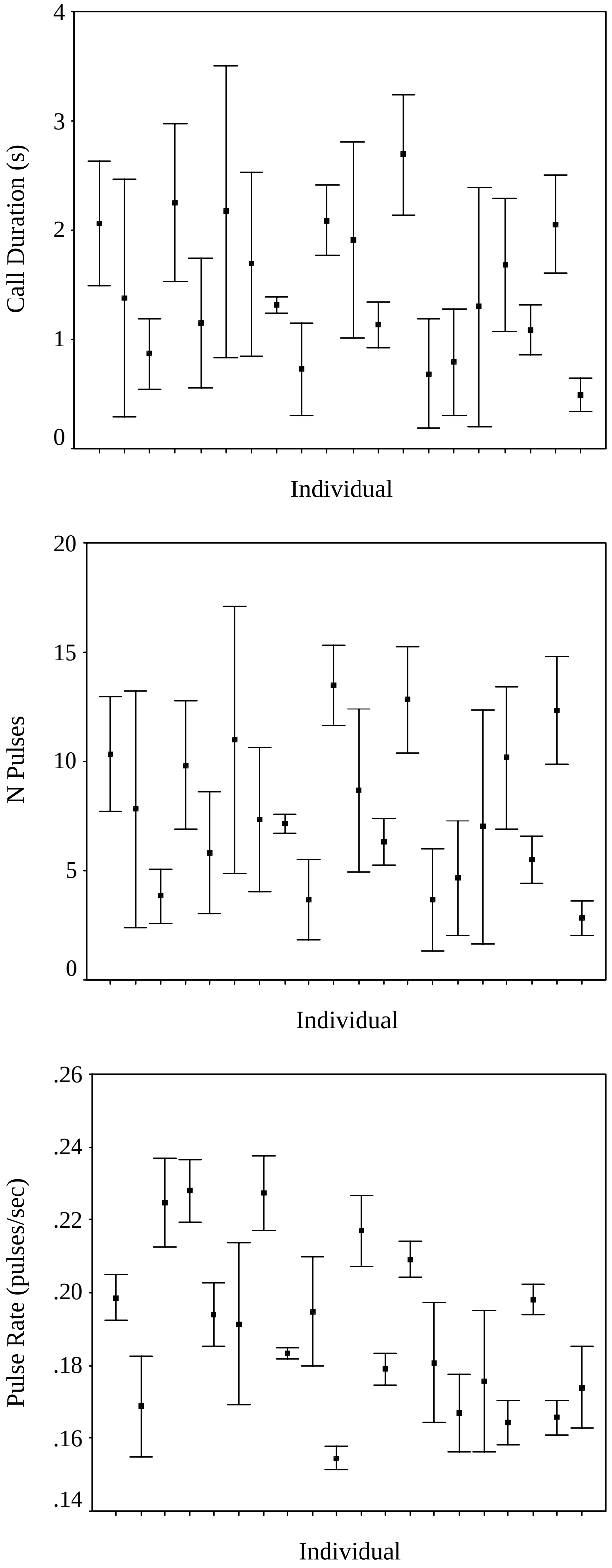


Figure 1. Waveform (A), spectrogram (B), and power spectrum (C) of a single advertisement call from a male *Rana okaloosae* in Okaloosa County, Florida.

Figure 2



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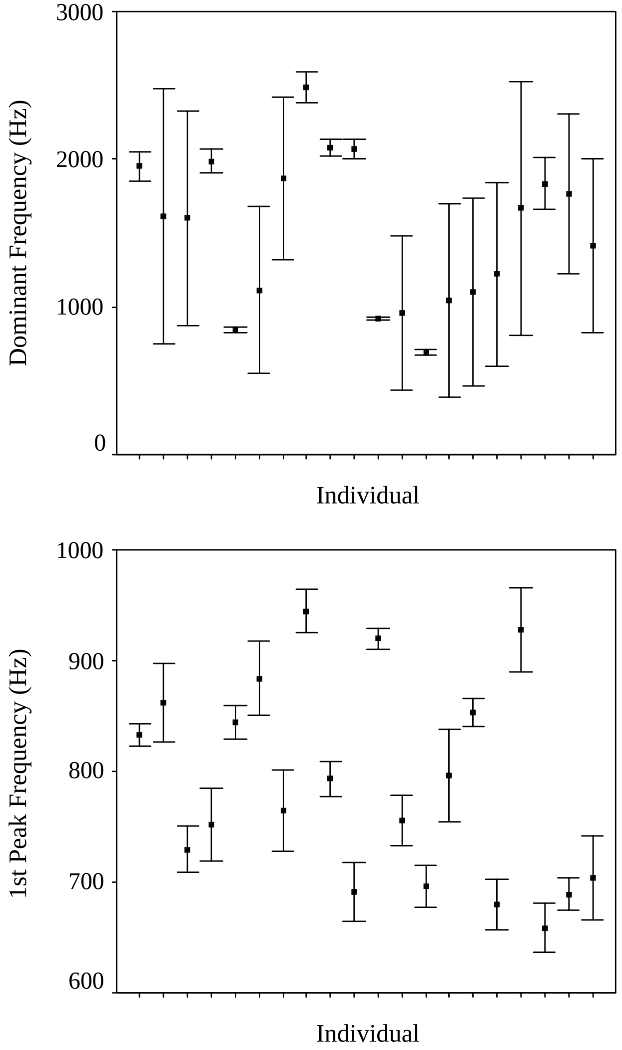


Figure 2. Overlap of call variables among individuals. Figures display the mean and 95% confidence intervals calculated from 6 advertisement calls from 20 *Rana okaloosae* males. Individual discrimination is highest for acoustic measurements containing tight confidence intervals within males and limited overlap among males.

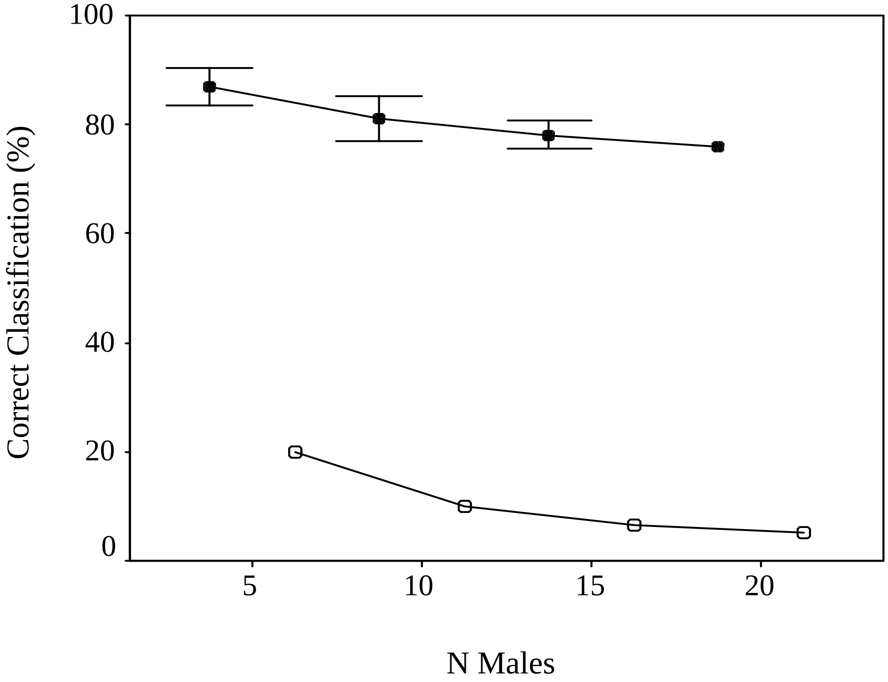
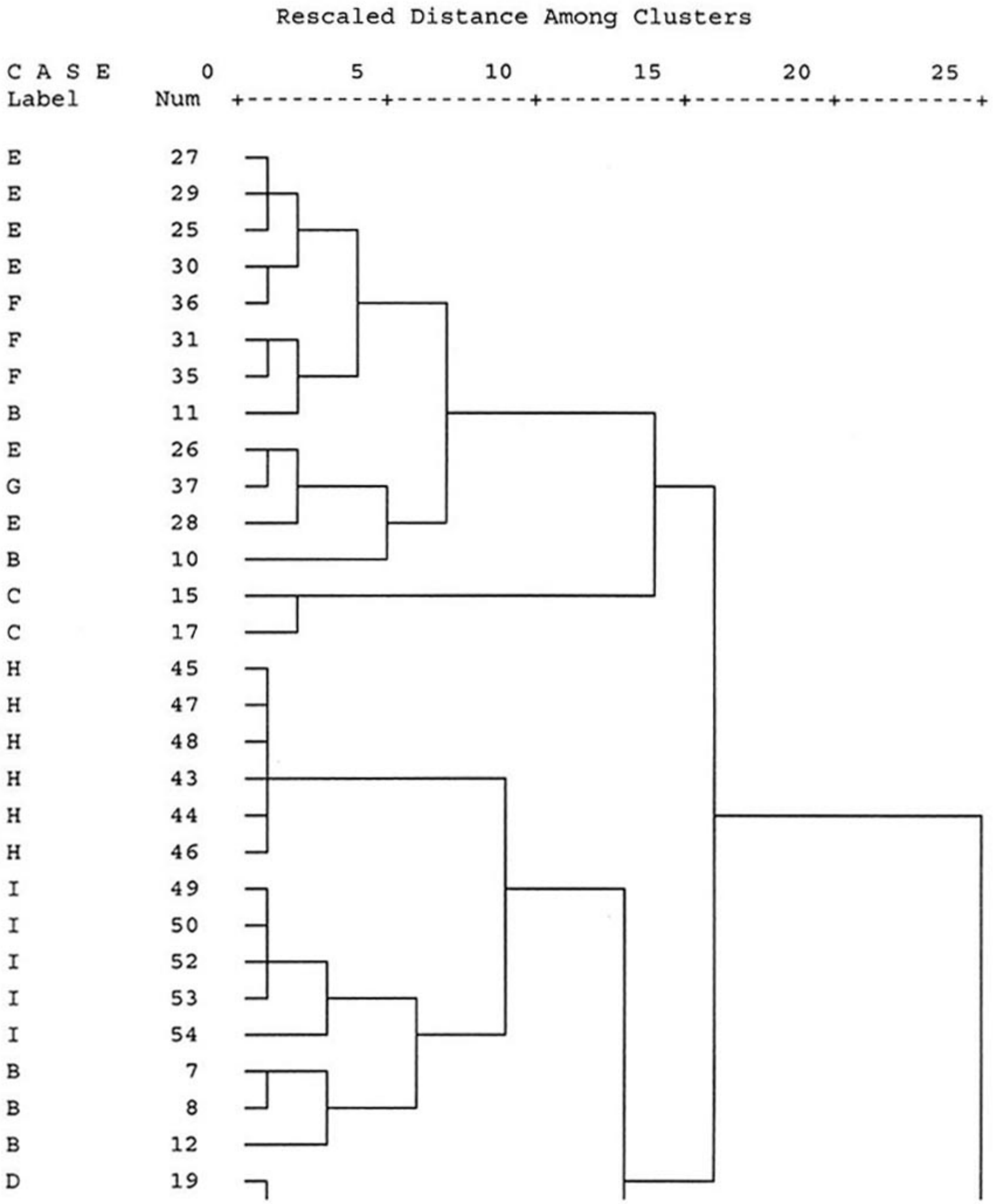


Figure 3. Percent of *Rana okaloosae* males classified correctly (closed circles) by discriminant analysis when 5, 10, 15, and 20 individuals were included in the data set. Bars indicate the 95% confidence interval calculated from averaging 10 trials for 5, 10, and 15 males. The lower line (open circles) indicates the classification success expected due to chance.

Figure 4



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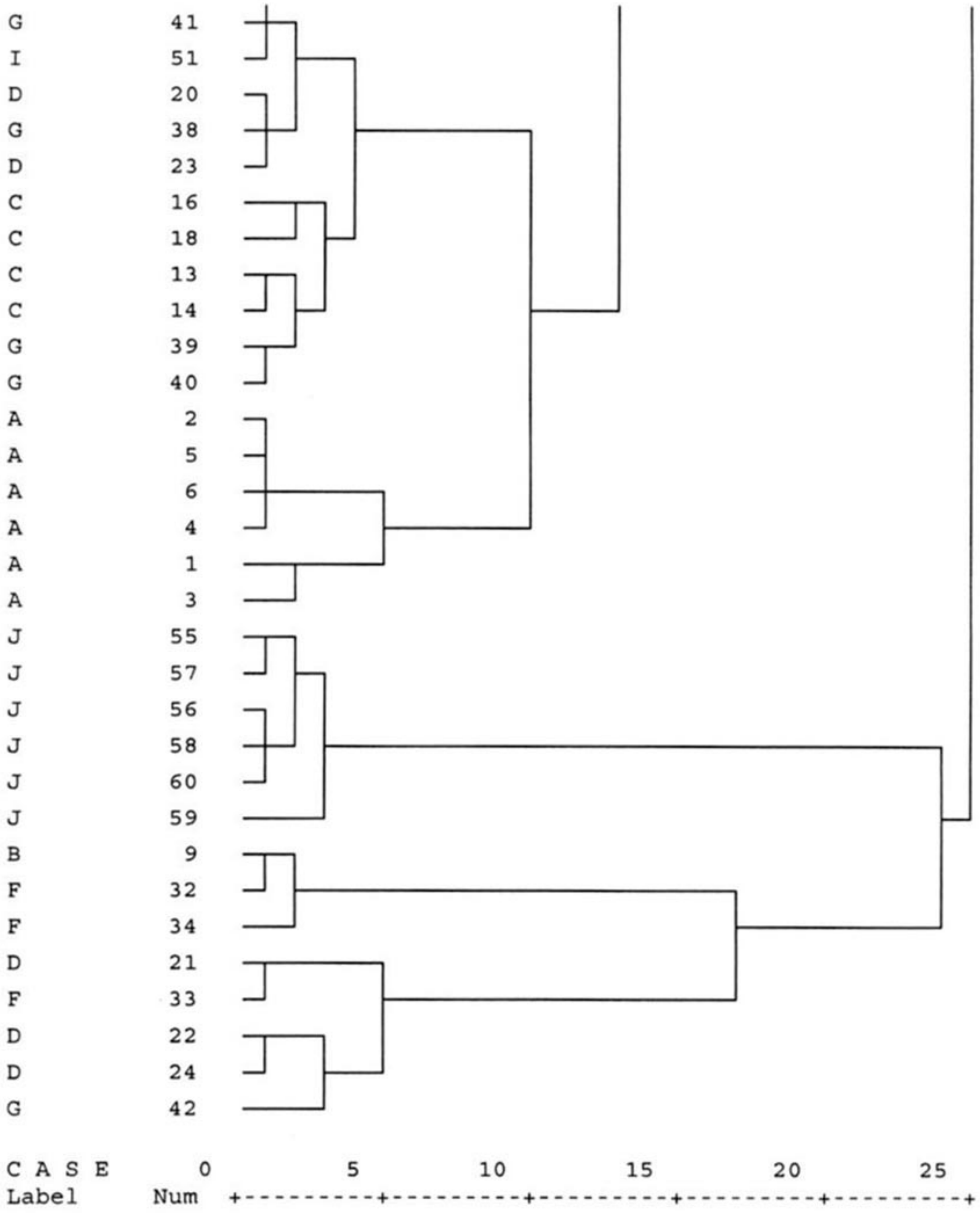


Figure 4. Example dendrogram resulting from cluster analysis of 6 calls from 10 *Rana okaloosae* males (labeled A–J). All 5 call variables were included in the dataset.

