Guidelines for the Marking of Frogs

Robert Johnson
South Penrith Veterinary Clinic
www.reptilevet.com.au

Introduction

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2. Reasons for marking frogs
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4. Current methods in use
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Introduction

- Need a reliable technique to identify individuals within populations
- Traditionally - toe-clipping, freeze and hotwire branding, & various methods of tagging
- Recent developments in technology & animal welfare
- Need to reassess currently acceptable means of marking frogs

Introduction

Aim

- Outline current and emerging methods of marking frogs and draw conclusions as to the most satisfactory method(s) to use

Introduction

Australian Society of Herpetologists guidelines:

- Negative consequences of marking technique - outweighed by the benefits
- Use the least harmful method for desired outcome
- Consider the welfare of the individual and population
- Consider the taxon-specific effects of toe-clipping
- Use the least number of animals for the desired outcome
- Ensure that only researchers with appropriate training and experience conduct potentially painful procedures such as toe-clipping

Introduction

Australian Code of Practice for the Care and Use of Animals for Scientific Purposes

- (i) the procedures performed in a clean area by competent persons, using clean equipment
- (ii) equipment and agents necessary to provide for the health and welfare of the animals and relief of pain or distress must be readily available
- (iii) sedated or anaesthetised animals should experience uneventful recovery to full consciousness in an observation area where they are able to maintain normal body temperature and are protected from injury and predation
- (iv) the methods and equipment used are appropriate to the species and cause the least distress and interference with normal behaviour

Reasons for marking frogs

- Population and behaviour studies
- To aid in the monitoring of life history parameters of individual animals
- Recording individual animals in collections, zoos etc.

Factors influencing the choice of a marking method

- Ease of application
- Cost
- Permanency of marking
- Tradition
- Amphibian anatomy, behaviour, and physiology
- Age and size of animals, metamorphs
- Physical factors
  - Risk of infection
  - Post marking inflammation, necrosis of digits
  - Post marking survival rates
- Skill of operator
- Available equipment
- Animal welfare considerations

Current methods in use

Historically :

- Toe-clipping
- Freeze, chemical and hotwire branding
- Tattooing

http://www.botany.uwc.ac.za/Presents/Focuson/frogs/freeze.htm

Current methods in use

More recently :

- PIT transponders (microchips)
- Polymers
  - Visible Implant Elastomer (VIE)
  - Visible Implant Alphanumeric (VIAlpha)
- Pattern mapping
- VIE (C) – combination of VIE and toe-clipping (C)

Toe-clipping

Toe-clipping of frogs for the purpose of marking:

- the amputation of one or more digits
- formulae have been developed which all involve the possible amputation of part of any digit

Advantages:

- Low cost
- Quick technique once mastered
- Well recognised technique
- Tissue can be retained for DNA analysis if required

Toe-clipping

Disadvantages

- Painful
- Skill required by operator
- Infection risk
  - to the individual
  - risk of disease transmission to other frogs
- Reduced survival
- Hinders amplexus (mating)
  - First 3 digits of the forelimb of the male are important for mounting the female
- Hinders ecdysis
  - Hindlimb – fourth digit of both sexes is used for removing shed skin

Toe-clipping

- *Should not be used on climbing species Hylidae (tree frogs)*
- *No more than two adjacent toes should be removed on the one foot*
- *The number of forelimb digits removed from males should be minimised - hinders amplexus*
- *Both left and right 4th hind digits should never both be removed – hinders ecdysis*

Toe-tipping

- A modified version of toe-clipping
- Removal of only the toe pad or disc from a hylid digit or the most distal phalanx from a non-hylid digit
- Toe-tipping is a reliable means of identifying individuals with minimal tissue regrowth

Lüddecke H & Amézquita A (1999)

