UNIVERSITY OF CANBERRA
AUSTRALIA'S CAPITAL UNIVERSITY

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Animal Ethics Committee

Standard Operating Procedures (SOPs) for the care and use of animals in laboratory studies, teaching and field research

This document has been developed and approved by the University of Canberra's Animal Ethics Committee.

PURPOSE OF THIS DOCUMENT

This document is designed to assist applicants in preparing applications for laboratory studies, teaching or field research projects involving animals that are to be submitted to the University of Canberra's Animal Ethics Committee. In addition, it is intended to assist Committee members when assessing applications. Therefore, this document is intended as an information source and guide for both applicants and Committee members. It is not intended to cover every possible scenario that may come up in a research, monitoring or survey program, nor is it appropriate to attempt to include project-specific details. These must be covered in individual project proposals.

This document is intended to continue to evolve, with additional techniques added from time to time, and other techniques modified in the light of new information and improved methodologies. To add a new technique or request a modification to an existing technique, please contact the Research Ethics & Integrity Unit.

Web:
http://www.canberra.edu.au/research/ucresearch/integrityandethics/animal_ethics

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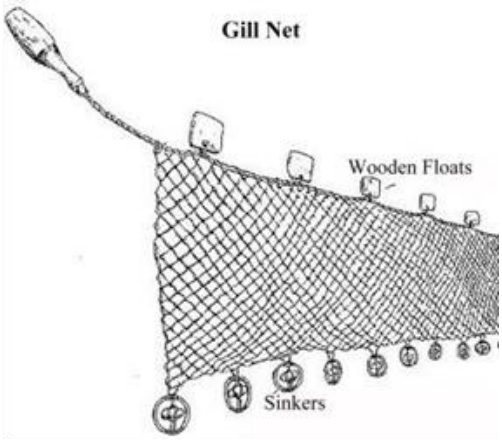
1. FISH

1.1. Capture Technique

1.1.1. Gill nets

Gill nets are constructed from multifilament nylon. These are un-weighted and attached to a float line. The drop of each net is 33-100 meshes deep (depending on the mesh size). Un-weighted, floating nets are used to minimise netting and drowning of platypus and other air breathing animals. Mesh sizes vary between 25 and 200 mm. One end of the gill net is attached to the bank and the other end to an anchor mid-stream.

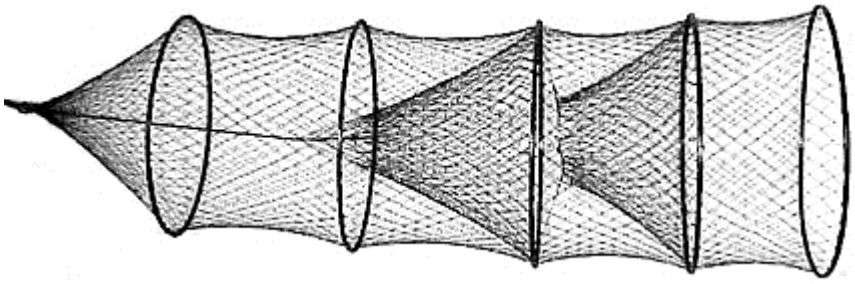
Gill nets are usually set between 15:30 and 16:00 hrs and retrieved between 21:30 and 22:00 hrs, giving a 6-hour soak time. The limited soak time reduces possible mortality of threatened fish species such as Macquarie perch or trout cod, as well as of non-target species such as platypus and Eastern long-necked tortoise. Gill nets must be monitored hourly from dusk to allow any animals to be removed. These operating procedures for gill nets and the manner in which they are set and patrolled are consistent with the technique outlined by Grant & Carrick (1974) to minimise platypus mortality.



1.1.2. Fyke nets

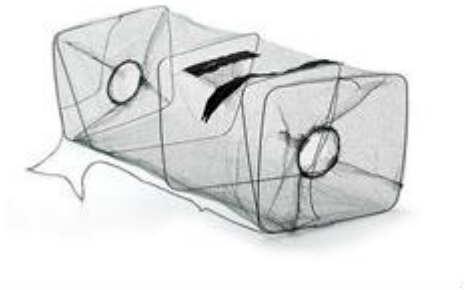
Fyke nets consist of a long "bag" containing 2 or 3 funnels and have a single or multiple wings extending from the mouth of the net. The net and wing are constructed from multifilament nylon mesh with the size correlated to the size of the target fish. The nets are attached to the bank at the cod-end (the upstream end, closed with a draw-string) and then angled downstream with a weight attached to the wing to hold the net securely. Each fyke net has a 150-mm diameter polystyrene float inserted in the cod-end to provide airspace to prevent mortality of non-target animals. Since captured animals are not enmeshed, there is little less stress.

Fyke nets are usually set between 15:30 and 16:30 hrs and retrieved the next day between 07:30 and 09:30 hrs.



1.1.3. Bait traps

A bait trap consists of a small collapsible mesh "box" with a single funnel entrance at each end. The mesh size is < 6mm and the funnel entrances are approximately 50mm in diameter. Bait traps are set at 18:00 hrs, are baited with a chemical light stick (Cyanolume Yellow, 12 hour), and attached to the banks with a short length of cord. Traps are retrieved at approximately 08:00 hrs the next morning. The small size of the mesh and funnels used virtually eliminates capture of non-target animals, and because captured fish are not enmeshed there is minimal stress.



1.1.4. Electrofishing

Electrofishing is used as a non-destructive method to capture fish in most habitat types dependent upon the method (e.g. Backpack for shallow wadeable habitats and boats for deeper water. It is also used for fish collection in circumstances where other techniques are ineffective.

Electro-fishing operations must be conducted in accordance with the Australian Code of Electrofishing Practice (Anon. 1997). Team leaders involved in electro-fishing must have appropriate training and experience in electric circuit and field theory, safety training, and

awareness of injuries to fish, and how to minimize these. Individuals must all have a medical prior to conducting electro-fishing and medicals need to be reviewed every two years.

1.1.4.1. Boat

Boat electro-fishing is carried out with a generator that produces an electric current that is passed through a rectifier unit, which produces a pulsed DC waveform (AC current should not be used without specific ethics approval). An electric field is produced in the water through a single or double electrode on a boom at the front of the boat (Cowx 1990; Cowx & Lamarque 1990). Output settings are dependent on water conductivity a. Settings should be chosen to maximise catch efficiency while minimising the risk of injury to fish.

Boats must be crewed by at least 2 people and observers should be kept to a minimum. At each site, a series of replicates or “shots” are carried out (generally 2 minutes long), with the boat slowly driven along the river, with one operator controlling the boat and electrofisher settings while the second person controls the passage of the electric current to the water and removal of immobilised fish. Stunned fish are dip-netted from the water and placed in an aerated tank of water to recover before measuring and release. All operators should wear polarising glasses to facilitate early sighting of affected fish and allowing such fish to be collected as quickly as possible, thus minimising their exposure to the electric field.

1.1.4.2. Backpack

Backpack electro-fishing is carried out using a portable unit such as the Smith Root LR 20 backpack electrofisher, powered by a 24 volt, 24 amp/hour battery which produces an electric current of pulsed DC waveform. Output options include a range of voltage settings and pulse frequencies and width. Output amperage ranges depending on water the conductivity and output settings used. Settings should be chosen to maximise catch efficiency whilst minimising the risk of injury to fish. At each site, a two-person team fishes a length of stream (usually 20-50 m). All operators should wear polarising glasses to facilitate early sighting of affected fish and allowing such fish to be collected as quickly as possible, thus minimising their exposure to the electric field. Stunned fish should be dip-netted from the water and placed in an aerated tank of water to recover before measuring and release. Backpack electrofishing should usually be carried out in the late afternoon or early morning.

1.1.5. Searching and capture by hand

1.1.5.1. Snorkelling - fish

In clear waters, small fish can be caught using a hand net while diving with a mask and flippers. Snorkelling is also used to observe fish in their environment.

1.1.6. Remote video/camera surveillance

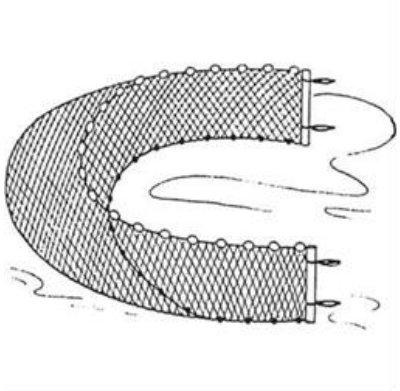
1.1.6.1. Underwater video surveillance - fish

A remote underwater video recording system that may include multiple cameras is used for filming fishes in aquatic ecosystems. Night-time footage can be obtained using infrared light. Cameras are placed in fixed positions either under water, above water or moved through the water and may be baited with raw fish or other bait to attract certain species.

1.1.7. Seine netting

Seine nets are commonly used in marine environment and can be useful in certain freshwater habitats. They are used in areas with hard and reasonably smooth substrates with few snags or litter. Hand pulled seine nets are generally short 10-30 m long and have a drop of up to 3 m with a float line at the top and a lead line at the bottom. (Larger seine nets of over 100 m can be used in particular circumstances but require specific approval) The mesh size is matched to the environment and target species. Small mesh seines for small species and juveniles have mesh sizes from 3-10 mm and large mesh seines for large bodied species have mesh sizes of 10-100 mm. Where possible they should be constructed from knotless mesh.

They are generally used by slowly encircling an area with the net and pulling or hauled the net through the habitat to the shore. Dependent upon the length of the seine net one, two or more people may be required to pull the net and a boat may be required to set the net. Fish and other animals encircled in net are pulled to the shore and place in to tubs of aerated water for processing. Care must be taken in the length of haul and mesh size to minimise the risk of crushing from excessive catch or detritus and reduce the capture and processing time.



1.2. Handling - general processing and release

All handling will be kept to the minimum required to obtain the necessary information according to predetermined objectives. Any methods of physical or chemical restraint and handling need to ensure that no physical injury occurs and minimal stress and handling is placed on the animals. The least amount of restraint and the shortest possible time necessary for the procedure or time need to handle the animal must be used. All persons intending to handle animals must be trained under the supervision of an experienced animal handler and must be able to demonstrate their ability to handle animals safely and responsibly prior to handling the animals.