CHAPTER 5. RESPONSE OF *RANA OKALOOSAE, R. CLAMITANS* AND *R. SPHENOCEPHALA* TADPOLES TO CHEMICAL CUES OF SNAKE AND FISH PREDATORS

ABSTRACT.—I investigated the behavioral response of Florida bog frog (*Rana okaloosae*), bronze frog (*R. clamitans clamitans*), and leopard frog (*R. sphenocephala*) tadpoles to chemical cues from 2 predators, the banded water snake (Colubridae: *Nerodia fasciata*) and the red fin pickerel (Esocidae: *Esox americanus*). Bog and bronze frogs are close relatives that breed in permanent–water habitats. The leopard frog is a more distant relative to *R. okaloosae* and typically breeds in ephemeral habitats. Predation pressure differs between permanent and ephemeral habitats. Banded water snakes occur in the habitats of all tadpole species used in this study. Large pickerel, however, are common only in the deeper sections of the permanent streams inhabited by *R. okaloosae* and *R. clamitans* and typically are absent from the ephemeral breeding wetlands of *R. sphenocephala*. Bog frog tadpoles were the least active (i.e., less time swimming) ranid in all predator treatments. Despite the close relationship and similar habitats of bog and bronze frogs, *R. clamitans* was the most active species. *Rana sphenocepha* tadpoles swam less in predator treatments than the control, a result that contrasts previous research that suggested species that breed in ephemeral wetlands should respond less to predators.

INTRODUCTION

Predators may affect community structure and distribution patterns of prey (Petranka et al. 1987, Sih et al. 1992, Hero et al. 2001, Case et al. 2005), but the effect that predators have on the behavior and distribution of their prey is difficult to evaluate in the field. Investigations involving pairwise interactions between predators and prey in small–scale laboratory experiments may help explain observed distribution patterns and community structure (Azevedo–Ramos and Magnusson 1999, Relyea and Yurewicz 2002). Because many tadpole species can detect chemical cues of predators, they frequently are used in predation experiments (e.g., Petranka et al. 1987, Lefcort and Blaustein 1995, Flowers and Graves 1997).

Upon detection of a predator, tadpoles typically decrease their movement rates or shift microhabitats to minimize predation risk (Semlitsch and Reyer 1992, Kiesecker et al. 1996). The antipredator behavioral response differs among tadpole species and depends on the selective pressures imposed by their primary predators (Lawler 1989, Schmidt and Amézquita 2001), the permanence of the larval habitat (Kats et al. 1988, Anholt et al. 2000), and their vulnerability as a result of body size (Semlitsch and Gibbons 1988, Puttlitz et al. 1999) or palatability (Kats et al. 1988, Hero et al. 2001). Tadpoles in ephemeral wetlands have limited time for growth and, therefore, are expected to spend more time searching for food (Woodward 1983, Kats et al. 1988) and respond less to predators than species that breed in permanent habitats (Chovanec 1992, Anolt et al. 2000).

I investigated the behavioral response of Florida bog frog (*Rana okaloosae*) tadpoles to chemical cues of 2 predators, the banded water snake (Colubridae: *Nerodia fasciata*) and the red fin pickerel (Esocidae: *Esox americanus*). For comparative purposes, I also tested the response of bronze frog (*R. clamitans clamitans*) and Southern leopard frog (*R. sphenocephala*) tadpoles to the same predators. The bronze frog is the closest relative of the bog frog (Austin et al. 2003) and occurs in the same permanent–water habitats (Chs. 1–4, Bishop 2004). The leopard frog is a more distant relative and typically breeds in ephemeral habitats. Banded water snakes, which eat tadpoles of bog frogs (Bishop 2003) and other ranids (Aresco and Reed 1998), occur in the habitats of all tadpole species used in this study. Large pickerel, however, are common only in the deeper sections of streams inhabited by *R. okaloosae* and *R. clamitans* and typically are absent from the ephemeral breeding wetlands used by *R. sphenocephala*. Because of their close relationship and similar habitats, I hypothesized that *R. okaloosae* and *R. clamitans* would react similarly to predators and would exhibit a greater response (i.e., reduce swimming) to predators than *R. sphenocephala*.

METHODS

Captures for experiment.—I conducted all sampling on Eglin Air Force Base in Okaloosa County, Florida. I dipnetted *R. sphenocephala* tadpoles from an ephemeral wetland (Site ID 16, Appendix 2, Bishop 2004) on 1 April 2004 and newly hatched *R. okaloosae* and *R. clamitans* tadpoles on 5 May 2004 from the Live Oak Creek study site. I maintained tadpoles between trials in aerated aquaria and fed them a mixture of spinach and rabbit chow *ad libitum*. I tested the response of *R. sphenocephala* tadpoles 1 week after capture and *R. okaloosae* and *R. clamitans* tadpoles 7–8 weeks after capture. All individuals were stages 23–25 when tested (Gosner 1960). I captured 4 fish (*Esox americanus*) and a single banded water snake (*Nerodia fasciata*) in Live Oak Creek to use as predators in this experiment. Between trials, I fed predators fish (primarily *Gambusia* sp.) *ad libitum*.

Palatability of tadpoles.—To verify that *Esox* would eat all 3 species of tadpoles, I placed a single fish in a 37.9–L (10 gal) aquarium for 72 h without food (e.g., Holomuzki 1995). I then placed 5 tadpoles of a given species in the aquarium for 24 h. At the end of the 24 h period, I checked for surviving tadpoles. I repeated this experiment with each species of tadpole, alternating among fish. I also tested the palatability of each tadpole species to *Nerodia* by placing 5 tadpoles in a water dish inside the aquarium after the snake fasted for 14 days. All tadpoles were eaten by both predators during the 24 h period.

Chemical cues.—After the 24–h palatability test, I placed the predator into a 37.9–L aquarium with clean water (7.6 L, 2 gal) for 24 h to saturate the water with chemical cues and eliminate the possibility that tadpole alarm cues (e.g., Summey and Mathis 1998) from the palatability test would affect tadpole behavior. Because tadpoles may react more to predators that recently have eaten larval conspecifics (Laurilla et al. 1997), I used the same predator from the palatability tests to condition the water.

Tadpole response.—After the 24–h saturation period, I removed each predator from the aquarium and positioned the tank over a poster board that was painted half–brown and half–white. The brown side matched the silt where *R. okaloosae* and *R. clamitans* typically are found. Because aquaria had clear glass bottoms, presumably the tadpole could see the difference and select either the white or brown half. All species of tadpoles have similar dorsal coloration and were more visible to me in the white half. A black blind was draped around the aquarium to remove any visual cues from the surrounding environment, and a video camera (Sony TRV128) mounted on a tripod was set above the aquarium.

I randomly assigned 20 tadpoles of each species to 1 of 3 predator treatments: control (no predator), fish (*Esox*), or snake (*Nerodia*). I released a single tadpole at the center of the assigned aquarium and videotaped for 8 min. After the 8–min taping period, I removed each tadpole and repeated the process for the remaining 19 tadpoles. Each individual was used only once in the experiment (total N = 60 tadpoles for each species). Using a stop watch, I recorded the time each tadpole was swimming during 2 time intervals (0–3 and 5–8 min). I averaged these two values to provide an average time spent swimming out of 180 s. I also calculated the average time each tadpole spent in the brown and white halves of the aquarium for the same time intervals.

Statistical analyses.—Data were analyzed using 2 methods. First, intraspecific comparisons were made to look for behavioral differences among predator treatments within each species of tadpole. Second, interspecific comparisons were made among tadpole species within the same predator treatment. Because data could not be normalized by transformations, I used a Kruskal–Wallis test with Tukey's HSD multiple comparisons procedure to identify which groups differed.

RESULTS

The average time spent swimming by each tadpole species differed among predator treatments (Figure 1, Table 1). *Rana clamitans* was most active in the *Esox* treatment. *Rana okaloosae* swam the most in the *Nerodia* treatment, and *R. sphenocephala* swam the most in the control treatment. There also were differences in the time spent swimming among species of tadpoles within the same predator treatment (Table 2). Overall, *R. clamitans* was the most active species of tadpole, followed by *R. sphenocephala*. There were no intraspecific (Table 1) or interspecific (Table 2) differences in time spent in the brown or white halves of the aquarium.

DISCUSSION

Tadpoles typically reduce movement rates when the potential for snake (Griffiths et al. 1998, Kupferberg 1998) or fish (Holomuzki 1995, Lefcort 1998) predation is high because movement rate typically is correlated with predation rate in amphibians (Lawler 1989, Azevedo–Ramos et al. 1992, Chovanec 1992). Kats et al. (1988) suggested that natural selection rather than phylogeny best explains antipredator behavior. Therefore, differences in behavior among *R. okaloosae*, *R. clamitans*, and *R. sphenocephala* tadpoles should reflect different selection pressures.

Because of their similar habitats and recent divergence, I expected the anti–predator behavior of *R. okaloosae* and *R. clamitans* to be similar. I expected both species to react strongly (i.e., reduce movement, select better camouflaged habitat) to snakes, which are common in the stream habitats where they are found, and possibly to predatory fish, which occur in the deeper sections of their stream habitats. Because leopard frogs typically breed in ephemeral wetlands, I expected less response to predator chemicals than the other two ranids. Species that breed in permanent–water habitats and have tadpoles that over–winter, such as *R. clamitans* and *R. okaloosae* (Moler 1985, Bishop 2004), can afford to be more cautious around predators than species that breed in ephemeral habitats, which must complete metamorphosis before dry–down (Anholt et al. 2000). If *R. sphenocephala* tadpoles did respond to predators, I expected the response to be greater to snakes, which are more common than large predatory fish in ephemeral wetlands.

Rana sphenocephala moved twice as much in the control treatment as they did in the predator treatments, a result that suggests they do respond to chemical cues of predators. *Rana okaloosae* was the least active tadpole species in all predator treatments, and despite their close relationship and similar habitats, *R. clamitans* was the most active tadpole. These results do not support previous work that predicted less response to predators in ephemeral wetland species than permanent–water species (Chovanec 1992, Anholt et al. 2000).

There is a trade–off associated with activity level; foraging opportunities may increase with activity but so does predation risk (Kats and Dill 1998, Anholt et al. 2000). Anholt et al. (2000) reported that ranid tadpoles (*R. catesbeiana*, *R. clamitans*, *R. pipiens*, and *R. sylvatica*) reduced movement rates when food was plentiful. Because tadpoles were well–fed prior to testing, perhaps they could be more cautious than if they were hungry. A better experiment would have been to limit the availability of food to tadpoles prior to testing, forcing individuals to choose between potential foraging opportunities and predation.

Presumably, bog and bronze frog tadpoles now experience similar selection forces because they occur in the same locations. Therefore, any behavioral differences between the two species may result from past adaptations to different habitats or differences in life history strategies. The variation in toe–webbing between the two species (Moler 1985) also suggests adaptations for different habitats. Behavioral differences between bog and bronze frogs may result in different predation rates (Lawler 1989). Water snakes (*Nerodia* spp.) hunt by a combination of chemoreception and vision (Drummond 1979, Balent and Andreadis 1998) and are more likely to attack moving prey (Drummond 1979). In addition, *Esox* spp. are ambush hunters (Craig 1996). Therefore, greater movement of tadpoles will increase the likelihood of encountering these stationary predators.

Anholt et al. (2000) also found *R. clamitans* to be the most active among 4 species of ranid tadpoles. Kats et al. (1988) suggested that *R. clamitans* tadpoles were unpalatable to sunfish (*Lepomis* sp.), a characteristic that may influence movement rates; however, both *Esox* and *Nerodia* readily ate bronze frog tadpoles in my palatability test. Failing to recognize or respond to potential predators may increase the vulnerability of tadpoles (Schmidt and Amézquita 2001).

Differences in activity between bronze and bog frogs also may have competition implications. If food is limited, for example, *R. clamitans* may be able to out–compete *R. okaloosae* if greater movement increases foraging opportunities. Variation in swimming behavior also may be related to differences in clutch size between the two species. Because clutch size in *R. clamitans* is an order of magnitude larger than *R. okaloosae* (Ch. 1), higher movement rates in bronze frog tadpoles may be adaptive if it increases dispersal away from oviposition sites and minimizes larval competition among siblings.

LITERATURE CITED

Anholt, B. R., E. Werner, and D. K. Skelly. 2000. Effect of food and predators on the activity of four larval ranid frogs. Ecology 81:3509–3521.

