Australian Society of Herpetologists Inc.

Position Statement No. 1

Toe clipping of lizards

The Australian Society of Herpetologist's (ASH) position on toe clipping

- The ASH recognises the importance of many studies that require the marking of reptiles, and that, at present, toe clipping is the only practical and reliable long-term technique for marking some taxa.
- It is incumbent upon researchers to demonstrate that the potentially negative consequences of their marking technique are outweighed by the benefits gained by the use of this technique in their research.
- The least harmful method that achieves the desired outcome should be applied.
- The welfare of the individual and population from which the individual has been drawn must always be considered prior to marking.
- The taxon-specific effects of toe clipping should be recognised by researchers, and where possible the life history and behaviour of the species should be a factor when deciding on the most appropriate marking technique (e.g., climbing lizards may be affected more adversely by toe clipping than fossorial species).
- As well as a technique for marking individual lizards, toe clipping presents a non-lethal method of obtaining bone segments for aging individuals via skeletochronology, and provides tissue samples for DNA extraction.
- In the event that toe clipping is necessary, negative effects should be objectively assessed, and the results of such investigations disseminated within and beyond the Society.
- The number of animals needed to be marked or sampled to achieve the desired outcome should be minimised, and statistically justifiable.
- Only researchers with appropriate training and experience should conduct toe clipping. Inexperienced people should be supervised by experienced researchers until they have demonstrated competency in this procedure.
- When toe clipping, the amount of digit removed, and the number of digits affected, should be minimised, but enough removed to ensure that the mark remains permanent and readable.
- The ASH cautions against anthropomorphic perceptions of the comparative effects of different research techniques.

Preamble

The Australian Society of Herpetologists Incorporated (ASH) is a professional body for practising herpetologists. The objectives of the Society are:

- to promote the scientific study of amphibians and reptiles;
- to provide opportunities for discussion and dissemination of information among its members by appropriate means, including meetings and publications; and

- to take an active interest in the conservation of amphibians and reptiles.

The ASH recognises that, for many studies of herpetofauna, there is a need to repeatedly recognise individuals within a population. Depending on the species (and sometimes the age / size of individuals), individual recognition may be achieved by recalling distinctive markings or patterns, by the attachment or insertion of identification tags, or by the marking of individuals.

There are various techniques for marking herpetofauna, ranging from temporary paint or ink marks, to the attachment of coloured beads, insertion of Passive Integrated Transponders (PIT tags), or permanent marking of body parts using techniques such as scale-, scute- and toe clipping, or heat and freeze branding. Toe clipping remains one of the most commonly-applied marking techniques for lizards.

In addition to serving as an individual identifying mark, toe clipping provides a non-lethal solution to obtaining bone segments for aging reptiles via skeletochronology, using lines of arrested growth within the bone to determine age. The suitability of toe clipping as a marking or sampling technique is dependent on the nature and reasons for the study, and on various attributes of the study species. As an invasive technique, toe clipping requires the approval of an official ethics committee, and is subject to some controversy in terms of the welfare of individual animals and the potential impact on the populations from which marked individuals are drawn. It is incumbent upon researchers to demonstrate that the potentially negative consequences of their chosen technique are outweighed by the benefits gained by the use of this technique in their research. Both researchers and those responsible for the approval or denial of research proposals should base their decisions on an objective assessment of the relative benefits of toe clipping, rather than being guided by anthropomorphic perceptions.

This Position Statement details the ASH's stance on the practice of toe clipping lizards. A separate ASH Position Statement will consider toe clipping of amphibians.

Why mark reptiles?

Reasons for marking wildlife, including reptiles, may include (from Beausoleil *et al.* 2004):

- determination of life history parameters of individuals, such as age at maturity, growth rates, longevity, etc.;
- identification of individuals in order to study demography and behaviour;
- to allow the estimation of population sizes, rates of increase and survival, reproductive output and recruitment levels;
- to monitor populations trends; or
- to identify particular stocks in populations undergoing conservation management.

Selection of an appropriate marking technique

There is a range of potentially suitable marking methods available. The properties of a hypothetical "ideal" identifying mark have been summarised by Beausoleil *et al.* (2004). They state that an ideal mark should:

- Allow the animal to be as free of pain and / or stress as possible.
- Identify the individual animal, if desired.
- Be easy to apply in both the laboratory and the field.
- Be easily and unambiguously read or observed.

- Be reliable over the duration of the study.
- Be cost-effective.
- Be suitable for the size of animal for which it will be used.
- Utilise materials that are easy to obtain.

