Toe-Clipping in Amphibians

Association of Reptilian and Amphibian Veterinarians

- ARAV recognizes the importance of marking amphibians as an essential ecological study field technique.
- Researchers should consider the following when selecting a method of marking amphibians and reptiles: species to be marked, amount of tissue to be removed and use of tissue, amount of pain to be incurred, potential behavioral impairment (i.e., inability to mate or climb), risk of predation resulting from the marking process, and risk of infection.
- Researchers should prove that the benefits of their research will outweigh the potential negative impact that their marking technique has on the population under research.
- Toe-clipping is a potentially painful procedure that may affect the survival and recapture rates of some species and should only be considered if less invasive techniques are not appropriate.
- The minimal number of toes should be clipped as deemed necessary by the study.
- The use of individual toes by different species of animals should be considered. For example, toes essential for mating or climbing should not be clipped.
- As evidence suggests that effects can be related to the species, the effects of toe-clipping on a particular species should be evaluated before its large-scale use.
- If toe-clipping is deemed necessary, it should be performed following strict protocols of cleaning and disinfection to minimize the risk of infection (guidelines can be found from the National Wildlife Health Center web site at http://www.nwhc.usgs.gov/publications/amphibian_research_procedures/toe_clipping.jsp).

The Association of Reptilian and Amphibian Veterinarians is a professional organization of veterinarians involved in the prevention and treatment of illness, research, and welfare of reptiles and amphibians. Here, we address the controversial technique of toe-clipping as a method to mark individual animals in field research settings.

Background

Herpetologists have been using marked amphibians for studies since the 1920s and specifically using toe-clipping as a method of marking individual animals since the 1940s in mark–recapture studies (Woodbury, 1956). Toe-clipping is the removal of part of all of one or more digits from an animal and is often more commonly performed in anurans than urodeles as urodeles tend to have better digit regeneration capabilities (Halliday, 1996). At the first capture, one or more toes are strategically removed to produce a unique code for the individual amphibian. Later, when the animal is recaptured, an identification key can be used to read the missing toe pattern and identify the specific animal. Mark–recapture is a valuable tool for ecological studies estimating population size and other demographic parameters such as survival rates. Toe-clipping became popular because of an inability to use more traditional identification systems. Amphibians tend to be small, do not have ears or other appendages suitable for placing tags, and the mucous nature and frequent shedding of their skin prevents other techniques from being effective (Nace, 1982).

The first adverse effects of toe-clipping reported were published in 1972, when Clarke described a decreased survival rate in Fowler's toads, *Bufo woodhousei fowleri*, associated with the number of toes removed (Clarke, 1972). This called into question the effectiveness of its use and future studies have attempted to refute or confirm these results. If a study is meant to determine death rates, for example, but the technique being applied to the animal changes the rate of death, then the results will be biased, and the true death rate will remain unknown. Therefore, it is imperative in such studies to fully understand the impacts of marking animals, so that its effect can be identified and correctly accounted for in the data analysis. In addition, when studying species or populations that are at risk of dying off or becoming extinct, it is even more critical to know the impact of any manipulation of the animal so that undue stress and death can be avoided.

Compared with other methods of marking animals, toe-clipping is relatively easy, inexpensive, and quick, and it can provide significant research value. Materials required for toe-clipping include sterile scissors; clean water; ethanol; Bactine® spray; and a method to collect the toe-clips, which will vary depending on the desired use. A description of the recommended protocol to follow to perform a toe-clip can be found at the National Wildlife Health Center's web site at http://www.nwhc.usgs.gov/publications/amphibian_research_procedures/toe_clipping.jsp.

It is important to understand what the ideal marking technique would be. Beausoleil *et al.* (2004), in the Department of Conservation in New Zealand, summarized characteristics in the following quotation that would make an ideal identification system:

The ideal mark should

- Allow the animal to be as free of pain and/or stress as possible.
- Identify the animal as an individual, if desired.
- Be easy to apply in both the laboratory and the field.
- Be easily and unambiguously read or observed.
- Be reliable over the duration of the study.
- Be cost-effective.
- Be adaptable to animals of different sizes.
- Utilise materials that are easy to obtain.

An ideal mark should not

- Cause death.
- Have sub-lethal effects on fitness, e.g. reduced growth or reproductive rates.
- Influence the behaviour of marked individuals.
- Influence the behaviour of other animals towards the marked individual.

- Affect the future probability of capturing marked individuals relative to unmarked individuals.

And although no specific marking system will meet all the criteria, a system should be selected that strives to meet each of these, while considering the species to be marked and ensuring that the objectives of the study are still met.

Value in Biological Research

Toe-clipping can be used as a simple method of identifying individual animals so as to prevent redundant sampling or to compare repeated measures over time at the individual level. It is also useful to study species at the population level. Mark–recapture techniques are used to estimate population sizes when all individuals cannot be counted directly. A portion of the population is marked, and then when resampled, the proportion recaptured that were previously marked are recorded. This number can be plugged into specific equations to estimate the population size, which can be compared over time to determine trends in growth or declines. Mark–recapture can also help estimate certain demographic parameters such as survival and birth rates. Again, one needs to bear in mind that this assumes that there is no effect of toe-clipping on these outcomes of interest.