Aresco, M. J., and R. N. Reed. 1998. *Rana capito sevosa* (Dusky Gopher Frog). Predation. Herpetological Review 29:40.

Austin, J. D., S. C. Lougheed, P. E. Moler, and P. T. Boag. 2003. Phylogenetics, zoogeography, and the role of dispersal and vicariance in the evolution of the *Rana catesbeiana* (Anura: Ranidae) species group. Biological Journal of the Linnean Society 80:601–624.

Azevedo–Ramos, C., and W. E. Magnusson. 1999. Tropical tadpole vulnerability to predation: association between laboratory results and prey distribution in an Amazonian savanna. Copeia 1999:58–67.

Azevedo–Ramos, C., M. Van Sluys, J.–M. Hero, W. E. Magnusson. 1992. Influence of tadpole movement on predation by odonate naiads. Journal of Herpetology 26:335–338.

Balent, K. L., and P. T. Andreadis. 1998. The mixed foraging strategy of juvenile northern water snakes. Journal of Herpetology 32:575–579.

Bishop, D. C. 2003. Florida Bog Frog (*Rana okaloosae)*. Predation. Herpetological Review 34:235.

Bishop, D. C. 2004. Summary of surveys for Florida bog frogs and flatwoods salamanders on Eglin Air Force Base through 2004. Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA.

Case, T. J., R. D. Holt, M. A. McPeek, and T. H. Keitt. 2005. The community context of species' borders: ecological and evolutionary perspectives. Oikos 108:28–46.

Chovanec, A. 1992. The influence of tadpole swimming behaviour on predation by dragonfly nymphs. Amphibia–Reptilia 13:341–349.

Craig, J. F. 1996. Pike: Biology and exploitation. Chapman and Hall, London, UK.

Drummond, H. M. 1979. Stimulus control of amphibious predation in the northern water snake (*Nerodia s. sipedon*). Zeitschrift für Tierpsychologie 50:18–44.

Flowers, M. A., and B. M. Graves. 1997. Juvenile toads avoid chemical cues from snake predators. Animal Behaviour 53:641–646.

Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183–190.

Griffiths, R. A., L. Schley, P. E. Sharp, J. L. Dennis, and A. Roman. 1998. Behavioural responses of Mallorcan midwife toad tadpoles to natural and unnatural snake predators. Animal Behaviour 55:207–214.

Hero, J. M., W. E. Magnusson, C. F. D. Rocha, and C. P. Catterall. 2001. Antipredator defenses influence the distribution of amphibian prey species in the central Amazon rain forest. Biotripica 33:131–141.

Holomuzki, J. R. 1995. Oviposition sites and fish–deterrent mechanisms of two stream anurans. Copeia 1995:607–613.

Kats, L. B., and L. M. Dill. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. Ecoscience 5:361–394.

Kats, L. B., J. W. Petranka, and A. Sih. 1988. Antipredator defenses and the persistence of amphibian larvae with fishes. Ecology 69:1865–1870.

Kiesecker, J. M., D. P. Chivers, and A. R. Blaustein. 1996. The use of chemical cues in predator recognition by western toad tadpoles. Animal Behaviour 52:1237–1245.

Kupferberg, S. J. 1998. Predator mediated patch use by tadpoles (*Hyla regilla*): risk balancing or consequence of motionless? Journal of Herpetology 32:84–92.

Laurilla, A., J. Kujasalo, and E. Ranta. 1997. Different antipredator behaviour in two anuran tadpoles: effects of predator diet. Behavioral Ecology and Sociobiology 40:329–336.

Lawler, S. P. 1989. Behavioural responses to predators and predation risk in four species of larval anurans. Animal Behaviour 38:1039–1047.

Lefcort, H. 1998. Chemically mediated fright response in southern toad (*Bufo terrestris*) tadpoles. Copeia 1998:445–450.

Lefcort, H., and A. R. Blaustein. 1995. Disease, predator avoidance, and vulnerability to predation in tadpoles. Oikos 74:469–474.

Moler, P. E. 1985. A new species of frog (Ranidae: *Rana*) from northwestern Florida. Copeia 1985:379–383.

Petranka, J. W., L. B. Kats, and A. Sih. 1987. Predator–prey interactions among fish and larval amphibians: use of chemical cues to detect predatory fish. Animal Behaviour 35:420–425.

Puttlitz, M. H., D. P. Chivers, J. M. Kiesecker, and A. R. Blaustein. 1999. Threat–sensitive predator avoidance by larval Pacific treefrogs (Amphibia, Hylidae). Ethology 105:449–456.

Relyea, R. A., and K. L. Yurewicz. 2002. Predicting community outcomes from pairwise interactions: integrating density– and trait–mediated effects. Oecologia 131:569–579.

Schmidt, B. R., and A. Amézquita. 2001. Predator–induced behavioral responses: tadpoles of the neotropical frog *Phyllomedusa tarsius* do not respond to all predators. Herpetological Journal 11:9–15.

Semlitsch, R. D., and J. W. Gibbons. 1988. Fish predation in size–structured populations of treefrog tadpoles. Oecologia 75:321–326.

Semlitsch, R. D., and Reyer, H.U. 1992. Modification of anti–predator behavior in tadpoles by environmental conditioning. Journal of Animal Ecology 61:353–360.

Sih, A., L. B. Kats, and R. D. Moore. 1992. Effects of predatory sunfish on the density, drift, and refuge use of stream salamander larvae. Ecology 73:1418–1430.

Summey, M. R., and A. Mathis. 1998. Alarm responses to chemical stimuli from damaged conspecifics by larval anurans: tests of three neotropical species. Herpetologica 54:402–408.

Woodward, B. D. 1983. Predator–prey interactions and breeding–pond use of temporary–pond species in a desert anuran community. Ecology 64:1549–1555.

Table 1. *Intraspecific* comparisons between predator treatments. Ranid tadpoles (N = 20) were assigned randomly to 1 of 3 predator treatments: control, fish (*Esox americanus*), or snake (*Nerodia fasciata*). The average time (out of 180 s) that each tadpole spent swimming, in the brown half, and in the white half of the aquarium was recorded. If there was a significant difference between treatments (Kruskal–Wallis Test), a Tukey's post hoc test was conducted to identify which treatments differed. Group differences are indicated by letter superscript(s).

Tadpole Species	Variable	Avg Time ± SE (s)			Kruskal–Wallis	
		Control	*Esox*	*Nerodia*	F	P
Rana clamitans	Swimming	20.7 ± 12.5^{A}	44.0 ± 19.3^{B}	17.2 ± 15.9^{A}	15.792	0.000*
	Brown half	92.5 ± 42.7	74.2 ± 34.4	72.0 ± 62.2	0.959	0.389
	White half	87.6 ± 42.3	105.9 ± 34.4	108.1 ± 62.2	0.959	0.389
R. okaloosae	Swimming	1.9 ± 1.2^{A}	2.0 ± 1.6^{A}	4.7 ± 6.0^{B}	5.144	0.009*
	Brown half	76.6 ± 84.9	107.2 ± 83.8	82.5 ± 79.5	0.755	0.475
	White half	103.4 ± 84.9	72.8 ± 83.8	97.5 ± 79.6	0.755	0.476
R. sphenocephala	Swimming	16.2 ± 10.5^{A}	8.6 ± 8.9^{B}	8.2 ± 6.5^{B}	6.212	0.004*
	Brown half	83.8 ± 53.3	87.0 ± 54.3	86.7 ± 56.0	0.068	0.934
	White half	96.2 ± 53.4	93.0 ± 54.3	93.3 ± 56.0	0.068	0.934

*$P < 0.05$

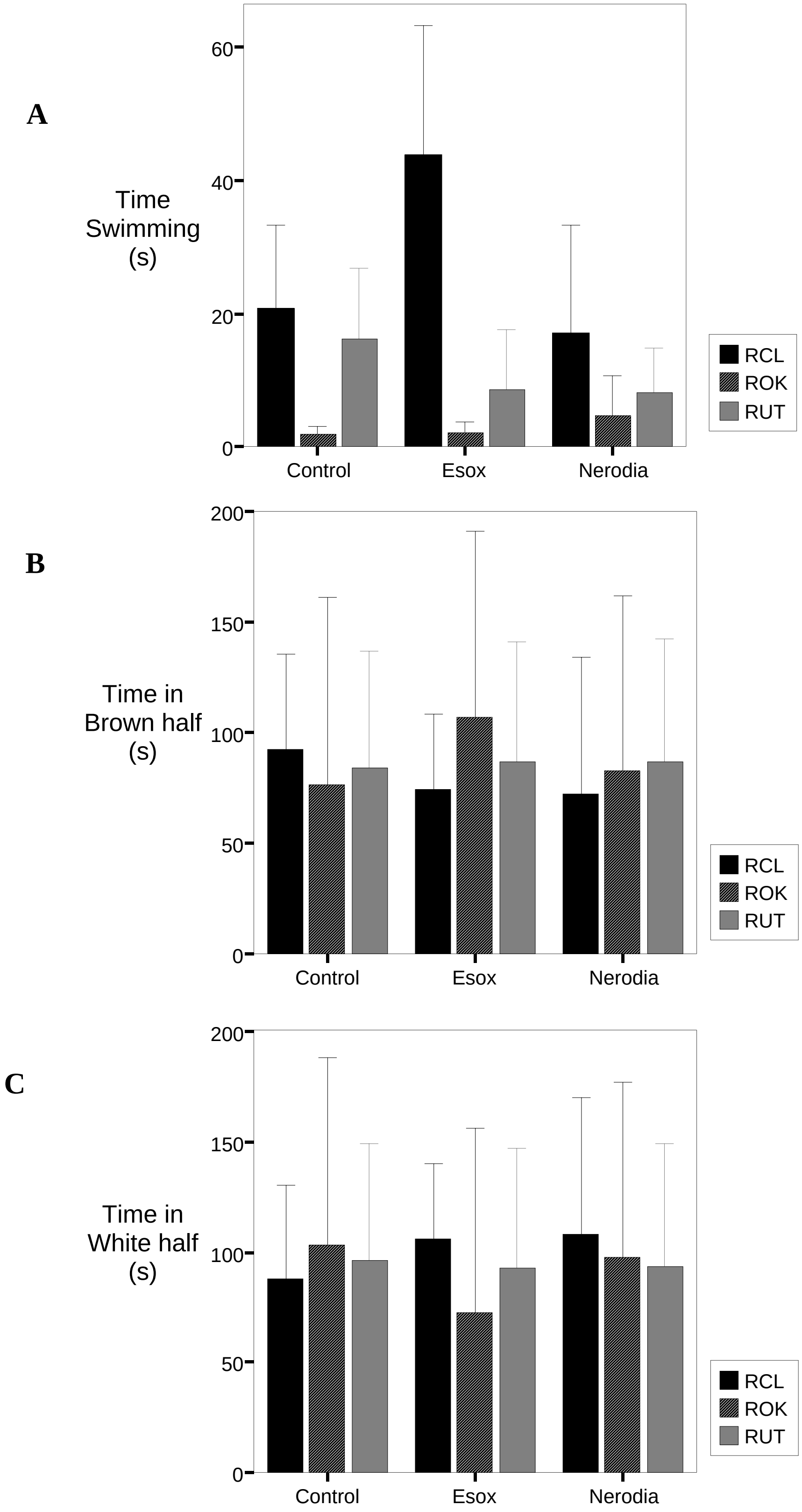
Table 2. *Interspecific* comparisons between predator treatments. Ranid tadpoles (N = 20) were assigned randomly to 1 of 3 predator treatments: control, fish (*Esox americanus*), or snake (*Nerodia fasciata*). The average time (out of 180 s) that each tadpole spent swimming, in the brown half, and in the white half of the aquarium was recorded. If there was a significant difference between treatments (Kruskal–Wallis Test), a Tukey's post hoc test was conducted to identify which treatments differed. Group differences are indicated by letter superscript(s).

		Avg Time ± SD (s)			Kruskal–Wallis	
Treatment	Variable	*Rana clamitans*	*R. okaloosae*	*R. sphenocephala*	F	P
Control	Swimming	20.7 ± 12.5^{A}	1.9 ± 1.2^{B}	16.2 ± 10.5^{A}	53.249	0.000*
	Brown half	92.5 ± 42.7	76.6 ± 84.9	83.8 ± 53.3	0.426	0.655
	White half	87.6 ± 42.3	103.4 ± 84.9	96.2 ± 53.4	0.426	0.655
Esox	Swimming	44.0 ± 19.3^{A}	2.0 ± 1.6^{B}	8.6 ± 8.9^{C}	78.376	0.000*
	Brown half	74.2 ± 34.4	107.2 ± 83.8	87.0 ± 54.3	1.156	0.322
	White half	105.9 ± 34.4	72.8 ± 83.8	93.0 ± 54.3	1.156	0.322
Nerodia	Swimming	17.2 ± 15.9^{A}	4.7 ± 6.0^{B}	$8.2 \pm 6.5^{A,B}$	9.318	0.000*
	Brown half	72.0 ± 62.2	82.5 ± 79.5	86.7 ± 56.0	0.213	0.809
	White half	198.1 ± 62.2	97.5 ± 79.6	93.3 ± 56.0	0.213	0.809

*P < 0.05

Figure 1. (next page) Average time (out of 180 s) (+ SE bars) each tadpole species spent swimming (A), in the brown half (B), and in the white half (C) of the aquarium in each treatment. Tadpoles (N = 20) were assigned randomly to 1 of 3 predator treatments: control, fish (*Esox americanus*), or snake (*Nerodia fasciata*). RCL = *Rana clamitans*, ROK = *R. okaloosae*, RSP = *R. sphenocephala*. See Tables 1 and 2 for data and comparisons.

Figure 1


A
Time Swimming (s)
0
20
40
60
Control
Esox
Nerodia
RCL
ROK
RUT
B
Time in Brown half (s)
0
50
100
150
200
Control
Esox
Nerodia
RCL
ROK
RUT
C
Time in White half (s)
0
50
100
150
200
Control
Esox
Nerodia
RCL
ROK
RUT

CHAPTER 6. BURNING TRENDS AND POTENTIAL NEGATIVE EFFECTS OF SUPPRESSING WETLAND FIRES ON FLATWOODS SALAMANDERS

Note to reader: A version of this chapter is in press in the Natural Areas Journal.