Live processing involves the identification of species, measurement of total weight and length and a visual inspection for deformities and external parasites. Live fish are always to be handled with wet hands to minimise stress and the removal of protective mucus, and for as short a period as is practical. Fish are then released at the point of capture. Fish needing to be euthanized must be treated in accordance with *1.10 Euthanasia.*

1.3. Handling techniques for sampling and returning animals

The principle aim of handling techniques is to minimize stress experienced by fish as far as possible and to prevent any further damage. The following handling techniques for sampling and returning animals are taken directly from ACP (NHMRC, 1997).

- The time for which the fish is held should be minimal and consistent with the aims of the study.

- Fish must be held in such a way as to minimize stress and/or injury. Knowledge of available information on the normal behaviour of the species and its likely response to captivity is essential and must form the basis for management practices.

- Wherever possible, fish must be sampled whilst still in the water. This is particularly relevant when using any trapping or netting sampling methods.

- Close confinement devices must:

  - allow fish to rest comfortably;
  - minimize the risk of escape or injury;
  - be adequately aerated;
  - maintain constant temperature; and
  - minimize the risk of disease transmission.

- Release should be at the site of capture, unless an alternative site is justified in the project proposal.

- The time of the release should be consistent with the species usual time of movement.

- Individuals must be released safely, particularly if the time of day for release is less than optimal.

- At the time of release all reasonable steps must be taken to protect animals from injury and predation.

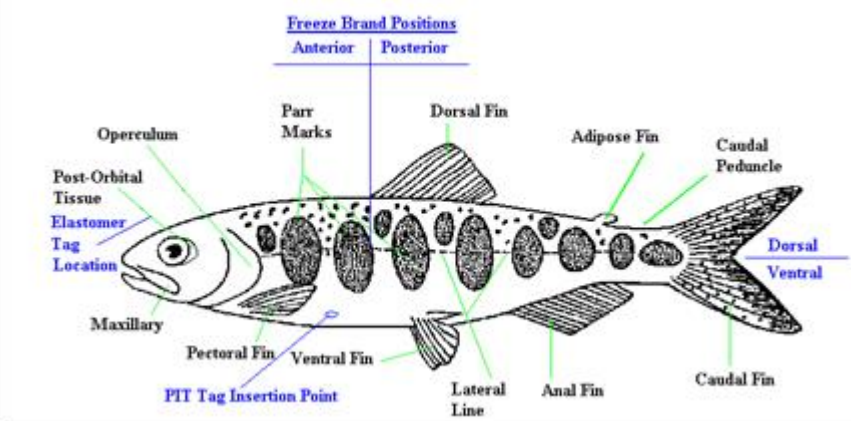
1.4. Marking

Fish marking, using a variety of techniques, provides one of the most important methods of analysing fish movements, abundance, and population dynamics. It is basic to all field studies. Important considerations in choosing a marking technique are suitability to answer the study's objective, likelihood to affect behaviour, physiology or survival of the target species Investigators must consider the nature and duration of restraint, the amount of tissue affected, whether distress is momentary or prolonged, whether the animal, after marking, will be at greater than normal risk or predation , whether the animal's desirability as a mate may be reduced, and whether the risk of infection or abscess formation is minimal. A literature review and careful testing of markers on preserved or captive animals before use on wild animals is recommended.

1.4.1. Important criteria for selecting marking method in any species

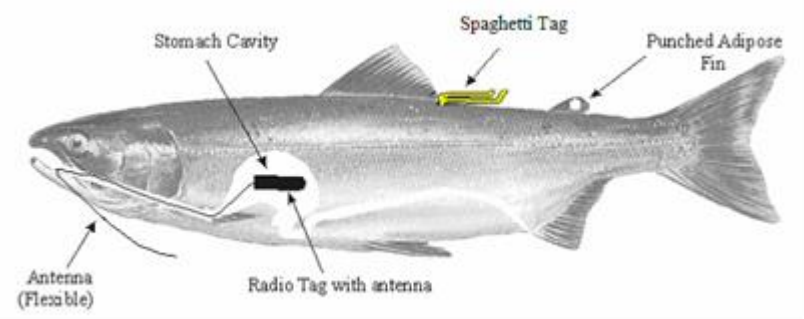
Criteria for acceptable methods

- Minimal suffering and pain during process and after
- Long lasting
- Easy and quick
- Economically viable



1.4.2. Fin clipping

Fin clipping is a relatively easy technique, may have minimal impact on survival and social structure of the marked fish, and is a recommended procedure for many studies. The fins selected for clipping or removal would depend upon the species. A small piece of fin (location and which fin is species-specific) is clipped with a pair of sharp scissors (dipped in 70% ethanol before and whenever a new animal is to be clipped) to indicate the individual has been caught. The fish is then released immediately.



1.4.3. Dart Tagging

Dart tagging involves the attaching of a commercially developed, individually numbered, external plastic tag to the body of the fish. Trained and experienced staff undertake and supervise all dart tagging. A specific tagging needle or gun is used to insert the dart end of the tag under the skin below the dorsal fin. The fish is removed from

the water onto a board or cradle and the tag inserted. Analgesic is not required and the procedure is no more technically demanding than ear tagging. Tag size is determined by fish size. These tags can stay with the fish for a number of years, often for the life of the fish.

1.4.4. Microchip implant/passive integrated transponder (PIT) tags

Microchip implants, as for mammals (Refer to 4.3.3 *Microchip implant/ passive integrated transponder (PIT) tags,* below), are widely used to permanently identify fish.

For fish, the PIT tag/chip is inserted in a suitable location beneath the skin, using a large-bore hypodermic syringe. No analgesic is required and the procedure is no more technically demanding than ear tagging or taking a blood sample. Care should be taken to avoid contamination of PIT tags prior to implantation. Once implanted, the PIT tag is "read" with a hand held or static, in river, scanning device that activates the tag. PIT tags can range in size from 5-24 mm in length and 2-3 mm diameter and last for the life of the fish. The smaller size is more appropriate for smaller species or juveniles and has a lower distance from which they can be read. In general, fish greater than >150 mm can be tagged with 12 mm tags.

Sedate fish first and ensure that the fish is immobile and kept very still. There are two places to insert the PIT tag in fish and it depends upon the size and species. Generally, in small fish the tag is placed into the gut cavity and in larger fish it is placed into the cheek or the dorsal muscle. Firstly, swab the area of insertion with a disinfectant (e.g. Betadine) and depress plunger to insert the tag. After withdrawal, lightly coat the incision area with a flexible cover of surgical adhesive (Vetbond) to further promote healing. After the operation place the fish in an aerated disinfectant salt bath to recover. Return the fish to point of capture. Before and after insertion ensure that you can read the pit tag.

1.4.5. Radio-telemetry and acoustic telemetry

Radio-telemetry and acoustic telemetry is a useful method of locating and tracking medium-sized and large fish and aquatic invertebrates whose movements are difficult or impossible to monitor by frequent live-trapping or direct observation. The transmitter normally is surgically implanted or fixed externally with sutures or surgical grade 'super-glue'. The total package weight (transmitter, battery, aerial and bonding material) for aquatic animals and invertebrates ideally should be less than 5% of body weight, and no greater than 10%.

1.5. Blood/tissue and stomach samples

1.5.1. Tissue samples - fin clipping

Fin clipping (refer to *1.4.2 Fin clipping,* above, for details), used for marking individuals is also suitable for providing tissue for molecular DNA analysis.

1.5.2. Stomach flushing

Stomach flushing is generally only used on threatened fish species such as Macquarie perch and trout cod where killing the fish cannot be justified. The procedure involves anaesthetising the fish with a solution of buffered MS 222 (or benzocaine or eugenol). Fish are placed in this solution until they lose stability and are unable to maintain an upright position. Fish are then removed from the anaesthetic and a small-gauge, flexible surgical catheter is inserted through the mouth and into the stomach. A gentle stream of water is passed into the stomach via the catheter and the stomach contents are expelled back through the mouth and collected in a gauze-lined sieve while holding the fish in such a way that the stomach contents are efficiently collected. Fish are then placed in aerated water in another container and allowed to recover. The diameter of the catheter depends on the size of the fish; fish smaller than 100 mm in length are considered too small to flush successfully. This must be done by a competent/trained person.

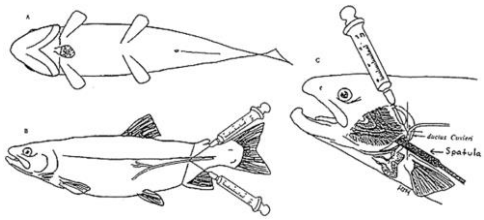
1.5.3. Blood Sampling

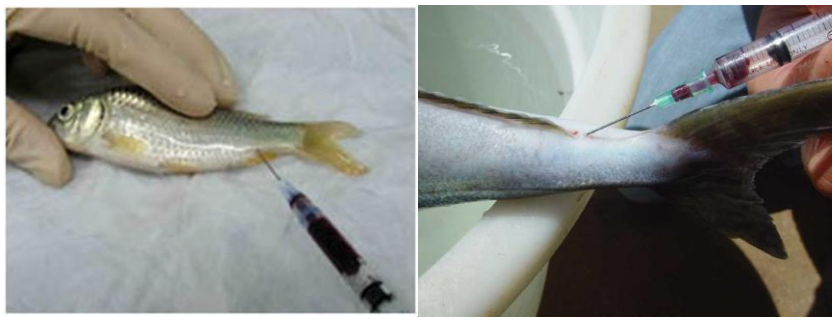
Blood sampling is used to obtain a sample for virilogical or metabolism investigations where killing the fish is not required. The procedure involves anaesthetising the fish with a solution of buffered MS 222 (or benzocaine or clove oil.). Fish are placed in this solution until they lose stability and are unable to maintain an upright position and unresponsive to stimuli. Fish are removed from the anesthetic and a small gauge syringe and needle is inserted into the caudal peduncle of the fish to penetrate the caudal artery (following Schreck & Moyle, 1990). A small sample of blood (1-15ml) is removed and the fish are then placed in aerated water and allowed to recover before release. Dependent upon the experience of the operator and the gauge of the needle, fish as small as 50-70 mm and as larger than 1000mm can be successfully sampled.

In general when blood is collected, sample size is recommended to be up to 1ml/kg body weight, although fish can sustain higher percentages of blood volume removal. The fish must be permitted to recover their haematocrit level prior to subsequent blood collection. Hematocrit recovery times are temperature dependent and highly variable between species.

