



Title: Toe-Clipping of Amphibians and Reptiles

SOP Number: 041

Purpose: The humane care of animals used in research is a recognized scientific and ethical responsibility endorsed by Texas Tech University (TTU) and encouraged or mandated by a variety of agency guidelines and regulations, including the Animal Welfare Act. “As with other vertebrate groups, the use of amphibians and reptiles in research and teaching raises ethical questions that must be carefully considered prior to the initiation of a project” (HACC, 2004). The role of the TTU Animal Care and Use Committee (ACUC) is to assist investigators to meet those standards. This SOP outlines the contexts in which toe clipping can be used in herpetological research at TTU. It also describes a safe, simple, sanitary and humane method to remove toe tips from amphibians and reptiles, primarily for the purpose of individually marking animals.

SCOPE

“Due to the large range of diversity represented by the over 12,280 species of amphibians and reptiles, no concise or specific compendium of approved or required methods for field and laboratory research is practical or desirable” (HACC, 2004). The procedure described below is limited to salamanders, fully metamorphosed anurans (frogs and toads), and lizards, but is NOT recommended for tadpoles, larvae, turtles, crocodylians, or fleshy lizards like Gila monsters, monitors, chuckwallas or komodo dragons.

BACKGROUND

Toe clipping is commonly used in herpetology when justified (Dunham et al., 1988; Ferner, 2007). The standard guidelines in the field (HACC, 2004) state that “Toe clipping, a ubiquitous technique (Dunham et al., 1988), may be used for general marking of free-ranging animals when toe removal is not judged (by observation of captives or of a closely-related species) to impair the normal activities of the marked animal.” Toe clipping of reptiles and amphibians meets legal and ethical expectations (Perry et al. 2011). Generally toe-clipping is conducted without anesthesia as these species often loose toes under normal conditions, stress (based on hormone analysis) is similar to that of other marking techniques (e.g. injection of elastomer, toe-clipping with anesthesia, and handling without marking (Kinkead et al., 2006)), and anesthesia can reduce survival do to susceptibility of anesthetized animals to predation. Possible negative effects should be objectively assessed before each study, given the biology of the species in question.

For endangered species, it may be appropriate to first evaluate the impact of toe-clipping on non-threatened relatives. Should an appropriate alternative method that is less invasive become available in the future, researchers should consider to switching to that method.

PROPER PROCEDURE FOR TOE-CLIPPING

A. Preparation:

1. If dirty, clean the foot gently by spraying a stream of clean water over the skin and toes.
2. The area of incision may then be prepared with an antiseptic agent such as Bactine or betadine; however, amphibian skin is sensitive; it may adversely respond to or absorb a wide variety of chemicals that are in common use on other taxa. Alcohols, phenolics, and iodine-based products should not be used on amphibians. At present, Bactine is the only preparation recommended for amphibians.
3. Use sharp, disinfected stainless steel, surgical-type scissors of a size appropriate for the species being studied. Scissors must be disinfected before use on each animal. An acceptable method of disinfection would be cold chemical sterilization (soaking in 0.5% chlorhexidine solution for 15 minute contact time). Blades should be allowed to dry or cool, or be thoroughly rinsed with water after respective treatments.

B. Procedure:

1. Always remove the smallest part of the digit that allows for proper marking.
2. Avoid removing digits that are known to have unique function, such as digit I ("thumb") of male frogs and toads, which is often used for clasping the female during mating, or the elongated forth toe of lizard hind limbs, which enhances locomotor performance in some species (Irschick, 2002).
3. Only clip the smallest possible number of digits possible, never removing more than two non-adjacent toes per foot and preferably limiting such cases. Numbering systems (e.g., Ferner, 2007; Donnelly et al., 1994) can assist in this.
4. Where possible, clip the toe at the interphalangeal joint (Wright, 2001).
5. Bleeding from the wound is usually minimal or non-existent; if blood flows from the wound for more than a few seconds, then absorbent material should be used to stop it.
6. Prior to release, use disinfectant (see above) on the site of the surgery unless there is evidence the disinfectant alters the species behavior.

