

Digital Recorders Increase Detection of *Eleutherodactylus* Frogs

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Improved methods for large-scale and long-term amphibian monitoring projects would aid species status assessments and identification of potentially declining species (e.g., Stuart et al. 2004). Acoustical surveys allow researchers to quickly determine species presence and calling activity level (Lips et al. 2001; Zimmerman 1994). The North American Amphibian Monitoring Program, a large-scale monitoring effort, uses acoustical surveys to monitor species in ponds and wetlands (United States Geological Survey 2004). A challenge of this approach is accurate species identification, which is particularly problematic in a tropical area with high anuran species diversity (e.g., anuran species richness in the Neotropics [2135] was greater than in the Neartic [90], Duellman 1999). Furthermore, abundant or loud species may lower the detection probability of other species.

An assumption that needs to be tested in acoustical surveys is that detection is highly correlated with the species presence or activity. Without an appropriate correction for detection probability, data will not accurately reflect the status of the species (MacKenzie et al. 2002). This may result in inappropriate management decisions that could waste time and money.

Digital recorders can be used as a standard method for species detection and determination of calling activity level in amphibian choruses in long-term monitoring projects. Digital recordings can be stored and transferred easily and can allow experts to analyze the recordings at their convenience. In addition, loud or abundant species can be filtered using computer software to increase the detection of other species. I tested the hypothesis that detection of species presence and calling activity level in anuran choruses will be higher when recordings are analyzed with the help of computer software compared to only hearing the recordings. The resulting increase in detection should result in improved population status data and reduced false negatives during species inventories (i.e., when a species is not detected due to interference).

Materials and Methods.—I recorded amphibian choruses at two sites in the Luquillo Experimental Forest (Tradewinds Trail site, 18.290°N, 65.798°W; Mount Britton Tower site, 18.303°N, 65.795°W), and at one site in the Carite State Forest (18.103°N, 66.035°W), Puerto Rico (Fig. 1). The Tradewinds and Mount Britton sites were located in the Lower Montane Wet Forest lifezone and the Carite State Forest site was located in the Subtropical Wet Forest lifezone (Ewell and Whitmore

1973). Recordings were made using an Automated Digital Recording System (ADRS; Acevedo and Villanueva-Rivera 2006). The recorder was a Nomad Jukebox 3 digital player and recorder (Model DAP-HD0003, Creative Labs, Inc, California), and was set to record in 16-bit wav files with a sampling rate of 48 kHz. Because the recorder had no microphone input, only a line level input, the microphone was connected to a portable preamplifier (Model SP-PREAMP, The Sound Professionals, Inc., New Jersey).

To test if digital recordings can be used to increase detection in tropical frog choruses, I compared one minute recordings, made at 2000, 2200, and 0000 h on five consecutive days, for a total of fifteen recordings per site. I analyzed these recordings using two methods: 1) listening to the recordings; and 2) listening and analyzing the recordings using computer software. For the first method I listened to the recordings with headphones, and each species heard was given an Amphibian Calling Index value (ACI; United States Geological Survey 2004). The ACI has four possible values: 0—no animals calling; 1—a few animals calling without overlap; 2—some overlap of animals calling; and 3—a full chorus of the species. For the second method, I listened to the recordings with headphones and analyzed them using computer software to detect the signal of each species' call by their sound and visual pattern in the auditory spectrum. There is little overlap in the call spectrum of the *Eleutherodactylus* frogs in Puerto Rico, and thus it was possible to distinguish each species (Drewry and Rand 1983). In addition, recordings of the calls of all the species have been published (Rivero 1998). Each recording was then filtered to remove the range of frequencies of the dominant species, *Eleutherodactylus coqui* (1–2.4 kHz), and re-analyzed. I gave each species a new ACI value for this second method. All recordings were coded and analyzed in random order to reduce bias. Each pair of results, for each recording, by species, were compared using Wilcoxon signed ranks tests.

