THE UNIVERSITY OF MELBOURNE

ANIMAL ETHICS

ANIMAL CARE AND USE STANDARD

The Animal Care & Use Standards are designed to provide guidance regarding good practice to institutional animal users and carers, as well as Animal Ethics Committees (AECs), on the care and use of animals for scientific purposes such as research and teaching. The Standards are evidence-based, reflecting current or accepted good practice and allow for the flexibility that is required in research and teaching activities using animals.

Working with Reptiles

This standard has been developed by the University of Melbourne Animal Care & Use Standards Committee, and approved by the University of Melbourne's Animal Ethics Committees.

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1. ASSOCIATED STANDARDS

This standard should be read in conjunction with the following University of Melbourne Animal Care & Use Standards:

- Humane Killing of Reptiles

2. SUMMARY

2.1 Research on reptiles may be conducted in their natural environment as field work, or in a captive setting using purpose bred animals or wild-caught specimens. Animals that are collected, handled and released in the field require careful and considered handling to limit the impacts of temporary capture and even greater care if they are being transported from their field site to a captive environment.

2.2 Specimens that are acquired from the wild for captivity face challenges associated with the stress of capture and transport, dietary transition to unfamiliar items and must rapidly adapt to an artificial environment. These events must be carefully managed to ensure there is minimal impact on their welfare and that stressors are minimized.

2.3 Captive-bred specimens do not face stress associated with a new environment, but as with all reptiles housed in captivity must be provided with exceptional, species-specific care and husbandry.

3. BENEFITS & RISKS

3.1 Field herpetological studies allow investigators to view the ecosystems in which a reptile lives and obtain information on their biology, diet, behaviour, social structure and longevity. This information may be used to improve the welfare of current or future captive colonies and to facilitate species management and conservation programs.

3.2 Maintaining captive reptile colonies for investigation allows comprehensive and long-term studies to be conducted, further increasing the available knowledge. Where possible, wild-caught animals should be encouraged to breed in captivity reducing the need to collect new specimens from the wild.

3.3 When in captivity, it is the investigator's responsibility to ensure these animals are suitably housed, fed an appropriate, nutritious diet and provided with everything they need to thrive. Substandard or inadequate husbandry practices can lead to very serious health consequences for these reptiles, and potentially death.

4. PROCEDURE/PROTOCOL

4.1 Considerations for collection of reptiles

4.1.1 There is a minimum number of people to be present for all field-based work as per the University of Melbourne fieldwork guidelines (http://safety.unimelb.edu.au). Required permits, certifications and other associated paperwork must be completed prior to undertaking collection of reptiles.

If the target species is venomous, poisonous or otherwise dangerous then investigators must consult with their faculty and University of Melbourne EH&S prior to undertaking field work. It is suggested that only personnel who have undertaken appropriate training in the handling of the target venomous, poisonous and dangerous species be permitted to catch them, and that an advanced certified first-aid officer (not participating) be present for the duration of the field trip with a first aid kit and oxygen kit (see 4.1.1).

4.1.2 Researchers should aim to conserve and protect the habitat they are working in by keeping disturbance of field sites to a minimum.

4.1.3 Where animals are to be removed from the wild, only the minimum number of animals required to produce scientifically valid results should be collected.

4.1.4 Special concern should be shown for species known to remain with nests or young during certain seasons (i.e. nesting season). Removal of individuals known to do this should be avoided during the nesting season, unless it is strongly justified by scientific reasons.

4.1.5 The investigator must be knowledgeable of all regulations pertaining to the animals under study, and must obtain all necessary federal, state, and local permits for the proposed studies. Researchers working outside of Australia should ensure they comply with all wildlife regulations of the country in which the research is being performed. This includes compliance with the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) regulations where applicable.

4.1.6 Any non-target species of reptiles during collection must be handled as per law, permit and other legislation required. Researchers should be aware of these prior to the collection of any reptiles and make provisions for any non-target species of reptiles caught during reptile collection.

4.2 Methods of capture

4.2.1 Trapping

4.2.1.1 The trap should be selected to accommodate the target species, and ideally be based on techniques from recent publications. It must be of adequate dimensions to ensure the animal can fit comfortably inside, and provide protection from the elements and predators once an animal is caught.

4.2.1.2 The interval between checks may be variable depending on species (including the target species or expected by-catch), weather, study objectives and type of trap. Traps of all varieties must be checked at least once daily, or more frequently in extreme weather conditions (heat or cold). Weather extremes (for the target species) may necessitate the cessation of trapping and closure of traps.

4.2.1.3 Pitfall traps must be equipped to prevent desiccation and dehydration during very dry periods of weather and to prevent saturation or drowning in very wet times.

4.2.1.4 Elliot traps containing a pressure-sensitive plate that releases a spring-loaded door when the animal enters may be used with or without bait.

4.2.1.5 Funnel traps made of wire mesh may be placed in trees for arboreal serpents or against fence lines. Animals are able to enter via the funnel but are unable to easily find the exit. Additional checking may be required for this type of trap as the level of protection provided to the animal may be reduced compared to other trap types.

4.2.1.6 Pit traps and other physical traps should be securely covered, removed or closed when not in use and removed at the conclusion of the research.

4.2.2 Direct capture methods

4.2.2.1 Investigators should select a method of direct capture (e.g. hand netting/noosing) that is appropriate to the size, type and environment of their target species. The capture technique should pose the least amount of risk and distress to the animals and the safety of researchers.

4.3 Handling and restraint

4.3.1 Investigators have a responsibility to determine and use the least amount of restraint necessary to perform their procedure in a humane manner, with minimal distress or suffering caused to the animal. In some cases, this may include the use of sedative or anaesthetic agents, especially for species which are venomous or capable of inflicting serious injuries on themselves or those handling them. Others may be more amenable to safe handling in a quiet, darkened and temperate environment.

4.3.2 When handling wild animals, including reptiles, there is the potential for personnel to be bitten or scratched. Some snake and lizard species are venomous and a bite from these may have potentially fatal consequences. Investigators should be familiar with the defensive strategies of their target species and other species that may be encountered in the field.

4.3.3 Maintaining a barrier between the researcher and the animal may help to reduce the risk of injury. The use of traps that allow the animal to be visible to researcher prior to opening it is encouraged. Thick gloves should be worn where practical, and items such as cloths or towels may also assist to reduce contact with claws. For potentially hazardous reptiles, such as very large crocodilians or venomous species, chemical restraint is strongly advised.

4.3.4 Lizards

4.3.4.1 Small lizards can often be handled safely for short periods of time and in captive situations may habituate to human interaction and examination. For small species, a one-handed grip with the head restrained by the thumb and index finger to prevent rotation of the skull (and potential bites) may suffice. Medium sized lizards will require a two-handed grip, with one hand around the base of the skull and the other around the body at the level of the pelvic limbs and tail base.

4.3.4.2 Large lizards such as monitors should be handled with great care, and chemical restraint may be necessary.

4.3.5 Snakes

4.3.5.1 Non-venomous snakes are generally handled by grasping the caudal end (tail) in one hand and its head controlled with a snake hook by an experienced handler. It should then be placed into a suitable bag to reduce the ongoing stress associated with capture.

4.3.5.2 Work with venomous snakes requires a minimum of two people and should never be done alone. Handlers should utilise a long plastic tube and snake hook to facilitate capture and restraint. The snakes head can be guided into the tube using the hook, and when the head has advanced far enough into the tube, the animal is secured by grasping the base of the tube and its junction with the snake’s body. This prevents the snake moving forward or backward, and ensures a solid barrier is between the snake’s head and personnel.

