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RUAREC Standard Operating Procedure

Capture and Handling Techniques For Animals Used In Research Or Teaching Activities At Rhodes University

Drafted by: Rhodes University Animal Research Ethics Committee (RUAREC)

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1. Background of this Standard Operating Procedure (SOP)

Ethical review and clearance/approval processes of research and/or certain teaching activities involving animals as defined below (referred to as research and teaching in further text of this SOP), must follow rigorous procedures, adhere to stringent standards, as well as fulfil national and international legal/accreditation requirements and best practices. This SOP and all its parts apply to activities in research and teaching that are conducted in/by:

- All academic departments of Rhodes University;
- All institutes affiliated with Rhodes University;
- All investigations conducted by affiliated researchers working with animals at Rhodes University (these are academic and support staff, undergraduate and postgraduate students, postdoctoral fellows, research associates and senior research associates).

The above-mentioned academic units and/or individuals are referred to as RU stakeholders in this SOP. Overall, all the unit operations and steps involved in the ethical review and clearance/approval processes of research and teaching at Rhodes University are aimed at achieving the following:

- To produce new knowledge as part of the academic project at Rhodes University that involves animals;
- To validate, review and continuously update the subject matter and content that are taught as subject matter in all disciplines at Rhodes University which have animal ethics implications;
- To ensure that the knowledge produced and/or validated must be of high standard, as to withstand the peer-review and all other review standards in a given academic discipline in which results of studies that involve animals are published;
- To achieve comprehension and understanding of the necessary and prescribed knowledge of animal physiology, behaviour and other related aspects of curriculum outcomes and/or degree requirements in a particular academic discipline;
- To conduct research and teaching according the principles of academic integrity, fairness and with respect and with the view towards the protection of animal rights;
- To contribute to the preservation of the habitat and the protection of populations of animals in South Africa, on the African continent and world-wide.

In light of the above principles and any other relevant ethical considerations, there are several stages to the animal ethics review process and ongoing monitoring of research and teaching. To ensure that the above-mentioned tenets are achieved, one of the steps in the ethical review and active process is the careful scrutiny to prevent unnecessary harm to all animals in any and all research and teaching activities by RU stakeholders.

In conjunction with the above, it is stated clearly here that there is an urgent and ongoing need to maintain competence and familiarity by RU stakeholders with the necessary procedure to properly handle and conduct research and teaching with all animals. To achieve this, this SOP is designed to achieve one main aim. The SOP is aimed at outlining procedures that are to be followed by all RU stakeholders with respect to the relevant research and teaching activities that involve animals. In that context, animals are defined in the SANS 10386:2008 as those organisms from the groups Vertebrata, Cephalopoda and Decapoda; however, we also include the lower invertebrates in the definition of "animal" in this SOP.

2. Philosophy and practical implementation/execution of the SOP

The philosophy of this SOP is derived from the principles of "*replacement, reduction, refinement, and responsibility*". Therefore, the use of animals that were not collected on permit, as defined above, in research and teaching by RU stakeholders is only sanctioned and authorised if no other alternatives are available to achieve the particular outcomes of research and teaching by RU stakeholders. These considerations must be based on a very careful weighing of risks and benefits with regard to research and teaching activities involving these animals. All uses of animals collected without permits in any research and/or teaching activities, performed by or involving RU stakeholders, is to be based and done on working knowledge with the relevant South African and international norms and standards that govern working with animals in general (see below); and based on input and in cooperation of animal welfare organisations, such as the National Council of the Societies for the Prevention of Cruelty against Animals (NSPCA) and WESSA. In line with these facts, all activities in research and teaching by RU stakeholders with animals imply and are aimed at maintaining compliance and adherence to the following South African legislation and international standards that are aimed at the protection of animal rights and welfare; and take the specific consideration of animals into account:

- South African National Standard no. 10386:2008
- Animals Protection Act no. 17 of 1962 as amended
- Performing Animals Protection Act no. 24 of 1935 as amended
- SPCA Act no. 169 of 1993
- Animal Matters Amendment Act of 1993
- Animal Protection Amendment Bill of 2017
- Professional Code of Ethics of the African Association of Zoos and Aquaria

- Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, Available at: www.cites.org/eng/disc/text.php; website accessed on 18th June 2019).

The above-mentioned legislation and international standards and working knowledge of them ultimately must result in making the RU stakeholders and RUAREC members aware of the fact that sentience of animals must be considered to be the same or at least very similar, as that of human beings (UKMRC, undated a,b). Therefore, the level of pain and distress that animals can and do experience during research and/or teaching, should be considered the same or very similar to the pain perception and distress that might/is experienced by human beings during engagement in the same research/teaching activities conducted on animals. These considerations must form a strong and fundamental part of all unit operations of the animal ethics review process at Rhodes University, which involves animals.

