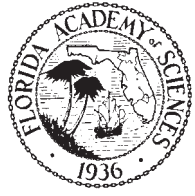


# *Florida Scientist*

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## COMPARATIVE MORPHOLOGY AND IDENTIFICATION OF FLORIDA BOG FROG AND BRONZE FROG TADPOLES

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**ABSTRACT:** *Identifying adult frog species in the field generally is straightforward; however, differentiating among tadpoles may be less clear and may complicate conservation and management activities. We described both the qualitative and quantitative morphological differences of tadpoles of a rare endemic species, Florida Bog Frog (*Rana okaloosae*), and a more common heterospecific, Bronze Frog (*Rana clamitans clamitans*). *Rana okaloosae* tadpoles had more pronounced tail spotting, greater pigmentation on the tail, and more defined ventral spotting than *R. c. clamitans*. The mean proportional tail height of fresh specimens and preserved specimens of *R. c. clamitans* was greater than *R. okaloosae* (fresh:  $P = 0.043$ ,  $t = -2.10$ ,  $n = 20/\text{species}$ ; preserved:  $P = 0.026$ ,  $t = -2.43$ ,  $n = 10/\text{species}$ ). The mean proportional length of the P3 tooth row was greater in *R. c. clamitans* than in *R. okaloosae* ( $P < 0.0001$ ,  $t = 9.58$ ,  $n = 10$  and  $5$ , respectively). In the field, qualitative assessment of tail coloration and ventral spotting patterns appear useful in identifying tadpoles of these species. Additionally, the differences in the length of the P3 tooth rows may help to positively identify the two species of tadpoles, but will only be feasible for anaesthetized or preserved specimens.*

**Key Words:** Florida, *Rana okaloosae*, *Rana clamitans clamitans*, Tadpoles

THE FLORIDA BOG FROG (*Rana okaloosae*) is endemic to northwestern Florida and occurs almost exclusively in seeps and bogs in Okaloosa, Santa Rosa, and Walton Counties (Moler, 1985, 1993; Bishop, 2005). This seepage stream habitat and the geographic range of *R. okaloosae* predominantly occur within the boundaries of Eglin Air Force Base (Moler, 1985). The impoundment and natural habitat succession of streams inhabited by *R. okaloosae* may be the two largest threats to the species (Moler, 1992). In addition to concerns related to habitat, hybridization with the Bronze Frog (*R. c. clamitans*) is considered a potential conservation concern (Bishop, 2005; Gorman et al., 2009). *Rana c. clamitans* is a more common species and is widely distributed across the southeastern United States. Although adults of the two species are easily distinguished (Bishop, 2005; Gorman et al., 2009), tadpoles of each are more difficult to identify to species (Bishop, 2005), especially early in development. Under field conditions, such as conducting surveys, identification is complicated further, but even in taxonomic studies younger tadpoles are

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more difficult to classify (Grosjean, 2005). Further, little research has described morphology of *R. okaloosae* tadpoles.

Several morphological features can be used to differentiate among species of tadpoles. For example, the oral surface features, the chondrocranium, and the hyobranchial anatomies have been used to examine the relationships among several species of frogs native to Chile and Argentina (Formas and Brieva, 2004). Further, external morphology and oral features have been examined for the *Scinax* group of frogs (Conte et al., 2007). Oral features that can be observed easily include soft mouthparts, buccopharyngeal cavity, oral discs and submarginal papillae, and the keratinized portions of the mouth (upper and lower jaw sheaths) (Altig, 2007). *Rana okaloosae* have labial tooth rows, of 1-2(2)/2-3; meaning 2-3 posterior tooth rows and 1-2 anterior tooth rows with a gap in the second row (Moler, 1985; Bishop, 2005). *Rana c. clamitans* have labial tooth row patterns of 1-2(2)/3; meaning 1-2 anterior tooth rows with a gap in the second row and always 3 posterior tooth rows (Altig, 1970; Bishop, 2005).

