Return rates of male hylid frogs *Litoria genimaculata*, *L. nannotis*, *L. rheocola* and *Nyctimystes dayi* after toe-tipping

Andrea D. Phillott[1,2,*], Keith R. McDonald[1,3], Lee F. Skerratt[1,2]

[1]Amphibian Disease Ecology Group and [2]School of Public Health, Tropical Medicine and Rehabilitation Sciences, James Cook University, Townsville, Queensland 4811, Australia

[3]Threatened Species Branch, Department of Environment and Resource Management, PO Box 975, Atherton, Queensland 4883, Australia

ABSTRACT: Toe-tipping is a commonly used procedure for mark-recapture studies of frogs, although it has been criticised for its potential influence on frog behaviour, site fidelity and mortality. We compared 24 h return rates of newly toe-tipped frogs to those previously toe-tipped and found no evidence of a stress response reflected by avoidance behaviour for 3 species: *Litoria genimaculata*, *L. rheocola* and *Nyctimystes dayi*. *L. nannotis* was the only studied species to demonstrate a greater reaction to toe-tipping than handling alone; however, return rates (65%) in the 1 to 3 mo after marking were the highest of any species, showing that the reaction did not endure. The comparatively milder short-term response to toe-tipping in *N. dayi* (24% return rate) may have been caused by the species' reduced opportunity for breeding. Intermediate-term return rates were relatively high for 2 species, *L. nannotis* and *L. genimaculata*, given their natural history, suggesting there were no major adverse effects of toe-tipping. Longer-term adverse effects could not be ruled out for *L. rheocola* and *N. dayi*, which had relatively moderate intermediate-term return rates (33 and 36%, respectively). We recommend that future studies directly observe the health effects of toe-tipping, are longer term and consider alternative marking methods.

KEY WORDS: Frog · Toe-tipping · Mark-recapture · Return rate

INTRODUCTION

Bogert (1947) described the procedure of excising digits in unique combinations from the fore-and hind-feet of amphibians to allow recognition of individuals. Excision occurs at the most distal inter-phalangeal joint (toe-tipping) or one more proximal (toe-clipping). This marking method has allowed some individual frogs to be recognised for more than 34 yr (Bell & Pledger 2005), and is one of the few reliable means of identifying small individuals or species lacking persistent, individually-identifiable natural markings (see Phillott et al. 2007, Heard et al. 2008).

Donnelly et al. (1994) recommended toe-clipping as being widely applicable to anurans, fast and inexpensive. However, May (2004) questioned the ethical application of the procedure after Parris & McCarthy (2001) and McCarthy & Parris (2004) modelled the effect of toe-clipping on return rates of frogs, and suggested that lower return rates are the result of mortality arising from digit/limb inflammation and infection, disturbance resulting in movement of animals away from the study site, or inability to recapture toe-clipped animals. Movement of animals away from the study site will not only influence return rates and survival estimates, but it can invalidate all basic mark-recapture models and may also contribute to the spread of diseases such as amphibian chytridiomycosis.

Few studies have directly measured the potential impact of toe-tipping/clipping on frog mortality rates

or behaviour. This is partly because of the difficulties of conducting such research in a natural environment, and the absence of a suitable non-invasive control marking technique for comparison to assess the effects of toe-tipping or toe-clipping on mark-recapture studies. Catching and toe-tipping/clipping frogs will result in both handling stress and injury. It is likely that pain is experienced by amphibians during these procedures, although it is uncertain if this is equivalent to that experienced by mammals (see Stevens 1992, Machin 1999). In frogs, the digits of the forefeet are innervated by branches of the ulnaris and radialis nerves, and those of the hind-feet by divisions of the tibialis and peroneus nerves (Ecker 1889). Pain receptors (slow-conducting, non-myelinated nerve fibres <1 μm diameter terminating as free nerve endings in the dermis) are present in all amphibians (reviewed by Heatwole 1998).

