Herpetological Journal FULL PAPER

Published by the British Herpetological Society

Tagging tadpoles: retention rates and impacts of visible implant elastomer (VIE) tags from the larval to adult amphibian stages

Loren Bainbridge, Michelle Stockwell, Jose Valdez, Kaya Klop-Toker, Simon Clulow, John Clulow & Michael Mahony

Conservation Biology Research Group, School of Environmental and Life Sciences, University of Newcastle, University Drive, Callaghan NSW 2308 Australia

Population demographics for amphibian larvae are rarely estimated due to marking technique limitations on small body size, morphological change (metamorphosis), and the associated habitat changes (aquatic to terrestrial environments). A technique that may meet some of these limitations is visible implant elastomer (VIE) tagging. In this study, we report on the efficacy of VIE tagging a tree frog (Hylidae) at the tadpole stage for cohort identification across metamorphosis to the adult stage, in a field environment. During our preliminary captive trial, post-metamorphosis tag retention was 100% over three months, with no adverse effects observed on survival, growth or time to metamorphosis. During our field study tag retention in recaptured *Litoria aurea* was 95% for tadpoles and 88% across metamorphosis. By 200 days post-tagging, retention declined to 75% in the adult stage and stabilised around 50% by 300 days. Post metamorphosis the retention rate was less reliable and dependent upon sex and life-stage. Females showed the highest retention rate (max. 62%, 760 days post tagging), followed by juveniles (max. 45%, 400 days post tagging) and males (max. 20%, 760 days post tagging). We conclude that VIE tagging is a viable method for studying cohort larval movements and population demographics of amphibians up to a 50 day post-metamorphosis stage.

Key words: Litoria aurea, metamorphosis, tadpole, tag retention, VIE tagging

INTRODUCTION

Amphibians are one of the most rapidly declining taxa globally, with over 30% of known species populations diminished or threatened (Wake, 1991; Stuart et al., 2004; IUCN, 2013). Capture mark recapture population studies play an important role in efforts to identify causal agents and quantify declines in the global amphibian crisis, and can ultimately assist in the development of management tools (Collins & Storfer, 2003; IUCN, 2013). Several successful marking techniques exist for adult amphibians; however few exist for tadpoles. This presents a challenge when trying to undertake longitudinal studies. The larval stage of development is the most vulnerable stage for many amphibian species (McDiarmid & Altig, 1999). Mark based population studies at this life stage could therefore be incredibly useful as rates of growth, metamorphosis, survival and recruitment are important drivers of amphibian population size and persistence (McDiarmid & Altig, 1999). However, due to marking technique limitations these important demographics are rarely estimated at the larval stage (Ferner, 2007). Tadpoles are a particularly unique case for identification, requiring a water resistant tag that is able to be retained throughout metamorphosis, ideally remaining recognisable into adulthood (Ferner, 2007). As marking can interfere with the physiology and behaviour of an individual, it is also important that the least invasive technique possible is employed (Melor et al., 2004).

Numerous studies have explored marking techniques for amphibian larvae, including pattern recognition by photographs (Ribeiro & Rebelo, 2011), coded wire tags (Martin, 2011), tadpole staining (Travis, 1981), and tail clipping (Turner, 1960). Whilst each of these techniques had some success, they did not show reliable tag retention rates post-metamorphosis. Marking techniques used on amphibians have also included the implantation of subcutaneous implants such as passive integrated transponder (PIT) tags (Christy, 1996; Pyke, 2005), visible implant alphanumeric (VIA) tags (Buchan et al., 2005; Courtois et al., 2013), and visible implant elastomer (VIE) tags (Moosman & Moosman, 2006). While these can be effective marking techniques for adult amphibians (Ferner, 2007), there are some limitations that prevent their use in larval stages. The size of PIT tags restricts their use to only larger individuals (Christy, 1996; Pyke, 2005), and VIA tags need to stay the correct side up through metamorphosis (Heard et al., 2008; Courtois et

Correspondence: Loren Bainbridge (loren.bainbridge@newcastle.edu.au)

al., 2013). However, high tag retention rates have been observed in previous studies of the VIE tagging technique in small aquatic organisms (shrimp: Godin et al., 1995; big bellied sea horse: Woods & Smith, 2003; juvenile red snapper: Brennan et al., 2006; earthworms: Butt & Lowe, 2006; reef squid: Zeech & Wood, 2007).

Visible implant elastomer tags seemingly meet the requisites for tag retention in tadpoles. They are practical for use in aquatic habitats (Buckley et al., 1994). Tag size can be controlled to small diameters (Frederick, 1997). The fluorescent tag pigmentation increases visibility in low light conditions (Bonneau et al., 1995). However, this technique does have its limitations and may not be reliable in all species, such as adult growling grass frogs (*Litoria raniformis*) with dark skin pigment and warty tubercules (Pyke, 2002) that could obscure the tag. For the reliable identification of individuals the VIE tag needs a combination of colours and positions to be used, which is limited (Anholt et al., 1998; Godin, 1995). As VIE tags can migrate from their original position (Brannelly et al., 2013) the identification of individuals also requires an isolated location that constrains tag movement. Whilst these limitations affect the reliability of individual identification, they still allow for batch-marking of cohorts. Cohort marking, using a single group colour and position (Godin, 1995), is useful for tracking tadpole releases at a specific pond/date.

