This document has been developed by The Australian National University's (ANU) Research Ethics Office. It has been endorsed by the ANU Animal Ethics Committee (AEC). It is designed to provide guidance regarding current best practice to institutional animal users and carers on the care and use of animals for scientific purposes. It has been prepared in consultation with the Australian code for the care and use of animals for scientific purposes 8th edition 2013.

Document 020 Standard Xenopus Husbandry and Surgery V2.0

Background

Any holding, experimentation or standard operating procedures involving the use of Xenopus frogs must have appropriate AEC approval.

The ANU and responsible research school(s) must ensure that Xenopus facilities are designed and managed based on current accepted husbandry guidelines for the keeping of laboratory Xenopus frogs. These include adhering to federal Approved Arrangement biosecurity requirements through appropriately sealed/restricted facilities and enclosures. Xenopus should always be handled wearing gloves, and thorough hand washing undertaken before exiting the quarantine area.

Procedures, analgesia, anaesthesia and euthanasia standards must be consistent with current veterinary practice. The aim must be to minimise pain and distress experienced by the animal at all times. Appropriate record keeping should be maintained and easily accessible.

Animal care staff and research staff must undertake appropriate post-surgical monitoring.

All staff and students working with Xenopus on campus must have a baseline knowledge of anatomy and husbandry of Xenopus and be able to apply this knowledge to maintain husbandry and surgery standards. **Animal health and wellbeing should be confirmed via a daily visual inspection.**

Definitions

General Anaesthesia: Loss of consciousness, reducing or preventing the perception of pain while the animal is unconscious.

Euthanasia**:** the act of inducing death using a method appropriate to the species that results in a rapid loss of consciousness without recovery and minimum pain and/or distress to the animal.

Analgesia: The use of medication to relieve pain

Sterilisation: To eliminate agents injurious to health on equipment and supplies used in aseptic surgery.

Surgical Procedure (as per NHMRC Guidelines): A surgical procedure is one that requires the incision of living tissue. In the scientific setting, the type of procedure will depend upon the scientific purpose but can range from a superficial cut-down to the penetration and exposure of a body cavity or extensive tissue dissection.

Aseptic technique: An approach that limits microbial contamination during a procedure.

General Information and Considerations

Space

The design of the space must meet the needs of Xenopus frogs in terms of stocking density, enrichment and filtration capacity as well as minimising the impact on the welfare of the individual animal.

Ideally, systems should be specifically designed for the stocking of Xenopus, with filtration adequate to support appropriately stocked tanks.

Holding spaces that are able to accommodate all handling and procedures within the room are highly desirable in order to prevent unnecessary movement of animals. As Xenopus species are exotic, the holding rooms must meet the Department of Agriculture, Fisheries and Forestry Approved Arrangements requirements, with added consideration to reduce the risk of cross contamination from other species and the introduction of pathogens.

Consideration must be given to other requirements of the space where restrictions to the housing of aquatic species may occur (such as within PC2 certified facilities).

Feeding Requirements

As per NHRMC guidelines, animals must receive *"nutritionally adequate food of a quantity and composition that maintain…normal weight of adult animals".* Feeding regimes must be at regular, set intervals, consistent with food measured, and feeding observed to prevent over or underfeeding. Any excess food must be removed to minimise contamination of the system from waste.

Pellets must be formulated to meet the nutritional needs of the animal and any importation must be organised and finalised well in advance of stock running low.

Technical staff must discuss the proposed introduction of new foods with veterinary and research staff. New foods should be introduced slowly over a period of weeks. Suggested pellet formations are trout pellets, salmon pellets or pellets based on the NASCO supplied diet. Additional feeds such as liver or other additives may increase the risk of tank contamination and therefore technical staff must discuss any plans to use these feeds with the veterinarians prior to use.

Maximum Stocking Density

Maximum stocking density must adhere to recommended guidelines. Based on current accepted practices, stocking should allow for at least 3L per adult frog. In a recirculating system, 10% of the system water should be exchanged daily over 24 hours.

Imports must take into account the holding capacities of the systems and planning for these needs to ensure that current frogs are appropriately isolated from newly introduced animals. It may be that all current animals must be utilised by the research team prior to the introduction of new animals to facilitate this requirement but this will be dependent on the stocks and facilities available. New animals must be isolated in a separate system and precautions established to manage any fluctuations in water parameters that may arise.

Water Quality and Environmental Parameters

Technical staff must maintain water parameters at values consistent with best current husbandry practice. Technical staff are responsible for regular monitoring of pH, temperature, ammonia, hardness and conductivity, with equipment calibration undertaken at regularly scheduled intervals. Direct exposure to very bright light should generally be avoided; a 12hr light/12hr dark lighting cycle is recommended.

