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Toe-Clipping as an Acceptable Method of Identifying Individual Anurans in Mark Recapture Studies

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Mark-recapture studies are an important and sometimes essential means for developing a detailed understanding of all aspects of amphibian natural history. Toe-clipping has historically been regarded as the most appropriate marking technique for use in many anuran population surveys and ecological research. Unique combinations of multiple digits are removed from the hands and/ or feet when the animal is first captured, to allow recognition of each individual (see Donnelly et al. 1994 for a summary). Bogert (1947) first described the procedure, and it has subsequently allowed some individuals to be followed for more than 34 years (Bell and Pledger 2005). Often toe-clipping has involved amputation above the toe base or webbing (Clarke 1972; Davis and Ovaska 2001; Funk et al. 2003; Lemckert 1996; Reaser and Dexter 1996; Waichman 1992). Current practice by authors Phillott, Skerratt, McDonald and Alford is to remove the toe pad/disc only from a hylid digit or the most distal phalanx from a non-hylid digit. This procedure is more accurately defined as "toe-tipping" rather than "toe-clipping", as there is a substantial distinction between the length of the digit removed. Frequent surveys can rely on toe-tipping rather than toe-clipping as a reliable means of identifying individuals with minimal tissue regrowth (Lüddecke and Amézquita 1999).

Recently, the appropriateness of toe-clipping frogs has been called into question (Funk et al. 2005; May 2004) when studies by Parris and McCarthy (2001) and McCarthy and Parris (2004) predicted decreased recapture rates following toe-clipping. This was considered due either to mortality arising from digit/limb inflammation and infection (see Golay and Durrer 1994; Lemckert 1996; Reaser and Dexter 1996; Williamson and Bull 1996), disturbance resulting in movement of animals away from the study site, or inability to recapture marked animals. Although Parris and McCarthy (2001) and McCarthy and Parris (2004) only used statistical models to project the effect of toe-clipping on amphibian recapture rates and did not conduct studies to quantify this potential effect, these predictions brought into question the scientific validity and ethical status of toe-clipping (e.g., May 2004) and have led some research and government organizations to refuse permission to use this method. However, there has been little consideration given to the impacts of alternative methods of marking anurans (Funk et al. 2005), no real discussion of how these impacts occur and if they may be overcome, and no assessment of the necessity of toe-clipping for carrying out ecological research that may be critical for species conservation.

The actual reasons for the reduced return rates after toe-clipping described by Parris and McCarthy (2001) and McCarthy and Parris (2004) need to be further explored. Reductions in survival rates (due to infection or limited mobility leading to starvation or increased predation risk) and recapture rates may both contribute to reduced return rates. Actual increases in mortality as a result of toe-tipping or -clipping have not been directly measured, and the validity of the assumption that this is the only factor affecting recapture rates following toe-clipping is questionable. Behavioral changes of several types could lead to decreased recapture rates following toe-clipping. Animals could alter activity patterns or locations within their home ranges, decreasing the probability of recapture. The degree of site fidelity of many amphibians is unknown, and at least some are known to be nomadic (Schwarzkopf and Alford 2002). If toe-clipping increases the probability of animals moving away from the location at which it occurred, it will appear to reduce survival, since survival and emigration rates are very difficult to separate using mark-recapture techniques. Distinguishing the mechanisms responsible for changes in recapture probability following the application of toe-clipping and other marking techniques should be a priority subject for future research.

Even if increased mortality is the cause of decreased returns, it is possible that these increases can be minimized through a more considered approach to toe-clipping. Frequent surveys can rely on toe-tipping rather than toe-clipping as a reliable means of identifying individuals with slow or no tissue regrowth. It has long been

TABLE 1. Alternative marking techniques to toe-clipping/tipping.

Marking Procedure	Advantages	Disadvantages
PIT (Passive Integrated Transponder) Tags	Should not effect mobility ¹	Invasive Requires extensive handling, anaesthesia and recovery ¹ Potentially suitable for inducing foreign body tumorigenesis ² Tags can migrate or be lost ¹ Cannot be applied to small frogs ¹
VIE (Visible Implant Elastomer) Tags	Relatively inexpensize ³ Should not effect mobility ⁴	Invasive Can be difficult to visualise ³ Requires extensive handling and anaesthesia ^{3,4} Easiest when amphibians tagged in groups ⁴
VIA (Visible Implant Alphanumeric) Tags	Relatively inexpensive Should not effect mobility ⁴	Invasive Requires extensive handling, anaesthesia and recovery time ³ Tags can migrate ³ Can be lost ³ Can be difficult to visualise ³
External Radio Transmitter	Non-invasive Allows researcher to find location of individual ^{5.6.7}	Normal movements and behaviour may be hindered ⁵ Skin lesions can occur ⁶ Restricted to medium to large amphibians ⁵ Time consuming ⁵ Limited battery life ⁵ Expensive ⁵ Tags can be lost ⁶
Internal Radio Transmitter	Allows researcher to find location of individual ⁵	Invasive Time consuming ⁵ Requires extensive handling, anaesthesia and recovery time ^{6.7} Tags can migrate ⁸ Tags can be lost ⁶
Pattern Mapping	Non-invasive Can be applied to small animals ⁹	Limited application due to lack of or limited patterns in many species ¹⁰ Temporal inconsistency of patterns in suitable species ^{9.11} Time consuming ⁹
Pressurized Fluorescent Marking	Tagged amphibians do not require capture to identify ¹²	Invasive Only reliable for few weeks ¹² Can result in immediate mortality ¹² Equipment heavy ¹²