Several studies have investigated post-metamorphic anurans for VIE tag retention and associated impacts (*Rana esculenta*: Nauwelaerts et al., 2000), tag misidentification, and movement or loss (*Rana sylvatica*: Moosman & Moosman, 2006; *Nectophrynoides asperginis* & *Lithobates pipiens:* Brannelly et al., 2013; *Psuedacris maculata*: Swanson et al., 2013), and tag effects on frog locomotion (*Litoria rheocola*: Sapsford et al., 2014). Far fewer have investigated VIE tagging in tadpoles and long-term tag retention past the metamorphosis stage. Two short-term studies have been undertaken in a lab environment with the aid of anaesthetic to administer VIE tags. Anholt et al. (1998) found an 85% tag retention rate in tadpoles at 8 days, though visibility was obscured by skin pigmentation post metamorphosis. Grant (2008) found a 100% tag retention rate in tadpoles at 20 days, though 50% of these had lost one of the two tags administered, and post metamorphosis there was a 79% tag retention rate with 67% of these missing one of the two tags. No negative impacts on survival, metamorphosis or body size were observed from VIE tagging (Anholt et al. 1998; Grant, 2008). Such results indicate that VIE retention rates in tadpoles warrant further investigation before it can be confirmed as a possible monitoring technique.

Our study aimed to evaluate the efficacy of VIE tagging for use in field population studies across the larval to the adult stages of frogs. We trialled the use of VIE tags in captive green and golden bell frog (*Litoria aurea*) tadpoles to identify any impacts on body size, metamorphosis and survival. We then investigated retention rates in a large cohort (*n*=9708) of free living *L. aurea* from the tadpole stage, through metamorphosis and into adulthood over 760 days in a field environment.

METHODS

Study species

Litoria aurea is a large lentic breeding anuran. The maximum size of tadpole snout to vent length (SVL) is 27 mm (Daly, 1995) and adults range in SVL between 35 and 108 mm for females and 47 to 77 mm for males (White, 1995). *Litoria aurea* is a frequently active species (Cogger 1992; Daly, 1995; White, 1995) with a large home range that can span between 90 m and 4 km (Murphy 1995; Pyke & Osborne, 1996). Today *L. aurea* has disappeared from more than 90% of its former range across Australia due to habitat loss, an introduced predatory fish (*Gambusia holbrooki*) and the chytrid fungus (Mahony, 1996; Pyke & White, 1996; Hamer & Mahony, 2007; Daly et al., 2008; Mahony et al., 2013). As a result, *L. aurea* has been declared a nationally vulnerable species that is being actively researched to manage for population declines (DEC, 2005; Pyke et al., 2008; Stockwell et al., 2008; Mahony et al., 2013; Pickett et al., 2013). However, investigations are currently limited by an inability to mark the larval life stage.

Marking technique

Visible implant elastomer tags (Northwest Marine Technology, Shaw Island, USA) were made following the manufactures instructions to achieve a fluorescent yellow, red or blue polymer. The tadpole was held in one hand with the ventral side up and the tail gently secured between the index and middle finger. A 0.3 cc syringe containing the polymer was then lightly inserted at a 90 degree angle to the epidermis on the right side of the lower lateral ventral surface of the abdomen. The syringe was then moved to a 180 degree position once under the subcutaneous layer, to position the tag away from the insertion point, and a 1–2 mm tag of polymer was injected. To distinguish between the cohorts released at different ponds a single colour tag was administered in this position, so that the pond of origin could be determined based on VIE tag colour. Tadpoles were held for a minimum of three days post marking to monitor well-being and tag retention before both the captive trial and the field study commenced.

Captive trial

To investigate the effect of VIE tagging on tadpole body size, time to metamorphosis and survival, 54 *L. aurea* tadpoles (Gosner stages 36–38) were randomly assigned to three groups (VIE treatment, handling control and control) and housed individually in 1L containers with dechlorinated tap water. All individuals had their SVL measured prior to the start of the experiment using a vernier dial caliper. Using the technique and marking scheme outlined above, all tadpoles in the VIE treatment group were removed from the water and implanted with a single VIE tag and then returned to the water. Tadpoles in the handling control group were removed from the water and handled as if they were being tagged, but no injections were made. Tadpoles in the control group were not removed from the water, handled or tagged.

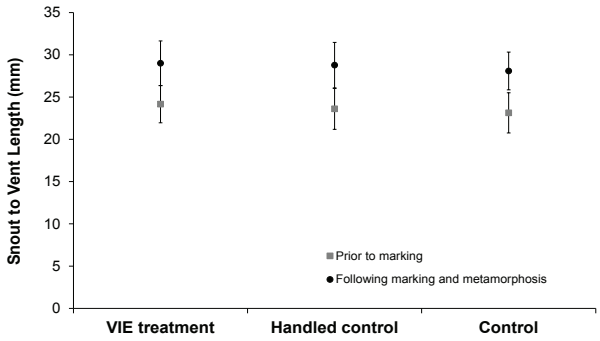

Fig. 1. Mean snout to vent length of green and golden bell frogs (*Litoria aurea*) that were implanted with a VIE tag (VIE treatment), handled but not tagged (handled control) or not handled or tagged (control) at the start of the captive trial as tadpoles (grey squares) and at the end of the trial as post-metamorphosis juveniles (black circles). Error bars show 95% confidence intervals.