Furthermore, Beausoleil *et al.* (2004) state that an ideal mark should not:

- Cause death.
- Have sub-lethal effects on fitness, e.g. reduced growth or reproductive rates.
- Influence the behaviour of marked individuals.
- Influence the behaviour of other animals towards the marked individual.
- Affect the future probability of capturing marked individuals relative to unmarked individuals.

No single marking method will meet all of these criteria. Consequently, the choice of marking method must be based on consideration of these factors, and application of the method that is most suitable for the particular study and species, and which minimises the negative impacts. The negative effects of toe clipping on individuals are likely to vary between taxa, depending on the functional significance of toes for particular species, their physiological and behavioural response to toe clipping, and their ability to recover from the procedure. Consequently, it is necessary to carefully consider marking options, and ensure that minimisation of negative consequences is a key factor in this consideration. For many species of small lizards, marking by toe clipping satisfies more criteria of an ideal mark than alternative techniques, and may prove to be the only method that does not cause unacceptable impacts.

Toe clipping to obtain tissue samples

The ability to age wild-caught individuals enables researchers to address important ecological questions regarding population dynamics and life history characteristics, as well as those critical to the management or conservation of many species. For species for which age cannot be determined using morphological characteristics, skeletochronology provides an alternative; age can be determined by reading the lines of arrested growth within histologically prepared bone sections. Toe clipping provides a non-lethal method of obtaining samples of bone for aging using this technique. Caudal (tail) bones are used for skeletochronological determination of age for snakes; however many lizards can autotomise (shed) their tails, reducing the value of these bones for aging. Additionally, longer bones provide more accurate age information. Although lizard femurs are commonly used for skeletochronology; toe-clips provide a non-lethal, and reliable, alternative. However, researchers planning on using this technique should familiarise themselves with the literature, especially studies that detail potential issues with accurately estimating age based on long bones from toes, particularly for longer lived (>10 years) species. In many studies, cross validation between skeletochronological and other methods are recommended.

In addition to providing bone for skeletochronology, toe clips provide tissue that can be used for genetic analyses. DNA from such tissue can provide a wealth of information that can be used to assess gene flow and diversity within and between populations, measure evolutionary change, assign paternity, and answer a myriad of other questions important to ecology and conservation. Potentially less invasive alternatives, such as obtaining blood samples, or tissue biopsies, should be considered (although in some cases toe-clipping may be considered the

least invasive procedure). If a study also requires individuals to be permanently marked, toe clipping can provide both a mark and a genetic sample in the one procedure.

What we know about the impact of toe clipping on lizards

- Natural toe and foot loss is observed in lizards in nature (e.g. Hudson, 1996), showing that toe-clipping is unlikely to generate effects not found in nature, and that lizards can survive such damage.
- To date, there has been little empirical investigation into the impacts of toe clipping on lizards. The few existing studies suggest that: 1) toe clipping does not reduce sprint speed of various terrestrial lizards (Huey *et al*., 1990; Dodd, 1993; Borges-Landaez and Shine, 2001); 2) toe clipping induces less stress, and for a shorter duration, than implantation of a PIT tag within a small scincid lizard (Langkilde and Shine, 2006; see Fig. 1); and 3) toe clipping reduces the clinging or climbing ability of some arboreal lizard species (*Anolis* – Bloch and Irschick, 2004; some geckos – Mahendra 2004), but not others (Paulissen and Meyer, 2000).

Evaluating alternative approaches

It is intuitive to many people that invasive techniques such as toe clipping must be more stressful and harmful to an animal than less invasive methods such as individual identification from a photographic library. This may or may not be the case depending on a range of factors such as the taxon involved, duration of handling, skill of the handler, and so on. When guided by intuition alone, assumptions on the comparative stress induced in an animal by differing procedures can be very inaccurate (Langkilde and Shine 2006). For example, for some species the lengthy handling time that is often necessary for effective identification from a photo library may be more stressful than a rapid marking / identification procedure such as toe clipping (and techniques such as photographic identification are not possible for many species that lack distinctive patterns or features). For this reason we caution against subjective anthropomorphising of animal's reactions to differing procedures, and encourage objective, comparative evaluation of various techniques. Ideally these evaluations will include assessment of both the short-term pain and stress inflicted on the animal, as well as the long-term impact of these procedures. Toe clipping has the advantage of having multiple functions, and acting as a permanent individual mark in addition to providing DNA for genetic analyses, and bone for skeletochronology. This permits the collection of large amounts of data with no additional handling. While this does not mitigate pain or distress on particular animals, it means that the animals are handled less than if separate actions were used for marking and sampling.