Studies of amphibian stress responses are limited but show variability in responses. Juvenile *Xenopus laevis* demonstrate significantly elevated plasma corticosterone between 30 min and 6 h after 1 min of vigorous shaking (Yao et al. 2004). However, handling-tachycardia is absent in *Lithobates catesbeiana* and *L. pipiens* (Cabanac & Cabanac 2000; formerly *Rana catesbeiana* and *R. pipiens*, see Frost et al. 2006), and emotional fever is absent in *Rhinella marina* (formerly *Bufo marinus*, see Frost et al. 2006) and *Bombina bombina* (Cabanac & Cabanac 2004) after gentle handling for 1 min.

Hormonal responses to stress are consistent across vertebrate taxa and involve complex pathways (Greenberg & Wingfield 1987). The typical acute vertebrate stress response of the hypothalamic-pituitary-adrenal (HPA) axis was summarised by Sapolsky et al. (2000). Within seconds to minutes, the sympathetic nervous system increases secretion of catecholamines (epinephrine and norepinephrine). Hypothalamic release of corticotropin-releasing hormone to the portal system stimulates enhances pituitary secretion of adrenocorticotropin. Subsequently, hypothalmic gonadotropin-releasing hormone and then pituitary gonadotropin release decreases. Pituitary secretion of prolactin and pancreatic secretion of glucagon also decrease. In response to haemorrhage, arginine vasopressin from the pituitary and renin from the kidney are secreted in relatively large amounts (Sapolsky et al. 2000). Within minutes of stress, glucocorticoid (GC) secretion is stimulated, and gonadal steroid secretion declines. The effect of these hormonal changes is not observed until about 1 h after release for GCs to several hours later for gonadal steroids. Major physiological changes that accompany stress are mobilisation of stored energy and diversion to active muscles, enhanced cardiovascular tone to increase substrate delivery to muscles, stimulated immune function, inhibition of reproductive physiology and behaviour, decreased appetite and feeding, and increased cerebral perfusion rates and glucose utilisation resulting in greater cognition. Haemorrhage (fluid loss) triggers renal and vascular mechanisms of water retention (Sapolsky et al. 2000).

The HPA stress axis may be manifested in either of 2 behavioural responses. The first, the 'flight or fight response', occurs within seconds of the threatening event and, after successful escape, allows normal activity to be resumed within seconds. The 'emergency response' may take minutes to hours to develop and interrupts normal behaviour for hours, days or weeks by initiating entry into the 'emergency life history stage' resulting in behaviour and physiology being redirected towards survival (Wingfield et al. 1998).

It is unknown whether toe-tipping/clipping induces either stress response. In the absence of endocrinological data, comparative behavioural responses may be used as indicators of the degree of stress experienced by toe-tipped frogs. Avoidance behaviour observed after toe-tipping/clipping may indicate that the marking method is an important stressor, while a high return rate would suggest absence of a significant stress response. We aimed to measure the effect of toe-tipping/clipping on emergency response behaviour of frogs by monitoring short-term return rates and therefore avoidance behaviour of 4 species of hylid frogs. All 4 sympatric species of frog in this study, *Litoria genimaculata*, *L. nannotis*, *L. rheocola* and *Nyctimystes dayi*, have demonstrated severe population declines due to amphibian chytridiomycosis (Berger et al. 1998, McDonald & Alford 1999, McDonald et al. 2005), and currently, the epidemiology of this disease in these populations is a topic of intense study (A. D. Phillott unpubl. data). Understanding the effect of this marking technique on frog behaviour and survival is of the utmost concern because it is necessary in order to be able to carry out these epidemiological studies.

MATERIALS AND METHODS

Surveys of *Litoria genimaculata, L. nannotis*, *L. rheocola* and *Nyctimystes dayi* at an unnamed creek in Tully Gorge National Park, Queensland, Australia (17° 46′ S, 145° 38′ E, elevation 100 m), were conducted for 2 successive nights per month from November 2005 to February 2006. This allowed short-term frog responses to toe-tipping to be determined by comparing them to frogs that had been previously marked during the period of this study. We searched a 200 m stream transect by spotlight after dusk and caught frogs by hand. Toe-tips (2 to 5 per animal) are excised

