Module 13 – Care and Use of Amphibians

Objectives

- to introduce the researcher to the proper handling and care of amphibians
- to discuss the use of various anaesthetic agents recommended for amphibians

Introduction

When working in the field, investigators should follow the Declining Amphibian Population Task Force (DAPTF) *Fieldwork Code of Practice* (http://www.mpm.edu/collect/vertzo/herp/Daptf/fcode_e.html) to reduce the risk of spreading diseases among study sites. Their recommendations acknowledge that the investigator will often be an authority on the biology of the amphibian species under study, and provide advice on techniques that are known to be humane and effective in the conduct of field or laboratory research. (http://www.asih.org/pubs/herpcoll.html]).

Amphibians are sensitive to heat, cold, dehydration and stress. Investigators must make every effort to avoid exposure to unfavourable temperatures, drowning, shock, predation and desiccation. However, since amphibians are ectotherms, they are less susceptible than small mammals to stress from lack of food.

Upon arrival in the laboratory each animal should be closely examined for deformities, injuries and parasites. To identify a parasite, it may be necessary to retain a live animal until the parasite has completed its lifecycle, as identification is often difficult or impossible when based on larvae alone. Investigators should be aware of the possibility of amphibians becoming thermally stressed (especially small amphibians that are handled extensively) due to the transfer of heat from the investigator's hand). As well, studies have indicated that handling time has a negative influence on hormone balance, and can upset the natural antibacterial properties of amphibian skin. It is therefore important that investigators handle amphibians quickly and efficiently.

When handling amphibians, investigators should thoroughly wash their hands or change their gloves between contact with different animals in order to avoid transferring noxious

secretions and pheromone-containing substances among animals.

Physical Restraint and Handling

The type of restraint applied should be appropriate for the particular organism, and handling times should not be any longer than necessary. Some amphibians do not tolerate physical restraint well, and attempts to restrain these species could cause injury to the animals or handlers. When there is a need to restrain amphibians, investigators should keep in mind that amphibians are capable of causing skin irritation or inflicting serious injury to those handling them.

Many amphibians are relatively small and slow moving and can be restrained by hand; however, they are easily injured if excessive force is used. Care must be taken to avoid removal of the protective mucus layer covering the skin of amphibians.

If handling amphibians with bare hands, it is extremely important for investigators to ensure that they have not applied insect repellents, perfumes, lotions, or other potentially toxic substances. Wearing latex gloves when handling amphibians will protect the animals' skin from abrasion, chemicals and the spread of infection; however, latex gloves containing talc should not be worn as they could irritate the amphibian's skin. Gloves should either be 'talc-free' or rinsed in warm water prior to use.

When handling highly toxic amphibians, latex gloves should be worn to protect the handler. Any contact with bare skin or mucus membranes should be avoided.

Frogs and toads

Medium and large frogs and toads (approximately 5 g and larger) should be grasped around the waist (immediately anterior to the hind limbs) with the hind limbs fully extended. The animals should not be allowed to flex their hip and knee joints, as this would allow them to kick. For larger animals, a second grip should be maintained around the forelegs.

Chemical Restraint and Anaesthesia

There is useful information on the CCAC web site regarding amphibian anaesthesia and in Module 10. However, a veterinarian experienced with the use of anaesthetics on amphibians should be consulted when planning their use in a new project. Certain chemicals produce initial

excitement before anaesthesia; the use of tranquilizers in conjunction with the aesthetic agent may be indicated.

General extrapolations for amphibians should not be made from aesthetic use in mammals. Ambient temperature and decreased metabolic rate can have profound effects on amphibian anaesthesia and lower doses may be needed. Injectable anaesthetics can often have prolonged effects in these animals and may take hours and the animal may take several days to recover. Since temperature will affect dose, rate of induction and recovery time, care should be taken to maintain the animal at its preferred body temperature.

Hypothermic anaesthesia is considered inappropriate for amphibians as it does not appear to induce rapid unconsciousness in these animals and the adequacy of this method of anaesthesia is difficult to monitor.

Amphibians

The skin of amphibians is highly permeable to many chemicals, and therefore soaking an amphibian in a solution of a soluble anaesthetic agent is an effective method to induce anaesthesia.

Tricaine methanesulfonate (TMS), also known as MS-222, is the agent of choice for anaesthetizing amphibians, providing the dose is calibrated for the species and size of the animal and the animal is carefully monitored. The US National Wildlife Health Center (NWHC) provides useful information on anaesthetizing amphibians in the field (http://www.nwhc.usgs.gov/research/amph_dc/sop_anesth.html). Amphibians are usually anaesthetized by submerging the animal in an aqueous solution of TMS. Because TMS is acidic when dissolved in water, the solution imposes stress on the animal and the majority of the tricaine converts into a form that cannot be absorbed. TMS should therefore be buffered with sodium bicarbonate.