For adult animals, it is recommended to use flood-and-flush tanks. These are self-cleaning tanks that are designed for automatic clearing of undissolved solids, such as leftover food and excretion products. Use of these tanks minimizes the time and effort required to net out the solids and in addition maintains cleaner water. Additional cleaning of tanks and surfaces should be conducted in line with manufacturer instructions of the relevant housing system. Xenopus are sensitive to chlorine and chloramines, and detergent will degrade the slime coating on their skin. These products should never be used when cleaning tanks.

Ideally, electronic automated parameters for water testing are established. For manual testing, water stability should be such that parameter testing be performed twice weekly and increased to daily if any issues are noted. All staff involved in the care of Xenopus should have knowledge on troubleshooting water quality issues.

- Avoid sudden and significant water changes.
- Minimise the number and amount of additives used in the system, as this will increase the risk of continual fluctuations that risk the ongoing stability of the system.
- System readings should be checked and recorded daily.

Technical staff can manage water quality using the following methods:

- Have available a holding tank of water that is 'pre-treated' and stabilised at temperature before use in the tank to avoid shock to the system.
- Monitoring of pH, temperature, ammonia, hardness and conductivity at regular intervals
- Prevention of overstocking through proper husbandry and experimental planning
- Prevention of overfeeding and removal of uneaten food after feeding
- Regular manual removal of large debris
- Regular maintenance of the life support system
- Detergents should never be used to clean any equipment used for housing and handling frogs

For guidelines on water parameters, please see Appendix. A.

Environmental enrichment should be included in each tank; enrichment is known to reduce to reduce fighting and cannibalism. Plastic tubing for hides is recommended. Artificial lilypads have not been shown to improve *X. laevis* welfare and contribute more surface area for algae to grow on. As such, they are an optional addition.

Anaesthesia, Analgesia and Humane Killing

All surgical procedures must be performed under general anaesthesia, with the provision of analgesia appropriate to the procedure.

Staff that are responsible for undertaking surgery must observe and monitor animals until they are recovered and able to move around their environment adequately.

The current accepted practice for anaesthesia is MS-222 (tricaine) immersion buffered with sodium bicarbonate. As anaesthesia is delivered via immersion in an aqueous solution (using tank water), for 10-15 minutes (checking on animal regularly). Anaesthetic solution must be prepared

fresh for each individual animal and the person undertaking the procedure must ensure it is appropriately buffered to a suitable pH for Xenopus frogs.

Unlike mammals, there is no reliable method of assessing grimace and pain in Xenopus frogs. Therefore, any procedures conducted must be done so with the assumption that levels of pain are comparable to their mammalian counterparts and must be adequately managed. It must be assumed that any illness or injury will result in the same amount of discomfort as a mammal and appropriate measures taken to minimise this.

The ANU Veterinary Services Team is available to provide extensive advice in the provision of adequate analgesia depending on the presentation of the animal. As per the NHMRC Guidelines

Pain and distress may be difficult to evaluate in animals. Unless there is evidence to the contrary, it must be assumed that procedures and conditions that would cause pain and distress in humans cause pain and distress in animals.

Humane killing must be species-appropriate and must be achieved with the intention of being performed quickly and to minimise pain and distress. Confirmation of death must be achievable either through assessment by the individual or via a secondary method. Methods of confirming death must be safe with minimal risk to the staff or student.

Acceptable methods of euthanasia include:

- Immersion in appropriate dose (5g/L) of buffered tricaine methane sulfonate solution (MS222) followed by pithing, decapitation, severing of the cervical spinal cord, exsanguination via ventral abdominal vein, or removal of the heart
- Intracoelomic injection of sodium pentobarbital (1100mg/kg)

Methods that are not acceptable for euthanasia include:

- Freezing or cold water stunning
- Electric shock
- Blunt force trauma
- Administration of caustic substances
- Carbon monoxide poisoning

Surgical Practice

Surgery technique and principles applied to Xenopus must be in line with current veterinary standards. This includes the use of appropriately sized sterilised surgical instruments, which are thoroughly cleaned and resterilised between surgeries. Surgical technique must comply with principles of aseptic technique, appropriate methods of tissue handling and instrument use to minimise trauma, the use of suture material and suture technique appropriate to the tissue layer and thickness. Sterile surgical gloves must be used for surgery, and an appropriate surgical area established which complies with current principles of aseptic technique.