through the inter-phalangeal joint proximal to the toe-pad, a procedure known as toe-tipping, rather than removing a larger proportion of the toe as occurs in toe-clipping. Animals are marked at the first capture only, according to the numbering scheme in Fig. 1, which allocates frogs a unique number based on the pattern of toes removed. When a previously marked frog is encountered, its unique number is recorded, and additional toes are not removed. This scheme was modified from Martof (1953) and has been used by K. R. McDonald at Tully Gorge since 1992.
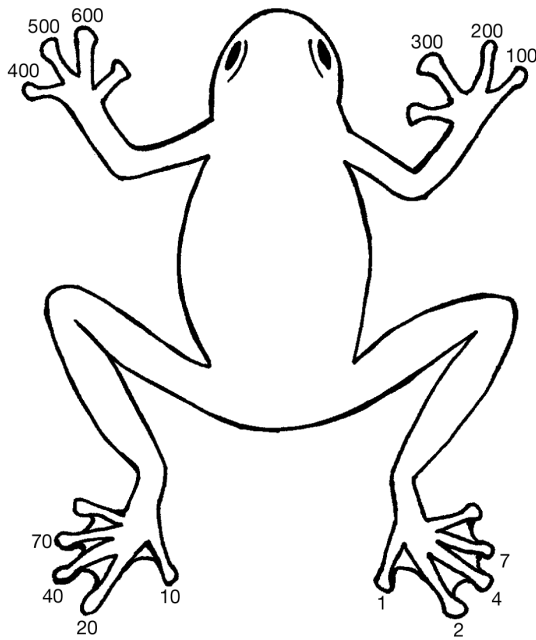

Fig. 1. Toe-clipping scheme for frogs, modified from Martof (1953), used at Tully Gorge National Park, Australia, since 1992. A minimum of 2 toes are removed so that injury is not confused with marking; a maximum of 5 toes are removed, with no more than 2 from each foot and 1 from either hand. The marking scheme, which provided each frog with a unique number, simply adds together the numbers allocated to each toe removed, beginning at 3 (toes 2+1) and finishing at 699 (toes 600+70+20+7+2)

Females of any species were rarely encountered along the stream transect, so only males (identified by nuptial pads and/or calling) were included in the analyses. Sample sizes varied for each species because of the differences in behaviour and population size (influenced by suitable habitat along the transect). The study period was restricted to summer (November to February) to reduce the confounding effects of changes in behaviour with season such as breeding, mortality due to chytridiomycosis in cooler months and the aftermath of Cyclone Larry in March 2006.

An individual was classified as newly toe-tipped at the initial encounter, but as previously toe-tipped in subsequent surveys in the following months. To avoid repeated measures for some individuals that were recaptured multiple times in the months after toe-tipping, only data for the first recapture event after toe-tipping were included. Low short-term return rates of animals did not allow for statistical analysis of the data, but intermediate-term return rates were compared among species using a chi-squared test.

RESULTS

Newly toe-tipped *Litoria nannotis* and *L. rheocola* were less likely to be recaptured in the 24 h after marking than *L. genimaculata* and *Nyctimystes dayi*. The return rate of *L. genimaculata*, *L. rheocola* and *N. dayi* did not vary greatly between newly marked and previously toe-tipped frogs within the same species. However, previously toe-tipped *L. nannotis* individuals had a much higher return rate than those newly marked (Table 1).

Likelihood of recapture in the months after marking was dependent on species (χ^2 = 12.629, df = 3, p = 0.006; Table 2). Return rates in the months following initial capture of *Litoria nannotis* were significantly higher than the expected ratio derived from the other species.

Table 1. Comparative return rate and return distance (i.e. the straight metre distance between successive captures on the transect) 24 h after capture of newly and previously toe-tipped frogs, November 2005 to February 2006. Return distance shows mean ± SD (where applicable) with range in parentheses

Species	Newly toe-clipped frogs			Previously toe-clipped frogs		
	No. captured	Return rate after 24 h (%)	Return distance (m)	No. captured	Return rate after 24 h (%)	Return distance (m)
Litoria genimaculata	22	14	37 ± 54 (2–100)	9	11	1 (1)
Litoria nannotis	31	3	1 (1)	20	25	1 ± 1 (1)
Litoria rheocola	33	6	2 ± 1 (1–3)	11	9	1 (1)
Nyctimystes dayi	74	24	3 ± 2 (1–10)	27	19	3 ± 4 (1–10)

