Am. Midl. Nat. 155:417–421

Notes and Discussion

An Evaluation of MS-222 and Benzocaine as Anesthetics for Metamorphic and Paedomorphic Tiger Salamanders (Ambystoma tigrinum nebulosum)

ABSTRACT.—Anesthesia is often used in amphibian studies, yet little information is available regarding the effectiveness of different anesthetics in the same species and across different life stages. We tested two popular anesthetics, benzocaine and MS-222 (tricaine methanesulfonate), in metamorphic (terrestrial adults) and paedomorphic (aquatic adults) Arizona tiger salamanders (*Ambystoma tigrinum nebulosum*). Benzocaine induced anesthesia more quickly than MS-222 in the concentration used here (0.02%) and was less variable in induction time. Metamorphic adults had higher and more variable induction times than paedomorphic adults. Recovery time was longer and more variable for animals subject to benzocaine rather than MS-222, but did not vary with morph. Our results suggest that benzocaine has higher per gram effectiveness than MS-222 for this species, which likely underlies the increased suggested dosages in the literature for the latter anesthetic. Such increased effectiveness at low concentrations, along with the monetary and time costs associated with MS-222, suggest that benzocaine is a more efficient and less costly anesthetic than MS-222. Because paedomorphic adults were more susceptible to anesthesia than metamorphic adults, paedomorphs may also be more affected by other aqueous chemicals, including pollutants.

INTRODUCTION

Anesthesia is an artificially produced unconsciousness used during surgery or other medical procedures to block sensations of pain. Different types of anesthesia are used routinely in the treatment and evaluation of amphibians (Wright, 2001). Reversible anesthesia causes total or partial loss of sensation and immobility, allowing for procedures such as implantation of passive integrated transponders (PIT tags), elastomers and radio transmitters (Madison, 1997; Madison and Farrand, 1998; USGS, 2001). Typically, the choice of anesthesia has been based on what other researchers have used rather than a critical evaluation of different methods.

Benzocaine and MS-222 are two well-known anesthetics that have been successfully used to anesthetize amphibians in both field and laboratory settings (Stouffer *et al.*, 1983; Werner, 1991; Colberg *et al.*, 1997; Goldberg *et al.*, 2002; Lowe, 2004). Both are powder anesthetics that are prepared with water, and animals are typically immersed in the resulting solution (USGS, 2001; Wright, 2001). Although the literature contains information on these and other anesthetics, no study, to the best of our knowledge, has compared the use of both anesthetics in a single species. In this study, we experimentally compared the effectiveness of benzocaine and MS-222 in Arizona tiger salamanders (*Ambystoma tigrinum nebulosum*).

Ambystoma tigrinum nebulosum is a facultatively paedomorphic species in which some larvae metamorphose while others retain their larval morphology to become paedomorphic adults (Semlitsch, 1985; Whiteman, 1994; Denoël *et al.*, 2002). Facultatively paedomorphic species provide an interesting comparison for anesthesia, because paedomorphic adults have gills and a more permeable epidermis than metamorphic adults (Whiteman, 1994), potentially facilitating the uptake of anesthesia. Thus, we predicted that both anesthetics would operate more quickly and effectively on paedomorphic adults than on metamorphic adults.

MATERIALS AND METHODS

Fifteen metamorphic tiger salamanders were collected from Kaichlen Pond and ten paedomorphic tiger salamanders were collected from Marshall Pass Pond #1, both in the Gunnison Basin of south-central Colorado, during July 2003. The experiment consisted of the two anesthetic treatments, using identical concentrations (0.02%). Although this concentration is below that recommended for MS-222 to induce anesthesia in terrestrial amphibians (0.1%; Wright, 2001), we chose it because it was recommended for larval (paedomorphic) forms using MS-222, and for terrestrial (metamorphic) forms using benzocaine (Wright, 2001). Additionally, preliminary studies revealed that paedomorphic forms would quickly succumb to both compounds at concentrations higher that 0.02%. Thus, we chose

