Flinders University

College of Science and Engineering

Standard Operating Procedure

For Working with Small Birds 18/06/19



Animal Facility

SOP Number	AWC Approval Date	
SOP-BIOL-3-Birds	18/06/2019	
Contact Person	**SOP prepared by**	**Review Date**
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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.

The following are a list of the main SOP's governing working with animals in the College of Science and Engineering. See the AWC webpage for all current versions of SOP's.

- **Standard Operating Procedures and Safe Work Procedures for the Use of the Animal Facility, Marine and Aquaculture Facilities**
- **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 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.
- 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.
- **Prior to animals arriving, your space must set up, housing ready, and food and equipment organised.**

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, birds may be moved-in consultation with the project C.I. and as per *"SOP - Working with Birds"* 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.**

Quarantine, Housing, and Monitoring

- Wash hands before and after handling birds.
- All animals must 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 small bird assessment checklist).
- Birds must 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).
- Cages to be used during quarantine period must be approved by Animal Facility Management before birds are transported to the facility.
- Room 019a in the Animal Facility is the designated quarantine room for birds. Large colonies/birds may be quarantined in the larger outdoor cages behind the Animal Facility if space is available. Only birds from the same location/capture group can be quarantined outside at any one time, to avoid cross contamination with other bird colonies.
- During the Quarantine period, all birds will be wormed and treated for external parasites, with medication such as Moxidectin (5ml/L drinking water, mixed daily, and given for 3 days). Birds will also be banded, and a blood sample taken for identification and sex determination.
- Records will be created for individual birds to maintain this information and ongoing health monitoring.
- Birds should be visually assessed daily for a two week quarantine period, as per the small bird assessment checklist.
- Check food and water daily, and replace as required. Outdoor water containers should be cleaned out once per week, and more often in warmer months if there is any sign of algal growth.
- Indoor water and feed bowls are rinsed/refilled/replaced daily.
- While under experimental conditions, investigators are responsible for keeping their animal's enclosures clean, and maintaining food and water supply.
- Paper substrate is generally changed daily, but this frequency may be affected by number of birds in cage and whether they are laying eggs.

Birds are monitored daily as follows:

(i) Clean and ample water supply.

(ii) Appropriate temperature and air circulation.

(iii) Any signs of discharge from eyes or beak.

(iv) Any signs of abnormal body shape.

(v) Swelling/fight injuries.

(vi) Abnormal movement/balance.

(vii) Significant change in appetite.

(viii) Abnormal level of activity/ socialising.

(ix) Abnormal respiration.

- 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.
- At the end of the quarantine period, animals must be confirmed to be in good health by experienced staff before they are allocated to a project.
- At the end of the Quarantine period, all tanks and benches must be cleaned with either 70% or 99% ethanol, F10, or bleach/sodium hypochlorite. Floors can be cleaned with bleach, F10, or hospital grade disinfectant.
- Cleaning equipment must not be shared between quarantine and conventional rooms. Each room has F10 spray, sponges, a dustpan/broom, and a bin.
- Surfaces and floors should be wiped down and mopped a minimum of once per week in conventional rooms. Quarantine rooms must be wiped down and swept daily, and mopped a minimum of once per week.
- Any variation in the enclosures currently available for housing must be approved as part of your application to the AWC, including a photo or detailed description. Cage design must not compromise animal health and welfare monitoring.

Transport

- Food and water should be available before and during transport. Water should be in a sturdy container designed for transport, or containing cotton wool to prevent water slop.

- All animals should be assessed by the Researcher 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 small bird assessment checklist).

- Any bird 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.

- Strongly sexually dimorphic birds should be transported individually or in established pairs/social groups (eg: budgies, finches). Unfamiliar birds should not be transported in the same carrier box.

- Birds should be transported in small bird carrier boxes, which are available from the Animal Facility. You may provide your own transport cages but they must be approved by Animal Facility Manager prior to use. They must be comparable to the standard Animal Facility Transport carriers regarding minimum size and design (eg: Ventilation panels, solid walls, a lid or a cover to protect from weather/draughts, and a secure door).

- Vehicle requirements:

    - The objective is to maintain the area in which the birds are being transported at an ambient temperature of between 20˚C and 30˚C. 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. Care must also be taken to arrange cages to provide ventilation for all birds while in the vehicle.

- Examples *(photo below)* of suitable small bird transport boxes (must include perch and locking pin for door).

(PHOTO: Examples of suitable small bird transport boxes)

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

- Where birds are transported to Flinders University College of Science and Engineering Animal Facility, they must be placed into prepared cages in a temperature-controlled quarantine room, and provided with food and water.

Release:

- Birds may only be released as per conditions of DEWNR permit, and transport to the release location must adhere to this Standard Operating Procedure.