Table 2. Return rates for newly toe-tipped frogs in the 1 to 3 mo period after marking, November 2005 to January 2006

Species	No. of frogs toe-tipped	Return rate (%)
Litoria genimaculata	22	41
Litoria nannotis	31	65
Litoria rheocola	33	33
Nyctimystes dayi	74	36

DISCUSSION

Our study suggests that toe-tipping has an effect on the 24 h return rates of *Litoria nannotis* but not of *L. genimaculata*, *L. rheocola* or *Nyctimystes dayi*. The latter 3 species showed similar return rates in the 24 h after capture to newly toe-tipped and previously marked frogs. However, there was a markedly greater return rate after 24 h of previously marked *L. nannotis* when compared to newly toe-tipped frogs.

The increase in *Litoria nannotis* return rates between newly toe-tipped frogs (3%) and previously toe-tipped frogs (25%) suggests that this species' adverse reaction to toe-tipping and handling is greater than that to handling alone. This species is relatively large and is amenable to other marking techniques such as passive integrated transponder (PIT) tags (R. Puschendorf pers. comm.). However, the effect of such techniques on behaviour has not been assessed. Of the 4 species studied, *L. nannotis* demonstrated the highest return rate (65%) in the 4 mo monitoring period and the smallest return distance. Therefore, it appears that toe-tipping does not have a major intermediate-term adverse effect on *L. nannotis*.

Litoria genimaculata demonstrated similar return rates immediately after toe-tipping (14%) and after handling alone (11%), so the marking technique is not perceived to cause any greater reaction in this species. The 41% return rate for *L. genimaculata* during the 4 mo monitoring period indicates that any reaction to toe-tipping and handling is brief. Considering the wide return distance of this species, already known for its extensive home range (Richards & Alford 2005, Rowley & Alford 2007) and its scansorial nature (Rowley & Alford 2007), we consider this intermediate-term return rate to be relatively high. Therefore, we do not believe toe-tipping has a major intermediate-term adverse effect on *L. genimaculata*.

There was a lesser chance of catching newly marked *Litoria rheocola* (6%) than other species, and the return rate was still low in previously marked frogs (9%). Again, our results suggest that this species does not show a greater reaction to toe-tipping than to handling alone. The intermediate-term return rate was 33%, and we cannot rule out a major intermediate-term adverse effect of toe-tipping.

The highest return rate immediately after toe-tipping was demonstrated by *Nyctimystes dayi* (24%), similar to the 24 h return rate in previously marked frogs (19%). While this suggests that *N. dayi* shows a similar response to handling and toe-tipping, there is the potential that the stress response was overridden by other, stronger life-history requirements and was therefore not detected. For example, the comparatively higher return rates of *N. dayi* 24 h after toe-tipping may be the result of a stronger influence of breeding behaviour compared to the other species. The breeding period of *N. dayi* is restricted to the wet season (October to April; Hodgkison & Hero 2002, K. R. McDonald unpubl. data). During this time, inter-male spacing is observed, possibly to maintain calling territories (Hodgkison & Hero 2002). Site fidelity and vulnerability to being caught, despite toe-tipping, could be the result of males defending calling territories and individuals avoiding being disadvantaged by movement away from the stream, and therefore adopting a tempered response to stress. Some breeding animals are able to endure stressors by desensitising the adrenocortical stress response and preventing the GC corticosterone from inducing defensive mechanisms that could prevent or reduce reproduction (Wingfield et al. 1998, Sapolsky et al. 2000). Specific stress-reduction mechanisms to maximise reproductive success have been shown in sea turtles (Jessop et al. 2004) and some birds (Wingfield et al. 1995) with a limited breeding period. The intermediate-term return rate was 36% and, similarly to *Litoria rheocola*, we cannot rule out a major intermediate-term adverse effect of toe-tipping.