All tadpoles were fed trout pellets (Ridley Aqua Feed) three times per week and half water changes were completed weekly. Following treatments, daily inspections of tadpoles were carried out until metamorphosis. We monitored their ability to keep their body upright and maintain their position in the water column, inspected their abdomen condition (if they were bloating/thin/distended), and whether there was any epidermal shedding or lesions. Any deaths or other abnormalities were also recorded.

At metamorphosis (defined as Gosner stage 45, when the front limbs emerge; Gosner, 1964) the presence or absence and position of the tag in the VIE treatment group was recorded and the retention rate calculated. Metamorphosed frogs were moved into group housing (18 per tank) according to their treatment group. This housing consisted of a 20L tank with gravel substrate that sloped into water and PVC pipes throughout for shelter. They were fed small meal crickets (Bio Supplies) twice a week and water changes were conducted weekly. Inspections for VIE tag retention in individuals were continued daily for a further 90 days and a retention rate calculated. Any VIE tags that moved or were obscured by skin pigment were also recorded.

Captive trial statistical analysis

SVL at metamorphosis was compared between experimental groups using a one-way analysis of variance (ANOVA). Time to metamorphosis and survival rate were also compared between groups using log-ranked mantel-cox tests in Kaplan-Meier survival analysis.

Field study

To investigate the efficacy and effect of VIE tagging in a field setting a trial was conducted at an established *L. aurea* re-introduction site on Ash Island, NSW Australia (a deltaic island at the mouth of the Hunter River, 32°51'13.11"S, 151°42'41.48"E). The site consisted of one semi-permanent existing water-body (maximum diameter 10m), four identically created permanent water-bodies (maximum diameter 8m), and six identical created ephemeral water bodies (maximum diameter 3m), each fringed by naturally established reed and grass vegetation within an area of 17,000 m^2. This site was situated over 2 km from one of the largest remaining *L. aurea* populations in NSW (Mahony, 1999), in an area where no *L. aurea* had been detected in annual surveys over the previous 15 years (JC pers. comm., 2013). A total of 9708 *L. aurea* tadpoles were captive-bred and raised to Gosner stage 36–38 (Gosner, 1964). These tadpoles were then marked with a VIE tag using the technique and cohort marking scheme previously outlined, with the fluorescent colours yellow, red or blue corresponding to the semipermanent / permanent pond of release.

To quantify the VIE tag retention rate in the tadpole population following release, capture and release surveys were performed by dip-netting daily for the first two weeks and then weekly until metamorphosis. This involved 20 sweeps, each 1 m in length, using a hand-held triangular dip-net, with a diameter of 40 cm and a mesh size of 0.9 x 0.3 mm. Tadpoles captured were inspected for the presence/absence of the according release-pond cohort colour VIE tag.

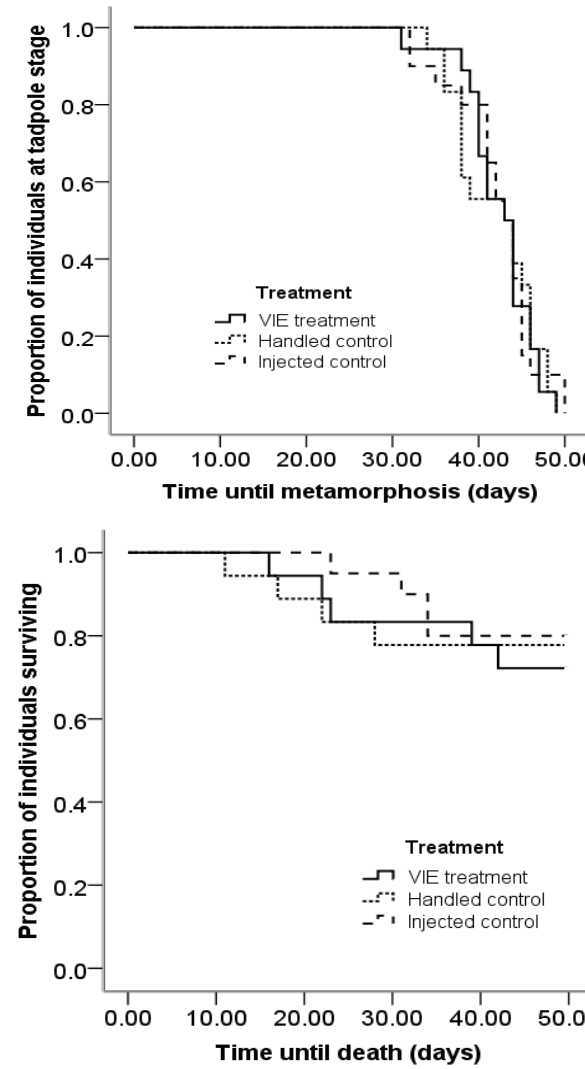

Fig. 2. The proportion of green and golden bell frog (*Litoria aurea*) tadpoles that (A) metamorphosed, and (B) survived, over time following VIE tagging (VIE treatment), handling without a tag (handled control) or not being handled or tagged (control).