C. Monitoring:

1. Follow the marked animals and carefully monitor for obvious distress or procedure-related mortality for as long as possible. If such an effect is observed, cease the marking program and report your findings in the peer-reviewed literature. In addition, peruse the literature for any new marking approaches.

Bibliography for justification and methods – not all cited in this SOP

- Arntzen, J.W., A. Smithson, and R.S. Oldham. 1999. Marking and tissue sampling effects on body condition and survival in the newt *Triturus cristatus*. *Journal of Herpetology* 33:567-576.
- Brunson, K. 1986. Some unusual injuries to snakes. *Kansas Herpetological Society Newsletter* 65:13-14.

- Association for the Study of Animal Behaviour/Animal Behavior Society. 2003. Guidelines for the treatment of animals in behavioral research and teaching. *Animal Behaviour* 65:249-255.
- Bloch, N. and D.J. Irschick. 2005. Toe-clipping dramatically reduces clinging performance in a pad-bearing lizard (*Anolis carolinensis*). *Journal of Herpetology* 39:288-293.
- Boone, J. and LaRue, E. (1999). Effects of marking *Uta stansburiana* (Sauria: Phrynosomatidae) with Xylene based paint. *Herpetological Review* 30: 33-34.
- Borges-Landáez, P.A., and R. Shine. 2003. Influence of toe-clipping on running speed in *Eulamprus quoyii*, an Australian scincid lizard. *Journal of Herpetology* 37:592-595.
- Brannelly, L. A., Berger, L., and Skerratt, L. 2014. Comparison of three widely used marking techniques for adult anuran species *Litoria verreauxii alpina*. *Herpetological Conservation and Biology* 9:428-435.
- Carothers, J. 1986. An experimental confirmation of morphological adaptation: toe fringes in the sanddwelling lizard *Uma Scoparia*. *Evolution* 40:871–874.
- Caorsi, V. Z., Santos, R. R., and Grant, T. 2012. Clip or Snap? An Evaluation of Toe-Clipping and Photo-Identification Methods for Identifying Individual Southern Red-Bellied Toads, *Melanophryniscus cambaraensis*. *South American Journal of Herpetology* 7:79-84.
- Davis, T.M., and K. Ovaska. 2001. Individual recognition of amphibians: effects of toe clipping and fluorescent tagging on the salamander *Plethodon vehiculum*. *Journal of Herpetology* 35:217-225.
- Dodd, C.K. 1993. The effects of toe-clipping on sprint performance of the lizard *Cnemidophorus sexlineatus*. *Journal of Herpetology* 27:209-213.
- Donnelly, M.A., C. Guyer, J.E. Juterbock, and R.A. Alford. 1994. Techniques for marking amphibians. In: Heyer WR, Donnelly MA, McDiarmid RW, Hayek LC, Foster MS (eds.) *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Smithsonian Institution Press, Washington DC, pp. 277-284.
- Dunham, A.E., P.J. Morin and H.M. Wilbur. 1988. Methods for the study of reptile populations. Pp. 330-386. In: *Biology of the Reptilia*, Vol. 16, C. Gans and R.B. Huey (eds). Alan R. Liss, New York, NY.
- FDA (Food and Drug Administration). 1989. Good Laboratory Practice Regulations; Minor Amendment. *Federal Register* Vol. 54, No. 75, Thursday, April 20, 1989.
- Ferner, J.W. 2007. A review of marking and individual recognition techniques for amphibians and reptiles. *Herpetological Circulars* 35. Society for the Study of Amphibians and Reptiles, Salt Lake City UT.
- Fisher, K. J., Guilfoyle, K.J., and Hatch, K.A. 2013. Stress induced by toe-clipping in cane toads (*Rhinella marina*). *Copeia*, 2013:539-542.
- Funk, W.C., M.A. Donnelly, and K.A. Lipps. 2005. Alternative views of amphibian toe-clipping. *Nature* 433:193.
- Ginnan, N. A., Lawrence, J.R., Russell, M.E., Eggett, D.L., and Hatch, K.A. 2014. Toe Clipping Does Not Affect the Survival of Leopard Frogs (*Rana pipiens*). *Copeia* 2014:650-653.
- Golay, N., and H. Durrer. 1994. Inflammation due to toe-clipping in natterjack toads (*Bufo calamita*). *Amphibia-Reptilia* 15:81-83.

