

Standard Operating Procedure

PERMANENT MARKING OF REPTILES BY SCALE MARKING

Prepared by: Species and Communities Branch, Science and Conservation, Department of Biodiversity, Conservation and Attractions

Prepared for: Animal Ethics Committee

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1 Purpose

Scale marking is a widely used method for long term individual identification of reptiles (such as snakes, crocodiles, and turtles). The advantage of scale marking is that it is lasting, fast and inexpensive. Tissue from notches may be used to genetically identify the animal and there is no extra weight or equipment, such as flipper tags, that potentially hinder the animal.

Scale marking requires a degree of practice, confident animal handling skills, good eyesight and a steady hand.

In deciding on appropriate reptile marking methods, consider the purpose and length of the study, data required and the biology of the animal. Temporary marking methods are also available utilising paints, dyes, tapes and threads (see Section 10). Where sufficient to achieve the desired purpose, temporary marking methods should be utilised over permanent methods.

This standard operating procedure (SOP) provides advice on permanently marking snakes, crocodiles and turtles using scale clipping, scute cutting and shell notching only. Microchipping may also be an option but this method is not covered in this SOP.

2 Scope

This SOP has been written specifically for scientific and education purposes, and endorsed by the Department's Animal Ethics Committee. However, this SOP may also be appropriate for other situations.

This SOP applies to all fauna survey and monitoring activities involving scale marking (scale clipping, scute cutting and shell notching) as a permanent marking method for reptiles, undertaken across the State by Department of Biodiversity, Conservation and Attractions (hereafter Department) personnel. It may also be used to guide fauna monitoring activities undertaken by Natural Resource Management groups, consultants, researchers and any other individuals or organisations with a need to take tissue samples. All Department personnel involved in scale marking of reptiles should be familiar with the content of this document.

Projects involving wildlife may require a licence under the provisions of the *Wildlife Conservation Act 1950* and/or the *Biodiversity Conservation Act 2016*. Personnel should consult the Department's Wildlife Licensing Section and Animal Ethics Committee Executive Officer for further guidance. In Western Australia any person using animals for scientific purposes must also be covered by a licence issued under the provisions of the *Animal Welfare Act 2002*, which is administered by the Department of Primary Industries and Regional Development. This SOP complements the *Australian code of practice for the care and use of animals for scientific purposes* (The Code). The Code contains an introduction to the ethical use of animals in wildlife studies and should be referred to for broader issues. A copy of the code may be viewed by visiting the National Health and Medical Research Council website (<http://www.nhmrc.gov.au>).

3 Definitions

Animal Handler: A person listed on an application to the Department's Animal Ethics Committee who will be responsible for handling animals during the project.

Carapace: A turtle or tortoise shell. The upper shell is referred to as the carapace and the underside the plastron.

Caudal: The tail or the posterior end of the body.

Distal: Situated away from the centre of the body/point of attachment (in terms of scales, the portion of the scale that is not attached to the body).

Lateral: Of or relating to the side or sides of the abdomen.

Ossified: To convert into or cause to harden like bone.

Permanent marker: A marker designed to stay on an animal for its lifespan. Permanent markers tend to leave marks that are less visible than temporary markings (therefore requiring recapture) and often involve tissue damage (Sharp et al., 2007).

Plastron: A turtle or tortoise shell. The upper shell is referred to as the carapace and the underside the plastron.

Scale marking: Involves all procedures consisting of permanent marking by removal of some part of tissue, being shell, scute, or scale. It includes scale clipping, scute cutting, and shell notching.

Scute: A bony plate or large shield like scale. Although similar in appearance to scales they have different origins and properties. Scutes on crocodiles are the bony ridged plates while in turtles and tortoises the entire upper shell is a structure of fused scutes.

Sterilising solution: A solution that sterilises equipment in a single step (e.g. Alconox®).

Ventral: Situated on or toward the lower abdominal plane of the body, or belly.

Verticils: A circular arrangement of parts about an axis (e.g. the projections on the tail of a crocodile).