To reduce the risk of anaesthesia complications, investigators should ensure that the animal is removed from the anaesthetic solution and given a brief but thorough rinsing in fresh water to remove residual anaesthetic as soon as the animal becomes unresponsive to the eyelid-touch and toe-pinch tests. Anaesthetic solutions should not be discarded into surface water.

Once the animal has been anaesthetized, and until it is fully recovered (which can take

over 30 min), care must be taken to ensure that it does not drown. Most amphibians respire through their skin in addition to lungs and gills. In anaesthetized animals, in which lung respiration is reduced or interrupted, the body skin should always be kept moist with a thin layer of wet tissue or gauze in order to allow respiration through the skin.

The length of time required for recovery from anaesthesia depends on the life stage, anaesthetic, temperature, species and depth of anaesthesia. Usually, amphibians recover from TMS within 30 to 90 minutes after being rinsed in fresh water. While recovering, amphibians should be placed in fresh water at ambient temperature and kept away from direct sunlight and temperatures greater than 25°C. When an animal can swim it may be released.

Marking

Tattoos, paints and dye markers. When required, tattooing has been used with success on amphibians. This method is very time consuming and requires wiping the skin of the animal clean. Some potential problems may be resolved prior to applying tattoos by ensuring that: 1) the dye contrasts with the normal skin pigmentation; and 2) the loss of tattoo legibility due to diffusion or ultraviolet degradation is minimized. When the toxicity of a marking agent is unknown, it should be reviewed in the literature or evaluated in laboratory trials before being applied in the field.

In general, the use of paints should be restricted to specific purposes (i.e. where quick visual identification is necessary) and routine use should be avoided. Paint should never be used to mark the moist and permeable skin of amphibians.

The injection of coloured inert plastics into the toe webs of amphibians has been used to mark individuals. Additionally, Visible Implanted Elastomer (VIE), a technique created for fish, is becoming commonly used to mark amphibians. Current research indicates that VIE will last for the life of the animal; however, problems associated with this technique include: migration of the mark when injected into the thigh, the lack of fluorescence of the mark due to the dark pigments of most amphibians, and the need to keep VIE cold until injected.

Toe clipping. Toe clipping should be avoided if at all possible. It is recommended that when less painful permanent marking methods are available, they should be used as long as they do

not influence survival or behaviour.

Banding and tagging. Marking methods should be chosen which are suited to the species, habitat and research goals. These methods are subject to the limitations mentioned for tagging in the general guidelines. In general, external tags are not recommended for amphibians. For amphibians, various coloured beads can be added to a stainless steel wire that is passed through the thigh and around the femur. With this method, there is a risk that the tags or wires could become entangled. Additionally, improper insertion of the wire could cause necrosis of the muscle and bone. Petersen disc-type tags have been used on some frogs by placing them in the web between the hind toes; however, only large frogs are able to accommodate even small disc tags.

Medical/Surgical Procedures

Pain perception in amphibians is likely to be analogous to that in mammals. Invasive, potentially painful procedures should be accompanied by both analgesia and anaesthesia. Appropriate standard surgical preparation protocols are recommended to prepare skin surfaces for any invasive procedures (e.g., biopsies, incisions or amputations). Any skin preparations must not contain alcohol as it is absorbed through the skin and dissolves surface secretions that provide protection from dehydration and infection. Iodine as a topical antiseptic is lethal to amphibians and should not be used. Tetracycline (topical) works well for external infections in captive animals, but it is powerful and must be handled carefully and injected intramuscularly. Injection into the dorso-lymphatic sacs in frogs provides a means of administering a dose rapidly; however, caution must be taken to ensure it is not too rapid.

Blood collection

For frogs and toads, blood may be taken from the femoral vein, the ventral abdominal vein or the lingual vein. Blood may also be collected using cardiac puncture; however, this is an invasive procedure and should be carried out under anaesthesia unless the animal is adequately restrained. It has been reported that anaesthesia may be more stressful to the amphibian than the cardiac puncture. There are concomitant risks in using anaesthetics if the animal is not

carefully monitored, but research is needed to determine the relative levels of stress for cardiac puncture, with or without anaesthesia.

Transportation

Individuals of endangered or threatened taxa should neither be removed from the wild (unless in collaboration with conservation efforts) nor imported or exported, except in compliance with applicable regulations.

During transportation, amphibians should be placed in containers that are closed, adequately ventilated, constructed of non-toxic materials, and insulated to protect the animals against temperature variations. The International Air Transport Association (IATA) *Live Animal Regulations* are a good source of information on container designs and appropriate animal densities within containers. All containers intended for re-use must be thoroughly cleaned and disinfected or sterilized.