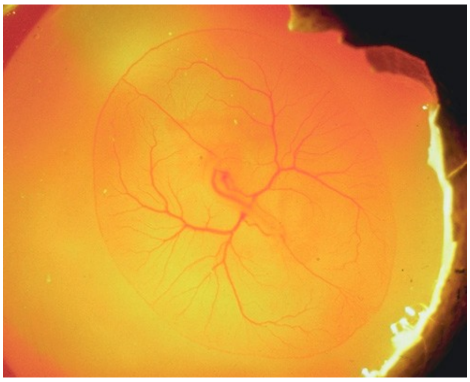
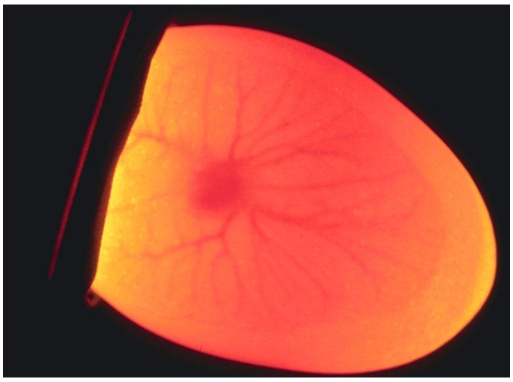
Egg Handling

General

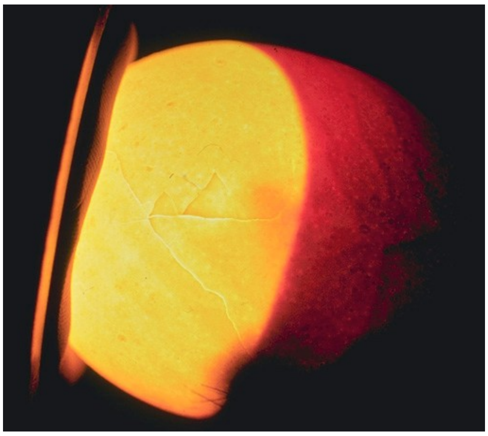
- Wash hands with disinfectant before and after handling eggs and birds.
- Refer to safe work procedure for administering medication/animal handling.
- **Record/check the nest ID and location via GPS, and using the appropriate data sheet.**
- Check for presence of eggs by inserting one finger inside the nest and gently touching the bottom of the nest (count the number of eggs by touch).
- To remove eggs, close hand gently around the egg and position the egg so it is sitting in the palm of the hand with the fingers slightly closed on top to protect the egg.
- Remove one egg at a time.
- Take extreme precaution not to apply to much pressure on the shell of the egg.
- To estimate the age of an egg, place the egg on the black soft seat of the egg candler *(see photo below)* and turn the light on. Alternatively, place the egg in the palm of hand with the hand as flat as possible and at eyes level. Direct a bright torch toward the inside of the eggs from the side.


(PHOTO: Egg candler)

- Four-six days old eggs, you should note a centrally located embryo and developing blood vessels *(see photos below).*

- When the eggs are close to hatching, you should note a light upper part enlarged air cell which is important for proper hatching. The dark, lower part contains the embryo *(see photo below).*

- **Do not remove eggs that are 1-2 days prior hatching time as this can disturb hatching.**

Bird 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 Rooms 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.

- Birds 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.

- For birds held indoors, switching off room lighting and having the background illumination of a nightlamp or red light globe appears to aid in keeping the bird calmer and less able to detect moment of capture (and thus less likely to fly about cage, increasing capture time and stress levels).

- If animals are to be handled for moving, try to encourage them to move themselves into a transport container, or alternatively use a net that will fit their size. For birds in particular, use a soft bird net to catch them and ask for help from the Animal Facility staff to reduce catching stress on the animal. If practicable, training to feed in transport container or suitable container in which to trap birds and allow easier capture for transfer to transport cages.

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

- After catching small birds, to safely hold them firmly place palm over upper back of the body, and fingers over the head, 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: Note head restraint to prevent biting, and gentle torso restraint so chest and keel are not compressed.
(Figure from "Everybird" Macwhirter, P. 1994)

Banding

- Wash hands with disinfectant before and after handling birds.
- Refer to Standard Operating procedure for Animal Handling and Safe work procedure for administering medication/animal handling.
- Researchers are provided with a banding kit containing:
    1. Bird bags,
    2. Metal rings,
    3. Plastic colour rings,
    4. Field logbook,
    5. Ringpliers,
    6. Balances and scales to measure weight,
    7. Stopped rulers for measuring wing and tail length, and
    8. Calipers for measuring bill and tarsus length.

1. After removing the bird from the bird bag, safely hold them by firmly placing the palm over upper back of the body, and fingers over the head. Use the thumb and index finger to restrain side to side head movement (this is known as the "banders grip").

2. Take care not to apply pressure to the chest and abdomen, as this will restrict their breathing.
3. Move the legs of the bird to one side so you can gently hold each individual leg to place the metal band *(see images below).*

(PHOTO: Bird banding)

4. Leg rings (metal ring & plastic colour rings) are fitted to the tarsometatarsus (below tarsus joint, not tibiotarsus above point).
5. Metal rings are fitted with special designed pliers.
6. Place one metal ring on the **left** leg of a bird.
    - (i) Hold the left leg of the bird and place the open metal ring around the tarsus.
    - (ii) Hold the open metal ring in place with your thumb and index finger.
    - (iii) Place the ringplier around the open metal ring, and gently close the metal ring.
    - (iv) Ensure there is no gap around the ring, and that the ends meet neatly, to reduce the risk of injury to the leg.
7. Plastic colour rings are fitted with a tool like a shoehorn *(a spoon, see image below).*
8. Place one colour ring above the metal ring on the **left** leg.
    - (i) Place the plastic ring on the spoon in such a way that it opens, and that the opening aligns with the hollow part of the spoon *(see image below).*
    - (ii) Place the spoon with the open plastic ring around the tarsus of the bird.
    - (iii) Gently slide the plastic onto the leg.
    - (iv) Check if the band is closed.