Although the use of mouthparts is standard for taxonomy and identification of tadpoles, this technique requires microscopy on dead or anaesthetized specimens. In order to identify live tadpoles in the field, other criteria are needed. Therefore, relationships among tail heights, tail lengths, snout-vent lengths, and total lengths may help distinguish among species of tadpoles. Further, the use of qualitative measurements such as colorations and markings also may assist the determination of tadpole species. *Rana okaloosae* tadpoles have olive brown coloration throughout the body with numerous buff spots along the tail, and numerous white spots on the ventral surface (Moler, 1985; 1993; Bishop, 2005). On *R. okaloosae* ventral spots are silver and overall body color can range from olive- brown to russet (Altig et al., 1998). Both *R. okaloosae* and *R. c. clamitans* are described as having ventral white spotting patterns, which can cause some confusion when trying to differentiate between the two species. However, the ventral spots of *R. c. clamitans* are reported to be less defined and not as clearly set against the black ventral surface (Bishop, 2005). Further, there are typically no spots on the tail of *R. c. clamitans*, but the posterior one-third of the tail may have black, haphazard, rectangular-shaped markings and the fin may be densely speckled (Altig et al., 1998).

Our goal was to test possible methods for differentiating between two sympatric tadpoles in an attempt to acquire identification tools for the laboratory and the field. In particular we were interested in differentiating between young tadpoles of these two species. Other researchers have described identifiable characteristics for larger more developed tadpoles (Moler, 1993; Bishop, 2005), but early in development these two species are more similar. Therefore we examined whether (1) *R. c. clamitans* had a greater proportional tail height than *R. okaloosae*, (2) the posterior P-3 rows of *R. okaloosae* were smaller (or not present) compared to *R. c. clamitans*, and (3) the coloration of the tails of each species and the spotting on ventral surfaces of the tadpoles were different.

**METHODS**—We used both quantitative and qualitative methods to study the differences between *R. okaloosae* and *R. c. clamitans*. We collected egg masses of *R. okaloosae* and *R. c. clamitans* on 28 May 2007 and raised both species in aquaria as part of a related experiment. *R. okaloosae* tadpoles hatched on 29 May 2007 and *R. c. clamitans* tadpoles hatched on 30 May 2007. Tadpoles were reared until specimens of both species were euthanized on 16 August 2007. We euthanized tadpoles with chlorotone (1,1,1-Trichloro-2-methyl-2-propanol) and immediately measured snout-vent length, maximum tail height, and tail length using an Olympus SZ61 dissecting microscope with an Olympus Q color 3 digital camera (Olympus America, Inc., Center Valley, PA) and ImagePro 6.1 software (Media Cybernetics, Inc., Silver Springs, MD). After measuring, tadpoles were fixed in a 10% formalin solution. We measured a subset of the fixed tadpoles on 22 January 2008 using the same microscope to confirm there were no substantial changes to tadpole morphology following fixation. For both comparisons, we divided the tail height by the total tail length to create a proportional measure of tail height to account for different sized individuals.

Secondly, we evaluated differences in posterior teeth rows on the oral disc of tadpoles. We measured lengths of the P1 and P3 tooth rows. We compared the P3 rows between species and corrected for individual size by dividing the P3 by the length of P1 row. In most individuals the oral discs were exposed by folding back the lower jaw, but some specimens required a clear plate to hold the oral disc in place while digital photographs and measurements were recorded. For all quantitative measurements, pictures were taken first and then measurements were made on the scaled digital photographs. We used t-tests to examine differences between *R. okaloosae* and *R. c. clamitans*.

We examined the qualitative differences in fresh specimens, by noting the differences in tail spotting patterns, ventral spotting patterns, and overall coloration in photographs. Additionally, in July 2008 we conducted a field test of our qualitative results. We presented 20 live tadpoles (10 *R. okaloosae* and 10 *R. c. clamitans*) one at a time to 3 observers and allowed them to review our qualitative results and photographs (Fig. 1) from 2007 with minimal training. We then asked each observer to identify tadpoles to species.

**RESULTS**—The mean proportional tail height of fresh specimens and preserved specimens of *R. c. clamitans* was greater than *R. okaloosae* (fresh:  $P = 0.043$ ,  $t = -2.10$ ,  $n = 20/\text{species}$ ; preserved:  $P = 0.026$ ,  $t = -2.43$ ,  $n = 10/\text{species}$ ). The mean proportional length of the P3 tooth row was greater in *R. c. clamitans* than in *R. okaloosae* ( $P < 0.001$ ,  $t = 9.58$ ,  $n = 10$  and  $5$ , respectively). Mean proportional tail heights were  $0.276$  ( $SE = 0.009$ ) for *R. c. clamitans* and  $0.252$  ( $SE = 0.007$ ) for *R. okaloosae* fresh specimens (Fig. 2) and after tadpoles were fixed in formalin the mean proportional tail heights were  $0.267$  ( $SE = 0.009$ ) and  $0.224$  ( $SE = 0.015$ ), respectively (Fig. 2). Mean scaled posterior tooth rows were  $0.658$  ( $SE = 0.026$ ) for *R. c. clamitans* and  $0.186$  ( $SE = 0.047$ ) for *R. okaloosae* (Fig. 2).

