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Visible Implant Alphanumeric (VIA) as a marking method in the lesser snouted treefrog *Scinax nasicus*

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Abstract. In this study we assessed the efficacy of Visible Implant Alphanumeric (VIA) for marking adults and juveniles of the Neotropical treefrog *Scinax nasicus*. We evaluated the success of this technique in the identification of individuals and the prevalence of tags in the field. As a control, we marked the same individuals through toe-clipping. Of 196 marked individuals, 57 were recaptured in a 7-month study period. Only one mark was unreadable because it was located too deep in the skin. We found one case of tag expulsion and two inverted tags. Almost complete regeneration of the adhesive disk was observed by the fifth month of the study in all recaptured frogs. We suggest VIA tagging method as suitable for *S. nasicus* over long term studies. Even though, a hybrid method for marking (VIA + toe-clipping) is recommended for species with dark and/or loose skin, or large frogs.

Keywords. Amphibian, mark-recapture, fluorescent elastomer, toe-clipping.

The recognition of individuals in a population is a key aspect in many amphibian ecology and conservation studies (Seber, 1982; Campbell et al., 2009; Clemas et al., 2009). Their identification over time and space helps researchers to estimate individual and demographic parameters such as growth and mortality rates, dispersal, population size and habitat use (Osbourne et al., 2011). In cases where individuals of a species do not possess characteristics that distinguish them from each other, like coloration and color patterns, and photo-identification is not suitable, it is necessary to use marking techniques (Donelly et al., 1994). For amphibians, the most commonly used are toe-clipping, Passive Integrated Transponder (PIT), Visible Implant Elastomer (VIE) tags, and more recently Visible Implant Alphanumeric (VIA) tags, the last two manufactured by North-west Marine Technology, Shaw Island, USA (Heard et al., 2008; Branelly et al., 2014).

Toe-clipping is the most widely used technique for marking anurans and salamanders (Ferner, 2010). However, this method is highly criticized because a decrease in the probability of recapture has been detected in some amphibian species (Clarke, 1972; Mccarthy and Parris, 2004; Waddle et al., 2008). Another disadvantage of toe-clipping in long term studies is the possibility of tissue regeneration, although this has been observed only in a few species (Ferner, 2007; Ursprung et al., 2011). PIT tags consist of an electromagnetic capsule with an alphanumeric code, which is read by a scanner. Insertion is done subcutaneously or in a body cavity (Ferner,

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2010). However, loss of tags and deleterious effects related to survival have been detected using this technique (Scherer et al., 2005; Guimaraes et al., 2014). VIE tagging is done through the subcutaneous injection of an elastomer mixed with a curing agent. This technique allows the individualization of many animals by creating different fluorescent color codes as well as placing the marks in various body locations (Moosman and Moosman, 2006). When comparing these three techniques, Branelly et al. (2014) mention that the least efficient is the VIE tagging, due to the migration, darkening and in some cases expulsion of the tag.

VIA tags are compacted elastomers in rectangular shape with an alphanumeric code in fluorescent colors visible under UV light (Heard et al., 2008). The insertion is done with an injector provided by the manufacturer, but in some cases the results are better making a previous incision (Buchan et al., 2005; Gower et al., 2006; Heard et al., 2008; Clemas et al., 2009; Kaiser et al., 2009). Despite being considered a reliable technique due to the capacity of individual identification, time of marking, handling of the individuals, durability of the marks and relatively low cost (Haw et al., 1990; Buchan et al., 2005; Gower et al., 2006), this technique also has disadvantages when used in amphibians: difficulty of tag insertion in species with loose skin, variation on tag retention among species and darkening of the tag when marking heavily pigmented species (Kaiser et al., 2009). Additionally, tag retention was low in studies of mark-recapture of tadpoles (Courtois et al., 2013).

We tested the VIA tags in the lesser snouted treefrog *Scinax nasicus* (Cope, 1862) in order to 1) evaluate the effectiveness of this technique in the identification of individuals, 2) determine the prevalence of tags in mark-recapture studies, and 3) provide some recommendations for further studies upon this species and others with similar morphology.

