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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 Whitman (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 amphibian 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

our stimulus, we removed animals from the anesthesia bath and rinsed them for 30 seconds in a de-chlorinated water bath. We then placed test animals on moist paper towels, observed, and recorded time until the first response to a toe pinch, which defined our "time until initial recovery." At this point, we visually monitored individuals until they exhibited behavior with no signs of sluggishness or disorientation associated with anesthesia. We defined this time period to be the "time until complete recovery." We chose to use a toe pinch as our stimulus as opposed to a typical righting response (Crook and Whiteman 2006) because our use of anesthesia occurs mainly during injections of visual implant elastomer (Northwest Marine Technology; e.g., Nauwelaerts et al. 2000), and animals frequently responded to elastomer injections (i.e., were not completely anesthetized) even when they failed to right themselves.

We analyzed our data using a MANCOVA (Minitab ver. 12.1) for each species. We used anesthesia type as the independent variable, mass of individual as a covariate, and time until induction, time until initial recovery, time until complete recovery, and the total time for all three stages of anesthesia as dependent variables. We evaluated significance at an $\alpha = 0.05$ level in all statistical tests. Because of the unexpected mortality of *B. fowleri*, we used logistic regression (Hosmer and Lemeshow 1989) to examine the effects of mass, snout–vent length, and treatment on mortality. We used a stepwise procedure with an inclusion/removal cutoff of $p = 0.10$. This analysis was conducted using the SAS statistical package (SAS v. 9.1, SAS Institute, Cary, North Carolina, USA).

Results.—We found that Orajel® required less time until induction in all species tested (MANCOVA: *A. crepitans*, $F = 46.38$, df = 1, 51, $p < 0.001$; *A. talpoideum*, $F = 24.21$, df = 1, 13, $p < 0.001$; *B. fowleri*, $F = 36.96$, df = 1, 36, $p < 0.001$; *D. fuscus*, $F = 20.99$, df = 1, 42, $p < 0.001$; Fig.1), produced longer times until initial recovery in *A. crepitans, B. fowleri,* and *D. fuscus* (MANCOVA: *A. crepitans*, $F = 148.73$, df = 1, 51, $p < 0.001$; *B. fowleri*, $F = 21.39$, df = 1, 36, $p < 0.001$; *D. fuscus*, $F = 70.77$, df = 1, 42, $p < 0.001$; Fig. 1a–c), but a shorter initial recovery period in *A. talpoideum* (MANCOVA: $F = 10.65$, df = 1, 13, $p = 0.006$; Fig. 1d). Anesthesia type had no effect on complete recovery times in *A. crepitans, A. talpoideum,* or *D. fuscus* (MANCOVA: *A. crepitans*, $F = 0.16$, df = 1, 51, $p = 0.69$; *A. talpoideum*, $F = 2.27$, df = 1, 13, $p = 0.156$; *D. fuscus*, $F = 0.24$, df = 1, 42, $p = 0.626$; Fig. 1 a, b, d), but anesthetization using Orajel® resulted in a longer period until complete recovery in *B. fowleri* (MANCOVA: $F = 14.55$, df = 1, 36, $p < 0.001$; Fig. 1c). Anesthesia using Orajel® took less time for the entire anesthesia process in *A. crepitans* and *A. talpoideum* (MANCOVA: *A. crepitans*, $F = 23.71$, df = 1, 51, $p < 0.001$; *A. talpoideum*, $F = 28.46$, df = 1, 13, $p < 0.001$; Fig. 2), but more time in *B. fowleri* and *D. fuscus* (MANCOVA: *B. fowleri*, $F = 24.74$, df = 1, 36, $p < 0.001$; *D. fuscus*, $F = 17.71$, dF = 1, 42, $p < 0.001$; Fig. 2). Mass significantly affected time until induction in