1.5.3.1. Removal of blood

Three main techniques have been devised for collecting blood from fish: cardiac puncture, venous puncture, and caudal bleeding (Blaxhall 1972; Stoskopf 1992a). The tail is the preferred site for blood sampling. The vessels running beneath the vertebrae of the fish can be sampled by using a lateral or ventral approach. Cardiac punctures from the ventral side are sometimes used in fusiform fishes or through the operculum in laterally compressed species.





Needles and syringe combinations for fish of various sizes (please dispose of needles thoughtfully):

- 5-20 cm fish - 25g x 5/8 inch needle, 1 ml syringe
- 20-60 cm fish - 21g x 1½ inch needle, 3 ml syringe
- > 60 cm fish - 18g x 1½ inch needle, 3 ml syringe

1.6. Holding animals for short periods (less than 24 hours)

1.6.1. Fish

Fish held for short periods must be held in water in plastic containers with an adequate aeration system until they are processed and released or transported to a permanent holding facility.

1.6.2. Non-target animals

Any non-target animal will be released immediately or when it is appropriate to do so. When gill netting to prevent recaptures any platypus captured are to be removed from the net and held in large plastic tubs/ bins until the nets are removed from the site. Dry plant material (usually tussock grass, *Poa sp.*) is to be placed in the tubs to

allow the animal to dry off and hide within the bin, thus minimising stress. All animals must be released at the point of capture following the removal of the nets.

1.7. Transport

The transportation container should be well insulated to minimize temperature changes during transport

Aeration or oxygenation systems should be installed to ensure adequate oxygen saturation of the water.

Small numbers of fish can be transported in plastic or polyethylene bags partially filled with water under an atmosphere of pure oxygen. These bags must be transported in an insulated box to maintain water temperature as close as possible to the fishes' initial/starting temperatures.

1.8. Holding facility

The single most important element for maintaining healthy fish in captivity is water quality. Aquarium care and maintenance is paramount if fish are to be held for extended periods. The main water quality parameters to be maintained are temperature, oxygen saturation, levels of nitrogen compounds, carbon dioxide, pH and salinity. Light and noise levels also need to be considered.

If the project requires animals to be kept of extended periods, applicants must provide species-specific requirements and details of when, where and for how long animals will be kept in a holding facility/ aquarium.

1.9. Release

In the field, fish must be released at the point of capture as soon as practicable and where ecologically appropriate. Upon completion of laboratory studies researchers are required to release wild-caught specimens whenever this is practicable and permit conditions have been approved. Exceptions are if national, state, or local laws prohibit release, or if release might be detrimental to the well-being of the existing gene pools of native fishes in a specific geographic area.

As a general rule, field-captured fishes may be released only:

- Only when specified and approved under relevant jurisdictional collection permit conditions (e.g. ACT Conservator licence to take and release under the nature conservation act).

- At the site of the original capture, unless conservation efforts or safety considerations dictate otherwise. Release should never be made beyond the native range of distribution of a fish without prior

approval of the appropriate state and/or federal agencies, and approved relocations must be noted in subsequent publication of research results.

- If their ability to survive has not been irreversibly impaired.
- Where it can be reasonably expected that the released animal will function normally within the population.
- When local and seasonal conditions are conducive to survival.
- When release is not likely to spread pathogens.

Captured animals that cannot be released or are not native to the site of intended release must be properly disposed of, either by distribution to colleagues for further study, or if possible, by preservation and deposition as teaching or voucher specimens in research collections.

1.10. Euthanasia

The AEC supports and adopts the euthanasia standards as outlined in the Euthanasia of Animals Used for Scientific Purposes 2001, Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART).

Applicants must identify the proposed euthanasia technique(s) and provide information on training and/or prior experience of all staff involved in the killing of animals.

1.10.1. Fish

Which method of euthanasia is used is dependent largely on the size of the fish involved, with small specimens (less than ~100 mm) euthanized with anaesthetic.

1.10.1.1. Recommended techniques - skin absorption

Halothane 5% can be bubbled through the tank water or Isoflurane at 0.75ml/l added to the water will anaesthetise and kill fish. Death should be ensured by destruction of the brain once the animal shows no sign of movement.

An overdose of tricaine methane sulphonate (MS-222) or benzocaine may be placed in the tank. However, death should be ensured by destruction of the brain once the animal shows no sign of movement.

A slurry of salted ice has also been shown to be effective in euthanasia of some species. However, death should be ensured

by destruction of the brain once the animal shows no sign of movement.

Eugenol or clove oil mixed with ethanol to assist dispersal applied to the water has been recommended as an anaesthetic agent prior to euthanasia.

1.10.1.2. Acceptable with reservations - physical method

Stunning by a sharp blow to the head with a heavy, blunt instrument (usually a length of steel bar) followed by destruction of the brain. The technique requires appropriate training and experience.

1.11. Disposal of dead animals and/or biological material

Whatever method is used, it is essential to ensure that the fish is dead before disposal. A fish can be considered to be dead ten minutes after the last sign of gill movement. Prompt and sanitary disposal of dead animals and waste materials must be in accordance with the jurisdiction's legislation and University standards.

Dead animals and biological sample material should, where possible, be made available for other teaching or research projects and/or lodged as museum specimens.

2. AMPHIBIANS

2.1. Capture technique

2.1.1. Pitfall trapping

It should be noted that the use of “wet pitfall” fall traps are considered unacceptable for vertebrates. Dry pitfall traps must be managed to minimise the impact on trapped animals by taking into account issues such as:

- time animals will spend in the trap,
- the possibility of trapping animals which may prey upon or parasitise other trapped animals,
- environmental effects such as dehydration and hyperthermia in hot weather, hypothermia or drowning,
- deprivation of food and water,
- deactivation of traps when no longer required,
- appropriate size of trap - diameter, depth,
- construction of trap - conformation of the walls, lids, covers or grids,
- possible non-target species - bearing in mind that small vertebrates may in fact be smaller than large invertebrates, and
- traps should not be set in areas where there is a possibility of them filling with water such as low lying areas or wetlands

Although generally used as a method of capturing reptiles and frogs, small mammals can also be caught using this technique. The methods described below have been designed to accommodate the requirements of amphibians.

Pitfall traps (pits) consist of metal or plastic buckets placed in the ground, with the top of the bucket open and at ground level. Pits can be temporarily closed at any point by fitting buckets with a metal or plastic lid. A site generally consists of a number of buckets (pits) arranged in a grid, line or cross-shaped pattern. Pits are usually linked by a drift fence, which channels animals toward the pits. A drift fence consists of a vertical strip of shade cloth or similar material (approximately 30-40 cm high), held up with wire uprights and with its lower edge lightly trenched into the ground.

The side of each pit should have an array of small holes approximately 1.5-2 cm from the bottom. This allows most accumulated rainwater to drain out, but retains a small amount of water at the bottom to prevent desiccation of any frog captured.

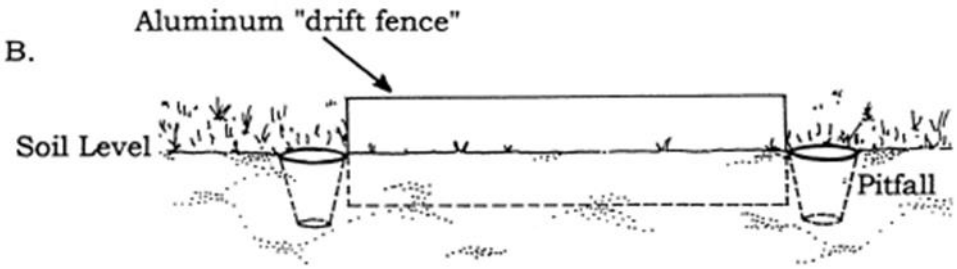
Each pit should also contain a short length of PVC pipe and a sponge cloth to provide shelter, moisture and an opportunity for a captured animal to climb out of the water (by climbing onto the sponge).

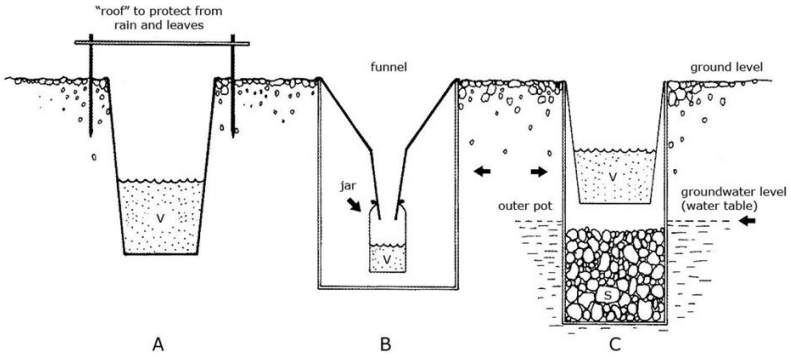
A portable metal roof mounted on wire legs, should be placed over each pit to provide shade and additional protection from predators.

Traps while open should be checked daily and any captured animal removed, and additional water added when necessary.

If excess water is accumulating in the pits (as sometimes happens after prolonged rain and saturation of the ground), additional material (usually wads of grass) should be put into the pits to allow animals to escape the water. Alternatively, traps can be closed by a tight fitting lid. When water has receded, this additional material is removed or the trap reopened.

All buckets and fences must be removed when the survey is complete and the holes filled in. If insufficient soil is available, holes should be filled with inert (weed free) material such as certified weed free soil or hardwood chips.





2.1.2. Searching by hand

Frogs can be actively searched for by hand beneath logs, rocks and other ground cover that can be easily removed and replaced, also refer to *3.1.6.2 Rock/ log rolling* below, for details.

2.1.3. Call census

This non-invasive technique is the most commonly used frog survey and monitoring technique. Two types of call census are used.

The number of frogs of different species calling at a breeding site can be estimated to provide an index of abundance. No direct contact is usually made with the frogs.

Calling loudly to some species of frog elicits a threat call response. The number of frogs responding can provide an index of abundance. In addition, a tape recording of frog calls can be played back loudly.

2.2. Handling

All handling must be kept to the minimum required to obtain the necessary information according to predetermined objectives.

Any methods of physical restraint and handling need to ensure that no physical injury occurs and minimal stress is placed on the animal. The least amount of restraint and the shortest possible time necessary for the procedure or to handle the animal must be used. All persons intending to handle animals should be trained under the supervision of an experienced animal handler and must be able to demonstrate their ability to handle animals safely and responsibly.

