

This Standard Operating Procedure (SOP) is applicable to all UniSQ Research Workers who care for and use Animals for Scientific Purposes. The procedure must only be performed by those persons who have been deemed competent, and who believe they remain competent to do so. Access to supervision by suitably qualified staff whilst undertaking this procedure is encouraged, where required.

Species

- *Litoria* species (tree frogs)
- Other arboreal vertebrates

Purpose

The purpose of this SOP is to provide information to people considering surveying arboreal vertebrates in research on wildlife populations, an alternate method of surveying arboreal species under-represented by traditional survey methods.

Population surveys and monitoring play a key component of many ecological research projects. Populations data is obtained through ecological survey techniques such as camera, cage, Elliott and pitfall trapping to obtain information about abundance from surveys to determine population sizes (and changes) and demographic information (e.g. different age classes). It is vital to obtain information on large numbers of individual animals to obtain sufficient data to obtain accurate and statistically meaningful results.

However, traditional trapping methods are designed primarily to survey terrestrial species and under-represent arboreal species in ecological surveys, resulting in inaccurate data on species abundance and diversity, particularly for tree frogs. Therefore, a method of surveying arboreal vertebrates is required to obtain data on arboreal vertebrate populations.

The use of an artificial refuge has been used as a trapping method in Hawaii to control pest populations of the coqui frog (*Eleutherodactylus coqui*) utilising PVC piping (College of Tropical Agriculture and Human Resources n.d.). Furthermore, studies conducted across Central and South America have found that artificial refuges are an effective method for sampling arboreal frog species (Glorioso & Waddle 2014).

This SOP describes a method of utilising 30 cm lengths of PVC piping and bamboo with a 3 cm side entrance and closed ends as artificial refuges to use as a sampling method for arboreal vertebrate species, particularly tree frogs.

Definitions

AEC	Animal Ethics Committee
PVC	polyvinylchloride, one of the world's most widely produced synthetic plastic polymers, is used in a wide range of products.

Linked SOPs

SOP ID number	SOP title
WL012	Dry pitfall trapping for vertebrates
WL003	Photographing small vertebrates

Potential hazard to Research Workers

UniSQ Risk Management Plan ID number	UniSQ Management Plan title
RMP_2020_4960	Wildlife research and teaching fieldwork

Personal Protective equipment required

- Disposal examination gloves, various sizes

Animal wellbeing considerations	
Perceived stressors	Management strategy
Extreme weather	Avoid trapping in extreme weather conditions. Plan ahead and monitor long-range and daily weather forecasts.
Handling of animals	Animals will be handled so as to cause minimal stress and released as soon as processing is completed.
Trap located near an ant nest	Pipe traps should not be placed in the vicinity of ant nests. Move trap to an area with no ant nest.
Disease risk from bags and equipment	All handling bags and equipment should be kept clean to minimise the risk of disease.

The overall perceived level of risk to an animal undergoing this procedure is:

High Medium Low

Substances to be administered			
Substance	Dose	Route	Purpose
Nil.			

Equipment/ materials required

- 30 cm length of 40 mm diameter PVC pipe
- 40 mm diameter PVC pipe caps
- Hand saw to cut pipe
- 30 cm length of bamboo culm (approximately 30-40 mm diameter)
- 3 cm hole saw drill bit and electric drill
- Sheets of fine grade sandpaper
- Armada Jumbo Trolley Liner garbage bags to make plastic squares to cover the end of the bamboo pipe traps
- Plastic bags for rubbish
- Rubber bands
- Tie-down straps
- Flagging tape
- Ziplock bags
- 1.5 m star pickets
- Datasheets
- Alcohol and alcohol wipes
- Disinfectant solution
- Boxes of different size disposal examination gloves
- Range of scales (10 g, 40 g, 100 g, 200 g, 1 kg, to weigh animals)
- Ruler (wood or steel) measured to 1 mm accuracy
- Permanent markers to label containers, i.e. plastic bags
- Zip-lock plastic sandwich bags

Site specification or location requirements

At locations/fields outlined in UniSQ AEC approved applications that includes the use of this SOP.

Waste disposal

Not applicable.

Duration of the procedure

- A day to purchase material, e.g. PVC pipe, caps, then cut PVC pipe into 30 cm lengths, and then drill entrance hole midway along the pipe.
- Several hours to source and organise the collection of 30 cm long 30-40 mm diameter bamboo.
- Several hours to purchase other equipment, e.g. garbage bags and rubber bands then cut garbage bags into 15 x 15 cm squares.
- Several days to leave pipe traps outside to be exposed to weather conditions to minimise the smell of plastic.
- Check trap for the presence of animals – 10 seconds.
- Removal of animal from the trap – 1 minute.
- Processing of animal removed from the trap – 1 to 5 minutes per animal.
- Monitoring of animal once released near trap – 1 to 2 minutes until the animal has moved out of sight.