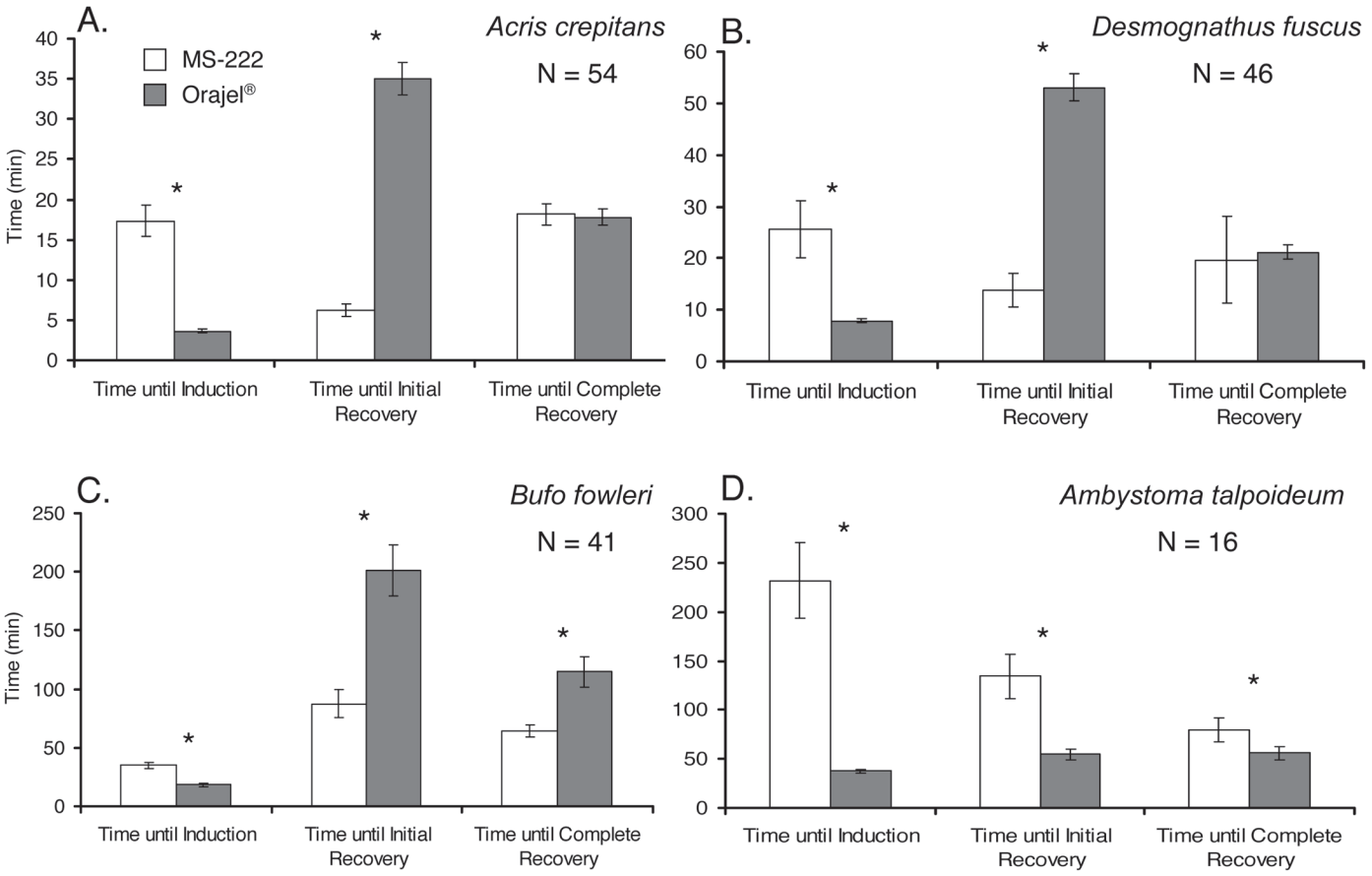

FIG. 1. Time (mean ± SE) required for the three stages of anesthesia using MS-222 and Orajel® in each test species. "*" indicates $p < 0.001$ (MANCOVA).

B. fowleri and *D. fuscus* (MANCOVA: *B. fowleri,* F = 16.91, df = 1, 36, p < 0.001; *D. fuscus*, F = 8.53, df = 1, 42, p = 0.006). We recorded relatively high mortality rates for *B. fowleri* in Orajel® (35%) and MS-222 (12%), but no mortality occurred in the other species tested. Results of our stepwise logistic regression identified mass as the best predictor of mortality, with heavier individuals having a higher probability of death (model likelihood ratio statistic = 4.9528, p = 0.026; parameter estimate for mass = -0.1193, p = 0.032).