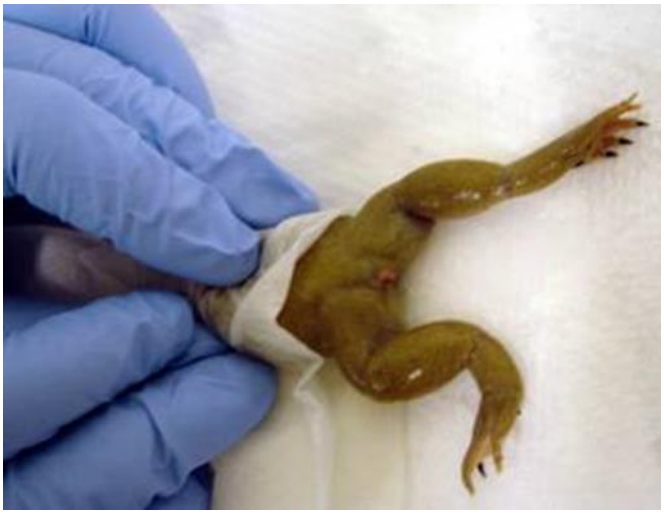
All frogs will be handled with disposable latex gloves that are changed between individual specimens, thus avoiding cross-contamination of specimens. If gloves are not available, frogs should be handled with clean, wet hands that are washed and disinfected between handling specimens. If handling amphibians with bare hands, it is extremely important for investigators to ensure that they have not applied insect repellents, perfumes, lotions, or other potentially toxic substances.

Frogs can be temporarily restrained by immobilising the back legs, with the body of the frog placed on a flat surface. If required to be restrained for any length of time, the frog should be placed in a clean, clear plastic bag with the inside moistened with water, and examined through the wall of the bag.

Nets, hooks, tongs, strong carrying boxes (not cardboard) or handling bags may be used to reduce injury and struggling in these animals. As well, small amphibians can be temporarily restrained in plastic bags containing a small amount of water and blown full of air, as the soft sides cushion the force of jumps. Care must also be taken to avoid removal of the protective mucus layer covering the skin of amphibians, and any nets that are used should be made of soft cloth materials.

Frogs should be grasped around the waist with the hind limbs fully extended to prevent kicking. Where possible, amphibians are best observed in clear enclosure such as a plastic Chinese container. Small frogs are best restrained with the hind legs extended and held securely between the thumb and index finger. Larger specimens may require support with two hands to the body between the front and hind legs. If

large enough they can be secured using the thumb and index finger around the frog under one front limb at thumb and over the other front limb and the index finger. Alternatively they can be wrapped firmly but not tightly in smooth damp cloth.



Where the frog is not required to be taken from the field, it should be released as soon as practicable, in an appropriate location at the site of capture.

2.2.1. Minimising introduction and spread of frog pathogens

The Chytrid fungus that has been associated with the decline of some amphibian populations is now widespread in Australia. Given the serious implications of this pathogen on frog populations, it is important to implement measures to minimise its spread (or the spread of new strains), as well as that of other pathogens. For example, laboratory testing should precede any release of amphibians or other aquatic animals that were kept or breed in captivity, particularly if captive individuals have been maintained outside the species normal range or kept in a facility that houses other frog species.

Guidelines developed by the NSW National Parks and Wildlife Service are intended for all persons involved with or likely to come in contact with frogs or their habitat. Investigators must ensure that they have read and implement the hygiene protocol in any proposed project where they are directly or indirectly encountering frogs and tadpoles.

A good hygiene protocol for the control of disease in frogs is found at: http://www.environment.nsw.gov.au/resources/nature/hyprfrog.pdf.

2.3. Marking

2.3.1. Toe clipping - frogs

Toe clipping is a commonly used method for individually marking frogs and reptiles. However, it is possible that it may slightly increase mortality rates of some species (particularly if more than one or two toes are removed). Furthermore, it is common for toes to regenerate, which may affect the identification of clipped individuals. Toe clipping should be used only as a last resort if other techniques (e.g. photographs) are not suitable. Please refer to *3.3.1 Toe clipping – lizards,* below, for details.

2.4. Blood/tissue and stomach samples

2.4.1. Tissue samples - frog toe clipping samples

Toe clipping (See *3.3.1 Toe clipping - lizards*) removes a frog's digit (or a combination of digits) up to the first joint (using sterile blades or instruments – if scalpels are used, disposable blades are recommended). Samples can be stored in liquid nitrogen or preserved in 70% ethanol.

2.4.2. Stomach flushing

Stomach flushing is a technique used to conduct dietary studies on frogs. The following procedure was described by Sole *et al.* 2005.

The stomach flushing needs to be conducted within 2 hours of capture. Frogs should be held by gently fixing forelimbs with one hand. The mouth can be opened using a small spatula before a pond-water filled syringe attached to a tube made of soft material (silicon) is introduced into the oesophagus and then the stomach. The contents of the syringe should be forced out gently and any material flushed from the stomach collected in a vessel. This flushing procedure may be conducted twice. The material collected in the vessel can be emptied into a sieve and relevant material picked out with forceps and stored in a vial of 70% ethanol for later analysis. Frogs should be released immediately following this procedure.

2.5. Holding for short periods (less than 24 hours)

Animals that are required to be held for short periods while being processed, identified or transported need to be cared for appropriately before being release at the site of capture or in approved holding facility.

2.5.1. Frogs

All frogs held for short periods (i.e. less than 24 hours) will be housed in plastic containers with adequate holes in the lid for ventilation. Containers must be sterilised. Several frogs (of the same species and of similar size) may be kept together in a container, but containers must not be over-crowded. A moistened paper towel, or vegetation from the site, should be placed in each container to ensure frogs remain hydrated. Frogs from different sites must always be housed in separate containers, and with vegetation from their respective collection sites only.

2.6. Transport

All frogs should be transported individually in either clean plastic containers or clean plastic bags. Appropriate measures are to be taken to ensure provision of adequate moisture and air during transport, and that the frogs are not overheated.

2.7. Holding facility

Frogs or tadpoles collected from different sites must not share water, equipment or filtration systems and care must be taken to avoid contact with them (directly and indirectly) or with other captive animals. Prior to housing animals in aquaria, ensure that the tanks and any associated equipment are sterilised. Also sterilise tanks and equipment immediately after the frogs have been removed.

Applicants must provide species-specific requirements and details of when, where and for how long animals will be kept in a holding facility, if the project requires animals to be kept for extended periods.

2.8. Release

Before release, all animals must be checked for injury and ill-health. Note that all animals must be released at or near their site of capture within 24 hours of capture unless being transported to a holding facility. Release of frogs back to the wild should follow the guidelines of Pessier and Mendelson (2010), which state that amphibians removed from the wild that have been taken outside the species range, or amphibians that have been kept at a facility which houses other amphibian species, are not to be released unless pathological screening for disease is undertaken to

ensure individuals are free from pathogens. An electronic copy of Pessier and Mendelson (2010) is available at:

http://amphibianark.org/pdf/Amphibian_Disease_Manual.pdf

Returning animals of unknown origin, or animals that have been kept in captive conditions poses a risk of introducing and spreading frog pathogens, in particular the Chytrid fungus.

2.9. Euthanasia

The AEC supports and adopts the euthanasia standards as outlined in the Euthanasia of Animals Used for Scientific Purposes 2001, Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART).

Applicants must identify the proposed euthanasia technique(s) and provide details of the project personnel and their appropriate experience, if the project requires animals to euthanised.

2.9.1. Amphibians (frogs and toads)

2.9.1.1. Recommended technique - injectable agents

Frog or toads to be euthanised should be injected with sodium pentobarbitone, at a dose rate of 60 mg/kg (euthanasia solution of 350-400 mg/ml). Only the intraperitoneal route is recommended; injections must **NOT** be made intra-cardially or into the lungs.

2.9.1.2. Recommended technique - skin absorption

The use of chloral hydrate is a recommended method for euthanasia of frogs and toads. Animals are placed in a container holding a 2-3 mm layer of a 3% solution of chloral hydrate. The chemical is absorbed through the ventral skin and the animal dies

in a relaxed state within a few minutes. This product is difficult to obtain and may require a script.

Benzocaine can be used in a similar fashion to chloral hydrate (dissolved in ethanol and buffered with sodium bicarbonate), or can be directly applied to the ventral surface of the amphibian (Chen and Combs 1999). The proprietary human dental medication “Oral-eze®” (an over-the counter medication for human toothaches) contains benzocaine 7.5%, clove bud oil 19% (which is used as an anaesthetic agent in amphibians) and ethanol 18%. “Oral-eze®” has been found to be effective in euthanizing amphibians by the senior veterinarian at Tidbinbilla Nature Reserve by placing 1 to 3 drops (depending on size of frog) of “Oral-eze®” into a small container and placing the frog into the container so that the liquid is in contact with the frog’s ventral surface. Loss of consciousness is typically rapid (60-90 seconds) and death occurs in a relaxed state usually within 2 minutes.

Tricaine methane sulphonate MS-222 (a S4 prescription drug) dissolved in water can be used in a similar fashion to chloral hydrate. The MS-222 reduces the pH of the solution in which it is dissolved. Thus it is recommended that the solution should first be neutralised with sodium bicarbonate before placing the animals into the solution, so as to reduce possible skin irritations (see 1.4.2 above).

Animals must be pithed or decapitated to ensure death.

2.9.1.3. Acceptable with reservations - physical method

Physical methods of euthanasia such as stunning and decapitation or stunning and pithing are acceptable with reservations.

Decapitation is not recommended unless a sharp blow to the head or neck has first stunned the animal. Use sharp equipment of an appropriate size for the species to be euthanized to ensure that the head is separated from the body rapidly and completely.

Stunning followed by pithing is also an option. A sharp blow to the head or neck stuns the animal first, then a rigid metal rod is inserted into the foramen magnum, which is identified by the slight midline skin depression posterior to the eyes when the neck is flexed. Both the brain and the proximal end of the spinal cord must be destroyed.

Both physical methods require the applicant to demonstrate that they have had appropriate prior training and experience.

2.9.1.4. Not acceptable - physical method

Immobilising the animal by placing in the fridge (4^{o} C), or covering it with crushed ice and then deep-freezing it is no longer an acceptable method, due to the likely suffering experienced as ice crystals form.

2.10. Disposal of dead animals and/or biological material

All animals must be confirmed as dead before being disposed of. Prompt and sanitary disposal of dead animals and waste materials must be in accordance with the jurisdiction's legislation and University standards.

Dead animals and biological sample materials should, where possible, be made available for other teaching or research projects and/or lodged as museum specimens.