4.3.6 Chelonians

4.3.6.1 The most suitable method of restraint for a chelonian will vary depending on its size. Smaller turtles or tortoises are easily immobilised by hand. A hand placed on the carapace and/or plastron will allow examination of the limbs, head and neck. A towel or cloth may also be used to immobilise the limbs if required.

4.3.6.2 Larger chelonians such as sea turtles or land tortoises may require multiple people for handling and restraint. These species feel most secure with a solid base beneath them and it is essential this surface be non-abrasive to avoid causing injury to feet or flippers.

4.3.7 Crocodilians

4.3.7.1 Small crocodilians and juveniles can usually be handled safely without the need for chemical restraint. Their primary defensive mechanism is to bite with their strong teeth and jaws, or to twist and roll in an effort to avoid a capture. Caution should be exercised around the long, muscular tail and claws.

4.3.7.2 Small crocodilians may be picked up by placing one hand behind the neck and another at the base of the tail. To hold the animal, its body should be supported underneath by one arm, with the hand secured behind the neck or under the pelvic girdle. The tail is then pinned against the body to prevent flicking or movement. For a two-handed grip, a hand is again placed behind the neck with the other around the pelvis and back legs or near the tail base.

4.3.7.3 A minimum of two people must be present if crocodilians over 1.2m long are to be handled. When it is essential to handle large animals, chemical restraint appropriate to the required task should be used.

4.3.7.4 The jaws should be secured using an elastic band (juveniles only), fabric loop or masking/duct tape applied caudal to the nostrils. The band needs to be moderately tight to hold the jaw closed. Adhesive tape can be used provided it is not too strong and could damage the skin on removal.

4.4 Procedures

4.4.1 Venepuncture

Investigators must be familiar with the anatomy and vasculature of their target species prior to attempting blood collection. Care should be taken after needle removal to give the reptile a chance to clot at the site of blood collection. If bleeding occurs from the site after this time, then it should be held off until haemostasis can occur and no blood is noted.

4.4.2 Lizards

4.4.2.1 The preferred site of blood sampling in most larger sized lizards is the caudal tail vein which is located along the ventral midline of the tail. It is important to note that in males, the hemipenes may extend from the cloaca toward the tail tip and should be avoided.

4.4.2.2 Method: restrain the animal on its back and ensure the hind limbs are held out of the way. Swab the ventral tail with alcohol, methylated spirits or chlorhexidine. Introduce the needle along the midline, perpendicular to the tail, and advance it until it contacts the ventral vertebrae. Withdraw the plunger slightly and gently withdraw the needle until blood enters the hub. Slowly draw the sample out, allowing breaks in the suction for the vessel to refill. Once the sample is acquired, remove the needle and place gentle pressure of the site for 30-60 seconds.

4.4.2.3 In many small and medium-sized lizards, blood samples can readily be taken from the sinus angularis vein, accessed at the corner of the mouth, using a sterile needle to puncture the vein then collect the blood with a heparinised capillary tube. However, in very small lizards' sub-orbital sinus sampling may be the only method available. This is a more technical procedure requiring additional training and be specifically noted in the animal ethics application for AEC approval

4.4.3 Snakes

4.4.3.1 Blood should be collected in snakes from the caudal tail vein, located along the ventral midline of the tail. The site of entry should be caudal to the cloaca in females, and caudal to the hemipenes in males.

4.4.3.2 The technique for snakes is similar as to the description for lizards in 4.4.2.2 however there is no ridge of bone to be felt in the vertebrae once the needle is inserted.

4.4.4 Chelonians

4.4.4.1 Blood should be collected from the jugular vein of smaller chelonians (note that the right side is generally larger), or the dorsal tail vein in large tortoises.

4.4.4.2 For venepuncture of large tortoises, they can be elevated from the floor by placing them on a bucket of equivalent diameter to the width of their plastron such that the flippers or feet are in the air. A stabilising hand may still be required on the carapace and to prevent forward or backward movement off the elevated area.

4.4.5 Crocodilians

4.4.5.1 The ventral tail vein is suitable for blood collection in many smaller crocodilians; however in larger ones the supraorbital vessel located just behind the skull can be used with care and suitable restraint.

4.4.6 Chemical restraint

4.4.6.1 When working with a new anaesthetic protocol or species it is advisable to anesthetize a few animals and follow these animals through to full recovery to ensure drug dosages and techniques are safe and provide sufficient anaesthetic depth for the intended procedures.

4.4.6.2 Where sedation is required for handling or other drugs are to be given, investigators must ensure they are familiar with the drug, dose, mode of delivery, expected effects and potential side-effects of its administration.

4.4.6.3 Further information can be sought from the Animal Welfare Officer (AWO) for specific anaesthetic protocols or drug information if required.

4.5 Acquisition of reptiles for laboratory research

4.5.1 Reptiles may be wild caught, obtained from existing captive collections and/or approved commercial suppliers.

4.5.2 Information needed for the development of research protocols should aim to include: body weight (including normal range and any sexual dimorphism if known), size (e.g. snout-vent length), diet, longevity, behaviour, social structure, aggression, habitat and thermal requirements (thermoneutral/ Preferred Optimal Temperature Zone (POTZ) if known), any unusual aspects of biology (e.g. parthenogenesis), relevant known diseases or health issues and any other special needs.

4.6 Acquisition from the wild for captivity

4.6.1 Prior to initiating field research, investigators and their team must ensure appropriate Licences are in place and be familiar with the target species and its response to disturbances, sensitivity to capture and restraint, and, any known requirements for captive maintenance. Establishing the captive environment and appropriate housing conditions for the target reptiles, prior to collection, is fundamental to ensure the animals' safety and survival in the laboratory. This is to be done in conjunction with the AWO as per the AEC recommendations for the housing of captive collections.

4.6.2 When planning a field expedition for the purpose of specimen collection, investigators and their team must develop a species-specific data sheet onto which data can be recorded. This is to ensure a clear plan is in place before an animal is captured, in order to reduce handling time and potential stress caused to the individual.

4.6.3 Field work applications must include a protocol with notes indicating environmental conditions suitable for safe capture from the native habitat. These may include: maximum and minimum temperatures, any weather events that might have an impact (e.g. drought), and maximum safe length of holding time prior to transport. Short term holding conditions or requirements should also include: housing type, temperature regime (preferred optimum temperature zone), necessary water and feeding regimes, and an indication of whether animals must be individually housed or if grouped the maximum number of animals per container/bag.

4.6.4 Field procedures

4.6.4.1 Following capture, an immediate and accurate record of body mass and length with an animal identification number may be collected. The data collected should be clearly ordered with records able to identify the species, animal number, capture site (preferably GPS co-ordinates), time of capture and habitat type.

4.6.4.2 A brief examination should be conducted to assess for external parasites such as reptile mites (*Ophionyssus natricis*) and to screen for obvious injuries or skin lesions that may reflect a known health issue, disease or pathogen. If abnormalities are found, then a decision must be made regarding the welfare of the animal and its ability to continue to survive in the wild. Investigators should refer to the intervention criteria sheet to assist with this decision making.

4.6.4.3 Reptiles held or enclosed in the field (i.e. caged or placed in a pen in their natural environment) should be monitored carefully for natural behaviours. Water should be provided by misting at least once daily or offered in appropriately sized shallow dish. Aquatic chelonians and crocodilians must have access to water deep enough to submerge at all times unless an exception is approved by an AEC on scientific grounds. If held for over 72 hours, then sufficient food resources should be made available.