Therefore, RUAREC must undertake their best efforts, in any and all research and teaching performed by RU stakeholders which involves animals, to adhere to the definition of animal welfare is defined as follows (SANS, 2005, page 5):

"*Provision of circumstances that contribute to wellbeing of the animal*".

In addition, the same South African National Standard defines well-being as follows (SANS 10386-2008, page 5):

"*Homeostasis – tendency towards a relatively stable equilibrium between inter-dependent elements, especially as maintained by physiological and psychological processes*".

Animal welfare must further be seen to include the freedom from "thirst and hunger", from "thermal and physical discomfort, pain, injury, distress" (SAAPAB, 2017). Unless otherwise stated in the text below, the following definitions for this SOP were extracted from section 1 of the Veterinary and Para-veterinary Professions Act no. 19 of 1982 (as amended; this act is designated as the Act in further text of this SOP). These definitions are as follows:

Veterinarian – any person who is registered or deemed to be registered in terms of the Act to perform the veterinary profession of a veterinarian. In addition, a veterinarian must be registered in the appropriate register operated by the SAVC (see section 18 subsections/paragraphs 1 and 2 of the Act). An extract of the register signed by the Registrar of the South African Veterinary Council (established in terms of sections 2-17 of the Act) or a certified copy thereof should be provided to RUAREC and filed by the Ethics Coordinator for all the Veterinarians, who are trained and/or have experience in working with specific animal groups (referred to as specialised veterinarians in further text). The specialised veterinarians must provide a proof of experience of previous professional previous work/research experience with these specific animals. The exact nature of the proof must be decided on by a majority of all RUAREC members in a regular or special committee meeting.

The specialised veterinarians must be involved in all research and teaching performed by or including the involvement of RU stakeholders, if such activities involve specific animal groups (e.g., higher primates). The role of the specialised veterinarians is to monitor and ensure/protect the wellbeing and maintenance of homeostasis of the animals in all the research and teaching activities with such animals that are performed by RU stakeholders. It's not necessary for the specialised veterinarians to be (standing) members of RUAREC. However, RUAREC, through its Chairperson and the Ethics Coordinator, should make every effort to maintain an ongoing engagement with the specialised veterinarians. This engagement is to be carried out in the form of activities such as workshops and/or lectures run by, or in collaboration with, the specialised veterinarians. The most fundamental aim of the engagement is to develop and keep up to date the knowledge and expertise of the RUAREC members in relation to the research and teaching involving specific animal groups. RUAREC and all RU stakeholders aim to achieve the fulfilment and maximum compliance with all South African and international regulations and guidelines for the research and teaching which involve the participation of specific animal groups, as defined above. To this effect, all academic units, institutes and/or laboratory facilities must make arrangements to have their facilities inspected for compliance with this SOP by a specialised veterinarian.

3. Capture and handling techniques for animals

Please note: much of this information is taken directly from the SANS 10386-2008.

3.1 Lower invertebrates

Kingdom Animalia

All animal groups not in subphylum Vertebrata, nor in Class Cephalopoda or Order Decapoda.

Lower invertebrates are not specifically covered in SANS 10386:2008. Nevertheless, following the principles from the document, research on lower invertebrates should follow the principles of reasonableness, and care should be taken to minimise harm and distress to invertebrate taxa as much as is possible.

- Capture techniques should not be environmentally harmful. Be mindful of bycatch of small vertebrates (when using aquatic traps/capture techniques (fish, amphibian tadpoles, frogs and toads), aerial netting techniques (small birds), pitfall traps (lizards, frogs, rodents)) and devise ways to mitigate this. Use of generalist pesticides / reagents to kill insects may be environmentally harmful, so other capture techniques are strongly recommended.
- The number of animals used in research should be kept to as small a number as possible. Doing power analyses and statistical assessments of the minimum sample number for the study prior to the application for ethical approval is strongly recommended, so as to determine the smallest sample number that is required for a successful outcome of a study using invertebrates.
- Handling of lower invertebrates should be undertaken with care. Where possible, use of a brush or squeegee or other tool to replace forceps or other methods that might lead to damage is encouraged.
- Where animals are to be maintained in culture, shelter, food, and water needs to be taxon appropriate and sufficient. Likewise, environmental conditions need to be maintained appropriately. The fate of the animals once the study is complete needs to be carefully considered – simple release of invertebrates into the environment without due consideration for potential invasiveness is not acceptable.
- Where data can be collected in the field without harm, it is recommended that this approach be followed, rather than translocating the animals to a laboratory.