For short periods of time, most amphibians may be accommodated in cotton bags, knotted at the neck, and transported in styrofoam coolers. The bags must be carefully inspected for holes. Proper ventilation and protection from temperature extremes are essential. Bags should be kept out of direct sunlight and away from hot surfaces, as amphibians can overheat quickly.

Amphibians

Amphibians need to be kept moist. This can be accomplished during transportation by keeping the bags wet if they are being transported in bags, or providing damp moss or moistened paper towel if they are being transported in plastic containers. Caution should be used, however, as the weight of a wet bag or substrate can smother a small amphibian. Amphibians may also be transported in a sealed plastic bag containing a small amount of water and blown full of air. In all cases, the bags or containers must be kept cool and out of direct sunlight.

For shipping amphibians, two distinct types of containers should be used: a packing container to house and contain the animal, and a rigid outer shipping container to hold the packing container. Packing containers should be made of water-resistant material that will not degrade when wet. Cardboard boxes cannot be used as they will weaken and break when wet by the packaging substrate or urine from the animals being shipped. New or cleaned plastic or styrofoam containers (deli or margarine containers) are suitable packing containers for

amphibians. Packing containers must be thoroughly washed and rinsed and allowed to air dry before use. Small holes (⅛ to ¼ in.) need to be made through the sides and tops of the containers for ventilation. The holes should be made from the inside out to avoid creating sharp edges on the inside of the containers that could cause abrasions on the animals.

The size of the packing container will depend on the size and activity level of the amphibian being shipped. Anurans tend to jump and collide with the container during shipping, causing injury to themselves. To prevent this, the animals' movement should be restricted by limiting the height clearance in packing containers. A cushioning substrate should be placed in packing containers to reduce traumatic injuries. The substrate can also provide a water source for the animal in transit. However, care must be taken to ensure the substrate is not abrasive as this would damage the sensitive skin of amphibians. Slightly dampened sphagnum or sheet moss that is pulled apart to create air spaces and refuges for the animals provides a good packing medium. The moss must not be saturated with water, as wet moss will settle and its weight can crush, trap or drown small animals. Moistened sponge pieces or chips can also provide a suitable substrate; however, dampened paper towel should not be used since it does not provide protective cushioning.

Damp cloth bags (30 x 45 cm) can also be used as packing containers in order to restrict movement; however, burlap or other abrasive cloths must not be used.

The lids or openings of packing containers must be closed tightly and securely sealed. The packing containers should then be placed in a rigid outer shipping container. To prevent jarring during transport, crushed newspaper or foam packing chips can be used to support the packing containers within the outer shipping container. Most species should be maintained between 16°C and 28°C. Insulated foam shipping containers (a styrofoam inner box placed in a cardboard outer box) are recommended to prevent sudden changes in temperature and to provide a buffer against temperature extremes. Boxes constructed from water resistant fibreboard or plywood and lined with insulating foam or polystyrene can also be used. Heat packs and cold packs may be placed inside the insulated shipping box to compensate for the external environment; however, these are of limited value and cannot be relied upon if the container and animals are to be exposed to extreme temperatures for an extended period of time. In most cases, it is advisable to avoid shipping if weather forecasts predict very hot or very cold temperatures.

In order to prevent cannibalism, inter-species aggression and toxic effects between certain species, different size classes (i.e. small and medium) or different species should never be mixed in the same packing container nor crowded together. Additionally, the following recommendations should be used in determining container sizes:

- Large amphibians (anurans with snout-vent length over 15 cm and other amphibians with a total length over 30 cm) should be packed individually. The minimum size for a packing container is 5 L and it should be large enough to allow the entire ventral surface of the animal to make contact with the bottom of the container. Cloth bags should be a minimum of 30 x 45 cm.
- Medium amphibians (anurans with snout-vent length of 6 to 15 cm and other amphibians with a total length of 15 to 30 cm) can be packed together to a maximum of 20 animals per container. Each animal should have a minimum of 250 ml of space. The container must be large enough to allow the entire ventral surface of every animal to make contact with the bottom of the container. Cloth bags should be a minimum of 30 x 45 cm.
- Small amphibians (anurans with snout-vent length of 3 to 6 cm and other amphibians with a total length of 6 to 15 cm) can be packed together to a maximum of 40 animals per container. Each animal should have a minimum of 100 ml of space. The container must be large enough to allow the entire ventral surface of every animal to make contact with the bottom of the container. Cloth bags should be a minimum of 30 x 45 cm.
- Very small amphibians (anurans with snout-vent length less than 3 cm and other amphibians with a total length less than 6 cm) can be packed together to a maximum of 50 per container. Each animal should have a minimum of 50 ml of space. The container must be large enough to allow the entire ventral surface of every animal to make contact with the bottom of the container. Cloth bags are not recommended for small amphibians.

