Visible implant elastomer tagging and toe-clipping: effects of marking on locomotor performance of frogs and skinks

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Marking for identification of previously captured animals is a critical aspect of many types of ecological studies. Marking animals may affect performance, which in turn could influence survival. We compared the effects of toe-clipping and elastomer tagging on the locomotor performance of frogs (jump distance) and skinks (running speed and endurance). We examined the immediate effect of marking, and the effect after a recovery period of two weeks. Jump distance decreased across all treatment groups in frogs immediately after marking, but toe-clipped individuals jumped less far in relation to their original jump distance than did elastomer tagged or control frogs. After two weeks, there was a relative increase in jump distance of the toe-clipped frogs, but for all groups performance was lower than at the start of the experiment. In skinks, both marking methods reduced skink endurance, and toe-clipping had a stronger negative effect on running speed than did elastomer tagging. After two weeks, skink endurance and running speed increased to above the initial measures for all treatment groups. Overall, toe-clipping had stronger immediate effects on locomotor performance, indicating that elastomer tagging may be a marginally better marking method.

Key words: Carlia pectoralis, endurance, Litoria nasuta, marking techniques, running speed

INTRODUCTION

Recognizing individuals is a critical aspect of many ecological studies (Otis et al., 1978; Pollock et al., 1990). Recapturing individuals over time and space allows for estimation of individual and demographic parameters such as mortality rates, population size, growth rates and dispersal, and facilitates genetic and behavioural studies of both wild and captive populations (Otis et al., 1978; Pollock et al., 1990; Lemckert, 1996; Brown, 1997).

Reliable but relatively unintrusive marking methods are required to observe populations in their natural habitat over long periods of time (Paulissen & Meyer, 2000). Ideally, individual marks should be permanent, without influencing the animal’s chance of survival due to changes in performance, behaviour or conspicuousness (Ricker, 1956), be easy to apply and read, cause minimum pain or stress to the animal, and be applicable to various sizes of animals (Lewke & Strood, 1974).

Toe-clipping is the most widely used method of identifying individual reptiles and amphibians, as it is a quick, easy and inexpensive way to create up to thousands of unique marks by varying the combination of toes clipped on each foot (Ferner, 1979). It may, however, cause stress and discomfort during the removal of phalanges, and may also affect individuals for extended periods afterwards (Golay & Durrer, 1994; Stanford, 1996). Concern that such effects may occur has led to questioning of the ethics of toe-clipping (May, 2004; McCarthy & Parris, 2004). The extent of the effects of toe-clipping may depend on the species (Lemckert, 1996), the physical state of the individual animal, and how clipping is carried out (Van Gelder & Strijbosch, 1996). In amphibians, toe-clipping may cause infection, which may lead to swelling, and in some cases even limb loss (Donnelly et al., 1994; Lemckert, 1996). Toe-clipping may also impede performance due to toes being absent (Clarke, 1972). Toe-clipping frogs may (Parris & McCarthy, 2001; McCarthy & Parris, 2004) or may not (Williamson & Bull, 1996; Phillott et al., 2007) reduce recapture rates of individuals; however, no studies so far have examined the influence of toe-clipping on locomotor performance.

Visible implant elastomer (VIE) tags have been widely used to tag fish (Catalano et al., 2001; FitzGerald et al., 2004; Astorga et al., 2005) as well as crustaceans (Godin et al., 1996; Clark & Kershner, 2006), amphibians (Pfennig & Murphy, 2000; Bailey et al., 2004; Belden, 2006), and to a lesser extent reptiles (Penney et al., 2001; Losos et al., 2004). They have become a moderately popular alternative to toe-clipping (Nauwelaerts et al., 2000; Davis & Ovaska, 2001; Marold, 2001; Lampert & Linsenmair, 2002; Heemeyer et al., 2007; Heard et al., 2008) and have also been used in combination with toe-clipping to reduce the number of toes clipped per individual (Hoffmann et al., 2008). There are, however, no studies comparing the relative effect on performance of elastomer tagging and toe-clipping. If a specific marking technique reduces the performance of marked individuals, it may reduce their survivorship in nature (Bloch & Irschick, 2004).

In this study we directly compare the effects of elastomer tagging and toe-clipping on the locomotor performance of frogs and skinks. Jumping ability is a good general measure of performance in frogs (Hirano & Rome, 1984; Navas et al., 1999; Tejedo et al., 2000). We quantified maximum jump distance of toe-clipped, elastomer tagged and control groups of frogs to compare perform-

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ance of these three groups. In skinks, we compared the effects of toe clipping and elastomer tagging on running speed and endurance. Because marking may have different effects immediately upon marking compared to a period of time after marking (Golay & Durrer, 1994), we looked at the immediate effects of marking and the effects of marking after two weeks.

