	Flinders College of Scier Standard Ope For Tawny Dra Captive Hatchin Releas	s University nce and Engineering erating Procedure agon Captivity and ng with Subsequent se 18/06/19	
			Animal Facility
SOP Number	RA Number	AWC Approval D	ate
SOP-BIOL-4-Captivity With Release	RA_	18/06/2019	
Contact Person	SOP	Prepared By	Review Date
Leslie Morrison	Leslie Morrison and Jessica Hacking		June 2021

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The SOP **Tawny Dragon Captivity and Captive Hatching with Subsequent Release** contains the following sections:

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## Legislation

- Australian Code for the Care and Use of Animals for Scientific Purposes 8<sup>th</sup> Ed.
- Animal Welfare Act 1985
- Animal Welfare Regulations 2012
- <u>Gene Technology Act 2000</u> (the Act)
- <u>Gene Technology Regulations 2001</u>
- 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.

<u>http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics\_home.cfm</u>

• 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) <u>http://www.environment.sa.gov.au/licences-and-</u> <u>permits/Animals in captivity permits</u>
- 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.
- 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.

#### Capture and Transport

- Individuals will be captured by noose according to procedures outlined in "SOP Capturing Small Dragon Lizards by Noose".
- After capture, lizards should be assessed for health and stress.
- Lizards should be secured in a clean calico bag, and placed in the shade out of direct sunlight (e.g. under a tree or under a car) until transport to the field station.

- In the time between capture and transport, lizards should be checked every half hour.
- During transport, lizards (in calico bags) should be placed in an esky with the lid held open slightly to keep them cool (~15°C) and thus less stressed. Lizards should be placed in a single layer at the bottom of the esky. The esky can be kept cool using freezer blocks wrapped in a towel (ensure that freezer blocks are secure and can't move as this could cause injury to lizards).
- Lizards should be checked every 2 hours during transport.

### Holding Procedures and Husbandry in Animal Facility

## Location:

• Flinders University College of Science and Engineering Animal Facility.

## **General Safety Precautions:**

- Only handle lizards when necessary.
- Wear appropriate closed-toe footwear.
- Use gloves when handling lizards with symptoms that indicate they are ill or diseased, or have broken skin.
- Use correct handling procedures to protect both lizard and handler (see "SOP Working with Lizards" and further details below).

## **Equipment and Maintenance:**

- Large metal and wooden storage racks for storage of lizard enclosures.
- Large plastic tubs approx. 60cm L x 45 cm W x 45 or 30 cm D with wire-mesh and plastic lids for adult enclosures.
- Small plastic tubs approx. 30 cm L x 20 cm W x 11 cm D with fly screen lids for hatchlings.
- UV lights.
- Regular lights (fluorescent tubes).
- 12V halogen light (heat lamps) 50W or 38W.
- Clip-on desk lamps with 60 W or 40 W incandescent bulbs for heat over hatchling tubs.
- Washed sand for substrate.
- Ceramic tiles to create hides (retreat sites).
- Disposable food bowls.
- Water bowls.
- 2-3 step safety step ladders to reach tubs stored on high shelves.
- A noosing pole to catch lizards in case of escape in room.

## Labelling:

- Animal ethics approval number with contact details of relevant people displayed on doors of rooms housing lizards.
- Individual lizard ID on each enclosure.
- Adults should have ID number painted on dorsal surface with a non-toxic paint pen.

## Handling:

- Only hold lizards at the chest/shoulders and the pelvis. Never hold lizards by the tail, stomach, or legs.
- To remove a lizard from its enclosure (usually they take refuge under the tile), lift the tile straight up (**never tilt**) and pick it up.
- Place the lizard in a calico bag and tie it off securely. Place the calico bag where it is clearly visible on a bench/self and **never** on the floor.
- Before returning the lizard to the enclosure, replace the tile.

# Feeding and Watering:

- > <u>Adults</u>
  - Approximately 5 (minimum 3) medium sized crickets every second day.
  - Bok choy, cos lettuce, or kale chopped finely offered once per week.
  - Crickets dusted with calcium and multivitamin powder once per week.
  - The tiles, underneath the tiles, and side of the enclosure, should be sprayed with rainwater when feeding as a source of water.
- Juveniles (> 3 months of age)
  - Minimum 5 small sized crickets every second day.
  - The tiles, underneath the tiles, and side of the enclosure, should be sprayed with rainwater when feeding as a source of water.
- Hatchlings (< 3 months of age)</p>
  - Minimum 5 extra small sized crickets every second day.
  - The tiles, underneath the tiles, and side of the enclosure, should be sprayed with rainwater daily as a source of water.