4.6.5 Equipment care

4.6.5.1 All equipment including boots, buckets, nets etc. should be cleaned at the site to remove debris, mud or dirt. After reducing the organic matter, they should initially be washed in soapy water to clean the remaining debris followed by soaking or spraying with an appropriate disinfectant. Disinfectants, such as F10sc®, bleach (sodium hypochlorite), and VirkonS®, require not only a clean surface to be effective, but also need the appropriate contact time and concentration to be used. This information can be obtained from the manufacturer.

4.6.5.2 Equipment should be checked prior to use to ensure it remains in good working order for animal and animal handler safety. Any issues with equipment should be rectified prior to use and if needed replaced. Disposables (i.e. nitrile gloves) should also be checked and researchers should ensure there is sufficient quantity available.

4.6.6 Transport

4.6.6.1 The specific type of transport container will depend on the size and species of reptile. Small lizards and snakes can be transported individually in cloth bags (inverted to place seams on outside to prevent entanglement), with multiple bags being placed into larger, insulated vessels (e.g. Cooler box or Esky®) modified to provide ventilation or opened at least once a day to provide fresh air. The exact number of individuals per vessel will depend on their sizes and species.

4.6.6.2 The transport container must not be left in direct sunlight, and investigators should aim to keep the ambient temperature as close to the reptile's usual environment as possible. Air conditioning of vehicles is required when transporting reptiles to avoid overheating, as there is no opportunity for the animals to move to an alternate thermal area.

4.6.6.3 Where overnight stays at field sites or camps are required, it is the investigators responsibility to ensure suitable housing is available with an appropriate degree of climate control. Water must be available during this time, either by misting or a water container (i.e. bowl), depending on the species preference.

4.6.6.4 The time from first capture to placement into an approved facility must not exceed 10 days.

4.7 Acclimation

4.7.1 Transport and/or capture are highly stressful events for reptiles and great care must be taken to mitigate the negative effects that may occur. An acclimation period is necessary to allow the animals to adjust to their surroundings, which can include a change in diet, lighting, temperature gradients and housing.

4.7.2 Reptiles should not be used for procedures or investigations until they have had adequate time to acclimate. This is to ensure they have time to recover from stress and to avoid inaccurate results that may be altered by a stressed physiological state.

4.7.3 The time required for acclimation will vary between and across species, and wild-caught reptiles will require additional time to acclimate compared to those born in captivity. At a minimum, captive-bred animals or those previously acclimated and housed in captivity should be allowed 7 days to adjust to the new facility before beginning experiments. Wild-caught animals should be allowed to acclimate over 3-4 weeks, unless experience with the species suggests a longer timeframe is required or there is a scientific/animal welfare justification for a shorter acclimation period.

4.7.4 Daily observations of the reptiles' body condition, food intake, activity, and behaviour is essential for any new animal. Body weight should be recorded a minimum of twice weekly.

4.7.5 Reptiles that are not feeding, losing weight and/or have decreased body condition over the first few weeks in the facility, may not be acclimating well. Additional interventions such as assisted feeding and increased monitoring may be required as per the Intervention Criteria Sheet (ICS). (See Appendix V)

4.7.6 The AWO can and should be utilised for additional advice if investigators have any concerns about the health and welfare of their reptiles.

4.7.7 Where a reptile fails to acclimate to captivity as identified by the ICS, then humane killing may be required to ensure it does not suffer.

4.8 Biosecurity: Health checks and Quarantine

4.8.1 Any incoming animals, including those from commercial suppliers, should be subjected to careful inspection for potential health problems or pathogens, prior to introduction to any existing laboratory colonies. A veterinarian with experience in reptiles and/or the AWO should be consulted if necessary.

4.8.2 Incoming animals should be treated as their own separate cohort for the purpose of quarantine. They should not be mixed with any animals currently housed at a facility until an appropriate quarantine time has been served.

4.8.3 Ideally, lizards and chelonians should be in quarantine for at least one month, while some snakes may require 6-9 months of isolation. This is rarely practical in a research setting; however, it highlights the need for continued health monitoring and good hygiene where shorter periods are implemented. A minimum of 7 days quarantine is recommended if bringing new reptiles into a facility with an existing reptile cohort.

4.8.4 Quarantine housing should provide appropriate light cycles, lighting, heat and diet; however the substrate may be altered for ease of cleaning and disinfection. Paper-based substrate should be changed at least every other day for quarantined animals.

4.8.5 A thorough examination for ectoparasites should be undertaken as part of the general health check and screening for endoparasites via a series of faecal floats is recommended (pooled samples may be used). If mites are detected, ivermectin at an appropriate dose may be used for lizards, snakes and crocodilians. Ivermectin must NEVER be used in chelonians as it is extremely toxic.

4.8.6 All incoming animals (excluding chelonians) should be treated for endoparasites using fenbendazole prior to transferring into the main facility holding areas. This requires an accurate weight measurement to within 1 gram to ensure a safe dose can be delivered, especially in small reptiles. Fenbendazole can be given orally at 25-50mg/kg once daily for 3 to 5 days, then repeated in 10 days if required, provided the animals tolerate this handling and are not excessively stressed by it. Even a once off dose at the higher dose range is likely to be beneficial if the 3 day course cannot be completed.

4.8.7 Weight should be monitored for animals in quarantine in case of failure to adapt to the captive diet or due to underlying illness. The frequency of body weight measurements will need to be balanced with the potential stress that may be caused to the animal. Therefore, body condition scores should also be considered to monitor body weight as this can be obtained without the need for handling.

4.8.8 Quarantined animals should be cleaned, fed and handled last in animal care facility regimes to reduce the risk of disease transmission.

4.8.9 Before and after servicing the quarantine area as well as between individual cages, staff must thoroughly wash their hands in warm water and soap for at least 20 seconds, or use an alcohol or disinfectant based gel rub.

4.8.10 Separate equipment must be used in the quarantine area and must not be used in the main facility. This includes cage furniture, bowls, scales and cleaning equipment.

4.9 Captive husbandry

4.9.1 The precise care requirements for reptiles are highly species-specific and must be tailored to the animal's ecology and habitat, behaviour, social structure of the species/taxonomic group and diet. The advice provided in this Standard should be considered a starting point for the groups mentioned unless specific species are referenced. It is not intended to supersede or provide expert guidance for individual species, but rather to specify the minimum considerations required to successfully house members of the taxonomic group.

4.9.2 A guide must be prepared for each incoming species that outlines the husbandry and housing requirements to ensure facility staff and investigators are aware of their species-specific needs. The species care guide should be prepared in consultation with the current literature available and with further assistance from herpetologists experienced in keeping the species such as zoo staff, investigators or faculty veterinarians with expertise in reptiles. The care guidelines should be made available for the AEC with the animal ethics application documents.

4.9.3 Individual enclosures or racks of enclosures (e.g. tubs housing lizards) must have their own data monitoring sheet. Investigators or technicians should note the following information: substrate (type, date added, replacement date, spot cleans), UV light (type, date added, replacement date), temperature (required range, hot spot temperature, time checked), reptiles housed (species name, number and sex of animal/s).

4.9.4 Animals in each enclosure should be individually identifiable, either by single housing or documentation of markings/patterns, tags (if large enough), or preferably using an implanted microchip. Note that microchips in reptiles are placed under the skin on the lower left quadrant of body.

4.9.5 Housing

4.9.5.1 The number of animals per enclosure will depend on the species, with the majority of adult reptiles being better suited to individual housing to avoid aggression between cage mates. Where pairs or groups of animals are housed together, then the care guides should note a maximum number of animals per cage, any special requirements for different sexes, and how to differentiate between sexually dimorphic animals where appropriate.

