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Visual implant elastomer (VIE) tags are an unreliable method of identification in adult anurans

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There has long been debate over alternatives to toe-clipping as an individual marking method in anurans. Alternative methods include visible implant elastomer (VIE) tags and passive integrated transponder (PIT) tags. VIE tags are low cost, easy to insert and have been used successfully in reptiles, fish and salamanders without tag loss or movement. In this study, we tested whether two species of VIE-tagged anurans (captive Kihansi spray toads, *Nectophrynoides asperginis*, and leopard frogs *Lithobates pipiens*) experienced tag movement or loss that could lead to errors in individual identification. VIE tag movement occurred in 50% of the tags implanted which caused 70.6% of individuals to be potentially misidentified. These results demonstrate that the use of VIE tags to individually mark anurans can be highly unreliable. We therefore recommend either verifying the reliability of VIE tags through species- and life stage-specific pilot studies, or choosing another method of marking.

Key words: capture-mark-recapture, individual identification, *Lithobates pipiens*, *Nectophrynoides asperginis*, VIE tagging

INTRODUCTION

Individual marking is often necessary in field and laboratory studies when individuals cannot be distinguished from each other based on physical features such as colour or pattern. Currently, there are four broadly used marking techniques for amphibians: toe-clips, passive integrated transponder (PIT) tags, visible implant alphanumeric (VIA) tags, and visible implant elastomer (VIE) tags. All four of these tagging methods have been successful in a variety of taxa, but their effectiveness on anuran species needs to be further assessed. The only reliable, non-invasive technique for individual identification is the photographic identification method. This method involves taking photographs of individuals and determining identity based on their unique colour and pattern (Donnelly et al., 1994). Photographic identification requires individuals to have unique markings that remain constant through time (Kenyon et al., 2009; Caorsi et al., 2012). Therefore, photographic identification is not an acceptable method for all species. In addition, it is often time consuming and in some species the misidentification rate can be higher than for other marking methods (Kenyon et al., 2009).

Toe-clipping has been used to mark individuals of a variety of organisms, including rodents, reptiles and amphibians. The method has been called "barbaric" (May, 2004) and is often questioned by animal ethics boards as causing unnecessary harm to study animals. Despite this criticism, it is currently the most widely used marking method for anurans, and has been defended by many because it is simple and cost effective (e.g., Phillott et al., 2007; Correa, 2013). This method has the added benefit of providing tissue for genetic analysis (Donnelly et al., 1994; Perry et al., 2011). Some limitations of toe-clipping are that it cannot be used on larval amphibians and some anurans regenerate toes, making misidentification more likely.

Although some studies have reported high levels of site infections after toe removal (Golay & Durrer, 1994), others have found infection to be rare (e.g., 6 of 500 individuals marked; Lemckert, 1996), and limited in occurrence to within the first 10 days after clipping. Infection is a risk for invasive marking techniques, including PIT, VIE and VIA tags. Rate of infection appears to be species dependent, and each species may react differently to different techniques (e.g., Lemckert, 1996). For all of these methods, marking should be performed with sterile instruments in order to minimize infection (Grafe et al., 2011). There is currently little evidence to suggest that toe-clipping are detrimental to the animals' survival (Grafe et al., 2011) or body condition (Hartel & Nemes, 2006). A Bayesian analysis found an effect on survival after eight toes had been clipped (McCarthy & Parris, 2004), although subsequent empirical data supporting this claim are minimal (Grafe et al., 2011). Waddle et al. (2008) found a 5.02% decrease in survival in one species of hylid frog when one or two toes were removed, and an 11.16% decrease in survival in individuals when three or four toes were removed.

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However, in a second, closely related species of hylid frog, no correlation was found between number of toes removed and survival (Waddle et al., 2008)

Evidence for short-term effects as a result of toe-clipping is limited in anurans. Kinkead et al. (2006) found that, in salamanders, stress hormone levels did not change as a result of different tagging methods (toe-clipping compared to VIE tagging), but increased with handling time. Schmidt & Schwarzkopf (2010) tested the effect of toe-clipping on anuran locomotion and found that although jumping distance decreased immediately after toe-clipping, locomotion in toe-clipped anurans did not differ from control animals after the wound healed. Lemckert (1996) found that gravid females and adult males of *Crinia signifera* altered their mating behaviour by leaving the breeding pond immediately after toe-clipping, but when they returned, they behaved normally. Previously toe-clipped individuals, in contrast, behaved normally throughout the breeding season.