# **Enclosure Layout:**

- Fine washed sand used as substrate.
- Each enclosure should have a cool end (same temperature at the room), and a warm end (up to 35°C), to allow behavioural thermoregulation. Heat at the warm end is maintained using a heat lamp.
- Each enclosure should have a hide (ceramic tile held off the ground by pegs glued to the bottom) at each end. The hide at the warm end also serves as a basking site under the heat lamp.

- The sides of the tubs should be painted on the outside with opaque paint to prevent lizards being disturbed.
- Hatchlings should be housed in plastic tubs (30 cm L x 20 cm W x 11 cm D) with fly screen lids.
- Adults should be housed in plastic tubs (60cm L x 45 cm W x 45 or 30 cm D) with wire-mesh and plastic lids.

# **Creating Egg-Laying Environment for Gravid Females:**

- Sand and peat moss mix (30% peat moss and 70% fine washed sand) should be piled in one corner of the container to provide an area for the female to dig a nesting hole in which to lay her eggs.
- Temperature and lighting conditions should be kept the same.
- The nesting sand pile should be kept moist by spraying every second day.

# Egg Incubation:

- Prepare containers for incubating eggs:
  - 1. Add 60ml of vermiculite and 20ml of water to the container.
  - 2. Write the hatchling ID on the container.
  - 3. Weigh eggs individually.
  - 4. Place each egg in an individual container.
  - 5. Seal lid onto the container.
  - 6. Place the container in the incubator at 30°C.
  - 7. Check eggs for desiccation three times per week.
  - 8. Check for hatchlings after 6-8 weeks.
  - 9. Allow hatchlings to emerge completely, and then place them in an enclosure.

# Monitoring:

• All individuals should be visually assessed twice daily for normal behaviour.

# • Normal behaviour includes:

- (i) Retreating to hide when startled or when the temperature is lowered during the night.
- (ii) Basking under heat lamp.
- (iii) Foraging chasing crickets.
- (iv) Eating greens.
- (v) Digging nesting hole in the sand (if gravid female).

# • Abnormal behaviour includes:

- (i) Constantly running around enclosure.
- (ii) Retreating to hide constantly and not basking.

- (iii) Not eating crickets or greens.
- (iv) Abnormal behaviour may indicate stress, sickness or injury.
- (v) If an individual exhibits abnormal behaviour for more than 2 days, the lizards enclosure should be moved to a quieter place that is likely to be less disturbed (e.g. a higher shelf on the container shelving). If abnormal behaviour continues for a further 2 days a physical assessment (see below) should be undertaken.
- (vi) Lizards will likely exhibit abnormal behaviour for the first couple of days after capture until they become accustomed to the enclosure.
- (vii) Each lizard should be physically assessed (see below) only when visual assessments indicate abnormal behaviour. If the assessment results in the discovery of sickness or injury, the advice of a vet should be sought.

## Physical Assessment:

- Signs of sickness/injury/stress include:
  - (i) Eyes closed.
  - (ii) Residue/secretions around the nose or eyes.
  - (iii) Abnormal body shape.
  - (iv) Swelling/fight injuries.
  - (v) Abnormal movement.
  - (vi) Abnormal respiration.
  - (vii) Abnormal level of activity.
  - (viii) Breathing very rapidly or holding breath (mild stress).
  - (ix) Holding eyes very wide open (mild stress).
  - (x) Biting (mild stress).
  - (xi) Shaking (moderate to high stress).

## Checking for Egg-Laying:

• Egg-laying behaviour involves digging in the sand pile provided. It will be known when a female has laid eggs because she will look like an 'empty sack' and no egg contours will be seen on the abdomen. Once a female has laid her eggs, she should be placed in a calico bag (placed in a safe place) while the eggs are removed from the enclosure and placed in the incubator. Replace the female to her enclosure.

## Monitoring Sheets:

• Monitoring sheets to record the results of each assessment for each individual, including the date, and time, should be kept up to date.

## **Cleaning and Maintenance:**

• Enclosures should be spot-cleaned (to remove scats) every second day, or as required.

