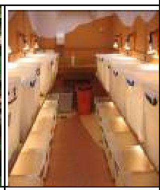
Flinders University

College of Science and Engineering

Standard Operating Procedure

For Working With Lizards 18/06/19



Animal Facility

SOP Number	AWC Approval Date	
SOP-BIOL-3-Lizard	18/06/2019	
Contact Person	**SOP prepared by**	**Review Date**
Leslie Morrison	Leslie Morrison	June 2021

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Legislation

- *Australian Code for the Care and Use of Animals for Scientific Purposes 8th Ed.*
- *Animal Welfare Act 1985*
- *Animal Welfare Regulations 2012*
- *Gene Technology Act 2000* (the Act)
- *Gene Technology Regulations 2001*
- *Work Health and Safety Regulations 2012*
- *Fisheries Management Act 2007* (Section 115)
- *South Australian National Parks and Wildlife Act 1972*

University Policy

- Work Health and Safety Policy 2013
- Responsible Conduct of Research Policy 2016
- NHMRC Guidelines

Local Policy

Use of the College of Science and Engineering Animal Facilities by all staff and students of the College of Science and Engineering, Flinders University, is subject to awareness of, and adherence to the following:

Research Involving Animals:

- The University holds a licence for the use of animals for teaching and research purposes. To satisfy the requirements of the licence, anyone wishing to undertake teaching and research using animals must submit a proposal to the Animal Welfare Sub-Committee (AWS-C). No work with animals may commence until written approval has been received from the Animal Welfare Committee (AWC). Standardised application forms for

Laboratory, Teaching and Wildlife work with animals can be found on the Flinders University Animal Welfare Committee website listed below. It is your responsibility to regularly check this site for updates to guidelines, forms etc.

http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics_home.cfm

- **All staff and students involved in animal research must complete Animal Ethics Online Training (AEOT) and must also regularly attend Animal Researcher Information Sessions (ARIS).**

Standard Operating Procedures

Refer to Risk assessments, Standard Operating Procedures and Safe Operating Procedures for chemicals, processes and plant equipment where appropriate. All projects must have an accompanying Risk Assessment signed by the Chief Investigator and submitted to the College of Science and Engineering OH&S Manager.

The following are a list of the main SOP's governing working with animals in the College of Science and Engineering. An extensive database of specific technique SOP's is also available from the Animal Facility Manager and on the AWC home page.

- **Standard Operating Procedures and Safe Work Procedures for the Use of the Animal Facility, Marine and Aquaculture Facilities**
- **Standard Operating Procedure for Working With Fish**
- **Standard Operating Procedure for Working With Lizards**
- **Standard Operating Procedure for Working With Birds**

Permits

- Any research to be undertaken in the field may require a permit from Department for Environment, Water and Natural resources(DEWNR) http://www.environment.sa.gov.au/licences-and-permits/Animals_in_captivity_permits

- Collection and live transport/holding of noxious species/declared pests will require a specific permit from The Department of Primary Industries and Resources of South Australia (PIRSA).

While your research may not involve animals as defined by the Australian Code, and therefore not require an application for the use of animals, it is necessary to provide details of organisms you propose to use to the AWC, so as to register their use and identify potential situations where an application will still be required. For example: marine or terrestrial invertebrate collecting which includes the 'by catch' of non-target animal species will require an application must be submitted to the AWC.

General

- Wash hands with disinfectant upon arrival at facility and before leaving.
- Refer to supporting Standard Operating Procedures and Safe Work Procedures.
- No Eating or drinking in areas housing animals.
- Wear closed shoes at all times (not thongs).
- Prior to submitting an application to the AWC, you must discuss space requirements with the Animal Facility Manager. Available facilities can then be matched to your project (with consideration of compatibility with other users, temperature, light cycle, housing type, length of project, etc).
- Report any health issues and animal incidents to the Animal Facility Manager and Animal Welfare Officer promptly (either in person, by phone, or email), and record details in the Communication Book.
- An Unexpected Adverse Event is an event that is not expected and was not foreshadowed in the application approved by the AWC.
- No animals can be housed in the facilities until your project has approval from the Animal Welfare Committee (if required) and *you* have a confirmed booking with appropriate housing for the animals, signed and submitted to the Animal Facility Manager.

Emergency Evacuation

- **In the event of an emergency evacuation, staff must move to local assembly points to await further instruction. If there is sufficient prior notice, lizards may be moved-in consultation with the project C.I. and as per *“SOP - Working with Lizards”* to a temporary new location that can maintain their husbandry requirements; until such time as they can be returned to the College of Science and Engineering Animal Facilities.**

Handling

- Wash hands and arms thoroughly before and after handling any animals to reduce risk of infection to animals, or transfer of zoonoses to users. Refer to the Animal Facility Safe Operating Procedures and Risk Assessment for any hazards or risks associated with animal handling.

- Hands must also be washed between handling unfamiliar animals (animals not housed in the same enclosure or from the same field site). Gloves and protective clothing should be worn where practical.

- The species being handled must be investigated by the researcher, and any additional risks like stings, bites, and potential zoonoses (not covered by the Animal Facility Risk Assessment) must be risk assessed in the project risk assessment prior to working with the particular species (e.g. snake bites, animals with sharp claws, etc).

- Lizards are often calmer when a small cloth/towel can be draped over the head to reduce visibility prior to and/or during handling. For larger animals, a calico bag can be used to cover the animals head.

- Ensure potential escape points such as doors and windows are firmly closed prior to handling.

- If animals are to be handled for moving, try to encourage them to move themselves into a transport container.

- To reduce stress on small lizards, a small mesh fish net can be used to capture them initially, before restraining by hand (Animal Facility staff will demonstrate this technique).

- After catching lizards, to safely hold them, firmly place palm over upper back of the body, and fingers over the head/shoulder area, and use the thumb and index finger to restrain side to side head movement. Take care not to apply pressure to the chest and abdomen, as this will restrict their breathing.

PHOTO: Support lower body of larger lizards, restrict head movement to prevent biting, net for initial capture of small lizards to help reduce stress.