3.2 Squid, cuttlefish, octopus, nautilus (Cephalopoda)

Kingdom Animalia

Class Cephalopoda

RUAREC follows the South African National Standards laid out in *SANS 10386:2008*, specifically Sections E.3 and E.4, for the handling of cephalopods in research and teaching at Rhodes University. Modifications to Section E.4 are given below (modifications are underlined):

E.4.3 For transport times of longer than 2 h, provision should be made for cool boxes and extra water <u>of the same temperature as the transport water</u>. Larger specimens may be contained in a polythene bag, filled one third with seawater and the remaining space filled with air (preferably oxygen). Only if poor oxygen has been added to the bag can the bags be sealed and kept cool, otherwise, they should be aerated while the bags remain open

NOTE: Survival of 8 h to 10 h is possible with this method. Small octopuses, hatchlings or egg masses can be transported by these methods over long distances as long as these transport times are not exceeded.

E.4.4 Animals captured at sea are best held in deck tanks of seawater, continuously pumped fresh from the open ocean. Pumping of harbour water is unacceptable because of high contamination levels. Squid will require larger tank space than octopuses. Containers should be covered so as to avoid that the animals are exposed to direct sunlight and to prevent escape.

In addition, use of generalist pesticides / reagents to kill cephalopods may be environmentally harmful, so other capture techniques are strongly recommended.

3.3 Crabs, lobster, crayfish, other decapod crustaceans (Decapoda)

Decapod handling, capture and transport are not specifically covered in *SANS 10386:2008*. Nevertheless, following the principles from the document, research on lower invertebrates should follow the principles of reasonableness, and care should be taken to minimise harm and distress to invertebrate taxa as much as is possible.

- Capture techniques should not be environmentally harmful. Be mindful of bycatch of small vertebrates (when using aquatic traps/capture techniques (fish, amphibian tadpoles, frogs and toads), aerial netting techniques (small birds), pitfall traps (lizards, frogs, rodents)) and devise ways to mitigate this. Use of generalist pesticides / reagents to kill decapods may be environmentally harmful, so other capture techniques are strongly recommended.
- The number of animals used in research should be kept to as small a number as possible. Doing power analyses and statistical assessments of the minimum sample number for the study prior to the application for ethical approval is strongly recommended, so as to determine the smallest sample number that is required for a successful outcome of a study using invertebrates.
- Handling of decapods should be undertaken with care. Where possible, use of a brush or squeegee or other tool to replace forceps or other methods that might lead to damage is encouraged.

- Where animals are to be maintained in culture, shelter, food, and water needs to be taxon appropriate and sufficient. Likewise, environmental conditions need to be maintained appropriately. The fate of the animals once the study is complete needs to be carefully considered – simple release of invertebrates into the environment without due consideration for potential invasiveness is not acceptable.
- Where data can be collected in the field without harm, it is recommended that this approach be followed, rather than translocating the animals to a laboratory.

3.4 Sharks, rays and skates (Chondrichthyes)

Kingdom Animalia

Class Chondrichthyes

Suitable capture techniques:

- *Gillnets.* Gillnets are useful to catch sharks; however, we **strongly** recommend the use of a different capture method due to the non-target nature of gillnets and the potential injury to the gills of captured fish and sharks. If gillnets are the intended capture method, strong motivation needs to be provided to the RUAREC committee detailing why other methods cannot be employed and detailing how injuries from the gillnets will be treated. Being a larger vertebrate, all injuries need to be tended to by a veterinarian. If the use of gillnets are approved, they must be continuously monitored and animals caught must be removed immediately on capture (gillnets may not be left unattended). Details on how bycatch will be treated and handled must also be provided. Gillnet mesh size needs to be carefully considered and must match the intended size of the shark to be caught.
- *Baited hooks*. Either 5–10 hook longlines or rod and line. Heavy line is required to prevent breakage when large sharks are hooked as longlines need to be anchored to the substrate, thus longlines are more effective at capturing sharks greater than 120 cm TL but may not provide a completely representative catch composition. Baited lines must be continuously monitored. For guidance on various baited hook longlines or rod and lines for shark capture, please see the guidelines provided by the Charles Darwin University (RIEL SOP 04.2018 Protocols for Surveying and Tagging Sawfishes and River Sharks). The weight of sinkers should be varied depending on tidal flow, but

should ensure that the bait is on or near the bottom. Ethics approval for the bait used needs to be obtained, if the bait used is a vertebrate, cephalopod, or decapod.