4 Approved Methods

General advice on suitable techniques for the permanent marking of reptiles by scale marking is contained below, however, training and supervision from experienced personnel in animal handling is required before a person may be considered competent.

There are different techniques for permanent marking by scale marking depending whether the animal to be marked is a snake, large lizard, a crocodile or a turtle (marine or freshwater). Methods for each are described below.

4.1 Scale clipping

Involves the clipping or cutting of ventral and adjoining lateral scales in order to create a unique notching pattern based on a numbering system. This technique is suitable for snakes and potentially for some larger lizards (e.g. blue tongues).

4.2 Scute cutting

Involves cutting of the double caudal and the single caudal verticils (i.e. the uppermost lateral scales along the tail that have crest) in order to create a unique identifier based on a numbering system.

This technique is suitable for estuarine or saltwater crocodiles (*Crocodylus porosus*) and Australian freshwater crocodiles (*C. johnstoni*).

4.3 Shell notching

Involves notching part of the marginal scutes of the carapace or plastron in order to create a unique notching pattern based on a numbering system.

This technique is only suitable for marine and freshwater turtles where the carapace consists of bony plates covered by epidermal horny scutes. (i.e. not suitable for turtles such as the pig-nosed turtle (*Carettochelys insculpta*)) (Georges *et al.*, 1993).

Permanence of shell notching is relative and should be assessed against the monitoring outcomes of a project. In some species, such as the oblong turtle (*Chelodina colliei*), notches tend to grow out after about 12 months, and therefore it is not a useful marking method for studies >12 months. Microchips are a better marking alternative for longer term studies on such species (see the Department SOP for *Permanent Marking of Vertebrates using Microchips*). In comparison, notches on adult western swamp tortoises (*Pseudemydura umbrina*) can remain visible for 10-25+ years. In general, hatchlings (5-6g) should not be notched, as the notches grow out in weeks. Juveniles can be notched but will need to be re-notched with growth. Photo identification can be used in addition to shell notches to help identify individuals.

5 Procedure Outline

5.1 Cleaning and sterilising

All equipment used to cut, file, or incise should be cleaned and sterilised between each animal and prior to returning the equipment for storage. Use of disposable single-use scalpel blades is also an option.

Flaming is the most common method for cleaning and disinfecting equipment but in fire risk areas it may not be possible or appropriate. Using 70% isopropyl alcohol medical swabs is a suitable alternative.

5.1.1 Flaming

(a) Dip the equipment to be used for cutting or filing into 70% ethanol and clean with a swab to remove dirt and any leftover tissue etc.

Note: Ethanol is a highly flammable substance. Care should be taken to not get ethanol on anything other than the equipment needing to be flamed. Ensure a clear workspace and that the ethanol container is in a stable position and unlikely to be knocked over.

Clean up any spillages immediately, including any ethanol on hands and clothing, and if required wait until the spilt ethanol has evaporated before continuing with the procedure.

- (c) Dip the equipment to be used for cutting or filing in ethanol and flame the cutting/filing part with a lighter or portable flame torch. *Note: the flame from ethanol is not visible in sunlight.*
- (d) Allow the equipment to cool before using it on an animal.
- (e) DO NOT allow contact with anything else before the next animal is sampled.

5.1.2 Cleaning and sterilising solutions

- (a) For single step disinfection, equipment can be soaked in a disinfectant solution (e.g. 10% bleach or other commercial disinfectant such as F10SC at an appropriate dilution) for 10 minutes followed by a rinse with deionised water.
- (b) For DNA tissue collection it is necessary to disinfect equipment between individuals to prevent cross contamination. It is also important to rinse equipment in water (preferably distilled water) after disinfecting to remove solutions that may destroy the DNA sample. Alternatively, use a new piece of equipment (e.g. disposable scalpel blade) for each individual and disinfect all equipment at the end of the sampling session. (Refer to the Department SOP for *Tissue Sample Collection and Storage for Mammals*).