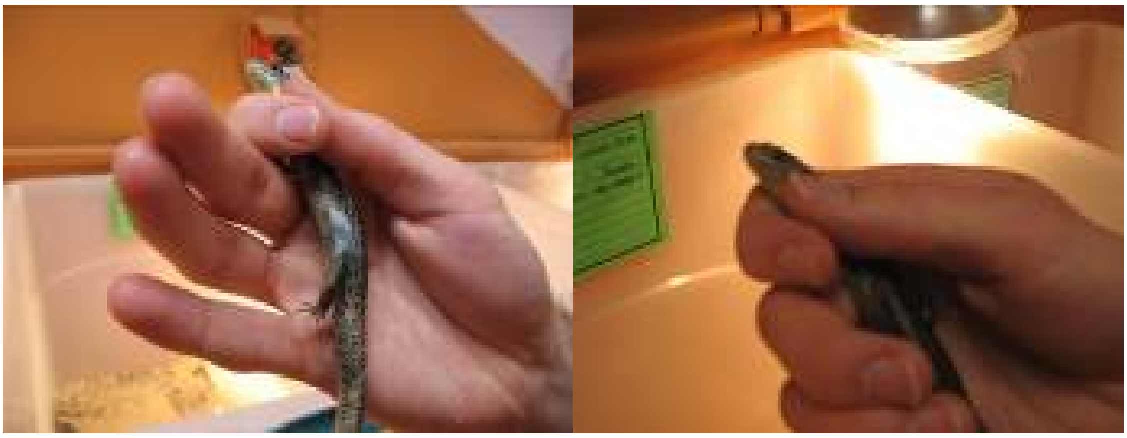

PHOTO: Gentle neck and shoulders grip so as not to compress lungs and abdomen.

- Animals should be immediately monitored (as per monitoring checklist) after handling, and then checked 1-2 hours after to assess signs of stress. Darkening the enclosure, the use of a red light, and/or providing a hiding spot for the animal can reduce post handling stress.

- Minimise handling whenever possible.

Capture Techniques

- If you observe any eye or nasal discharge from lizards captured in the field (that are to remain in the field), when practical, take a swab of the discharge for later diagnosis.

By Hand

- If you are capturing lizards in rock crevices, do not move any rocks that you are not able to lift and move to a secure location adjacent to the crevice. There is a risk you may encounter a venomous snake when lifting a rock or lizards that move due to the disturbance. In either case, you need to be able to safely position the rock back in its original position without injuring animals beneath, or move it adjacent to free up your hands for capturing lizards.

- Hand capture restraint is as illustrated in the “Handling” section of this SOP.

By Mealworm Lure

- This capture method only works in warm conditions when lizards are alert and active. Because lizards are ectothermic, they cannot be captured in this way unless conditions are warm enough, as they will not respond to lures in cold conditions.
- Small to medium skink: up to 150mm snout-to-vent length.
- Equipment: Fishing rod (6ft or 180cm extendible rod works well), thin polymer fishing line or heavy duty cotton thread (e.g. 12lb; 0.30mm), and mealworms.

1. Cut a 10-15cm length of fishing line, and tie one end onto the fishing rod.
2. Tie a small loop around a mealworm (this is the bait).
3. For pygmy bluetongues, find potential home sites by looking for spider burrows.
4. Once a burrow has been spotted, check for lizards with an optic fiberscope camera by carefully inserting scope into hole. Be careful not to insert too fast and to stop once a lizard is seen.
5. Move back 2 meters from the burrow and use fishing rod to tempt lizards to emerge from its burrow by dangling the tied meal worm on the fishing line at the burrow entrance.

6. As the lizard bites onto the mealworm, carefully withdraw the fishing rod with the lizard, which clings to its food reward, and capture the lizard, handling according to the “Handling” section of this SOP.
7. Preferably process (weigh, measure, and take samples (e.g. blood/tissue)) immediately after capture to reduce handling time to 5 minutes.
8. Place the lizard in a calico bag, or a breathable container, in a cool area out of direct sunlight if not processing immediately.
9. If lizards are being transported to the field house for extended periods, record the capture location via GPS or other method (e.g. flagging tape). Seal the burrow with jam jar lid and place a rock over the lid to prevent predators (e.g. centipedes or spiders) from entering the burrow.
10. Release lizards at the capture location. The pygmy bluetongue (*Tiliqua adelaidensis*), for example, retreats into burrows. If released away from the burrow from which it was captured, it may flee far from its home range in fright where it will be highly vulnerable to predation.
11. Only in warm conditions when the lizards are alert and active will they be able to respond rapidly and appropriately when they are released.

By Noose

- <u>Small</u> dragon: up to 150mm snout-to-vent length.
- Equipment: Fishing rod (6m extendible rod works well), and thin polymer fishing line (e.g. 12lb; 0.30mm).

1. Cut a 10-15cm length of fishing line, and tie one end onto the fishing rod.
2. Tie a small loop (few millimetres wide) and then pull the fishing line through to make a larger loop (this is the noose).
3. Once a lizard has been spotted, approach to within the length of the fishing rod, moving slowly.
4. Slowly extend the fishing rod and place the noose so it is around the lizards’ neck. Pull up gently until the noose is secured around the lizards’ neck.
5. Once the lizard is secure, place the fishing rod on the ground. Do not hang the lizard in the air by the noose as this may cause injury. Remove the lizard from the noose by holding the lizard in one hand and loosening the noose with the other. Lizards should be held according to the “Handling” section of this SOP.
6. Immediately place the lizard in a calico bag, or a breathable container, in a cool area out of direct sunlight.
7. If releasing, record the capture location via GPS or other method (e.g. flagging tape).
8. Release lizards at the capture location. Consider the behaviour and biology of the particular species captured. For example, the tawny dragon (*Ctenophorus decresii*) retreats into rock crevices close to basking sites. If released on top of the rock upon which it was captured, it may flee far from its home range in

fright. This could be avoided by releasing lizards into the nearest crevice to the capture location, after safely checking for predators (such as snakes).

9. Careful considerations should be given to environmental temperature, and conditions for capture and release.

- Monitor or other large lizard: up to 360mm snout-to-vent length.
- Equipment: Fishing rod (6m extendible rod works well), and shoe lace or twine (do not use polymer fishing line) dependent on size of lizard (the larger the lizard, the thicker the string required).

1. Cut a 40cm length of twine, or use a boot shoe lace, and tie one end onto the fishing rod.
2. Tie a small loop (few millimetres wide) and then pull the twine/shoe lace through to make a larger loop (this is the noose).
3. Once a lizard has been spotted, approach to within the length of the fishing rod, moving slowly.
4. Slowly extend the fishing rod and place the noose so it is around the lizards' neck. Pull up gently until the noose is secured around the lizards' neck.
5. Once the lizard is secure, hold on to it, but do not hang the lizard in the air by the noose as this may cause injury. The fishing rod can be placed on the ground, once the lizard has been secured in one hand. Remove the lizard from the noose by holding the lizard in one hand and loosening the noose with the other. Lizards should be held according to the "Handling" section of this SOP.
6. Immediately place the lizard in a calico bag, or a breathable container, in a cool area out of direct sunlight.
7. If releasing, record the capture location via GPS or other method (e.g. flagging tape).
8. Release lizards at the capture location. Consider the behaviour and biology of the particular species captured.
9. Careful considerations should be given to environmental temperature, and conditions for capture and release.