4.9.5.2 The size of the enclosure will depend on the size and behaviour of the reptile. Arboreal species need tall cages compared to burrowing or ground dwelling species that need horizontal space. The cage should be sufficient to allow a thermal gradient, but not so large that smaller reptiles will have difficulty with thermoregulation.

4.9.5.3 Snakes must be housed individually unless specifically paired for breeding purposes.

4.9.5.4 Aquatic chelonians and crocodilians require access to water at all times for swimming and feeding. Tanks or enclosures and the volume of water required will vary with the species and its size.

4.9.5.5 Chelonians may be group housed successfully up to 6 months of age but should be kept individually or in male-female pairings after this time.

4.9.5.6 Crocodilians require a minimum land area of 1.5 times their snout-to-vent length (SVL) on all sides, with enough room to stretch out fully and turn around comfortably when on land. If 2 or more individuals are kept together, the sides must be 2.5 times SVL of the largest animal. Ponds must be large enough that all individuals can be submerged simultaneously without touching, and covered by at least 15cm of water.

4.9.5.7 Hatchling crocodiles must be housed individually due to their high rates of intra-species aggression and tendency to injure each other. Adults or sub-adults can be housed in a group; however, will require close monitoring for aggression or dominance issues. All measurements for enclosure and pond sizing should be based on the largest animal in the group and will need to consider any potential growth of the animals.

4.9.5.8 Housing of hazardous species such as crocodilians and venomous snakes requires special care to avoid escape. Hazardous species should be kept in locking cages (i.e. cages with locking mechanisms that do not rely on weighted lids), which should in turn be isolated in locked, escape-proof rooms. Such rooms should be inspected carefully and any potential routes of escape (including air vents, drains, exposed fixtures, or cracks under doors) appropriately blocked. Signage identifying the enclosed animals as venomous is mandatory.

4.9.6 Substrate, cage furniture and enrichment

4.9.6.1 Substrates vary according to the needs of the species. For most terrestrial lizards and snakes, newspaper, butcher's paper or recycled paper cat litter may be used. Paper based substrates are cheap and can be disposed of when soiled, whilst synthetic mats can be used in rotation with thorough disinfecting between uses. For some desert species, sand may be an appropriate substrate choice, though should be avoided in large Agamids such as Bearded Dragons. Though considered more natural, some species will inadvertently ingest sand, which may cause a life-threatening gut impaction or cloacal prolapse. Substrates such as sand, bark chips or dirt cannot be adequately disinfected, and provide a growth media for bacteria, fungi and parasites. Further advice should be sought from the AWO.

4.9.6.2 Aquatic chelonians should have a 3-5cm deep substrate layer in their tank comprised of aquarium gravel and/or river pebbles.

4.9.6.3 The specific substrate for crocodilians will depend on their size and age, but can include aquarium gravel, pebbles, rocks and logs.

4.9.6.4 Furnishings for the enclosure will vary with the species being housed but typically includes at least one hiding area or structure along with other items for environmental/behavioural enrichment. Rocks, logs, branches, small plants, ceramic tiles, PVC pipe or empty pots may all be used to provide both shelter and enrichment. Investigators should try and choose items that can be easily cleaned and disinfected.

4.9.6.5 Water bowls should be bottom heavy-based to prevent spillage and made of non-porous material to facilitate cleaning and disinfection without the risk of absorbing residues from cleaning products. Many arid species will not drink from water bowls and will only drink when misted.

4.9.7 Heating

4.9.7.1 All poikilothermic reptiles rely on their environmental temperature to support their body temperature and metabolic processes thus they must have access to their Preferred Optimal Temperature Zone (POTZ). However, this POTZ may vary depending on the seasonal conditions (i.e. overwintering). This typically should be provided as a thermal gradient (i.e. a 'hot' end and 'cool' end) with a basking area provided by a heat source for access to their optimal temperature.

4.9.7.2 Enclosures may be heated using radiant heat sources (heat lamps or ceramic globes) or convective heat (heat mats or tape). Arboreal snake species, lizards, turtles and crocodilians rely on radiant sources of heat to thermoregulate and must be provided with a basking spot under the heat source. Ground dwelling snake species and some geckos utilise conductive heat, and other species may require a combination of both heat types.

4.9.7.3 The reptile must not be able to directly touch the heat source. It must be covered with a cage or mesh barrier and/or placed at a suitable distance away for non-climbing species.

4.9.7.4 Water in tanks for aquatic chelonians or crocodilians should be heated with a submersible aquarium heater and positioned near water currents to facilitate the movement of the warm water. The output of the heater (wattage) should be appropriate for the volume of water. In large tanks, the use of two smaller heaters at either end of the water source may be beneficial.

4.9.7.5 An extremely comprehensive guide outlining the thermal gradient, POTZ, day length and UVB requirements of over 250 species is available as an open access publication online (Baines *et al*, 2016, How much UV-B does my reptile need? The UV-Tool, a guide to the selection of UV lighting for reptiles and amphibians in captivity). Investigators and animal technicians are strongly encouraged to read this paper to ensure they understand the crucial principles of UV access and heat provision in reptiles.

4.9.8 Lighting and Ultraviolet Light access

4.9.8.1 Reptiles should be provided with a day-night cycle appropriate to the time of year and their natural environment. This can be achieved using artificial lighting in the form of UVA/B emitting lamp on a timer.

4.9.8.2 A heat source must always be provided in addition to a source of UV light (or another suitable source), as warmth is required for reptiles to synthesise vitamin D_3 and other metabolic pathways. (See section 4.9.7)

4.9.8.3 UVA (320-400nm) and UVB (in particular the 290-320nm which is present on the earth's surface) are both required by reptiles for normal activity, feeding and breeding behaviour. UVB is also used in vitamin D_3 metabolism to help with calcium absorption, and is important to prevent metabolic bone disease.

4.9.8.4 The Ferguson Zones match the typical behaviour of a reptile in a given habitat to their likely UVB exposure. A summary of these zones is found in Table 1.

Table 1. The Four Ferguson Zones (Table adapted from Baines *et al*, 2016)

Zone	Characteristic of Typical Reptile Found in Zone	UV lamp type	Species Examples
1	Crepuscular or shade dweller	Fluorescent lamp or globe	Crepuscular pythons (e.g. Children's Python, Geckos)
2	Partial sun or occasional basker	Fluorescent lamp or globe	Diurnal pythons (e.g. Carpet python)
3	Open or partial sun basker	Mercury Vapour/ Halide lamp	Blue-tongued lizard, Bearded dragon, Eastern Water Dragon
4	Mid-day baskers	Mercury Vapour/ Halide lamp	'Extreme' desert species (e.g. Painted Dragon). Note that shade is vital even for these species.

4.9.8.5 Reptiles in Zone 1 and 2 should be provided with low-level UVB over the enclosure to resemble being outdoors in a shaded area. This is best provided by fluorescent UVA/B tubes or globes.

4.9.8.6 Reptiles in Zone 3 or 4 should be offered a smaller, more concentrated basking area of UVB to mimic the effects of a sunlight patch. This is best achieved with a mercury vapour or metal halide lamp, although in small enclosures, a high-output fluorescent lamp may be needed.

4.9.8.7 UV lamps should be positioned above the reptile to avoid any light shining sideways or from below them. The quality of UV radiation is reduced as distance from the lamp increases, so a maximum distance of 45cm between the lamp and animal is suggested for most species and enclosures where appropriate. Excessive UV exposure, by way of an inappropriate wavelength or insufficient shade, can result in illness and disease, such as skin tumours or eye problems. As the UV lamp in the enclosure mimics the sun in nature, it must be switched on only during the daytime cycle.