5.2 Animal handling

If an animal is seriously injured during handling or marking, refer to the flowchart in the Department SOP *Humane Killing of Animals under Field Conditions* to make the decision on whether or not to euthanase or seek veterinary care.

5.3 Scale clipping (snakes and lizards)

5.3.1 Materials required

The following equipment is needed to undertake scale clipping (sterilised prior and between each marking, see Section 5.1):

- A sharp-pointed pair of good dissecting scissors (for large snakes/lizards) or micro-surgical scissors (for small snakes/lizards) or scalpel
- Fine tipped tweezers or jewellery forceps
- A vial of 70% ethanol and cigarette lighter or portable blow torch for flaming or sterilising solution (see Section 5.1)
- 240V soldering iron or medical cautery unit.

5.3.2 Task

- (a) Restrain the snake/lizard, with the help of a second person, making sure that the head is immobile. (Refer to the Department SOP for *Hand Restraint of Wildlife* for different restraining methods.)
- (b) The scales need to be cleaned prior to cutting with an alcohol swab, as dirt/bacteria can be pushed into open wounds as the knife moves through the tissue.
- (c) If the tissue is being collected for DNA purposes, it is important that the handler does not touch the area where the sample will be taken from to avoid cross contamination of DNA.
- (d) At half the width of the ventral scale (i.e. the middle), insert the tip of the scissors under the posterior edge of the scale to be clipped, push it forward beneath the width of

the entire scale, and cut. Make another such incision, either on the left or the right of the first excision. Insert the scissors under the entire section and cut. If using a scalpel, insert the blade under the scale and, holding the blade parallel to the body, undertake a single clean cut.

(e) In addition to the ventral scale, three to four adjoining lateral scales can be clipped. To remove ventral scales insert the blade under the scale and, holding the blade parallel to the body, use a gentle sawing motion to undertake a single clean cut. The lateral scales should be clipped following the natural scale alignment down the side of the snake (see Figure 1). Ventral scale clipping distinguishes marking from natural variations on the lateral scales. Individual identification can be achieved following the basic marking plan described below (see Figure 2). Ventral scales are counted anteriorly from the anal scale. The first complete scale directly above the vent counts as 0, and is never cut. Scales can be cut consistently on either right or left side, depending on the marker’s preference (i.e. right or left handed). Up to four such marks may be used to number animals into the 000s.

(f) Cutting to the white connective tissue under the scale is sufficient if adjacent scales are also taken out. Another way to assure good visibility of marking is to cauterize the wound using the tip of a soldering iron or medical cautery unit. The wounds will heal over and leave a small black scar which will be visible for >10 years.

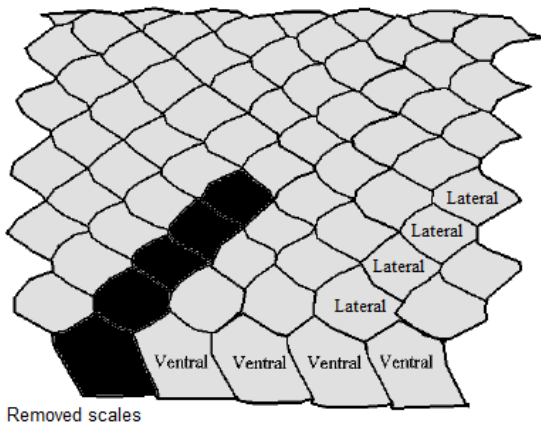


Figure 1 The pattern of ventral and adjoining lateral scales to be removed.

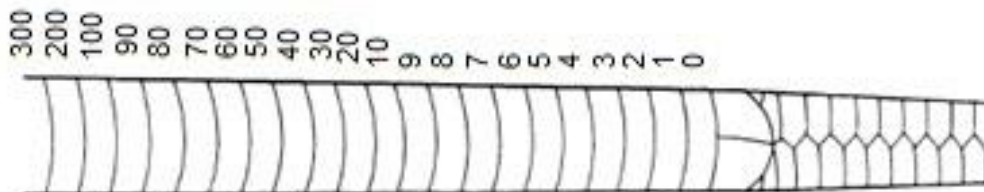


Figure 2 The ventral scale clipping system for marking snakes. The enumeration of ventrals proceeds anteriorly from anal scute. Image: adapted from Brown & Parker (1976).