3. REPTILES

3.1. Capture Technique

Pitfall trapping for reptiles involves dry trapping, in contrast to amphibians' wet trapping.

3.1.1. Pitfall trapping

The methods described below have been designed to accommodate the requirements of reptiles.

Pitfall traps (pits) consist of metal or plastic buckets placed in the ground, with the top of the bucket open and at ground level. Pits can be temporarily closed at any point by fitting buckets with a metal or plastic lid. A site generally consists of a number of buckets (pits) arranged in a grid, line or cross-shaped pattern. Pits are usually linked by a drift fence, which channels animals toward the pits. A drift fence consists of a vertical strip of shade cloth or similar material (approximately 30-40 cm high), held up with wire uprights and with its lower edge lightly trenched into the ground.

The bottom of each pit bucket has small holes to allow rainwater to drain out.

Each pit bucket should also contain a short length of PVC pipe and a sponge cloth to provide shelter, moisture and an opportunity for a captured animal to climb out of the water (by climbing onto the sponge).

A portable metal roof mounted on wire legs, should be placed over each pit to provide shade and additional protection from predators.

Pitfall traps should be checked daily while open and any captured animal removed.

If excess water is accumulating in the pits (as sometimes happens after prolonged rain and saturation of the ground), additional material (usually wads of grass) should be put into the pits to allow animals to escape the water (if only a small amount of water is present). The traps should be closed by a tight fitting lid if there is a risk of reptiles drowning.

All buckets and fences must be removed when the survey is complete and the holes filled in. If insufficient soil is available, holes should be filled with inert (weed free) material such as certified weed free soil or hardwood chips.

3.1.2. Spider tubes (artificial burrows)

Spider tubes were developed as a technique for surveying and monitoring grassland earless dragons (*Tympanocryptis pinguicolla*). This tube is not a trap but an artificial home site that lizards can enter and leave at will. It consists of an outer PVC tube buried in the ground into which fits an inner sleeve that can be removed for inspection. The inner assembly has a drainage hole at the bottom and the inner surface is painted with dark brown acrylic paint to which dry beach sand is attached to assist the lizards to climb in and out. The inner sleeve has an internal diameter of 20 mm and is 125 mm in height and can be replaced upside down to close the “burrow”.

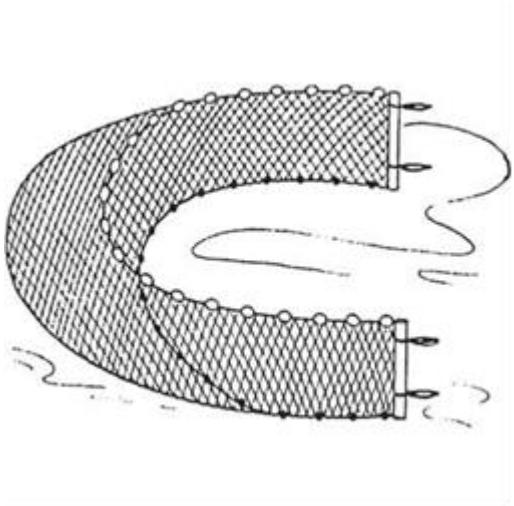
Apart from the target species, spiders and other arthropods, frogs and other lizards are also known to use these “burrows”.

3.1.3. Hoop traps (cathedral turtle traps)

Baited hoop traps (originally described by Legler, 1960; modified by Georges, Guarino & White, 2006) are used for capturing turtles in turbid waters. They consist of a single compartment made from netting held in place by a metal frame, with one or two funnel entrances. The funnels allow easy entry but prevent exit. Sardines, bread or meat can be used as bait depending on the species sought. The traps are now commercially available. Hoop traps are checked within 2 hours of setting to prevent drowning of captured animals. They have floats attached and are individually numbered to ensure that they are all examined during each trapping session, and all retrieved at the end of trapping.

3.1.4. Seine nets

Seine nets are used to capture turtles/ tortoises from discrete ponds, sections of rivers or farm dams. They are typically 10 m or less in length, and are dragged through the water by two people positioned at each end of the net. Captured turtles must be secured immediately to prevent drowning.



3.1.5. Artificial shelters

This refers to a technique where artificial shelters; e.g. roof tiles, timber or iron, are placed on the ground. At regular intervals after placement, the shelters are turned over and animals sheltering beneath them caught and/or identified.

To ensure animals are not trapped or squashed when replacing the artificial shelter, animals caught should be released back under the shelter after it has been returned to its original position.

If artificial shelters are to be removed at the end of a project, animals sheltering beneath need to be encouraged to shelter in adjacent vegetation or natural cover such as logs or rocks and not left exposed in the open.

3.1.6. Searching and capture by hand

3.1.6.1. Snorkelling - turtles

In clear waters, a diver wearing mask and flippers can catch turtles by hand.

3.1.6.2. Rock/log rolling

This refers to a technique where rocks and logs are turned over and animals sheltering beneath are caught and identified.

All logs and rocks must be replaced as they were found with appropriate care taken to ensure that animals are not injured when replacing rocks and logs, i.e. animals must be caught or moved out of the way before replacing rocks and logs. Disturbance to the animal and its habitat should always be kept to a minimum.

3.1.6.3. Stalk and hand capture (grabbing)

Larger lizards, particularly the larger dragons such as *Pogona* spp. can be captured opportunistically by hand if weather conditions are cool. The lizards should be approached slowly and then secured either directly by hand or by the use of a noose extended on a pole. Care needs to be taken not to strangle the animal with the noose. Animals must not be lifted from the ground using the pole and noose. Once restrained by the noose, animals must be caught and secured by hand as quickly as possible.

3.1.6.4. Fibre-optic survey of burrows

This technique requires a fibre-optic scope/probe (an industrial version of an endoscope or laparoscope) to be inserted carefully into a burrow to detect the presence or absence of the animal of interest.

The fibre-optic scopes should use a cool light source, cause no disturbance to the animal and do not come into direct contact with the animal. Animals are not extracted from the burrow and the burrow is not destroyed.

3.2. Handling

Any methods of physical restraint and handling need to ensure that no physical injury occurs and minimal stress is placed on the animal. The least amount of restraint and the shortest possible time necessary for the procedure or time need to handle the animal must be used. All persons intending to handle animals should be trained under the supervision of an experienced animal handler and must be able to demonstrate their ability to handle animals safely and responsibly.

Reptiles that are susceptible to tail shedding (e.g. skinks, geckos and legless lizards) must **NOT** be caught or held by the tail.

Non-venomous snakes (e.g. blind snakes - family *Typhlopidae*) can be caught and/or removed from the trap by hand. However, no snake should be caught by hand unless the investigator has (or others present have) the appropriate skills in the identification of snakes and snake handling. Snakes caught in pitfall traps should be removed either by providing a means of allowing the animal to exit the trap without handling or by lifting the snake out with the aid of long tongs, or a similar device that will not harm the animal.

All handling should be kept to the minimum required to obtain the required information.

Animals not required to be taken from the field must be released as soon as practicable in an appropriate location at or near the site of capture.

3.3. Marking

3.3.1. Toe clipping - lizards

Toe clipping is a common method used for individually marking lizards. It involves the removal of the top joint of one or more digits from an animal. Only sharp, surgical scissors are to be used and these should be sterilised before and after each use, e.g. by immersing in 70% ethanol or by other suitable methods. Animals should be monitored to ensure bleeding has stopped before being released. See 3.4 below: Tissue samples - lizards (toe clipping).

3.3.2. Microchip implant/passive integrated transponder (PIT) tags

Microchip implants are widely used to permanently identify domestic animals and livestock. Microchips may also be used in reptiles to replace ear tags, ear notching, or tattooing.

The PIT tags are small microchips (about the size of a grain of rice) inserted in a suitable location beneath an area of relatively loose skin, using a large-bore hypodermic syringe. No analgesic is required and the procedure is no more technically demanding than ear tagging or taking a blood sample. Care needs to be taken to avoid contamination of PIT tags prior to implantation (needles should not be re-used). Once implanted, the PIT tag can be “read” with a scanning device that activates the tag. PIT tags are expensive in terms of both the tags and the reader; however, they are generally more reliable than ear marking or ear tagging.

3.3.2.1. PIT tags - turtles

Carettochelys can be marked using a pit tag inserted beneath the skin of the rear limb using the hypodermic supplied by the manufacturer for the purpose.

3.3.2.2. PIT tags - water dragons

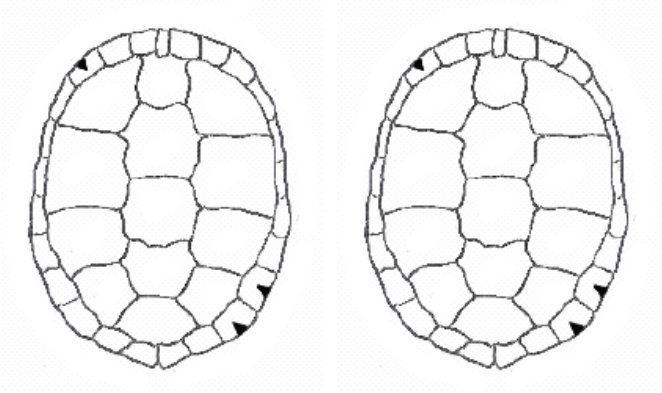
Water dragon PIT tags can be injected into the sub-dermal layer. The tags should be injected in a downward direction on the left hand side of the body approximately 3 cm anterior to the hind limb. The syringe opening is then closed with a drop of tissue adhesive.

3.3.3. Heat branding

Heat branding is a long-term marking technique used on limbless reptiles that allows animals caught in previous seasons to be identified.

Heat branding involves using a fine, tipped-heated soldering iron to mark an individual scale or scales on the underside of the tail of the reptile. In some instances, the dorsal surface may be used. The hot iron is applied for approximately one second or until the scale is visibly affected.

3.3.4. Shell notching



Chelid turtles can be marked using a unique pattern notches in the marginal scutes of the carapace. Turtles can be notched using a small triangular file to file a 2-3 mm notch in the shell. Nail clippers can also be used to notch the shell, particularly in small turtles.

3.4. Blood/tissue samples and egg collection

3.4.1. Blood samples - turtles

Blood can be taken from turtles using a fine needle (23-gauge for adults and sub-adults; 26-gauge for juveniles) attached to a heparinised syringe. Needles and syringes must not be re-used.