By Elliott-Style Box Traps

- Elliott (and Sherman) traps are collapsible aluminium or galvanised steel box traps. When the animals enters far enough inside the trap, their weight releases a latch, and the door closes behind them.
- Both Elliott and Sherman traps are manufactured in a range of sizes, suitable for both small and medium lizards.

- You **must** use either shade cloth, or place the traps in shade, when temperature is predicted to be over 30°C.

Box Trap Guide for the Capture of Small and Medium Sized Lizards

	Small Lizards (e.g. Gidgee Skinks) ≤ 22cm TL	**Medium Lizards** (e.g. Cunningham's Skinks) ≤ 35cm TL
Sherman	"Medium Type A" 30 x 10 x 8 cm	"Large Type F" 38 x 12 x 11 cm
Elliott	Medium Aluminium 33 x 9 x 10 cm	Large Aluminium 46 x 15 x 15.5 cm

- Your experimental design and Animal Ethics Proposal must clearly state:

1. An indicative number of traps you will be setting, including a clearly marked map of their locations and/or GPS coordinates.

2. The number of people checking traps, and anticipated time to check and clear all traps.
3. If the traps will be baited and (if so) how ants will be minimised. Generally, traps are unbaited for crevice dwelling animals such as gidgee skinks.
4. The time traps will be set and subsequently checked and either closed/collected or reset.
5. Consideration must be given to behaviour of the species (when they are active, basking, looking for food, etc), potential predators, forecast temperatures (extreme heat, heavy rains), placement of traps (do they need to be shaded), and cut off points (length of time, temperature, etc) provided to ensure minimal stress to the animals.
6. First check what kind of animal is in the trap. The first clue will be the weight of the trap when picked up. Gently push in one end of the trap so that you can see inside but can quickly close the trap if the animal attempts to escape.
7. If it is a non-target animal, then choose a sheltered location to release the animal.
8. To release smaller non-target animals, hold the trap upright and push down on the opening, then and lay the trap on the ground and point the opening away from you. The animal should run out. If not, place the trap on the ground and pull the pin on the trap. Large (i.e. too large to depress flap on the trap) non-target species may need to be realised in this manner.
9. If the animal is venomous, take particular care. Choose a location where it is possible to place the trap on the ground in a vertical position, pull the pin in the side of the trap to release the animal, and quickly move away from the trap.
10. To remove target animals from the trap, depress the door until it clicks open (or manually hold it open), and then gently capture the lizard with your hand. Care must be taken to ensure that the occupant of the trap is not crushed when the door is opened to extract the animal.
11. If the animal is large, such that there is little room to open the door, then place the entire trap in an appropriate calico bag and pull the pin on the side of the trap. Ensure that the animal is at the bottom of the bag and extract the trap before securing the top of the bag.
12. There is some variation in Elliott-style box trap design. Ensure you are using a trap that allows you to thoroughly check the entire trap. Small skinks/juveniles will potentially hide under the open flap of the trap.
13. Traps should be cleaned before being used again.

Transport

General:

- Transport must be supervised at all times by a researcher, or other person suitably familiar with the species of animal being transported.
- Where transport occurs between remote locations, and the distance travelled necessitates an overnight stop or stops, animals must be supplied with water and maintained within their ideal environmental temperature range.

- All carrier boxes should be labelled with the following:
  1. Project approval number,
  2. Species and numbers,
  3. Name and contact details of person responsible for transport, and
  4. Emergency contact details.

- Feeding prior to transport where lizards/snakes are being held within a controlled environment:

  (i) Feeding should be ceased three days prior to transport where it is anticipated that the temperature can be maintained between 25˚C and 30˚C. This could be expected to occur in the instance where animals are being transported between local institutions.

  (ii) Feeding should be ceased 1 week prior to transport where it is anticipated that the temperature <u>CANNOT</u> be maintained between 25˚C and 30˚C. This could be expected to occur in the instance where animals are being transported from remote locations, or where they are to be released back into the wild, and require acclimatization to a daily temperature between 20˚C and 25˚C.

- All animals should be assessed by the Chief Investigator, or other person suitably familiar with the species of animal being transported, and confirmed as healthy prior to transport. A visual examination is sufficient (examination should be consistent with parameters on animal monitoring records and lizard assessment checklist).

- Any animal with a Clinical Record Sheet should not be transported without consultation first with the Animal Welfare Officer and the Animal Facility Manager. Contact details are listed at the end of all Standard Operating Procedures (including this SOP).

- Large lizards (greater than 200g) should be transported individually, or in groups of up to 3 (to reduce stress if they are a social species). Carrier boxes for transport are available from the Animal Facility. No other transport boxes can be used without prior approval from the Manager of the Animal Facility.

- Small lizards (less than 200g) are transported individually in tied calico bags (available from the Animal Facility), which are then placed in an esky. Bags should only be placed in a single layer on the bottom of the esky.

- Snakes will be placed in securely tied calico bags, and these bags will then be placed in clip-lock containers. A sign will be fixed to the top of the transport box notifying all persons that the box contains venomous snakes (Sign text reads "*WARNING! VENOMOUS SNAKES*" along with investigator contact details). Any transport box containing a snake will remain with the investigator at all times. *At no time will a transport box containing a snake be left unsupervised by the investigator*.

- Vehicle requirements:
    - The objective is to maintain the area in which the animals are being transported at an ambient temperature approximately between 20˚C and 30˚C (some species prefer slightly higher temperatures and you must confirm preferred conditions prior to transport).Where temperatures are expected to exceed this range, the vehicle must be capable of effectively heating and cooling this area to maintain the temperature within this range.
    - **Animals must be transported in the temperature controlled section of the vehicle (not the boot, tray of a ute, etc). Containers stacked in vehicles must allow for adequate ventilation and temperature control of each container**. **A temperature probe must be used.**

Admission into the Flinders University College of Science and Engineering Animal Facility:

- Where animals are transported to Flinders University College of Science and Engineering Animal Facility, they must be placed into prepared cages in a temperature-controlled room, and provided with ONLY water upon arrival. Animals must not be offered food until they have acclimatized for at least 24 hours.

- Animal Facility staff must be notified in advance of expected arrival day and time. Space booking and housing with appropriate environmental parameters must be confirmed prior to transport commencing.