Procedure

Before field – manufacture and preparation of pipe traps

1. Purchase appropriate amounts of 40 mm diameter PVC pipe and 40 mm diameter PVC end caps.
2. Cut PVC pipe into 30 cm lengths and use 3 cm hole saw drill bit to drill a hole in the middle of the pipe section (Figure 1).
3. Sand edges of entrance holes to ensure they are smooth.
4. Push pipe caps onto both ends of the PVC pipe (they are a tight fit) to allow their removal so that animals in the pipe trap can be removed.
5. Obtain bamboo segments, cut each end underneath the node, and use the 3 cm hole saw drill bit to drill the hole in the middle section (Figure 1).
6. Use rubber bands to secure the plastic squares over the open ends of the bamboo.
7. Leave pipe traps outside for a week to be exposed to weather conditions to remove any artificial odours.



Figure 1: PVC and bamboo pipe traps showing the side entrance.

In field – installation of pipe traps

1. Secure pipe traps vertically in a tree or to a star picket at the selected location using tie-down straps with the side entrance hole in the pipe trap 0.7 m from ground level. Ensure the entrance hole into the pipe trap is facing outward.
2. Use flagging tape to designate the position of pipe traps.

NOTE: All pipe traps need to be cleaned with disinfectant after they have been removed from one site prior to the installation in a new site. After disinfecting, the pipe traps should be well rinsed.

In field – checking traps and removing animals

1. Pipe traps should be checked twice daily, within an hour of dawn and at dusk. Gently remove the loose pipe cap from the PVC pipe and observe any animals within the pipe trap.
2. If the trap empty, replace the pipe cap and repeat the procedure for the bamboo pipe trap.
3. If the trap has been successful and an animal has been caught, with a permanent marker pen, label a new zip-lock sandwich bag with the trap number. Weigh and record the plastic bag's weight on the datasheet in the row where you wrote the trap number, in the column labelled 'Wt bag (g)' on the 'Trap data record sheet Amphibians'.
4. Put on clean disposable gloves.
5. Remove the plastic covering (bamboo) or pipe cap (PVC) at one end of the pipe trap and hold the zip-lock bag firmly over the open end of the pipe trap and then remove the plastic covering or pipe cap from the other end and use your hand to cover the side entrance.
6. Gently blow into the pipe trap to encourage the animal to move out of the pipe trap and into the zip-lock bag, closing the zip-lock bag once the animal has emerged from the pipe trap. Ensure that processing of the frog is undertaken in less than 5 minutes.
7. Record the species of the animal in the first column of the data sheet in the row where you wrote the trap number in the column labelled 'Species name'.
8. Weigh the animal in the zip-lock bag and record its weight on the datasheet in the row where you wrote the trap number, in the column labelled 'Wt bag + animal (g)'. From this, you can determine the weight of the animal and write this number in the column labelled 'Wt animal (g)'.
9. While the animal is still in the zip-lock bag, measure the snout-vent length (from the tip of the nose to the cloaca = head and body length) to the nearest millimetre using the ruler while the animal is placed on a flat surface (e.g. clipboard) and record this number in the column labelled 'Head and body (mm)'. See Figure 2 for details of these and the following measurements.
10. While the animal is still in the zip-lock bag, measure the hind leg length (groin to the tip of the longest toe but do not include the nail) to the nearest millimetre using the ruler while the animal is placed on a flat surface and record this number in the column labelled 'Leg (mm)'. The nail, if present, is not included in this or foot length as they can be of varying length due to wear etc.
11. While the animal is still in the zip-lock bag, measure the hindfoot length (heel to the tip of the longest toe but do not include the nail) to the nearest millimetre using the ruler while the animal is placed on a flat surface and record this number in the column labelled 'Foot (mm)'.
12. The last data to be recorded is in the column labelled 'Fate (1-4)' write the appropriate number; where Fate: 1 = released unharmed; 2 = escaped while being handled; 3 = died in the trap or while handling; 4 = to be euthanised. For most animals, this should be 1, unless the animal has escaped, died or has a reason to be euthanised (e.g. obvious major trauma or evidence of fungal infection). If you select 4 you also need to record clearly why an animal has been euthanased. Ensure any other reporting requirements as per USQ AEC policy and procedures are met if animals are euthanased or found deceased in the traps.
13. In the vicinity around the pipe trap (ideally within a 1 to 2 metre radius) find vegetation that would offer safe refuge to the animal and undo the zip-lock bag and release the animal into that vegetation. The animal may sit for a few minutes and then move into the vegetation or disappear into the vegetation the moment it is released. Observe the animal until it has moved into the vegetation and if there appears to be a problem, e.g. unstable movement or jumping, record this in the column labelled 'Remarks'.
14. If there was more than one animal in the pipe trap, replace gloves and repeat steps 3 to 13 (checking traps and removing animals) for each additional animal until the pipe trap is empty of animals.
15. If there are no more animals in the pipe trap, place all zip-lock bags in the rubbish bag, remove gloves and put them in the rubbish bag. Gloves mustn't be used between animals, i.e. a new pair of gloves must be used for each animal. It is important not to use the same gloves/bags between animals to prevent the spread of diseases. Ensure all equipment is cleaned with alcohol wipes after use.
16. Move onto the next pipe trap and repeat this procedure until all the pipe traps have been checked and all animals processed.