ABSTRACT.—The federally threatened flatwoods salamander (*Ambystoma cingulatum*) inhabits the fire–adapted longleaf pine flatwoods and savannas in the southeastern coastal plain. It breeds in ephemeral wetlands that typically dry during summer and refill in fall and winter. If prescribed fires are conducted only in winter, fires are less likely to burn through water–filled wetlands, potentially affecting larval habitat. I measured canopy cover, dissolved oxygen (DO), water temperature, and water depth in 13 breeding wetlands with different fire histories and compared burned and unburned halves of a partially–burned wetland on Eglin Air Force Base in northwest Florida. In general, areas that burned more recently had more open canopies, higher DO concentrations, higher water temperatures, more understory vegetation, and were shallower than unburned areas. I also collected data on burning trends of *A. cingulatum* populations across the range. Rangewide surveys indicate that prescribed fires typically are applied in winter and early spring. Based on what is known about the natural history of the species, the historical burning regime of the longleaf ecosystem, and the effects of fires on ephemeral wetlands, I suggest that land mangers diversify their fire–management strategy to increase the likelihood of burning the breeding wetlands of flatwoods salamanders.

INTRODUCTION

The impacts of forest management activities on amphibian communities have received more attention in recent years. Most studies have focused on the negative effects of timber harvesting on amphibian populations (e.g., Petranka et al. 1994, Ash 1997, Grialou et al. 2000, Knapp et al. 2003). However, other activities, such as ditching and bedding often used in coastal plain silvicultural operations (Pechmann et al. 1989, Means et al. 1996), and fire suppression (deMaynadier and Hunter 1995, Russell et al. 1999, Pilliod et al. 2003), also may affect amphibian diversity and abundance.

The federally threatened flatwoods salamander (Ambystomatidae: *Ambystoma cingulatum*) has declined throughout its range. Major threats to the species include the degradation and loss of habitat from development, agriculture and silviculture, modification of breeding wetland hydrology, and fire suppression (Means et al. 1996, Palis 1996, USFWS 1999). Flatwoods salamanders inhabit fire–adapted longleaf pine (*Pinus palustris*) flatwoods and savannas in the southeastern coastal plain. They breed in ephemeral wetlands (Means 1972, Anderson and Williamson 1976, Palis 1997a), which typically dry in summer and refill in fall and winter (Palis 1997b, Stevenson 1999).

The historical fire regime of the longleaf pine included frequent summer fires from lightning strikes and Native Americans (Ware et al. 1993, Battle and Golladay 2003). Now, fires often are prescribed in winter, the season when weather conditions are favorable for controlled burns (Wade and Lunsford 1989). If prescribed burns are applied only in winter, fires are less likely to burn through wetland basins, except in drought years. Fire exclusion may impact vegetation composition, solar radiation, water temperature, water chemistry, hydrology, nutrient cycling, and productivity of wetlands (Kirkman 1995, Battle and Golladay 2003, Pilliod et al. 2003).

The primary purpose of this paper is to relate what is known about the natural history of flatwoods salamanders to the role of fire in structuring their breeding wetlands.

I compared canopy coverage, DO concentrations, water depth, and water temperatures among 13 known breeding wetlands with different fire histories. I also compared canopy and understory differences between halves of a partially–burned breeding wetland. In addition, I surveyed management lands that contain historical breeding populations of *A. cingulatum* to identify burning patterns across the range of the species. Although evidence of a direct effect of fire suppression on flatwoods salamanders is lacking, I suggest fire–management decisions be based on what is known about the natural history of the species, the historical burning regime of the longleaf ecosystem, and the effects of fires on ephemeral wetlands

METHODS

Study area.—All field data were collected on Eglin Air Force Base in Okaloosa County, Florida. The breeding wetlands used by flatwoods salamanders on Eglin AFB are located in mesic longleaf pine flatwoods (see Bishop 2004 for locations of wetlands). Wetlands are dominated by pond cypress (*Taxodium ascendens*), slash pine (*Pinus elliottii*) and occasionally black gum (*Nyssa sylvatica*). Chapman's St. John' wort (*Hypericum chapmanii*) and myrtle–leaf holly (*Ilex myrtifolia*) dominate the midstory, where the latter often reaches tree size and contributes the most shade to a wetland. The understory consists of a mixture of grasses and sedges and varies in coverage from occurring only around the edge to extending throughout the basin. Surrounding each wetland is an ecotone dominated by bunch grasses (*Aristida beyrichiana* or *Calamovilfa curtissii*). Palis and Jensen (1995) provide greater detail on these wetlands.

Partially–burned wetland.—In August 2000, a wildfire partially burned through one breeding wetland (Site ID 30, Appendix 2, Bishop 2004). Because of the close proximity to a residential neighborhood, fire fighters plowed through the center of the dry wetland to contain the fire, leaving a half–burned wetland. I established a 100–m transect through the wetland, extending between the two sides, to compare overstory and understory vegetation between burned and unburned halves. I set poles at 10–m intervals (4 per side), allowing a 10–m buffer on each side of the fire–break. At each pole, I measured the coverage and height of herbaceous understory vegetation in two 0.5–m^2 plots (pvc frame), positioned at north and south cardinal directions. I estimated percent understory coverage (0–5, 6–25, 26–50, 51–75, 76–95, 96–100%), with midpoints of each cover class averaged for each plot. I also recorded the maximum height of herbaceous vegetation at 10 locations within each 0.5–m^2 plot and calculated an average maximum understory height. At each sample location, I photographed the canopy using a Nikon 995 Coolpix™ digital camera equipped with a fisheye (180°) lens and leveled on a tripod at a height of 1.5 m. I took digital photographs on overcast days and calculated percent canopy coverage using HemiView™ software. I recorded all data in July 2002, 23 months after the fire.

Comparisons among breeding wetlands.—I explored the relationship between fire history, canopy coverage, DO concentration, water temperature, and depth in 13 known breeding wetlands. I established a transect through the middle of the long axis of each wetland and set poles at 20–m intervals to mark sample locations. The number of poles in each transect ranged from 2–11, depending on the size of the wetland. I recorded DO concentration and water temperature at a depth of 5 cm at each sample location along each transect. Between 19 November 2002 and 15 January 2003, I recorded DO levels at dawn (N = 3) and in late afternoon (N = 4), times corresponding to likely minimum and maximum DO levels.

At each sample location, I photographed the canopy the first week of January 2003 as described above, a measurement that represents minimal cover. I measured water depth once in each wetland in late December 2002 to get a relative measure of depth and averaged the individual depths recorded along the transect. I estimated the number of months since the last fire burned through the wetland using historical fire records, satellite images, and field observations (e.g., fire scars, standing deadwood). January 2003 was used as the starting date to determine the number of months elapsed since the last fire. I separated the wetlands into two groups, those in which the basin burned within the last 5 years (N=7) and those that had not burned in the previous 10 years (N=6). I used principal components analysis (PCA) to summarize the variation among habitat variables for each wetland.

Rangewide burning patterns.—I collected fire histories on state and federal lands that contain breeding populations of *A. cingulatum* from across the range of the species. Participants in the survey (Table 1) provided months and years of fires (Total N = 228) for an 11–year period (1 January 1992 – 31 December 2002). These data were used to evaluate the frequency of fires in surrounding uplands, not whether fires actually burned through wetlands. Data indicating whether each fire burned through the wetland basin were not available. I also did not have sufficient data to distinguish between wildfires and prescribed fires.

I calculated the average fire interval (i.e., the average number of years between fires) by dividing the total number of years of fire history by the number of fires. For wetlands in compartments that never burned within the observed period, I set the burn frequency at the next year. For example, if a compartment containing a wetland never burned during the 11–year period, I set burn frequency at the default value of 12 (i.e., the area burned an average of once every 12 years). Thus, the mean fire interval may slightly overestimate the actual fire frequency. However, only 9 of 154 wetlands were located in compartments that never burned in the observed period.

RESULTS

In the partially–burned wetland, the burned half had a more open canopy and greater herbaceous understory than the unburned half (Table 2). When comparing among wetlands (N = 13), those burned more recently had more open canopies, higher DO concentrations, and were shallower than unburned wetlands (Figures 1 and 2, Table 3). The first principal component accounted for 63.8% of the variation in the data set (Table 4). Other components were not extracted because they had eigenvalues < 1 and could not be interpreted. Management areas containing historical breeding wetlands (N = 154) burned an average (± SD) once every 4.4 ± 2.9 years (range: 1.2–12 years). Fires were >3x as common in winter and early spring (December–April) than in summer (May–August) (Figure 3).

DISCUSSION

Adult flatwoods salamanders move to breeding wetlands from October–January on rainy nights associated with cold fronts and decreasing atmospheric pressure (Means 1972, Anderson and Williamson 1976, Palis 1997a, Safer 2001). They typically breed in open–canopy wetlands (Anderson and Williamson 1976, Palis 1997b). Larvae can be captured December–April (Means 1972, Palis 1996) by dipnetting herbaceous vegetation (Palis 1996, Sekerak et al. 1996, personal observations), which typically is more abundant in open–canopy wetlands and in open sections within a wetland (Bishop, unpublished data). It is unknown why larvae choose areas with high understory coverage and open canopies within a wetland, but they potentially offer greater protection from predators, higher water temperatures, higher DO concentrations, and possibly more prey items. All of these factors likely increase growth rates and the chances of surviving to metamorphosis.

The potential that exclusion of fire negatively affects flatwoods salamanders has been suggested (Means et al. 1996, Palis 1997b, Safer 2001). However, because there are no long–term monitoring datasets and it is difficult to capture adequate numbers of individuals for research, there is limited direct evidence that suppression of wetland fires is harmful to *A. cingulatum*. Populations occasionally persist in fire–suppressed areas, but casual observations suggest that breeding sites within fire–suppressed areas produce fewer larvae than those that burn frequently (D. Printiss, Apalachicola National Forest, personal communication). In the absence of direct evidence, we should rely on indirect evidence to determine appropriate management strategies and, whenever possible, mimic the natural processes that shaped the evolutionary history of the species.

Ephemeral depression wetlands in the southeastern coastal plain are structured by two primary factors, hydrology and fire frequency (Kirkman et al. 2000). Fire frequency declines as hydroperiod increases; however, fire frequency also may affect hydrology by altering evapotranspiration rates. In flatwoods communities, removal of woody vegetation can alter the hydrology of uplands and wetlands (Sun et al. 2000, Bliss and Comerford 2002). Cypress domes, limesink ponds, Carolina bays, and other southeastern ephemeral wetlands naturally burn when dry (Ewel and Mitsch 1978, Sutter and Kral 1994, Ewel 1995), but managers have been reluctant to incorporate burning wetlands in fire–management plans. As a result, many depression wetlands in the southeast likely are overgrown after years of fire suppression (Huffman and Blanchard 1991).

In other ecosystems, canopy overgrowth has lead to local population extinctions (Skelly et al. 1999) and loss of diversity (Werner and Glennemeier 1999) of amphibians. Closed canopies diminish growth, development, and survivorship rates in some species, presumably because shading decreases the amount of understory vegetation, lowers dissolved oxygen (DO) concentration, limits the prey base, and decreases water temperature (Werner and Glennemeier 1999, Skelly et al. 2002, Halverson et al. 2003). Larval amphibians are highly sensitive, both behaviorally and physiologically, to changes in DO and water temperature in the field and lab (Costa 1967, Noland and Ultsch 1981, Nie et al. 1999). Fire also affects nutrient and pH levels in depression wetlands in longleaf pine ecosystems (Battle and Golladay 2003), factors that may alter habitat composition and larval development rates. Amphibian species that breed in ephemeral habitats must complete metamorphosis before dry–down; hence, growth and survivorship rates need to be maximized. Slow–growing individuals often metamorphose at smaller sizes, a factor that may affect adult fitness in *Ambystoma* (Semlitsch et al. 1988, Taylor and Scott 1997).

My data suggest that wetland fires decrease canopy coverage, and lead to increases in understory vegetation, DO concentrations, and water temperatures in *A. cingulatum* breeding wetlands. However, identifying the cause and effect relationships can be challenging without experimental manipulation. For example, I was unable to evaluate whether fire or hydroperiod was the primary factor affecting observed vegetation patterns in the 13 wetlands compared in this study. Percent canopy openness appeared to be more strongly related with both mean DO and mean water temperature than with mean water depth, suggesting that hydrology (at least as measured by water depth) alone could not account for the differences. Casual observations suggest that water depth is related to hydroperiod in these wetlands; i.e., deep wetlands retain water longer than shallow wetlands. I originally planned to relate wetland habitat variables to larval abundance and body size; however, I captured few larvae on Eglin AFB during the course of this study (Bishop 2004).

Land managers recognize that fire is necessary maintain the longleaf ecosystem, but there is considerable debate on the importance of seasonality of fires. Some suggest that fires during the growing season are better than those in winter for maintaining the longleaf vegetation community (e.g. Brockway and Lewis 1997), but others believe that fire frequency is more important than season (e.g. Hiers et al. 2000). The success of a prescribed burning program often is evaluated by the number of acres burned, the frequency of fires, or the seasonality, rather than the ecological effects.

In the Southeast, prescribed fires typically are applied in winter and early spring, principally because winter weather conditions often are more favorable for controlling burns than summer fires (Wade and Lunsford 1989). Winter and early spring fires, however, are unlikely to burn through water–filled wetlands, except in drought years (Battle and Golladay 2003, personal observations). Land managers in many locations try to conduct growing–season fires, although months that are considered part of the growing season do not necessarily correspond to the peak lightning season (May–August, Robbins and Myers 1992). Fire personnel sometimes are reluctant to burn wetlands because they believe they are protecting them and will attempt to avoid 'muck' fires by plowing fire–breaks around their perimeters. Water–filled wetlands often are used intentionally as natural fire–breaks in prescribed burns.

