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 clasp 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