PIT tags are an alternative to toe-clipping and have been used in a variety of taxa including small mammals, fish, amphibians and reptiles. Standard PIT tags have a size of 2 x 10 mm and are therefore too large for use with small amphibians (Funk et al., 2005). PIT tags do not appear to effect the health or survival of amphibians, and rates of misidentification and tag loss are low (Christy, 1996; Brown, 1997; Unger et al., 2012). With PIT tags, up to 34 billion unique codes are possible, but the method is expensive and requires a portable scanner (Donnelly et al., 1994). Although new technology is being developed to make PIT tags smaller, the cost of PIT tagging will likely continue to be high compared to other marking methods.

An alternative to PIT tags is visual implant alphanumeric (VIA) tags. These tags are injected into the epidermis of the amphibian, are relatively inexpensive and do not rely on permanent position for correct identification. VIA tags are illuminated with a handheld UV light and can supply thousands of codes. The downside of using these tags is that if the tag migrates to a darkly pigmented portion of the body, the tag might be difficult to find again. As long as the tag is visible, rates of misidentification for VIA tagging are reportedly low (Heard et al., 2008). VIA tags are commonly used in fish (e.g., Isely & Grabowski, 2011) and have been used with success in some amphibian species (Heard et al., 2008; Osbourn et al., 2011).

Visible implant elastomer (VIE) tagging is a fourth option that has been successfully used for individual marking in salamanders, reptiles, crustacean and fish (Davis & Ovaska, 2001; Woods & James, 2003; Curtis, 2006; Grant, 2008; Waudby & Petit, 2011). This method involves the subcutaneous injection of coloured silicone beads, generally on the ventral surface of the limbs because those areas tend to be least pigmented allowing a clearer view (Waudby & Petit, 2011). VIE materials are relatively inexpensive and a portable UV light is used to visualize the pigmented beads. This method has been used successfully on very small animals (<1g, Waudby et al., 2011) and studies in fish (Curtis, 2006) and reptiles (Waudby et al., 2011) suggest that tags are rarely displaced or lost. One study found that misidentification was lower in VIE-tagged than toe-clipped salamanders (Davis & Ovaska, 2001). Lastly, VIE tagging is one of the few reliable methods for marking tadpoles, with tags being retained through metamorphosis (Grant, 2008).

Few studies have assessed VIE tag loss and misidentification in anuran species. In the present study, we aim to determine the efficacy of using VIE tags in adult anurans, and the rate of misidentification based on tag loss, movement and breakage. Specifically, we examined (i) the movement of VIE tags one week post-implantation in a small species of frog, *Nectophrynoides asperginis,* and (ii) the rate of misidentification due to tag movement or loss in a larger species of frog, *Lithobates* (=*Rana*) *pipiens*.

MATERIALS AND METHODS

We assessed the movement of VIE tags using a sample of 17 adult Kihansi spray toads (*Nectophrynoides asperginis*). Toads were housed communally (3–9 individuals per terrarium) in 36 x 21 x 21 cm terraria on a moss substrate at the Detroit Zoo in Royal Oak, Michigan. The animals were uniquely tagged by injection of VIE beads (Northwest, Marine Technologies, Inc.) under the dermis with a sterile 29-gauge needle. Beads were applied using 2–3 mm injections halfway between the knee and the pelvis on either or both hind limbs, for a total of 28 implanted tags. Combinations of three fluorescent silicone bead colours (pink, yellow and green) were used to tag individuals. The locations of the tags, as identified using a handheld UV light, were recorded upon injection and again one week post-injection.