To evaluate the differences in the level of the recorded sound of the species, which may explain why some species were not heard, I obtained the average signal level by frequency using the values of decibels full scale (dBFS), which corresponds, on a logarithmic scale, to the signal in the digital file. On this scale the maximum is 0 dB and the minimum is -96 dB, which corresponds to the maximum and minimum levels, respectively, of sound that the

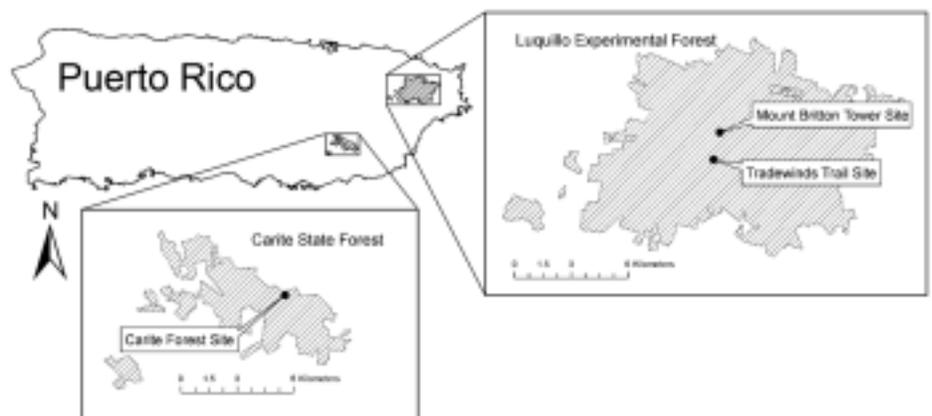


FIG. 1. Location of the three study sites in Puerto Rico.

digital file can store (Fries and Fries 2005). The recordings were analyzed using the program AUDITION (ver. 1.0, Adobe Systems, Inc., California, USA).

Results.—A total of eight species of *Eleutherodactylus* frogs were detected. Seven species were detected at the Tradewinds Trail site (Fig. 2a), where there was no difference between the methods