In addition to using toe-clips to identify animals, the actual toe pieces can be a useful source of study material if appropriately collected. In species that cannot be aged morphologically, toe-clips provide a nonlethal source of bones that can be used to estimate age through skeletochronology. Growth rings on the phalanges can be counted, giving an estimate of age (Bastien and Leclair, 1992). This information can provide valuable information about the population dynamics and life history characteristics of the population, although some studies suggest that it might not be a reliable method (Yilmaz *et al.*, 2005). It can also provide genetic material and tissues for histopathology. Gonser and Collura (1996) found that toe-clips can provide suitable total cellular DNA for polymerase chain reactions (PCR) in genetic studies, although alternative samples such as blood or skin or mucosal scrapings might provide similarly useful material. Toe-clips might also serve as a diagnostic sample for infectious diseases that might localize in the bloodstream or tissues of the toes (Gonser and Collura, 1996). As examples, St. Ainour and Lesbarreres (2007) found that *Ranavirus* can be detected in toe-clips in antemortem testing, and Goldberg *et al.* (2007) diagnosed chytrid fungus in toe-pads by using PCR. Again, other tissues such as blood and skin scrapings might be able to provide similar diagnostic specimens, negating this benefit of the toe-clip method.

Opposing Arguments

There are two significant long-term outcomes that have been researched in amphibians regarding the effects of toe-clipping. The first outcome relates to overall fitness and measures items such as body composition, feeding ability, and mating. It is felt that amphibians that have had toes removed may not be able to ambulate, capture prey items, and generally thrive as well; thus, body composition is evaluated as an outcome of interest. A study comparing toe-clipping with the fluorescent tagging technique in salamanders found that toe-clipped *Plethodon vehiculum* gained less weight within a month after tagging compared with initial weights than did the fluorescent tagged or control salamanders (Davis and Ovaska, 2001). In 2006, Hartel compared snout–vent length and wet body mass in marked and unmarked yellow bellied toads, *Bombina variegata*, and found no significant differences.

Recapture rate is the second significant outcome of interest. A decrease in recapture rates suggest that either the animal died or the animal moved out of the study area, possibly as a result of the stress induced by the technique. These reasons are very different and should be kept in mind when interpreting results of any mark–recapture study. There has been increasing research on whether there are adverse effects of toe-clipping in marked amphibians since research first discovered that it may affect survival rates (Clarke, 1972). A review by Parris and McCarthy (2001) identified three studies of *Crinia signifera* in which one study found an association between decreasing returns of frogs with increasing numbers of toe-clips, one study showed no significant effect, and a third study showed variable results. Waddle *et al.* (2008) used modeling to determine the effect of toe-clipping on treefrog survival and found that results varied by species; an association between decreased survival with increasing numbers of toes missing existed in green treefrogs but not in squirrel treefrogs, although there was still some effect on recapture rates in the squirrel treefrogs.

The ethics of toe-clipping were questioned in an article in *Nature* (May, 2004) in response to research showing that previously conflicting studies, when reanalyzed with Bayesian statistics, showed a 4–11% reduction in return rates of frogs with each subsequent toe clipped after the first, assuming a similar effect for each toe (McCarthy and Parris, 2004). This study used data from four previous studies of three species (*C. signifera*, *B. fowleri*, and *Hyla labialis*). When analysis was performed on the specific number of toes removed, there was an inverse association between the number of toes clipped and the recapture rate: as the number of toes clipped increased, there was a decreasing chance of recapture. There was a 96% return rate of frogs with two toes missing compared with frogs with one toe missing, and only a 28% return rate of frogs with eight toes missing compared with one toe missing. McCarthy and Parris (2004) suggest that sample sizes might have limited the power of the other individual studies, where pooling the studies provided sufficient power in their study to measure the true effect. In response to May's article, Funk *et al.* (2005) returned with an alternative view, pointing out that several other studies such as that of Ott found no effect, despite sufficient statistical power (Ott and Scott, 1999). Funk *et al.* (2005) also suggest that species plays an important role that must be considered and that other techniques often either cannot be used or may have more harmful effects than that of toe-clipping. The gains of any research must be weighed against the potential risks, and all studies should be performed under the approval of an Institutional Animal Care and Use Committee.

Although this remains a controversial topic, research continues to attempt to determine whether in fact toe-clipping has such adverse effects. One factor that is often not mentioned is the method by which toes are clipped. Researchers probably vary in their methods of preparing

the digits for removal, which may influence the outcome. Until standardized approaches are used for comparison or methods are more fully described, results between studies may not be comparable. In addition, as has been pointed out previously, species differences may play a role, as well as environmental stressors in different geographic regions.

In addition to the obvious detrimental effects as a result of toe-clipping, there are other concerns that arise, when relying on clipped digits for animal identification. Animals can lose toes as a result of predation or trauma, which could bias the results of a study if an unmarked animal is suddenly included as a marked individual. In addition, it has been observed that some amphibians can regenerate their toes. Although some data exist on the amount of time that is required for this to happen, there is probably significant variability between species and even individuals. This could result in significant loss of information, as well as biasing population estimates if marked individuals are later being counted as new individuals.