Metamorphosed individuals and adults were observed for a further 25 months to calculate tag retention, growth (SVL) and sex. This was done through weekly nocturnal standardised visual encounter surveys (Crump & Scott, 1994) combined with standard capture and release methods across the ponds and terrestrial environments. Frogs were detected using head torches, and were captured by hand adhering to chytrid hygiene protocols (DECC, 2008); where the hand was covered in a disposable plastic bag inverted and sealed to contain the individual. Captured individuals were inspected for a release-pond cohort VIE tag by inspecting the tagged region and the body with a UV-light. The SVL was then measured and sex was determined using secondary sexual characteristics; >45 mm SVL with nuptial pads were identified as male, >45 mm SVL without nuptial pads were identified as female, <45 mm SVL were identified as juveniles (Hamer et al., 2007). Each individual was returned to its point of capture immediately following these observations.

Statistical analysis

Generalised additive mixed models (GAMM) were used to fit a smoothed time effect with a binomial distribution and a logit link function to measure the tag retention rate as the proportion recaptured each survey that retained a tag from the total number captured, across time in days post marking. A model establishing tag retention across the: i) tadpole stage through to post-metamorphosis (0 to 100 days), and ii) juvenile to adult stages (50 to 760 days) were first run. Size (SVL) across the juvenile to adult stages was then investigated as a second explanatory variable to determine if it had an effect on tag retention over time. Similarly, across the juvenile to adult stages the sex/life-stage was added as a second explanatory variable in a separate model to determine if there was an interaction (with time). From this, three independent models were run with the data for each recapture divided by: i) juveniles across 50 to 420 days, ii) females across 50 to 760 days, and iii) males across 50 to 760 days. Chi squared statistics were reported for the significance of the smoother terms in each model. Models were performed in the program R using package gamm4 (v. 3.0.1, R Development Core Team, 2013).

RESULTS

Captive trial

All tagged tadpoles were found to retain the VIE tags through to metamorphosis (100%). As the tail was resorbed at metamorphosis the tags consistently moved

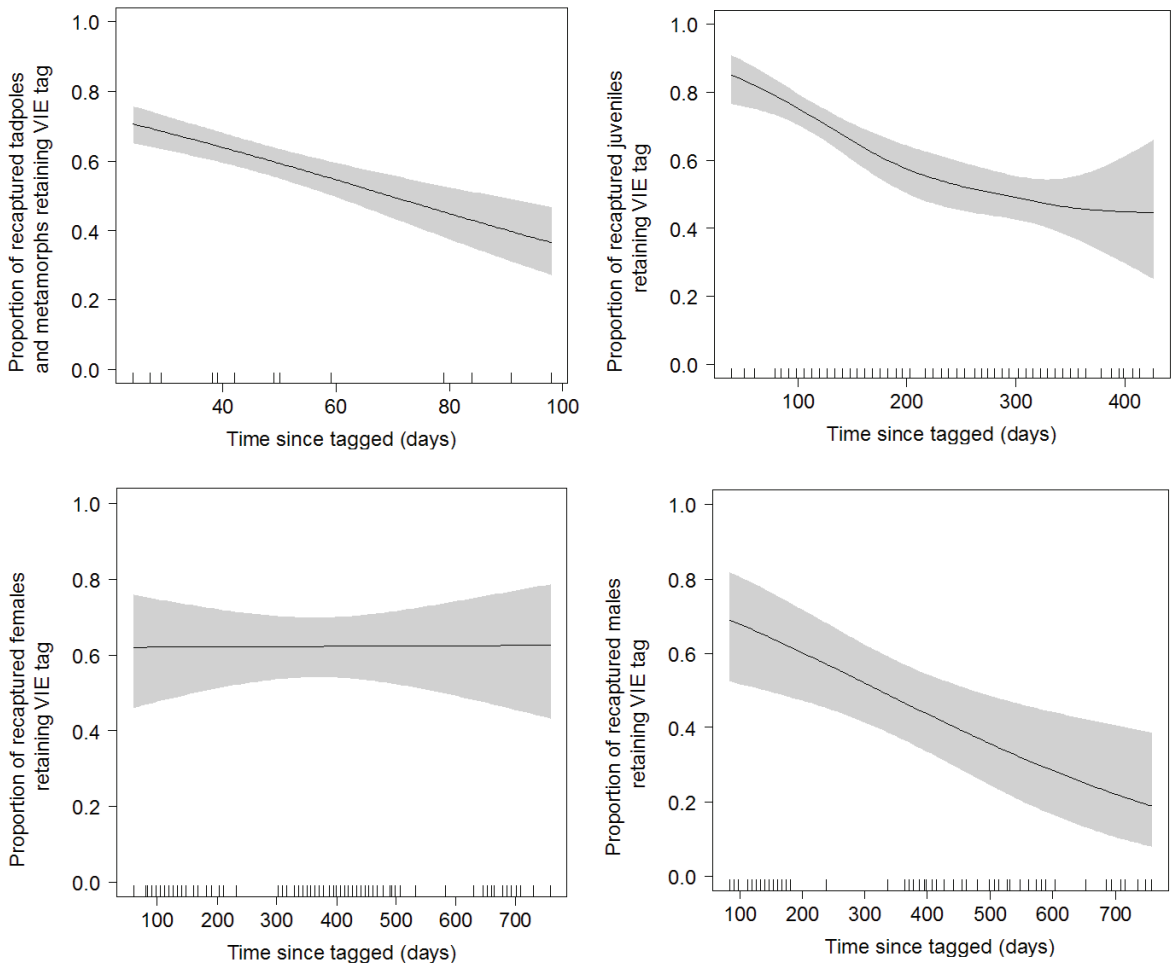

Fig. 3. The modelled proportion of green and golden bell frogs (*Litoria aurea*) retaining a VIE tag over the maximum 760 day experimental time period for (A) tadpoles to post-metamorphosis (0 to 100 days); (B) juvenile frogs (50 to 420 days); (C) female frogs; and (D) male frogs (all adults 50 to 760 days). The shaded areas show 95% confidence intervals. The tick marks indicate when recaptures were encountered.