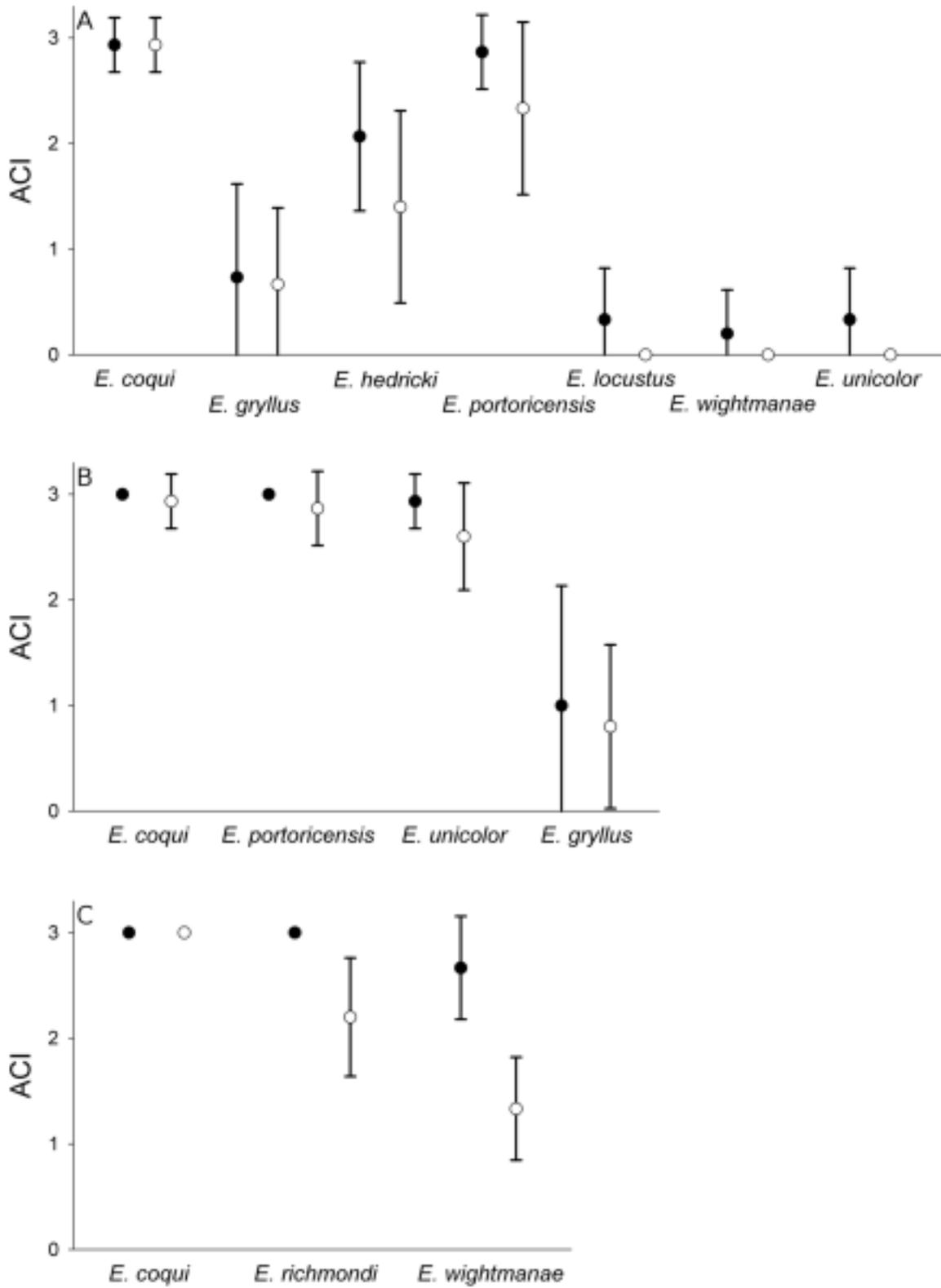


FIG. 2. ACI values (mean ± SD) per species using two methods at: A) Tradewinds Trail site; B) Mount Britton Tower site; and C) Carite Forest site. Filled circles = values for computer analysis method. Open circles = values for the listening method.

for *E. coqui* and *E. gryllus*. The ACI values of two species were higher on the computer-analyzed recordings: *E. hedricki* ($P=0.016$) and *E. portoricensis* ($P=0.022$). Three species were detected only

when the signals of their calls were seen in the spectrogram and the recordings were filtered: *E. locustus*, *E. unicolor*, and *E. wightmanae*. I detected *E. locustus* and *E. unicolor* in five of the