4.9.8.8 No glass is to be placed between the lamp and the reptile, as glass will dissipate the UV and prevent it reaching the animal. Mesh covers on enclosures are acceptable.

4.9.8.9 UV lamps must be replaced at least every 12 months, unless regular testing with a UV meter is conducted to assess its continued output.

4.9.9 Diet and feeding

4.9.9.1 Food for reptiles should only be acquired from reputable sources and preferably via commercial suppliers that offer a quality guarantee. Any fresh produce offered should be washed before use and not fed if it is wilted or rotting.

4.9.9.2 Food should only be offered to warm and active reptiles to avoid problems with digestion.

4.9.9.3 *Lizards*: Juvenile lizards typically need to eat daily or at least every second day, whilst adult lizards can be fed every 2-3 days. Where animals are housed on sand it is especially important to place food onto a suitable surface (i.e. basking tile) or feed container (i.e. bowl) to limit the potential for sand ingestion. If housed as a group, then more than one feeding container is advised. It is also worth noting that some lizards, such as Geckos, are nocturnal and may only eat at night during their active period.

4.9.9.4 Insectivores may be offered crickets, meal worms (sparingly, these are high in fat), silkworms, moths, beetles, cockroaches etc. The size of the insect should ideally be no longer than the width between the lizard's eyes.

4.9.9.5 Vegetable matter can include dark leafy greens, broccoli, carrot, capsicum, etc. and should be raw and in large cubes (approx. $10mm^2$). For native species such as Bearded dragons, native desert grasses or plants from their home range may be beneficial for the long-term prevention of dental disease.

4.9.9.6 Any insects fed to reptiles must be fed a nutritious diet until such time as they are offered as reptile food. An appropriate diet for crickets can be as simple as carrots and oranges. A more comprehensive diet can include a mix of fresh fruit, vegetables and legumes (Example diet carrot, oranges, sweet potato, broccoli, romaine lettuce, raw seeds and nuts).

4.9.9.7 Insects with low calcium content, such as crickets, must be gut-loaded (see glossary) prior to their use as a food source. The types of insects are offered commercial gut-loading formula or fed a combination of high nutrient foods including items high in calcium (e.g. Spinach) within 4-6 hours of feeding them to reptiles.

4.9.9.8 Insects can also be gut loaded weekly with a reptile specific multi-vitamin powder prior to feeding, and twice weekly with calcium powder. Juveniles may require different frequencies gut loading depending on the species and their species-specific needs.

4.9.9.9 **Snakes:** Hatchling snakes should be offered food every 4-5 days, while adults may be fed every 1-2 weeks depending on the species. Snakes should be fed prey at a size and frequency appropriate to their age, size and development.

4.9.9.10 A range of frozen prey items are available from commercial suppliers including pinkie mice/rats, adult mice/rats in numerous sizes, quail of varying ages and rabbits that are suitable for feeding snakes. Prey items should be frozen for at least one month to eliminate parasites. These items must be thawed prior to feeding by placing the prey in warm water and should be gently towel dried before offering to the snake. A microwave should never be used for this purpose.

4.9.9.11 Where applicable live mammalian prey must never be offered to snakes as food. This represents a huge welfare concern for the prey species and often results in the prey species causing severe injuries to the snake. In rare cases an exemption may be granted by the AEC with strong scientific justification and animal welfare implications.

4.9.9.12 Chelonians: Juvenile chelonians should be offered food daily, with adults fed 2-3 times per week. This section will deal with Australian freshwater chelonians (*Chelodina*, the long-necked turtle and *Emydura*, the short-necked turtles), and investigators should consult the specific care guide for other species such as tortoises or marine turtles.

4.9.9.13 Aquatic turtles are ambush feeders and normally feed only when in the water. They may be fed in their enclosure, or placed into a separate feeding tank to facilitate observation of feeding habits and reduce debris in the main tank. The feeding tank should be filled using water from the main tank to reduce any physiological stress that may accompany a sudden change in water chemistry or quality.

4.9.9.14 Members of *Chelodina* are carnivorous, whilst *Emydura* are more omnivorous. A captive diet should be based on live feeder fish (fish should be fed on high-quality commercial pellets), yabbies and invertebrates along with aquarium plants for the short-necked turtles. If this is not an option, whole frozen-thawed whitebait, bloodworms and thawed seafood mix (eg. Marinara mix) can be offered. Meat, such as beef or chicken, and frozen turtle blocks should not be offered due to their potentially limited nutrient contents and unknown formulations respectively.

4.9.9.15 Crocodilians: As a general guide hatchling freshwater crocodiles should be fed 2-3 times weekly, juveniles fed 1-2 times weekly and adults fed once a week.

4.9.9.16 Diets for juvenile freshwater crocodiles are initially based on insects (crickets, cockroaches, silkworms), pinkie mice and whitebait but progresses to yabbies, larger fish and larger rodents as they grow. As adults, their diet is predominantly made up of whole mammalian or avian prey, larger fish and crustaceans.

4.9.9.17 Calcium dusting or injection of calcium into food items is suggested at least weekly for all stages of growth and age to prevent calcium deficiency. This must be used in conjunction with exposure to unfiltered sunlight for a minimum of 4 hours per week.

4.9.10 Drinking water

4.9.10.1 A source of freshwater for drinking should be provided for all reptiles, at all times, including desert species. This may be provided in bottom-heavy, non-porous shallow dish for larger species or a smaller sized container (i.e. bottle caps) for animals under 10-20g or via misting (see 4.9.10.2).

4.9.10.2 Alternative sources of freshwater may be more appropriate such as misting (providing a spray of fresh water from a bottle) to allow droplets to form in the environment and/or on the animal should be considered for species that utilise rain or other water droplets for hydration in the wild. This should be done 2-3 times weekly as a minimum, but may need to be more frequent in tropical species.

4.9.11 Cleaning

4.9.11.1 A disinfectant capable of destroying bacteria, viruses, fungi and parasite eggs should be selected for use in the facility. Suitable options include household bleach (sodium hypochlorite), hydrogen peroxide (PerDiem®), Quaternary Ammonium Compounds (F10sc®) or combination products (e.g. VirkonS®).

4.9.11.2 Commercially available disinfectants should always be made up according to the manufacturer's instructions and allowed adequate contact time based on their concentration. Household bleach can be used at a 1:30 dilution of bleach: water. Personal protective equipment should be used as per EH&S guidelines.

4.9.11.3 Most disinfecting agents have reduced efficacy if applied onto surfaces or equipment with organic material (i.e. faeces, urates, and urine or food matter) so surfaces and equipment should first be cleaned with warm, soapy water to reduce the build-up of organic matter.

4.9.11.4 Spot cleaning of faecal material should be done at least every other day for sand, synthetic grass mats or other particulate substrates. Paper-based substrates should be disposed of and replaced once soiled.

4.9.11.5 Synthetic grass mats should be removed from the enclosure every 4-6 weeks for thorough cleaning and disinfecting. These mats can be hung outdoors in natural sunlight for drying, which has the added benefit of UV exposure to further reduce pathogen numbers.

4.9.11.6 Sand and particulate substrates should be completely replaced every 3 months to limit the accumulation of pathogens inside the enclosure.

4.9.11.7 Water bowls or containers and the water inside them should be cleaned and changed at least twice per week or more frequently if the reptile has been soaking in it. Cage equipment and enrichment should be cleaned and disinfected every 3 months, and spot-cleaned as needed.