¹Lane 2005a, ²Vogelnest et al. 1997, ³Nauwelaerts et al. 2000, ⁴Lane 2005b, ⁵Richards et al. 1994, ⁶Werner 1991, ⁷Weick et al. 2005, ⁸Gray et al. 2005, ⁹Donnelly et al. 1994, ¹⁰Lemckert and Shine 1993, ¹¹Reaser 1995, ¹²Schlaepfer 1998

recognized that toe-clipping could negatively affect anurans, and there are current guidelines for amphibian toe-clipping that provide guidance regarding how best to undertake these procedures (e.g., Herpetological Animal Care and Use Committee HACC [2004] and National Wildlife Health Centre NWHC [2001]). Some of the studies reviewed by Parris and McCarthy (2001) and McCarthy and Parris (2004) did not use, or at least did not report, the use of aseptic techniques such as those recommended by the National Wildlife Health Centre (2001) and NSW National Parks and Wildlife Service (2001). These recommend the use of singleuse gloves, sterilized stainless steel scissors, and application of the antiseptic Bactine[®] to minimize infection (though the latter may not be effective for some species such as stream dwelling frogs that immediately wash off the antiseptic when they return to the water after capture). Higher infection rates are likely to occur if unsterilized instruments are used, and diseases may be transferred between multiple individuals if equipment and handling surfaces are not disinfected. The prevention of contamination and infection should be a primary concern in studies using any tagging technique, and toe-tipping/clipping is less likely to result in infection if the above standards are followed.

Alternatively, some increased mortality may result from a loss of mobility due to the loss of toes. This aspect does not appear to have been explored, and we recommend that controlled studies should be undertaken to compare the mobility and survival of clipped versus unclipped frogs. However, even given this lack of

knowledge, steps can be taken to minimize any possible effects on mobility. The effect of toe-tipping/clipping on populations can be minimized by careful consideration of frog activities. Species heavily reliant on toes (e.g., arboreal or burrowing species) should be given a minimal number of clips. Removal of the toe pad only, instead of the entire toe, still allows a reliable means of recognition. It is already recommended that toes essential for burrowing, climbing, amplexus or nest excavation should not be not removed (HACC 2004).

May (2004) describes the practice of toe-clipping as "casually barbaric." The relative pain of toe-tipping/clipping has not been assessed or compared with alternative marking procedures. We have rarely observed a clinical pain response after toe-pad removal. While invasive procedures should not be applied without due consideration of the pain and stress caused to the animal, it must be remembered that mammals have a greater capacity to perceive pain than do other vertebrates (Stevens 1992). Toe-tipping/clipping amphibians cannot be considered as causing the equivalent distress and pain as digit amputation in a human or other mammal. We acknowledge that amphibians are able to perceive pain, however we regard the use of anaesthetics, analgesics and sedatives as far more likely to negatively affect an amphibian's homeostasis because it may be difficult to localize effects of applications of chemicals associated with toe-clipping. No single anaesthetic is effective for all amphibians due to their species-specific responses (Fellers et al. 1994) so their use can be both difficult and harmful. Chemical intervention during minor procedures increases handling and recovery time, and is thus likely to impose further stress on animals. In addition, the HACC (2004) regards pain as an adaptive response that reduces the use of an injured limb during recovery, and describes the use of anaesthetics in procedures such as toe-clipping as undesirable. This reduces handling time and allows post-operative, field-released animals to adapt their activity to compensate for the affected limb/s.

While toe-tipping/clipping is the least expensive of the marking procedures suitable for amphibians, the decision to use this method should not be based on cost alone. We believe that toe-tipping/ clipping is fast, reliable, and toe-tipping possibly the least stressful marking method for anurans. Furthermore, we note that in many countries, toe-tipping/clipping cannot occur without the approval of animal ethics committees at research institutions.

In recent times a range of alternative techniques have been proposed as suitable means to mark individuals over extended periods. Proponents of alternatives (e.g., May 2004) have provided no evaluation of impacts of the procedures on marked individuals. The only entirely non-invasive technique successfully used to identify individual anurans is pattern mapping by photograph or sketch. Alternative invasive techniques to toe clipping or toe tipping have included dye markers (e.g., Visible Implant Elastomer [VIE] tags and pressurised fluorescent marking techniques), individual recognition tags (e.g., Visible Implant Alphanumeric [VIA] Tags and Passive Integrated Transponder [PIT] tags), radio telemetry transmitters, and branding (using tattoos, silver nitrate, heat, or freezing). The advantages and disadvantages of all procedures are summarised in Table 1. It is clear that all marking techniques carry risks and may have adverse effects on marked animals, so all techniques violate assumptions related to population estimation models. However, more study is required to determine which techniques have the least adverse effects.

All invasive marking techniques have ethical considerations. The value of the work to the species as a whole should be weighed against the potential impacts on individuals. The stress imposed on a limited number of individuals needs to be balanced against the increased knowledge that can be gained for the species. Management plans must be based on an understanding of the natural history of the animal, including site fidelity, home range and longevity, and factors such as disease, which affect survival. Assured recognition of individuals is essential to determine such parameters.

As extinctions of frog species occur with the global emergence of chytridiomycosis, threatened and potentially-threatened populations must be studied to understand the epidemiology of this disease. Prevalence, incidence, survival and transmission rates cannot be calculated without absolute certainty of individual recognition. In the absence of another widely applicable tagging technique, known to have at most small effects on return rates, we suggest that toe-clipping is the only feasible option that can be applied to many species and to all post-metamorph members of a community. Additional research is urgently needed to evaluate the impacts of all marking techniques, and to develop new ones with lower impacts. Until this has been done, we believe that it is more ethical to use toe-clipping in studies aimed at understanding and preventing further loss of anuran diversity than it would be to stand by, leaving many species unstudied, or to use alternative techniques that have not been studied and which may have greater impacts on amphibians.

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