(PHOTO: Plastic colour ring)

(PHOTO: Plastic colour ring)

9. Place two plastic colour rings on the **right** leg of the bird as described above.

Example: YXYY

Band	L Left up	Alu (X) left down	R right up	R right down
Colour Code	*Yellow*	*X*	*Yellow*	*Yellow*

Mist Netting

General:

- In your Animal Ethics application, you must stipulate the size of the nets to be used, your target species, and expected numbers of both target and non-target species that you anticipate catching.
- You must detail how you will observe the nets, and the maximum time birds will be in the nets before retrieval.

Setting up Mist-Nets

1. Place nets close enough to each other that a person can visit all net locations in a maximum of 10-15 minutes walking, preferably less, if no birds are caught. On flat, level terrain, this array would be about 0.5-0.6 miles (800-1000 m) in length.
2. In order to operate nets properly, the trammels (the horizontal shelf strings that support the net) should be taut horizontally. These can be arranged at 120° angles to the net, with one end secured to the pole and the other to nearby rocks, bushes, or stakes.
3. When operated, the netting material should not be stretched apart to its full extent, but should allow some slack between the trammel lines; otherwise birds will bounce off the tight net.
4. When closing a net, spin it to keep it from unravelling.
5. Nets are commonly put in cloth bags. To take down the net, it is rolled up on small folds and put into the bag, as the biologist moves from one end of the net towards the other.

Time of Day and Number of Checks

- Nets should be opened within 15 minutes of local sunrise, and closed within 15 minutes before sunset.
- Nets should be checked every 20 minutes (more often in inclement or very hot weather).
- The nets should not be operated in rain, wind, and extreme heat. If already open when these conditions occur, they should be closed, because precipitation is heavy enough for the birds' feathers to become wet enough to lose their insulation.
- Be aware of predators (e.g. ants or birds of prey) around nets to ensure trapped birds are not at risk of injury or death from predators.

Removing Birds from Nets

1. First take the time necessary to figure out exactly how the bird went into the net.
2. Observe carefully from which side the bird entered the net, and between which trammels it went, in order to find the opening of the pocket the bird made.
3. Start on the side of the net that the bird entered; part the trammels and netting loosely, and look into the pocket caused by the weight of the bird. Because the tail is the last to enter, look at its position to get a clue about how the bird entered the net.
4. After determining where the bird entered, remove the bird with the first feet method.

Feet First Method:

1. Find out from which side of the net the bird entered. Find the opening of the pocket caused by the weight of the bird.
2. If you (the bander) are right-handed, grasp both tibiae (the tibia is the feathered part of the leg above the bare tarsus) from the rear of the bird using your left hand, so that your fingers point towards the bird's head. The bird should be upside down in the net when you have your grip (*see image below*).

(PHOTO: Feet first method)

3. Put your index finger between the tibiae, and press your thumb against the bird's right tibia, and your middle finger against the left tibia. This leaves your right hand free to remove net strands from the entangled legs and feet.
4. Most importantly, make certain that all threads are pulled down and off tibiae and thighs below the heel joint, the prominent joint between the tibia and tarsus. These threads are sometimes high up on the thigh at the flank.
5. Untangle the toes by pulling strands gently. You will notice that if the heel joint is straightened out, the bird's toes have a tendency to relax, so that the netting can be more easily removed. If the bird is clutching the net firmly, extract the feet by:
    (i) First freeing the opposable toe (the "thumb") by sliding the threads over it, and lifting it away from the other toes,
    (ii) With the fingers, straightening the other three toes out, and
    (iii) Sliding the netting over the toes with repeated strokes.
6. Pull the bird up and away from the net, still holding the bird upside down by the feathered tibiae, above the bare tarsus. Flick net threads from the bend of the wings, working from the underside. Generally, the thumb should be placed under the thread(s) on the underside of the wing, and the forefinger placed on the outer bend of the wing as a fulcrum to flick the thread over. Often at this stage it is helpful to pull gently on the exposed portions of the still tangled threads in order to free them, or to see where they are caught.
7. When both wings are free, pull remaining loops from around the neck, working from the back of the head forward. Be sure to secure the bill by placing the thumb against the tip while pulling the net over the head, in order to protect the delicate neck.

Processing Birds

1. After removing the bird from the net, safely hold them by firmly placing palm over upper back of the body, and fingers over the head, and use the thumb and index finger to restrain side to side head movement (this is known as the "banders grip").
2. Take care not to apply pressure to the chest and abdomen, as this will restrict their breathing

PHOTO: Note that restraining the head will prevent biting, gently hold torso so that the chest and keel are not compressed.
(Figure from "Everybird" Macwhirter, P. 1994)

3. Once the birds are removed from the nets, put each individual in a separate small cloth bag, and transport to the processing site.
4. Have a central processing site, rather than processing birds at each net as they are captured, because (1) a biologist rapidly circulating around the nets can better monitor the captures, in case of an influx of birds that might necessitate shutting down some nets temporarily; and (2) it lessens the disturbance in the vicinity of the nets. Further, if processing becomes delayed, it is always preferred to have the birds out of the nets and stored in bags.
5. Do not hold for longer than 60 minutes. If there appears to be adverse effects from the capture, such as apathy, gently drip glucose water across the bill.
6. Bags should be made from opaque cloth, and sewn so that the seams (and possible loose threads that can catch toes) are outside.
7. Hang bags from hooks or branches to prevent them from being stepped on, and out of direct sunlight.
8. Bags should be washed regularly.

