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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, or crocodilians.

BACKGROUND

- A. The Guide for the Care and Use of Laboratory Animals and numerous books on research methods stress the ongoing need for reliable identifications of individual animals in sound research and humane care of animals. Toe-clipping has historically been used to individually mark a variety of organisms, but the Food and Drug Administration (FDA, 1984) “determined that toe clipping for animal identification is a potentially painful procedure and its use in animal studies should be discouraged.” Although the Interagency Research Animal Committee did not recommend prohibiting the use of toe clipping for *any* taxonomic group, the use of the procedure in many groups is declining because of concerns about its humaneness.
- B. This is not the case in herpetology, where the method has long been, and still remains, very common (Dunham et al., 1988; Ferner, 2007). The standard guidelines in the field (HACC, 2004) recommend that “The IACUC should be receptive to reasonable justification of such procedures”. Specifically, they 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.”
- C. HACC (2004) states that “the high incidence of natural toe loss among small lizards suggests that for small species at least, toe clipping, when prudently applied, may



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result in only minimal impact.” Several studies (e.g., Brunson, 1986; Hudson, 1996) document the incidence of toe or similar tissue loss, indicating that experimental removal of toes and similar minor procedures does not create a situation not frequently encountered and survived under natural conditions. Most studies examining this issue have empirically likewise reached the conclusion that toe-clipping, when properly conducted in herpetological research, poses few risks in most herpetofauna (Ferner, 2007; Phillott et al., 2007). Most publications that do document adverse effects lack precise information on the methods used, making it difficult to assess the appropriateness of the procedure evaluated. Finally, alternative methods may not always be feasible (Funk et al., 2005), and studies that examine standard alternatives to toe-clipping also show drawbacks (e.g., Boone and LaRue, 1999; Phillott et al., 2007; Caorsi et al., 2012; Brannelli et al., 2014). This led Perry et al. (2011) to conclude that “toe clipping of reptiles and amphibians meets legal and ethical expectations and should remain acceptable where it meets study needs.” Below is a brief review of current literature on toe-clipping in each of the three major taxonomic groups covered in this OP.

TAILED AMPHIBIANS

- A. Despite potential for toe regeneration in some species, toe-clipping remains a standard method for marking urodels (Ferner, 2007). Davis and Ovaska (2001) found slower weight-gain in toe-clipped individuals, and this appears to be the only study reporting negative impacts in this group. Studies showing no impact of toe-clipping in salamanders include Ott and Scott (1999), Arntzen et al. (1999), and Kinkead et al. (2006). The last study is especially insightful because they specifically evaluated stress and behavioral responses to toe-clipping and alternative methods. The authors found that toe-clipping without anesthesia produced similar levels of stress hormones and behavioral response as injection of elastomer, toe-clipping with anesthesia, and even handling without marking (Kinkead et al., 2006).

FROGS AND TOAD

- A. Toe-clipping is a standard method for marking anurans, and most evaluations have shown no negative impacts. However, some concerns over its use have been raised in recent years (Funk et al., 2005; Ferner, 2007). Specifically, Parris and McCarthy (2001) and McCarthy and Parris (2004) used statistical models to reevaluate some previous work and concluded that removal of multiple toes results in low but measurable reduction in return rates in mark-recapture studies. However, “the relative pain of toe-tipping/toe-clipping has not been assessed or compared with alternative marking procedures,” which carry their own disadvantages (Phillott et al., 2007). The authors go on to point out that “mammals have a greater capacity to perceive pain than do other vertebrates (Stevens 1992)” and that they “have rarely observed a clinical pain response after toe-pad removal.” They suggest that “toe-tipping/clipping amphibians cannot be considered as causing the equivalent distress and pain as digit amputation in a human or other mammal.” Further, “the use of anaesthetics,



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analgesics and sedatives [is] far more likely to negatively affect an amphibian's homeostasis because it may be difficult to localize effects of applications of chemicals ... chemical intervention during minor procedures increases handling and recovery time, and is thus likely to impose further stress on animals." Finally, they conclude that "we believe that toe-tipping/clipping is fast, reliable, and toe-tipping possibly the least stressful marking method for anurans" (Phillott et al., 2007). Similarly, the more recent work of Grafe et al. (2011) concludes that toe-clipping is "a viable method for marking anurans" and Guimarães et al. (2014) stated that "Based on our results, but also on logistical and cost issues associated with PIT tagging, we suggest the use of toe clipping with anurans like the blacksmith tree frog." Ginnan et al. (2014) also could not find a reduction in survival of frogs that were toe-clipped. Narayan et al. (2011) showed that toads showed more prolonged stress response following toe clipping than following capture alone, but effects on white blood cell proportions were actually less pronounced following toe-clipping than capture and handling alone. More recently, Fisher et al. (2013) studied the same species and found "no difference in corticosterone levels between Cane Toads that were handled only and Cane Toads that had been handled and had their toes clipped. We therefore conclude that toe-clipping does not cause a greater stress response than does handling."