S. nasicus is a small sized hylid frog distributed in northern and central Argentina, Paraguay, Uruguay, eastern Bolivia, and central and southern Brazil (Frost, 2019). The species is commonly found in open areas of the Atlantic Forest, Cerrado, Chaco, Pampa, and Pantanal domains (Dalmolin et al., 2017). We captured individuals of *S. nasicus* using 173 PVC pipes as a refuge in an area of approximately 3 km^2 of wetland and associated riparian forest in Benjamín Aceval, Presidente Hayes department in Paraguay (-24.960522S, -57.359425W). Field work was done during the months of November 2017, January, March and May 2018.

The individuals were tagged both with VIA tags and toe-clipping. Additionally, measurements of the snout-vent length (SVL) were taken with a digital caliper Mitutoyo Absolute AOS Digimatic. The VIA tags used were of standard size (1.2 × 2.7 mm) in fluorescent green with black letters. The insertion site was the ventral region of the right thigh following Buchan et al. (2005) (Fig. 1). The implantation site was sterilized with ethanol 90%, then the tag was inserted under the skin using the injector provided by NMT following the manufacturer protocol and no veterinary glue was used. All marking equipment was sterilized between frogs by immersion in 90% ethanol for several minutes. Toe-clipping was carried out as control following the method of Martof (1953). As suggested by Kinkead et al. (2006), no anesthesia was used in either both procedures. Frogs showed no signs of discomfort, e.g., emitting distress calls or abnormal movements of the affected limb and/or foot. Frogs were put under observation for 24 hours in plastic bags filled with air to observe presence of redness, edema or bleeding on the treated foot or at the injection site. Subsequently they were released on the same PVC pipe where they were captured. We marked 196 individuals of *S. nasicus* (55 females, 46 males, and 95 juveniles). Frogs averaged 25.9 mm (SD = 5.34 mm) of SVL. We obtained a total of 57 (29%) recaptures, of which 2 individuals were recaptured three times, 14 individuals twice, and 41 individuals only once. The recapture rates in juveniles was 47%, males 16% and females 37% (Fig. 2). We did not find significant differences in the recapture rates of juveniles and adults ($t = 0.3487$, d.f. = 4, $P = 0.7449$); therefore, it can be suggested that the VIA implants did not have negative effects on the survival of juveniles.

Fig. 1. Location of the VIA tag in the inner thigh of *Scinax nasicus*. VIA tag was injected just below the skin.

Every time the label was observed, the code could be identified without ambiguity with the help of the UV light lantern provided by the kit, in 98% of the times. In one case, the label was located too deep under the skin and could not be read properly. At the same time, by January 2018, we observed tissue regeneration in recaptured

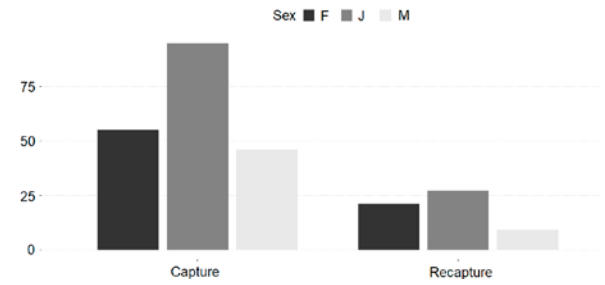

Fig. 2. Number of capture and recapture males, females and juveniles of *Scinax nasicus*.

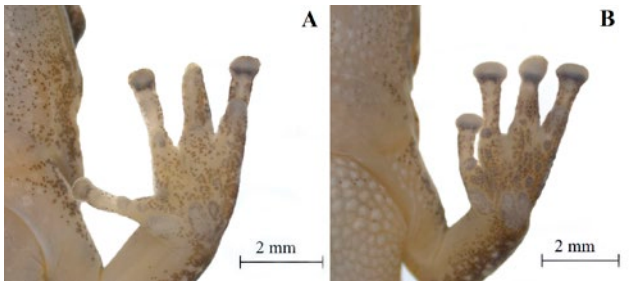

Fig. 3. Toe pad regeneration in *Scinax nasicus*. A: after a week of clipping, B: after two months of clipping.

frogs where the beginning of the growth of the adhesive discs was noticeable, and by March 2018 the discs had similar diameters to those that were not clipped (Fig. 3). In general, discs presented similar shape and coloration to those not clipped and no cases of aberrant growth were observed, such as those described by Hoffman et al. (2008).