Releasing Birds

1. Close the nets before releasing the birds to prevent recapturing already processed birds.
2. Release birds immediately after processing at the site of capture, no more than three metres away from the net.

Administering Medication

Anaesthesia

Considerable variation between strain and animal variation may occur when using anaesthetics. Investigators must research the species they are working with for current recommendations. Veterinary recommendations listed below.

Species	**Dose and Route**	**Chemical Restraint Agent**
Most species	Facemask	Isoflurane in O_2
Most species	Intramuscular or Intraperitoneal	Alfaxalone 10-30 mg/kg

Analgesic and Anti-inflammatory Drugs

Considerable difference between strain and animal variation may occur when using drugs. Investigators must research the species they are working with for current recommendations. Veterinary recommendations listed below.

Drug	**Dose and route**	**Comments**
Butorphanol	0.5 – 4.0 mg/kg IM q2-4h	Little is known regarding dosing frequency
Buprenorphine	0.01- 0.05 mg/kg IM q8-12h	
Carprofen	1-4 mg/kg SC, PO q12h	Has been associated with haemorrhage
Flunixin meglumine	0.5-1 mg/kg IM q24h	Nephrotoxic Need good hydration
Ibuprofen	5-10 mg/kg PO q8-12h	Use paediatric suspension
Meloxicam	0.1-0.5 mg/kg SC, PO q24h	Unknown efficacy Longer half-life in chickens and pigeons
Phenylbutazone	3.5-7.0 mg/kg PO q8-12h	Psittacines

Euthanasia

- Euthanasia must only be undertaken by trained personnel, and should be in consultation with the Animal Welfare Officer, if being undertaken outside of approved application (eg: emergency situations).

- The NH&MRC *Guidelines to promote the wellbeing of animals used for scientific purposes*, p H6, Part III, deems the following as recommended forms of euthanasia:
  - Carbon dioxide inhalation for chicks. Based on laboratory animal practice, the chamber should initially contain air, and carbon dioxide then delivered at a rate of 20 to 30% of the volume of the container per minute. The chicks should remain in the container at least 5 minutes after signs of respiration have cessed.
  - Intraperitoneal injection of sodium pentobarbitone (60mg/mL) for all birds at a dose rate of 180 mg/kg. Once the bird is anaesthetised, additional pentobarbitone (60 mg/mL) can then be given IP or IV.
- The NHMRC *Guidelines* deem the following methods as acceptable with reservations due to either the need for specialised equipment or training required:
  - Inhalants:
    - Isofluorane in air – bell jar or other enclosed container in which the isoflurane is contained in an absorbent material, and not physically accessible to the bird.
    - Carbon dioxide delivered in the same manner as for chicks described above.
  - Cervical dislocation**

    ** Physical methods of euthanasia should only be performed by an appropriately trained operator.
- Forms of euthanasia that do not involve sedation and anaesthesia by injection must be performed in a quiet area, away from other animals.

Blood Sampling

General:

- Wash hands with disinfectant before and after handling birds.
- Refer to Standard Operating Procedure for Animal Handling and Safe Work Procedure for administering medication/animal handling.
- Persons without previous experience sampling must receive training and supervision from an individual experience in blood sampling small birds, including chicks. How and when this will be achieved must be included in your application to the Animal Welfare Committee.
- You must provide (from previous experience and/or references) information as to whether there is any risk to the health of chicks, from blood sampling, or the potential for parental rejection.

Small Birds:

- Blood may be taken from the right-sided jugular vein, the medial metatarsal vein, and the brachial vein.

- It is recommended larger volumes are taken from the jugular (e.g. for hormone or nutrition analysis), due to its accessibility and size. The left-sided jugular is not suitable due to its relatively small size.
- Smaller amounts (e.g. for DNA analysis) can be collected by puncturing the brachial or median metatarsal vein, and collecting blood directly into a microcapillary tube.
- Feathers should not be plucked to locate the vein as this may tear the skin. Dampening the feathers with alcohol solution is sufficient to expose the skin.
- Great care must be taken to avoid haematoma and bleeding in very small birds, as the loss of a couple of extra drops of blood can represent a significant proportion of the circulating blood volume, and hence prove fatal.
- Haemostatic agents to control excessive bleeding must be readily available.

Equipment

1. Disinfectant hand soap;
2. 25-27 gauge needles for brachial and metatarsal sampling and 29-30 gauge needles for jugular sampling;
3. 0.5-1ml syringes or insulin syringes;
4. Calico bird bags;
5. Alcohol swabs;
6. Cotton wool;
7. FTA paper and/or eppendorf tubes; and
8. Heparinised capillary tubes.

From the Jugular

1. Prepare a 29-30 gauge needle and 1mm diameter heparinised capillary tube so they are accessible with one hand.
2. Prepare FTA paper or eppendorf tube.
3. Prepare a piece of cotton wool.
4. Remove the cap of the needle, and place both cap and needle on a sterile surface.
5. Bend needle to a 45’ angle with the bevel facing up for easier access to the vein.
6. Ensure there is no air in syringe.
7. Hold the bird’s head between index and middle finger, and use the thumb to control the body and wing.
8. To reduce the risk of haematoma formation, ensure that the bird is carefully restrained so it cannot struggle, causing the vein to tear.
9. Use cotton wool & alcohol to swab feathers away from the jugular, to make it clearly visible.