LIZARDS

- A. "Toe clipping is the most popular technique used in marking lizards" (Ferner, 2007). Nearly all existing studies suggest that toe clipping does not reduce locomotor performance (Huey et al., 1990; Dodd, 1993; Borges-Landáez and Shine, 2001) and the ability of some arboreal species (Bloch and Irschick, 2004; Mahendra 2004) to climb or cling. However, other climbing species showed some effect (Paulissen and Meyer, 2000), and Carothers (1986) showed an effect of removing the toe fringes of *Uma scoparia* on some substrates, but not others. Most relevant to the issue of humaneness had been the recent work of Langkilde and Shine (2006), which focused on stress and showed that toe clipping induces less stress, and for a shorter duration, than use of a PIT tag. Moreover, "Toe-clipping also was less stressful than superficially trivial manipulations such as housing the animal in an unfamiliar enclosure" (Langkilde and Shine, 2006).

CONCLUSION

- A. Inflicting high levels of stress on research organisms is ethically generally considered indefensible (Association for the Study of Animal Behaviour, 2003), and this is also the position of the TTU ACUC. The Food and Drug Administration (FDA, 1984) "determined that toe clipping for animal identification is a potentially painful procedure and its use in animal studies should be discouraged." However, the TTU ACUC also recognizes the need for the individual marking of animals in field studies, and the fact that each method has advantages and disadvantages that are often species- and/or situation-specific. It is incumbent upon investigators to choose the methods most appropriate, humane, and painless for the particular study and to justify this choice to



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the ACUC. However, “the relative impact of different techniques often diverged from our *a priori* expectations (Langkilde and Shine, 2006). Most studies show little or no adverse ecological, behavioral, or physiological effects from properly conducted toe-clipping in herpetofauna.

- B. The TTU ACUC recognizes that any research method that involves animals also carries some potential risk of some level of stress to that animal, and works to minimize such undesirable effects. Where directly assessed, the stress caused by properly conducted toe-clipping has experimentally been shown to be equivalent to, or lesser than, those caused by alternative marking methods such as PIT-tagging and even of non-invasive handling (Kinkead et al., 2006; Langkilde and Shine, 2006). Thus, for many species, marking by toe clipping appears to satisfy more criteria of an ideal mark than alternative techniques available. Furthermore, the method is ethically accepted and broadly used by the herpetological community (HACC, 2004; Ferner 2007). Consequently, the TTU ACUC follows Perry et al. (2011) and more recent studies and endorses the use of properly conducted toe-clipping, as detailed below, in salamanders, frogs, and lizards.

PROPER PROCEDURE FOR TOE-CLIPPING

Following FDA guidelines, the TTU ACUC regards toe-clipping as a potentially painful procedure that should be conducted only when less invasive methods are not appropriate. In addition, toe-clipping may affect the survival of some species, and consideration should be given to this possibility before it is used on a large scale. For endangered species, it may be appropriate to first evaluate the impact of toe-clipping on non-threatened relatives. Should an appropriate alternative that is less invasive become available in the future, researchers should consider to switching to that method. Below, this SOP will present a safe, simple, sanitary, and humane method to remove toe tips from approved organisms. This procedure should eliminate or greatly reduce any adverse effects of toe clipping. When it is employed, investigators should conduct the toe-clipping in the most painless and humane manner possible by following the procedures set below.

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. Acceptable methods of disinfection include soaking in 70% or higher ethanol; flaming the scissors after soaking in ethanol; or bleach



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solution. Blades should be allowed to dry or cool, or thoroughly rinsed with water, after respective treatments.

4. Adults of species with fleshy toes, such as Gila monsters (*Heloderma* spp.) and iguanas (*Iguana* and *Cyclura* spp.) should not be marked by toe-clipping, as the procedure is more likely to result in infection.

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. Possible negative effects should be objectively assessed, given the biology of the species in question.
4. Only clip the smallest possible number of digits possible, never removing more than two non-adjacent toes per foot and limiting even such cases. Numbering systems (e.g., Ferner, 2007; Donnelly et al., 1994) can assist in this.
5. Where possible, clip the toe at the interphalangeal joint (Wright, 2001).
6. 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.
7. 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.

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