TABLE 1.—Effects of MS-222 and benzocaine on metamorphic and paedomorphic tiger salamanders. Means are ± 1 SE; sample sizes are in parentheses. *See* text for statistics

	Anesthetic		Morph	
Time (min)	MS-222	Benzocaine	Metamorph	Paedomorph
Induction	106.6 ± 18.1 (13)	21.5 ± 14.1 (12)	89.3 ± 18.6 (15)	30.5 ± 6.1 (10)
Initial Recovery	4.7 ± 1.1 (8)	60.5 ± 2.6 (12)	42.4 ± 8.9 (10)	34.0 ± 9.6 (10)
Final Recovery	9.7 ± 1.6 (8)	73.8 ± 4.5 (12)	51.0 ± 10.2 (10)	45.36 ± 12.0 (10)

a concentration that would protect the safety of paedomorphic adults while allowing comparison of the effectiveness of the two agents at the same concentration.

Both anesthetics were purchased from Sigma-Aldrich (St. Louis, Missouri). The MS-222 solution was prepared by dissolving 0.2 g of MS-222 into 1000 ml of aged water. Benzocaine was first dissolved in 10 ml of 95% ethanol and then added to 990 ml of aged water (Wright, 2001). Salamanders of each morph were divided by sex and then randomly assigned to an anesthetic treatment. We measured snout-vent length (SVL) using a metric ruler and mass with a portable balance. For each individual, we utilized two rectangular plastic containers (approx. 20 cm × 10 cm × 5 cm), one with 1 l anesthetic treatment and one with 1 l aged water. The solutions and aged water were used in only one replicate, after which the containers were cleaned thoroughly with soap and water, rinsed multiple times and filled with new solution or water. All solutions and containers were at room temperature (20–22 C) and salamanders were allowed to equilibrate to room temperature before use in the experiment.

At the beginning of each trial, a salamander was placed in its respective anesthetic solution such that it was completely submerged. A stopwatch was used to record induction time, defined as the time required for the animal to fail to execute a righting reflex after being turned on its back. Salamanders were observed constantly and, once an individual showed signs of anesthesia, it was turned on its back for testing. Once incapacitated, the individual was moved to aged water, still on its back, and recovery time was recorded. Initial recovery time was recorded when the animal could execute the righting reflex. Final recovery time was recorded when the animal could move freely on its own and was showing no obvious visual effects from the anesthesia, such as slowed movement and reactions. Finally, the mass of each individual was recorded after the animal had fully recovered.

All analyses were conducted with StatView (SAS Institute, 1999). Because of low sample sizes, we used rank-order transformed ANOVA (Zar, 1999) to control heteroscedastic variances. In all analyses, sex of the experimental animals was not a significant factor and was, thus, removed for final analysis to increase the power of our tests.

RESULTS

Paedomorphic adults utilized in this experiment were significantly larger than metamorphic adults [SVL (mm): paedomorphs (mean ± 1 SE), 103.2 ± 2.5; metamorphs, 98.2 ± 1.7; $F_{1,21} = 4.7$, $P = 0.04$; mass (g): paedomorphs, 36.0 ± 2.0; metamorphs, 25.3 ± 1.3; $F_{1,21} = 22.3$, $P < 0.0001$]. However, in only one case might these differences have affected the experiment, in that there was a marginally significant morph × treatment effect ($F_{1,21} = 4.0$, $P = 0.06$), with paedomorphic adults larger in SVL than metamorphic adults in the MS-222 treatment but similar in size during the benzocaine treatments. There were no significant sex effects on initial body size.

