Canadian Council on Animal Care
Conseil canadien de protection des animaux

CCAC species-specific recommendations on: AMPHIBIANS AND REPTILES

1. General Considerations

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. Additionally, the guidelines of the American Society of Ichthyologists and Herpetologists (ASIH) should be consulted. Their recommendations acknowledge that the investigator will often be an authority on the biology of the species under study, and provide advice on techniques that are known to be humane and effective in the conduct of field research (ASIH *et al*., 1987 [http://www.asih.org/pubs/herpcoll.html]).

2. Killed Specimens

Collecting techniques and collection management practices for amphibians and reptiles have been compiled by Simmons (2002). Specimens should be euthanized according to CCAC recommendations (see Section 11. Euthanasia) before preservation. Whenever amphibians or reptiles are collected (e.g., for museum deposition), specimens should be fixed and preserved according to accepted methods (Pisani, 1973) in order to ensure maximum utility of each animal and to minimize the need for duplicate collecting. Where possible, photo vouchers should be considered as an alternative.

3. Live Capture

Amphibians and reptiles are sensitive to heat, cold, dehydration and stress. Investigators must make every effort to avoid trap deaths from such causes as exposure to unfavorable temperatures, drowning, shock, predation and desiccation. In general, traps for amphibians and reptiles should be checked early in the day and at least daily when weather conditions threaten the survival of the trapped animals. The season and abundance of the target species should also be considered when determining the frequency of trap checks. However, since amphibians and reptiles are ectotherms, they are less susceptible than small mammals to stress from lack of food.

Each animal that is captured or handled 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 (Barnett *et al.*, 2001). As well, studies have indicated that handling time has a negative influence on hormone balance (Coddington & Cree, 1995; Moore & Jessop, 2003) and can upset the natural antibacterial properties of amphibian skin (Matutte *et al*., 2000; Nascimento *et al.*, 2003). 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 (Barnett *et al*., 2001).

3.1 Capture on land

Traps should be shaded, or positioned to avoid exposure to direct sunlight, and a lean-to roof over the trap is advisable. Suitable cover, such as moss under which the animals can hide, should be provided in traps.

Pitfall traps set during dry periods should contain a source of moisture in order to prevent desiccation of captured amphibians. Synthetic sponges can supply a useful source of water if rewet by researchers when necessary, and they also serve as flotation devices if the traps become flooded. Natural sponges can be used, but they will degrade faster. In the field, moss (such as sphagnum moss) is a useful temporary alternative to sponges; however, it does not float well and cannot protect captured animals from drowning if the traps fill with water.

Care should be taken to reduce predation in pitfall traps by using deep buckets or by including a baffle over the trap. Additionally, pitfall traps require a stick to allow small mammals to climb out (however, this may provide a means of escape for tree frogs with friction toe-pads).

Field capture of snakes can be done with special snake hooks that allow the handler to safely lift a snake and transfer it to a collection container or bag. Special long-handled tongs are also used. Models with a wide flattened grip spread the pressure over a wider area and reduce the chance of injuring a snake.

Alternatively, snakes can be captured using drift fences and pitfall traps. The fence line and traps should be checked daily and early in the day to avoid predation and exposure. These traps should also provide cover to shelter captured snakes from weather extremes and predators.

Female turtles can be captured for marking at nesting sites, but preferably after laying. Care must be taken when approaching nesting turtles not to interfere with their normal behavior. If a female is approached early in her nesting behavior, she may be scared away from the nesting site and expend additional energy searching for a new site, digging a new nest, etc., and be exposed to potential predators.

3.2 Capture in water

The capture of marine and aquatic species must be conducted in a manner that prevents accidental drowning. Marine turtles may be captured at the water's surface using breakaway nets of cotton mesh, guided carefully over the animals (Eckert *et al*., 1999). Submerged tangle nets should only be used under extreme circumstances; if used, they must be constantly monitored to prevent turtles from drowning. Turtles can also be captured by hand during free or scuba diving.

Freshwater turtle traps should be positioned so that captured turtles have access to air. Similarly, if minnow traps are used for amphibians, access to the air-water interface must be maintained to avoid drowning or asphyxiation. The provision of floating perches in these traps for turtles to cling to is advised, but may not be necessary depending on the species and the frequency with which the traps are monitored. Precautions should be taken to accommodate possible changes in water depth due to precipitation.