Besides excluding fires, there are other concerns about fire–breaks surrounding wetlands. First, they may alter hydrology. Second, adult flatwoods salamanders deposit eggs terrestrially prior to inundation (Anderson and Williamson 1976). The environmental cues that a female uses to decide where to deposit her eggs are unknown. If females search for a depression area in the general vicinity of the wetland, a plow–line may be sufficient to trigger oviposition. Lastly, fire–breaks are potential death traps to larval amphibians. When a wetland is filled completely, a fire–break may be submerged within the boundaries of the wetland. As the wetland dries, the plow–line becomes an isolated moat, typically drying well before the interior of the wetland and leaves trapped amphibians less time to complete metamorphosis. In January 2002, I counted more than 500 dead leopard frog (*Rana sphenocephala utricularia*) tadpoles in a recently dried plow–line surrounding a breeding wetland used by *A. cingulatum*.

The accumulation of organic material in fire–suppressed wetlands concerns fire personnel who wish to avoid lingering fires and resulting smoke (Miller et al. 1998). An increase in organic material also may increase successional change within a wetland (Russell et al. 1999) and alter DO concentration (Werner and Glennemeier 1999). This usually is not a concern in many wetlands used by *A. cingulatum* because of frequent dry periods (e.g., Battle and Golladay 2001); however, some have substantial organic layers. Continued exclusion of fires, however, allows organic matter to accumulate, possibly leading to catastrophic fires in the future as well as modifying the habitat of larval flatwoods salamanders. Even if fires only burn through the outer edge of wetlands, larval salamanders likely will benefit from greater understory growth, higher water temperatures, and higher dissolved oxygen levels. Larvae often are captured in the inundated wiregrass ecotone (personal observations), which flowers after growing season fires (Seamon et al.1989).

It is difficult to know the appropriate range of canopy and understory coverage in ephemeral depression wetlands, if such a range exists. Likewise, no one knows what the optimum fire frequency in wetland basins should be. The preferred fire frequency actually may vary among different amphibian species (e.g., Schurbon and Fauth 2003, Means et al. 2004). If the longleaf pine ecosystem historically burned every 1–4 years (Clewell 1989, Stout and Marion 1993, Frost 1995) and primarily in summer, fires likely would have burned through ephemeral wetlands. The mean fire interval for management areas surveyed in this study was 4.4 years in known breeding areas. However, fires were applied mostly in winter and early spring, and some compartments that contained breeding wetlands never were burned in the 11–year period for which I had data.

Because breeding wetlands typically are inundated during winter and early spring, they likely are being burned at an unnaturally low frequency where reliance on prescribed applications exists. In addition, most private lands do not burn as frequently as the government lands surveyed in this study, many of which actively are managed for the federally endangered red–cockaded woodpecker (*Picoides borealis*). In the absence of certainty of what good salamander habitat ‘should’ look like, the best approach may be a strategy that alters both the frequency and seasonality of fires (Hiers et al. 2000).

Pilliod et al. (2003) provided a thorough review of the wide–reaching effects of fire on amphibians. More long–term research is needed to understand the relationship between fire history and the abundance, distribution, and reproductive success of *A. cingulatum*. With a declining species, however, management decisions must be made now. Management strategies should be based on what is known about the biology of the species, the hydrology of their breeding wetlands, studies of other amphibian taxa with similar life histories, and the historical importance of lighting–season fires in the longleaf ecosystem. Until new data suggest otherwise, current information indicates we should burn these wetlands periodically. At the very least, land managers need to stop intentionally excluding fires from them. We may, in fact, be required to by the Endangered Species Act (White 1989).

LITERATURE CITED

Anderson, J. D., and Williamson, G. K. 1976. Terrestrial mode of reproduction in *Ambystoma cingulatum*. Herpetologica 32:214–221.

Ash, A. N. 1997. Disappearance and return of plethodontid salamanders to clearcut plots in the southern Blue Ridge Mountains. Conservation Biology 11:983–989.

Battle, J., and S. W. Golladay. 2003. Prescribed fire's impact on water quality of depressional wetlands in southwestern Georgia. American Midland Naturalist 150:15–25.

Bishop, D. C. 2004. Summary of surveys for Florida bog frogs and flatwoods salamanders on Eglin Air Force Base through 2004. Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA.

Bliss, C. M., and N. B. Comerford. 2002. Forest harvesting influence on water table dynamics in a Florida flatwoods landscape. Soil Science Society of America Journal 66:1344–1349.

Brockway, D. G., and C. E. Lewis. 1997. Long–term effects of dormant–season prescribed fire on plant community diversity, structure and productivity in a longleaf pine wiregrass ecosystem. Forest Ecology and Management 96:167–183.

Clewell, A. F. 1989. Natural history of wiregrass (*Aristida stricta* Michx., Gramineae). Natural Areas Journal 9:223–233.

Costa, H. H. 1967. Avoidance of anoxic water by tadpoles of *Rana temporaria*. Hydrobiologia 30:374–384.

deMaynadier, P. G., and M. L. Hunter, Jr. 1995. The relationship between forest management and amphibian ecology: a review of the North American literature. Environmental Reviews 3:230–261.

Ewel, K. C. 1995. Fire in cypress swamps in the southeastern United States. Proceedings of the Tall Timbers Fire Ecology Conference 19:111–116.

Ewel, K. C., and W. J. Mitsch. 1978. The effects of fire on species composition in cypress dome ecosystems. Florida Scientist 41:25–31.

Frost, C. C. 1995. Presettlement fire regimes in southeastern marshes, peatlands, and swamps. Proceedings of the Tall Timbers Fire Ecology Conference 19:39–60.

Grialou, J. A., S. D. West, and R. N. Wilkins. 2000. The effects of forest clearcut harvesting and thinning on terrestrial salamanders. Journal of Wildlife Management 64:105–113.

Halverson, M. A., D. K. Skelly, J. M. Kiesecker, L. K. Freidenburg. 2003. Forest mediated light regime linked to amphibian distribution and performance. Oecologia 134:360–364.

Hiers, J. K., R. Wyatt, and R. J. Mitchell. 2000. The effects of fire regime on legume reproduction in longleaf pine savannas: is season selective? Oecologia 125:521–530.

Huffman, J. M., and S. W. Blanchard. 1991. Changes in woody vegetation in Florida dry prairie and wetlands during a period of fire exclusion, and after dry–growing–season fire. Pages 75–83 *in* S. C. Nodvin and T. A. Waldrop, editors. Fire in the Environment: Ecological and Cultural Perspectives. General Technical Report SE–69, Southeastern Forest Experiment Station, Asheville, North Carolina, USA.

Kirkman, L. K. 1995. Impacts of fire and hydrological regimes on vegetation in depression wetlands of southeastern USA. Proceedings of the Tall Timbers Fire Ecology Conference 19:10–20.

Kirkman, L. K., P. C. Goebel, L. West, M. B. Drew, and B. J. Palik. 2000. Depressional wetland vegetation types: a question of plant community development. Wetlands 20:373–385.

Knapp, S. M., C. A. Haas, D. N. Harpole, and R. L. Kirkpatrick. 2003. Initial effects of clearcutting and alternative silvicultural practices on terrestrial salamander abundance. Conservation Biology 17:752–762.

Means, D. B. 1972. Notes on the autumn breeding biology of *Ambystoma cingulatum* (Cope) (Amphibia: Urodela: Ambystomatidae). ASB Bulletin 19:84–85.

Means, D. B., J. G. Palis, and M. Baggett. 1996. Effects of slash pine silviculture on a Florida population of flatwoods salamander. Conservation Biology 10:426–437.

Means, D. B., K. D. Dodd, Jr., S. A. Johnson, and J. G. Palis. 2004. Amphibians and Fire in Longleaf Pine Ecosystems: Response to Schurbon and Fauth 18:1149–1153.

Miller, S. J., K. J. Ponzio, M. A. Lee, L. W. Keenan, and S. R. Miller. 1998. The use of fire in wetland preservation and restoration: are there risks? Proceedings of the Tall Timbers Fire Ecology Conference 20:127–139.

Nie, M., J. D. Crim, and G. R. Ultsch. 1999. Dissolved oxygen, temperature, and habitat selection by bullfrog (*Rana catesbeiana*) tadpoles. Copeia 1999:153–162.

Noland, R., and G. R. Ultsch. 1981. The roles of temperature and dissolved oxygen in microhabitat selection by the tadpoles of a frog (*Rana pipiens*) and a toad (*Bufo terrestris*). Copeia 1981:645–652.

Palis, J. G. 1996. Flatwoods salamander (*Ambystoma cingulatum* Cope). Natural Areas Journal 16:49–54.

Palis, J. G. 1997a. Breeding migration of *Ambystoma cingulatum* in Florida. Journal of Herpetology 31:71–78.

Palis, J. G. 1997b. Distribution, habitat, and status of the flatwoods salamander (*Ambystoma cingulatum*) in Florida, USA. Herpetological Natural History 5:53–65.

Palis, J. G., and J. B. Jensen. 1995. Distribution and breeding biology of the flatwoods salamander (*Ambystoma cingulatum*) and gopher frog (*Rana capito*) on Eglin Air Force Base, Florida. Final Report. Florida Natural Areas Inventory, Tallahassee, Florida, USA.

Pechmann, J. H. K., D. E. Scott, J. W. Gibbons, and R. D. Semlitsch. 1989. Influence of wetland hydroperiod on diversity and abundance of metamorphosing juvenile amphibians. Wetlands Ecology and Management 1:3–11.

Petranka, J. W., M. P. Brannon, M. E. Hopey, and C. K. Smith. 1994. Effects of timber harvesting on low elevation populations of southern Appalachian salamanders. Forest Ecology and Management 67:135–147.

Pilliod, D. S., R. B. Bury, E. J. Hyde, C. A. Pearl, and P. S. Corn. 2003. Fire and amphibians in North America. Forest Ecology and Management 178:163–181.

Robbins, L. E., and R. L. Myers. 1992. Seasonal effects of prescribed burning in Florida: a review. Tall Timbers Research Station Miscellaneous Publication No. 8, Tallahassee, Florida, USA.

Russell, K. R., D. H. Van Lear, and D. C. Guynn, Jr. 1999. Prescribed fire effects on herpetofaunal: review and management implications. Wildlife Society Bulletin 27:374–384.

Safer, A. 2001. Natural history and ecology of the flatwoods salamander, *Ambystoma cingulatum*, on the Atlantic coastal plain. M.S. Thesis, Georgia Southern University, Statesboro, Georgia, USA.

Schurbon, J. M., and J. E. Fauth. 2003. Effects of prescribed burning on amphibian diversity in a southeastern U. S. National Forest. Conservation Biology 17:1338–1349.

Seamon, P. A., R. L. Myers, L. E. Robbins, and G. S. Seamon. 1989. Wiregrass reproduction and community restoration. Natural Areas Journal 9:264–265.

Sekerak, C. M., G. W. Tanner, and J. G. Palis. 1996. Ecology of flatwoods salamander larvae in breeding ponds in Apalachicola National Forest. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 50:321–330.

Semlitsch, R. D., D. E. Scott, and J. H. K. Pechmann. 1988. Time and size at metamorphosis related to adult fitness in Ambystoma talpoideum. Ecology 69:184–192.

Skelly, D. K., L. K. Freidenburg, and J. M. Kiesecker. 2002. Forest canopy and the performance of larval amphibians. Ecology 83:983–992.

Skelly, D. K., E. E. Werner, and S. A. Cortwright. 1999. Long–term distributional dynamics of a Michigan amphibian assemblage. Ecology 80:2326–2337.

Stevenson, D. J. 1999. The herpetofaunal of Fort Stewart, Georgia: habitat occurrence, status of protected and rare species, and species diversity. Unpublished Report. Fort Stewart Fish and Wildlife Branch, Georgia, USA.

Stout, I. J., and W. R. Marion. 1993. Pine flatwoods and xeric pine forests of the southern (lower) coastal plain. Pages 373–447 *in* W. H. Martin, S. G. Boyce and A .C. Echternacht, editors. Biodiversity of the Southeastern United States: Lowland Terrestrial Communities. John Wiley and Sons, Inc., New York, New York, USA.

Sun, G., H. Riekerk, and L. V. Kornhak. 2000. Ground–water–table rise after forest harvesting on cypress–pine flatwoods in Florida. Wetlands 20:101–112.

Sutter, R. D., and R. Kral. 1994. The ecology, status, and conservation of two non–alluvial wetland communities in the south Atlantic and eastern Gulf coastal plain, USA. Biological Conservation 68:235–243.

Taylor, B. E., and Scott, D. E. 1997. Effects of larval density dependence on population Dynamics of *Ambystoma opacum*. Herpetologica 53: 132–145.

USFWS. 1999. Endangered and threatened wildlife and plants: final rule to list the flatwoods salamander as a threatened species. Federal Register 64:15691–15704.

Wade, D. D., and J. D. Lunsford. 1989. A guide for prescribed fire in southern forests. USDA Forest Service, Southern Region, Technical Publication R8–TP11, Atlanta Georgia, USA.

Ware, S., C. Frost, and P. D. Doerr. 1993. Southern mixed hardwood forest: the former longleaf pine forest. Pages 447–493 *in* W. H. Martin, S. G. Boyce and A. C. Echternacht, editors. Biodiversity of the Southeastern United States: Lowland Terrestrial Communities. John Wiley and Sons, Inc., New York, New York, USA.

Werner, E. E., and K. S. Glennemeier. 1999. Influence of forest canopy cover on the breeding pond distributions of several amphibian species. Copeia 1999:1–12.

White, D. H. 1989. Legal implications associated with use and control of fire as a management practice. Proceedings Tall Timbers Fire Ecology Conference 17:375–384.

Table 1. Fire histories of federal and state management areas. The number of breeding wetlands indicates the number of wetlands used historically by *Ambystoma cingulatum* for breeding.