Handling:

- Sharks are dangerous to handle: Consider both the welfare of the animal and the safety of the field crew.
- Minimise handling time as much as possible.
- Do not lift animals only by their tail or one fin. Always use two hands. Animals larger than 1.5 m length will require two people for handling.
- **Do not gaff sharks, rays, or skates.**
- Use a large landing net to lift smaller sharks, rays and skates on board for processing.
- **Do not handle or drag sharks by inserting hands in the gill slits.** Use gloves to handle fish in order to improve grip and provide protection from abrasive skin. Secure small sharks with one hand around the caudal peduncle, and one on the body or by holding a pectoral fin.
- Exposure to the sun must be minimised.
- Whilst on board, hold animals in a large tub with frequent water changes (every 1-2 minutes) or continuous irrigation. Fish should initially be placed in a large tub with water taken from the source of capture. This tub can hold animals during any preparation required prior to processing and tagging and returned to the tub for pre-release recovery.

Removal from fishing gear

Removal of fish from fishing gear should be undertaken as quickly as possible. If the capture method involved the use of nets, an initial visual assessment should occur to determine the extent of entanglement. If entanglement is moderate, the fish can be removed by hand; however, if the fish is more severely entangled, net snips should be used to free the fish from the net quickly. It is far better that the net is cut than spend the time trying to disentangle the fish from the net, causing undue stress for the fish. If the capture method involves the use of a hook (longline or rod and line), the animal must be secured well and the hook be removed only when it is safe to do so – a dehooking device or heavy duty long-handled pliers should be used to remove the hook, do not remove hooks by hand.

3.5 Fish (Osteichthyes)

Phylum: Chordata

Superclass Osteichthyes

RUAREC follows the South African National Standards laid out in *SANS 10386:2008*, specifically Sections G.3, G.4 and G.5, for the handling of fish in research and teaching at Rhodes University. Modifications to those sections are given below (modifications are underlined):

G.3 Capture and acquisition

G.3.1.4 The choice of collection method should take into account the welfare of the animals, worker safety, research objectives, seasonal conditions, <u>required estimated sample size</u> and the type of habitat.

G.3.2 Representative samples

Poor handling of large numbers of captured fish can result in high and unnecessary mortalities <u>in the field or post-transport mortalities in the research facility or post-release mortalities in the field</u>.

G.3.3 Collection of imperilled species

G.3.3.1 Imperilled species applies to those animals officially listed as threatened or endangered. It also applies to those animals identified as candidates for listing. It is important to know if an area or a habitat supports imperilled species and how to identify <u>and sex</u> them.

G.3.3.4 Translocation of imperilled species might require specialized equipment and conditions. This will include the transport used for their return into the wild. Biosafety and biosecurity issues <u>must</u> ~~should~~ be considered.

G.3.4 Wild fish and captive-bred fish

G.3.4.1 Fish caught in the wild may be captured by the research team (with necessary Conservation or CITES permits) or be bought from suppliers. Collection techniques shall be declared. Where dead fish, fish products and eggs are collected, it is <u>important</u> ~~wise~~ to ascertain the disease status and disease transmission risks before transporting to the laboratory.

G.3.4.2 Captive-bred fish are available from hatcheries and other laboratory supply houses, aquaria, and hobbyists. <u>Disease transmission risk must be considered for these animals in the same way as it applies to wild-caught specimens.</u>

G.3.5 Killed and museum specimens

G.3.5.1 The collection of fish from natural populations, for preservation, is necessary for:

a) understanding basic biology, evolution and life history;

b) documenting and recording biodiversity;

c) establishing reference collections;

d) environmental impact assessments and ecological surveys (voucher specimens); and

e) geographic variation and delineation of new species.

f) understanding the spread of infectious diseases, in particular notifiable diseases.

G.3.6 Acquisition of hatchery fish

G.3.6.2 Hatcheries that regularly supply fish to laboratories should be encouraged to develop husbandry and management practices for those fish that are allocated for research consistent with those of the laboratories that receive them.

G.4 Transport

G.4.2 Important considerations are water quality, oxygen, temperature, and ammonia levels. Cooling the water will reduce the metabolic rate and thus reduce the amount of ammonia excreted into the water as well as the oxygen requirement. Species-specific requirements must be considered. Fish excreta lowers pH value levels and fish that have been kept at a low pH value must be acclimatised to an environment with a high pH-value to avoid ammonia autointoxication.

G.4.4 Transport boxes are usually made of cardboard lined with polystyrene (styrofoam) panels for insulation and protection. Ideally, fish should be packed into square-bottomed plastics bags that provide better protection. Bags should be half-filled with original aquaria housing water. The bag should be inflated to balloon capacity with oxygen and sealed off with an elastic band. If possible, water temperature should be lowered to a level that can be tolerated by the species that is being transported. Newspaper should be used to isolate bags from each other and to absorb any excess water. Spiny fish have the capacity to puncture a plastic bag, and shall be transported in more durable containers. If air-breathing species are transported the water surface must never be covered with any materials that can prevent them from gulping air.