For chelid turtles, blood should be taken from the jugular vein that is clearly visible beneath the skin on the side of the neck. For *Carettochelys*, blood should be taken from a facial vein immediately behind the junction of the upper and lower jaw.

The sampled blood volume needs to be limited to 2.5 ml per adult and sub-adult animals (400-2,000 g), and scaled down appropriately for smaller animals. Samples should be transferred into duplicate tubes and can be stored in liquid nitrogen or in ethanol with a concentration of 70% to 80%.

3.4.2. Tissue samples - turtles

For chelid turtles, skin samples can be taken from the trailing flaps of the vestigial toe of the rear foot with a sharp scalpel. This causes no long-term incapacity to turtles. For *Carettochelys*, skin samples should be taken from the webbing of the first toe of the rear limb. Samples can be stored in liquid nitrogen or preserved in 70% ethanol. For each animal, a new and sterile blade must be used.

3.4.3. Tissue samples - reptiles (tail samples)

For molecular analyses, a small (< 1cm) section of the tip of tail can be removed from an animal using sharp dissecting scissors. This causes no long-term incapacity to the lizards. Samples can be stored in liquid nitrogen or preserved in 70% ethanol. Scissors must be sterile, and re-sterilised for each animal.

Taking tail snips from turtles is specifically prohibited. It involves severing vertebrae and the tail tip is used by males to locate the cloaca during copulation.

3.4.4. Tissue samples - lizards (toe clipping)

Toe clipping as described above (*1.3.1. Toe clipping*) removes a lizard's digit (or a combination of several digits) to the first joint. In case of arboreal lizards, climbing toes should be spared. Samples can be stored in liquid nitrogen or preserved in 70% ethanol. A sterile instrument must be used, and a new blade or re-sterilised instrument for each animal.

3.4.5. Egg collection - lizards

The state of gravidity can be determined by external examination and palpation. Oviparous reptiles should be held for 10 days to ensure that eggs are shelled, and egg laying can then be induced with an intramuscular injection of oxytocin (2-10 units per kg body weight), preferably after an injection of calcium borgluconate 100mg/kg i/v, i/m or s.c or gluconate 50-100 mg/kg/i/m or s.c. This should be repeated no more than 3 times at 90-min intervals or greater.

3.5. Holding for short periods (less than 24 hours)

Animals that are required to be held for short periods while being processed, identified or transported should be cared for appropriately before being released at the site of capture or transported to an approved holding facility.

3.5.1. Turtles

Chelid turtles can be held without risk of injury in open weave bags, tied at the top. Open weave bags allow free flow of air, even when wet.

However, *Carettochelys* is prone to injury, and therefore they must always be held singly in individual hessian bags (nylon rope bags are unsuitable) or better, singly in individual tubs with a damp foam underlay.

All turtles in holding bags need to be kept in a cool, shaded place while they wait to be processed before being released. Turtles held in this way should not be kept for longer than 24 hours.

3.5.2. Other reptiles

All reptiles (other than turtles) should be held individually in secure containers or calico/ open-weave bags. Appropriate measures need to be taken to ensure animals are provided with adequate moisture and air while being held, and are not overheated (note that bags should not be moist for most arid species). These measures include provision of moist paper towelling, air holes in containers and the use of insulated boxes. All reptiles should be kept in a cool, shaded place while they wait to be processed before being released. Reptiles held in this way should not be kept for longer than 24 hours.

3.6. Transport

All reptiles should be transported in individual secure containers or calico/ open-weave bags. Appropriate measures must be taken to ensure the provision of adequate moisture and air during transport, and that the animals are not overheated. These measures include provision of moist paper towelling, air holes in containers and the use of insulated transportable containers.

3.7. Holding facility

Because the biological needs of each species and the nature of individual projects vary widely, only general recommendations can be made on housing wild reptiles and captive-reared reptiles. When dealing with an unfamiliar species, it may be necessary to test and compare several methods of housing to find the method most appropriate for the needs of the animal and the purpose of the study. Restraint and ease of maintenance by animal keepers should not be the prime determinant of housing conditions; however, researchers often can infer from knowledge of the biology of a particular species, what the requirements are for that species to thrive. Such information needs to be applied whenever possible.

Investigators must ensure that all facilities are approved by their institution for holding reptiles, are appropriately staffed, designed, constructed, equipped and maintained to achieve a high standard of animal care.

Applicants must provide species-specific requirements and details of when, where and for how long animals will be kept in a holding facility if the project requires animals to be kept of extended periods (i.e. longer than 24 hours).

3.8. Release

All animals need to be checked for injury and released at or near their site of capture immediately or within 24 hours of capture unless being transported to a holding facility. Animals relocated to the investigator's institution should be housed appropriately until the project is complete.

If animals are removed from the wild or are involved in research that continues for longer than **two weeks,** the animals are required to be killed and not released back into the wild because reintroducing these animals could have detrimental implications to the individual and/ or the population as a whole. Exceptions may apply if the animal(s) can be guaranteed to be free of disease or if the animal is a member of a threatened species.

3.9. Euthanasia

The AEC supports and adopts the euthanasia standards as outlined in the Euthanasia of Animals Used for Scientific Purposes 2001, Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART).

Applicants must identify the proposed euthanasia technique(s) and provide details of the project personnel and their appropriate experience, if the project requires animals to be killed.

3.9.1. Tortoises and turtles

3.9.1.1. Recommended technique - injectable agents

Tortoises or turtles required to be euthanised should be injected with sodium pentobarbitone at a dose rate of 60 mg/kg (euthanasia solution of 350-400 mg/ml). The intravenous route is recommended where possible; otherwise the intraperitoneal route can be used. Injections are **NOT** to be made intra-cardially, or into the lungs.

3.9.2. Snakes and lizards

3.9.2.1. Recommended technique - injectable agents

Lizard or snakes required to be euthanised should be injected with sodium pentobarbitone, at a dose rate of 60mg/kg. The intravenous route is recommended where possible; otherwise the intraperitoneal route can be used. Injections are **NOT** to be made intra-cardially, or into the lungs.

3.9.2.2. Acceptable with reservations - physical method

The method of euthanasia by stunning and separation or destruction of the brain is only acceptable with reservations. A sharp blow behind the head should be followed by decapitation or destruction of the brain. Such techniques require appropriate training and experience.

3.9.2.3. Not acceptable - Physical method

Immobilising the animal by placing it in the fridge at 4^{o}C or covering it with crushed ice and then deep-freezing the animal is not an acceptable method due to the potential for suffering as ice crystals form.

3.10. Disposal of dead animals and/or biological material

All animals must be confirmed as dead before being disposed of.
Prompt and sanitary disposal of dead animals and waste materials must be in accordance with the jurisdiction's legislation and University standards.

Dead animals and biological sample material should, where possible, be made available for other teaching or research projects and/or lodged as museum specimens.

3.11. Automatic infra-red triggered cameras - reptiles

See equivalent section for mammals. Cameras set for reptiles have often been used on time lapse, e.g. taking a picture every few seconds. In common with NSW, if no attractant is involved such as a food odour, AEC approval is generally not sought.

4. MAMMALS

The AEC distinguishes between laboratory animals and wild life. The following procedures mostly describe dealings with wild mammals.

4.1. Capture techniques

4.1.1. Trapping (Elliot traps)

Elliot traps are used for the survey and monitoring of small ground dwelling mammals. The Elliot trap is essentially an aluminium rectangular box into which bait is placed. An animal entering the trap triggers the door to close, and once triggered, no other animals can enter the trap. Although a variety of sizes of Elliot traps exist, the most commonly used is the small Elliot trap (33 cm long x 8 cm wide x 9.5 cm high). This trap is used to target animals up to about 340 grams in body weight. Where larger animals are targeted, larger traps must be used.

Dacron fibre should placed into each trap as bedding material for any captured animal, and the trap should be placed in a plastic bag to prevent it getting wet from rain and heavy dew. Traps are then placed into sheltered positions, e.g. near a log or under a shrub. Traps must be checked daily.

Important logistical considerations in a trapping program include the numbers of traps set per night and their positioning. The researcher must ensure that all traps can be inspected (at least once/day) and cleared within a reasonable period of time to avoid animals being confined for long periods. A further consideration is the breeding season. Where possible, trapping should not be conducted when dependent young are likely to be present, either attached or in a nest.

Traps may be set for no more than 3 consecutive nights to minimise the potential impacts on individuals that are re-captured.

4.1.2. Trapping (cage traps)

Cage trapping utilises wire cages with trap door mechanisms and bait to capture animals. The type of bait used and the size of the cage trap are determined by the type of animal being targeted. Traps should be placed in sheltered positions and, if possible, leaf litter and bark should be placed on top of the cage to provide protection from the weather.

Although a variety of sizes of cage trap exist, the most common dimensions are 60 cm long x 30 cm wide x 30 cm high. This size is used to target animals up to 6 kg in body weight. Where larger animals are targeted, larger traps must be used.

Traps are inspected as early as practicable in the morning if nocturnal animals are targeted. Depending on the target species, objectives of the trapping exercise and location of traps, traps may be need to be checked more frequently than once per day. Details should be provided with the project application.

Animals are released from cages by simply opening the cage and coaxing an animal out. If an animal is required for inspection and/or tagging it can be released into a hessian or similar fabric bag, which is placed over the mouth of the cage.

Animals do experience distress while trapped, and some may butt the walls of the cage with their head. In some cases, e.g. when trapping wallabies, this can be reduced by the addition of sponge rubber or similar material to the inside of the trapdoor but in other cases this approach is ineffective or impractical.

Efforts should be made to avoid trapping non-target species, e.g. the catch of currawongs in possum traps is reduced by addition of cinnamon or cinnamamide to the bait.

4.1.3. Tracking tunnels

Tracking tunnels consist of a rectangular, open-ended tunnel (with dimensions similar to a standard small Elliot trap) made of Corflute. The tunnels have a centrally mounted non-toxic inkpad on the floor and two flanking papers. Animals are attracted into the tunnel by a smear of peanut butter on the roof above the inkpad, and leave inky tracks on the paper as they exit the tunnel. Tracks on the paper can then be identified as to genus or species.

4.1.4. Harp traps - bat trapping

The collapsible bat-trap (Tidemann and Woodside, 1978) referred to as a harp trap, consist of two banks of nylon lines strung onto an aluminium frame and a collection bag with protective flaps where the bats are confined until they are released. Four adjustable legs support the frame and bag. The trap is designed to capture bats attempting to fly through the nylon lines.