Alternatives

There are many methods of marking amphibians that have been described in the literature, as researchers look for the most reliable means of identifying animals in long-term studies that cause the least amount of stress to apply and have the least impact on their natural existence. This list is by no means exhaustive. All methods require some manipulation, which can affect the natural behaviors of the animals and research study results. Much research remains to be done to determine the effects that these methods have on survival, recapture rates, and fitness, and that also offers alternative methods of marking animals that can be considered.

Passive Integrated Transponder (PIT) Tags: PIT tags are used extensively in marking free-ranging animals. The PIT tags are small, cylindrical devices containing a bar code consisting of a unique set of numbers and/or letters that can be read by an electromagnetic scanning device (Ott and Scott, 1999). The tag is inserted under the skin or into the peritoneal cavity. The insertion of the PIT tags requires disinfection at the site of injection, because there is still a risk of infection whenever the skin is penetrated. Other potential complications include migration of the tag in the body and ejection of the tag out of the body, either through skin or through the wound used to insert the tag before it has time to heal. Many species of amphibians are very small, making PIT tagging impossible. In addition, there is a higher cost associated with the individual tags, as well as the scanning device that is not incurred with clipping toes. Ott and Scott (1999) compared PIT tagging and toe-clipping in *Ambystoma opacum* and found no significant differences in short-term growth or survival.

Visible Implant Tags: Two methods of implanting visible tags are becoming increasingly studied as a result of studies suggesting the negative effects of toe-clipping. The visible implant elastomer (VIE) tag is a mixture of two silicone-based materials that are mixed immediately before use (see web site for Northwest Marine Technologies at www.nmt.us). The resulting colored mixture is injected as a liquid that then solidifies into a rubber-like material under the skin where it remains. The VIE tag can be either observed with the naked eye in animals with light-colored skin or observed with the use of a blacklight that causes the marker to fluoresce in darker pigmented animals. A combination of color and body location enables several unique identification codes. Recommended injection sites include between the toes and on the upper hind legs of frogs, and at the base of the limbs on the ventral side of salamanders. They cannot be injected under the skin in other locations as the tags tend to migrate. One study using VIE tags to mark salamander egg masses found no effect on egg survival or hatching rates or body size or developmental stage of hatchlings (Regester and Woosley, 2005). Although more research is needed in amphibians, some potential complications of this method seen in seahorses were misreading the implant color, not being able to visualize the implant, and partial or total implant loss (Curtis, 2006).

A second method using a visible implant is the visible implant alpha tags. Similar to VIE tag, these implants are premixed and contain an alphanumeric code on one side, which increases the number of identification codes and eliminates the need to use different locations for identification. Codes can be read either directly through the skin or with the aid of a light. Similar concerns such as tag migration/ejection and visualization apply to these tags as occur for VIE tags. The advantage to visible implant alpha tags is that they can be applied in legless as well as legged amphibians.

Skin Patterns/Photographic Identification: Identification of skin patterns or natural markings can be a useful means of differentiating animals in some species, whereas less applicable to others. This method is often easier on larger animals and those that have more distinctive skin markings. This method, however, might not be very reliable, because it relies on the ability of the observer to correctly identify the animal at recapture.

Tattoos and Dyes: Various methods of tattooing, chemical branding, freeze branding, and applying dye markers have been suggested for short- and long-term marking strategies (Clark, 1971; Nace, 1982; Taylor and Deegan, 1982; Davis and Ovaska, 2001). Several factors need to be considered when choosing one of these methods. First, to visualize any marks, appropriate dye colors need to be selected that contrast with the natural pigmentation of the skin. Second, amphibians have very permeable skin, so any dyes or agents to be applied on the skin need to be thoroughly tested for toxic properties in the laboratory setting before being used as a method of marking animals in the field. Third, degradative properties of the dye need to be considered. Finally, branding must reach the deep layers of the skin to ensure that regeneration does not occur. Depending on the duration of the desired mark, and the frequency with which observations will be made, some of these methods may not suffice. Nace (1982) found that marking procedures such as tattooing and chemical branding only lasted a few months.

Summary

Research suggests that the use of toe-clipping may have an effect on survival and recapture rates of amphibians.

However, this is still debated and may be species and location specific. Alternative methods of identification should always be evaluated against the toe-clipping technique when designing a research study that weighs the potential benefits of the research results against the potential adverse effects.

The ARAV recommends that, when possible, toe-clipping be avoided. If a toe-clipping technique is to be used, pain and stress on the individual animal should be minimized, proper disinfection techniques should be used (http://www.nwhc.usgs.gov/publications/amphibian_research_procedures/toe_clipping.jsp), and the minimal number of toes is clipped. Toes that are essential for normal behaviors, such as mating, should be avoided. Effects of toe-clipping should be monitored throughout the study and accounted for in the analysis. Toe-clipping should never be used on a large scale in critical populations or endangered species without prior studies of the effect on that species. In addition, all studies should be approved under an Institutional Animal Care and Use Committee before commencing.

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