Standard Operating Procedure

SC25-07 PERMANENT MARKING OF REPTILES BY SCALE MARKING

Animal welfare is the responsibility of all personnel involved in the care and use of animals for scientific purposes.

Personnel involved in an Animal Ethics Committee approved project should read and understand their obligations under the *Australian code for the care and use of animals for scientific purposes*.

Version 1.2

June 2025

GOVERNMENT OF WESTERN AUSTRALIA

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Revision History Log

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1.0	21/07/2017	Minor revisions	G. Anderson and M. Page	M. Page	August 2017
1.1	08/06/2022	Revision of content & clarification of procedures	D. Pearson, A. Robey and L. Povh	M. Dziminski	June 2023
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Approved by the DBCA Animal Ethics Committee:

Dr Jacqui Richards

Chairperson, Animal Ethics Committee

Department of Biodiversity, Conservation and Attractions

Contents

1 Acknowledgements

This standard operating procedure was originally compiled by Myrto Robert, and Rebecca Bloomfield based on information provided by Peter Mawson, Jacqueline Styants, David Pearson, Winston Kay and Gerald Kuchling.

2 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 method, consider the purpose and length of the study, data required and the biology of the animal. Temporary marking methods are also available utilising non-toxic and xylene free marker pens, paints, dyes, nail polish, tapes and threads. Where sufficient to achieve the desired purpose, temporary marking methods should be utilised over permanent methods. For information on temporary marking see department SOP *Temporary Marking of Mammals, Reptiles and Birds.*

This Standard Operating Procedure (SOP) provides advice on permanently marking snakes, crocodiles and some large lizards using scale clipping and scute cutting only. Microchipping may also be an option; further detail on this method can be found in the department SOP *Permanent Marking of Vertebrates Using Passive Integrated Transponders*.

3 Scope

This SOP has been written specifically for scientific and education purposes, and approved by the Department of Biodiversity, Conservation and Attractions' (DBCA) Animal Ethics Committee (AEC). 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 and scute cutting) as a permanent marking method for reptiles undertaken across Western Australia by DBCA (hereafter department) personnel. It may also be used to guide fauna related activities undertaken by Natural Resource Management groups, consultants, researchers and any other individuals or organisations. All department personnel involved in scale marking of reptiles should be familiar with the content of this document.

This SOP complements the *Australian code of practice for the care and use of animals for scientific purposes* (The Code). The Code provides the ethical framework and governing principles to guide decisions and actions of all those involved in the care and use of animals for scientific purposes and should be referred to for all AEC approved projects. A copy of the code may be viewed by visiting the National Health and Medical Research Council website (https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes).

4 Animal Welfare Considerations

To reduce the level of impact of scale marking on the welfare of animals, personnel must consider, address and plan for the range of welfare impacts that may be encountered. Strategies to reduce impacts should be identified during the planning stage to ensure that they can be readily implemented during scale marking, and to ensure that contingencies for managing welfare issues have been identified. All personnel should be aware of the range of issues that they may encounter, the options that are available for reducing impact and improving animal welfare, and the process for managing adverse events.

Departmental projects involving permanent marking of reptiles using scale marking will require approval from the department's AEC. Key animal welfare considerations that should be considered when permanently marking reptiles using scale marking are listed below and are highlighted throughout the document.

4.1 Injury and unexpected deaths

If adverse events including injury, unexpected deaths or unplanned requirement for euthanasia occur then it is essential to consider the possible causes and take action to prevent further issues. Adhering to the guidance in this SOP will assist in minimising the likelihood of adverse events. For projects approved by the department's AEC, adverse events must be reported in writing to the AEC Executive Officer as soon as possible after the event by completing an *Adverse Event Form*. Guidance on first aid and field euthanasia procedures are described in the department SOPs *First Aid for Animals* and *Euthanasia of Animals Under Field Conditions*. Where infectious disease is suspected, refer to the Department SOP for *Managing Disease Risk and Biosecurity in Wildlife Management* for further guidance.