- The Animal Facility staff will have allocated space to quarantine the animals for 2 weeks, so they must not come into contact with other animals housed at the university. This will help reduce the transfer of disease. Quarantined animals should have their separate nets, water bottles, and cleaning equipment allocated.

Release:

- Once lizards have been held captive at the Flinders University College of Science and Engineering Animal Facility, they are NOT routinely released back into the wild. However, Flinders University does have permission to release a limited number of specific species.

- Lizards/snakes that have been captured at remote sites and transported to a “sterile” holding area may be routinely released back into the wild. Notwithstanding this, the release must follow the AWC Approved Project protocol, and transport to the release location must adhere to this Standard Operating Procedure. Where lizards/snakes have been provided supplemental heat to raise the temperature to between 25˚C and 30˚C, and the maximum forecast temperature is anticipated to fall between 20˚C and 25˚C, the lizards will need to be acclimatized to the lower temperatures for a week prior to release.

Quarantine/Health Monitoring

- All animals should be assessed by experienced staff and confirmed as healthy prior to transport to the Animal Facility. A visual examination is sufficient (examination should be consistent with parameters on animal monitoring records and lizard assessment checklist).
- Lizards should be quarantined individually, or in pre-established pairs/social groups (eg: they were housed together at the facility they have come from, or were observed to be part of a pair/social group in the wild).
- Lizards may be quarantined in their individual tank in any of the designated lizard rooms.
- During quarantine, reptiles should be weighed and photographed for individual identification. If they are permanently being housed, either individually or in a pair where each individual can be clearly identified visually, tank number is sufficient identification. Records will be created for individual reptiles to maintain this information and ongoing health monitoring.
- During quarantine, reptiles will be acclimatised to their preferred temperature range and standard diet.
- During quarantine, reptiles will be monitored daily as per the checklist in the Animal Health Management SOP.
- At the end of the Quarantine period, all tanks and benches must be cleaned with either 70% ethanol or F10. Floors must be cleaned with bleach or hospital grade disinfectant.

<u>Lizards/Snakes and environment are monitored daily as follows:</u>

(i) Clean and ample water supply.
(ii) Appropriate room temperature and air circulation.
(iii) Any signs of discharge from eyes or nose.
(iv) Any signs of abnormal body shape.
(v) Swelling/fight injuries.
(vi) Abnormal movement.
(vii) Significant change in appetite.
(viii) Abnormal level of activity.
(ix) Abnormal respiration.
(x) Treatment.

- All health observations must be reported in the Communication Book in the Animal Facility hallway. Staff must be notified of urgent health issues as soon as possible, and in emergencies the vet can be contacted on the phone number listed next to the Animal Facility Office door or at the end of this SOP.

- A medication book in the Animal Facility lists all current treatments.

- Reptiles with shedding issues may be given a once off spray with Aloe Vera (kept in the fridge), and monitored/resprayed as required. Restrain by catching in a fish net and spray through net to minimise capture stress, if the species requires it.

Adverse Event Reporting

- An Unexpected Adverse Event is an event that is not expected, and was not foreshadowed in the application approved by the AWC.
- You must advise the Animal Facility Manager and the AWO as soon as possible when such an even occurs, but within 24 hours of the event.
- You must submit a report to the Animal Welfare Committee within 3 working days.
- The reporting form can be found here: http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/resources/forms.cfm
- All unexpected deaths must be necropsied.
- The Animal Facility Manager and AWO will work with you in the short term to stabilise the situation and maintain the animals, until the AWC has reviewed the incident and decided whether the incident is:
    1. An unexpected adverse event and the project may continue unmodified,
    2. An unexpected adverse event and the project may continue with modifications, or
    3. An expected adverse event and whether or not the project can continue, and if modifications are required.

Cleaning

Key points	Equipment/Stage
1. To clean lizards housed, you will require the following: (i) F10 in a spray bottle, (ii) Gloves, (iii) Squeezy bottle of water,	

(iv) Washcloth, (v) Bucket of warm water, (vi) Optional lab coat/face mask, and (vii) Newspaper.	
2. Put on gloves, and encourage lizard into the shelter.	
3. Remove or fold newspaper in half of tank where shelter is. Clean the exposed section of tank (including walls) with damp cloth to remove solid matter, and spray with F10. Thoroughly rinse cloth. Place new paper in this section, move shelter to clean paper, and repeat process with the remaining dirty section of tank and rinse cloth.	
4. Tip out old water, rinse or replace water bowl, and **refill to the top** with fresh water. Disinfect weekly by soaking in F10.	
5. Clean shelter or replace entirely.	
6. Check basking rocks and replace any dirty ones with fresh ones from store room.	
7. Put dirty basking bricks in a bucket of F10 to soak for a couple of hours. Scrub off any remaining poos and rinse thoroughly. Leave outside to dry off.	
8. Check lizards while cleaning and note any health issues in Communication Book.	

- For lizards kept on a sand substrate, clean water bowls, shelters, and tank walls as per steps above. Put on gloves and place petri dish in tank, and place poos in dish as collected, discarding into bin when finished.
- F10 is used to clean enclosures/housing and can also be used to spray hands in between handling different animals.

Housing

(PHOTO: Example)

- Standard Housing must provide a safe secure environment for the animals. Lizards are routinely housed on green matting to reduce incidence of respiratory infection. They must also be provided with a shelter and stable water supply *(see previous photo)*.

- Smaller species are also routinely provided with off cuts of vegetation for environmental enrichment.

- Each tank must also have access to a basking rock and heat lamp (the rock and water bowl holder also provide opportunities for the lizard to rub against a rough surface to aid shedding).

- All enclosures should be labelled with the following:
    1. Project approval number,
    2. Species and numbers,
    3. medications/recent health history, and
    4. Name and contact details of person responsible.

Outdoor Semi-Natural Housing

- Standard Housing must provide a safe secure environment for the animals.

(PHOTO: An example of outdoor semi-natural enclosure)

- Each enclosure of 3m x 3m will have two sets of crevices made from cement slabs *(see photo below – single layer but normally a double layer would be used).*

(PHOTO: Typical cement slab crevice (left photo) and underside of the slab showing permanently attached wood to prevent dislodgment of slab (right photo))

- One set of crevices should be under a heat lamp, which will be located in one corner. The other set of crevices should be away from the heat source to allow the lizards to choose a cooler site if required. Each tank must also have access to water.

- All enclosures should be labelled with the following:
  1. Project approval number,
  2. Species and numbers,
  3. medications/recent health history, and
  4. Name and contact details of person responsible.