G.5 Quarantine and acclimatization

G.5.6 Fish arriving in transport bags should be acclimatized by placing the bags in the tank water to equilibrate temperatures between the bags and the tank water (normally for 30 min). Bags may be clamped to the side of the tanks so that they can be opened for aeration but air stones must be used or pure oxygen must be supplied at all times once the bags have been opened. To reduce stress, the bags should be handled as little as possible, and lighting levels kept low. Transfer of fish should be done gently, using appropriate fine mesh nets.

G.5.7 On entering the quarantine facility, fish should be inspected for any abnormalities and external lesions. Appropriate samples should be taken. The lesions may be ~~routinely~~ treated with approved broad-spectrum antibiotics for fish, ~~anti~~parasiticides, or~~and~~ antifungal agents or any therapeutics acceptable for use in fish.

G.5.11 Lighting provision should be adequate for inspection purposes. Dimmer systems may need to be incorporated.

G.5.14 All wastewater, when discharged from the facility, should enter directly into an approved municipal sewer system. It might be necessary to treat wastewater. This water should be chlorinated for a period of 20 hours with a~~n available~~ chlorine level of not less than 200 mg/L.

3.6 Amphibians (Amphibia)

Class: Amphibia:

All Orders within the Class Amphibia

RUAREC follows the South African National Standards laid out in *SANS 10386:2008*, specifically Sections B.3 and B.5, for the handling of amphibians in research and teaching at Rhodes University.

3.7 Reptiles (Reptilia)

Class: Reptilia:

All Orders within the Class Reptilia

Suitable capture methods for reptiles:

- *Pitfall Traps*: Generally, these are a trap setup with buckets dug into the ground, sometimes with a small fence(s) to direct animals into the trap. Be sure to check the trap several times per day, as pitfall traps can become hot, or flooded, or overrun with ants, and predators could prey on trapped animals. When deciding where to place traps, avoid placing the trap near ant colonies. To avoid heat stress for the trapped animals, place shelter in the trap (in the form of soil, leaf litter or large leaves or bark) and ensure that there is moisture in the trap (by using a wet cloth or small lid of water under leaf litter).

- *String/rope noose:* a pole (often extendable) with a string noose on the end can be used effectively to capture small sized lizards (<30cm SVL). Ensure that the string used is wax-coated or is thick nylon “gut”, so as to avoid injuring a struggling reptile once caught. Larger lizards are more easily caught in pitfall traps, or by hand, or with specialised strong rope nooses. Very large reptiles, such as crocodiles, require specialised nooses and nets, and researchers must be adequately trained to capture these animals. String nooses are not as effective for snakes.
- *Snake-tongs and snake hooks*: Specially made poles with gripping heads (tongs) or metal blunt ended hooks are effective for catching snakes and are the recommended method of capturing venomous snakes. The researcher must be adequately trained in this method of snake capture, as venomous snakes are extremely fast and incompetent handling of a venomous snake could result in a bite and envenomation of the handler. It is highly recommended that researchers intending to work on venomous snakes attend and pass a snake-handlers course (such as the one presented by the African Snakebite Institute, ASI).
- *By hand*. Capture by hand is recommended for smaller-sized, slower reptiles, such as limbless, burrowing lizards, tortoises and terrapins. An attempt to capture iguanas by hand is commendable, but difficult, and other methods may be recommended for these reptiles. The faster, smaller lizards can be caught by hand, but greater success may be had with string nooses or pitfall traps.
- *For crocodiles specifically*: Please follow the guidelines detailed by the SANParks document: “*Standard Operating Procedure for the Monitoring, Capture and Sampling of Nile Crocodiles (Crocodylus niloticus)*.” This document can be obtained either from the Chair of the RUAREC or at this link: https://www.researchgate.net/publication/311452145_Standard_Operating_Procedure_for_the_Monitoring_Capture_and_Sampling_of_Nile_Crocodiles_Crocodylus_niloticus

Handling of reptiles:

RUAREC follows the South African National Standards laid out in *SANS 10386:2008*, specifically Section N.20, for the handling of reptiles in research and teaching at Rhodes University. It is, however, recommended that non-venomous reptiles can be handled by hand, but for venomous reptiles, appropriate handling equipment (such as snake tongs and snake hooks) must be used and holding the venomous reptiles by hand is not recommended. Researchers that work on venomous reptiles must obtain adequate training before handling the animals. Care must be taken with lizards that have autotomous tails – when capturing or handling the lizard, do not hold it by the tail, as it will likely break off.