4.2 Level of impact

Scale clipping and scute cutting are considered invasive procedures as they involve physical restraint and removal of live tissue.

Potential animal welfare impacts when scale marking reptiles include:

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

Project planning must involve the identification and mitigation of all potential welfare risks to minimise their impacts as much as possible. Note 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. Investigators must be aware that the effects of a series of stressors, such as trapping, handling, transportation, sedation, anaesthesia and marking can be cumulative.

5 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 on whether the animal to be marked is a snake, large lizard, or a crocodile. Methods for each are described below.

ANIMAL WELFARE: 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 highly stressed animal can lead to physiological disturbances, such as hyperthermia, shock, capture myopathy and lactic acidosis (for large crocodiles). In the case of an acutely stressed individual, the animal should be rested until calm or released if handling is likely to cause harm to the animal.

5.1 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 (see Section 6.2). This technique is suitable for snakes and potentially for some larger lizards (e.g., blue tongues).

5.2 Scute cutting (Crocodiles only)

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 (see Section 6.3).

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

6 Procedure Outline

6.1 Cleaning and disinfection

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

(b) Flaming is the most common method for disinfecting equipment, but in fire risk areas dipping the equipment in disinfectant solutions is advised (refer to Section 6.1.2).

(c) If you are collecting tissue for DNA, it is important to rinse equipment in water (preferably distilled water) after cleaning and disinfecting to remove solutions that may destroy the DNA sample (See department SOP *Tissue Sample Collection and Storage for Genetic Purposes – Vertebrates and Cephalopods).*

6.1.1 Flaming

(a) Wipe the equipment to be used for cutting or filing with an ethanol swab to remove any dirt or tissue.

(b) Dip the equipment into 70% ethanol. *Note: ethanol is a highly flammable substance, and care should be taken to not get ethanol on anything other than the equipment to be flamed.*

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

(d) Remove equipment from the ethanol and flame the cutting part with a lighter or portable flame torch. *Note: the flame from ethanol is not visible in sunlight*. Allow the equipment to cool before using it on an animal.

(e) Ensure sterility of equipment to avoid contamination especially in the case of DNA analysis. DO NOT allow contact with any other biological material, including human fingers, before the animal is marked.

6.1.2 Disinfectant solutions

(a) For single step disinfection, the equipment can be dipped in a disinfectant solution (e.g., 10% bleach or other commercial disinfectant, such as F10 SC (1:100 concentration)) for 10 minutes followed by a rinse with distilled water (See SOP *Tissue Sample Collection and Storage for Genetic Purposes – Vertebrates and Cephalopods* for methods relating to tissue collection for genetic purposes).

6.2 Scale clipping (snakes and lizards)

Materials required

The following equipment is needed to undertake scale clipping (disinfected prior to and between each marking, see Section 6.1):

- 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 disinfecting solution (Section 6.1)
- 240V soldering iron or medical cautery unit.

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 (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 (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. Be aware that scale anomalies (such as divided scales rather than entire scales) may occur just anterior to the vent and can confuse scale counts. A rule is needed to avoid such confusion, such as scale anomalies are noted, and the count commences above the scale anomalies where the ventrals resume normal configuration.

(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.

(g) Refer to the department SOP for *Tissue Sample Collection and Storage for Genetic Purposes – Vertebrates and Cephalopods* for details on storing the scale for genetic identification.