(g) Refer to the Department SOP for *Tissue Sample Collection and Storage for Mammals* for details on storing the clipped scales for genetic analyses.

(h) If the animal is bleeding apply pressure with a dry gauze swab until the bleeding stops or cauterize the wound to seal. Apply a topical antiseptic spray to the area that has been clipped to prevent infection.

(i) Re-secure the animal in the handling bag/bucket and allow it to recover before releasing.

5.4 Scute cutting (crocodiles)

5.4.1 Materials required

The following equipment is needed to undertake scute cutting:

- Scalpel. Advantages of using scalpels include: scalpels come individually wrapped and so are always sterile, of single use application and able to be disposed of in a 'sharps' container.
- Fine tipped tweezers or jewellery forceps (optional)
- A vial of 70% ethanol and cigarette lighter or portable blow torch for flaming or sterilising solution (see Section 5.1); unless using disposable single-use scalpel blades.

5.4.2 Task

(a) Restrain the crocodile:

- Securely tie the jaws to prevent injury to handlers and to the animal.
- Cover the eyes to reduce visual stimulation by taping a wet sack (hessian bag)/cotton wool pad/moistened crepe to the head
- Depending on the size of the crocodile and handling time, restrain the legs by tying them alongside the body and off the ground in the natural swimming position (feet to the rear) using flat webbing (cords and ropes can restrict circulation and cut the skin easily)

Capture, restraint and marking of a crocodile should never be attempted alone and should be conducted by people with appropriate training and experience. Several people are often required, depending on the size of the animal.

(b) Dirt/bacteria can be pushed into open wounds as the scalpel moves through the tissue, so the scutes need to be cleaned with an alcohol swab prior to cutting

(c) If the tissue is being collected for DNA purposes, it is important that the handler does not touch the area where the sample will be taken from to avoid cross contamination of DNA.

(d) The scutes to be cut are the double caudal verticils and single caudal verticils (i.e. the uppermost lateral scutes with a crest along the top of the tail). To permanently mark an individual, it is necessary to completely remove the distal portion of the scute by making the cut near the base of the scute with a sharp knife or scalpel. This should leave the scute just proud of the surrounding skin surface. Only white connective tissue should be visible while cutting the scute but the wound will bleed. If red tissue is visible while cutting the scute, the incision has been made too deep.

Note: These scutes can re-grow and obliterate any marks if not cut properly, especially in small animals.

(e) Refer to the Department SOP for *Tissue Sample Collection and Storage for Mammals* for details on storing the scute for genetic identification.

(f) With an antiseptic spray apply topical antiseptic to the area that has been cut to prevent infection. If the animal is bleeding apply pressure with a dry gauze swab or tissue until the bleeding stops.

(h) Figure 3 shows the scute pattern numbering system. The numbering system can be applied to both saltwater crocodiles (*C. porosus*) and freshwater crocodiles (*C. johnstoni*).