10. Use thumb to compress vein proximally and cause it to distend.
11. Use 29-30 gauge needles on 1ml or 0.5ml insulin syringe (Heparinised) *(see image below).*
12. Insert needle with the bevel facing up in an upward direction at an angle of 45’ to the skin, and gently draw back with the thumb on the syringe to collect blood.
13. Take no more than 1% of the birds body weight by volume within a 24 hr period (preferably less frequently).
    - The logic being that a bird is about 10% blood, and you don’t want to take more than 10% of that blood volume (0.10*0.10 = 0.01).
    - So for a 5g zebra finch, you would take no more than (0.01*5g =) 0.05ml of blood.

(PHOTO: FTA card)

14. Store the blood (e.g. FTA card or eppendorf tube).
15. Do not recap needle, place the needle directly into a sharps container.
16. Compress vein with thumb as the needle is drawn-out and hold thumb in position for at least 30 seconds.
17. Allow blood to clot by applying gentle pressure with cotton wool to stop bleeding effectively.
18. Monitor the bird for subsequent bleeding.

From the Brachial Vein

1. Prepare a 25-27 gauge needle and 1mm 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. Prepare FTA paper or eppendorf tube.
3. Prepare a piece of cotton wool.
4. Hold the bird with wing extended *(see image below).*

(PHOTO: Blood collection from Brachial Vein)

5. To reduce the risk of haematoma formation, ensure that the bird is carefully restrained so it cannot struggle, causing the vein to tear.
6. Use cotton wool and alcohol to swab feathers away from the brachial vein to make it clearly visible.
7. Use thumb to compress vein proximally to make it distend.

8. A 25-27G needle will be used to puncture the vein, and collect blood using a heparinised microcapillary tube.
9. The brachial is too small in small passerines to do more than prick the vein with a needle and draw blood from the skin surface. This is often all that is needed to get a drop of blood onto FTA paper for DNA analysis.
10. Store the blood (e.g. FTA card or eppendorf tube).
11. Do not recap the needle, place the needle directly in a sharps container.
12. Allow blood to clot by applying gentle pressure with cotton wool to stop bleeding effectively.
13. Put pressure on the vein for at least 30 seconds once enough blood has been obtained to prevent superfluous bleeding.
14. Monitor the bird for subsequent bleeding.

From the Metatarsal Vein

1. Prepare a 25-27 gauge needle and 1mm 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. Prepare FTA paper or eppendorf tube.
3. Prepare a piece of cotton wool.
4. Hold the leg of the chick between your thumb and index finger *(see image below).*
5. Use cotton wool and alcohol to swab sterilize the area.
6. Use thumb to gently compress the vein to make it distend.
7. A 25-27G needle will be used to puncture the metatarsal vein, and collect blood using a heparinised microcapillary tube.

(PHOTO: Blood collection from Metatarsal Vein of chicks)

8. The metatarsal vein is too small in small passerines to do more than prick the vein with a needle and draw blood from the skin surface. This is often all that is needed to get a drop of blood onto FTA paper for DNA analysis.
9. Store the blood (e.g. FTA card or eppendorf tube).
10. Do not recap the needle, place the needle directly in a sharps container.
11. Allow blood to clot by applying gentle pressure with cotton wool to stop bleeding effectively.
12. Put pressure on the vein for at least 30 seconds once enough blood has been obtained to prevent superfluous bleeding.
13. Monitor the bird for subsequent bleeding.

Heart Rate Monitoring

General:

- Wash hands before and after handling birds.
- Refer to Safe Work Procedure for handling animals/administering medication.

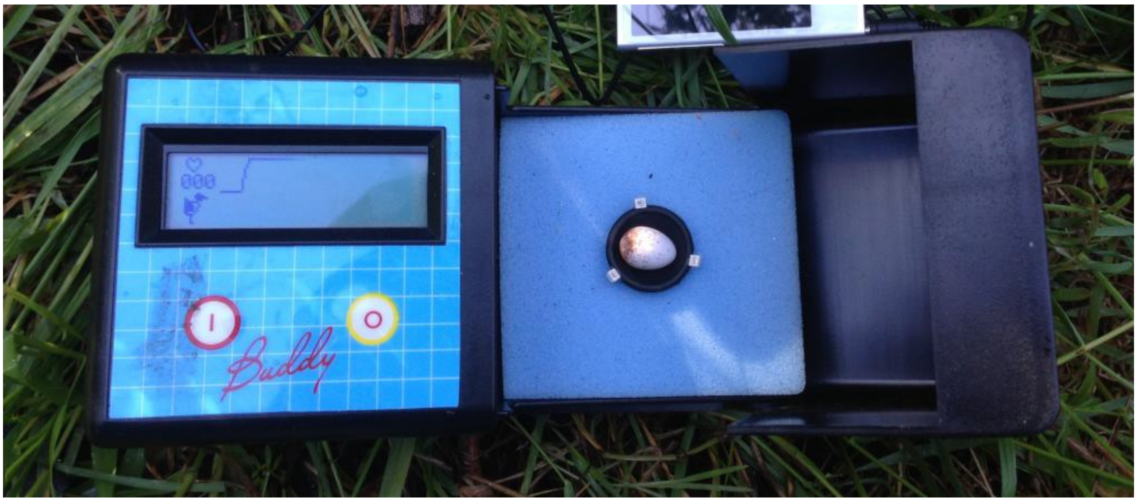
In Eggs

1. Record/check the nest ID and location via GPS, and using the appropriate data sheet.
2. Prepare the heat pack by flexing disc to activate heat instantly *(see photo of heat pack below).*