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

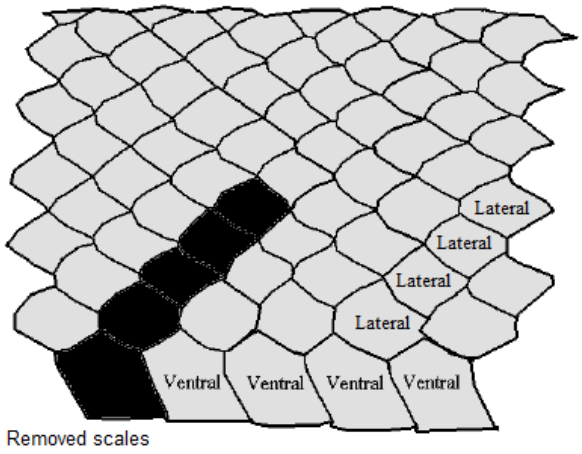

Figure 1 The pattern of ventral and adjoining lateral scales to be removed.

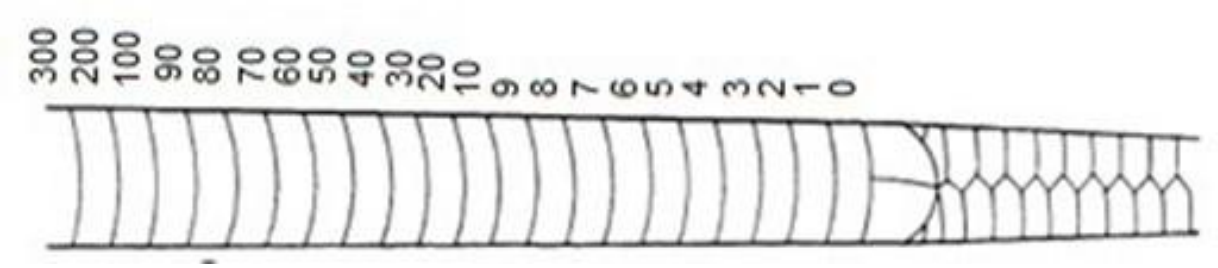

Figure 2 The ventral scale clipping system for marking snakes. The enumeration of ventrals proceeds anteriorly from anal scute. Image: adapted from Brown & Parker (1976).

(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.

6.3 Scute cutting (crocodiles)

Materials required

The following equipment is needed to undertake scute cutting:

- Scalpels, which are individually wrapped and are always sterile, for 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 disinfecting solution (Section 6.1); unless using disposable single-use scalpel blades.
- Sharps disposal container.

Task

(a) 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) Securely tie the jaws to prevent injury to handlers and to the animal.

(c) Cover the eyes to reduce visual stimulation by taping a wet sack (hessian bag)/cotton wool pad/moistened crepe to the head.

(d) 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).

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

(f) 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.

(g) 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.

(h) Refer to the department SOP for *Tissue Sample Collection and Storage for Genetic Purposes – Vertebrates and Cephalopods* for details on storing the scute for genetic identification.

(i) 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.

(j) The numbering system can be applied to both saltwater and freshwater crocodiles (Figure 3).