**MATERIALS AND METHODS**

**Study species**

The species used in this study were the striped rocket frog, *Litoria nasuta*, and the open litter rainbow skink, *Carlia pectoralis*. *Litoria nasuta* were chosen because jumping is an important part of their ecology, and a reduction in jumping distance could have negative consequences such as increased capture by predators (Wassersug & Sperry, 1977). *Carlia pectoralis* are active-foraging skinks (Stuart-Fox et al., 2002), and therefore both running speed and endurance are important to food acquisition and predator escape success.

**Marking and performance measurements**

This study was conducted between 19 December 2007 and 4 May 2008 at James Cook University, Townsville, Queensland. Animals were collected from the University campus (19°19'53"S, 146°45'41.76"E), the Town Common Conservation Park (19°13'13.21"S, 146°45'27.57"E) and Hervey’s Range Road (19°19'17.76"S, 146°36'06.37"E). Frogs were kept in groups of up to four in 20-litre containers at a constant temperature (23±1 °C). Each container had a moist sand and leaf-litter substrate. Skinks were kept outdoors in groups of up to five in 1200-litre cattle watering tanks, with a sand, rock and leaf-litter substrate, and a shelter for each skink, covered with shade cloth. Frogs and skinks were provided with water and fed with commercially supplied crickets (*Acheta domestica*).

Frogs and skinks were marked by toe-clipping or visible implant elastomer (VIE) (Northwest Marine Technology™). The two-part silicon elastomer material was mixed and loaded into 0.3 cm³ insulin syringes with a 29 gauge (or 0.17 mm inner bore-diameter) needle, and injected subcutaneously at four body locations. For frogs, tags were inserted ventrally into the proximal anterior and posterior legs; for skinks, tags were inserted ventrally into the proximal anterior and posterior legs (Losos et al., 2004).

Body locations for tag insertion were selected after tag retention had been observed at various body locations, in several species of frogs and skinks over a period of 75 days in the laboratory. Six species of frog, *Limnodynastes tasmaniensis*, *L. convexiaculus*, *Opisthodon ornatus*, *Litoria nasuta*, *Cyclorana alboguttata* and *C. novaehollandiae*, and two species of skink, *Carlia pectoralis* and *C. jarnoldae*, were used during the trial experiments. Tags were inserted at eight different locations in frogs: four posteriordorsally, and four ventrally into the posterior legs. For skinks, there were ten possible locations: six ventrally between anterior and posterior legs and four into the legs. Tags were best retained at the four locations chosen for this study. Some of the tags at the other locations were lost or moved so that the original position could not be determined.

No anaesthetic was used, and animals did not appear distressed during injection. After injection the elastomer hardened, and was detectable externally through the skin. Toe clips were made with sharp sterilized scissors by removing the first phalange of one toe per foot, the combination of toes removed being different for each frog. The injection needle and scissors were sterilized in 70% alcohol between individuals and clips to reduce the likelihood of infection.

Locomotor performance of all animals was measured before assigning individuals to one of three treatment groups (elastomer tagged, toe-clipped and control). After measuring jump distance for frogs and running speed and endurance of skinks, individuals were weighed (to the nearest 0.01 g using a digital balance), snout–urostyle length measured for frogs (with digital callipers, ±0.01 mm), and snout–vent length measured for skinks (with a ruler, ±1 mm). The animals were distributed evenly among groups with respect to size, performance level, and sex for skinks.

After initial trials, animals were given five (frogs) or three (skinks) days rest before marking. To determine the immediate effect of each marking technique, performance was measured within 15 mins of marking. Individuals in control groups were not marked, but were handled for the same amount of time required for tagging. Animals were allowed to recover in captivity for two weeks, after which each experiment was repeated.

**Maximum jump distance in frogs**

Maximum jump distance of 63 (22 elastomer tagged, 21 toe-clipped, 20 control) frogs was measured before and immediately after marking (Day 1). The experiment was conducted at 0730 in a constant-temperature room. To measure jump distance, the hindquarters of each frog were dipped in non-toxic food dye, and the frog was encouraged to jump on paper. Jump distances were measured three times for each frog, and the length of the longest jump by each individual was recorded (to the nearest cm). After two weeks, two of the frogs ceased to jump (one control and one toe-clipped), and eight had died (two control, five toe-clipped and one elastomer tagged). Because these individuals were approximately evenly distributed among groups, their original jump distances were still used to compare with their performance immediately after marking. On Day 15, data were collected from the remaining 53 frogs to analyse the longer-term effects of marking.