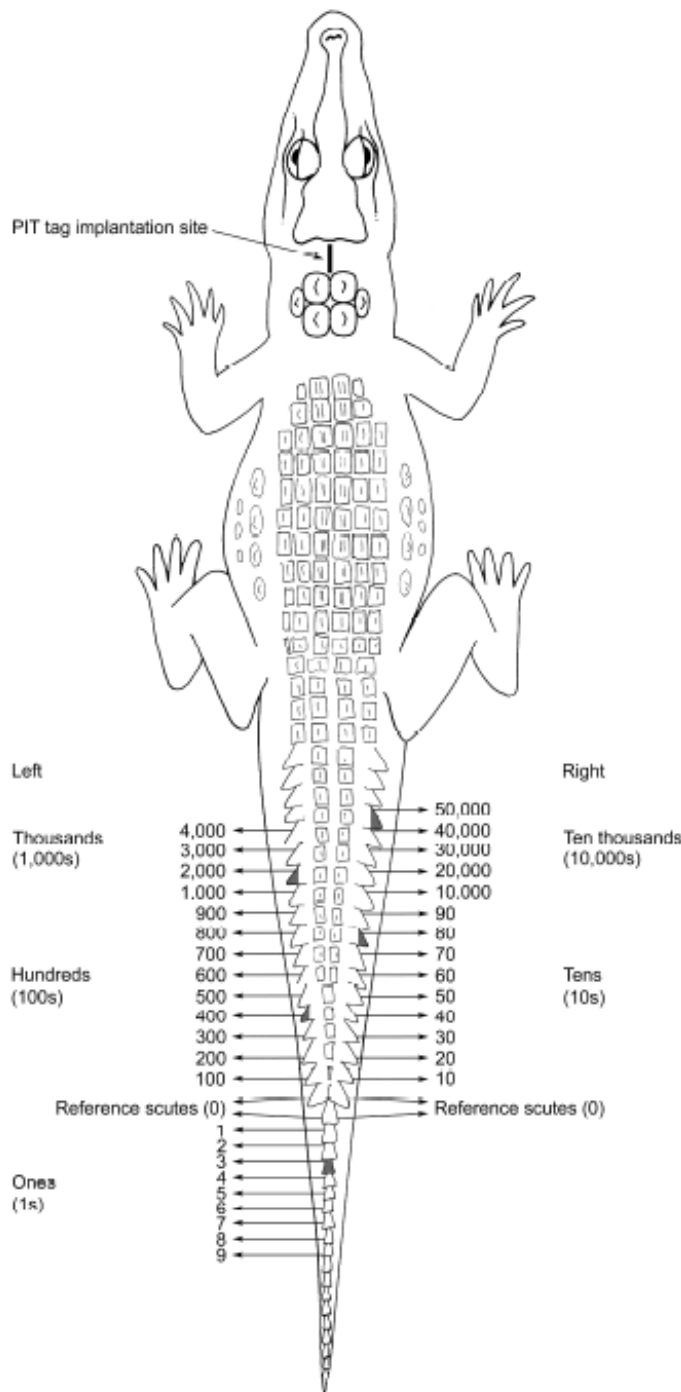


Figure 3 An example of a scute cutting numbering system for marking crocodiles.

- (i) There are several scute cut combinations which can be used to get each number, the best approach is to cut the least number of scutes.
- (j) In the case of marked crocodiles, release the animal as close as possible to the site of capture. If the animal has been processed on a river bank, release it close to the water's edge. Most processing should occur during the evening, early morning or late afternoon. Be mindful of extremes of temperature during the middle of the day and use a shady spot for processing and release where possible. Do not allow the animal to overheat.

5.5 Shell notching (turtles and tortoises)

5.5.1 Materials required

The following equipment is needed to undertake shell notching:

- A small saw or scalpel
- A file
- A bronze wire brush, for cleaning the files
- Fine tipped tweezers or jewelry forceps
- A vial of 70% ethanol and cigarette lighter or portable blow torch for flaming or sterilising solution (see Section 5.1).

5.5.2 Taking a notch

(a) Turtles should always be held immobile at the bridge between the carapace and the plastron, or held by tail end, palm on plastron and thumb on carapace. Turtles have very sharp and powerful jaws, so keep hands and fingers clear of the head region.

(b) The marginal scales may need to be scrubbed, cleaned and swabbed with alcohol prior to notching, as dirt, bacteria and algae can be pushed into open wounds as the saw or file moves through the tissue.

(c) Using the saw or scalpel, cut a triangular notch in the centre of the scute of the marginal scales. Markings should be predetermined to encode the unique number for individual identification. The depth of the notch will depend on the size of the turtle and how long the marking needs to remain visible for, but generally not deeper than 3mm. Retain the tissue being collected for DNA purposes - it is important that the handler does not touch the area to be sampled to avoid cross-contamination. File the newly created notches with a round file to obtain a semi-circular indent (see Figure 4).