We assessed the rate of misidentification resulting from VIE tag movement or loss using a sample of 36 adult male northern leopard frogs (*Lithobates pipiens*). These frogs were uniquely tagged using one or two 2–3mm injections of VIE beads into eight body regions, either the upper or lower half of the fore or hindlimb, with no more than one tag being implanted in each limb segment. Only one colour tag, yellow, was used in this species. Tag positions were chosen based on standard practice in lizard species (S. Lailvaux personal communication), that have also been shown to have the least movement

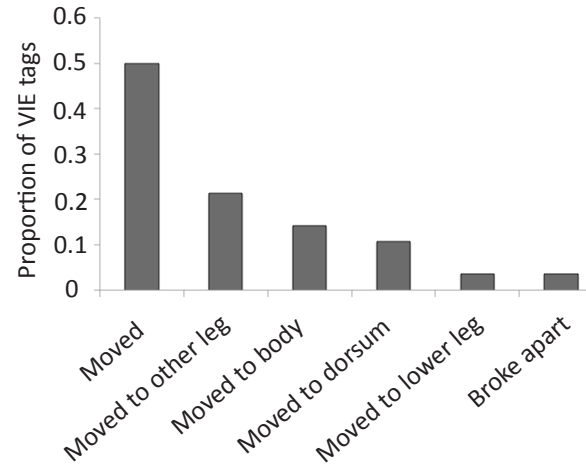

Fig 1. The proportion of VIE tags that moved after one week in *Nectophrynoides asperginis*, compared to the total number of tags implanted.

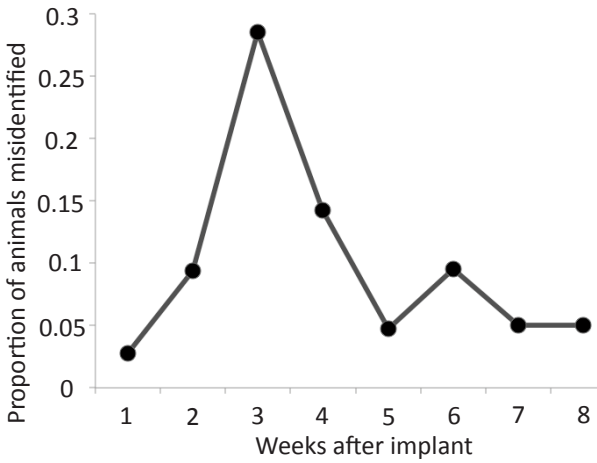

Fig 2. The proportion of *Lithobates pipiens* misidentified per week.

in anurans (Mooseman & Mooseman, 2006). Animals were housed communally with 16 individuals per 255 x 35 x 34 cm enclosure, at University of New Orleans in New Orleans, Louisiana. Each enclosure was filled with 10 litres of water and a dry refuge of 36 x 21 x 16 cm was placed in the centre of the enclosure. Due to animal deaths over the course of the 8-week experiment, the number of animals dropped to 10 individuals per enclosure. The number of identity checks performed over the course of the experiment was 228, with each animal in each enclosure being checked once per week.

In the *L. pipiens* study, instances of misidentification were recorded, not individual tag movement. Animals with tags that had been lost or migrated so that the animal's identity was indistinguishable from another animal in the same enclosure were recorded as misidentified. For example, if two animals in the same enclosure had VIE tags in the lower portion of each hindlimb we would not know which animal had a tag that originated in the upper portion of the hindlimb but had moved to the lower portion and which animal's tags had not moved. While only one of the two animals' tags had moved, in this case we would consider both animals misidentified as we could not reliably distinguish between them. However, if an animal showed two tags in the lower portion of a hindlimb, one of those tags was assumed to have originated in the upper portion of its hindlimb, and thus it was not considered misidentified (because no other frog was marked with two tags in the lower hindlimb). An individual's identity was determined based on the location of its tags in comparison with the tag locations of the other individuals in the communal enclosure. For this reason, we consider the number of animals misidentified over the eight-week period to be conservative.

RESULTS

Of the 17 *N. asperginis* marked, only five individuals had markings that maintained their original placement. Within one week of implantation, 70.6% (12 of 17) of individuals had one or both marks moved, so that the animals were potentially misidentified. Fifty percent of the individual tags injected into the animals moved (Fig. 1). The most common movement pattern was to a different section of the body (either the other hindlimb or the body cavity, Fig. 1). Another common observation was the migration of a tag from the ventral to the dorsal surface where the mark was obscured by overlying skin pigmentation (Fig. 1). In one individual, a tag broke apart, and one half moved to the dorsal surface while the other half moved to the opposite hindlimb.