Surgeries must be performed at appropriate intervals with enough time provided for complete healing and rest before another surgery is undertaken. The animals may only receive a maximum of six surgeries within their lifetime at intervals of no shorter than 6 months and must be euthanased on the final surgery. Methods to ensure the identification of the animals is adequate to manage these restrictions are required.

Monitoring, Intervention and Reporting

Post Surgical Monitoring and Care

Animals should be housed in tanks of a lower stocking density that are easily viewable and accessible for post-surgical monitoring and to minimise complications. Surgical wounds must be monitored by way of a score card and any concerns reported to Veterinary Services. Scoring should take into account the animals appetite, level of activity and visual assessment of the surgical wound.

Record Keeping and Identification

Animals must be individually identifiable and their husbandry and surgery records easily tracked. Records of animal numbers and movements must be available in the facility and be easily accessed if required. Any animal undergoing monitoring or treatment should be assigned an individual score card which must be assessed and updated at regular intervals depending on the circumstances.

Acceptable methods of ID include microchipping, pattern identification and visible implant elastomers, with microchipping being the preferred method. Toe clipping is not considered an acceptable method of identification.

Knowledge and Training

All staff and students working with Xenopus must have a clear understanding of the basic husbandry requirements of Xenopus and the principles behind water quality testing and maintenance. Those undertaking surgery must have an understanding of the anatomical configuration of Xenopus and how they are relevant to surgery and anaesthesia, as well as knowledge in managing potential complications. In order to work with Xenopus unsupervised, staff and students will be required to undergo a competency assessment, which will assess baseline knowledge of the system and principles of surgery and anaesthesia. All persons working with Xenopus (including husbandry staff) should undertake husbandry training, including post-surgical monitoring.

Animal Welfare

ANU is committed to providing the highest standard of animal welfare through best practice. This includes regular training, research and review of methods and techniques as they apply to the care and management of laboratory Xenopus. Any concerns by staff or students should immediately be brought to the attention of the Veterinary Services team so that they may act swiftly to address these concerns and uphold animal welfare.

Minimum Requirements

- Table 1 (Appendix 1) outlining the water parameters to be maintained
- Stocking density of no greater than one frog per three litres of water
- Use of system water for housing and anaesthetising animals
- Competency assessment for all staff working with husbandry and surgery of animals (including casual/part time staff).
- Humane killing via one of the acceptable methods only
- Provision of pain relief where indicated
- Post-surgical animal housing and monitoring must allow for easy visualisation of frogs and ensure good record keeping.

- No less than 6 months must be allowed for between surgeries and a maximum number of 6 surgeries over the lifetime of any individual animal.
- Feed must be assessed to be suitable for xenopus species and must be provided in measured quantities.
- Record keeping tracking individual animal histories and maintaining compliance with Quarantine regulations.

Appendix I: Guidelines for Xenopus Husbandry Parameters

Table 1: Guidelines for Xenopus Husbandry Parameters

Parameter	Frog (Xenopus Laevis) Holding Requirements
Ammonia	0.0-0.5ppm
PH	7.0-8.5
Nitrite	<1ppm
Nitrate	<40ppm
Alkalinity	>40ppm
Hardness (mg/L)	175-300
Conductivity (µS)	1200-1800
Water Temperature (C)	18-21
Room Temperature (C) (Dependent on whether supplementary in water heating provided)	As above
Lighting - Lux	No recommendation - must provide full spectrum lighting
Lighting - Cycle	12:12

References and Resources

The Australian code for the care and use of animals for scientific purposes 8th edition. 2013

Procedure for Managing & Reporting Unexpected Adverse Events

Green, S.L. (2010) The Laboratory XENOPUS sp. CRC Press, Taylor & Francis Group, Florida, USA

McNamara, S, Wlizla, M & Horb, ME. (2018) Husbandry, General Care, and Transportation of Xenopus laevis and Xenopus tropicalis, Methods Molecular Biology, 1865:1-17.

doi: 10.1007/978-1-4939-8784-9_1. PMID: 30151755; PMCID: PMC6421069.

Torreilles, S.L., McClure, D.E. & Green, S.L. (2009) Evaluation and refinement of euthanasia methods for Xenopus laevis, Journal of the American Association for Laboratory Animal Science,48(5): 512-516.

https://animal.research.uiowa.edu/iacuc-guidelines-euthanasia

https://www.animalethics.org.au/animal-ethics-committees/animal-ethics-seminars/aec-meetings/Session-2-Frog-Marking-Guidelines-R-Johnson.pdf

https://www.med.hku.hk/images/document/04research/culatr/04_Xenopus_RSPCA%20Guidelines_05.pdf