Freeze and Hotwire Branding

Advantages
- Low cost

Disadvantages
- Painful
- Infection risk
- Excessive scarring
- Difficult to avoid dee
- tissue damage

http://www.botany.uwc.ac.za/Presents/Focuson/frogs/freeze.htm

Freezing and hotwire branding are no longer recommended for marking amphibians

Tattooing

Advantages
- Low cost

Disadvantages
- Painful
- Loss of legibility – not permanent
- Infection risk
- Scarring
- Difficult to avoid deep tissue damage

Tattooing

- *Tattooing is no longer recommended in amphibians due to the availability and increased efficacy of alternate methods of marking*

Passive Integrated Transponder Tags (PIT)

- Permanent method of marking
- Insertion sites for frogs include
  - Intracoelomic – injected into the left caudal body cavity
  - Subcutaneous – into the dorsal lymphatic sacs

Passive Integrated Transponder Tags (PIT)

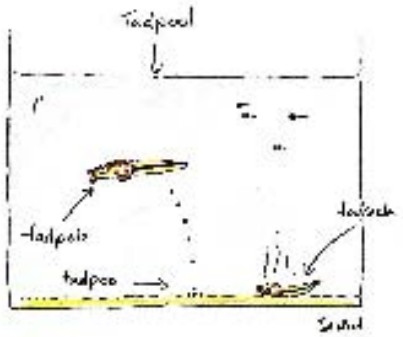
Advantages:
- Unlimited number of codes
- Permanent

Disadvantages
- Painful
- High cost
- Requires handling and tissue penetration
- Limited to use in larger frogs
  - Animals in lower allowable size class (40-50 mm snout vent length [SVL]) occasionally show signs of distress
    - A Stauber, *pers. comm.*

Passive Integrated Transponder Tags (PIT)

- *PIT tags should only be used for frogs >50mm SVL.* e.g. *Heleioporus and Mixophyes* spp.

Heleioporus australiacus



Visible Implant Fluorescent Elastomer Tags

- VIE tags - 2 silicon-based components - mixed just prior to use
- A range of colours and injection sites combine to produce a large number of individual codes
- The technique has been used on tadpoles (e.g. marking a cohort for future ID) as well as adult frogs with some success
  - A Stauber, *pers. comm.*

http://www.nmt.us/products/vie/vie.htm

Visible Implant Fluorescent Elastomer Tags (VIE)

- A pilot study testing the capacity for VIE tags to be retained and visible in metamorph *Litoria booroolongensis*
- Tagged as mid stage tadpoles
- VIE tag could be identified in 60% of individuals post-metamorphosis
  - D. Hunter, *pers. comm.*

Soft Visible Implant Alphanumeric Tags

- VI Alpha tags same material as the VIE tags
- Pre-cured with individual alphanumeric codes printed on one side
- Two sizes: standard (1.0 mm x 2.5 mm) and large (1.5 mm x 3.5 mm)
- Variety of colours

http://www.nmt.us/products/via/via.htm

Pattern Mapping

- Non invasive
- Ventral patterns - great potential for individual recognition in genera with individually distinct markings. *Adelotus, Crinia, Pseudophryne, Uperoleia* spp., *Limnodynastes spp.* and some species of the *Litoria* genus
- Preprinted forms - describe patterns on the ventrum or dorsum of frogs
- Potential to use digital photography and pattern recognition software

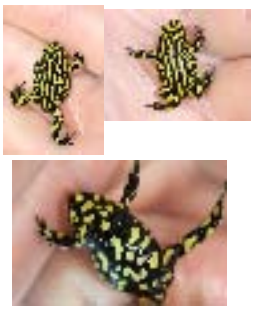
Pattern Mapping

- Successfully used to monitor individual *Pseudophryne corroboree*, although over a two year period there was substantial changes in individual belly pattern
  - D. Hunter, *pers. comm.*
- Temporal changes in patterns during early life-history stages (metamorph through to sub-adult) may limit the use of this technique during these periods
- Double blind study on museum specimens to validate technique?
  - Fading of patterns on alcohol-preserved specimens
  - Difficult to discern individual patterns?
    - T. Leary, *pers. comm.*