Amphibian larvae must be transported in water that is sufficiently aerated. This could be in the form of blown-up bags that contain a large amount of air relative to the amount of water. Aeration should be provided as necessary. If especially toxic species (e.g., pickerel frogs and

West Coast newts) are to be kept in a container for a few days, which is not a desirable situation, the water will need to be changed during the holding period.

Housing and Husbandry

The living conditions of animals held in captivity should be appropriate for the species and contribute to their health and well-being. In particular, care should be taken to provide for the social and behavioural needs of the animal. Amphibians that are destined to be kept long-term should be cared for according to chapter II of the CCAC *Guide to the Care and Use of Experimental Animals*, vol. 2 (1984).

Housing

Captive amphibians are prone to abrasions from attempting to dig out of their cages, and therefore, proper cage design should incorporate rounded internal corners and edges, and a smooth lining. A tight fitting lid is also important.

Many amphibian species benefit from measures to duplicate their natural environment if housed over an extended period of time, and such enrichment can often be very simple cleanliness.

Amphibians should always have access to fresh water. Animals kept in captivity indoors during the winter are particularly at risk of dehydration due to the low humidity that results from central heating. Even desert species, which in the wild take refuge in high humidity (60 to 70%) retreats such as burrows, can dehydrate if they are kept in a low humidity environment without access to a usable form of fresh water. Daily misting should be offered, and if the amount of water needed to ensure adequate hydration causes the substrate to become too wet, the substrate should be changed after misting.

If larval amphibians are to be housed past metamorphosis, dry areas or floating platforms must be provided so that the amphibians do not drown or prolong their larval life stage.

Disinfectants should not be used on containers while amphibians are being held. After use, however, appropriate disinfectants should be used on all containers, tools, and non-porous furniture.

Food

The use of live prey has benefits and risks that need to be considered prior to providing it as a food source, and if amphibians are to remain in captivity, efforts should be made to adapt them to accept previously killed food items. Many insectivore/predator species of amphibians prefer live, moving food and do not readily accept dead food items or pellets.

Amphibians should be fed at a time that is appropriate to their individual activity patterns: diurnal animals should be given food in the morning and early afternoon, while nocturnal animals should be fed early evening. If food is presented when the animal is not hungry, the effectiveness of any vitamin and mineral supplements may be reduced or, in the case of live prey, the animal may be subjected to wounds from the prey animals as mentioned above.

Euthanasia

Euthanasia should result in rapid loss of consciousness, followed by respiratory and cardiac arrest and ultimate loss of all brain function. For ectothermic animals, euthanasia must take into account differences in metabolism, respiration and tolerance to cerebral hypoxia.

Adult amphibians may be humanely killed through an overdose of anaesthetics such as injectable sodium pentobarbital for reptiles or solutions of buffered TMS (MS-222®) for amphibians. Anaesthetics such as TMS may be used for very small or larval animals. TMS is acidic, and in concentrations ≥ 500 mg/L it should be buffered with sodium bicarbonate to result in a solution of pH 7 to 7.5. TMS may also be injected into lymph spaces and pleuroperitoneal cavities. There are standard protocols for the use of TMS (e.g., http://www.nwhc.usgs.gov/research/amph_dc/sop_anesth.html). However, TMS should only be used by those who have received training in its use for euthanasia.

Sodium pentobarbital, 60 to 100 mg/kg, can be administered intravenously, intra coelomically, intra-abdominally or intrapleuroperitoneally. Subcutaneous lymph spaces may also be used in frogs and toads. Barbiturates other than pentobarbital can cause pain on injection.

Benzocaine hydrochloride may also be used in a bath for amphibians. Benzocaine itself is not water soluble and needs to be prepared as a stock solution in ethanol. Benzocaine hydrochloride is water soluble and can be used directly at a concentration > 250 mg/L for euthanasia. The use of benzocaine is also discussed on the NWHC website but it is not specifically recommended by the NWHC for post-metamorphic anurans.

Some amphibians can hold their breath and survive long periods of anoxia for up to 27 hours. Therefore, euthanasia of some amphibians and reptiles using inhalation agents, such as CO2, is difficult.

Decapitation does not lead to rapid unconsciousness, and therefore should not be used unless followed by pithing to instantaneously destroy the brain and not merely sever the spinal cord to render the animal insensitive to pain. Propofol and short acting barbiturates can be used to produce rapid general anaesthesia prior to pithing.

Cooling or freezing is generally not a recommended method of euthanasia as formation of ice crystals on the skin and in the tissues of an animal may cause pain and distress. Quick freezing of deeply anaesthetized animals is acceptable.

Human Safety Considerations

Salmonella and a host of other pathogens can be transmitted between amphibians and reptiles and humans. Handling protocols should include hand washing between handling individuals and immediately after handling the animals.