Transport of reptiles:

- Lizards can be transported individually in cloth bags. Lizards must not remain in the cloth bags for longer than 12 hours, after which they must be placed in a sealed (aerated) container with appropriate food and water.
- Snakes can be transported in specialised snake "tubes" (PVC tubes with screw lids) or plastic containers (with holes for allowance of air into the box) with lockable lids. Provide some shelter within the plastic container to minimise the stress of the snake in transit.
- Similarly, tortoises and terrapins can be transported in plastic containers (aerated, with lockable lids).
- Iguanas can be transported in plastic buckets/containers that are appropriate for their size. Their body and tail should not be forced to bend less than 90 degrees (angle), so ensure that the container is not too small for the animal being transported.
- Crocodiles can be transported in boxes appropriate for their size, with a cloth covering their eyes and their jaws taped shut so as to minimise the stress of the animal and reduce the danger to the researchers transporting them. Please refer to the *SANS 1884-3:2008* for specific procedures (especially clause 6; http://www.sava.co.za/wp-content/uploads/2015/10/SANS-Carnivore-transport1.pdf).

3.8 Birds (Aves)

Class: Aves:

All Orders within the Class Aves

RUAREC follows the South African National Standards laid out in *SANS 10386:2008*, specifically Sections C.4, C.5, C.6, C.9 and C.10, for the handling of birds in research and teaching at Rhodes University.

3.9 Mammals (Mammalia)

3.9.1 Non-human primates and monkeys)

Class: Mammalia:

Mirorder Primatomorpha; Order Primates (excluding *Homo sapiens*)

Please see the ***RUAREC Standard Operating Procedure: Research and Teaching Activities with Higher Primates at Rhodes University*** for specific information pertaining to these animals.

3.9.2 Rodents (e.g. rats, mice and hamsters) and lagomorphs (e.g. rabbits)

Class: Mammalia:

Order Rodentia
Order Lagomorpha

RUAREC follows the South African National Standards laid out in *SANS 10386:2008*, specifically Section L.3.9, for the handling of rodents in research and teaching at Rhodes University. Guidelines for housing and use of rodents in research and teaching is provided in a separate SOP.

3.9.3 Bats

Class: Mammalia:

Order Chiroptera

General procedures for decontamination of field gear, equipment and clothing.

RUAREC follows the guidelines from the Ministry of Environment and Ministry of Forest Lands and Natural Resource Operations, British Columbia, Canada (https://www2.gov.bc.ca/assets/gov/environment/plants-animals-and-ecosystems/wildlife-wildlife-habitat/wildlife-health/wildlife-health-documents/white-nose_syndrome_decontamination_standard_operating_procedures_march_2017.pdf) for decontamination procedures for bat work.

To reduce the risk of introduction and spread of pathogenic agents (fungal, viral or bacterial), particular handling of bats is required. The agent that causes White Nose Syndrome in bats, *Pseudogymnoascus destructans* (*Pd*, formerly known as *Geomyces destructans*) is particularly destructive in North America. While it has not been found in South Africa, standard handling procedures employed in North America to prevent the transmission of this fungal agent are recommended for handling of bats and decontamination of equipment in South Africa – as this will prevent transmission of other pathogenic agents (that could potentially lead to zoonosis). Additionally, if bat work is conducted near fresh water bodies, there is a potential for the spread of amphibian diseases (including *Batrachochytrium dendrobatidis* (*Bd*), the causal agent of Chytridiomycosis, and ranavirus strains) among wetlands and streams.

Before proceeding with decontamination processes, thoroughly wash all mud and other debris from equipment - these can reduce the efficiency of the disinfection procedure.

Various disinfecting procedures can be utilised, depending on the equipment:

- Soaking in disinfectant containing at least 0.26% ammonium quaternary compounds for 10 minutes.
- Soaking in 10% bleach (1:10 or one part bleach to 9 parts water) for 10 minutes (approximately half cup bleach in a litre of water).
- Submersing in water at a temperature of at least 55ºC for > 20 minutes.
- Steam-cleaning of large pieces of equipment may be used where other methods are impractical.
- Wipe down of equipment using either ethanol (60% or greater), isopropanol (60% or greater), isopropyl wipes (70%), or hydrogen peroxide wipes (3%). The latter two wipes have been found effective against *Pd* on contact and do not require soaking. Use of ethanol and isopropanol requires at least one minute of soaking.

Submersion in the above decontamination liquids for the advised time is recommended for smaller equipment (such as boots, ropes, climbing harnesses, mist nets), then rinsed and dried. Equipment larger in size or non-submersibles (such as headlamps and acoustic equipment) can be wiped down or sprayed (where possible) with decontamination liquids or wipes, with time allocations as indicated above. Clothing should be washed in hot water with bleach in a washing machine, hand washed with a pre-soak of 10 minutes in the decontamination liquids above or immersed in a >15-minute pre-soak in water ≥ 60º C. When bats are captured, at the end of each night, or between uses, all nets and equipment that has been in contact with bats must be decontaminated. Any bags or covers for mist nets need to also be decontaminated if they have been in contact with a potentially contaminated net.