Management Area	State	Breeding Wetlands	Fire History
Apalachicola National Forest	FL	50	1992–2002
Eglin Air Force Base	FL	18	1992–2002
Fort Stewart (Army)	GA	18	1992–2002
Francis Marion National Forest	SC	5	1992–2002
Holley Field (Navy)	FL	3	1992–2002
Hurlburt Field (Air Force)	FL	13	1992–2002
J. W. Jones Research Center (Univ. Georgia)	GA	4	1993–2002
Mayhaw Wildlife Management Area	GA	1	1992–2002
Pine Log State Forest	FL	1	1992–2002
St. Mark’s National Wildlife Refuge	FL	41	1992–2002

Table 2. Mean canopy and understory coverage in a half–burned wetland used for breeding by *Ambystoma cingulatum* on Eglin Air Force Base in Okaloosa County, FL, summer 2002. Measurements were taken 23 months after the fire.

	Canopy Openness (%)		Understory Coverage (%)		Understory Height (cm)	
Section	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Burned	52.9 ± 9.3	42.3 – 64.9	44.7 ± 24.6	15.0 – 62.25	64.9 ± 23.2	26.9 – 90.0
Unburned	17.3 ± 4.1	12.3 – 22.3	2.5 ± 0.0	2.5 – 2.5	8.0 ± 8.8	0.0 – 27.3

Table 3. Physical and chemical attributes of wetlands with different fire histories used by *Ambystoma cingulatum* for breeding on Eglin Air Force Base in Okaloosa County, Florida, winter 2003. Burned wetlands had a fire within the last 5 years. Unburned wetlands had not burned in over 10 years.

		Dissolved Oxygen (mg/L)		Water Temperature (°C)		Water Depth (cm)		Canopy Openness (%)	
	N	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Burned	7	6.7 ± 0.6	4.8 – 9.5	13.0 ± 0.6	10.7 – 15.8	17.3 ± 3.2	9.3 – 29.7	53.4 ± 4.3	36.3 – 73.2
Unburned	6	4.8 ± 0.6	2.6 – 6.7	12.1 ± 0.7	9.8 – 14.3	27.9 ± 3.9	15.4 – 39.2	27.6 ± 2.9	18.2 ± 38.2

Table 4. Results of principle components analysis of habitat variables in 13 wetlands used by *Ambystoma cingulatum* for breeding on Eglin Air Force Base in Okaloosa County, Florida, winter 2003.

Eigenvalues and Eigenvectors	Principal Component
	1
Eigenvalue	2.553
% of variance	63.82
Eigenvectors	
Mean water temperature	0.596
Mean water depth	– 0.651
Mean DO	0.957
Percent canopy openness	0.926

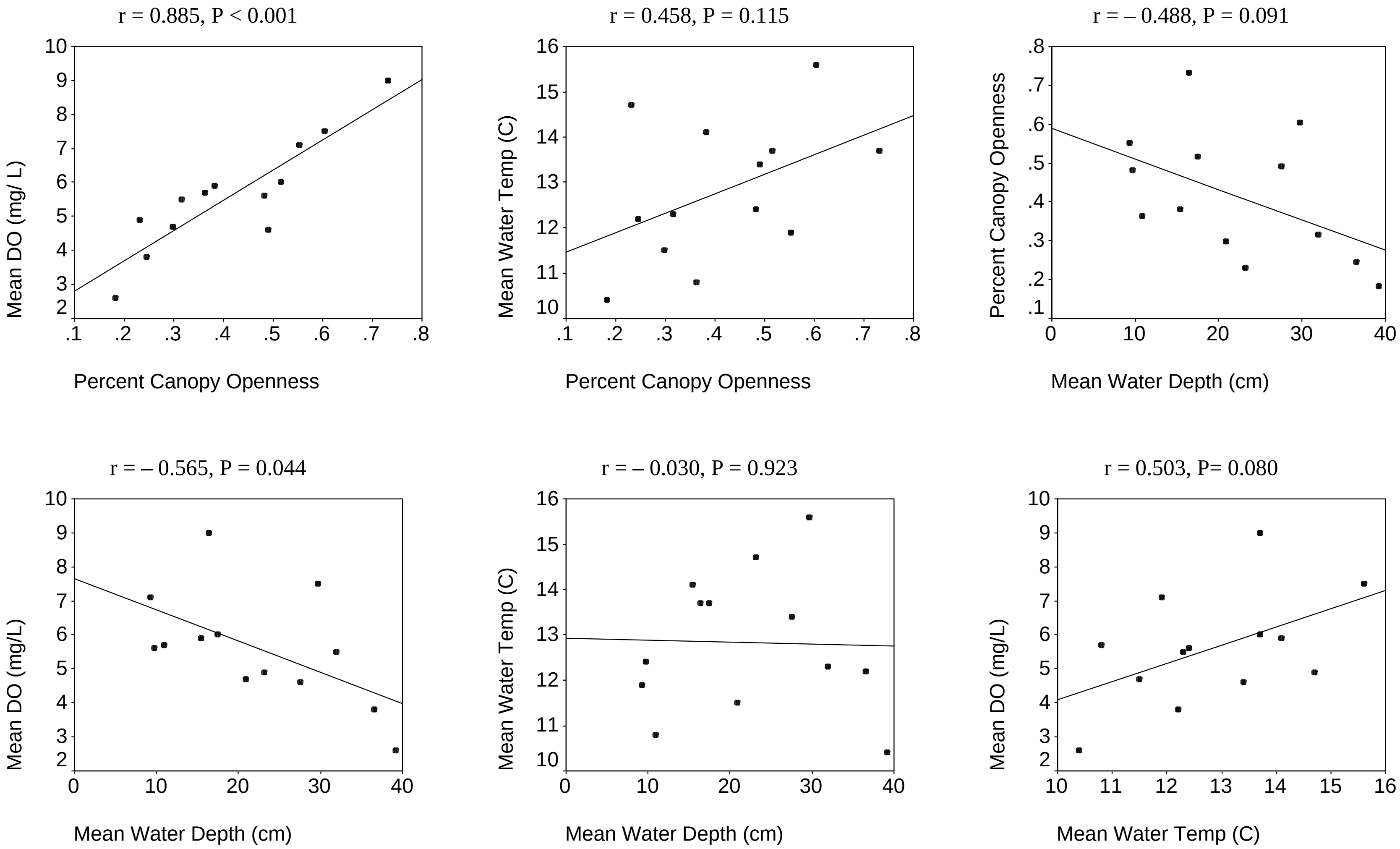


Figure 1. Correlations between dissolved oxygen (DO) concentration, water temperature, water depth, and canopy coverage in 13 wetlands used by *Ambystoma cingulatum* for breeding on Eglin Air Force Base in Okaloosa County, Florida, winter 2003.

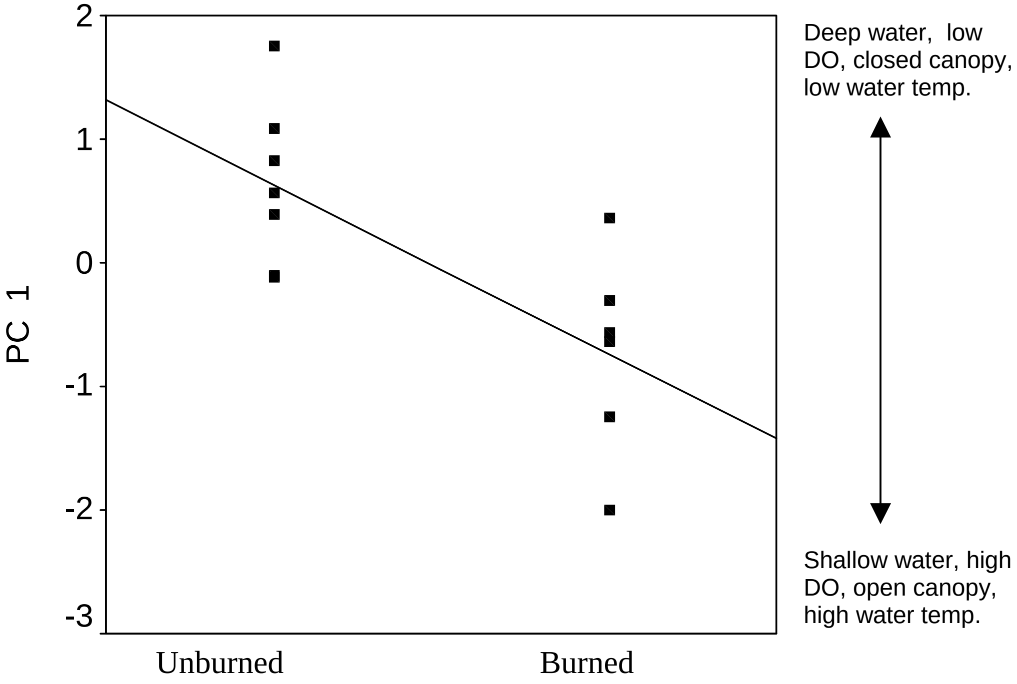


Figure 2. Principle component weightings between burned (N = 7) and unburned (N = 6) wetlands used for breeding by *Ambystoma cingulatum* on Eglin Air Force Base in Okaloosa County, Florida, winter 2003. Burned wetlands had a fire within the last 5 years. Unburned wetlands were not burned in over 10 years.

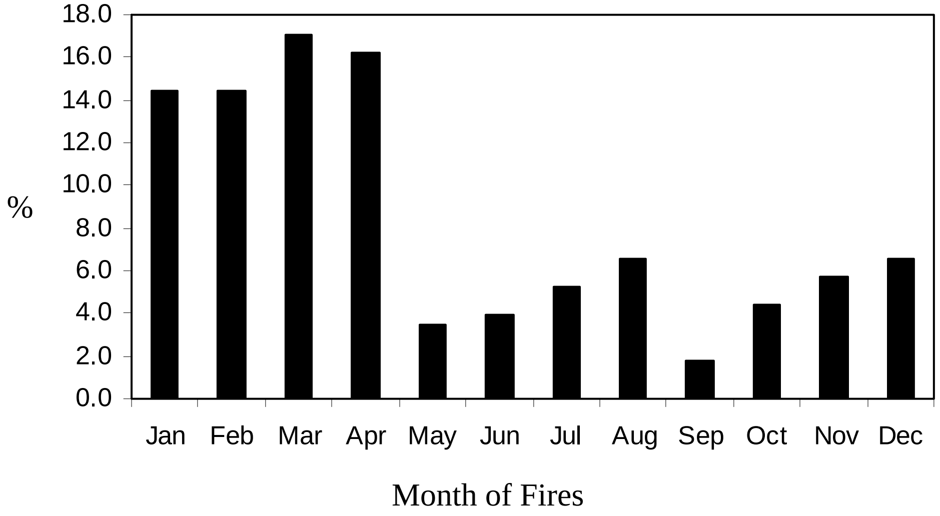


Figure 3. Percentage of fires (Total N = 228) by month over an 11–year period in locations containing flatwoods salamander breeding wetlands (see Table 1 for wetland locations).

SUMMARY

Florida bog frog: species profile

The Florida bog frog (Ranidae: *Rana okaloosae*) was discovered in 1982 on Eglin Air Force Base (AFB)(Moler 1985a). Moler classified *R. okaloosae* in the *R. catesbeiana* group, a conclusion later supported by Austin et al. (2003) using mitochondrial DNA analysis. Austin et al. (2003) found little genetic divergence between the bog frog and its closest relative, the bronze frog (*R. clamitans clamitans*), and suggested a recent Pleistocene origin. Suspected hybrids between the two species have been found (Austin et al. 2003, personal observations).

Prior to my dissertation, no previous ecological research had been conducted on *R. okaloosae*. Besides the species description (Moler 1985a), available data were limited primarily to surveys (Moler 1986, Moler 1993, Printiss and Hipes 1999, Enge 2002, Bishop 2004), a phylogenetic analysis of the *Rana catesbeiana* group (Austin et al. 2003) and a natural history note on larval predation (Bishop 2003). Other publications included field guides (e.g., Ashton and Ashton 1988, Conant and Collins 1991, Moler 1992, Bartlett and Bartlett 1999, Hipes et al. 2001) and popular science articles (e.g., Moler 1985b, Stone 1986, Matthews 1996), but they contained little new information. The Florida Fish and Wildlife Conservation Commission maintains an online herpetological database (http://wildflorida.org/herpbibl/default.asp) that lists all citations for *R. okaloosae* and other species.

Range.—The current documented range of *R. okaloosae* is restricted to the East Bay, Yellow, and Shoal River drainages in Okaloosa, Santa Rosa, and Walton counties in northwest Florida (Bishop 2004). Bog frogs have been verified at 58 locations on Eglin

AFB (Bishop 2004) and 3 locations outside of Eglin (Moler 1986). Most of the locations within Eglin AFB occur at different road crossings of a limited number of drainages. There has been little effort to survey for *R. okaloosae* outside of Eglin AFB. The limited distribution of the bog frog provides support for a recent speciation event for the bog frog (Austin et al. 2003).

Morphological description.—Morphological descriptions of *R. okaloosae* can be found in Moler (1986, 1993), the field guides listed above, and Jackson (2004). Bog frogs have reduced webbing between their toes (Moler 1985a), a characteristic that enables consistent differentiation from small *R. clamitans*, a species with similar coloration. Bog frogs have a brown dorsum and a lighter–colored dorsolateral ridge. The venter is white and typically contains dark worm–like markings. The throat is yellow in both sexes, but the amount of yellow varies among individuals. The tympanum is slightly larger in males than in females, but is not substantially larger than the eye as is typical in many other male ranids. Males can be distinguished from females by applying slight pressure to the abdomen and observing the paired external vocal sacs (Appendix, Figure 1). The bog frog is the smallest ranid in North America. The largest male I measured was 53.9 mm SVL and 14.0 g, and the largest captured female was 48.8 mm SVL and 9.8 g. Females may reach larger sizes, but finding them is more difficult than finding males because they rarely vocalize.