Figure 4 Round filing notches on the marginal scutes of a western swamp tortoise. Photo: G. Kuchling.

(d) Figure 5 shows a marking system used to identify individuals of western swamp tortoises (*P. umbrina*) (Burbidge, 1976). Different marking systems can be found in Georges *et al.* (1937) or Cagle (1939). The animal handler administering the shell notching technique must be experienced in this method. Incorrect application of this technique (e.g. cutting too deep into the shell surface) can result in harm to the animal, especially in the bridge area and with smaller turtles.

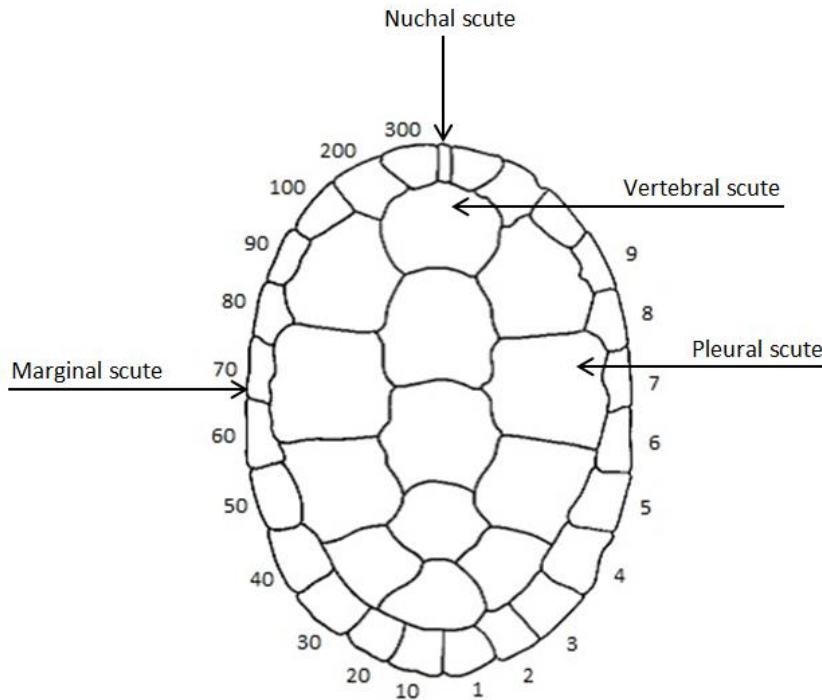


Figure 5 An example of a shell notching numbering system for marking turtles. Image: adapted from Burbidge, 1967

(b) Apply antiseptic spray to the area that has been notched to prevent infection. Once you hit live tissue, there will be a change in tissue coloration (white to pink) or flecks of blood. Filing usually smothers and closes blood vessels. Cutting with a scalpel or saw causes more bleeding than filing and should therefore always be followed up with filing. If filing does not stop the bleeding, apply pressure with a dry gauze swab until the bleeding stops and rinse the notch with a topical antiseptic.

(c) Re-secure the animal in a dry area and preferably allow the wound to completely dry before releasing; ideally overnight.

5.5.3 Cleaning the file

Cleaning files can be difficult as the bone dust impregnates the file teeth pattern. It is therefore recommended to wash off debris, then use a wire brush (preferably bronze), in combination to the flaming technique to maximise sterility of the file.

Refer to the Department SOP for *Tissue Sample Collection and Storage for Mammals* for details on storing the notch for genetic identification.

5.6 Labelling and recording data

It is important to keep a record of the numbering system being used.