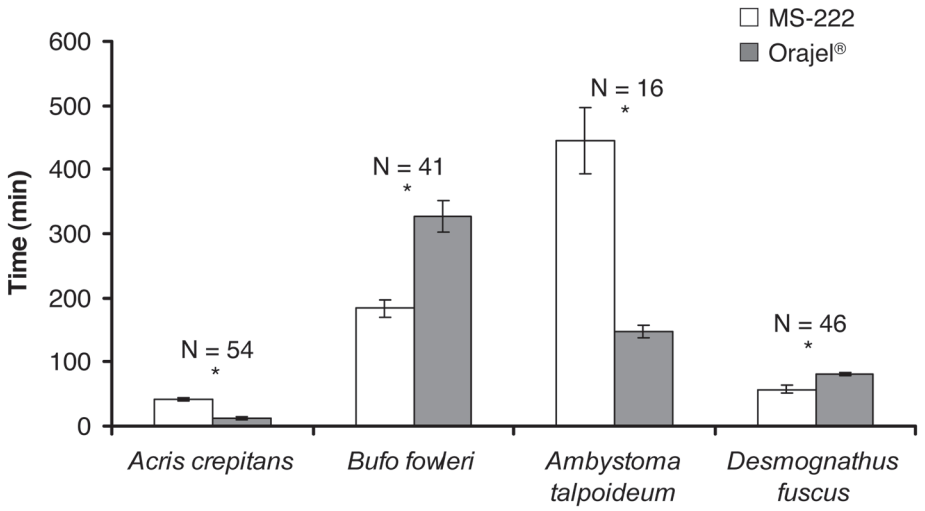

FIG. 2. Time (mean ± SE) required for the entire anesthesia process using MS-222 and Orajel® on our 4 test species. "*" indicates p < 0.001 (MANCOVA).

Discussion.—We observed that for most of the amphibian species we tested, anesthetization using Orajel® required less time for induction and produced a longer anesthetization period with variable recovery periods than recommended doses of MS-222 (Fig.1). The effect of anesthesia type on the time required for the entire anesthesia process varied among species (Fig. 2). We attribute inconsistent effects of anesthesia to variation in the physiologies of the species we tested. Factors such as methods of gas exchange, differing metabolic rates, or variation in water absorption rates likely impacted the reactions of species to each anesthesia (Feder and Burggren 1992).

Differences in time required for the three periods of the anesthetization process for each species also may have been a function of mass. We detected a positive effect of mass on induction time in *B. fowleri* and *D. fuscus*. Our sample contained individuals with masses ranging from 2.1 to 27.3 g in *B. fowleri* and 0.5 to 6.5 g in *D. fuscus*. Conversely, we were unable to detect any effect of mass in *A. crepitans* and *A. talpoideum*, which might be a result of testing similarly sized individuals (masses ranged from 0.4 to 1.6 g in *A. crepitans* and 3.9 to 7.6 g in *A. talpoideum*). Contrary to Lowe (2004) but similar to Peterman and Semlitsch (2006), we found that heavier individuals of some species required more time for induction than individuals that weighed less.

An unexpected result of our study was mortality experienced by *B. fowleri*. Orajel® and MS-222 have been used for euthanasia, but typically concentrations are higher or applied differently (Altig 1980). The mortality we observed might be attributed to behavioral and physiological responses of toads to physical handling. During handling, toads frequently released water from their cloacas. Toads might have rapidly absorbed water through their "pelvic patch" (Brekke et al. 1991) in response to water loss. Thus, they might have absorbed more anesthesia than necessary for anesthetization, effectively acquiring a lethal dose before showing signs of reduced motor coordination. Results from our logistic regression suggest that these effects may be most pronounced in heavier individuals. Despite the mortality we witnessed in toads, we suspect that Orajel® and MS-222 are safe for many non-bufonid species. For example, although not included in this study, we also have anesthetized several other species of larval and adult amphibians, including *Ambystoma maculatum, Eurycea cirrigera,* and *Pseudotriton ruber*, safely in Orajel® and have experienced no mortality. Yet, our study and others suggest the importance of experimenting with anesthesia type prior to anesthetizing many individuals because effects may vary between different species and life stages (Crook and Whiteman 2006).

Researchers should consider several factors when choosing an anesthesia. For example, researchers needing to work quickly in a field setting may want to consider the anesthesia that requires the least amount of time for the entire anesthesia process. Conversely, if total time or recovery time is less of a concern, researchers may choose to use the anesthesia that produces the shortest induction period, which was Orajel® in all tested species. Investigators choosing anesthesia may also consider the procedures they are conducting such as visual implant elastomer injections or implantation of radio transmitters, because a long anesthetization period may be required. For these longer procedures, Orajel® appears to be a better option for many species (Fig. 1).

Various other factors also may affect a researcher's choice of anesthesia. Although MS-222 must be purchased from a chemical supply company (Brown et al. 2004), Orajel® is a common oral analgesic, can be found at most convenience stores, and is slightly less expensive per dose than MS-222 (Orajel®: CVS Pharmacy in Davidson, NC, US $0.65/dose; MS-222: Sigma Aldrich US $0.87/dose). We also observed that, similar to Crook and Whiteman (2006), Orajel® anesthesia baths anesthetized more individuals than MS-222 baths. Furthermore, although there are no known negative side effects of low doses of Orajel® on amphibians, MS-222 may decrease natural cutaneous gram-negative bacterial growth (Fedewa and Lindell 2005).

Based on our study, researchers should prioritize their needs while choosing anesthesia for amphibians. Orajel® appears to be a relatively safe, quick, and convenient anesthesia, but MS-222 may be a better choice when the study organism requires less time for the entire anesthesia process, or the study organism's mortality risk due to anesthesia is high and/or the study species is of special conservation concern.

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