- Enclosures should be cleaned thoroughly every 6 weeks using F10 disinfectant.
- Work benches and food preparation areas should be disinfected daily.
- Before cleaning, lizards must be removed from the enclosure and placed in a calico bag. Place the calico bag where it is clearly visible on a bench/self, and **never** on the floor.
- Replace pegs separating tile shelters if needed.
- Replace light bulbs and fittings as required.

# Temperature and Lighting Conditions:

- Temperature and lighting resembles that experienced in the wild:
- Summer (November 1<sup>st</sup> to February 23<sup>rd</sup>)
  - Room temperature: 28°C
  - UV and fluorescent lights: 7am 8pm
  - Heat lamps (35°C): 8am 6pm
- Autumn (February 23<sup>rd</sup> April 27<sup>th</sup>)
  - Room temperature: 23°C
  - UV and fluorescent lights: 8am 6pm
  - Heat lamps (35°C): 9am 5pm

# > <u>Hibernation preparation</u>

- 1) **Stage 1:** April 27<sup>th</sup> June 2<sup>nd</sup>
- Room temperature: 20°C
- UV and fluorescent lights: 9am 5pm
- Heat lamps (35°C): 11am 4pm
- 2) **Stage 2:** June 2nd June 5th
- Room temperature: 18°C
- UV and fluorescent lights: 9am 4pm
- Heat lamps (35°C): 12pm 2pm
- Stop feeding, water only
- 3) Stage 3: June 6th June 9th
- Room temperature: 18°C
- UV and fluorescent lights: 10am 2pm
- Heat lamps (35°C): no heat lamp

# > <u>Hibernation (June 9th – August 5th)</u>

- Into incubator: 12°C
- No food, no water, no light

• Ensure substrate is moist

# > <u>Hibernation Emergence</u>

- 1) Stage 1: August 5<sup>th</sup> August 9<sup>th</sup>
- Room temperature: 18°C
- UV and fluorescent lights: 10am 2pm
- Heat lamps (35°C): no heat lamp
- No feeding, water only
- 2) Stage 2: August 9th August 12th
- Room temperature: 18°C
- UV and fluorescent lights: 9am 4pm
- Heat lamps (35°C): 12pm 2pm
- Begin feeding again
- Spring (August 12<sup>th</sup> November 1<sup>st</sup>)
  - Room temperature: 23°C
  - UV and fluorescent lights: 8am 6pm
  - Heat lamps (35°C): 9am 5pm

## **Quarantine Conditions**

- Strict quarantine conditions are required so that pathogens are not spread among tawny dragon individuals, or between tawny dragons and other species, at the Flinders University Animal Facility.
- Signage should be placed on the door of each room explaining quarantine and entry conditions.
- Tawny dragon lizards should be kept in separate enclosures in a separate secure room to other species at the Flinders University Animal Facility.
- While in captivity, tawny dragons should never come into direct contact with each other or any other animal.
- Pathogen cross-contamination via carers and equipment should be avoided by:
  - 1. Using good personal hygiene (washing hands before and after handling lizards).
  - 2. Wearing protective clothing (e.g. laboratory coat and boots).
    - ✓ Use new disposable booties to enter each room housing Dragons, and wear the lab coat labelled and allocated to each specific room.
  - 3. No transfer of equipment between rooms.

4. Tawny dragon water bowls should not be transferred between enclosures, and food bowls should be disposed after each use.

#### Release to Field

- All adult (wild-caught) individuals should be released at the point of capture according to "SOP Capturing Small Dragon Lizards by Noose".
- All hatchlings (hatched in lab) should be released in a manner resembling natural dispersal by young dragon lizards.
  - Healey et al. (2007) found that young Ctenophorus pictus (same genus as tawny dragons) dispersed at an average rate of 26cm per day. Given this information, tawny dragon juveniles should be released in random directions from the capture location of the mother at a rate of 26cm per day since hatching (e.g. a hatchling that is 3 months and 1 week old will be released approximately 9.6m from where its mother was captured).
- Care should be taken to release lizards in a safe crevice (checked for predators – including adult lizards in the case of hatchlings).

#### 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
- <u>http://www.adelaide.edu.au/ANZCCART/</u>
- <u>http://www.environment.sa.gov.au</u>
- <u>http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics\_home.cfm</u>