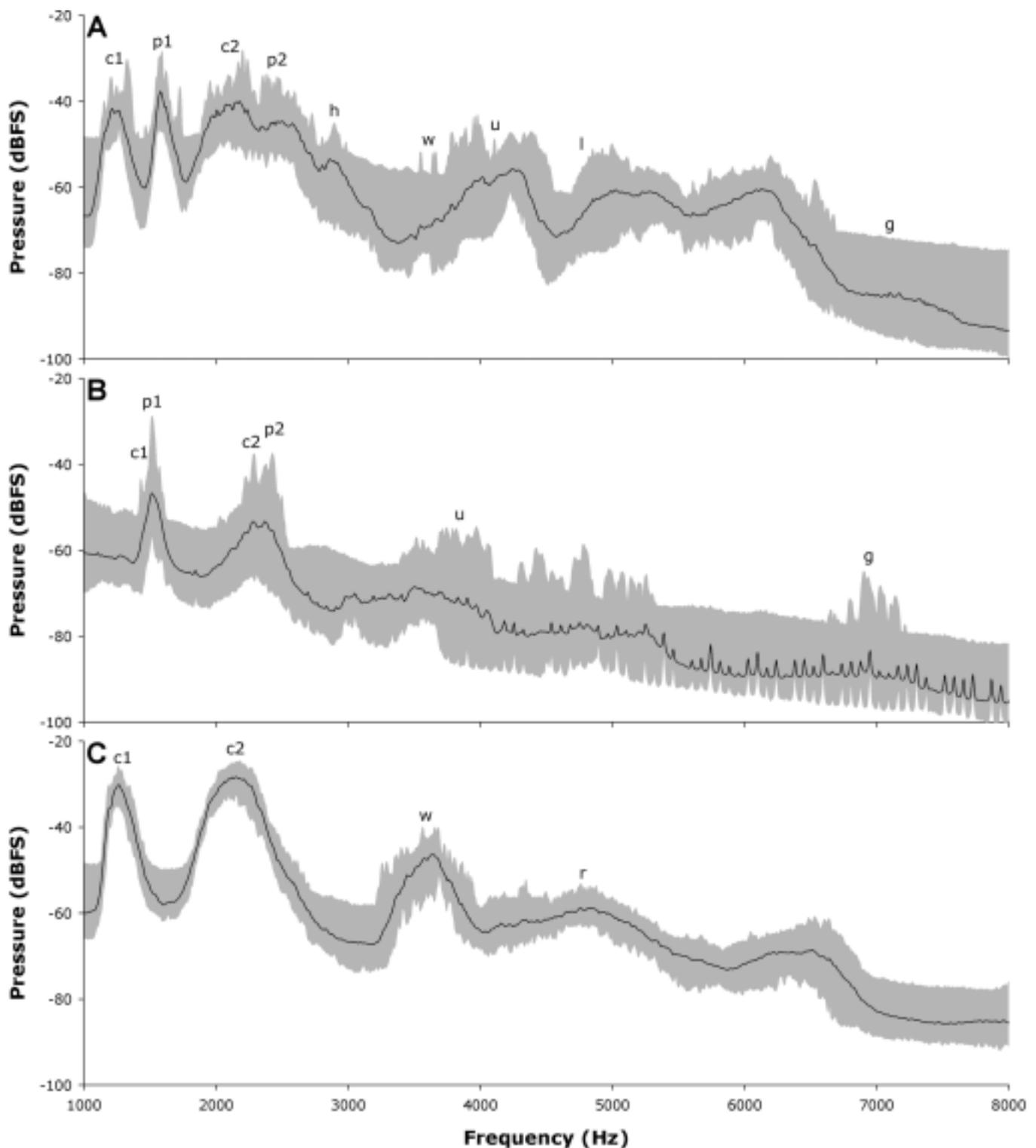


FIG. 3. Sound pressure by frequency of species of *Eleutherodactylus* frogs at the (A) Tradewinds Trail site, (B) Mount Britton Tower site, and (C) Carite Forest site. Sound pressure is measured in decibels full scale (dBFS). The black line represents the average for the fifteen recordings at each site and the shaded area encloses the range of values for each frequency. Letters above the graphs represent the dominant frequency for the species. c1 = first note of *E. coqui*; c2 = second note of *E. coqui*; p1 = first note of *E. portoricensis*; p2 = second note of *E. portoricensis*; h = *E. hedricki*; w = *E. wightmanae*; u = *E. unicolor*; l = *E. locustus*; r = *E. richmondi*; g = *E. gryllus*. Other peaks represent sounds made by insects.

fifteen recordings and *E. wightmanae* in only three of fifteen recordings. Two species had the highest sound pressures, *E. coqui* and *E. portoricensis* (Fig. 3a). The average level of the signal was -41.7 dB and -40.0 dB for the first and second notes of *E. coqui*, respectively, and -37.7 dB and -44.7 dB for the first and second notes, respectively, of *E. portoricensis*. The average maximum signal level for *E. locustus* was -50.7 dB, 13.0 dB less than the average of the loudest species, *E. portoricensis*. The average maximum signal level of *E. unicolor* was -44.5 dB and for *E. wightmanae* was -43.3 dB; these values were 6.8 dB and 5.6 dB lower than the loudest species, respectively.