Notwithstanding this, researchers should always seek the least harmful technique for marking or sampling reptiles, and that which has the least effect on the behaviour and ecology of the study organism. Aside from the impact on survival, and the ethical implications of alternative techniques, their influence on the scientific integrity of resulting data should be of considered. If a technique affects the movement patterns of an organism, data from mark-recapture or dispersal analyses may under- or over-represent natural patterns (e.g. Langkilde and Alford, 2002). The choice of an appropriate technique should be based on a thorough review of the literature, consideration of the specifics of the taxa to be marked, consultation with people who have experience in these techniques, and, where necessary, a preliminary evaluation of the technique on a small, pilot sample of individuals. For highly-threatened taxa, it may be prudent to evaluate the impact of techniques on non-threatened analogue taxa.

Marking lizards

Photographic identification. Identifying individuals from photographs or hand rendered drawings on blank template line illustrations (e.g., drawing dorsal markings of individuals) can be an effective means of individually identifying lizards. This should be explored before more invasive procedures are considered; however, the reliability or this technique, as well as the handling time required to identify individuals should be taken into account. This method may be particularly suitable for larger species (e.g., *Tiliqua* spp. and some *Egernia* with distinctive markings), but less suitable for taxa that have few distinctive markings (e.g., some scincid species from genera such as *Eulamprus, Lampropholis,* or *Niveoscincus*), or for larger scale programs where, in some cases, hundreds of individuals need to be rapidly identified in field conditions.

Branding. Numbers or letters can be applied via the application of electricity, heat, or cold, whereby deep layers of skin are cauterised to prevent regeneration. One potential problem with employing this technique on lizards is that their thick scales make it difficult to determine the final appearance of the mark until several days after application; cauterisation that is too brief will result in a feint, difficult to read mark, whereas prolonged application can cause deep tissue damage. Additionally, reptile scales impede the action of local anesthetics, suggested to reduce the levels of pain associated with this procedure (Guidelines for use of live amphibians and reptiles in field and laboratory research, 2004). This success and applicability of this technique is likely to be size-dependent – attempting to brand scales of very small lizards is not recommended.

Tattooing. Tattooing has been successfully used on some lizard species. As with all techniques, there are certain considerations that must be considered before employing this marking technique: 1) a dye must be selected that will contrast with the natural skin pigmentation, 2) tattoo marks may become illegible due to ultraviolet degradation of the dye or diffusion over time, 3) dyes can cause secondary problems (Boone and Larue, 1999), so care should be taken to avoid dyes containing toxic ingredients. Preliminary laboratory studies should be undertaken in cases where toxicity is unknown.

Paint or other temporary marks. In some studies, it may not be necessary to permanently mark animals (for example in field studies to prevent recapture of the same animals within a narrow time-frame). In these cases, paint (or other non-toxic marks) can be simply applied to the skin. A problem with this technique is that it is effective for a very time – the mark readily rubs or washes off (particularly with smooth-scaled species), and as skin is sloughed the mark is lost. This technique may be considered the least invasive method for temporary marking lizards within populations (in the field or laboratory).

Banding and tagging. Bands and tags can be placed around the legs, or sewn through head or tail crests, or dorsal tail musculature of lizards. The size, shape and placement of bands and tags must be selected to minimise the risk of physical impairment or entanglement in undergrowth. Generally, the use of external tags is not recommended for lizards that are likely to be impeded by their presence, such as burrowing or fossorial species. Brightly colored tags and bands could compromise an animal's camouflage, increasing its risk of predation. The pain caused by sewing tags through body parts, and the potential for them to be torn out if they become entangled, must be considered. This procedure should only be conducted by researchers with appropriate training and experience, as improper insertion of the attaching thread or wire could cause necrosis of the muscle and bone (Wright, 2001).

PIT tagging. Passive integrated transponders (PIT tags), or microchips, are small, implantable devices that allow the permanent identification of individuals, and are a technique often suggested as a preferred alternative to toe clipping by animal ethics committees. PIT tags are typically injected subcutaneously using a 12-gauge hypodermic needle and syringe; the trauma of insertion and size of the tags make this method unsuitable for small reptiles (see Fig. 1). Other potential complications include: 1) migration of transponders, 2) breakage of the tags, and 3) ejection of the tags before the wound heals, all of which can prevent retrieval of identifying information, and may contribute to the prolonged stress caused by this technique (when compared to toe clipping, Langkilde and Shine, 2006). The application of tissue glue to the wound after insertion can help to prevent ejection. Specialised equipment is required to read the tags.