(PHOTO: Heat pack)

3. Place the heat pack on the ground in a shady area, under the buddy monitor.
4. Open the buddy monitor.

5. Collect one egg from the nest.
6. Place the egg in soft seat in the buddy monitor *(see photo below)* using the thumb and the grooming fingers (careful not to apply to much pressure on shell). The egg should not be moved up and down too much.



(PHOTO: Buddy monitor - open)

7. Close the buddy monitor and record heart rate on data sheet.

(PHOTO: Buddy monitor - closed)

8. Wash hands before and after handling birds.
9. When finished, open buddy monitor, collect the egg, and return it to its original nest.
10. If collecting data from more than one egg per nest, remove second egg before returning the first egg.

In Adults and Nestlings of Larger Birds

1. Prepare the heat pack by flexing disc to activate heat instantly (see photo of heat pack above).
2. Prepare the microphone, cloth, calico bag, and testing box.
3. Use a clean cardboard box the size of a shoes box (30x15 cm) for the small adult passerine birds and quails, and a larger cardboard box (50x50 cm) for the little penguins (both adults and nestlings).
4. The bottom of the box will be covered with newspapers to protect the bottom of the box. All boxes will have a lid to prevent the birds from escaping. Some small holes (1-2mm) will be made on all sides of the box to allow air to pass through. A larger hole will be made at the back to allow passage for a cable to connect the microphone to the recorder.
5. Place the testing box on the ground in a shady area.
6. Catch the bird.
7. Place the microphone on the chest area of the birds (connected to the recorder), and listen through the recorder to detect heart rate. Always use a microphone that has a wind protective cover so it has a soft surface.
8. Once the heart rate is detected, hold the microphone and secure it with a piece of cloth surrounding the bird body around the chest area, and held in place with a strap of Velcro that is not too tight to prevent breathing.
9. Wings (for small passerines and quails) and flippers (for little penguins) will also be held by the piece of cloth, which will prevent the birds from moving too much once in the testing box.
10. For small passerine species, place the bird in a small calico bag.
11. Place the bird in the testing box, disconnect the cables from the recorder, pass it though the appropriate hole at the back of the box, and reconnect the microphone to the recorder.
12. Close the lid and start recording heart rate.
13. When finished, open the box and remove the bird from box (for small passerine species, remove the bird from the calico bag).
14. Gently remove the cloth around the body.
15. Release the bird into its original nest/burrow or territory.

In Nestlings of Small Birds

1. Wash hands before and after handling birds.
2. Record/check the nest ID and location via GPS, and using the appropriate data sheet.

3. Prepare the heat pack by flexing disc to activate heat instantly *(see photo of heat pack above).* Place the heat pack on the ground in a shady area, under the artificial nest *(see photo below of artificial nest below)*. Use a plastic container (10-15 cm in diameter) as the base for the artificial nest.
4. Nest should have a round bottom and nesting material (e.g. cotton balls/wheat bags) to support the nestling.
5. A lid that has an archway cut out of the front but is solid roofed is essential to protect the nestling and simulate the conditions of the natural nest. The nest also has a tiny archway at the back for the cable connecting the microphone to the recorder.

(PHOTO: Artifical nest)

6. Wash hands before and after handling birds.
7. Remove lid.
8. Hide condenser microphone in the middle of the cotton balls in the artificial nest, so that only the top of the microphone will be visible.
9. Check for presence of nestling by inserting one finger inside the nest and gently touching the bottom of the nest (count the number of nestlings by touch).
10. To remove nestlings, close hand gently around the nestling and position the nestling so it is sitting in the palm of the hand, with the fingers slightly closed on top to protect the nestling.
11. Remove hand from nest and place the nestling in the artificial nest resting against the microphone.
12. Replace the protective lid on top of the nest and start recording heart rate.
13. When finished, open nest, collect the nestling, and return it to its original nest.
14. If collecting data from more than one nestling per nest, remove second nestling before returning the first nestling.

Audio Recording and Playback

- Check and test all audio recording equipment (microphone, audio recorder, charged batteries, headphones, adequate space on hard drive, SD card, CF card) before going into the field.
- Check and test all playback gear (iPod, speakers, Fox pro Scorpion playback system) before going into the field.

Audio Recording

1. Make sure sound recording equipment is on and working, and select an appropriate sample rate and bit depth (e.g. 24-bit 48kHz .wav files).
2. Turn on the audio recorder and check the recording levels on the recorder using the level meters to obtain recordings at -3 to -6dB if possible, otherwise the S/N ratio will be too low.
3. Note the location, time, day, weather, bird ID, etc, on the recorder.