up to a ventral position above the right thigh, where the skin pigment was pale. Ninety days after metamorphosis, the tags in 72% of animals were still visible to the naked eye. The remaining 28% of tags were obscured by skin pigment that developed following metamorphosis, but were still visible when fluorescing under a UV-light. No significant impact of VIE tagging or handling was detected on tadpole SVL (ANOVA: F=0.53, df=2, p=0.59; Fig. 1), time to metamorphosis (Kaplan-Meier survival analysis: χ^2=0.09, df=2, p=0.95; Fig. 2A), or survival rate (Kaplan-Meier survival analysis: χ^2=0.35, df=2, p=0.84; Fig. 2B).

Field study

Based on Chi squared statistics of the smoother terms for independent GAMMs, declines in VIE tag retention were observed. Of the total 9708 *L. aurea* tadpoles tagged, there was a significant decline in tag retention in tadpoles and post-metamorphs recaptured between 0 and 100 days (χ^2=52.35, df=7.58, p<0.001). Of the tadpoles recaptured, 95% retained the tag at 10 days and 88% retained the tag across metamorphosis (Fig 3). Of the juveniles and adults recaptured 75% retained the tag at 200 days, this rate continued to decline to 51% at 300 days and stabilised around 46% at 400 to 760 days (χ^2=48.82, df=2.47, p<0.001). No effect of SVL (χ^2=0.12 df=1, p=0.73) was found on the tag retention rate across the juvenile to adult stages. Sex and life stage however, effected tag retention over time (GAMM: time df=3, AIC=1202; GAMM: time*sex/life stage df=9, AIC=1195). From independent models of each sex/life-stage (Fig. 3), the females had a consistent retention rate over time (χ^2=0.001 df=1, p=0.97), whilst juveniles and males each had retention rates that declined over time (juveniles: χ^2=41.636 df=1, p<0.001 males: χ^2=9.486 df=1, p=0.002).

The three different colours (fluorescent: yellow, red or blue) could not be compared for retention independently as juveniles and adults moved across the ponds freely. There were 91 occasions of frogs captured in ponds other than their release pond (5.7% of captures showed movement), and therefore the colour a VIE tag should be could not be predicted based on the pond that they were sighted in. However, recaptures retaining each of these colours were observed across the duration of the study, with the blue last seen at 759 days, the yellow at 709 days and the red at 686 days. Through-out this study there was no evidence of drastic tag movement observed around the tagged area. Nor, where any tags were spotted by UV-light elsewhere across the body.

DISCUSSION

Our study shows that VIE tagging cohorts of *L. aurea* tadpoles is a reliable marking technique, however followed by a tag loss from the juvenile to adult stages. Whilst VIE tag retention was reliable in a lab setting (100% retention in tadpoles), including across metamorphosis (100% retention observed at up to 90 days post-metamorphosis), it endured various rates of loss in a field environment from metamorphosis onwards. Tag loss after metamorphosis was dependent upon sex and life-stage, with females showing the greatest retention rate, followed by juveniles, then males. Still, the 88–95% retention rate observed in tadpoles through to newly metamorphosed juveniles was high, and considered reasonably reliable for use in population studies. Particularly as after metamorphosis juveniles can be recaptured and marked individually using an applicable technique for the species. Furthermore, as no significantly negative effects on the growth or survival of tadpoles from VIE tagging were observed, this method may be suitable for meeting the requirements of amphibian larval population studies in the future.

Our study found larval mark retention rates within the range of those observed by Anholt et al. (85%: 1998) and Grant (79%: 2008). However, there was no tag migration, separation and dispersal observed in our lab trial, unlike observations by Grant (2008) and Swanson et al. (2013). This may be due to the application of a very small tag (2 mm diameter). Tag visibility may have been obscured instead of completely lost in the recaptured adults but this was considered to be less likely, as no tag movement was observed around the initial area or was found across the body under UV-light. As *L. aurea* are free from tubercules, have light ventral skin pigmentation and dorsal skin pigmentation coloured contrastingly to those selected for VIE, it did not share the problems reported for VIE tagging *L. raniformis* (Heard et al., 2008; Clemas et al., 2009). Similar to Heard (2008), negative effects of tagging were not observed, with no differences in size, metamorphosis and survival found between tagged and untagged groups in the captive trial and no signs of poor wellbeing observed in the field study. The growth rates observed in the field study were comparable to those reported in PIT tagged free-living *L. aurea* (Hamer & Mahony, 2007).

Loss and movement of a tag is common for subcutaneously injected tags, and generally occurs when physical pressure is applied (e.g., Moosman & Moosman, 2006; Grant, 2008; Brannelly et al., 2013). Tag movement and loss may therefore occur from activities such as general body movements from dispersal, foraging, predator avoidance and physical pressure from breeding behaviours, though this was not directly observed in this study. Different activity levels could explain the differences of the tag retention rates across the sexes and life-stages. Female *L. aurea* are more solitary than males (Hamer, 2002; Bower et al., 2012) and may engage in fewer activities such as intraspecies breeding congregation competition that inflict pressure on the tag or cause it to move. Males had the lowest tag retention rate, which could be attributed to their more active lifestyle and breeding behaviours, particularly their position in amplexus (Christy, 2000; Hamer, 2002). Juveniles are also more active as they disperse away from the natal pond and encounter greater predatory threats (Hamer, 2002; Bower et al., 2012).