There were four species at the Mount Britton Tower site: *E. coqui*, *E. gryllus*, *E. portoricensis*, and *E. unicolor*. There was no significant difference between methods for the four species (Fig. 2b).

I detected three species at the Carite State Forest site (Fig. 2c). There was no difference between methods for *E. coqui*, but the other two species had higher ACI values when analyzed by computer: *E. richmondi* ($P = 0.004$) and *E. wightmanae* ($P = 0.002$). The highest signal level was made by *E. coqui*, with -30.1 dB and -28.4 dB for the first and second notes, respectively (Fig. 3c). The average signal level for *E. richmondi* was -58.8 dB, 30.4 dB less than *E. coqui*, and for *E. wightmanae* it was -46.3 dB, 17.9 dB less than the loudest species.

Discussion.—The loudest species at the study sites, *E. coqui* and *E. portoricensis*, had a continuous chorus. In addition, the sound pressure of these species was higher than the species that were not as easily detected, up to 30 dB louder (Fig. 3). The continuous loud chorus of *E. coqui* and *E. portoricensis* caused interference in the detection of the other species.

The detection and level of activity measured using the ACI increased at two of three sites when recordings were filtered and the spectrogram was evaluated in the computer compared to only listening to the recordings. Of particular importance was the detection of three species, *E. locustus*, *E. unicolor*, and *E. wightmanae* at one of the sites only after filtering. These three species appear to be threatened or endangered since their range is restricted and few populations are known (Burrowes et al. 2004; pers. obs.).

Digital recordings, and their analysis using software, can be used as a tool to search for populations with apparently limited distributions, in areas where limited herpetological work has been conducted, and where loud species interfere with the detection of others. The ACI of four species was higher when the recordings were filtered to remove the common and loud *E. coqui*. Two of these four species, *E. hedricki* and *E. portoricensis*, have limited distribution in the highlands of Puerto Rico, and the two other species, *E. richmondi* and *E. wightmanae*, are thought to have only a few small populations (Burrowes et al. 2004; Villanueva-Rivera 2006).

Furthermore, recordings and computer analysis of choruses may advance our knowledge of anuran breeding phenology. For example, Bridges and Dorcas (2000) found that *Rana sphenocephala*, a species that was thought to be breeding only during the spring and fall, was also calling during the summer between 0200 and 0500 h. This finding, made with a cassette-based automated recording system, indicated a calling period that was missed by most herpetological work done in the area (Bridges and Dorcas 2000).

The use of digital recordings has important implications for the current discussion of amphibian declines and the need for long-

term monitoring projects. In a study that tested the use of recorders to detect species of birds, several experts agreed on the identifications of the species present in the recordings, so the method was recommended as an effective avian monitoring method (Rempel et al. 2005). In another study, the number of species identified with recordings made with an ADRS was higher when compared to point counts for birds and transects for amphibians (Acevedo and Villanueva-Rivera 2006). For amphibian monitoring projects, technicians can deploy ADRS and record choruses to be analyzed by experts in the laboratory at another time. This can reduce personnel costs, especially for areas with a small temporal window of amphibian reproductive activity, areas that have high frog diversity, species that do not congregate, and species with low detection probabilities. Another important advantage is that a recording is a permanent record of the presence of a species. Lastly, as demonstrated here, digital recorders and use of computer software may help detect rare species or calls that are difficult to detect due to interference.