Suitable trapping methods include:

- *by hand.* Bats can be caught by hand, by e.g. carefully extracting them from a roosting spot. Captors must wear soft leather gloves (or gloves akin to leather), to prevent the bats' teeth penetrating the glove while also having the flexibility to be able to handle the animals without injuring them. Once in hand, bats' wings should be folded against the body and gently held immobile to prevent injury from bats flapping their wings.
- *mist nets.* Mist nets of an adequate mesh size for the species anticipated should be set up/opened up at dusk and monitored continuously during the capture effort. Once a bat flies into the nets and becomes entangled, the bat should be removed by hand (gloved) immediately and handled as in the point above. Any bycatch (e.g., nocturnal birds) should be immediately extricated from the nets by hand and released. The mistnets should be closed/taken down during the day (i.e., at dawn), to prevent diurnal birds and other animals from becoming entangled. If a bat in a mist net seems stressed or has been entangled for a long time, cut it out of the net. It is better to damage and repair a net than to risk an animal death.
- *harp traps*. As captured bats will roost within the trap, harp traps may be set up at dusk, left unattended at night, and checked early in the morning (at dawn). Captured bats are easily removed from the trap by hand. Harp traps used over consecutive nights in the same location may attract predators, so be aware of the potential for predation on the trapped bats.

Handling of bats

- When handling bats, researchers must wear PPE (personal protective equipment), especially face masks and soft leather gloves. All equipment should be wiped with disinfectant wipes/liquids between each bat being handled, to prevent any transference of pathogens between individual bats.
- Once in hand, bats' wings should be folded against the body and gently held immobile to prevent injury from bats flapping their wings. If measurements of bat wings, legs, and other appendages are required, the bat should be gently held immobile in hand and only the required appendage stretched out for measurement. This pertains to other procedures (like injections, ringing, PIT-tagging etc) too.

Transport of bats

- To transport bats, each individual should be placed in a soft, cloth bag. Release of the bat should be at the site of capture (or at a new location if that is what the research calls for) after it is dark, or if the release site is a cave, any time during the day or night.

3.9.4 Medium and large carnivores

Class: Mammalia:

Order Carnivora (including the herbivorous giant panda *Ailuropoda melanoleuca*)

RUAREC follows the South African National Standards laid out in *SANS 1884-3:2008* (http://www.sava.co.za/wp-content/uploads/2015/10/SANS-Carnivore-transport1.pdf).

For all wild carnivore handling and capture it is recommended that an experienced veterinarian be consulted and present as the majority of these animals will require chemical restraint in order to protect the safety of the animal and the people working with them.

Suitable trapping methods include:

- *free-ranging chemical restraint* (i.e., anaesthetic dart administered by a registered veterinarian). The main advantage of this is there is no bycatch and the animal is not left waiting in a trap for any period of time. The veterinarian must plan the anaesthetic procedure specifically for the required period of restraint. The veterinarian is responsible for the storage and administering of the chemical restraint.
- *cage traps* (sometimes known as box traps). The setting and management of the trap does not require a veterinarian and non-target individuals or species can be released without the presence of a veterinarian as long as safety precautions are taken. Cages must be monitored very regularly (it is recommended that a camera is set up to monitor the trap remotely, as this allows for a fast response time when animals are caught in the trap). This reduces the amount of time any non-target catch spends restrained. These traps are usually baited with food and operate using a pressure pedal, releasing the trap door as the animal steps on the plate. Once caught, large carnivores must be darted (anaesthetised) quickly by a registered veterinarian before transport or handling. This is particularly true for species such as leopards and caracals, which have a tendency to become very stressed and injure themselves in their efforts to

escape. Some evidence of the use of cage traps does indicate that a panicked carnivore within the cage trap can lead to damage to paws and mouths as the animal tries to claw and bite its way out of the trap. Foot loops are recommended over cage traps for large felids, specifically.

- *Foot loop traps* (using stainless steel rope). These are not to be confused with illegal snares. These are a very safe, humane, and widely used method for even the largest of wild felids. When used correctly they can greatly reduce the risk of injury often associated with cage traps.
- *Soft catch leg hold traps or Soft traps.* Less commonly used, not to be confused with the traditional metal “gin trap”, which should be condemned. This type of trap does NOT include the Schneekluth ‘Terminator’ leghold trap.