Radiotelemetry. Radiotransmitters can allow both the relocation and individual identification of individuals, and remote recording of additional variables, such as temperature. This method requires specialised transmitters and equipment to detect the signal. Transmitters can be fitted either externally or internally (implanted or ingested). As for banding and tagging, care must be taken to ensure that externally fitted transmitters do not interfere with the movement and behaviour of individuals (Langkilde and Alford, 2002). It is generally accepted that the maximum ratio of transmitter weight to body weight is 10%. The size of a transmitter is limited by the size of the battery needed to power it, making this method unsuitable for small reptiles.

Scale clipping. Clipping or removing subcaudal or ventral scales provides a good permanent marking system for snakes, but this technique is inappropriate for most lizard species due their lack of enlarged scales.

Obtaining tissue/bone samples

Toe clipping is not recommended as the primary technique for obtaining tissue samples. Blood samples or skin biopsies can provide suitable genetic material without the potentially negative effects of toe clipping. However, if permanent individual identification is also required as part of a study, toe clipping will provide both while minimising handling time and the number of procedures to which an individual is exposed. For lizards, toe-clips provide a non-lethal method of obtaining bone samples for aging via skeletochronology.

Best practice for toe clipping

If, after careful consideration of the costs and benefits of alternate marking techniques, toe clipping is determined to be the most appropriate technique for a particular study, researchers should make efforts to minimise the impact of this technique. A number of factors should be taken into consideration:

1) the number of digits to be clipped should be minimised. This can be achieved by using numbering systems that minimise the number of toes that need to be removed for the expected sample size (see Ferner, 1979 or Donnelly *et al*., 1994 for a minimal-removal coding method), marking the least number of animals necessary to achieve the desired outcome while remaining statistically justifiable, or allocating batch-specific marks (for example, when information about the cohort to which an individual belongs provides adequate data, allocating a cohort-specific mark, requiring fewer combinations, will result in fewer toes being clipped than individual marks). When toe clipping is used to obtain tissue samples, the minimum number of digits needed to obtain adequate amounts of tissue should be clipped;

2) characteristics of the species to be marked should be taken into account when determining which toes to include in a toe clipping scheme, and the number of toes to be clipped. For example, avoiding certain digits that

may have particular functions, such as the elongated forth toe of the hind limb, purported to enhance sprint performance (Irschick, 2002), and clipping no more than one digit per foot from strongly arboreal species, which rely on toes to enhance climbing ability (Paulissen and Meyer, 2000), may minimise the impact of toe clipping in these species. Toes should be clipped using sharp, sterile dissecting scissors or a razor blade and, where possible, should be clipped at the interphalangeal joint (Wright, 2001).

Continuing investigations into less invasive techniques

Advances in technology and methods can help reduce or eliminate the harmful effects of individual recognition procedures and tissue sampling. The ASH encourages continuing investigations into, and refinement of, techniques that reduce potentially harmful effects on individuals and populations, and urges researchers to disseminate new knowledge on these topics rapidly and widely.

Evaluation of the effects of toe clipping

At all possible times the effects of marking on individuals and populations should be monitored and factored into analyses. In the event that obvious distress or mortality is observed, the marking program should cease and the methods should be re-evaluated.

Additionally, assessing the relative impact of differing marking techniques on a range of taxa will provide valuable, objective criteria with which to guide choices on the most ethical techniques for a given species. There is very little information available on the negative impacts of alternative marking techniques on lizards, making it difficult to objectively determine the most appropriate technique. It is incumbent upon researchers to investigate and publish any observed effects of toe-clipping with respect to these impacts. Evaluating alternative marking techniques generally requires studies specifically designed for this purpose, and these studies may be more informative than imposing this task on every study that requires individual recognition.

Preparation and revisions of this statement

This statement was prepared by Nick Clemann, Tracy Langkilde and Erik Wapstra, incorporating comments and suggestions from many members of the Australian Society of Herpetologists. It is expected that these guidelines will be periodically revised; researchers are encouraged to send constructive criticisms or applicable new information to the committee members of the Australian Society of Herpetologists.

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Figure 1. Standard PIT tag applicator syringe with adult spotted snow skink (*Niveoscincus ocellatus*). This adult male is approximately 60 mm snout-vent length and ~4 g. Offspring of these and similar species are < 1.0 g and measure only 30-40 mm (photograph courtesy of Sue Jones).