The traps should not be set for more than two consecutive nights at any one site and must be checked as early as possible the following morning.

Captured bats can be identified using weight and forearm measurements and then confined to a secure cloth bag in a cool, dark, quiet location until the evening when they should be released at dusk at the capture location.

Bats may be marked in order to allow subsequent identification. This should be done by clipping off a small amount of fur from the back of the bat. Refer to *4.3.4 Temporary marking,* below, for details.

Where possible, and where the objectives of the project allow, trapping should not be conducted during the time of year when the majority of bats is pregnant or lactating.

Ultrasound detectors, such as Anabat^{TM} detectors are often used in conjunction with the harp traps at the site. The device is designed to record and analyse the echolocation calls of bats (usually beyond human hearing). The device does not interfere in anyway with bat behaviour.

Bat trapping can pose serious risks to human health (e.g. through the transmission of viruses); refer to *4.2.1 Health Precautions,* below, for details.

4.1.5. Spotlighting nocturnal mammals

Spotlighting is a popular technique used when surveying nocturnal mammal populations. It involves the use of a spotlight, either being carried by an observer on foot, or alternatively from a vehicle. The light is simply used to locate target species. Because shining a spotlight into the eyes of a nocturnal mammal may impair the animal's vision for a short period and may make it briefly more vulnerable to its natural predators, animals should not be held in the beam for long periods of time.

4.1.6. Automatic video/camera surveillance

4.1.6.1. Infrared-triggered digital cameras - mammals

An infrared-triggered digital camera is a battery-operated system, housed in a compact, weatherproof container that can be easily fixed to a tree or post to photograph animals that trigger the mechanism. These 'wildlife cameras' or 'trailcams' typically record colour images in daylight and use either an invisible infra-red flash at night (producing monochrome images) or a visible (white) flash for colour images. The white flash, although it might startle an

animal at first, is unlikely to cause any harm or distress to the animal.

Wildlife cameras may be deployed either ‘passively‘ or with an attractant such as a food odour. In common with NSW practice, AEC approval is not sought for passive deployment of cameras. Surveys using attractants require AEC approval. Animals rarely make excessive visits to attractants but operators should be vigilant for this particularly for surveys involving species not previously surveyed. Vigilance should also be exercised for the possibility of predators using attractants to aid their predation.

4.1.7. Hair tubes – small mammals

Hair tubes are generally polyvinyl chloride (PVC) pipes that are baited to attract animals. The tubes are constructed to target a particular species, so the size and design can vary. The entrance must be small enough such that animals entering the tube come into contact with adhesive tape within the tube, but do not actually get trapped. The adhesive tape is placed on the top and/ or sides of the tube entrance to collect guard hairs from the animal trying to enter the tube. It is important to ensure that the floor of the tube is free of adhesive tape to prevent small lizards and frogs becoming stuck. If an animal does become stuck to the tape, do not try to pull the tape off, as this may seriously damage the skin. Either carefully trim the tape on the animal to as small a size as possible (the remaining tape will be shed during normal skin replacement) or gently ease vegetable oil under the tape and slide it off. Species are identified from the hair samples left behind. This is based on the physical characteristics of the hair or on the genetic make-up.

4.2. Handling

Any methods of physical restraint and handling need to ensure that no physical injury occurs and minimal stress is placed on the animal. The least amount of restraint and the shortest possible time necessary for the procedure or time need to handle the animal is used. All persons intending to handle animals should be trained under the supervision of an experienced animal handler and must be able to demonstrate their ability to handle animals safely and responsibly.

4.2.1. Health precautions

All people working with mammals need to minimize the chances of zoonotic infection from contact with body fluids or being bitten or scratched and where practical wear gloves. Latex gloves must be worn to avoid unnecessary exposure to blood or other body fluids and faeces, which may contain parasites or pathogens that affect humans.

All field workers should maintain up-to-date tetanus immunisations.

In studies on bats, all persons handling bats must be made aware of the possible exposure to bat Lyssa virus and Henipa viruses. Vaccinations against lyssa virus infection are compulsory for any person proposing to handle or come in close contact with bats and or their faeces. A vaccine is under development for henipa virus infections and, once available, vaccination will be made compulsory as well.

Special care must also be taken to avoiding needle stick injuries when using syringes and similar devices.

A key component of safety in the field is common-sense personal hygiene. Investigators need to wash their hands frequently in the approved manner (see web site on Hendra virus) and also wash their field clothes and any other materials that come in contact with bats, their blood or body fluids. They need to also take precautions to prevent contamination of food and living areas with faeces and urine. All animal handlers should seek the advice of the appropriate health professionals if they are unsure of the risks involved in handling a particular species.

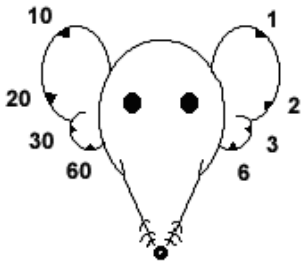
4.2.2. Responsibility for dependent offspring

Field study of mammals is most frequently carried out during warmer months of the year, which corresponds to the reproductive season of many species. As a consequence, there is a high probability that captured females may be lactating and, therefore, have dependent young, which are at risk. Investigators need to recognise the dependent relationship between suckling infants and their mothers, and wherever possible design sampling procedures to minimise the possibility of removing or killing lactating females. When this cannot be avoided or when orphaned young are found, the investigator will assume responsibility for such young, most commonly by killing them quickly and humanely. Live trapping will be designed to minimise the time lactating females are removed from their dependent offspring.

Applicants must provide species-specific details on how they propose to deal with dependent offspring.

4.3. Marking

4.3.1. Ear notching



Ear notching is a standard technique used to mark small mammals in capture/ recapture surveys. The technique is used when the ears of target animals could be injured by numbered metal tags (e.g. *Antechinus* spp.) or are too small for metal tags. It involves removing a small wedge from predetermined locations, from the margin of one or both ears. The number of notches placed in an individual's ear depends on the identification number allocated.

The maximum number of notches to be placed in an ear is two; see Figure for a description and example of the numbering technique. A notch is created using a pair of sterilised surgical scissors (he scissors are sterilised in 70% ethanol before if used on more than one animal). The illustration represents an example of the ear notch numbering technique. An animal given an identification number 5 would have notches at positions 2 and 3, while an animal given the identification number 99, would have notches at positions 30, 60, 6 and 3.

4.3.2. Ear tagging/marking

These are required only when the identity of an individual animal is required for a subsequent survey or monitoring event. A wide variety of commercially made tags are available for a variety of species. Considerations for ear tagging include placement in a part of the ear that minimises the opportunity for tearing the ear or causing excessive bleeding and choosing the appropriate size of tag for the size of the animal.

4.3.2.1. Ear tagging - small mammals

Small stainless steel numbered tags are available suitable for rat-sized mammals. The tag is inserted into the ear with a tag applicator.

4.3.2.2. Ear tagging - possums and macropods

If visual identification of an animal is required from a distance at a later time, coloured tags similar or identical to commercial sheep and cattle ear tags may be used for animals above 1,500 g body weight.

4.3.2.3. Ear marking - tattooing

Tattooing is widely used to permanently identify that pet cat and dogs have been desexed. Tattoo irons or applicators are commercially available that contain spike-like projections that form a number in a dot-to-dot format. Tattoo ink is applied to the spikes and the applicator then closed firmly on the ear. The applicator must always be cleaned, and sterilised with 70% ethanol before it is used.

4.3.3. Microchip implant/ passive integrated transponder (PIT) tags

PIT tags are also used for permanently marking mammals. Refer to *1.3.2 Microchip implant transponder (PIT) tags,* above, for detail.

4.3.3.1. PIT tags - possums and macropods

The PIT tag is inserted under the skin, midway between the base of the neck and the shoulder blades.

4.3.4. Temporary marking

Temporary marking with non-toxic dyes or dry fluorescent pigments, by spot-shaving or by cutting fur/ hair can be employed when practical, if the study is short-term or seasonal.

4.3.5. Radio-telemetry

Radio-telemetry is a useful method for locating and tracking medium-sized and large mammals where the animal's location is difficult or impossible to monitor by frequent live-trapping or direct observation. This method is appropriate for use on mammals that can carry a transmitter and antenna with minimal impact on the individual. The transmitter is normally incorporated into a collar or harness that should be secured without restricting the animal's movement or causing abrasions. Collars placed on young, growing mammals should be of an expandable or break-away type if there is a low probability of recapturing the mammal for later removal of the collar. Surgical grade 'super glue' can be used to attach transmitters to smaller species; gluing it the directly to the animal's fur/ hair/skin ensures that the attachment is only temporary. For terrestrial mammals, the total package weight (collar, transmitter, battery, aerial and bonding material) should ideally be less than 5% and certainly must be no greater than 10% of the body weight.

Applicants must provide species-specific requirements and details of placement, attachment technique(s) and for how long the animal(s) will be carrying transmitters if the project requires the use of radio telemetry for locating and tracking mammals.

4.4. Blood/tissue samples

The techniques used for sampling blood and tissue from live mammals are usually invasive procedures. Thus, the advice of a veterinarian is essential as to the appropriate technique to use. Only trained, experienced personnel are to take blood and tissue samples from live animals.

Applicants must provide species-specific requirements and detail descriptions of any chemical(s), including dose rate(s) if the project requires the use of chemicals prior to taking blood or tissue samples.

4.4.1. Tissue samples - ear notching

Ear notching, (Refer to *4.3.1 Ear notching,* above, for detail) Tissue samples obtained as a result of marking small mammals should, were possible, be kept for molecular DNA analysis to minimise the need for subsequent additional tissue sampling.

4.4.2. Tissue samples - biopsy punch

A biopsy punch can be used to take tissue samples from live animals for molecular DNA analysis. An area is cleaned with cotton swabs soaked in 70% ethanol and allow to air-dry. Sterile disposable 2-3 mm diameter skin biopsy punches are used to aseptically obtain biopsy samples. Biopsied tissue is removed from the biopsy instrument and placed in 75% ethanol for storage.

4.4.2.1. Ear biopsy

The biopsy punch is used to take tissue samples from the ear of small to mid-sized mammals (e.g. mice, possums, quolls). Up to 2 samples can be taken, usually one from each ear. The biopsy punch is placed so as to avoid obvious veins in the ear. Pressure is applied to the ear to stop any minor bleeding and the animal is released immediately after processing. No anaesthesia is required.