5. MONITORING & INTERVENTION

5.1 Captive Monitoring

The following sheets will be required:

A - Species-specific care guide (per project) – See Section 4.9

B - Reptile assignment list (per facility or per room) – Appendix II

C - Cage card (per enclosure/cage) – Appendix I and Section 5.1.1

D - Temperature monitoring sheet (per enclosure) – Appendix IV

E - Reptile monitoring sheet (per individual animal or animals in one cage) – Appendix III

F - Intervention Criteria Sheet (per project) – Appendix V

5.1.1 Cage cards

5.1.1.1 Each enclosure should be labelled with a cage card containing information pertaining to the study and the animals. It should display the investigators initials, Animal Ethics ID, cage/enclosure number, total animals in the cage, date of acquisition, place of origin (i.e. location for wild caught specimens, name of supplier, hatched in-house), the Latin name and common name of the species, sex of the animals (if known), initial body weight on entry, diet required, feeding frequency and day(s) of feeding. (See Appendix I for example)

5.1.2 Record keeping and monitoring sheets

5.1.2.1 One central assignment list must be maintained per reptile facility or room that identifies total number of animals, their species, date of entry, place of origin and date of exit. (See Appendix II)

5.1.2.2 Individual animals or cages should have their own monitoring sheet that notes the date and time of checks, observations of the animal and its ID. Weight, any abnormal findings, faecal droppings and procedures performed should be recorded here. All entries must be initialled by the person undertaking the check. (See Appendix III for example). These may be kept in a folder or log book within the room.

5.1.3 Feeding

5.1.3.1 The diet for the species should be noted on the cage card for easy reference, with full details available in the species care notes.

5.1.3.2 The amount of food offered (e.g. number of crickets, weight or volume of mixes) along with the amount of uneaten food found at the next feeding should be monitored and recorded on the individual animal monitoring sheet.

5.1.4 Weighing

5.1.4.1 All reptiles must be weighed on arrival and admission to the facility, and ideally the body condition score noted.

5.1.4.2 Reptiles show no or very subtle signs of illness when unwell. Body weight and condition is an excellent indicator of potential health issues, and downward trends in weight can be used to alert investigators to potential underlying disease or animal husbandry deficiencies.

5.1.4.3 The frequency of weighing will vary depending on the species and age of the animal. Hatchlings and juveniles may require daily or twice weekly checks, whilst weekly or fortnightly may be sufficient for adults.

5.1.4.4 Where animals have been wild caught and transported to a captive facility, an increased frequency of monitoring will be necessary after arrival. A minimum of 2-3 times a week is suggested, taking care not to further increase animal stress due to handling.

5.1.4.5 Digital scales with 1-2 decimal places should be used to weigh reptiles, with small kitchen scales being suitable to most species under 500g. It is recommended that wild-caught or flighty animals be placed inside an inverted pillow case (seams on the outside) or darkened container with a lid for their weigh-in.

5.1.5 Observations

5.1.5.1 Prior to handling reptiles for weighing and other procedures, the investigator or carer must first observe them in the enclosure.

5.1.5.2 Animals should be assessed for their body condition, gait, behaviour and activity levels, social interactions and respiratory effort. When stimulated prior to handling, their response should be noted in case it is abnormal (i.e. a typically calm animal is suddenly restless or vice-versa).

5.1.6 Temperature

5.1.6.1 The temperature of each enclosure should be observed and recorded twice daily for the first 3 days, then twice per week thereafter onto monitoring sheets.

5.1.6.2 A recording of the hot end, cool end and basking/ hot spot temperatures should be made. For aquatic animals such as chelonians and crocodiles, the water temperature should also be checked.

5.1.6.3 An infra-red temperature gun or digital thermometer with probe (held in required area for at least 20 seconds) should be used to measure the temperature of air and/or water.

5.1.6.4 Use of a 24-hour data logger is suggested to track the temperature of newly set up enclosures and should be used periodically in existing ones to ensure correct temperature ranges are occurring throughout the day.

5.1.7 Reproductive activity

5.1.7.1 The birth of any animals, both live and stillborn, must be recorded. They should be weighed as soon as safe to handle and this recorded on their cage card and monitoring sheet.

5.1.7.2 Thorough records should be kept when eggs are laid and when/if these offspring hatch on the reptile's monitoring sheet. If the reproductive activity is part of a breeding program, it is essential to record when pairs are placed together and/or mating is observed. Any overt displays of courtship behaviour should also be noted.

5.2 Intervention points

5.2.1 The Intervention Criteria Sheet (ICS) should provide guidance to investigators when abnormalities are noted in the health or behaviour of reptiles maintained as part of their project.

5.2.2 Where any moderate signs listed on the ICS are identified, investigators should complete the Troubleshooting Checklist and implement increased once daily visual monitoring and twice weekly weight recording for the animal.

5.2.3 If one or more severe signs from the ICS are noted, then the AWO must be contacted immediately. If not already implemented, commence once daily visual inspections and twice weekly weighing. The AWO may instruct the investigator to monitor the animal more frequently following a discussion of the individual case.

5.2.4 A single missed feed is acceptable provided the animal is bright and active otherwise. If, however the reptile does not eat for 3 feeds or 21 days (lizards, chelonians) or 60 days (snakes), then the AWO must be contacted.

5.2.5 Investigators who are concerned about the health or welfare of reptiles in their care at any time must contact the AWO or AFM to seek advice as soon as the concern arises.

5.3 Unexpected adverse events and humane killing

5.3.1 Where an animal suffers from an unexpected adverse event, that is not anticipated, and the animal's behaviour indicates pain, distress or dies of a cause other than humane killing, the body must be preserved appropriately until a necropsy is performed. The AFM and AWO should also be contacted immediately and correct preservation instructions will be given. An adverse event that may be anticipated may include; a reptile escaping from its housing; a reptile that is bullied by other reptiles; reptiles trapped by housing furnishings; or natural mortality.

5.3.2 A necropsy must be performed on any animal whose illness or death constitutes an unexpected adverse event. The body of an animal found deceased or humanely killed as a consequence of an unexpected adverse event must be refrigerated and the necropsy performed in a timely manner to provide for accurate and reliable results. A full necropsy report as well as any relevant photographs and external Laboratory results should be submitted to the AWO alongside the adverse event report.

5.3.3 Competency to conduct a necropsy should be a listed skill on the AEC application, with investigators noting if they are 'Training' or 'Competent'. Training for this procedure can be provided by the AWO or a competent investigator if required.

5.3.4 Where humane killing is required, investigators should refer to the following Standard: Humane killing of Reptiles.

5.3.5 Humane killing must only be performed by trained and competent individuals and should not be carried out in the view of other animals.

5.3.6 If preservation of a deceased reptile is indicated, then the body should be placed in 10% neutral-buffered formalin, or if unavailable 70% w/v ethanol can be used. The use of formalin ensures tissues are preserved sufficiently for follow up pathology testing if required.

5.4 Medications for use in reptiles

5.4.1 It is important to note that the majority of drugs used in reptiles are considered "off-label" as these products may not be registered for use in reptilian species. As such, toxicological and pharmacokinetic studies are not always available in reptiles and those that have been studied are unlikely to have been performed in the particular species of interest. It is accepted amongst reptile veterinarians that extrapolation of doses may be used as a guide across the group under veterinary advice and if required veterinary prescription.

6. ADDITIONAL INFORMATION

6.1 Where investigators are looking to acquire a species of reptile they are not familiar with or which has limited published data to support husbandry decisions, then they are advised to contact the AWO to discuss alternative, reputable sources of information and expertise. This may include zoological or wildlife veterinarians and reptile keepers, herpetological societies, academics or other references.