Tadpoles have a brown dorsum, often containing dark spots, and a black belly with white spots (Moler 1985a, 1993). Differentiating between small *R. okaloosae* and *R. clamitans* tadpoles is difficult because ventral spots are not fully developed in small individuals (Bishop 2004). Larger tadpoles of both species have white belly spots, but *R.*

okaloosae tadpoles typically have spots that are separated, whereas the spots on *R. clamitans* merge together (Appendix, Figure 2). Another possible distinguishing characteristic between tadpoles may be differences in mouthparts (Appendix, Figure 3).

Habitat.—Bog frogs occur in several different permanent–water habitats, including the bends of streams, in overflow areas adjacent to streams, in headwaters of streams, in isolated seepages, downstream of impoundments, and rarely on the edge of ponds above impoundments (Bishop 2004). I also have captured individuals in an ephemeral cypress wetland and in a ditch, but these are atypical habitats. Within all of these habitats, individuals select shallow, low–flow sites, typically near emergent structure (e.g., vegetation, woody debris, stream bank) and cover. Other descriptions of the habitat and associated vegetation can be found in Moler (1985a), Printiss and Hipes (1999), and Jackson (2004). Tadpoles occur in the same habitats as adults and can be captured year–round by dipnetting the low–flow, heavily silted areas (Bishop 2004).

Ecology.—The Florida bog frog has a prolonged breeding season, where males call from March through August or September. I have found egg masses March–August, with the peak of the reproductive season occurring in mid–summer. Egg masses are small and consist of a few hundred eggs, usually deposited as a single surface layer. Gravid females can be identified by shining a concentrated light source through the abdomen.

Males choose calling positions away from other males, although there may be nearby satellite males who can be identified by their lack of calling and crouching posture. Males may fertilize several clutches in a season; the number of clutches fertilized is correlated positively with the number of nights spent calling. It is unknown if female bog fogs lay multiple clutches in a season, but given their long breeding season and their close relationship with *R. clamitans*, a species that does have multiple clutches (Wells 1976), they likely do. Limited observations suggest that females must initiate amplexus, upon which they deposit eggs in a location near the calling site of the male. It is unknown if females choose oviposition sites based on the quality of the male, the quality of his territory, or a combination of the two factors.

The behavior, growth, and distribution of bog frog tadpoles have not been studied in great detail. Tadpoles can be captured year–round in the same locations used as breeding sites. It is unknown if tadpoles must over–winter prior to metamorphosis. Streams potentially provide a corridor system through which tadpoles could disperse. However, limited laboratory observations suggest that *R. okaloosae* tadpoles swim very little. Juvenile bog frogs can be captured during the summer months. The few (N = 4) metamorphs (i.e., still have tail bud) I measured were 18–20 mm SVL.

Adult bog frogs move little during the breeding season. I estimated home ranges for bog frogs, but my data are limited due to the small number of recaptures per individual. The winter ecology of bog frogs is unknown. A single individual has been found buried in moss adjacent to a stream. Bog frogs likely remain in the same location throughout the year, possibly seeking refuge from cold temperatures under the banks of the stream and surrounding vegetation. There is limited evidence that dispersal into and out of breeding sites may occur.

Male bog frogs have 3 common call types, plus a release call that occasionally is given by captured individuals. Females have a call similar to the type II chuck call of males, but this call is rare and its function is unknown. When an actively–calling male is broadcast a call from an unfamiliar conspecific male, he typically decreases the number of type I advertisement calls and increases the number of type II chuck calls. Bog frogs also have a response call (type III), generally given in simultaneously with the calls of other conspecific males but also directed occasionally to calling *R. clamitans*. The limited movements of bog frogs, the spatial separation of calling males, the prolonged breeding season, the vocal response of males to the playback of conspecific calls, and the close relationship with *R. clamitans* suggest that *R. okaloosae* likely is territorial.

The sex ratio at breeding locations is assumed to be 1:1, although, as mentioned previously, males are captured more frequently than females because of their vocalizations. Predation events on adults have never been observed, but tadpoles are believed to be depredated heavily by snakes (Bishop 2003). The number of individuals present at a location differs among years, as does the size distribution. It is unknown if observed yearly differences at my study site were the result of natural population changes or observer–induced mortality, but the high recapture rate of toe–clipped individuals throughout the season suggests it is the former.

Status and threats.—The bog frog is listed as a species of special concern in the state of Florida. It was enrolled in the species at risk program, a partnership with the U.S. Fish and Wildlife Service and the Department of Defense. It was selected as the candidate species for the Air Force because most known locations are within the boundary of Eglin AFB. Jackson's (2004) management plan for the bog frog on Eglin AFB reviews potential threats and management suggestions. There are no known threats to this species. Potential threats to the species include: fire suppression, feral pigs, erosion from roads and borrow pits, flooding from impoundments or inadequate bridge crossings, invasive plants, military maneuvers, pollution, and diseases.

Management and research recommendations

Below are potential management issues for the Florida bog frog and flatwoods salamander. For each issue, I make management recommendations and suggest future research questions related to that issue. Many of the recommendations are based on logic and the best available supportive evidence, rather than my own research. For recommendations based directly on my research, I indicate the chapter from which these data are presented. I also list other research ideas that address interesting behavioral, ecological, or evolutionary issues.

I. Florida bog frog

Fire

Management recommendations

- Continue an aggressive prescribed burning program on Eglin AFB, preferably with a burning regime that includes summer fires. Based on the natural fire frequency in longleaf pine habitats, I suggest burning all *R. okaloosae* habitats a minimum of once every 5 years. Upland fires should be allowed to burn into riparian and other wetland habitats.
- Avoid plowing fire–breaks adjacent to bog frog habitats. Rehabilitate fire–breaks that are sufficient to exclude fires or alter hydrology. Avoid soil compaction and rut formation from mechanical equipment during the wet season.

Potential research questions

- Does fire in riparian areas create habitat (e.g., open canopy, warm water temperatures) that bog frogs prefer?
- Does fire (or fire suppression) in surrounding uplands alter the hydrology in bog frog sites?
- Does adding canopy coverage (e.g. tent) affect the distribution of frogs and egg masses within a site?

Protection areas

Management recommendations

- Avoid heavy foot traffic (e.g., troop movements) and all vehicle traffic in bog frog habitats. Egg masses can be found March–Aug and tadpoles can be found year-round at breeding sites (Ch. 1, Bishop 2004) and may be at risk to foot or vehicle traffic in wetland areas. A minimum of 75 m up and downstream (total 150 m) should be protected at bog frog breeding locations (Ch. 2). Whenever possible, the entire stream should be protected.

Potential research questions

- Where do bog frogs seek refuge in the winter? Are my movement estimates adequate to serve as a recommendation for core protection areas? More research into the home range and habitat use of bog frogs will provide greater information on which areas should be protected.

- Are streamside management zones (SMZ) needed at bog frog sites? Does logging in riparian areas affect bog frog habitat? Do bog frogs use riparian areas for refuge in the winter?

Feral pigs

Management recommendations

- Continue the eradication program of feral pigs. Pigs destroy the banks of streams, locations used for refuge by bog frogs (personal observations).

Potential research questions

- What is the extent of feral pig damage in bog frog sites?
- Do bog frogs routinely use the microhabitats disturbed by foraging pigs? More research into the home range and habitat use of bog frogs will provide greater information on habitat use.

Chemicals and water quality

Management recommendations

- Avoid chemicals (e.g., herbicides, fire retardants) near aquatic habitats that may affect development, growth, and survivorship of amphibians.
- Control invasive plant species through mechanical removal. If herbicides are absolutely necessary for plant eradication, use spot applications.
- Continue to monitor the water quality through various indices (pollution, fish, invertebrates, etc.) in Eglin’s streams.

Potential research questions

- Do herbicides affect tadpoles, egg masses, or adult bog frogs? Some bog frog sites occur where power–lines intersect drainages (Bishop 2004). How does mechanical mowing of power–line corridors affect frogs? At what time of year should power–line corridor maintenance be done?
- Is there a minimum SMZ needed around bog frog sites to protect against fertilizer, herbicide, or pesticide runoff?

Erosion and Siltation

Management recommendations

- Minimize erosion at road–crossings and borrow pits. There is some debate about whether silt actually is good for bog frogs because it increases the shallow water microhabitat they prefer. However, unless future data suggest otherwise, erosion should be controlled to increase the overall water quality.

Potential research questions

- Does rehabilitation of heavily eroded sites affect the relative abundance or distribution of bog frog adults or larvae?

Monitoring and distribution

Management recommendations

- Continue the annual monitoring program (Bishop 2004) to detect any changes in the relative abundance or distribution of bog frogs on Eglin.

Potential research questions

- How does calling activity correlate with environmental variables (e.g., rain, lunar phase)? Can frog–loggers and call surveys consistently estimate relative abundance and reproductive activity of *R. okaloosae*?
- Where are bog frogs located outside of Eglin AFB?
- What factors limit the distribution of bog frogs? There appear to be distinct gaps in the distribution of bog frogs on Eglin (Bishop 2004). Are there habitat characteristics associated with the occurrence of *R. okaloosae* within a stream and among streams?
- What is a bog frog population? Jim Austin (currently a post-doc at Cornell; future faculty member at the University of Florida) currently is developing genetic markers for *R. okaloosae*. His work may verify the hybridization between bog and bronze frogs and lay the genetic foundation for future population structure studies.
- What is the distribution, habitat, and movement patterns of tadpoles? Do tadpoles use streams as corridors to move between locations?

<u>Other potential research questions</u>

- How long do bog frogs live? Can adults be aged using skeletochronology?
- Do bog frogs distinguish between familiar and unfamiliar calls; do they exhibit a ‘dear enemy’ effect?
- What is the function of type III response calls?
- Do tadpoles select areas with less predation risk?

- Do males choose calling sites or females choose oviposition areas with less potential for competition or predation?
- Do bog and bronze frog tadpoles compete with each other? Do growth or survivorship rates differ between the two species?
- Does the limited toe–webbing of *R. okaloosae* affect performance (e.g., swimming, predator escape) and distribution? Can morphological differences between bog and bronze frogs provide any information about their evolutionary history?
- Do bog frogs differ in body size, growth rates, and mortality rates at different locations?

II. Flatwoods salamander

Because the species profile for flatwoods salamanders (Ambystomatidae: *Ambystoma cingulatum*) has been reviewed elsewhere (Palis 1996), I will not review the same information here. A recovery plan for the flatwoods salamander is in preparation (Linda LaClaire, U.S. Fish and Wildlife Service, pers. comm.). Once available, it will provide more detailed management guidelines.

<u>Fire</u>

Management recommendations

- Continue an aggressive prescribed burning program on Eglin AFB, preferably with a burning regime that includes summer fires. Locations that contain salamanders should be burned a minimum of once every 3–4 years (see Ch. 6). Upland fires should be allowed to burn into riparian and other wetland habitats.

- Avoid burning known and potential breeding areas October–January, the time when adults are migrating to breeding wetlands. Similarly, avoid burning March–April, the time when metamorphs are emerging. Both of these factors support a burning regime focused on lighting season prescribed fires. However, if these months are the only times when prescribed fires are possible in fire–suppressed areas, I suggest burning rather than not.
- Avoid plowing fire–breaks adjacent to salamander breeding habitats. Rehabilitate fire–breaks that are sufficient to exclude fires or alter hydrology. Avoid soil compaction and rut formation from mechanical equipment during the wet season.

Potential research questions

- How do winter and summer fires affect the water chemistry of breeding wetlands? Fires affect pH, nitrates, and other nutrients, variables which may affect developing larvae.
- If fires are conducted in summer, how frequently do they burn through the breeding wetlands? What changes does fire have on the wetland?
- Are larvae more abundant or do they grow faster in burned wetlands or burned sections within a wetland?
- Does mechanical removal of canopy affect larval microhabitat, distribution, or abundance?

Protection areas

Management recommendations

- Keep all vehicle traffic, troop movements, and forestry operations out of salamander breeding wetlands and surrounding uplands. Until the recovery plan is published, guidelines for buffer areas (450 m) can be found in U. S. Fish and Wildlife Service (1999).

Potential research questions

- How big are adult home ranges and microhabitat? This eventually may become possible with technological advances in transmitter size. Current guidelines for buffer areas are based on limited data and other taxa.

Chemicals and water quality

Management recommendations

- Avoid chemicals (e.g., herbicides, fire retardants) near aquatic habitats that may affect development, growth, and survivorship of amphibians.
- Control invasive plants by mechanical removal. If herbicides are absolutely necessary, use spot applications when wetlands are dry.

Potential research questions

- How big of a buffer zone is needed around wetlands to protect against fertilizer or herbicide runoff?
- Do commonly used pesticides or fertilizers affect growth or survivorship of larvae?

Monitoring and distribution

Management recommendations

- Continue the annual monitoring program (Bishop 2004). Long–term monitoring datasets are needed to understand the population dynamics and conservation needs of *A. cingulatum*.
- Work with other biologists and agencies to develop a standardized monitoring, management, and research protocol.

Potential research questions

- What is flatwoods salamander population? The current method of delineating population structure is based on limited data on movements. Genetic samples could be collected from larvae and compared across locations to determine unique populations. This information eventually may be important if reintroductions are planned.

LITERATURE CITED

Ashton, R. E., Jr., and P. S. Ashton. 1988. Handbook of reptiles and amphibians of Florida, part 3: The amphibians. Windward Publishing, Inc., Miami, Florida.

Austin, J. D., S. C. Lougheed, P. E. Moler, and P. T. Boag. 2003. Phylogenetics, zoogeography, and the role of dispersal and vicariance in the evolution of the *Rana catesbeiana* (Anura: Ranidae) species group. Biological Journal of the Linnean Society 80: 601–624.

Bartlett, R. D., and P. P. Bartlett. 1999. A field guide to Florida reptiles and amphibians. Gulf Publishing Company, Houston, Texas.

Bishop, D. C. 2003. *Rana okaloosae* (Florida bog frog): predation. Herpetological Review 34:235.

Bishop, D. C. 2004. Summary of surveys for Florida bog frogs and flatwoods salamanders on Eglin Air Force Base through 2004. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Conant, R., and J. T. Collins. 1991. A field guide to reptiles and amphibians: eastern and central North America, third ed. Houghton Mifflin Co., Boston, Massachusetts.