A single marking technique rarely meets all of the required criteria for reliable identification (Ferner, 2007). Therefore, the tag to use depends upon the requirements of the study and the traits of the species. Alternative methods such as double VIE tagging and VIA each had similar retention rates reported to our

study (Grant, 2008; Heard et al., 2008). The advantage of the technique we employed is that the application is relatively inexpensive (ca. US$ 0.15 per mark: Hoffmann et al., 2008). Furthermore, tag application is quick and is able to be done in a field environment (Sapsford et al., 2014).

Researchers interested in vital rates of early development such as time to metamorphosis and juvenile growth could use VIE tags to study cohorts over periods >50 days. The biased estimates from tag loss could be mitigated through large sample sizes, such as the one used in this study, or by using models that account for rates of tag loss (McDonald et al., 2003). This can be done by estimating the probability of tag loss in a closed population and correcting the demographic parameter estimates based on this probability (Pollock, 1981).

Our study shows no effect of tagging on body size, time to metamorphosis and survival rate, although VIE tagging may still negatively affect amphibian well-being and behaviour in ways that were not considered here. For example, tagging or the handling processes involved could result in increased vigilance and stress that in turn could affect capture probabilities and disease susceptibility (Moberg, 1985; Pollock et al., 1990; Fisher et al., 2013; Antwis et al., 2014; Sapsford et al., 2014). This could be further assessed by monitoring stress hormones with marking methods (Langkilde & Shine, 2006; Fisher et al., 2013). It has been recently suggested that invasive injections used in PIT tagging disrupt cutaneous microbial communities that could increase susceptibility to chytrid infection (Antwis et al., 2014), a replicate of this study specific to VIE injections is therefore warranted. Furthermore, a fluorescent colour tag may increase the detection probability by predators, which could be assessed through a lab based study (Carlson & Langkilde, 2012). Investigations into tag retention and impacts in other amphibian species would also be beneficial in order to quantify inter-species variability that may not otherwise be incorporated into studies. As species behave differently it is recommended that a trial should be undertaken before committing to any one marking technique.

From our study it is evident that VIE tagging has reliable retention rates for cohort marking at the larval amphibian stage, up to 50 days post-metamorphosis. However, tag loss after this period suggests that VIE tagging is not reliable as a sole marking method through juvenile development. A second mark with established retention rates should therefore be administered (Fellers & Kleeman, 2007; Hoffmann et al., 2008; Campbell et al., 2009) in juveniles re-captured around 50 days post-metamorphosis. Thus, VIE tagging may be useful in monitoring demographic parameters, from encounter histories, across the early developmental stages of amphibians.

ACKNOWLEDGEMENTS

We would like to thank Kim Colyvas for statistical advice, the many volunteers that assisted with field surveys, and the three reviewers that provided valuable feedback. This project was funded by BHP-Billiton. All work was conducted according to the Australian Government Code of Practice for the Care and Use of Animals for Scientific Purposes, under approval from the University of Newcastle Animal Care and Ethics Committee, with project number A-2010-145. All work was carried out under National Parks and Wildlife Service scientific licence number S13227.

REFERENCES

Anholt, B., Negovetic, S. & Som, C. (1998). Methods for anaesthesizing and marking larval anurans, *Herpetological Review* 29, 153–154.

Antwis, R., Garcia, G., Fidgett, A. & Preziosi, R. (2014). Passive integrated transponder (PIT) tagging frogs causes disruption to the cutaneous bacterial community and proliferation of opportunistic fungi. *Applied and Environmental Microbiology*, doi: 10.1128/AEM.01175-14.

Bailey, L. (2004). Evaluating elastomer marking and photo identification methods for terrestrial salamanders: marking effects and observer bias. *Herpetological Review* 35, 38–41.

Bonneau, J., Thurow, R. & Scarnecchia, D. (1995). Capture, marking, and enumeration of juvenile bull trout and cutthroat trout in small, low-conductivity streams. *North American Journal of Fisheries Management* 15, 563–568.

Bower, D., Stockwell, M., Pollard, C., Pickett, E. et al. (2012). Life stage specific variation in the occupancy of ponds by *Litoria aurea*, a threatened amphibian, *Austral Ecology* 38, 543–547.

Brannelly, L., Chatfield, M. & Richards-Zawacki, C. (2013). Visual Implant Elastomer (VIE) tags are an unreliable method of identification in adult anurans, *Herpetological Journal* 23, 125–129.

Brennan, N. Leber, K. Blackburn, B. (2006). Use of coded-wire and visible implant elastomer tags for marine stock enhancement with juvenile red snapper *Lutijanus campechanus*. *Fisheries Research* 83, 90–97.

Buchan, A., Sun, L. & Wagner, R. (2005). Using alpha numeric fluorescent tags for individual identification of amphibians. *Herpetological Review 36,* 43–43.

Buckley, R., West, J., & Doty. D. (1994). Internal micro-tag systems for marking juvenile reef fishes. *Bulletin of Marine Science* 55, 850–859.