- **In the Field:**

1. Point the microphone in the direction of the focal bird.
2. Approach the bird quietly and keep far enough from the bird so not to disturb it.
3. Back off when their behaviour changes.
4. Note the bird ID on the recording when the focal bird vocalises e.g. “that’s male 1”.
5. Regularly check the recording levels on the recorder using the level meters to obtain recordings at -3 to -6dB if possible.
6. Stop recording if:
    (i) Sufficient number of vocalisations have been recorded,
    (ii) Focal bird is not vocalising,
    (iii) Focal bird’s behaviour changes, or
    (iv) Poor weather conditions (windy, raining, etc).

- **At the Nest:**

1. Put the audio recorder in or next to the nest (e.g. under the nest), with the microphone facing the nest.
2. Make sure the audio equipment does not block the entrance to the nest.
3. Cover the recorder with vegetation and/or camouflage cloth to ensure it is not noticed by the parent birds.
4. Monitor the nest after setting up the recorder until the parent birds return to ensure the nest is not disturbed.

5. If the audio recorder is not accepted after 20 minutes (parent birds have not returned to the nest), remove the recorder and do not try again on the same day (a second attempt will be made on the following day).
6. Leave the audio recorder for an appropriate time to record the vocalisations of the focal birds.
7. Remove the audio recorder from in, or next to, the nest if:
    (i) Sufficient number of vocalisations have been recorded,
    (ii) Audio recorder has been at the nest for an appropriate time,
    (iii) Parent birds and/or nestling behaviour changes, or
    (iv) Poor weather conditions (wind, raining, etc).

Audio Playback

1. Record and/or select vocalisations with good signal-to-noise ratio and no overlapping sounds to use as playback stimuli.
2. Create stimulus tracks using an appropriate program (e.g. Amadeus, Raven).
3. Select an appropriate sample rate and bit depth for the playback stimulus (e.g. 16-bit 44.1kHz .wav files) and ensure the stimulus track is an uncompressed wave file.
4. Upload playback stimulus onto playback gear (e.g. iPod, Fox pro Scorpion playback system).
5. Test all playback gear and stimulus to ensure it works and is set at the appropriate volume before going into the field.

- **In the Territory:**

1. In the field, locate the focal bird(s) in the territory.
2. Observe the behaviour of the birds for approximately 3 minutes before placing the speaker down in the territory.
3. Place the speaker on the ground at an appropriate distance from the focal bird(s) (10 – 20 metres).
4. Observer(s) remain at least 10 – 20 metres from the speaker, hidden in vegetation.
5. Start the playback stimulus using a remote control (e.g. Fox pro Scorpion remote, iPod with bluetooth speaker).
6. Observe and record the behaviour of the focal bird(s).
7. Playback stimuli are usually 3 - 6 minutes long.
8. Remove speaker from territory once playback trial is over.
9. Models or mounts of birds may also be used in playback trials.

- If so, the bird model will placed on top of, or next to, the speaker before the playback stimulus starts, and removed along with the speaker once the playback trial is over.

- **At the Nest:**

1. In the field, locate the nest.
2. Place the playback gear (e.g. iPod, cable, and speaker) under the nest.
3. Monitor the nest after setting up the playback gear until the parent birds return to ensure the nest is not disturbed.
4. If the playback gear is not accepted after 20 minutes (parent birds have not returned to the nest), remove the playback gear and do not try again on the same day (a second attempt will be made on the following day).
5. When observing behavioural responses to the playback stimulus, remain at least 10 – 20 metres from the speaker and nest, hidden in vegetation.
6. Playback stimuli are usually 30 – 180 seconds long played at a natural rate for a predetermined length of time, depending on the experimental design and stimulus type.
7. Remove playback gear from under the nest once playback trial is over, or if parent birds and/or nestling behaviour changes.

Radio Tracking

- In order to identify how individuals use and move within their environment, some captured birds will be tracked using radiotelemetry.
- It is generally assumed that radio-marking will have some effects on the animal, but efforts can be made to minimise marking effects so that they do not disrupt the normal movements and behaviour of the marked individual.
- Detrimental effects of radio-marking can be reduced by:
    1. Minimising capture and handling time;
    2. Using the smallest possible radio transmitter suitable for the objectives of the study; and
    3. Using the most inconspicuous and best fitting attachment method available.
- Ensure the entire transmitter and attachments do not exceed 5% of the individual’s body mass.

Equipment Familiarisation

- Radio-tracking involves the use of three devices:
  1. Transmitter: This is attached to the animal and emits a VHF signal at a pre-set frequency (usually within the range of 150MHz to 152MHz).
  2. Receiver: This device is tuned to the frequency of the transmitter and the strength of the signal will indicate the proximity to the animal. If an appropriate antenna is attached, the direction of the signal can be determined.
  3. Antenna: This receives the signal from the transmitter. The antenna needs to be matched to the frequency range (i.e. 150MHz to 152MHz) of the transmitters being used. A lead is usually permanently attached to the antenna that is used to connect it to the receiver.

- You will need to be familiar with how to tune your receiver to the frequency of the transmitters and make adjustments. Consult the user manual.
- It is a good idea to carry back-up equipment (antenna, lead, and receiver) and spare batteries.
- You will also require datasheets to record your observations.
- A GPS is often carried to take a reading of the exact location of listening points, or of the located transmitter. If using a GPS, you should familiarise yourself with the functions required by consulting the user manual prior to venturing into the field.