Labelling is of the utmost importance when taking biological samples for genetic analysis. It is important to ensure that handwriting is legible (O'Meally and Livingston, 2002). All individual samples MUST be labelled with the following as a bare minimum:

- Date
- Species/possible species
- Location (GPS reading is preferable)

- Individual ID (to be linked to additional metadata)
- Collector's initials

Other information (metadata) can be added to the label or provided in a spreadsheet referencing the individual ID:

- Collector's name
- Sex of animal
- Other observations (age, weight, size, breeding status, etc.)
- Translocation source/destination

Do not write on greasy, dirty or wet tubes. Permanent markers can rub off when they come into contact with ethanol; therefore, as a precaution it is advised to insert a waterproof label written with pencil inside the tube. If possible, labels should be prepared before collecting the sample. Also inform the laboratory what liquid was used to store the sample (e.g. 100% ethanol, DMSO).

6 Level of Impact

Scale clipping, scute cutting and shell notching are considered invasive procedures as it involves physical restraint and removal of tissue.

Potential animal welfare impacts when scale notching reptiles include:

- Distress (caused by handling, discomfort),
- Trauma (possible injury to animal during restraint e.g. scratching itself, biting itself),
- Bleeding from notching (rare),
- Infection at site of marking.

It should be noted that whilst these impacts are specifically associated with the procedure of taking tissue samples for genetic identification, an animal may also experience other impacts from associated procedures such as trapping and capture.

7 Ethical Considerations

To reduce the level of impact of scale marking on the welfare of animals there are a number of ethical considerations that should be addressed throughout projects involving these procedures. Department projects involving scale marking to permanently mark reptiles will require approval from the Department's Animal Ethics Committee.

7.1 Animal Handling

To minimise stress to the reptiles, they should only be handled for as long as required to mark them and to collect any necessary measurements. This can usually be completed in a few minutes. Improper restraint, especially when dealing with a stressed and frightened animal can lead to physiological disturbances, such as hyperthermia, stress, shock, capture myopathy and lactic acidosis (for large crocodiles). In the case of a stressed or frightened animal, the animal should be rested until calm, or released if handling is likely to cause harm to the animal.

7.2 Pain and infection

Although hygiene is difficult in the field, cleanliness of all surgical and notching techniques is essential to minimise the potential for infection and to provide reliable DNA samples. All equipment should be kept sharp and clean to minimise tearing, bruising, infection and transmission of disease. Also refer to the Department SOP for *Managing Disease Risk in Wildlife Management*.

In general, within a few weeks the horny layer grows into the notch and seals it. However any recaptured animals must have their notches inspected closely to assess whether any infection has occurred. Appropriate anaesthetic, anti-septic and measures of pain control must be used when and if required.

7.3 Bleeding

Although scale marking does not result in excessive bleeding, should it occur, it needs to be controlled prior to the animal being released. Refer to the Department SOP for *First Aid for Animals*.

7.4 Injury and unexpected deaths

If injury, unexpected deaths or euthanasia occur then it is essential to consider the possible causes and take action to prevent further deaths. For projects approved by the Department's Animal Ethics Committee, adverse events such as injury, unexpected deaths or euthanasia must be reported in writing to the AEC Executive Officer on return to the office (as per 2.2.28 of The Code) by completing an *Adverse Events Form*. Guidance on field euthanasia procedures is described in the Department SOP for *Humane Killing of Animals under Field Conditions*. Where disease may be suspected, refer to the Department SOP for *Managing Disease Risk in Wildlife Management* for further guidance.

8 Competencies and Approvals

Department personnel, and other external parties covered by the Department's Animal Ethics Committee, undertaking projects that involve permanent marking of reptiles by scale marking require approval from the committee and will need to satisfy the competency requirements detailed in Table 1. This is to ensure that personnel involved have the necessary knowledge and experience to minimise the potential impacts of scale marking on the welfare of the animals. Other groups, organisations or individuals using this SOP to guide their survey and monitoring activities are encouraged to also meet these competency requirements as well as their basic animal welfare legislative obligations.

It should be noted that details such as intensity of the study being undertaken will determine the level of competency required and Table 1 provides advice for basic monitoring only.