Pattern Mapping

Advantages

- Low cost - unless software needs to be purchased
- Allows individual identification of very small frogs, juveniles
- Permanent ?
- Non invasive
- No risk of infection or spread of disease
- No pain



Pattern Mapping

Disadvantages

- Useful only in species with unique individual markings
  - Released corroboree frogs belly patterns have changed to the point of "hard-to-be-sure" after 4 -6 years
    - D Hunter, *pers. comm.*
- Need to handle frogs (as with most techniques)
- Technology still being developed, i.e. pattern recognition software
- Limited by numbers – cumbersome with a large population
- Time consuming
- Potential temporal shifts in pattern

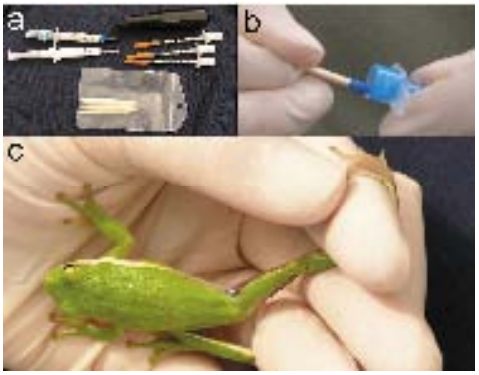
Pattern Mapping

- *The application of pattern mapping is limited by the number of species that display unique individual markings*

Combined VIE-C technique

- More recently - hybrid marking technique (VIE-C)
  - Visible Implant Elastomer (VIE) + toe-clipping (C)
  - Tree frogs (Hylidae)
  - The number of toes clipped is reduced (one per frog)
  - Injection of the elastomer in the plantar surface of the digit
  - Decreases the chance of elastomer migration.
  - More user friendly - a light-weight marking kit easily carried in the field
  - Relatively inexpensive
  - Duration of marks is unknown at this stage, however legible marks were found on frogs more than a year after marking

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



Hoffmann K, McGarrity ME & Johnson SA (2008)

What does it all mean?

- Microchips are the preferred option for larger frogs (> 50mm SVL)
  - M. Mahony, *pers. comm.*
- The majority of Australian frogs are less than 60 mm SVL
- How to mark smaller frogs other than by toe-clipping?

What does it all mean?

- Traditionally toe-clipping has been the favoured method of marking frogs worldwide
- Losing favour as researchers opt for less invasive techniques of marking??
- Restrictions should be placed on the amputation of specific toes and on the number of toes
- Amputation of certain digits would appear to affect behaviour
- No more than two adjacent toes should be removed
- Anaesthesia prior to toe-clipping?

What does it all mean?

- If behaviour or survival of the animal is likely to be seriously impaired, alternate methods to toe-clipping should be considered
- Toe-clipping has been demonstrated to influence the return rate of marked animals
- Operators of this technique need to have a high degree of manual dexterity in order to carry out the technique quickly and accurately, thereby reducing the stress to the animal
- Given the negative aspects of toe-clipping it still appears that it is the only feasible option for marking many amphibian species

What does it all mean?

- *It is more ethical to use toe-clipping in studies aimed at understanding and preventing further decline in frog population, than it would be to fail to undertake the research, or use an alternative method that has not been studied and may potentially have a greater impact on the population.*
  - Phillott *et al.*(2007)

What does it all mean?

- A basic assumption in all mark-recapture studies
  - The marking technique does not increase mortality or impact upon competitive ability
- Difficult to know if this assumption has been violated
- Ideally, each marking technique should be subjected to a pilot study to gauge any negative impacts on the individual animal and the population as a whole
- For obvious reasons unmarked controls could not be used in such a study; as a result survivorship can only be compared between cohorts that are marked using several different systems

Conclusion

- *There is currently unlimited scope for researchers to develop new techniques or refine existing ones in order to minimise pain and suffering to individual animals, and limit any negative effects on the welfare of populations*

Pattern Mapping

http://www.doc.govt.nz/conservation/native-animals/reptiles-and-frogs/frogs/docs-work/photo-stage/

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