Butt, K. & Lowe, C. (2006). A viable technique for tagging earthworms using visible implant elastomer. *Applied Soil Ecology*, 35, 454–457.

Campbell, T.S., Irvin, P., Campbell, K.R., Hoffmann, K.,et al. (2009). Evaluation of a new technique for marking anurans. *Applied Herpetology*, 6, 247–256.

Carlson, B. & Langkilde, T. (2012). A common marking technique affects tadpole behaviour and risk of predation, *Ethology* 119, 167–177.

Christy, M. (1996). The efficacy of using Passive Integrated Transponder (PIT) tags without anaesthetic in free-living frogs. *Australian Zoologist* 30, 139–142.

Christy, M. (2000), The ecology and conservation biology of the green and golden bell frog *Litoria aurea* (Lesson) (Anura:Hylidae), PhD Thesis, School of Biological Sciences, University of Sydney.

Clemas, R., Germano, J., Speare, R. & Bishop, P. (2009). Use

of three individual marking methods in Australian frogs (Genus: Litoria) with notes on placement of visible implant alphanumeric tags. *New Zealand Natural Sciences* 34, 1–7.

Collins, J. & Storfer, A. (2003). Global amphibian declines: sorting the hypotheses. *Diversity and Distributions* 9, 89–98.

Cogger, H. (1992). *Reptiles and Amphibians of Australia* (5th ed.). Reed Books, Chatswood, Australia.

Courtois, E., Lelong, C., Calvez O., Loyau A. & Schmeller D. (2013). The use of Visible Implant Alpha tags for anuran tadpoles. *Herpetological Review 44*, 230–233.

Crump, M. & Scott, Jr. N. (1994). Visual encounter surveys, in: *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians* (eds. Heyer, W. Donnelly, M. McDiarmid, R. Hayek, L. & Foster, M.). Smithsonian Institution Press, Washington, D.C. pp 84–92.

Daly, G. (1995). Observations on the green and golden bell frog *Litoria aurea* (Anura: Hylidae). *Herptofauna* 25, 2–9.

Daly, G., Johnson, P., Malolakis, G., Hyatt, A. & Pietsch, R. (2008). Reintroduction of the green and golden bell frog *Litoria aurea* to Pambula on the south coast of New South Wales. *Australian Zoologist* 34, 261–270.

DEC, Department of the Environment and Conservation, NSW. (2005). *Draft recovery plan for the L. aurea (Litoria aurea)*. Australian Government, Department of the Environment and Conservation (NSW), Hurtsville.

DECC, Department of Environment and Climate Change, NSW. (2008). *Hygiene protocol for the control of disease in frogs: Information Circular Number 6.* Australian Government, Department of the Environment and Conservation (NSW), Sydney South.

Ferner, J. (2007). A review of marking and individual recognition techniques for amphibians and reptiles. *Herpetological Circular* 35, Society for the Study of Amphibians and Reptiles, Salt Lake City.

Fellers, G. & Keelman, P. (2007). California red-legged frog (*Rana draytonii*) movement and habitat use: implications for conservation. *Journal of Herpetology* 41, 276–286.

Frederick, J. (1997). Evaluation of fluorescent elastomer injection as a method for marking small fish. *Bulletin of Marine Science* 61, 399–408.

Fisher, K.J., Guilfoyle, K. & Hatch, K. (2013). Stress induced by toe-clipping in cane toads (*Rhinella marina*). *Copeia*, 3, 539–542.

Godin, D., Carr, W., Hagino, G., Segura, F., et al. (1995). Evaluation of a fluorescent elastomer internal tag in juvenile and adult shrimp *Penaeus vannamei*. *Aquaculture* 139, 243–248.

Gonser, U., Grant, R., & Kregzde, J. (1964). Mössbauer effect in haemoglobin with different ligands. *Science* 143, 680–681.

Grant, E. (2008). Visual Implant Elastomer mark retention through metamorphosis in amphibian larvae. *Wildlife Management* 72, 1247–1252.

Hamer, A. (2002). Ecology of the endangered green and golden bell frog *Litoria aurea:* roles of habitat determinants, spatial dynamics, population demography and threatening processes, PhD Thesis School of Environmental and Life Sciences, The University of Newcastle.

Hamer, A. & Mahony, M. (2007). Life history of an endangered amphibian challenges the declining species paradigm, *Australian Journal of Zoology* 55, 79–88.

Heard, G., Scroggie, M. & Malone, B. (2008). Visible Implant Alphanumeric tags as an alternative to toe-clipping for marking amphibians – a case study. *Wildlife Research* 35, 747–759.

Hoffmann, K., McGarrity, M. & Johnson, S. (2008). Technology meets tradition: A combined VIE-C technique for individually marking anurans. *Applied Herpetology* 5, 265–280.

IUCN, Global Amphibian Assessment. (2013). Conservation International, and Nature Serve. Available from: <http://www.amphibians.org/redlist/rla/>. Accessed: 4 March 2014.

Langkilde, T. & Shine, R. (2006). How much stress do researchers inflict on their study animals? A case study using a scinid lizard, *Eulamprus heatwolei*. *The Journal of Experimental Biology* 209, 1035–1043.

Mahony, M. (1996). The decline of the green and golden bell frog *Litoria aurea* viewed in the context of declines and disappearances of other Australian frogs. *Australian Zoologist* 30, 143–149.