Enge, K. M. 2002. Herpetofaunal drift-fence survey of steephead ravines and seepage bogs in the western Florida panhandle. Final Performance Report, Florida Fish and Wildlife Cons. Comm., Tallahassee.

Hipes, D., D. R. Jackson, K. NeSmith, D. Printiss, and K. Brandt. 2001. Field guide to the rare animals of Florida. Florida Natural Areas Inventory, Tallahassee.

Jackson, D. R. 2004. Management guidance document for species at risk on Eglin Air Force Base, Niceville, Florida: Florida bog frog (*Rana okaloosae*). Final report to U.S. Fish and Wildlife Service, Panama City, Florida. Florida Natural Areas Inventory, Tallahassee, USA.

Matthews, J. 1996. Delicate balance: Florida bog frog (*Rana okaloosae* Moler). Florida Wildlife 50:19.

Moler, P. E. 1985a. A new species of frog (Ranidae: *Rana*) from northwestern Florida. Copeia 1985b:379-383.

Moler, P. E. 1985b. Delicate balance. Species: Bog frog (*Rana okaloosae*). Florida Wildlife 39 (July-August):31.

Moler, P. E. 1986. Distribution of the Florida bog frog. Florida Game and Fresh Water Fish Commission, Bureau Wildlife Research Final Report, Tallahassee, Florida.

Moler, P. E. 1992. Florida bog frog, *Rana okaloosae* Moler. Pages 30-33 in P. E. Moler, editor. Rare and Endangered Biota of Florida, Vol. 3: Amphibians and reptiles. University Press of Florida, Gainesville.

Moler, P. E. 1993. *Rana okaloosae* Moler. Florida bog frog. Catalogue of American Amphibians and Reptiles 561:1-3.

Printiss, D. J., and D. L. Hipes. 1999. Rare amphibian and reptile survey of Eglin Air Force Base, Florida. Final report to U.S. Dept. of Defense. Florida Natural Areas Inventory, Tallahassee.

Stone, L. M. 1986. “New” frog in the bog. Animal Kingdom 89:40-43.

USFWS. 1999. Endangered and threatened wildlife and plants: final rule to list the flatwoods salamander as a threatened species. Federal Register 64:15691–15704.

Wells, K. D. 1976. Multiple egg clutches in the green frog (*Rana clamitans*). Herpetologica 32:85–87.

APPENDIX

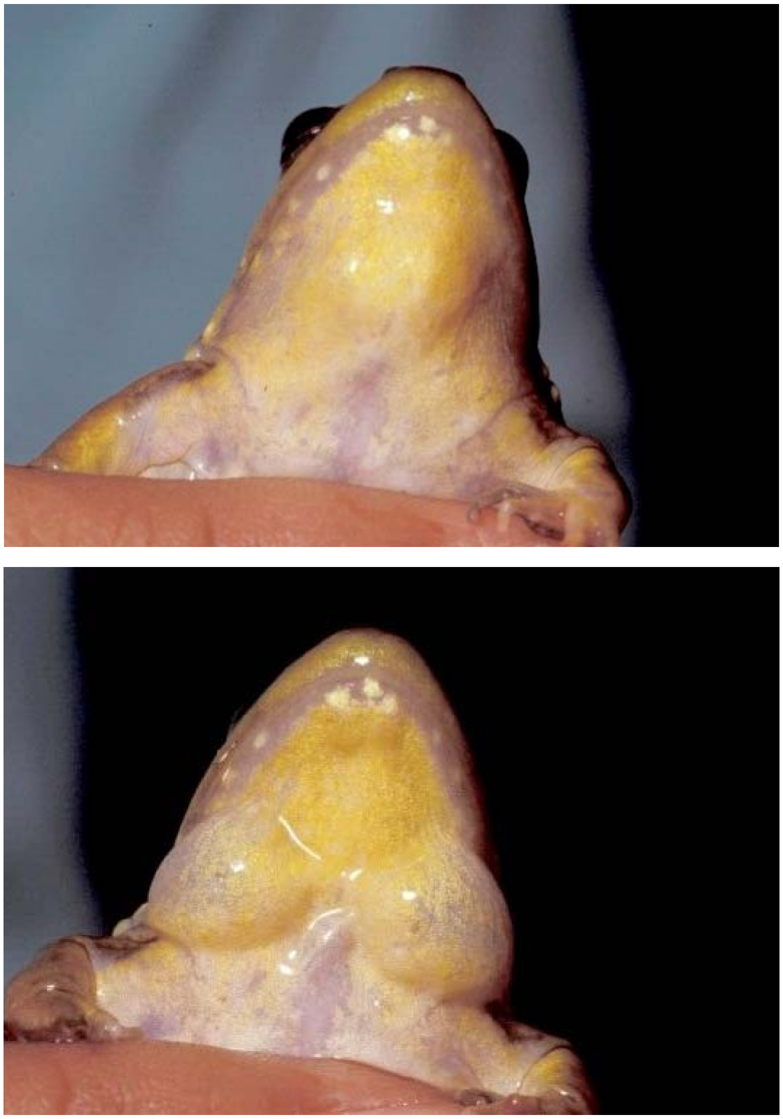


Figure 1. Technique used to identify male *Rana okaloosae*. Vocal sacs are external on males and can be seen when slight pressure is applied to the abdomen. Females lack external vocal sacs.

IDENTIFYING *RANA OKALOOSAE* AND *R. CLAMITANS* TADPOLES

The Florida bog frog (*Rana okaloosae*) and bronze frog (*Rana clamitans*) are close relatives (Austin et al. 2003) that are sympatric throughout the entire range of the former (Moler 1985, 1993, Bishop 2004). The breeding seasons and oviposition locations overlap between the species (Ch. 1). Tadpoles of both species can be found throughout the year (Moler 1985, Bishop 2004). Moler (1985, 1993) suggested that *R. okaloosae* and *R. clamitans* tadpoles can be distinguished by the presence of white ventral spots on the former. However, this can be misleading because both species have white ventral spots in locations where they are sympatric.

On larger tadpoles, the ventral spots are well–formed and typically are a good identification characteristic. The spots on *R. oklaloosae* are clearly separated against a black background, whereas in *R. clamitans*, the spots typically merge together (Figure 2). Ventral spots fade in preserved specimens. The spotting pattern in hybrids is unknown. I had limited success identifying small tadpoles (Bishop 2004). Ventral spots are not well developed in small tadpoles and, therefore, cannot be a reliable identification characteristic. In this note, I provide greater detail and pictures on the posterior tooth rows, a characteristic that may provide a reliable method for identification of tadpoles.

Moler (1985) provided a diagram of the oral disc of *R. okaloosae* and identified the labial tooth rows as 1–2(2) / 2–3 (following Altig 1970), i.e.; there are 1–2 anterior rows of teeth with the 2nd row having a gap and 2–3 posterior rows of teeth. *Rana clamitans* has a similar tooth pattern of 1–2 (2) / 3 (Fanning 1966, Altig 1970). I collected 10 tadpoles of each species (stages: 23–38) from Live Oak Creek (Site ID 125, Appendix 1, Bishop 2004) on Eglin Air Force Base in Okaloosa County, Florida in 2002 and 2004. I preserved specimens in formalin and stored them in 70% ETOH. I looked at the oral disc of all tadpoles to evaluate whether the number and relative size of the posterior tooth rows could be used to distinguish between the two species.

As mentioned by Moler (1985), the P–3 row may be missing in *R. okaloosae*. If it is present, however, it is very short and less obvious than the P–3 row in *R. clamitans* (Figure 3). The P–3 row always was present in the bronze frog tadpoles I observed. With a sufficient hand lens, these differences may be able to be detected in the field or in preserved specimens. More individuals should be examined to verify this is a consistent distinguishing characteristic. The tooth row pattern of hybrids is unknown.

LITERATURE CITED

Altig, R. 1970. A key to the tadpoles of the continental United States and Canada. Herpetologica 26:180–207.

Austin, J. D., S. C. Lougheed, P. E. Moler, and P. T. Boag. 2003. Phylogenetics, zoogeography, and the role of dispersal and vicariance in the evolution of the *Rana catesbeiana* (Anura: Ranidae) species group. Biological Journal of the Linnean Society 80: 601–624.

Bishop, D. C. 2004. Summary of surveys for Florida bog frogs and flatwoods salamanders on Eglin Air Force Base through 2004. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Fanning, S. A. 1966. A synopsis and key to the tadpoles of Florida. M. S. Thesis. Florida State University, Tallahassee, Florida.

Moler, P. E. 1985. A new species of frog (Ranidae: *Rana*) from northwestern Florida. Copeia 1985:379–383.

Moler, P. E. 1993. *Rana okaloosae* Moler Florida bog frog. Catalogue of American Amphibians and Reptiles 561:1–3.

Figure 2. Comparison of belly spots between living *Rana okaloosae* (left) and *R. clamitans* (right) tadpoles. Note the greater separation of spots on the belly of *R. okaloosae*.

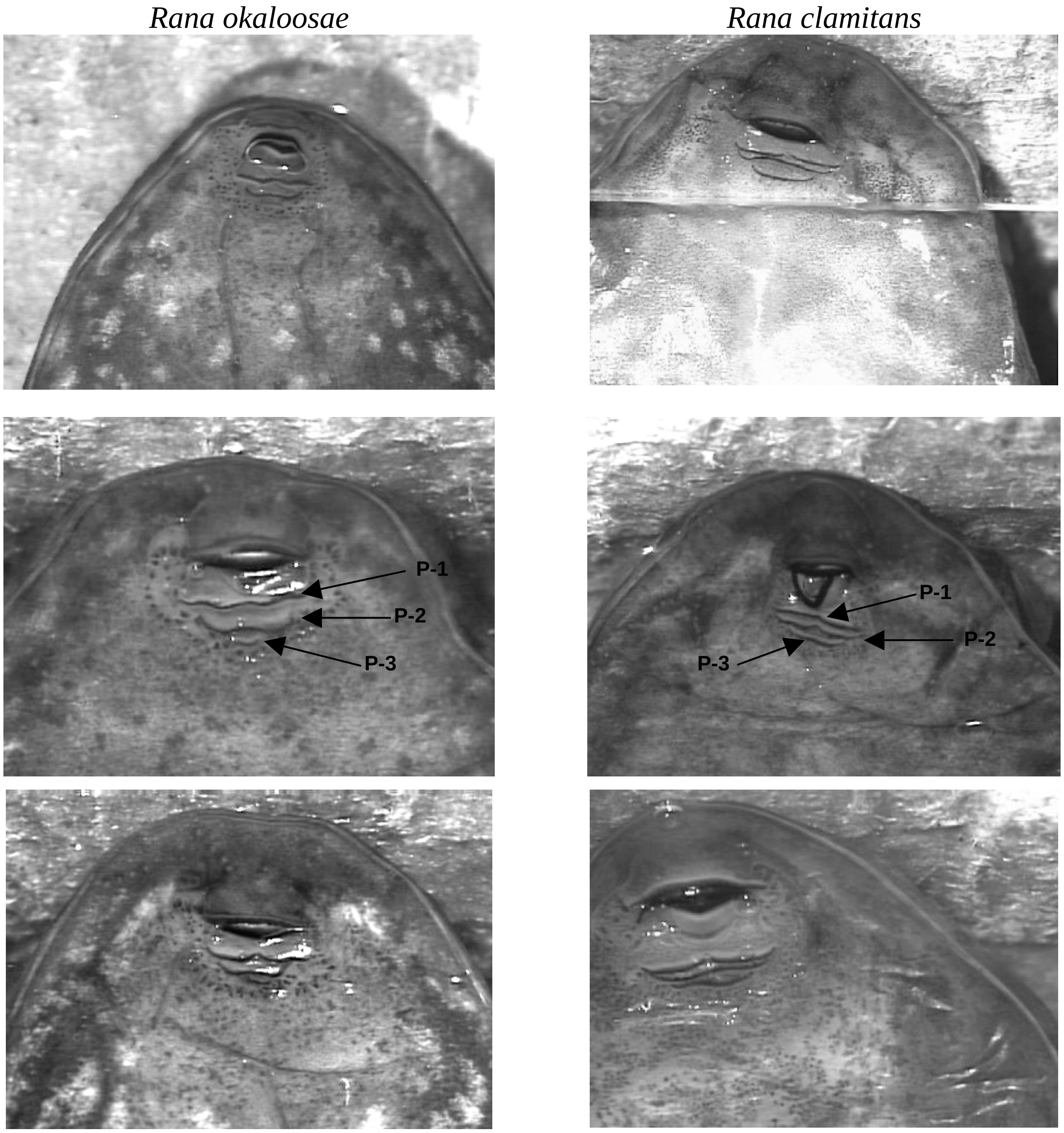


Figure 3. Comparison of posterior tooth rows between Florida Bog frog (*Rana okaloosae*) and bronze frog (*R. clamitans*) tadpoles. Nomenclature follows Altig (1970). The P–3 row is longer in *R. clamitans* than in *R. okaloosae*. The P-3 row may be missing in *R. okaloosae*. Pictures are slightly distorted because a glass cover was used to make mouth parts visible.

VITA

David Christopher Bishop was born May 9, 1974 in Florence, South Carolina. He graduated from South Florence High School in 1992, after which he attended Presbyterian College, in Clinton, South Carolina. He received a B.S. in biology in 1996. After graduation, he taught biology and physical science at Gilbert High School in Lexington County, South Carolina for two years. He then enrolled in graduate school at the University of Tennessee in Knoxville. For his master's research, he studied the winter ecology of *Anolis carolinensis* in the foothills of the Smoky Mountains. He was awarded the M.S. degree in Ecology and Evolutionary Biology in December 2000. He enrolled in the Ph.D. program in the Department of Fisheries and Wildlife Sciences at Virginia Tech in August 2001. For his doctoral degree, he researched the Florida bog frog (*Rana okaloosae*) and the flatwoods salamander (*Ambystoma cingulatum*) on Eglin Air Force Base in northwest Florida. The Ph.D. was awarded in July 2005.