4. 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 and reptiles do not tolerate physical restraint well (such as small plethodontids which easily overheat in the hand or large turtles which tend to bite), and attempts to restrain these species could cause injury to the animals or handlers. When there is a need to restrain amphibians and reptiles, investigators should keep in mind that many species are capable of causing skin irritation or inflicting serious injury to those handling them.

Many amphibians and reptiles are relatively small and slow moving and can be captured and restrained by hand; however, they are easily injured if excessive force is used. Nets, hooks, tongs, strong carrying boxes (not cardboard) or handling bags may be used to reduce injury and struggling in these animals. As well, small amphibians can be temporarily restrained in plastic bags containing a small amount of water and blown full of air, as the soft sides cushion the force of jumps. Care must also be taken to avoid removal of the protective mucus layer covering the skin of amphibians, and any nets that are used should be made of soft cloth materials.

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 or where bites from animals are possible, latex gloves should be worn to protect the handler. Any contact with bare skin or mucus membranes should be avoided.

4.1 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.

4.2 Lizards and salamanders

When restraining small lizards or salamanders, an open flat hand should be used to apply even pressure over the animal's entire body. Pressure on the tail should be avoided. Medium and large salamanders (approximately 5 g and larger) should be grasped in the middle of the body between the forelimbs and hind limbs. Larval and neotenic salamanders should never be grasped around the head or neck because their gills can be easily damaged.

Under no circumstances should lizards or salamanders be grasped or picked up by the tail, as tail shedding can occur. Although this is not a serious injury, it will influence future growth and reproduction by depriving the animal of fat stores (Derickson, 1976; Bellairs & Bryant, 1985), and cause damage to the integrity of the specimen. It may also affect the behavior of the animal (Ballinger, 1973; Bellairs & Bryant, 1985). Tail loss can be avoided through proper handling techniques, for example, by using squeeze-boxes for some species. A few species of geckos and specialized lizards may 'voluntarily' drop their tails if approached by a predator; however, others do so only when there is an external threat and pressure.

4.3 Turtles

Placing a dark cloth or hood over the eyes of many marine turtles will immobilize them. This will also calm other large turtles, such as snapping turtles. Leatherback turtles (*Dermochelys coriacea*) may be restrained by holding them in the capture net with the front flippers folded in along the body (M. James, pers. comm.). Hard-shelled marine turtles, such as the loggerhead (*Caretta caretta*), are more aggressive and need to be

carefully handled to prevent injury to investigators. Placing these turtles in a large box in a darkened area will aid in their restraint (M. James, pers. comm.).

4.4 Snakes

During handling, snakes should be supported at multiple locations; supporting the entire body weight at just one location can cause vertebral damage.

Venomous snakes, such as rattlesnakes, are potentially dangerous and require special methods of restraint. The particular method chosen will vary with the species and the purpose of the project. Procedures should be chosen to minimize handling time and reduce or eliminate contact between the handler and the animal. Any anesthetic poses a degree of risk and delays the snake's return to its habitat and territory. Therefore, during general handling and 'processing' of venomous snakes in the field for health assessment, morphological measurements, etc., the use of anesthetic agents is not always necessary or recommended if appropriate restraining devices are used by well trained and experienced personnel.

Venomous snakes can be safely restrained in squeeze boxes or clear plastic tubes. When necessary to restrain a venomous snake for hands-on procedures such as blood collection or sexing, it is recommended that the snake be directed into a clear plastic or acrylic tube using a snake hook. This can be done on a worktable, on the floor, in a bucket or directly from a snake bag. The tube should be approximately 60 cm (24") long and the diameter should be wide enough to accommodate the thickest part of the snake but not enable the snake to turn in the tube. Once approximately one third of the snake has entered the tube, the tube and snake are grasped together and held firmly so that the snake cannot move further into the tube or back out.

Only experienced personnel should handle dangerous species and they should never work alone. A second person, knowledgeable of capture, handling techniques and emergency measures, should be present at all times. For venomous species, adequate supplies of antivenin appropriate for the species and within the expiry date should be available, and written snakebite procedures should be posted or carried if working in the field. It is also advisable to notify local medical authorities of the potential risks and familiarize them with snakebite protocols if necessary (see Section 12. Human Safety Considerations).