In all the above-mentioned trapping methods, it is of the utmost importance that trap checks are done at very high frequency and that traps are checked regularly for any failures or damage that may cause injury to a trapped animal. Once a wild carnivore has been trapped, it must be anaesthetised by a registered veterinarian before being handled.

Transport of carnivores

- Wild carnivores shall be transported only in containers specifically designed and prepared for that purpose, with the few allowable exceptions (see next point).
- Wild carnivores which have been anaesthetized, or chemically immobilized, may be transported on, or in, a conventional vehicle, provided that the procedure shall be supervised by a veterinarian who is competent in this type of procedure.

3.9.5 Large herbivores - Terrestrial

Class: Mammalia:

Order Perissodactyla; Family Equidae, Family Tapiridae, Family Rhinocerotidae (rhinoceros)
Order: Artiodactyla; Tylopoda (camel, llama, alpaca), Family Hippopotamidae (hippopotamus), Ruminantia (chevrotain, pronghorn, giraffe, deer, Moschidae, Bovidae)
Infraclass Marsupialia; Family Macropodidae (kangaroo)
Clade Paenungulata; Order Proboscidea (elephants)

RUAREC follows the South African National Standards laid out in *SANS 0331:2000* (http://www.wtass.org/research/sans%20standards/files/SANS-0331-2000.pdf) and *SANS 1884-2:2007* (http://www.wtass.org/research/sans%20standards/files/SANS-1884-2-2007.pdf) for capture, transport, and handling of large herbivorous mammals. Please refer to these National Standards for appropriate procedures for use of large herbivorous mammals in research and teaching at Rhodes University.

Specifically for rhinoceros work, please refer to the IUCN *Guidelines for the in situ Re-introduction and Translocation of African and Asian Rhinoceros* (http://awsassets.panda.org/downloads/rhinotransguidelines.pdf). Rhinoceros boma design and other information pertaining to the biological management of rhinos can be obtained from the *Rhino Managers Handbook* (https://rhinos.org/wp-content/uploads/2020/10/rhino_managers_handbook.pdf).

Specifically for elephant work, please refer to the IUCN *Guidelines for the in situ Translocation of the African Elephant for Conservation Purposes* (https://portals.iucn.org/library/sites/library/files/documents/2003-089.pdf).

3.9.6 Equines

Class: Mammalia:

Order Perissodactyla; Family Equidae (horses, donkeys, zebras)

RUAREC follows the South African National Standards laid out in *SANS 10386:2008*, specifically Section H.3.9, for the handling of horses (specifically) in research and teaching at Rhodes University. Guidelines for housing, biosecurity, transport, and veterinary medication can be found in the document produced by the South African Equine Federation (https://saef.org.za/wp-content/uploads/2019/06/SAEF-Veterinary-Regulations-UPDATED-VERSION-2019-June-ABF-Final-a-without-index.pdf).

3.9.7 Aquatic mammals (all sizes)

Animals included in this group (aquatic species of the following taxonomic groups):

Class: Mammalia:

Order Sirenia (sirenians); Family Trichechidae (manatees)
Order Cetartiodactyla: Suborder Whippomorpha (including Hippopotamidae (hippopotamuses) and Cetacea (dolphins, porpoises, & whales)
Order Carnivora; Family Mustelidae (Subfamily Lutrinae, Subfamily Mustelinae), Family Phocidae (Genus *Pusa*)
Order Rodentia; Suborder Hystricomorpha (capybaras; including Family Castoridae: (beavers), Family Cricetidae)
Order Monotremata
Order Perissodactyla; Family Rhinocerotidae (Javan rhinoceros *Rhinoceros sondaicus*; Indian rhinoceros *Rhinoceros unicornis*)
Order Afrosoricida; Giant otter shrew (*Potamogale velox*)
Order Eulipotyphla; Family Soricidae (shrews); Family Talpidae (moles and relatives)
Order Didelphimorphia (opossums); Family Didelphidae

The SANS10386:2008 does not explicitly mention protocols and guidelines for the care and use of aquatic mammals. We therefore recommend that researchers interested in studying aquatic mammals refer to the excellent document produced by the Canadian Council on Animal Care (CCAC) entitled: "Recommendations for the care and maintenance of marine mammals". A link to this document can be found here: LINK. Additional guidelines produced by the European Association for Aquatic Mammals can be found here: LINK.

4. Animal carcasses, excrement, blood (or other bodily fluids)

Please refer to the *RUAREC Guidelines for use of animals not collected on permit* for the use of these samples in research at RU.

5. References

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South African Veterinary and para-veterinary professions act no. 19 of 1982 as amended (SAVPVA, 2004-2007). Published in the South African Government Gazette as notice no. 26311 in 2004 and updated as notice no. 30184, South African Government Printing Works, Pretoria/Cape Town, South Africa.