Treatment and morph had significant effects on induction time (both $F_{1,21} > 26.5$, both $P < 0.0001$), but the interaction effect was not significant ($F_{1,21} = 1.0$, $P = 0.32$). Animals treated with MS-222 took significantly longer to reach incapacitation and were more variable in their response than those treated with benzocaine (Table 1). Metamorphic adults required significantly more induction time and were more variable in their response than paedomorphic adults, irrespective of the anesthetic (Table 1). Five metamorphic adults had not reached induction after 180 min of immersion in MS-222. When these animals were removed from the analysis, the results remained the same, with significant treatment and morph effects (both $F_{1,16} > 11.9$, both $P < 0.004$), but no interaction effect ($F_{1,16} = 0.003$, $P = 0.96$).

Initial and final recovery time varied only by treatment (both $F_{1,16} > 44.8$, both $P < 0.0001$), with animals treated with benzocaine requiring significantly longer recovery times and being more variable in their response than MS-222 (Table 1). Morph effects and the morph × treatment interaction were not significant for either response variable (all $F_{1,16} < 1.49$, all $P > 0.24$).

There were significant effects of treatment and the morph × treatment interaction on change in mass (as a function of mass; treatment: $F_{1,16} = 5.3$, $P = 0.04$; interaction: $F_{1,16} = 21.3$, $P = 0.0003$). On average, benzocaine treatments functionally produced zero loss of mass in salamanders (0.00005 ± 0.006 SE g/g), while MS-222 treatments produced significantly different, but minimal loss (−0.013 ± 0.003 g/g). Similarly, the morph × treatment interaction revealed that metamorphic adults exposed to benzocaine gained mass, while the other morph-treatment combinations lost mass.

DISCUSSION

Our results suggest that there are substantial differences in the response of *Ambystoma tigrinum nebulosum* to benzocaine and MS-222 at the same concentrations. This is not surprising, given that the recommended dosage for MS-222 in terrestrial amphibians is higher than the concentrations used here (0.02 vs. 0.1% in Wright, 2001), and those recommended for benzocaine (0.02–0.03%; Wright, 2001). Recommended concentrations for larval forms are much lower (MS-222: 0.02%; benzocaine: 0.005–0.01%; Wright, 2001). Thus, comparisons of induction or recovery times are biased against MS-222, which was necessary because of the inclusion of paedomorphic adults in the experiment. Nonetheless, our results allow comparison of the per gram effectiveness of each anesthetic.

Benzocaine was less variable in the induction response of salamanders than MS-222, with the reverse being true for recovery times. For example, five metamorphic adults exposed to MS-222 had not been induced after 180 min of testing, while others of this morph took much less time. Such variation suggests that MS-222 may be less reliable for induction, particularly in metamorphic adults, at such low doses, which may be one reason why higher levels are recommended for terrestrial amphibians. Such variation in response might also be due to the fact that our MS-222 solutions were unbuffered, potentially decreasing the effectiveness of the anesthetic (Wright, 2001; Lowe, 2004) and, thus, perhaps increasing its variability. Future investigations should utilize buffered MS-222 solutions to provide more insight into the effectiveness of this anesthetic.

Although there were statistically significant differences in the change in mass as a function of initial mass between treatments and the morph × treatment interaction, it is unclear whether such differences, because they were so minimal, are biologically relevant. MS-222 exposure reduced mass in both morphs, but benzocaine was more variable in that paedomorphic adults lost mass while metamorphic adults gained mass. Although the loss of mass via metabolic processes while under anesthesia was expected, the mechanism by which metamorphic adults increased in mass is unclear. It is possible that metamorphic adults osmoregulate to reduce the concentration of anesthetic within their tissues, the result of which leads to increased mass via water retention. However, one would expect that such osmoregulatory abilities would be more evident among paedomorphic adults, because of their gills and more permeable skin (Whiteman, 1994; *but see* Currens *et al.*, 2002). Because our concentrations of MS-222 were below those typically used for terrestrial amphibians (Wright, 2001), it is likely that more substantial weight loss could occur at higher concentrations, particularly in unbuffered solutions.