Feeding

Under general holding conditions, lizards housed indoors are fed twice weekly. One of these feeds should be a veggie mix, and the other can be either a veggie mix meal or live crickets and mealworms.

- If no heat lamp/rock is present in the aquarium/pen, the room or outside temperature should be a minimum of 26 degrees on the day of feeding and for 2 to 3 days afterwards. The lizard will not be able to digest the food properly at lower temperatures.

Making up food:

- The aim is to provide a balanced diet. It is not necessary to use a food processor to blend food or use frozen mixed vegetables if you don't have access to this equipment. Substitute more of the other listed ingredients in this case. This consists of a mix of cooked frozen mixed vegetables, watermelon, spinach, banana, broccoli, apple, boiled eggs (shell removed), and a scoop of reptile supplement.

- Food processing ingredients is ideal for small skinks as it allows them to lick at the food.
    - Alternatively, finely chop food (raw carrot can be grated).
    - Remove shell from boiled eggs.
    - Add reptile supplement as per instructions on packet (if instructions no longer present, a small pinch on each lizards plate of food is fine).

- As a guide, food should be chopped to dice sized pieces for sleepy lizards, and progressively smaller for smaller lizard species.

- When all ingredients have been chopped up or processed, mix together in a tub and keep covered and refrigerated.

- Approximately 1 heaped table spoon of food per Sleepy lizard per feed is sufficient, a standard tablespoon measure for Gidgee skinks, and a heaped teaspoon for small skinks.

- Space feeds a couple of days apart (eg. Mondays and Thursdays). It is not necessary to feed more than twice per week.

Attaching Ticks to Lizards

- This procedure is used for investigating transmission of ticks within a lizard social network. The aim of this procedure is to attach a certain number of ticks to a lizard, and the lizard will either be previously fitted with a GPS unit or transmitter. The procedure may need to be repeated (as described below) in order for the required number of ticks to attach to the lizard.

- Prior to capturing lizards (which have already been fitted with GPS units or transmitters in previous surveys), ticks should be counted and housed in small sample jars (~50ml sized containers) with a small amount of tissue as a substrate.

1. Wash hands with disinfectant prior to handling any lizards.
2. Next, capture the lizard from the field site and bring it back to the field house (following SOP for capture and transport of lizard) or Flinders University Animal Facility. This is usually done once the lizard has become inactive if GPS Units are attached, or at any time if the lizard only has a radio tag attached. Lizards are normally held in captivity for just 24 hours, but it may take longer if the required number of ticks do not attach on the first day.
3. To reduce stress to the animal, and to prevent the escape of ticks, use a tub and a calico bag. The bag must be clean and used for only one lizard between washes, and the tub must be sterilised with 70% ethanol between lizards. Place the lizard in the bag, over the tub.
4. Ensure that tick larvae have climbed onto the tissue substrate provided, and count the larvae. Place the tissue in with the lizard, preferably placing the tissue so as to touch the lizard. Repeat until the required number of larvae are in the bag with the lizard (e.g. 200).
5. Fold down the top of the lizard bag, and zip tie shut to ensure the lizard and ticks cannot get out.
6. The lizard is then kept in its bag, in an individual box (from any other lizards held), either overnight (GPS) or for at least 8 hours (transmitter only), depending on the purpose of the lizard in the study.
7. After this time, open the bag and remove the tissue. If ticks are loose in the bag, manually place larvae onto the lizard. Fold and zip tie the bag again.
8. Hold for another 3 hours.
9. Over the tub, remove the lizard from the bag and conduct a tick count, making note of location of larvae on the lizard’s body. Again manually place any unattached larvae onto the lizard before zip tie-ing the bag again.
10. Lizards are then released back to the field site, to the location of capture (before the lizards are active if GPS units attached).
11. The lizard bag is then zip tied and placed into a large zip lock bag so that it can be further inspected.
12. Once back at the field house, open the zip lock bag over a tub and invert the lizard bag into the tub.
    a) Make note of live and dead larvae seen in the bag.

b) These larvae are then collected in 99% ethanol for genetic analysis.

c) Ensure tub is disinfected between tick clutches, using 70% ethanol to ensure removal of all ticks.

d) The lizard bag is then returned to the zip locked bag to ensure that it is clear that this bag is used and needs cleaning.

❖ **Example of data recorded when attaching larvae:**

Lizard Number:

Capture time:

GPS Location:

Larvae clutch id:

Number of larvae initially in bag:

Number of larvae not attached to lizard: Live: Dead:

Number of larvae seen attached to the lizard:

Time lizard released:

Taking Nasal and Oral Swabs

- Nasal and oral swabbing is a relatively non-invasive technique used to collect tissue for DNA extraction, and/or collect parasite/pathogen samples.

- Careful consideration should be given to environmental temperature and conditions for capture and release.

1. Place a sterile swab (3 mm x 10 mm) and the preservative solution (100 mM Tris pH 8.0, 100 mM EDTA, 10 mM NaCl, 0.5% sodium dodecyl sulfate), or RNALater, nearby to ensure it can be easily accessed.
2. Secure the lizard in your hands (can use a calico bag if it is a medium to large lizard).
3. Gently open the lizard's mouth (for oral swabs) and hold open with one hand. Using the other hand, gently run the swab on the roof of the mouth at the back for about 2 seconds. For nasal swabs, rub the swab gently around the nostril area picking up any mucus that is there.
4. Place the swab in the preservative/buffer solution.
5. Secure the lizard in the calico bag and place in the shade, out of direct sunlight, for a maximum of 30 minutes. Before being released, the animal should be assessed as normal. If after 30 minutes of rest in a bag any signs of distress are noted, then you must consult with the AWO.

Taking Cloacal Swabs

- Cloacal swabbing is a relatively non-invasive technique used to collect tissue for DNA extraction, and/or collect parasite samples.

- Careful consideration should be given to environmental temperature and conditions for capture and release.

1. Place a sterile swab (3 mm x 10 mm) and the preservative solution (100 mM Tris pH 8.0, 100 mM EDTA, 10 mM NaCl, 0.5% sodium dodecyl sulfate) nearby to ensure it can be easily accessed.
2. Secure the body of the lizard in a calico bag, and control the legs in one hand. Allow the lizards back legs and tail to protrude out of the end of the calico bag. Lizards are likely to feel safer if their head is covered.
3. Turn the lizard onto its side, with the ventral side facing inwards.
4. Clean the area surrounding the cloaca with gauze and a watered-down (50% water) betadine solution.
5. Using the sterile swab, push down gently at the base of the tail, just posterior to the cloaca, to create an opening. Insert the cotton swab ~ 10 mm inside the cloaca, and carefully rotate before removing.
6. Place the swab in preservative.
7. Secure the lizard in the calico bag and place in the shade, out of direct sunlight, for a maximum of 30 minutes. Before being released, the animal should be assessed as normal. If after 30 minutes of rest in a bag any signs of distress are noted, then you must consult with the AWO.