**Endurance and running speed in skinks**

We measured endurance and running speed of 22 (seven control, seven elastomer tagged and eight toe-clipped) skinks. Performance was measured at 1100, in a constant-temperature room at 32±1 °C. Each animal was encouraged to walk on a constant-speed, motorized treadmill moving at 1 cms⁻¹ until it became exhausted (i.e. would not right itself when overturned). Time to exhaustion was recorded to the nearest 0.01 sec. After experimentation, all
skinks recovered their righting response within a few minutes.

We measured running speed one hour after the endurance experiment. Running speed was measured three times for each individual, with a 45-min rest between trials. The wooden race-track was 1.1 m long, with 12 cm walls. A video camera was set directly above the track for recording. The track was marked every 10 cm, and the number of seconds required to cross each 10-cm section of track was used to calculate speed (in ms⁻¹) from video footage. Speed was always calculated from the longest continuous run along the track, and maximum speed achieved in any trial was used as the estimate of running speed for each individual.

**Statistical analyses**

The difference between log-transformed maximum jump distance before treatment (Before) and on Day 1 for individual frogs in each treatment was calculated to conduct ANOVAs on mean differences between the largest initial jump and the longest jump immediately after marking. For skinks, we also calculated differences between log-transformed endurance times, as well as running speeds. By using differences in performance, we controlled for individual variation among frogs and skinks, because each individual acted as its own control. Separate ANOVAs were conducted to examine the effect of recovery time on performance of the animals, using the differences in performance of individuals between Day 1 and Day 15. If ANOVAs were significant at α=0.05, a Tukey’s Honestly-Significance-Difference Test using least-square means was used to examine the source of the difference among the treatment groups. All statistical analyses were carried out using Systat v. 12.

**RESULTS**

**Maximum jump distance in frogs**

Maximum jump distance decreased across all treatment groups immediately after marking (Fig. 1), but the extent of this decrement differed among groups (ANOVA, $F_{2,63}=3.264$, $P=0.045$). Upon marking, the maximum jump distance of the toe-clipped frogs decreased significantly by 5% (CI 4–6%) as compared to a decrement of 1% (CI 0–2%) in the control group (Tukey’s test, $P=0.037$, df=60).

Two weeks after marking, there was no statistically significant change in jump distance, but it remained lower than at the start of the experiment (ANOVA, $F_{2,53}=2.251$, $P=0.116$).

**Endurance and running speed in skinks**

**Endurance.** Endurance times of skinks decreased in both treatment and control groups immediately after marking (Fig. 2). Endurance of the elastomer tagged group decreased by 19% (CI 11–27%) while that of the toe-clipped group decreased by 28% (CI 19–37%). Endurance then increased to greater than the original value over the next two weeks, with the largest increase being in the control group, at 58% (CI 42–74%). However, none of these differences were statistically significant (ANOVA, $P>0.05$).

**Running speed.** Immediately after marking, running speed of the toe-clipped group decreased significantly to approximately 90% of its original value (CI 2–14%), while that of the other two treatment groups increased (Fig. 3), a difference which was significant (ANOVA, $F_{2,22}=4.335$, $P=0.028$). Running speed of the control animals increased by 20% (CI 8–32%, Tukey’s test, $P=0.023$, df=19). Running speed remained higher than the original values for the control and elastomer tagged groups, and increased.

Fig. 1. Relative maximum jump distance (as a percentage of initial value) of frogs, *Litoria nasuta*, immediately after marking (Day 1) and 14 days after marking (Day 15), compared to before marking. Statistical analyses were conducted on the differences between Before and Day 1 values, and differences between Day 1 and Day 15 values, rather than on raw values. Data are presented as means ± SE.
significantly by 43% (CI 17–69%) for the toe-clipped animals (Tukey’s test, \( P=0.018, \) df=19).

**DISCUSSION**

Locomotor performance, measured as jump distance in frogs and as running speed and endurance in skinks, decreased for both toe-clipped and elastomer tagged groups immediately after marking, but all measures recovered to some degree after a two-week period. In frogs, jump distances in the three groups decreased over the two-week trial period, while performance in skinks increased to above the initial measures over the two weeks.