- Grafe, T. U., Stewart, M.M., Lampert, K.P., and Rödel, M.O. 2011. Putting toe clipping into perspective: a viable method for marking anurans. *Journal of Herpetology* 45:28-35.
- Guimaraes, M., Corrêa, D.T., Sérgio Filho, S., Oliveira, T.A., Doherty, P F., and Sawaya, R.J. 2014. One step forward: contrasting the effects of Toe clipping and PIT tagging on frog survival and recapture probability. *Ecology and evolution* 4:1480-1490.
- HACC (Herpetological Animal Care and Use Committee of the American Society of Ichthyologists and Herpetologists). 2004. Guidelines for use of live amphibians and reptiles in field and laboratory research, Second Edition.
- Huey, R.B., A.E. Dunham, K.L. Overall, and R.A. Newman. 1990. Variation in locomotor performance in demographically known locations of the lizard *Sceloporus merriami*. *Physiological Zoology* 63:845–872.
- Hudson, S. 1996. Natural toe loss in southeastern Australian skinks: implications for marking lizards by toe clipping. *Journal of Herpetology* 30: 106-110.
- Irschick, D.J. 2002. Evolutionary approaches for studying functional morphology: examples from studies of performance capacity. *Integrative and Comparative Biology* 42:278-290.
- Kinkead, K.E., J.D. Langham, and R.R. Montanucci. 2006. Comparison of anesthesia and marking techniques on stress and behavioral responses in two *Desmognathus* salamanders. *Journal of Herpetology* 40:323-328.
- Langkilde, T., and R. Shine. 2006. How much stress do researchers inflict on their study animals? A case study using a scincid lizard, *Eulamprus heatwolei*. *Journal of Experimental Biology* 209:1035-1043.
- Mahendra, B.C. 2004. Contributions to the bionomics anatomy, reproduction and development of the Indian House-Gecko, *Hemidactylus flaviviridis* Rupel. Part II. The problem of locomotion. *Proceedings of the Indian Academy of Science, Section B* 13: 288-306.
- McCarthy, M.A., and K.M. Parris. 2004. Clarifying the effect of toe clipping on frogs with Bayesian statistics. *Journal of Applied Ecology* 41:780-786.
- Narayan, E.J., Molinia, F.C., Kindermann, C., Cockrem, J.F., and Hero, J.M. 2011. Urinary corticosterone responses to capture and toe-clipping in the cane toad (*Rhinella marina*) indicate that toe-clipping is a stressor for amphibians. *General and comparative endocrinology* 174:238-245.
- Ott, J.A., and D.E. Scott. 1999. Effects of toe-clipping and PIT-tagging on growth and survival in metamorphic *Ambystoma opacum*. *Journal of Herpetology* 33:344-348.
- Parris, K.M., and M.A. McCarthy. 2001. Identifying effects of toe clipping on anuran return rates: the importance of statistical power. *Amphibia-Reptilia* 22:275-289.
- Paulissen, M.A., and H.A. Meyer. 2000. The effect of toe-clipping on the gecko *Hemidactylus turcicus*. *Journal of Herpetology* 34:282-285.
- Perry, G., Wallace, M.C., Perry, D., Curzer, H., and Muhlberger, P. 2011. Toe Clipping of Amphibians and Reptiles: Science, Ethics, and the Law. *Journal of Herpetology* 45:547-555.
- Phillott, A.D., L.F. Skerratt, K.R. McDonald, F.L. Lemckert, H.B. Hines, J.M. Clarke, R.A. Alford, and R. Speare. 2007. Toe-clipping as an acceptable method of identifying individual anurans in mark recapture studies. *Herpetological Review* 38:305-308.

- Wright, K.M. 2001. Surgical techniques. Pp. 273-283 in: Wright, K. M. and Whitaker, B. R. (eds.) Amphibian medicine and captive husbandry. Krieger Publishing Company, Malabar, Florida.