Taking Cloacal Temperature Probe Measurements

- Using a temperature meter and probe (e.g. Instrument Choice, IC-YC-821 2 channel K-type temperature meter and TP-01 K-type probe) inserted into the cloaca is an accurate way of measuring lizard body temperature. The K-type probe has a diameter of approximately 1mm.

- Careful consideration should be given to environmental temperature and conditions for capture and release.

1. First, sterilise the end of the probe with a 90% ethanol solution to ensure pathogens are not transmitted among lizards.
2. Secure the body of the lizard in a calico bag and control the legs in one hand. Allow the lizards back legs and tail to protrude out of the end of the calico bag. Lizards are likely to feel safer if their head is covered.
3. Turn the lizard onto its back, with the ventral side facing upwards.

4. Clean the area surrounding the cloaca with gauze soaked in a watered-down (50% water) betadine solution.
5. Using the end of the probe, gently pull back the skin covering the cloaca until the cloaca is observed. Insert the probe 2-3 mm inside the cloaca and hold until the temperature is recorded.
6. Secure the lizard in the calico bag and place in the shade, out of direct sunlight, for a maximum of 30 minutes. Before being released, the animal should be assessed as normal. If after 30 minutes of rest in a bag any signs of distress are noted, then you must consult with the AWO.

Identification

- Lizard identification will depend on the project location and design, and the nature of the species (eg: social, solitary).
- Common and minimally invasive methods include individual housing with labelled enclosures, and nontoxic paint marking. Paint marking is not suitable for long term studies, as it will come off when lizards moult and may also affect behaviour in species. The labelled tape in the illustration below can also be used for temporary marking. A small, labelled strip can be placed along the side of the lizards body, behind the front leg.

http://www.tesa.com/consumer/adhesive_tapes/cloth_tapes/tesa_extra_power_perfect,c.html

- Toe clipping, microchipping, and blood sampling are all methods that may be used for identification, and may also provide an opportunity to gather other valuable data, thus reducing the overall number of procedures on the animals.

Toe Clipping

- The purpose of this procedure is to identify lizards when no other suitable, less invasive method is available.
- Toe clipping must not be used on climbing species without detailed justification as to why there are no suitable alternatives.

1. Prepare topical anaesthetic (0.5% w/v solution of Lignocaine):
    a. 7.5 ml of 2% lignocaine, and
    b. 22.5 ml saline from a nasal saline spray bottle.
2. Wash hands with disinfectant before handling lizards.
3. Restrain lizard as per “Handling” section of this SOP.
4. Toe clip scissors must be cleaned with 70% ethanol prior to use.
5. Scissors should be positioned to take no more than the terminal joint.
6. Post clipping, spray toe with the lignocaine mix, taking care to point at a slight downward angle so that spray does not contact lizards face.
7. No more than two toes per foot on each of four feet may be clipped to identify.
8. Toe clip scissors must be thoroughly cleaned before clipping next lizard.
9. Hands must be disinfected before handling next lizard, or gloves replaced if lizards have been captured from different sites.
10. Upon completion of all toe clipping, hands must be washed with disinfectant and toe clip scissors must be disinfected with 70% ethanol.

Attaching Radio Transmitter’s and GPS Units to Lizards

- Prior to attaching a GPS unit or transmitter, it is important to ensure that the selected lizard is of a suitable body condition/health for the procedure (the unit should not exceed 10% of lizard mass). Lizards which are underweight (low body mass for SVL or thin tail), or show signs of illness (sluggish, discharge from eyes or mouth, visible abnormalities to body), should not be used.
- Some species that are crevice dwelling or live in holes may not be suitable for attaching GPS units to (For example: gidgee skinks or pygmy bluetongues).
- Ensure that your hands have been disinfected prior to handling lizards, and that lizards are handled and capture as per the animal “Handling” and “Capture Techniques” sections of this SOP.

1. Place the lizard’s body into a calico bag, so that only the tail is exposed/out of the bag (this makes attaching the transmitter/GPS unit easier and also reduces stress for the lizard).

2. Provide a surface for the lizard to sit on in the bag, such as a lap, leg, or clipboard, and rest the bag containing the lizard on this surface.
3. Hold the transmitter/GPS unit (on dorsal surface of lizards tail) and antenna (parallel to the body, facing caudally so as to move in the direction of the tail) in place, and wrap leucoplast (2.5cm or 5cm width adhesive tape) around the tail up to 2.5 times (~20cm of tape). (For sleepy lizards ~ 25g (35mmX25mmX10mm) GPS unit/transmitter should be chosen (or less than 4% of the average body weight of an adult lizard for other species)).
4. Ensure that the tape is wrapped close enough to the scales to ensure large debris does not get caught under the tape, but not tight enough to put pressure on the lizards tail. Also make sure the vent is clear.
5. Minimize exposure to hot sun (to prevent overheating).
6. Once the transmitter/GPS unit is attached, release the lizard at the location where it was captured, and monitor its movement for its first few steps to ensure that it is able to move freely.
7. When the experiment is completed, generally after several months but definitely before the end of the active season, the units and tape should be carefully removed. To avoid stress to the lizard, place the lizard head and cranial half of its body in a calico bag. Use a sharp pair of scissors to cut the tape off the lizard. The tape should be cut off on the dorsal side of the animal to avoid the softer underbelly. Gently prise the tape upwards and cut off.

- **Note:** If the lizard’s body condition declines more than expected during the season, and the lizard appears to be in poor condition (underweight, sluggish, discharge from eyes or mouth), the lizard is no longer suitable for tracking and should have the unit removed. A monitoring sheet should record this intervention.

Obtaining Bite Force Data From a Lizard

- Bite force is used as a measurement of lizard body condition.
- Bite force performance can be measured using a various devices. Often these are custom built from a Kistler transducer that can accurately measure bite forces between 0.2 and 50 N ±0.1 N. The bite plate surface should be covered in leather to protect the animal’s jaw and provide grip.
- This procedure is best done with two people.

1. Hold the lizard in a relaxed grip so its forelegs are supported by your index finger.
2. Present the bite force transducer in front of the lizards nose.
3. The lizard will likely then bite the leather covered plates.