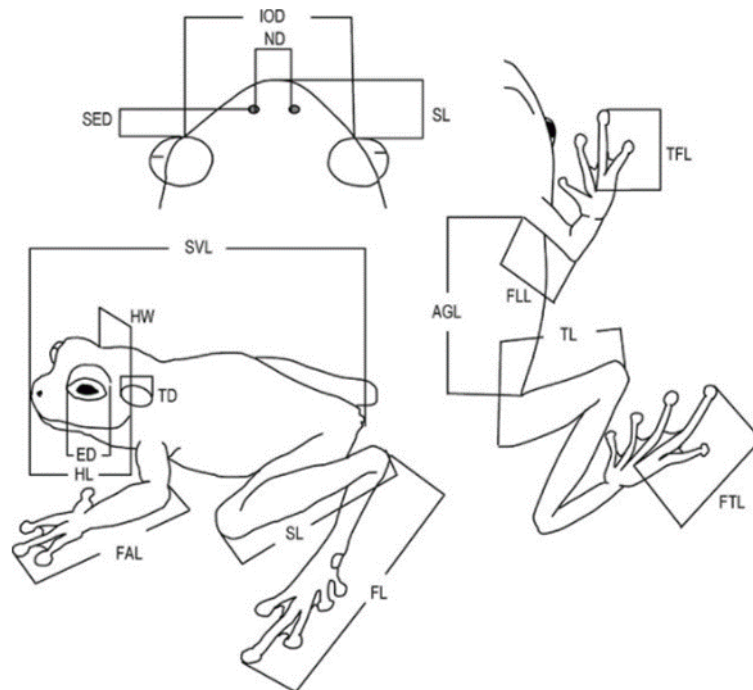


Figure 2: Morphometric measurements taken from frogs: snout–vent length (SVL); head length (HL); head width (HW); snout length (SL); internasal distance (ND); interorbital distance (IOD); eye diameter (ED); snout–eye distance (SED); tympanum diameter (TD); forelimb length (FLL); forearm length (FAL); third finger length (TFL); axilla–groin length (AGL); thigh length (TL); shank length (SL); foot length (FL); fourth toe length (FTL). Redrawn from Duellman (1970).

Training, qualifications or competencies required

Researchers with relevant experience or qualification can only undertake this SOP to complete the procedures required.

Student researchers must receive appropriate training and supervision from UniSQ research supervisors or qualified individuals prior to undertaking procedures.

References

- College of Tropical Agriculture and Human Resources n.d., *Control of Coqui Frogs in Hawaii*, University of Hawai'i at Manoa, viewed 12 June 2020, <<https://www.ctahr.hawaii.edu/coqui/refugia.asp>>.
- Duellman, W. E. 1970. *The Hylid Frogs of Middle America*. Monographs of the Museum of Natural History, University of Kansas, Lawrence.
- Glorioso, B. & Waddle, J. 2014. A review of pipe and bamboo artificial refugia as sampling tools in anuran studies. *Herpetology Conservation Biology*, 9, 609-625

Licences and permits

Any required licences and/or permits to undertake the procedure(s) under this SOP must be obtained before undertaking this SOP.

SOP approval and review history

Date	Version	Review Pathway	Notes
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17/12/2020	0.0	3/12/2020 UniSQ AEC "Subject to Modifications". 17/12/2020 Reviewed and approved by the UniSQ AEC Executive.	Approved
23/06/2021	0.1	23/06/2021 Inserted under "Licences and Permits", the words: "Any required licences and/or permits to undertake the procedure(s) under this SOP must be obtained before undertaking this SOP.	Update
16/09/2022	0.2	16/09/2022 Converted SOP to new UniSQ branding.	UniSQ 2022 Rebrand
15/09/2023	0.3	11/05/2023 UniSQ AEC "Subject to Modifications" with the revised SOP to be reviewed by the UniSQ AEC Executive. 15/09/2023 Revised SOP reviewed and approved by the UniSQ AEC Executive.	Approved