Table 1 Competency requirements for Animal Handlers of projects involving permanent marking of reptiles by scale notching.

| Competency category | Competency requirement | Competency assessment |
|--|---|---|
| Wildlife licences | Licence to take fauna for scientific purposes (Reg 17) OR Licence to take fauna for educational or public purposes (Reg 15) | Provide licence number |
| Formal training <i>Note: Suitable levels of skills/experience can substitute for formal training requirements</i> | Department Fauna Management Course or equivalent training | Provide course year |
| General skills/experience | Relevant knowledge of species biology and ecology | Personnel should be able to correctly identify the likely species to be encountered at the site/s being studied. This knowledge may be gained by sufficient field experience and/or consultation of field guides and other literature. Estimated total time in field: Min1 year involved in similar projects. |
| Animal handling and processing skills/experience | Experience in handling terrestrial herpetofauna | Personnel should be confident at handling reptiles in the application of permanent marking techniques likely to be used in fauna monitoring and surveying. At least some of this experience must be obtained under the supervision of more experienced personnel. Estimated total time in field: Min 2-5 years involved in similar projects. |

9 Occupational Health and Safety

Always carry a first aid kit in your vehicle and be aware of your own safety and the safety of others as well as the animals when handling.

A job safety analysis is recommended prior to undertaking tissue sampling. This safety analysis should include the following considerations.

9.1 Animal bites and scratches

This technique applied to species that can cause serious harm and injury to handlers. Experience with all likely species to be encountered is essential. All inflicted injuries (even

superficial ones) should be appropriately treated as soon as possible to ameliorate possible allergic reaction, prevent infection and promote healing.

To improve safety, field personnel should be aware of the treatment for snakebite and carry appropriate pressure bandages. Personnel should also have up-to-date tetanus vaccinations. Department personnel must not capture bats unless fully vaccinated against Australian Bat Lyssavirus.

If Department personnel or volunteers are injured, please refer to the Department's Health and Safety Section's 'Report a Hazard, near-miss or incident' intranet page, which can be found at http://intranet/csd/People_Services/rm/Pages/ReportingHazards,Near-MissesandIncidents.aspxZoonoses.

9.2 Zoonoses

There are a number of diseases carried by animals that can be transmitted to humans (i.e. zoonoses such as Toxoplasmosis, Leptospirosis, Salmonella). All personnel must take precautions to minimise the risk of disease transmission to protect themselves, their families and wildlife populations.

Advice on minimising disease risk is contained in the Department SOP for *Managing Disease Risk in Wildlife Management*

9.3 Allergies

Some personnel may develop allergies when they come in contact with animal materials such as hair and dander. Personnel known to develop allergies should wear gloves when handling animals and long sleeved pants/shirt.

People with severe allergies associated with animals, with immune deficiency diseases or on immunosuppressant therapy should not engage in the handling of wildlife.

9.4 Chemicals

Personnel should be aware of the dangers of the chemicals they use in the field. Refer to the *Material Safety Data Sheets* (MSDS) relevant to the chemical(s) they may be using.

9.5 Fire risk

Personnel intending to clean and sterilise equipment by flaming should be aware of the associated fire risk and take appropriate action to reduce this risk.

9.6 Sharp equipment

The sharp points of scissors and blade of the scalpel are hazardous and can easily puncture or cut a finger. Care must be taken at all times to avoid such injuries and personnel must be diligent in the correct disposal of sharps.

10 Further Reading

The following SOPs have been mentioned in this advice and it is recommended that they are consulted when proposing to undertake permanent marking of vertebrates by scale marking:

- Department SOP *Tissue Sample Collection and Storage for Mammals*
- Department SOP *Hand Restraint of Wildlife*
- Department SOP *First Aid for Animals*
- Department SOP *Permanent Marking of Vertebrates using Microchips*
- Department SOP *Permanent Marking of Mammals using Ear Notching*
- Department SOP *Temporary Marking of Mammals, Reptiles and Birds*
- Department SOP *Humane Killing of Animals under Field Conditions*
- Department SOP *Managing Disease Risk in Wildlife Management*

11 References

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