6.2 There is huge a diversity of species within the reptilian group along with a myriad of laboratory and field research situations. This has led to the frequent use of the word 'should' in the preparation of this Standard, as the information may not be available to provide specific information and techniques that can be applied to multiple species, field sites or projects. We recognise that context is important when developing protocols for each project, but above all else the aim of the Standard is to ensure optimal welfare standards for reptiles are maintained across all species, disciplines and research projects.

7. ENFORCEABLE REQUIREMENTS

7.1 Completion of a species-specific husbandry guide for review by the AEC, prior to acquiring the animals.

7.2 Provision of a thermal gradient, heat source, UVA/B lighting, appropriate substrate and enrichment in the cage set up.

7.3 Provision of an appropriate diet with nutritional supplements as needed.

7.4 Use of a monitoring and intervention sheet for experimental projects as documented in the approved ethics application.

7.5 Monitoring is completed by trained personnel as listed in the approved ethics document.

7.6 Adherence to monitoring protocols and monitoring frequency as listed in this Standard.

7.7 Any welfare and health issues are escalated promptly by reporting to the appropriate personnel (see Section 5.1) and are immediately recorded on the daily/routine monitoring sheet.

7.8 Necropsies to be performed by appropriately trained staff for all unexpected adverse events and for any animal that dies of a cause other than humane killing. Necropsy records must be kept (this may include photos and/or samples) and forwarded to the AWO as part of the Unexpected Adverse Event report.

8. EXEMPTIONS

Where adherence to this Standard conflicts with proposed work, the University's AECs may grant exemptions to all or part of the Standard. To seek exemption, applications should clearly outline how the proposed work deviates from the Standard, and justify the need for this. Before seeking exemption, it is recommended that you consult with the University's AWO.

9. UNEXPECTED ADVERSE INCIDENTS

An unexpected adverse event is any event, which impacts negatively on the wellbeing of animals, and which was not anticipated, or has occurred at a frequency or severity in excess of what was anticipated in line with the AEC approval. This can be a single or cumulative event, and will normally involve unexpected mortality, morbidity or injury. Anyone identifying an unexpected adverse event must act to remove and/or minimise any immediate risk to animals. Immediately thereafter, the University's AWO and relevant Animal Facility Manager must be notified of the event. The AWO will advise researchers of the appropriate response.

10. GLOSSARY

Scientific Term	Lay Description
Acclimation	A period of time when a reptile that has been moved from one location to another, typically from the wild to captivity, is allowed to adjust to its new surrounds with minimal disturbance. Starting experiments during this time is not generally permitted as to avoid the effects of stress altering results.
Agamid	Lizards of the Agamidae family, often referred to as 'dragons' or 'dragon lizards'.
Chelonians	A reptile of the order Chelonia such as a turtle, terrapin, or tortoise
Caudal	At or near the tail or the posterior part of the body
Cloaca	A common cavity at the end of the digestive tract for the release of both excretory and genital products of reptiles
Dysecdysis	Problems with shedding; may be due to poor humidity or underlying disease process.
Ecdysis	Shedding of skin. Snakes shed one single piece, Lizards and Chelonians shed in smaller pieces over several days.
Endoparasites	Internal parasites
Ectoparasites	External parasites
Fenbendazole	A drug used to treat internal parasites such as round worms and flat worms. Considered quite safe in reptiles and widely used for this purpose.
Gut loading	Describes the process of feeding crickets on a specific high calcium and high nutrient diet a few hours before offering them as food, to increase the insects overall nutrition value to the lizard.
Hemipenes	The paired male reproductive organs in snakes and lizards
Herpetology	The study of reptiles and amphibians

Ivermectin	A drug used to treat parasites such as mites or round worms. Must not be used in turtles as it will kill them.
Quarantine	A period of isolation when new animals can be closely observed and checked for potential illness.
Pelvic Girdle	The pelvis at the lower part of the body between the abdomen and the thighs and/or where that portion of the pelvic bones is embedded in the body
Poikilothermic	An organism that cannot regulate its body temperature internally, so uses behavioural means such as basking or burrowing to maintain warmth.
POTZ	Preferred Optimal Temperature Zone refers to the environmental temperature at which a given species has optimal metabolic function
Supravertebral vessel	A blood vessel in large crocodiles; located just behind the rounded, back part of the skull (occiput) and above the spinal cord.
Sexual dimorphism	Where the two sexes of the same species exhibit different characteristics beyond the differences in their sexual organs
Venepuncture	The puncture of a vein as part of a medical procedure, typically to withdraw a blood sample or for an intravenous injection
UVA	Ultraviolet Light A waves
UVB	Ultraviolet Light B waves

11. REFERENCES & RESOURCES

The following source material contributed to the development of this Standard:

- Baines, F., Chattell, J., Dale, J., Garrick, D., Gill, I., Goetz, M & Swatman, M. (2016). How much UV-B does my reptile need? The UV-Tool, a guide to the selection of UV lighting for reptiles and amphibians in captivity. *Journal of Zoo and Aquarium Research*, *4*(1), 42.
- Carmel, B and Johnson, R. 2014. *A guide to…Health and disease in Reptiles & Amphibians*. Reptile publications, QLD, Australia
- Pough FH. 1992. Recommendations for the care and use of amphibians and reptiles in academic institutions. National Academy Press, Washington, DC.

The following resources may provide additional or supplementary information:

- Canadian Council on Animal Care (CCAC). 2004. CCAC species-specific recommendations on: Amphibians and Reptiles. http://www.ccac.ca/Documents/Standards/Guidelines/Add_PDFs/Wildlife_Amphibians_Reptiles.pdfAmerican Veterinary Medical Association (AVMA). 2007. AVMA Guidelines on Euthanasia. https://www.**avma**.org/KB/Policies/Documents/**euthanasia**.pdf
- Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists. 2004. Guidelines for the Use of Live Amphibians and Reptiles in the Field and Laboratory Research (2nd ed). www.asih.org/files/hacc-final.pdf
- The Code of Practice for the Care and Use of Animals for Scientific Purposes 8th Ed 2013: https://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/ea28_code_care_use_animals_131209.pdf

- Wildlife Act 1975: http://www.legislation.vic.gov.au/Domino/Web_Notes/LDMS/LTObject_Store/LTObjSt4.nsf/DDE300B846EED9C7CA257616000A3571/85925CC790ACABB8CA257761002DA8F2/$FILE/75-8699a076.pdf

APPENDIX I: Cage card template

NB. Where multiple choices are listed, circle the appropriate option.

CAGE CARD		
Investigator: (initials)		Ethics ID:
Cage #	Total animals:	Animal ID(s):
Date of entry: DD/MM/YY		Species: *Genus species* (Common name)
Sex: M/ F/ Unknown		Weight on entry:
Place of origin: internal/ wild / external		Frequency of feeding: Daily/ weekly / fortnightly / monthly
Diet:		Day of feeding: M Tu W Th F Sa Su
Substrate: *(type)* Date substrate to be replaced:		UV lighting: *(brand/type)* Date UV globe to be changed:

APPENDIX II: Reptile assignment list (per facility or per room)

Date of entry	Animal ID	Species	Investigator (initials)	Ethics ID	Date of exit

APPENDIX III: Reptile Monitoring Sheet

Monitoring sheet for (Ethics Application #):

Principal Investigator: **Contact details (BH):** **Emergency contact details (AH):**

Secondary contact person: **Contact details:**

Cage or animal number(s) covered by this sheet: **Species:**

Frequency of monitoring: Daily/ Weekly **Frequency of feeding:** Daily/ Weekly/ Fortnightly/ Monthly **Feeding schedule:** M Tu W Th F Sa Su

Intervention: See Intervention Criteria Sheet attached.