Mahony, M. (1999). Review of the declines and disappearances within the bell frog species group (*Litoria aurea* species group): *Declines and Disappearances of Australian Frogs* (eds. Campbell, A.). Environment Australia, Canberra. pp. 81–93.

Mahony, M., Hamer, A., Pickett, E., McKenzie, D., et al. (2013). Identifying conservation and research priorities in the face of uncertainty: A review of the threatened bell frog complex in Eastern Australia. *Herpetological Conservation and Biology* 8, 519–538.

Martin, R. (2011). Evaluating a novel technique for individual identification of anuran tadpoles using coded wire tags, *Herpetological Conservation and Biology* 6, 155–160.

McDiarmid, R. & Altig, R. (1999). *Tadpoles: the biology of anuran larvae*. University of Chicago Press. pp 183–211.

McDonald, T., Amstrup, S. & Manly, B. (2003). Tag loss can bias Jolly-Seber capture-recapture estimates. *Wildlife Society Bulletin* 31, 814–822.

Mellor, D.J., Beausoleil, N.J., Stafford, K.J. (2004). *Marking amphibians, reptiles and marine mammals: animal welfare, practicalities and public perceptions in New Zealand*. Department of Conservation, Wellington, New Zealand. 55 pp.

Moberg, G. (1985). Biological response to stress: key assessment of animal well-being? In *Animal Stress* (Ed. Moberg, G.), American Physiological Society: Bethesda, MD. pp. 27–49.

Moosman, D. & Moosman Jr., P. (2006). Techniques-subcutaneous movements of Visible Implant Elastomers in wood frogs (*Rana* sylvatica). *Herpetological Review* 37, 300–301.

Murphy, M. (1995). A capture/recapture study of the endangered hylid frog *Litoria aurea*. *Herpetofauna* 25, 19–21.

Nauwelaerts, S., Coek, J. & Aerts, P. (2000). Visible Implant Elastomer as a method for marking adult aurans. *Herpetological Review* 31, 154–155.

Pickett, E., Stockwell, M., Bower, D., Garnham, J., et al. (2013). Achieving no net loss in habitat offset of a threatened frog required high offset ratio and intensive monitoring. *Biological Conservation* 157, 156–162.

Pollock, H. (1981). Capture-recapture models allowing for age-dependent survival and capture rates. *Biometrics* 37, 521–529.

Pollock, H., Nichols, D., Brownie, C. & Hines, E. (1990). Statistical inference for capture-recapture experiments. *Wildlife Monographs* 107, 1–97.

Pyke, G. & Osborne, W. (1996). The green and golden bell frog *Litoria aurea*: Biology and conservation. *Australian Journal of Zoology* 30.

Pyke, G. & White, A. (1996). Habitat requirements for the green and golden bell frog *Litoria aurea* (Anura: Hylidae). *Australian Zoologist* 30, 224–232.

Pyke, G. (2002). A review of the biology of the southern bell frog *Litoria raniformis* (Anura: Hylidae). *Australian Zoologist* 32, 32–48.

Pyke, G.H. (2005). The use of PIT tags in capture-recapture studies of frogs: a field evaluation. *Herpetological Review* 36, 281–284.

Pyke, G., Rowley, J., Shoulder, J. & White, A. (2008). Attempted introduction of the endangered green and golden bell frog to Long Reef Golf Course: a step towards recovery? *Australian Zoologist* 34, 361–372.

R Development Core Team (2008), R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ribeiro, J. & Rebelo, R. (2011). Survival of *Alytes cisternasii* tadpoles in stream pools: a capture-recapture study using photo-identification. *Amphibia-Reptilia* 32, 365–374.

Sapsford, S.J., Roznik, E.A., Alford, R.A. & Schwarzkopf, L. (2014). Visible implant elastomer marking does not affect short-term movements or survival rates of the treefrog *Litoria rheocola*. *Herpetologica* 70, 23–33.

Stockwell, M., Clulow, S., Clulow, J. & Mahony, M. (2008). The impact of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* on a green and golden bell frog *Litoria aurea* reintroduction program at Hunter Wetlands Centre Australia in the Hunter Region of NSW. *Australian Zoologist* 34, 379–386.

Stuart S., Chanson J., Cox N., Young B., et al. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science* 306, 1783–1786.

Swanson, J., Bailey, L., Muths, E. & Funk, C. (2013). Factors influencing survival and mark retention in postmetamorphic boreal frogs. *Copeia* 4, 670–675.

Travis, J. (1981). The effect of staining on the growth of *Hyla gratiosa* tadpoles. *Copeia* 1981, 193–196.

Turner, F. (1960), Population structure and dynamics of the western spotted frog, *Rana p. pretiosa* Biard and Girard, in Yellowstone park, Wyoming, *Ecological Monographs* 30, 251–278.

Wake, D. (1991). Declining amphibian populations. *Science* 253, 860.

White, A. (1995). The green and golden bell frog. *Frog Facts* 5, 1–4. Tadpole Study Group of NSW Inc., Sydney, Australia.

Woods, C. & Martin-Smith, K. (2003). Visible implant fluorescent elastomer tagging of the big-bellied seahorse, *Hippocampus abdominals*. *Fisheries Research* 66, 363–371.

Zeech, K. & Wood, J. (2007). Impact of visible implant elastomer tags on the growth rate of captive Caribbean reef squid *Sepioteuthis sepioidea*. *Fisheries Research* 95, 362–364.

Accepted: 20 August 2014