4. Video the bite from a side angle (it is important that you can clearly see the external ear and a fairly straight line to the tip of the snout). Verbally read out the baseline reading on the handheld recording device (usually 0.0 – 1.0).

PHOTO: Lizard biting a bite force transducer.

PHOTO: Lizard biting a bite force transducer.

PHOTO: Handheld recording device.

PHOTO: Lizard biting a bite force transducer.

5. If the lizard does not readily bite the transducer, gently tap it on the nose to illicit a defensive gape. Then position the transducer in front of the snout and allow the lizard to bite.
6. The lizard will normally release its bite after 2-3 mins. If the lizard continues to hold on to the device, place a calico bag over the lizard and the device, and the lizard will often then release its grip.

7. Place the lizard in a calico bag for up to 30 minutes after the procedure, and check on its wellbeing before releasing the lizard.

For more information, see *Lappin AKL, Jones MEH. 2014. Reliable quantification of bite-force performance requires use of appropriate biting substrate and standardization of bite out-lever. Journal of Experimental Biology 217: 4304-4312. DOI:10.1242/jeb.106385.*

Determining Lizard Body Temperature Using an Infrared Handheld Thermometer

- A lizards body temperature can be taken with a handheld infrared thermometer. Care must be taken not to shine the laser into a lizards eyes on devices that have a laser pointer to guide the pointing of the thermometer. Users should be supervised by an experienced user, or the Animal Welfare Officer (AWO), upon first use.

  1. To take a temperature without catching the lizard, carefully point the laser spot at the body, or neck, of the lizard.
  2. Take measurement.
  3. If the lizard moves, release the button to ensure the lizards eyes are not contacted by the laser.
  4. Minimise the possibility of shining the laser into the lizards eyes by taking temperatures from behind the lizard at a distance of 1-2 meters.

Blood Sampling

- Lizards should be calmed by placing them in a cool area out of direct sunlight for up to 30 minutes before sampling.
- Careful consideration should be given to environmental temperature and conditions for capture <u>and</u> release.

- **<u>Site to use:</u>** The ventral caudal vein is recommended for blood collection in most lizards. For those prone to tail loss, the abdominal vein can be used, but the vein is fragile. The jugular venipuncture is possible in monitor lizards. Sampling from the abdominal vein should be conducted under anaesthetic.
- **<u>Needle:</u>** A 23-27G needle depending on the size of the animal.

- **<u>For a once off bleed:</u>** The blood volume of a lizard is about 7% of total body weight, and you don't want to take more than 10% of that blood volume (0.07*0.10 = 0.007). E.g. If an individual weighs 30g (0.007*30g), no more than 0.21ml (210µl) of blood should be taken.
- **<u>For repeat bleeds:</u>** You don't want to take more than 7% of that blood volume per week (0.07*0.07 = 0.0049). E.g. If an individual weighs 30g (0.0049*30g), no more than 0.147ml (147µl) of blood should be taken.

From the caudal vein:

1. Prepare a 23-27 gauge needle so it is accessible with one hand. Remove the cap of the needle and place both cap and needle on a sterile surface.
2. Remove lizard from bag and hold with ventral surface up, and lizard tail towards your body. Covering lizards head in a calico bag can sometimes help to calm lizards.
3. Swab tail area with betadine below cloaca to half way down tail.
4. Insert needle under scales in line with the middle of the tail, approximately a third way down the tail.
5. Hold needle near vertical and gently push into tail until from the resistance from the backbone is felt.
6. Carefully withdraw plunger a small way and note if blood comes into needle body. If no blood comes, move needle gently to one side or the other whilst in the tail and try again.
7. Keep drawing back if successful until desired volume of blood is withdrawn.
8. Withdraw needle and store blood (e.g. FTA card).
9. Secure the lizard in the calico bag and place in the shade, out of direct sunlight, for a maximum of 30 minutes. Before being released, the animal should be assessed as normal. If after 30 minutes of rest in a bag any signs of distress are noted, then you must consult with the AWO.

*(PHOTO: Approximate starting position of needle for **caudal vein blood collection**).*

From the *Sinus Angularis* (e.g. for small dragon lizards):

- Blood may be taken by puncturing the *sinus angularis* (accessed in the corner of the mouth), and collecting blood using a heparinised capillary tube.
- Careful consideration should be given to environmental temperature and conditions for capture and release.

1. Prepare a 23-26 gauge needle and 1 mm diameter heparinised capillary tube, so they are accessible with one hand. Remove the cap of the needle and place both cap and needle on a sterile surface.
2. Hold the body of the lizard and control the legs, in one hand. Allow the head to protrude past the index finger *(use left hand if right-handed).*
3. To open the mouth: Use thumb of the hand holding the lizard to gently press on the base of the lower jaw, pulling towards the anterior of the lizard (this will usually cause the lizard to open its mouth).
4. To keep the mouth open: As soon as the lizard opens its mouth, place the cap of the needle (should be malleable plastic) in one corner of the lizards mouth. Hold the cap in place using the thumb and index finger of the hand holding the lizard.
5. Look for a dark redish/purplish area past the jaw muscles deep in the corner of the mouth (this is the *sinus angularis).*
6. To puncture the *sinus angularis*, hold the needle parallel to the bottom jaw and enter the needle tip 1-3 mm into the *sinus angularis*. Remove the needle and dispose.
7. Collect the blood quickly, before clotting occurs, using a 1 mm diameter heparinised capillary tube held on a 50-60 degree angle to the bottom jaw to ensure blood flows. Place the capillary tube in a sterile tube/container.
8. Gently apply pressure to the site with a sterile moist swab for 3-5 seconds to ensure blood flow has stopped.
9. Remove the cap from the corner of the lizards’ mouth and dispose.
10. Store the blood (e.g. FTA card or eppendorf tube).
11. Secure the lizard in the calico bag and place in the shade, out of direct sunlight, for a maximum of 30 minutes. Before being released, the animal should be assessed as normal. If after 30 minutes of rest in a bag any signs of distress are noted, then you must consult with the AWO.

*(PHOTO: Left - Opening mouth with cap and site of **sinus angularis).***

(PHOTO: Right - Blood flow into capillary tube).

Microchipping

- Lizards can be permanently marked using microchips for larger lizards, such as gidgee skinks. Note that juveniles may be too small to microchip and should be individually identified by another method, such as toe clipping. The DEWNR Policy on the use of microchips for marking wildlife is used as a reference for this modification/procedure.

- A sterile storage and application procedure should be adopted. Pre-sterilised microchips will be used, and chips will be contained within a dispenser that is packaged and sealed in a plastic vial and submersed in cold sterilisation solution.