Paedomorphic adults had significantly lower induction times than metamorphic adults, as predicted, but we found no difference in recovery times between morphs. The larger body size of paedomorphic adults, primarily in the MS-222 treatments, provided a conservative test of our hypotheses, because the lower surface area:volume ratio of these animals should have made them less susceptible to induction and recovery than the smaller metamorphic adults. However, it is possible that body size differences between the morphs affected both the recovery and induction times by leading to equal recovery times and, perhaps, a smaller difference in induction time than would have occurred had the two samples been more similar in mean size. Nonetheless, our induction results suggest that paedomorphic adults are more susceptible to both anesthetics and, thus, must be treated differently from metamorphic adults when establishing anesthesia protocols.

Our results also support the hypothesis that paedomorphic forms might be more susceptible to uptake of a variety of other chemicals, including pollutants (Whiteman and Howard, 1998). Such increased exposure could affect the distribution and abundance of these morphs, and provides an alternative hypothesis, along with the introduction of fish (Whiteman and Howard, 1998; Denoel *et al.*, 2005) for why paedomorphic forms are rare in areas subject to human encroachment, particularly in the Midwestern United States (Whiteman and Howard, 1998). The increased exposure of larval forms to lampricide in Great Lakes tributaries led to mortality and subsequent population declines in the obligately paedomorphic mudpuppy, *Necturus maculosus*, (Gilderhus and Johnson, 1980; Matson, 1990). Pollution is also thought to be the major factor affecting the critically endangered Axolotl, *Ambystoma mexicanum* near Mexico City (Griffiths *et al.*, 2004). Thus, paedomorphic forms, with their increased susceptibility to chemical uptake, may provide ideal bioindicators of habitat quality and anthropogenic disturbance (Whiteman and Howard, 1998; Denoel *et al.*, 2005).

Significantly quicker induction, and significantly longer recovery times by benzocaine at the same concentration, indicates that less chemical needs to be utilized during experiments to provide effective anesthesia, whereas the higher recommended concentrations of MS-222 suggest that substantially more MS-222 would be needed to induce sufficient anesthesia. Because the costs of MS-222 are considerably higher than benzocaine ($0.90/g vs. $0.20/g for 100g; pers. obs.), cost may be a factor in the use of each anesthetic. Additionally, there are costs associated with providing an adequately buffered MS-222 solution, including the cost of chemicals and the extra time required to create buffered solutions. Finally, benzocaine powder can be conveniently stored at room temperature rather than the freezing temperatures required for some brands of MS-222 (Wright, 2001), and benzocaine appears to remain effective for a longer amount of time than MS-222 when in solution (pers. obs.).

In sum, our results suggest that the use of anesthetics should not only be based on previous usage but also preliminary testing to determine which protocols are most useful for successful anesthesia in a specific species and/or life stage. Such decisions will also depend on the goal of the study, the length of time required or needed for induction and recovery, and the time and money available for the research.

Acknowledgments.—We thank A. Benson and J. Boynton for assistance with the capture and care of study animals, J. Doyle-Pittsnogle and B. Kobylarz for manuscript review, W. Cowher for support and the NSF for funding (DEB 0109436). This research was conducted with approval from the MSU and RMBL IACUC. Animals were collected under Colorado Division of Wildlife Permit HP339.

LITERATURE CITED

COLBERG, M. E., D. F. DENARDO, N. A. ROJEK AND J. W. MILLER. 1997. Surgical procedure for radio transmitter implantation into aquatic, larval salamanders. *Herpetol. Rev.*, **28**:77–78.

CURRENS, C. R., P. H. NIEWIAROWSKI AND H. H. WHITEMAN. 2002. Effects of temperature and time of day on the resting metabolic rates of paedomorphic and metamorphic mole salamanders, *Ambystoma talpoideum. Copeia,* **2002**:489–495.

DENOËL, M., G. DZUKIC AND M. L. KALEZIC. 2005. Effect of widespread fish introductions on paedomorphic newts in Europe. *Conservation Biology,* **19**:162–170.