During the eight weeks of the *L. pipiens* experiment, 7.9% of identification checks resulted in misidentifications (18 individual checks across 228 identity checks; Fig. 2). Eight individuals were misidentified at least once throughout the experiment. Three individuals were misidentified in more than one week. One individual was misidentified for two weeks, correctly identified for one week, and misidentified for the final three weeks of the experiment. This individual was most likely misidentified because its tag migrated to an area of the skin that was too heavily pigmented to permit tag detection. An increase in misidentification in week 3 (Fig. 2) is partially explained by the fact that three separate individuals' marking patterns converged. The variation in number of animals misidentified ranged from 2.7% to 28.6% in any given week.

DISCUSSION

VIE tagging has been successful in a broad range of taxa including salamanders, lizards, fish and crustaceans (Davis & Ovaska, 2001; Woods & James, 2003; Curtis, 2006; Grant, 2008; Waudby & Petit, 2011). VIE tags in these taxa have been reported to remain in place and, therefore, misidentification rates have been low. Our results suggest that despite their success in some groups, the use of VIE tags in adult anuran amphibians may have limited effectiveness.

Our results indicate that, for the species of adult anurans used in the present study, VIE tags are not a reliable method for identifying individuals. In the *N. asperginis* study, where only two body regions were used, 50% of tags migrated from their original position within one week. This suggests that in tagging schemes where body position is important to individual identification, high rates of misidentification are likely. In the *L. pipiens* study where eight distinct body regions were used, misidentifications due to tag migration occurred 7.9% of the time. In this study, animals were housed in relatively small groups of 10–16 individuals per enclosure. Rates of misidentification are expected to be higher if frogs have to be identified from within a larger group of marked individuals, as is often the case in field capture-mark-recapture studies.

VIE tags appear to move easily under the skin of adult anurans. It is possible for a researcher to recreate this natural tag movement and cause tags to migrate between body regions by gently massaging the animal's skin in one direction. The size of the animal appears to have an effect on the direction of tag movement. In the smaller *N. asperginis*, tags rarely migrated from the thigh, over the knee joint and into the lower portion of

the limb, presumably because the tag was large in size compared to the knee joint in this species. In the larger *L. pipiens*, there was no discernible movement pattern.

Misidentifications in *L. pipiens* ranged from 2.7% to 28.6% across weeks. In this study, individual tag movement was not quantified, as was done in the *N. asperginis* study. Instead, we quantified the number of instances in which tag movement resulted in confusion with other marked individuals within the enclosure. This large range of misidentification does not necessarily reflect changes in the rate of tag movement, but rather the potential for misidentification of individuals once tags have moved. The results of this pilot experiment suggest that the potential for misidentification is likely to be affected by the number of animals to be identified and the tagging regime chosen. Given the large range of misidentification rates we observed, even among groups of only 10 to 16 marked animals, larger VIE studies run the risk of suffering similar or even greater problems with individual identification.

If the VIE tagging technique is to be used for individual identification in adult anurans, tag placement as well as the size and activity level of the species should be taken into consideration. Our observations suggest that injecting the tag into the lower leg might result in less tag movement, especially in smaller individuals. Nauwelaerts et al. (2001) suggest tagging frogs in the webbing of the hind feet. Using this technique is likely to result in less tag movement than implanting the tags in the limbs, but in small animals or ones with minimal webbing, this may not be feasible. We suggest injecting VIE tags into multiple body regions in a subset of test animals and monitoring their detectability before settling on a tagging scheme.

As an alternative to strictly VIE tagging, a mixed method of VIE tagging and toe-clipping could be considered. This combination would result in fewer toes needing to be removed than toe-clipping alone, but would still require permanent placement for VIE tags. Hoffmann et al. (2008) suggest removing one toe and injecting a VIE tag into the plantar surface of the digits. Such a method would also increase the number of potential combinations.

We found potential misidentification in 70.6% of *N. asperginis* individuals due to tag movement. This high rate of misidentification would not be acceptable in a capture-mark-recapture study where all recaptures must be identified with a high degree of certainty. Misidentification rates may be lower with other methods. For example, misidentification rates for PIT tags can be as low as 0% for anurans (Brown, 1997), as long as the tag is not expelled, and less than 7.7% in capture-mark-recapture studies that use toe-clipping (Kenyon et al., 2009; Caorsi et al., 2012). Given the high rates of tag movement and subsequent misidentification that we observed in this study, the VIE method appears to be the least reliable of the individual marking options for adult anurans. Our findings reinforce the need to test marking methods for efficacy and adverse impacts on study species prior to use in laboratory or field studies.

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