4.5. Holding animals for short periods (less than 24 hours)

Captured mammals to be retained for brief periods (less than 24 hours), must be placed in appropriate holding cages that provide adequate space, ventilation, food, and a source of moisture. Furthermore, cages should have appropriate padding and bedding to ensure the comfort of the captive animals. Cages holding the animals need to be kept out of the sun, wind and rain; animals should always be kept at a comfortable temperature. Caged animals must be checked frequently to assess their wellbeing. Care also must be taken to minimize stress, by shielding cages from excessive light, noise, and human activities.

Applicants must provide species-specific requirements and details of when, where and for how long animals will be held for short periods if the project requires animals to be kept for less than 24 hours.

4.6. Transport

Captured mammals to be transported to a laboratory or holding facility must be placed in appropriate holding cages, which provide adequate ventilation, food, and a source of moisture, and will provide sufficient space with appropriate padding and bedding to ensure the comfort of captive mammals. Cages for transporting mammals must be kept out of the sun, wind, and rain and at a comfortable temperature. Captive animals must be checked frequently for well-being. It should be borne in mind that most field vehicles are not mobile laboratories and conditions in such vehicles may not be suitable for keeping animals. Again, care should be taken to minimise stress, by shielding cages from excessive light, noise, and human activities.

Applicants must provide species-specific requirements and details of when, where and how animals will be transported if the project requires animals to be transported.

4.7. Holding facility

Cages or enclosures constructed to house wild-caught mammals and their offspring in a holding facility should be designed to accommodate all important features of their ecology, morphology, physiology and behaviour.

Applicants must provide species-specific requirements and details of when, where and for how long animals will be kept in a holding facility if the project requires animals to be kept of extended periods.

4.8. Release

Field-caught mammals must be released only at the sites where they were captured. To do otherwise would potentially upset natural populations and reduce the chances for survival of released animals. More importantly, animals must be released as soon as possible after capture to minimise the stresses resulting from being in captivity. Also, consideration must be given to releasing mammals at times consistent with their normal daily and seasonal activity patterns.

4.9. Euthanasia

The AEC supports and adopts the euthanasia standards as outlined in the Euthanasia of Animals Used for Scientific Purposes 2001, Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART).

Applicants must identify the proposed euthanasia technique(s) and provide details of individuals and their appropriate experience, if the project requires animals to euthanised.

4.9.1. Rats and mice

4.9.1.1. Recommended technique - inhaled agents

Carbon dioxide (food, industrial or medical grade) from compressed cylinders can be passed through a pressure-reducing valve and piped into either a plastic bag enclosing cages or directly into a container into which the rats or mice have been placed. An optimal flow rate is one that displaces approximately 20% of the chamber volume per minute. This technique should be followed by cervical dislocation. The technique requires appropriate training and experience in the use of inhaled agents. Adequate ventilation must be provided in the room where this procedure is being carried out to avoid excess build-up of CO_2 in the room.

4.9.1.2. Recommended technique - injectable agents

Sodium pentobarbitone solution (350-400 mg/ml) is used at a dose rate of 10-15 mg/100g body weight. The preferred route of administration is intravenous but this may not be feasible unless the animal is already anaesthetised. Sodium pentobarbitone solution given by the intraperitoneal route may produce irritation of the peritoneum and pain prior to unconsciousness due to the solution's high alkalinity. It is suggested that a fast acting local anaesthetic solution be added to the barbiturate solution immediately prior to use. The technique requires appropriate training and experience in the use of injectable agents.

4.9.1.3. Acceptable with reservations - physical method

Cervical dislocation involves holding the animal prostrate on a bench with the thumb and forefinger of the operator firmly squeezing the neck behind the head of the animal. The hindquarters of the animal are grasped firmly with the free hand and pulled caudally. An instrument such as the blade of a pair of scissors, or a firm steel rod can be used instead of the thumb and forefinger. The technique requires appropriate training and experience.

The method requires that the applicant has demonstrated that they have had appropriate training and experience prior to applying this technique.

4.9.2. Mammals (other)

4.9.2.1. Recommended technique - injectable

Sodium pentobarbitone solution (350-400 mg/ml) is used at a dose rate of 150 mg/kg body weight intravenously whilst the animal is under anaesthetic or heavy sedation.

Applicants must provide species-specific detail descriptions of any chemical(s), including dose rate(s), or physical technique(s) proposed to euthanize mammals.

4.10. Disposal of dead animals and/ or biological material

All animals must be confirmed as dead before being disposed of.
Prompt and sanitary disposal of dead animals and waste materials must be in accordance with the jurisdiction's legislation and University standards.

Dead animals and biological sample material should, where possible, be made available for other teaching or research projects and/or lodged as museum specimens.

5. BIRDS

5.1. Capture Technique

5.1.1. Trapping – raptors

Nestlings are captured by climbing up to the nest, and secured by hand. Fledglings can also be hand-caught while in their day roost. Adult raptors of some species can be caught by using a nylon noose mounted on a surfcasting rod to slip around the body or feet of the bird at their roost. These techniques were developed for rare and threatened owls by Forsman (1983) and continue to be used in North America because they are safe and effective (Bull 1987) and have subsequently been used for threatened Australian owls (Hill & Lill 1998).

Common trapping methods for raptors include the 'Swedish Goshawk Trap' and Bal-chatri trap whose use is well described by Bloom, Clark and Kidd (2007) 'Capture Techniques' *in* Raptor Research and Management Techniques (2nd Edn) *Eds* David Bird and Keith Bildstein, Publ. Raptor Research Foundation – available from www.raptorresearchfoundation.org.

Swedish Goshawk Traps must be monitored at least twice per day and Bal-chatri traps at least hourly, preferably continuously. Trap status radio devices which signal the operator when triggered can be ideal and 'back to base' wildlife cameras which provide a continuous view of the trap from a computer if there is cell phone coverage.

Raptors are vulnerable to various injuries when handled e.g. talons piercing the opposite foot, and the talons of larger species can do serious harm to the researcher if the bird is not handled correctly. The hazards of working at height around nests also need to be properly managed, if applicable.

Applicants must provide details and a justification of the technique to be used for capture of a particular species

5.2. Handling

Any methods of physical restraint and handling need to ensure that no physical injury occurs and minimal stress is placed on the animal. The least amount of restraint and the shortest possible time necessary for the procedure or need to handle the animal is used. All persons intending to handle animals should be trained under the supervision of an experienced animal handler and must be able to demonstrate their ability to handle animals safely and responsibly before they handle animals on their own.

Handling, where possible, should be kept to a minimum. The bird's head should be covered in such a way as to cover the eyes and allow free air movement in order to reduce stress during capture and handling. The feet

of the bird will be secured and the wings will be held against the body in a position that will not harm the bird or personnel.

5.3. Marking

5.3.1. Banding

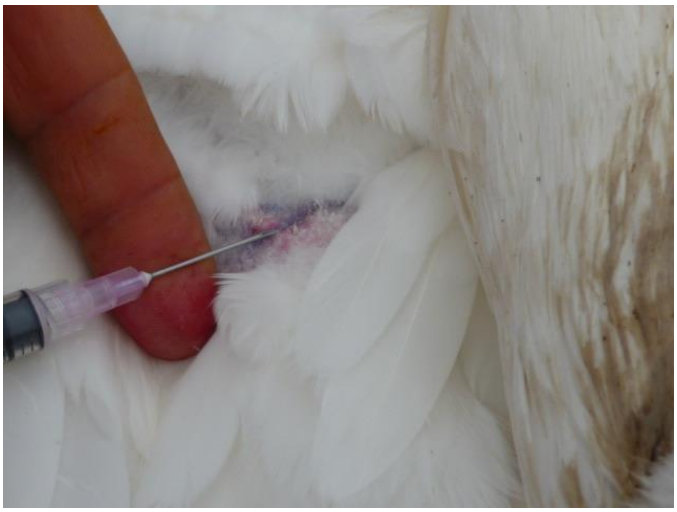
Nestlings and adults can be banded using stainless steel numbered Australian Bird and Bat Banding Scheme bands (Lowe 1989). Adults are often also fitted with an additional plastic coloured-band sealed with super-glue. Raptors often remove the plastic and so are generally fitted with a coloured aluminium band attached with two rivets.

5.3.2. Radio-transmitters

Birds can be fitted with a back-pack style Sirtrack® single-stage transmitter, attached to a string harness that fits like a vest and allows free movement. The string harness has a weak link designed to break if the bird becomes entangled by its transmitter and or harness (Karl & Clout 1987). Radios weigh 5.4 grams and 1g for the harness is 2.4 and 1.9% of total body weight for male and female respectively. Batteries will last 10-12 months.

5.4. Blood/tissue samples

There are currently no published standard procedures approved for taking of blood, tissue or other bodily fluids or samples from birds although wing vein is recommended as a method but requires two people and can cause haematoma very easily. Applicants must provide species-specific requirements and details of why, when, where tissue or fluid samples will be taken if the project requires birds to have samples taken.



5.5. Holding for short periods (less than 24 hours)

As a general rule, birds must not be captured and then housed. They should only be held for the time taken to complete an approved procedure. Birds are to be caught and held by hand until procedures (banding or fitting transmitters) are complete, then released within 15-20 minutes.

5.6. Transport

There are currently no published standard procedures approved for the transport of birds.

Applicants must provide species-specific requirements and details of why, when, where animals will be transported to if the project requires birds to be transported.

5.7. Holding facility

Applicants must provide species-specific requirements and details of when, where and for how long animals will be kept in a holding facility if the project requires animals to be kept for extended periods.

5.8. Release

Birds should be released where they were captured.

5.9. Euthanasia

The AEC supports and adopts the euthanasia standards as outlined in the Euthanasia of Animals Used for Scientific Purposes 2001, Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART).

Applicants must identify the proposed euthanasia technique(s) and provide details of individuals and their appropriate experience, if the project requires animals to be euthanized.

5.9.1. Recommended technique - Injectable agents

Sodium pentobarbitone solution (350-400 mg/ml) at a dose rate of at least 150 mg/kg is given by the intraperitoneal route in all birds. The intravenous route can be used in larger birds. The technique requires appropriate training and experience in the use of injectable agents.

5.10. Disposal of dead animals and/or biological material

All animals must be confirmed as dead before being disposed of.
Prompt and sanitary disposal of dead animals and waste materials must be in accordance with the jurisdiction's legislation and University standards.

Dead animals and biological sample material should, where possible, be made available for other teaching or research projects and/or lodged as museum specimens.

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