Use of three individual marking methods in Australian frogs (Genus: *Litoria*) with notes on placement of Visible Implant Alphanumeric tags

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Abstract

With controversy over toe-clipping as an ethical and effective approach to individual marking methods, finding a method that least impacts anurans needs to be a priority. Our aim was to evaluate handling and processing time plus recapture rates for fluorescent Visible Implant Alphanumeric (VIA) tags, toe-clipping and photographic identification techniques that would be suitable for use with *Litoria ewingii* and *L. raniformis*. VIA tags were initially trialled in these two species in captivity, using a range of insertion points. They were most effective in *L. ewingii* when inserted in the interfermoral sac. We found VIA tags were ineffective for *L. raniformis,* both because of skin pigmentation and subcutaneous lymph sacs were relatively large for the tag size so they migrated. Therefore we suggest VIA tags may be useful in juvenile *L. raniformis* only. Marking trials to compare the handling and processing times of the VIA tags, toe-clipping and photographic identification in the wild for *L. ewingii* were carried out in three ponds in Dunedin, New Zealand. VIA tags were significantly faster for recapture handling times, with toe-clipping having a significantly lower handling time on initial capture of an animal. When processing time was incorporated, photo identification took the longest out of the three marking methods tested. Our results indicate VIA tags to be a reliable method, toe-clipping to be effective but we would not support photo identification as a technique for *L. ewingii*.

Keywords: *Litoria ewingii* - *L. raniformis* - amphibian - toe-clipping - photographic identification - fluorescent visible implant alphanumeric tags - population research.

Introduction

With amphibians in global decline, understanding populations at the spatial and temporal level is crucial (Houlahan *et al*. 2000). Individual marking methods are essential tools for amphibian conservation management

and population research. Toe-clipping has been the traditional method used for individual marking of amphibians but the efficacy of this method has recently been discussed (Davis & Ovaska 2001; Funk *et al*. 2005; May 2004; McCarthy & Parris 2004). In response to concerns about increased mortality rates, effectiveness, and the ethical treatment of animals, numerous other marking methods have been developed and utilised (Brown 1997; Buchan *et al*. 2005; Davis & Ovaska 2001; Mossman & Mossman 2006).

One such method is the use of photographic identification using natural markings. This method has been used for three endemic anuran species in New Zealand with mixed results depending on the species (Beausoleil *et al*. 2004; Germano pers. obs.).

Another recently developed method is the use of small soft biocompatible fluorescent Visible Implant Alphanumeric tags placed under the skin (VIA tags; Northwest Marine Technology Inc., Shaw Island, Washington, USA). These tags were initially designed for use on fish, but have been used for several amphibian species including *Gegeneophis ramaswamii, Pseudacris regilla, Taricha granulosa, Bufo boreas, Rana cascadae, Rana luteiventris,* and *Spea intermontana* (Buchan *et al.* 2005; Gower *et al*. 2006; Measey *et al.* 2001). Anurans are unique among vertebrates because they have well demarcated subcutaneous lymph sacs defined by septa (Carter 1979). Knowledge of the anatomical location and size of the anuran subcutaneous lymph sacs is important during the placement of VIA tags, elastomers or PIT tags. If the marker is inserted into a relatively large sac, it can move within the confines of the sac or alternatively, could migrate into adjacent sacs via connecting foramina. This is the likely explanation for the changing position or occasional disappearance of markers such as injected elastomers in some studies (e.g., Davis & Ovaska 2001).

Finding a method with minimal impact at the individual level for amphibians should be a priority. Though initial physical invasiveness is one part of this, repeated handling time of individuals should also be considered, as handling time and capture has been shown to raise corticosterone levels and cause stress in amphibians (Coddington & Cree 1995). Additionally, the processing time outside the field associated with some methods should be taken into consideration as this can significantly increase the time and resources required.

The brown tree frog (*Litoria ewingii*), an introduced species from Australia, was used as a model species for this study. *L. ewingii* is common and widespread throughout New Zealand with a snout-vent length ranging between 25-45 mm. It is similar in size to the endangered, endemic Leiopelmatid frogs and so results from this study may have implications for monitoring techniques used in native frogs. Additionally we trialled VIA tags on *L. raniformis* (snout-vent length 55-100 mm), another introduced Australian frog which is common in New Zealand but endangered in Australia with a declining population trend (IUCN 2007).