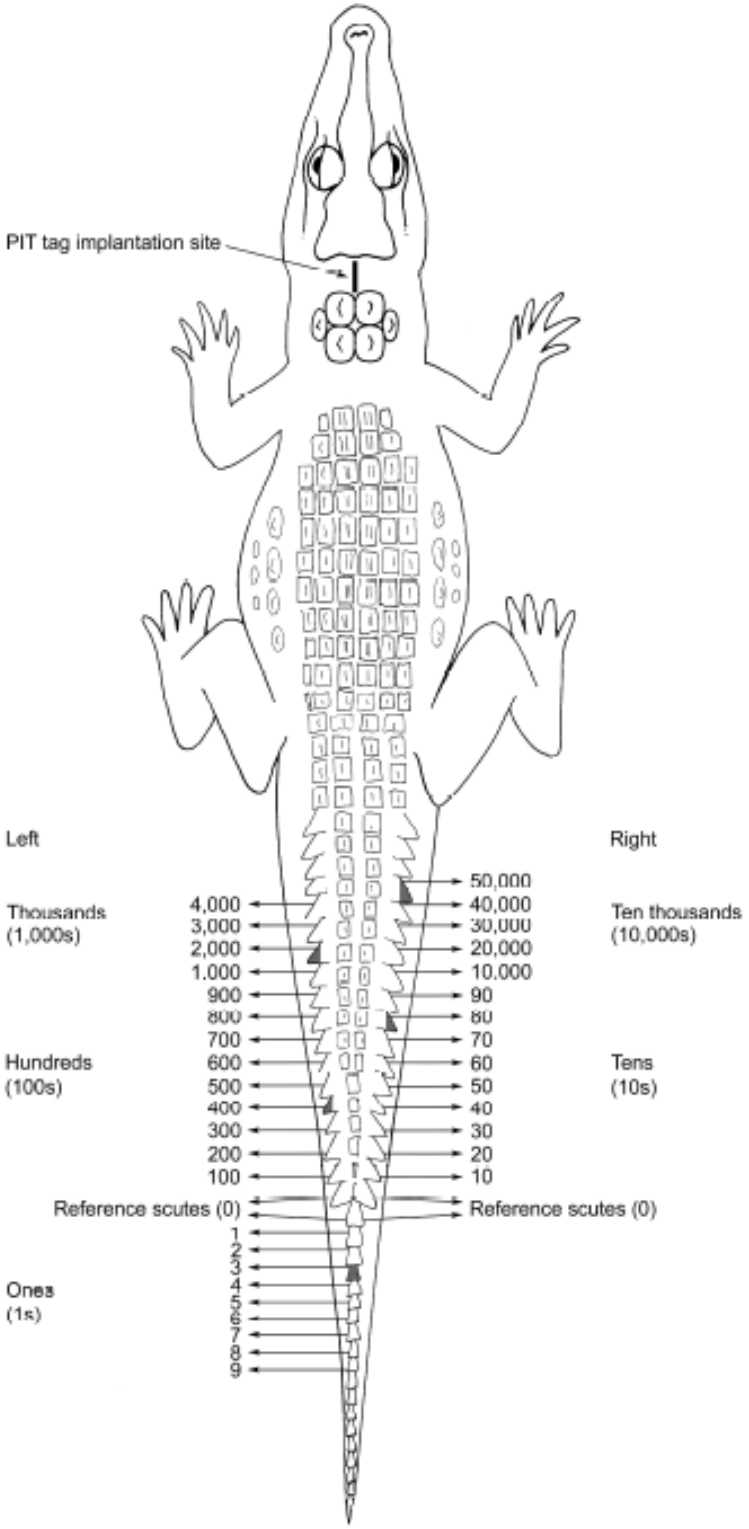

Figure 3 An example of a scute cutting numbering system for marking crocodiles.

(k) 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.

(l) 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 riverbank, 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.

ANIMAL WELFARE: Do not allow the animal to overheat.

7 Labelling and recording data

It is important to keep a record of the numbering system 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, 2011). 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 in 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).

8 Competencies

A person who is competent has the knowledge, skills, and experiences that allow them to capture and handle animals successfully, and appropriately manage adverse events as required. Department personnel, and other external parties covered by the department's AEC, undertaking projects that involve permanent marking of reptiles by scale marking require approval from the committee and will need to satisfy the competency requirements (Table 1). Other groups, organisations or individuals using this SOP to guide their fauna monitoring activities are encouraged to also meet these competency requirements as well as their animal welfare legislative obligations.

It should be noted that sampling design details such as intensity and scope of the study undertaken will determine the level of competency required and Table 1 provides advice for