The ventral spotting pattern of *R. okaloosae* was defined by a dark background with individual white spots more discernible than on the ventral surface of *R. c. clamitans*. *Rana c. clamitans* had white ventral spots characterized by jagged, undefined edges and were not well defined against a much lighter brown colored background. *Rana c. clamitans* had a lighter colored tail with rectangular speckles and lighter flecks of color, whereas, *R. okaloosae* had a tail that was much less transparent with an orange tint that included mottled dark colors in undefined shapes (Fig. 1). Tail coloration was apparent within days of hatching; however ventral coloration and spotting were not apparent until 5–6 weeks post hatching.

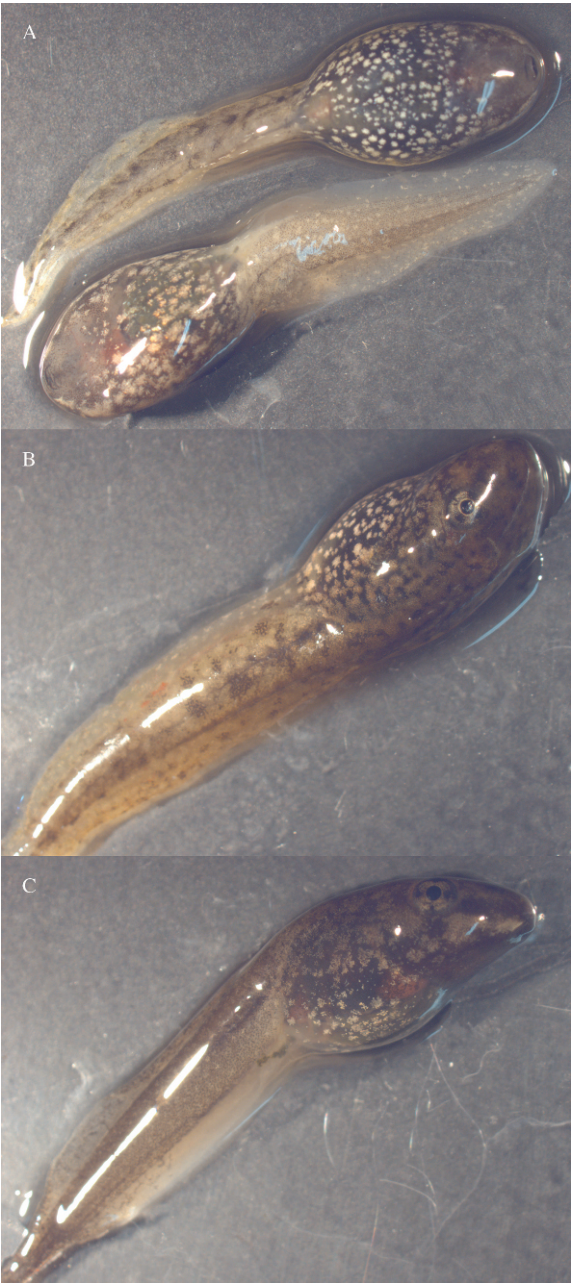


FIG. 1. (A) Comparison of the ventral surface and tail of *R. okaloosae* (top) and of *R. c. clamitans* (bottom) tadpoles. (B) *R. okaloosae* tadpole showing the tail and side view. (C) *R. c. clamitans* tadpole showing tail markings and side view.