				Observations (Tick any that apply): Add specific signs appropriate to study									Comments/Food consumed/Procedures performed/Actions taken (include fate):	Initials:
Date:	Time:	Animal ID:	Body-weight (g):	Food offered	Normal	Weight loss	Abnormal body condition & posture	Abnormal behaviour	Abnormal response to stimulation	Abnormal activity or movement/ paralysis	Abnormal respiration	Other signs (describe)		

				Observations (Tick any that apply): Add specific signs appropriate to study									Comments/Food consumed/Procedures performed/Actions taken (include fate):	Initials:
Date:	Time:	Animal ID:	Body-weight (g):	Food offered	Normal	Weight loss	Abnormal body condition & posture	Abnormal behaviour	Abnormal response to stimulation	Abnormal activity or movement/ paralysis	Abnormal respiration	Other signs (describe)		

APPENDIX IV: Reptile Enclosure Temperature Monitoring Sheet

Temperature monitoring sheet for (Ethics Application #):

Principal Investigator:

Cage number:

Species:

Temperature range:

Hot spot temperature: Frequency: Temperatures are to be checked twice daily for the first 3 days, then twice per week thereafter.

			Temperature (°C)	
Date:	**Time:**	**Cool end**	**Warm end**	**Hot spot**
21/3/16	*13:00*	*19*	*25*	*37*

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Intervention Criteria Sheet for reptiles

Intervention Criteria	
Criteria are based on the severity of signs, as classified by the table below:	
Observations (see Severity Table below)	**Action required**
No/Mild signs	Twice weekly visual observation unless project specifies more frequent checks are required (e.g. for hatchlings)
1 or more "Moderate" signs	Visual inspection once daily, weigh twice a week. Complete "Troubleshooting Checklist".
1 "Severe" sign	Consult AWO immediately and seek advice
2 or more "Severe" signs (Criteria 1 to 5)	Euthanasia

Please amend table to account for species-specific differences

	Severity Table		
	No or mild	**Moderate**	**Severe**
	Non-specific sign(s):		
1. Appearance	Alert with normal posture, moving around enclosure or across thermal zones	Quiet with low posture, staying close to ground. Minimal movement within enclosure, excessive time in one spot.	Dull with body flat to ground, has not moved from same place in 48 to 50 hours.
2. Body condition *See Appendix*	In good body condition	Reduced body condition, vertebrae more visible and/or pelvic bones easily palpated.	Poor body condition; prominent vertebrae and/or pelvic bones. Muscles reduced over skull.
3. Body weight	Body weight stable or increasing.	Body weight reduced by 5-15%.	Body weight decreased by 15-20%.
4. Behaviour	Normal behaviour, responds to handling (no sign of distress)	Subdued but responsive, decreased interaction on handling or with cage mates	Minimally responsive or unresponsive to activity and provocation
5. Integument **(Scales, skin and/or scutes)** *See Appendix*	No lesions; integument is clean, normal colouration for species; Shedding skin for <7 days	Small lesions present. Integument is dull or dirty. Abnormal or darker colour of skin (esp. Agamids). Shedding >7 days.	Large, ulcerated or bleeding lesions including major fight wounds.
	Intervention criteria: Specific conditions or abnormal clinical sign(s):		

6. Not eating*	Eating all or most of food offered; Does not eat for one feed.	Food intake reduced by 50% or does not eat for 2 consecutive feeds	Not interested in food at all. Does not eat for 3 feeds or 21 days (lizards, chelonians) or 60 days (snakes)
7. Dysecdysis**	Normal shedding; Snakes in one whole piece (including spectacles), Lizards/ Chelonians in small sections	Retained shed over body or around toes, retained spectacles in snakes (seen as opaque or blue eyes)	Toes and/or tail tip constricted or necrotic due to retained shed
8. Injection site reactions	No visible reaction	Mild to moderate swelling, discomfort when site touched or not using limb (for IM injections)	Site of injection cracked, bleeding or has discharge. Significant swelling of limb.
9. Other signs	Seek AFM/AWO advice re: appropriate action or euthanise if animal is in severe pain or distress		

***6. Not eating** Reptiles who have been eating well and either gradually eat less, or suddenly refuse food should be monitored carefully. A single missed feed or reduced appetite is not generally cause for alarm if the animal is otherwise bright and alert, however if the problem persists then monitoring should increase and intervention is warranted. If multiple animals are affected, then the AWO and AFM must be notified.

Common reasons for not eating, or eating less:

- Normal variation in appetite
- Seasonal or hormonal cues
- Inappropriate food offered (unlikely if animal has been eating the food previously without issue)
- Chronic low temperatures or other husbandry not species appropriate
- Underlying illness

ACTION REQUIRED

1. Complete Troubleshooting Checklist and make corrections as necessary. If an issue is identified, then allow the animal 24 hours to readjust before attempting to feed again.
2. If the reptile is group-housed, place it in a different enclosure on its own to facilitate closer monitoring. Label the enclosure with a new cage card and a yellow “Health Monitoring” card. A Temperature monitoring sheet should be started and the animals individual Reptile Monitoring Sheet should be moved to the appropriate area.
3. If after 3 days the animal has not shown signs of improvement, then the AWO should be contacted for further advice and veterinary care as needed.

****7. Dysecdysis** If animals have retained pieces of skin during their shedding events, they may require intervention to enable them to finish the process.

Common reasons for difficulty or an incomplete shed include:

- lack of available water for soaking in
- insufficient humidity
- lack of abrasive surfaces in the enclosure
- Previous scars or healing wounds
- Underlying illness

ACTION REQUIRED

1. Place a shallow dish of water into the enclosure that is large enough for the animal to sit in and soak themselves. A small rock or branch may be added to provide an abrasive surface for the reptile to rub against. Allow 24 hours for the reptile to sort itself out with no handling, but observe at least twice over the day.
2. A high humidity retreat may be created by placing moistened vermiculite or a wet sponge in or on the bottom of a plastic container with an entry port. It may be helpful to provide either option 1 or 2 to any animal that is actively shedding to prevent problems, and is recommended for those with a history of dysecdysis.
3. If after 3 days the retained shed is still present, then the animal should be taken out of the enclosure and placed in a shallow bath of tepid water for 15-30 minutes, provided they are not stressed by this. The head should not be submerged.
4. Retained skin can be removed by gently lifting away with tweezers/forceps once it has been softened. It is not advisable to simply pull or remove the skin prior to allowing the reptile access to humidity and moisture, as damage may occur to underlying new skin in some cases.

Troubleshooting Checklist

1. Is the enclosure size appropriate for the size of the reptile?
2. Is the heat source working correctly? Check the temperature of the hot end, cool end, basking spot and any water for aquatic reptiles.
3. Is the UV light working? When was it installed and when is it due for replacement?
4. Is the day length cycle (day being the time the UV lamp is on) appropriate for the species and time of year?
5. What is the humidity like? Is this species appropriate?
6. When did the substrate get added? How long since it was changed over entirely?
7. Have any new animals been added to the enclosure? What about any new reptiles in to the facility?
8. How has the reptile been feeding? When and what did it last eat? Was this a new or familiar diet?
9. When were the last recorded droppings? Were there normal faeces, urates and urine?
10. When did the reptile last shed? Were there any issues with this?
11. Has the reptile's behaviour changed at all?