———, F. HERVANT, R. SCHABETSBERGER AND P. JOLY. 2002. Short- and long-term advantages of an alternative ontogenetic pathway. *Biol. J. Linn. Soc.*, **77**:105–112.

GILDERHUS, P. H. AND B. G. JOHNSON. 1980. Effects of sea lamprey (*Petromyzon marinus*) control in the Great Lakes on aquatic plants, invertebrates, and amphibians. *Can. J. Fish. Aqu. Sci.*, **37**: 1895–1905.

GOLDBERG, C. S., M. J. GOODE AND C. R. SCHWALBE. 2002. External and implanted methods of radiotranmitter attachment to a terrestrial anuran (*Eleutherodactylus augusti*). *Herpetol. Rev.*, **33**: 191–194.

GRIFFITHS, R. A., V. GRAUE, I. G. BRIDE AND J. E. MCKAY. 2004. Conservation of the axolotl (*Ambystoma mexicanum*) at Lake Xochimilco, Mexico. *Herpetol. Bull.*, **89**:4–11.

LOWE, J. 2004. Rates of tricaine methanesulfonate (MS-222) anesthetization in relation to pH and concentration in five terrestrial salamanders. *Herpetol. Rev.*, **35**:352–354.

MADISON, D. M. 1997. The emigration of radio-implanted spotted salamanders, *Ambystoma maculatum. J. Herpetol.*, **31**:542–551.

——— AND L. FARRAND, III. 1998. Habitat use during breeding and emigration in radio-implanted tiger salamanders, *Ambystoma tigrinum*. *Copeia*, **1998**:402–410.

MATSON, T. O. 1990. Estimation of numbers for a riverine *Necturus* population before and after TFM lampricide exposure. *Kirtlandia*, **45**:33–38.

SAS INSTITUTE. 1999. StatView. SAS Institute, Inc., Cary, North Carolina.

SEMLITSCH, R. D. 1985. Reproductive strategy of a facultatively paedomorphic salamander *Ambystoma talpoideum*. *Oecologia*, **65**:305–313.

STOUFFER, R. H., J. E. GATES, C. H. HOCUTT AND J. R. STAUFFER. 1983. Surgical implantation of a transmitter package for radio-tracking endangered hellbenders. *Wild. Soc. Bull.*, **11**:384–386.

USGS. 2001. Anesthesia of amphibians in the field. USGS National Wildlife Health Center ARMI SOP #104. www.nwhc.usgs.gov.amph_dc/sop_anesth.html.

WERNER, J. K. 1991. A radiotelemetry implant technique for use with *Bufo americanus*. *Herpetol. Rev.*, **22**:94–95.

WHITEMAN, H. H. 1994. Evolution of facultative paedomorphosis in salamanders. *Q. Rev. Biol.*, **69**: 205–221.

——— AND R. D. HOWARD. 1998. Conserving alternative amphibian phenotypes: is there anybody out there?, p. 317–324. *In:* M. J. Lannoo (ed.). Status and conservation of Midwestern amphibians. University of Iowa Press, Iowa City, Iowa.

WRIGHT, K. M. 2001. Restraint techniques and euthanasia, p. 111–122. *In:* K. M. Wright and B. R. Whitaker (eds.). Amphibian medicine and captive husbandry. Krieger Publishing, Melbourne, Florida.

ZAR, J. H. 1999. Biostatistical analysis, 4th ed. Prentice Hall, Englewood Cliffs, New Jersey.

AMANDA C. CROOK AND HOWARD H. WHITEMAN[1], Department of Biological Sciences, Murray State University, Murray, Kentucky 42071 and Rocky Mountain Biological Laboratory, P.O. Box 519, Crested Butte, Colorado 81224. *Submitted 23 May 2005; accepted 25 September 2005.*

[1] Corresponding author: Telephone: (270) 762-6753; e-mail: howard.whiteman@murraystate.edu