Many frogs of both species, when both newly toe-tipped and previously marked, were not recaptured on successive nights. However, this could be the result of low detection rates due to frogs remaining hidden. Most frogs that were seen were usually caught, apart from *Litoria nannotis*, which moved quickly to hide when spotlit. True absence from the transect could be the result of foraging periods away from the stream for terrestrial prey (Hodgkison & Hero 2003). Calling is energetically expensive (reviewed by Wells 2001, 2007, McLister 2003) and would require males to forage, venturing away from the original stream capture site. Call territories may also be left voluntarily in response to environmental fluctuations in territory quality (Wells 1977), to avoid predators (Howard 1978b), during amplexus (Fellers 1979) or as a result of male-male aggression (Howard 1978a, Rowley 2007, Phillott 2008). Predation, disease and permanent dispersal may also lower the return rates on successive nights (Howard 1978a).

In the absence of information on the return rates of *Litoria genimaculata*, *L. nannotis*, *L. rheocola* and *Nyctimystes dayi* after marking with PIT tags, visible implant alphanumeric tags or visible implant elastomer tags, no comparisons can be drawn on the efficacy of each method. However, our study contributes to the understanding of the effect of toe-tipping on the behaviour of these 4 frog species.

CONCLUSION

Our results, indicating relatively low to moderate return rates immediately (24 h) and relatively moderate to high return rates intermediately (1 to 3 mo) after toe-tipping, help support the validity of this procedure as a marking tool for amphibians. Toe-tipping does not appear to adversely influence the short-term behaviour of these species, with the exception of *Litoria nannotis*, any more than handling alone, and therefore should not contribute to the movement of animals away from a study site. *L. nannotis* showed relatively high intermediate-term return rates, suggesting that marking did not greatly contribute to its movement away from the study site. In 2 species, *L. nannotis* and *L. genimaculata*, we believe a major adverse effect of toe-tipping is unlikely, given their relatively high intermediate-term return rates, after taking into account the natural history of each species. We could not rule out a major intermediate-term effect of toe-tipping on *L. rheocola* or *Nyctimystes dayi*. To validate toe-tipping as an ethical marking procedure for amphibians, future studies need to determine potential inflammation rates of digits and mobility after varied numbers of toe-tips are removed. In addition, the effects of toe-clipping, where the entire toe is removed, may differ from those of the more conservative toe-tipping, and should be considered separately.

LITERATURE CITED

Bell BD, Pledger S (2005) Does toe tipping/clipping affect the return rates of the terrestrial frog *Leiopelma pakeka* on Maud Island, New Zealand? NZ J Zool 32:219–232

Berger L, Speare R, Daszak P, Green DE and others (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. Proc Natl Acad Sci USA 95:9031–9036

Bogert CM (1947) A field study of homing in the Carolina toad. Am Mus Novit 1355:1–24

Cabanac A, Cabanac M (2000) Heart rate response to gentle handling of frog and lizard. Behav Process 52:89–95

Cabanac A, Cabanac M (2004) No emotional fever in toads. J Therm Biol 29:669–673

Donnelly MA, Guyer C, Juterbock JE, Alford RA (1994) Appendix 2. Techniques for marking amphibians. In: Heyer WR, Donnelly MA, McDiarmid RW, Hayek LC, Foster MS (eds) Measuring and monitoring biological biodiversity: standard methods for amphibians. Smithsonian Institution Press, Washington, DC, p 277–284

Ecker A (1889) The anatomy of the frog. Clarendon Press, Oxford

Fellers GM (1979) Aggression, territoriality, and mating behaviour in North American treefrogs. Anim Behav 27: 107–119

Frost DR, Grant T, Faivovich J, Bain RH and others (2006) The amphibian tree of life. Bull Am Mus Nat Hist 297:1–291

Greenberg N, Wingfield JC (1987) Stress and reproduction: reciprocal relationships. In: Norris DO, Jones RE (eds) Reproductive endocrinology of fishes, amphibians and reptiles. Wiley, New York, NY, p 389–426

Heard GW, Scroggie MP, Malone B (2008) Visible implant alphanumeric tags as an alternative to toe-clipping for marking amphibians — a case study. Wildl Res 35: 747–759

Heatwole H (ed) (1998) Amphibian biology, Vol 3. Sensory perception. Surrey Beatty & Sons, Chipping Norton

Hodgkison S, Hero JM (2002) Seasonal behavior of *Litoria nannotis, Litoria rheocola* and *Nyctimystes dayi* in Tully Gorge, north Queensland, Australia. In: Nattraus AEO (ed) Frogs in the community. Proc Brisbane Symp 13–14 February 1999. Queensland Frog Security, Brisbane, p 29–39