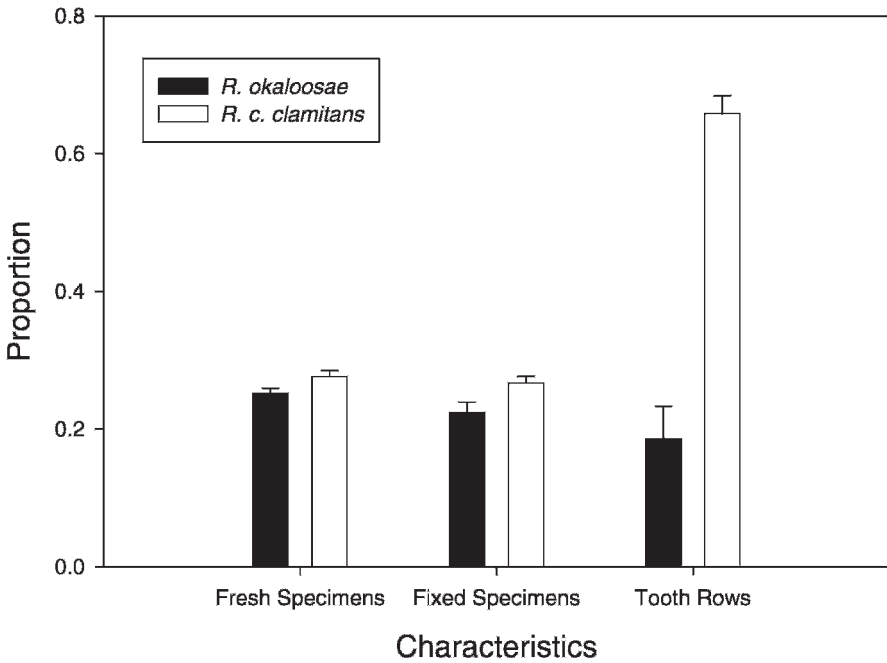


FIG. 2. Proportional means with 1 standard error of tail heights scaled by tail length of fresh specimens and fixed specimens of *R. okaloosae* and *R. c. clamitans*, and proportional means of P3 tooth rows scaled by the length of the P1 tooth rows of fixed specimens from Eglin Air Force Base, Okaloosa County, Florida.

Our test of our qualitative description and photographs (Fig. 1) revealed that 2 blind observers were able to identify 100% of tadpoles correctly and 1 observer identified 85% of the tadpoles correctly.

**DISCUSSION**—Morphological characteristics can be used to distinguish between tadpoles of *R. okaloosae* and *R. c. clamitans*. Spotting patterns on the ventral surface, tail coloration, and tail spotting patterns of each species can be used in the field. Differences in tail coloration were the first discernible qualitative trait as tadpoles developed post-hatching, followed by tail spotting, and lastly ventral spotting. Detecting variation in spotting patterns of tadpoles from both species becomes more feasible as they grow, and spotting patterns become more distinct. For definite identification in the laboratory on euthanized or anesthetized specimens, posterior teeth row proportions can positively identify the species, as can tail height proportions.

Previous studies have demonstrated that in the presence of predators, tail heights of tadpoles become taller and overall body lengths become shorter (Van Buskirk et al., 1997, 2003; Van Buskirk and McCollum, 2000; Vences et al. 2002; Kraft et al. 2005, 2006). Kraft and co-workers (2006) also found that tadpoles with this increased tail-height survived predation 25% more than

those without increased tail heights. Similarly, Vences and co-workers (2002) suggested that taller tail heights may be more beneficial in larger bodies of water where longer daily distances are moved than in smaller bodies of water. This is possibly due to thermoregulatory functions and possibly feeding activity (Vences et al., 2002). Thus, the greater tail heights of *R. c. clamitans* may be a result of their ability to exploit more habitat types as opposed to *R. okaloosae* that only selects small bogs and seeps in northwestern Florida (Moler, 1985, 1993; Bishop 2005). Additionally, the shortening or lack of the P-3 tooth in *R. okaloosae* may be an adaptation related to their habitat preferences; however, more work in this area is needed.

Overall, our results suggest that differentiating between the two sympatric tadpoles using qualitative features is possible. The ability to distinguish between these species will prove useful when conducting field surveys and will allow researchers to positively differentiate from other species that may be studied in relation to either of these tadpoles. *Rana okaloosae* is a rare species of frog and is a species of special concern in Florida due to the small size of its geographic range (Florida Fish and Wildlife Conservation Commission, 2007). Identifying this species of frog while in the larval stages has many benefits in the field, because it may allow for surveying in seasons when adults are not active (e.g., winter). In addition, identifying *R. okaloosae* tadpoles from sympatric species will be useful in monitoring populations of *R. okaloosae* (Bishop, 2005) and could prove useful in monitoring future management actions conducted for this species.

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