Equipment Care and Maintenance

- Radio-tracking equipment is subject to failure. Actions to prevent problems include:
  1. Protect equipment from rain.
  2. Take care with connectors when attaching and detaching the antenna from the receiver, as they are easily damaged.
  3. Take care to not bend or stress the arms of the antenna. The arms of the antenna must remain straight and parallel to one another to ensure maximum receiving efficiency (Resources Inventory Committee, 1998).
  4. Do not twist or kink leads. This includes tightly wrapping them around the antenna. The lead can be loosely wrapped around the antenna for storage or transport. It is important to ensure that the lead is not slammed in the vehicle door during storage or transport.
  5. Remove batteries from the receiver when storing for an extended period of time.
  6. Take note of care instructions for transmitters from manufacturers. Battery life of some stored transmitters can be prolonged by periodically switching on the transmitters and/or storing the transmitters in a fridge.

Attachment of Transmitter

1. Apply the radio transmitter at the time of mist-netting.
2. Cut the feathers in the immediate attachment area to ~1mm long with round-nosed scissors.
3. Glue radio transmitters to the feathers of the bird in the inter-scapular region *(see image below)* using a non-irritant adhesive eyelash glue (ie. Manicare®, Goth & Johns 2001).

(PHOTO: Attachment of transmitter to inter-scapular region)

4. Hold the bird in the holding bag that was used at the time of capture for a period up to 5-10 minutes, to allow the bird to become accustomed to the transmitter.
5. Release the bird at the site of capture.
6. Follow the birds for a period of 60 minutes to check that they are accustomed to the transmitter.
7. If there is any sign that the transmitter is causing adverse effects (such as reduced mobility, apathy, or incapability of flight), recapture the bird immediately and remove the transmitter.
8. After the transmitter has been accepted, follow the bird every 30 minutes over a 12 hour period to sample detailed patterns of habitat use.
9. For the next 14 days, sample birds twice per day for 30 minutes (morning & afternoon) to sample detailed patterns of habitat use.
10. If any sign that the transmitter is causing adverse effects during this sampling period, recapture bird immediately and remove the transmitter.
11. Record the time and location of the bird two or three times a day for up to two weeks.
12. The transmitter will fall off and can be recovered by the researcher after two weeks, as the eye-lash glue will lose its hold.

13. When necessary (to replace transmitter batteries and remove transmitters), recapture birds using mist-nets.
14. Remove all transmitters and attachments from birds at the completion of tracking.

Finding a Radio Tagged Individual

1. Keep antennas at least 2m away from other objects, especially those that are large or metal as these objects will cause detuning of the antenna.
2. Use high points in the landscape wherever possible, including hills or even tree stumps.
3. Be familiar with your study area.
4. Avoid sources of interference (e.g. rocky outcrops, cliffs, radio communication towers, large metal structures, or objects, etc).
5. Navigate to a listening point, preferably a high point in the landscape. It is usually best to begin listening for a transmitter where it was last known to be present.
6. Connect the antenna to the receiver, and ensure the arms of the antenna are perpendicular to the main shaft.
7. Listen for a signal by holding the antenna up and swinging slowly around 360 degrees.
8. If a signal can't be heard:
    - (i) Check that the frequency is tuned correctly.
    - (ii) Try holding the antenna in different positions (vertical and horizontal).
    - (iii) Check your equipment is working properly by listening for a known working transmitter.
    - (iv) Check your receiver has the correct settings.
    - (v) Try listening from a higher elevation. Even a rock or tree stump will help.

9. Once a signal is heard, you will need to find the direction of the strongest signal. There are three main techniques for finding a peak signal:
    - (1) Swing the antenna slowly around for a full 360 degrees. Decide the general direction of the strongest signal (peak signal), and swing back and forth narrowing the arc each time until you have settled on a direction.
    - (2) Swing the antenna around to determine roughly the direction of the strongest signal, then point the antenna in particular directions and compare the signal strength. Each time you point and decide which direction is stronger, you can home into the strongest signal to narrower and narrower slices of the circle. Using this technique

means that each time you attempt to narrow down the signal direction you are comparing two signal strengths and deciding if one is stronger than the other, rather than listening to a gradient which can be difficult to distinguish where the signal is strongest.

(3) If fitted, it is possible to use the signal strength meter on the receiver to determine the direction of the transmitter, but it is recommended that this is only used in conjunction with listening for the strongest signal.

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 on the AWC website.
- 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:

    (i) An unexpected adverse event and the project may continue unmodified,

    (ii) An unexpected adverse event and the project may continue with modifications, or

    (iii) An expected adverse event and whether or not the project can continue, and if modifications are required.

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.pir.sa.gov.au/fishing/permits_and_exemptions
- http://www.nhmrc.gov.au
- http://www.adelaide.edu.au/ANZCCART/
- http://www.environment.sa.gov.au
- http://www.environment.sa.gov.au/licences-and-permits/Animals_in_captivity_permits
- http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics_home.cfm
- http://www.birds.cornell.edu
- McGregor PK (2000) Playback experiments: design and analysis. Acta Ethologica 3 (1):3-8.
- Kroodsma DE (1986) Design of song playback experiments. Auk 103:640-642.
- Kroodsma DE (2005) The singing life of birds: the art and science of listening to birdsong. Houghton Mifflin Company, USA.