- Chips will be removed with injector, which will be stored in the dispenser with the cold sterile solution (70% isopropyl alcohol). This procedure makes the use of antibiotics unnecessary.

- The implant site will be the left body side just in front of hind leg. Before implantation, the site will be swabbed with dilute topical antiseptic (betadine). Microchipping should occur in September/October, several months prior to the end of the active season, to allow the warm weather to facilitate full healing prior to cooler weather.

- Careful consideration should be given to environmental temperature and conditions for capture and release.

Procedure:

1. Prepare a microchip applicator with chip, so it is accessible with one hand.
2. Remove lizard from bag, and hold with ventral surface up and lizard tail towards your body. Covering lizards head in a calico bag can sometimes help to calm lizards.
3. Swab scales just above cloaca on left side of lizard with betadine.
4. Insert applicator under scales on left side (some pressure needs to be applied). Gently rock the applicator tip up and down to cut the skin.
5. Push the applicator in and dispense the microchip.
6. Remove applicator and apply a small amount of pressure with a sterile wipe.
7. Hold the animal for a few minutes to check any bleeding has stopped.
8. Place animal in calico bag once the bleeding has stopped and check on it in 15 minutes.
9. Secure the lizard, once the bleeding has stopped, in the calico bag and place in the shade, out of direct sunlight, for a maximum of 30 minutes. Before being released, the animal should be assessed as normal. If after 30 minutes of rest in a bag any signs of distress are noted then you must consult with the AWO.

Tail Clipping

- Careful consideration should be given to environmental temperature and conditions for capture and release.

Procedure:

1. Sterilise a sharp pair of surgical scissors in 90% ethanol, and place on a sterile surface in preparation.

2. Secure the body of the lizard in a calico bag and control the legs in one hand. Allow the lizards tail to protrude out of the end of the calico bag. Lizards are likely to feel safer if their head is covered.

3. In regards to tail-dropping species, take extreme care not to apply too much pressure to the tail.

4. Apply betadine to the end 10 mm of the tail using a swab or gauze.

5. Use the scissors to remove </= 5 mm of tail tip.

6. Store the tail tip (e.g. 90% ethanol).

7. Apply betadine to the wound using a new swab or piece of gauze and make sure blood has coagulated at the site.

8. Secure the lizard in the calico bag and place in the shade, out of direct sunlight, for a maximum of 30 minutes. Before being released, the animal should be assessed as normal. If after 30 minutes of rest in a bag any signs of distress are noted, then you must consult with the AWO.

Administering Medication

- Administering medication to animals may require handling. Users must specifically refer to the “Handling” section of this SOP.

- All medications and how they are administered must be approved by the AWC in the animal welfare application, when using animals that come under the animal ethics guidelines.

- Medication must only be administered by trained personnel.

- Prior to administration of medication, ensure you have a quiet location and all equipment required (eg: Appropriate gauge needles, alcohol swabs, collection vials, sharps bin, protective clothing, etc).

Analgesic and Anti-Inflammatory Drugs

Drug	Dose and Route	Comments
Carprofen	Initial dose 2-4 mg/kg Maintenance dose 1-2 mg/kg IV,IM,SC.PO q24-72h 1-4 mg/kg IV,IM,SC.PO q24h	Rehydrate chelonians before administration. Follow with ½ dose at 24 hours. Maximum treatment duration 3-5 days.
Meloxicam	0.1-0.3 mg/kg IV, IM, SC, PO q24-48h 0.2-0.4 mg/kg IV,IM,SC,PO	Rehydrate chelonians before administration. Maximum treatment 3-5 days.

Meloxicam/Metacam (commonly used in ACU for lizards) can be given:

- orally at a rate of 0.013ml per 100g.

  or

- by subcutaneous injection at a rate of 0.004ml/100g.

Dilution of 1 ml of Meloxicam (aqueous preparation for injection) in 9 ml of sterile saline is useful in smaller lizards, as it allows a more easily measureable volume to be administered.

Anaesthesia

Animal	Route	Anaesthetic	Dose
Lizards	Facemask	Isoflurane	5% in O_2
	Intraperitoneal (I/P)	Alfaxalone	10 – 30 mg/kg

Euthanasia and Humane Killing

Emergency Euthanasia

- For emergency field euthanasia of lizards (other than monitors, genus *Varanus*) that do not need to remain intact, destruction of the central nervous system by skull crush, followed by decapitation, is recommended.

- **For emergency field euthanasia of members of the genus *Varanus* (monitor lizards), use of a captive bolt or firearm is preferred, where the use of injectable anaesthetics may not be possible.**

Planned Humane Killing

- For planned humane killing of lizards, an intraperitoneal injection of pentobarbitone sodium (60mg/ml) should be given at a dose rate of 180mg/kg – This is equivalent to 0.3 mL/100g bodyweight.
    - For example, with a 300g lizard:
        - **0.3kg** (weight of lizard in kilograms) x **180mg/kg** (dose rate) divided by **60 mg/ml** (concentration of the pentobarbitone)
        - **= 0.9ml**

- **For very small lizards, such as those weighing less than 5 grams, oral pentobarbitone at a concentration of 6 mg/mL should be used. This may avoid undue pain associated with injections and prevent muscle spasms after death, which may distort voucher or anatomical specimens.**

- **Planned humane killing by skull crush followed by decapitation requires special justification in applications to the Animal Welfare Committee.**

- Placing lizards on a heat mat or in a warm room prior to procedures involving the use of pentobarbitone will facilitate the action of this drug.

Disposal

- Euthanised animals must be placed in a plastic bag and put in the carcass bin in the level 1 freezer (biology room 130). Disposal will be treated as medical waste and will be incinerated at Flinders Medical Centre.

SOP Review

This SOP currently applies to the animals housed in the College of Science and Engineering Animal Facility and field sites. This SOP will be reviewed 3 yearly, but also updated more frequently as policies, techniques and animal care requirements change.

Any questions regarding the above guidelines and any technical advice/ assistance required can be directed to Animal Facility Manager.

Position	Name	Contact Details
Animal Facility Manager	Leslie Morrison	X 12196 Office in Animal Facility Leslie.morrison@flinders.edu.au
Animal Welfare Officer	Lewis Vaughan	0450 424 143 awo@flinders.edu.au

Useful References:

- http://www.nhmrc.gov.au

- http://www.adelaide.edu.au/ANZCCART/
- http://www.environment.sa.gov.au
- http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics_home.cfm **(Link for Animal Incident Report forms, Teaching and Research Application Forms and all animal welfare related matters)**