We wished to evaluate the potential of VIA tags as a monitoring technique for *L. ewingii* and *L. raniformis*. VIA tags were initially assessed for their suitability in these species. We then compared the handling and processing times of three individual marking techniques for *L. ewingii*; VIA tags, toe-clipping and photographic identification. This focussed on three aspects; 1) initial handling times during first capture and marking; 2) handling times during repeat capture of

marked individuals; and 3) the combined time required for handling and processing individuals.

Materials and Methods

Alphanumeric tag insertion

In December 2006, we tested the suitability of fluorescent Visible Implant Alphanumeric (VIA) tags for use in *Litoria ewingii* (n = 3) and *L. raniformis* (n = 4). The VIA tags had a dimension of 1.0 mm by 2.5 mm. The number on the tag could be read in normal light and fluoresced green under blue light (Northwest Marine Technology Inc., Shaw Island, Washington, USA).

Location of the subcutaneous lymph sacs was used to guide choice of likely insertion sites (Carter 1979). Knowing that *L. raniformis* has the same septal patterns as *L. ewingii* (Carter 1979), we inserted tags in the interfemoral lymph sac, under the eye, in between the nostrils, and on the forearm. The ideal subcutaneous lymph sac should be large enough to accommodate the tag but small enough to prevent the marker moving or flipping over to any extent. For *L. ewingii* the tags were inserted in the interfemoral sac as it was small enough to prevent the tag from moving or flipping over and its position on the ventral aspect of the upper leg allowed easy visual access.

Initially, attempts were made to insert tags using the needle-like injector provided by the manufacturer. However, this proved difficult without incising the skin. Access was achieved via a 3 mm incision made transversely to the long axis approximately at the proximal 10 % of the leg (Figure 1) using cuticle scissors. Scissors were sterilised prior to each use with 70 % ethanol or medical isopropyl alcohol (Briemarpak Skin Cleansing Swab). Tags were carefully inserted under the skin, number up, using the injector provided with the kit. The incision was then sealed with Liquid Bandaid® (Johnson & Johnson) to promote healing and the frogs were kept in captivity for three weeks for observation prior to the main study.

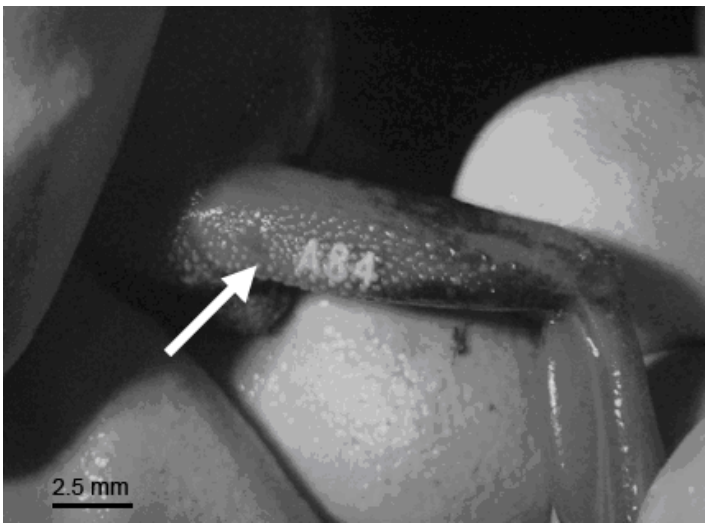

Figure 1: The VIA tag in the interfemoral subcutaneous lymph sac of the left hind limb of *L. ewingii* viewed under blue light. Arrow indicates the transverse incision at point of insertion on the medial thigh, not yet sealed with wound acrylic.