Hodgkison S, Hero JM (2003) Seasonal, sexual and ontogenetic variations in the diet of the 'declining' frogs *Litoria nannotis, Litoria rheocola* and *Nyctimystes dayi.* Wildl Res 30:345–354

Howard RD (1978a) The evolution of mating strategies in bullfrogs, *Rana catesbeiana.* Evolution 32:850–871

Howard RD (1978b) The influence of male-defended oviposition sites on early embryo mortality in bullfrogs. Ecology 59:789–798

Jessop T, Sumner J, Lance V, Limpus C (2004) Reproduction in shark-attacked sea turtles is supported by stress-reduction mechanisms. Proc Biol Sci 271(Suppl):S91–S94

Machin KL (1999) Amphibian pain and analgesia. J Zoo Wildl Med 30:2–10

Martof B (1953) Territoriality in the green frog, *Rana clamitans.* Ecology 34:165–174

May RM (2004) Ethics and amphibians. Nature 431:403

McCarthy MA, Parris KM (2004) Clarifying the effect of toe tipping/clipping on frogs with Bayesian statistics. J Appl Ecol 41:780–786

McDonald KR, Alford RA (1999) A review of declining frogs in northern Queensland. In: Campbell A (ed) Declines and disappearances of Australian frogs. Environment Australia, Canberra, ACT, p 14–22

McDonald KR, Mendez D, Muller R, Freeman AB, Speare R (2005) Decline in the prevalence of chytridiomycosis in frog populations in north Queensland, Australia. Pac Conserv Biol 11:114–120

McLister JD (2003) The metabolic cost of amplexus in the grey tree frog (*Hyla versicolor*): assessing the energetics of male mating success. Can J Zool 81:388–394

Parris KM, McCarthy MA (2001) Identifying effects of toe tipping/clipping on anuran return rates: the importance of statistical power. Amphib-Reptilia 22:275–289

Endang Species Res 11: 183–188, 2010

Phillott AD (2008) An observation of interspecific aggression by a male *Nyctimystes dayi* toward a sympatric male *Litoria rheocola* (Anura: Hylidae). Herpetofauna 38:75–77

Phillott AD, Skerratt LF, McDonald KR, Lemckert FL and others (2007) Toe-clipping as an acceptable method of identifying individual anurans in mark recapture studies. Herpetol Rev 38:305–308

Richards SJ, Alford RA (2005) Structure and dynamics of a rainforest frog (*Litoria genimaculata*) population in northern Queensland. J Zool (Lond) 53:229–236

Rowley JJL (2007) Observations of physical interactions between conspecific male rainforest stream frogs *Litoria genimaculata* and *Nyctimyestes dayi* (Anura: Hylidae). Herpetofauna 37:11–15

Rowley JJL, Alford RA (2007) Movement patterns and habitat use of rainforest stream frogs in northern Queensland, Australia: implications for extinction vulnerability. Wildl Res 34:371–378

Sapolsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev 21:55–89

Stevens CW (1992) Alternatives for the use of mammals for pain research. Life Sci 50:901–912

Wells KD (1977) Territoriality and male mating success in the green frog (*Rana clamitans*). Ecology 58: 750–762

Wells KD (2001) The energetics of calling in frogs. In: Ryan MJ (ed) Anuran communication. Smithsonian Institution Press, Washington, DC, p 45–60

Wells KD (ed) (2007) The ecology and behavior of amphibians. The University of Chicago Press, Chicago, IL

Wingfield JC, O'Reilly KM, Astheimer LB (1995) Modulation of the adrenocortical responses to acute stress in arctic birds: a possible ecological basis. Am Zool 35: 284–294

Wingfield C, Maney DL, Breuner CW, Jacobs JD, Lynn S, Ramenofsky M, Richardson RD (1998) Ecological bases of hormone-behavior interactions: the 'emergency life history stage'. Am Zool 38:191–206

Yao M, Westphal NJ, Denver RJ (2004) Distribution and acute stressor-induced activation of corticotrophin-releasing hormone neurones in the central nervous system of *Xenopus laevis.* J Neuroendocrinol 16:880–893