**Locomotor performance in relation to marking in frogs**

Locomotor performance of frogs has not previously been assessed in relation to marking. Conclusions about the effects of marking have been based on recapture rates, where the probability of being recaptured can decrease with number of toes removed (Williamson & Bull, 1996; Parris & McCarthy, 2001; McCarthy & Parris, 2004). Our study suggests that one possible reason for increased mortality in toe-clipped frogs may have been an immediate reduction in performance. This may be due to the wound itself, stress associated with marking, or the absence of toes may simply be a physical hindrance to the animals. Recovery after two weeks may indicate that once the wound has healed, missing toes do not have much effect on jump performance. Alternatively, if missing toes present a physical hindrance, the recovery we observed could mean that individuals adjust to toe loss and learn to move normally within two weeks.

Maximum jump distance decreased over time across all groups, but was lowest in the toe-clipped frogs. The decrease in jump distance of the unmarked control group (as well as the two marked groups) may have several possible explanations. It may indicate that handling frogs and keeping them in captivity reduced their performance (Houlihan & Mathers, 1985). Alternatively, frogs may have become less motivated to jump when the experiment was repeated over time, for example, if they habituated to the experimental situation and no longer felt threatened. Toe-clipping had a larger effect on frogs in our experiment than did elastomer tagging, which did not lower locomotor performance significantly below the performance of control animals even immediately after marking. The immediate reduction in physical performance may reduce survival of toe-clipped frogs in the field relative to frogs that have been elastomer tagged.

**Locomotor performance in relation to marking in skinks**

Endurance of skinks was affected in all three treatment groups, whereas only toe-clipped animals showed a reduction in running speed immediately after marking. Although endurance time decreased in all treatment groups, the decrease was almost 10% greater in toe-clipped compared to elastomer tagged individuals. Handling may explain the initial decrease of approximately 20% in endurance time shown by all treatment groups, and marking apparently increased this impact, especially in the toe-clipped group. Two weeks after marking, endurance of all three groups increased to above the initial measures, particularly in the control group. In this case, unlike the frogs, captivity seemed to have a positive effect on the animals. Whereas the control group showed a dramatic improvement in endurance, the marked individuals increased endurance to only slightly above 100%
(their initial endurance), indicating that both forms of marking were stressful over time. Endurance improved in both the toe-clipped and elastomer tagged groups, suggesting that if tagging is stressful, the effect is not lasting. Other studies have also observed no long-term effect of toe-clipping on running speed in lizards (Dodd, 1993; Borges-Landaez & Shine, 2003), and have suggested that removal of one toe per foot probably does not affect activities in skinks (Borges-Landaez & Shine, 2003).

Toe-clipping reduced running speed of the skinks immediately after marking, but two weeks after marking the speed of the toe-clipped skinks increased relative to the initial measure. Counterintuitively, increases in performance of the toe-clipped group could be caused by the stress of the marking method. For example, in a study examining the impacts of PIT tagging in the alpine newt Triturus alpestris, stress due to PIT tagging led to an increase in reproductive output (Perret & Joly, 2002). It is not clear from our study whether relatively small decrements in locomotor performance might have a negative effect on fitness, but it is conceivable that they may.

**Which technique is best?**

Our findings provided evidence that elastomer tagging could be a viable alternative to replace toe-clipping. This conclusion was moderately well supported in frogs, for which toe clipping was detrimental to locomotor performance immediately after the procedure. This result may be generalized to other frogs, for which jumping is an important aspect of locomotion. Effects of toe-clipping on skinks were not as clear, but the greater decrease in locomotor performance after clipping indicates that elastomer tagging may also be a better option. This could apply to other species of skinks with similar characteristics.

Elastomer tagging is more time consuming than toe-clipping and requires relatively expensive equipment. Although it is preferable to avoid even small decrements in performance, toe-clipping might still be a more suitable marking method if it does not cause much detriment to other species. Our study certainly suggests that it is important to avoid assuming that any particular marking method is detrimental, or that it is more detrimental than another; instead tests should always be conducted (e.g. Langkilde & Shine, 2006). Future studies on the effects of marking could include various species from different functional groups, to determine if the effects of toe-clipping on locomotor performance are shared by different species.

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**Fig. 3.** Relative maximum running speed (as a percentage of initial value) of skinks, Carlia pectoralis, immediately after marking (Day 1) and 14 days after marking (Day 15), compared to before marking. Statistical analyses were conducted on the differences between Before and Day 1 values, and differences between Day 1 and Day 15 values, rather than on raw values. Data are presented as means ± SE.
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