Comparison of marking techniques

Over the austral 2007/2008 summer (December 2007 – February 2008), we captured a total of 110 *L. ewingii* from three distinct frog populations in Dunedin, New Zealand, for use in one of three identification methods: toe-clipping, photographic identification, and VIA tags. Each site differed in size; from an area directly around a 0.125 m^2 bath to a pond with 380 m^2 of suitable frog habitat. Initially all the frogs in each site were caught and held in plastic bags before being randomly allocated to a particular marking method. The initial timing only started once a method was to be applied to the frog. A new pair of disposable gloves was worn for each individual frog. As per Kinkead *et al.* (2006) anesthesia was not used. Toe-clipping required a maximum of two unique toe clips to be made, ensuring the first digit was not removed, and the scissors were cleaned in-between each frog as per standard procedure. Photographic identification required photographing the frog from several different angles. The time taken to do this was combined with the time required to download digital images from camera to computer and reformat them to create photographic identities and added to the initial handling time. Fluorescent VIA tags were inserted subcutaneously in the interfemoral sac as per the method described above. Frogs were released back into their native pond after marking.

Subsequent visits ascertained if an individual had been previously marked, and if not, photographs for identification were taken. The time taken for this process was also added to overall handling time.

Statistical analysis was carried out using JMP 5.0.1a (SAS Institute Inc). Differences in the time required for the three marking methods were assessed using ANOVA, with post hoc Tukey Tests on the initial handling time to determine where the differences were. Non-parametric Kruskal-Wallis Tests were used to compare the recapture handling times and the combined recapture handling and processing times between methods due to small sample sizes.

Results

Application of alphanumeric tags

Both species of frogs, *L. ewingii* and *L. raniformis,* had 100 % survival in the captive population for the tagging process, with no negative effects on the health of frogs observed post-tagging. In *L. ewingii*, VIA tags were easily read through the skin of the frogs (Figure 1) with no movement or deterioration of the tags. No infection or reaction to the tags was observed in any frog. In the larger *L. raniformis*, VIA tags were obscured by pigmentation and thickness of the skin. The outline of the tags could be seen with the aid of blue light, but the characters on the tag remained unreadable or only partially readable. In addition, the tags moved location within these larger subcutaneous sacs in *L. raniformis*, consequently making them harder to find.

Comparison of marking techniques

The initial handling times when marking individuals at first capture were significantly different for the three marking methods ($F_{2, 102} = 34.3$, $P < 0.0001$), with toe-clipping the most time-efficient method (Figure 2a). In comparison, VIA tags had significantly faster handling times upon repeat capture ($\chi^2_2 = 7.6$, $P = 0.0219$; Figure 2b). Significant differences were noted between marking methods when initial handling

time was combined with processing time ($F_{2, 102}$ = 701.4, P < 0.0001; Figure 3a), with toe-clipping the fastest method. A similar analysis of combined recapture handling and processing time found that VIA tags were significantly faster (χ^2_2 = 10.7, P = 0.0047; Figure 3b). In both cases, photographic identification was the slowest method. There was no significant difference due to the pond location ($F_{2, 101}$ = 0.17, P = 0.843), nor an interaction between method and location ($F_{4, 101}$ = 1.61, P = 0.178).

Discussion

VIA tags

VIA tags worked well in *L. ewingii*. In choosing an insertion site the size and location of the underlying subcutaneous lymph sac is a critical factor, as is the degree of pigmentation and opacity of the overlying skin. For adult *L. ewingii,* the interfemoral lymph sac appears to be ideal because the lymph space is sufficiently large enough for the tag, yet small enough to inhibit any migration. The tag is easily

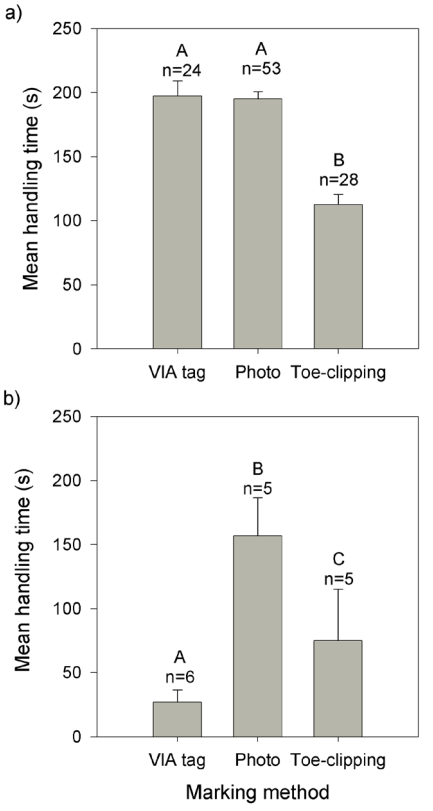

Figure 2. Comparison in the mean handling times (± SE) required for the three methods of marking *Litoria ewingii* (visible implant alphanumeric (VIA) tags, photographic identification and toe-clipping) for a) initial captures and b) recaptures. Significant differences indicated by different letters above the bars.