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LITERATURE CITED

- ACEVEDO, M. A., AND L. J. VILLANUEVA-RIVERA. 2006. Using automated digital recording systems as effective tools for the monitoring of birds and amphibians. *Wildlife Soc. Bull.* 34:211–214.
- BRIDGES, A. S., AND M. E. DORCAS. 2000. Temporal variation in anuran calling behavior: implications for surveys and monitoring programs. *Copeia* 2000:587–592.
- BURROWES, P. A., R. L. JOGLAR, AND D. E. GREEN. 2004. Potential causes for amphibian declines in Puerto Rico. *Herpetologica* 60:141–154.
- DREWRY, G. E., AND A. S. RAND. 1983. Characteristics of an acoustic community: Puerto Rican frogs of the genus *Eleutherodactylus*. *Copeia* 1983:941–953.
- DUELLMAN, W. E. 1999. Global distribution of amphibians: patterns, conservation, and future challenges. In W. E. Duellman (ed.), *Patterns of Distributions of Amphibians: A Global Perspective*, pp. 1–30. Johns Hopkins University Press, Baltimore, Maryland.
- EWELL, J. J., AND J. L. WHITMORE. 1973. The ecological life zones of Puerto Rico and the U. S. Virgin Islands. Forest Service Research Paper ITF-18, Institute of Tropical Forestry, U. S. Forest Service, Río Piedras, Puerto Rico.
- FRIES, B., AND M. FRIES. 2005. *Digital Audio Essentials*. O'Reilly Media, Inc., Sebastopol, California. 357 pp.
- LIPS, K. R., J. K. REASER, B. E. YOUNG, AND R. IBÁÑEZ. 2001. *Amphibian Monitoring in Latin America: A Protocol Manual*. Society for the Study of Amphibians and Reptiles, New Haven, Connecticut. 115 pp.
- MACKENZIE, D. I., J. D. NICHOLS, G. B. LACHMAN, S. DROEGE, J. A. ROYLE, AND C. A. LANGTIMM. 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology* 83:2248–2255.

- REMPEL, R. S., K. A. HOBSON, G. HOLBORN, S. L. VAN WILGENBURG, AND J. ELLIOTT. 2005. Bioacoustic monitoring of forest songbirds: interpreter variability and effects of configuration and digital processing methods in the laboratory. *J. Field Ornithol.* 76:1–11.
- RIVERO, J. A. 1998. *Los Anfibios y Reptiles de Puerto Rico*. Editorial de la Universidad de Puerto Rico, San Juan. 2nd edition. 510 pp.
- STUART, S. N., J. S. CHANSON, N. A. COX, B. E. YOUNG, A. S. L. RODRIGUES, D. L. FISCHMAN, AND R. W. WALLER. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306:1783–1786.
- UNITED STATES GEOLOGICAL SURVEY. 2004. North American Amphibian Monitoring Program. Accesible at <http://www.pwrc.usgs.gov/naamp/index.html>.
- VILLANUEVA-RIVERA, L. J. 2006. Calling activity of *Eleutherodactylus* frogs of Puerto Rico and habitat distribution of *E. richmondi*. Master's thesis, University of Puerto Rico, Río Piedras. 65 pp.
- ZIMMERMAN, B. L. 1994. Audio strip transects. In W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek and M. S. Foster (eds.), *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*, pp. 92–97. Smithsonian Institution Press, Washington, DC.

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A Comparison of the Effectiveness of Recommended Doses of MS-222 (tricaine methanesulfonate) and Orajel® (benzocaine) for Amphibian Anesthesia

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Traditionally, tricaine methanesulfonate (Ethyl 3-aminobenzoic methanesulfonate salt), commonly known as MS-222, has been used to anesthetize amphibians for a variety of procedures including surgery, marking, and photography (Anholt et al. 1998; Kaplan 1969; Kaiser and Green 2001). Recently, Orajel®, a widely used analgesic for oral pain in humans, has been suggested as an effective alternative (Brown et al. 2004; Chen and Combs 1999). Previous studies also have suggested that Orajel® may be a more convenient choice (Altig 1980; Chen and Combs 1999) because it may be purchased from pharmacies and convenience stores, is relatively inexpensive (Crook and Whiteman 2006) and may be easier to transport (Kaiser and Green 2001; Wright 2001).