standard 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
Knowledge	Broad understanding of the framework governing the use of animals in research and environmental studies in Western Australia	Training (e.g., DBCA Fauna Management Course or equivalent training). In applications, provide details on the course provider, course name and year.
	Understanding species biology and ecology	Personnel should be able to correctly identify the likely species to be encountered at the site(s) being studied and understand the species' biology and ecology. This knowledge may be gained through sufficient field experience and consultation of field guides and other literature.
	Understanding environmental conditions	Personnel should be aware of the environmental and seasonal conditions that may be expected on the project and understand how these may impact animal welfare.
Animal handling and tagging skills/experience required	Experience handling herpetofauna	Personnel should be experienced at hand restraint of species being marked. This experience is best obtained under supervision of more experienced personnel. In applications, provide details on the longevity, frequency & recency of experience.
	Experience in permanent marking using scale marking	Personnel should be familiar with the animal welfare principles of permanent marking using scale marking. Personnel should be familiar with how to operate marking equipment. This experience is best obtained under supervision of more experienced personnel.
	Experience managing disease risk in wildlife management	Personnel should be familiar with hygiene procedures. This knowledge may be gained through sufficient field experience and consultation of literature.

In conjunction with possessing the required understanding and knowledge of the permanent marking technique and animal welfare requirements, a guide to the experience and skill

requirements for an animal handler to be considered competent to permanently mark reptiles using scale marking is as follows: (noting that some personnel with experience may still require initial supervision in unfamiliar locations or with species that they have not encountered previously):

- Recency of time in field: within the past 10 years.
- Minimum 5 individuals of similar species handled.

9 Approvals

In Western Australia any person using animals for scientific purposes must also be covered by a licence issued under the *Animal Welfare Act 2002*, which is administered by the Department of Primary Industries and Regional Development. Projects involving wildlife may require a licence/authorisation under the *Biodiversity Conservation Act 2016* (examples below).

- Fauna taking (scientific or other purposes) licence (Reg 25)
- Fauna taking (biological assessment) licence (Reg 27)
- Fauna taking (relocation) licence (Reg 28)
- Section 40 Ministerial Authorisation to take or disturb threatened species.

Personnel should contact the Department's Wildlife Licensing Section for more information. It is your responsibility to ensure you comply with the requirements of all applicable legislation.

10 Occupational Health and Safety

The following departmental SOPs for wildlife survey and monitoring activities are relevant to occupational health and safety:

- SOP *Managing Disease Risk and Biosecurity in Wildlife Management*
- SOP *Hand Restraint of Wildlife*

Departmental personnel, contractors and volunteers have duties and responsibilities under the *Occupational Safety and Health Act 1984* and Occupational Safety and Health Regulations 1996 to ensure the health and safety of all involved. Fieldwork is to be undertaken in line with the department's corporate guidelines, policies and standard operating procedures, including but not limited to, risk management and job safety analyses. Further information can be found at

https://dpaw.sharepoint.com/Divisions/corporate/people-services/HS/SitePages/SOPs.aspx

If department personnel or volunteers are injured, please refer to the departmental Health, Safety and Wellbeing Section's 'Reporting Hazards, Near-misses and Incidents' intranet page, which can be found at

https://dpaw.sharepoint.com/Divisions/corporate/people-services/HS/SitePages/Reporting-Hazards,-Near-Misses-and-Incidents.aspx

11 Further Reading

The following SOPs have been mentioned in this advice and it is recommended that they are consulted when proposing permanently mark mammals using ear notching:

- Department SOP *Tissue Sample Collection and Storage for Genetic Purposes – Vertebrates and Cephalopods*
- Department SOP *Hand Restraint of Wildlife*
- Department SOP *Permanent Marking of Vertebrates using Passive Integrate Transponders*
- Department SOP *Permanent Marking of Mammals using Ear Notching*
- Department SOP *Temporary Marking of Mammals, Reptiles and Birds*
- Department SOP *Managing Disease Risk and Biosecurity in Wildlife Management*
- Department SOP *First Aid for Animals*
- Department SOP *Euthanasia of Animals Under Field Conditions*

For further advice refer also to:

National Health and Medical Research Council (2013) *Australian code for the care and use of animals for scientific purposes*, 8th edition. Canberra: National Health and Medical Research Council.

12 References

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13 Glossary of Terms

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.

Disinfecting solution: A solution that disinfects 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).