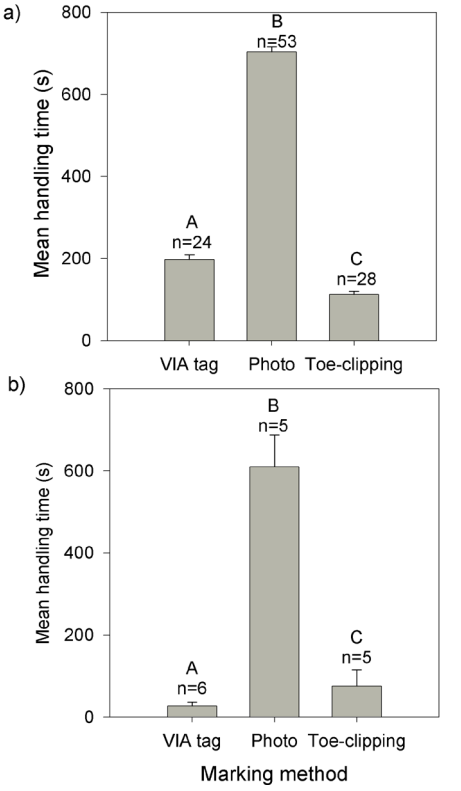

Figure 3. Comparison in the mean combined handling and processing times (± SE) required for the three methods of marking *Litoria ewingii* (visible implant alphanumeric (VIA) tags, photographic identification and toe-clipping) for a) initial captures and b) recaptures. Significant differences indicated by different letters above the bars.

inserted and easy to read, even without the aid of the blue light provided with the Northwest Marine kit. *L. ewingii* are small enough that an experienced researcher can hold them with one hand, while inserting the tag with the opposite hand. In the larger *L. raniformis*, VIA tags were unreadable due to the skin opacity and possibly the skin thickness.

Our results show VIA tags are a reliable method for marking and identifying individual adult *L. ewingii*, but unsuitable for use with adult *L. raniformis.* We recommend the use of this technique for other taxa whose lymph sacs are relatively similar in size to the tag and have minimal skin pigmentation.

A possible novel use for this method in larger species of amphibian could be to follow individual animals in longitudinal studies from the metamorph or juvenile stage. This may enable individuals of these larger species to be monitored after emergence using the tags initially, with substitution of other marking methods, such as PIT tags, at a larger size when the VIA tags may become unreadable.

Marking techniques

With toe-clipping under recent scrutiny to determine whether it is ethical, justifiable or even effective (Funk *et al.* 2005), our study supports the opinion that it is a time and cost efficient method (Phillott *et al*. 2007). Toe-clipping took the least amount of initial handling time when marking individuals and was the least expensive method. It is quick compared to the photographic identification, particularly when dealing with recaptures as there is extensive computer photograph processing time involved.

Photographic identification is dependent on the camera type and skill of the photographer to create ideal identification photos in the field. Although *L. ewingii* individuals did have some markings and shade differences, most were very similar which made assessing the photographs time consuming and difficult. There is a significant time investment beyond what was already discussed to match up photos and that if this method is used for management then the cost of resources to do this must be considered before the onset of the project. This increased the processing time taken and this technique is not recommended for this species, especially as it is open to subjective operator error. Further complications could arise if there are temporal changes in shading and patterns.

The tag insertion technique requires considerable skill and its extended initial handling time was attributable to waiting for the wound glue to dry. However it was the quickest recapture method since the tags were easily read.

Although the number of recaptures was low, they were even numbered across method types, possibly due to two ponds having dried out and then one of these became polluted. Consequently no more frogs were sighted in the latter pond. In addition, it is unknown the extent of site-fidelity in this species, which may contribute to the low return rates. Further research needs to focus on differences in recapture time and rates for the different methods. However, each method will have particular strengths depending on the desired aim of future research.

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