Few studies have examined the responses of amphibians to either MS-222 (Anholt et al. 1998; Kaplan 1969; Lowe 2004) or Orajel® (Brown et al. 2004). Crook and Whiteman (2006) found that benzocaine, the active ingredient in Orajel®, was more effective than MS-222 for anesthetizing *Ambystoma tigrinum*, and Cakir and Strauch (2005) found that benzocaine had more associated health risks than MS-222 in *Rana pipiens*. No other studies have compared the effectiveness of MS-222 and Orajel® among am-

phibian groups with dissimilar physiology that may affect their responses to anesthesia (Fellers et al. 1994). For example, factors such as rate of gas exchange across the skin vary among groups (e.g., plethodontid and ambystomatid salamanders), and may alter rates of anesthesia uptake.

We examined the effectiveness of recommended doses of MS-222 and Orajel® on four North American amphibian species (Northern Cricket Frogs [*Acris crepitans*], Mole Salamanders [*Ambystoma talpoideum*], Fowler's Toads [*Bufo fowleri*], and Northern Dusky Salamanders [*Desmognathus fuscus*]) by measuring the length of time required until induction, initial recovery, complete recovery, and the entire anesthesia process.

Methods.—We collected 54 adult *A. crepitans*, 41 adult *B. fowleri* and 46 adult *D. fuscus* from various localities within the western Piedmont of North Carolina, USA, and 16 adult *A. talpoideum* were collected on the Savannah River Site in the upper Coastal Plain of Aiken and Barnwell counties, South Carolina, USA. The snout-vent lengths ranged: 18–27 mm for *A. crepitans*; 29–64 mm for *B. fowleri*; 28–78 mm for *D. fuscus*; and 47–61 mm for *A. talpoideum*. After capture, we minimized stress by housing animals in dark containers with paper towels wetted with aged tap water. We housed *A. crepitans* and *D. fuscus* in same-species pairs in 18 × 18 × 7 cm plastic containers and housed *A. talpoideum* and *B. fowleri* in species-specific 75 × 32 × 30 cm aquariums with no more than 20 individuals per aquarium. *Acris crepitans*, *B. fowleri*, and *A. talpoideum* were kept at room temperature (ca. 21°C), and *D. fuscus* individuals were kept at 4°C but allowed to equilibrate to room temperature 3 h prior to testing. Individuals were kept no longer than a week prior to testing and monitored for at least 24 h before release. We prepared anesthesia solution by adding the recommended doses, 0.50 g/L for MS-222 (0.05%, Fellers et al. 1994) and 1.0 g/L of maximum strength Orajel® (Active ingredient: 20% benzocaine, Brown et al. 2004), to 1 L of 20–22°C, de-chlorinated tap water prepared by allowing chlorine evaporation overnight. We chose not to use a pH buffer with MS-222 as recommended by Lowe (2004) because we did not detect substantial pH change during use, as measured initially by a pH meter (YSI pH100; MS-222 pH = 6.53 ± 0.14, N = 6, Orajel® pH = 7.13 ± 0.08, N = 6) and by hydrion test strips (Micro Essential Laboratory, Inc.) following the last use of a solution (MS-222 pH = 7, N = 6, Orajel® pH = 7, N = 6). Baths were prepared in containers that allowed *D. fuscus* and *A. talpoideum* to completely submerge within the anesthesia solution. *Acris crepitans* and *B. fowleri* were placed in containers that allowed them to maintain their head above the solution until anesthetized.

After we prepared the solutions, individuals were arbitrarily assigned to two groups, either MS-222 or Orajel®, and no more than three individuals at a time were placed in their respective anesthesia solutions (Peterman and Semlitsch 2006). Animals were removed from the anesthetic solution when they failed to respond to our stimulus. We used a toe pinch as our stimulus and administered the pinch every minute in the anesthesia bath and every 2 minutes after induction until complete recovery. All amphibian species groups were tested separately, replacing anesthesia solutions after 15 animals were tested or after 1.5 h of testing. We defined “time until induction” as the period of time necessary for an individual to fail to respond to the toe pinch after being placed in the anesthesia bath. When the animal no longer responded to