Kaiser et al. (2009) studied the possibility of using VIA tags as a marking method that does not require the recapture of animals (by placing the tags on the back of a small frog species). They concluded that it is not possible to read the codes on the labels without having the animal in hand. When comparing the handling times between VIA tags and toe-clipping, Clemas et al. (2009) found that toe-clipping took slightly less amount of initial time of handling and marking than time with the VIA tags, but the last one took less time during identification. Despite results showed by Clemas et al. (2009), by using VIA tags in adults and juveniles, we had success identifying individuals of *S. nasicus* through this methodology.

Regarding the second objective of this work, we observed the retention of tags on 97% of the cases. Only on one occasion the label was expelled and, in another opportunity, as a result of an inflammation caused in a frog's leg, the tag had to be removed when the animal was recaptured. Also, in two cases the tags were inverted but could be easily rotated by prodding with a finger, and on one occasion a small piece of the tag broke when removed from the tag block but was not discarded.

When marking individuals at the caudal end of the dorsum, Kaiser et al. (2009) noted that very often the labels were turned over and migrated ventrally, but they do not mention if this affects frog's survivor or movement capacity. They also mention that when tags were not easily detected they remarked animals, and this constituted an extra effort and a waste of resources. Contrarily, Heart et al. (2008) compared tag migration placing them in the thigh and dorsolateral region of the thorax, finding that tag retention was higher in the latter site. Although, movement of tags in dorsal regions of the body is less common, this area tends to be more pigmented, making reading difficult (Moosman and Moosman, 2006). We opted for the ventral thigh location because this area is not heavily pigmented in *S. nasicus*, being almost translucid, thus we did not observe any noticeable migration, which may arise when the target species is large and allows movements of the tag in the interfemoral sac (Clemas et al., 2009). We also consider that the use of veterinary glue is not necessary since only in one case we observed the expulsion of the tag. When we purchased the product, manufacturers mentioned that the injection needle was re-designed and that it should not be necessary to make an initial incision. However, for marking large or thick-skinned species as well as when marking a large number of individuals this may be necessary, in order to maintain the instrument sharp and in shape to not cause discomfort to the animals.

The estimation of population and demographic parameters in ecological studies are based on assumptions that depend on the marking technique; these are: (1) no loss of marks; (2) no misidentification of marks; and (3) marking procedures do not alter survival or capture probabilities (Seber, 1986; Pollock et al., 1990). This last assumption is the most controversial because in most techniques' negative effects on both survival and recapture probability have been observed, being toe-clipping the most deleterious technique and VIE and VIA tagging the least, although this last one has been scarcely studied (Heard et al., 2008; Schmidt and Schwarzkopf, 2010; Sapsford et al., 2014, 2015).

VIA tags are an interesting method to test in amphibians due to its relatively low cost, lower invasiveness when compared to other techniques such as toe-clipping, and straightforward code interpretation. In our study, the rate of success of the VIA tag method suggests that it is suitable for *S. nasicus*, as it was easy, safe, rapid and effective to carry out, as well as easy to detect. Moreover, the method is well advised for the species

if long term studies are made, due to the pad regeneration observed in clipped toes. Still, we suggest keeping a hybrid marking method (i.e., VIA tags + toe-clipping) when working with other species than *S. nasicus*, since VIA tags can have a distinct rate of success depending on several factors, such as the degree of stretch in the skin, size of the leg or other body part, transparency. We also advise to take time to inspect carefully the animals to detect VIA tag migration or loss, so there is no resource waste and unnecessary animal stress.

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