5. Chemical Restraint and Anesthesia

Wright (2001a) and Mader (1996) provide useful information on amphibian and reptile anesthesia, including various anesthetic protocols that have been used with different species. However, a veterinarian experienced with the use of anesthetics on reptiles and amphibians should be consulted when planning their use in a new project or a new species. Certain chemicals produce initial excitement before anesthesia; the use of

tranquilizers in conjunction with the anesthetic agent may be indicated (ASIH *et al*., 1987).

General extrapolations for reptiles and amphibians should not be made from anesthetic use in mammals. Ambient temperature and decreased metabolic rate can have profound effects on reptilian anesthesia, and lower doses may be needed. Injectable anesthetics can often have prolonged effects in reptiles; induction may take hours and the animal may take several days to recover (Bennett, 1991; 1996). Since temperature will affect dose, rate of induction and recovery time, care should be taken to maintain the animal at its preferred body temperature.

Additionally, animals of different species and even different individuals may respond differently to anesthetics. For example, Blouin-Demers *et al*. (2000), in examining the use of inhalant anesthetics in three snake species, suggest there is substantial variation in response to anesthetic agents among species. It is therefore necessary to consult the published literature and/or persons experienced with the species.

Hypothermic anesthesia is considered inappropriate for amphibians and reptiles as it does not appear to induce rapid unconsciousness in these animals and the adequacy of this method of anesthesia is difficult to monitor (Martin, 1995).

5.1 Amphibians

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

Tricaine methanesulfonate (TMS), also known as MS-222®, is the agent of choice for anesthetizing 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 anesthetizing amphibians in the field (http://www.nwhc.usgs.gov/research/amph_dc/sop_anesth.html). Amphibians are usually anesthetized 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 (Wright 2001a). TMS should therefore be buffered with sodium bicarbonate. Wright (2001a) provides information on recommended dosages and suggested buffering procedures.

To reduce the risk of anesthesia complications, investigators should ensure that the animal is removed from the anesthetic solution and given a brief but thorough rinsing in fresh water to remove residual anesthetic as soon as the animal becomes unresponsive to the eyelid-touch and toe-pinch tests (NWHC, 2001). Anesthetic solutions should not be discarded into surface water.

Once the animal has been anesthetized, 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 anesthetized 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 anesthesia depends on the life stage, anesthetic, temperature, species and depth of anesthesia. 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 or walk normally, it may be released. If possible, amphibians should be released in cool dark places to minimize detection by predators (NWHC, 2001).

Injectable anesthetics such as propofol have been tried on frog species (von Esse & Wright, 1999 [http://www.arav.org/Journals/JA014223.htm]) and may prove useful where an injectable agent is required.

5.2 Reptiles

Among the variety of injectable and inhalation anesthetics successfully used in reptiles, propofol provides a rapid and smooth induction, minimal accumulation from repeat injections, relative freedom from repeat injections and excitatory side effects, and rapid recovery with little residual effect. Propofol is an injectable anesthetic that must be administered either intravenously or by intraosseous injection. It is rapidly metabolized and is noncumulative. However, it does produce a dose-dependent cardiopulmonary depression. Apnea (stopped breathing) is common after initial administration. This apnea is dependent on speed of injection and dose. For longer procedures, once anesthesia by injectable anesthetics has been achieved, many animals can be intubated and maintained on gas anesthesia.

Volatile anesthetic gases, such as isoflurane, are particularly useful in reptiles if a rapid recovery is desired. However, volatile anesthetics are slower to take effect in reptiles because of their ability to hold their breath. The rate of induction and recovery for these gases is also temperature dependent, with faster rates being achieved under higher temperatures.

Local anesthesia may provide a good alternative to general anesthesia in reptiles that are easily restrained; however, the toxic dosage of local anesthetics does not appear to have been investigated in reptiles. It is recommended that small volume syringes and small gauge needles be used.

For snakes, if no information on the use of anesthetics is available for the species concerned, isoflurane is recommended as the agent of choice. Blouin-Demers *et al*. (2000) successfully recovered all snakes anesthetized with isoflurane, in contrast to those anesthetized with halothane.

6. Marking

6.1 Tissue marking

Photo identification and hand rendered drawings on blank template line illustrations (e.g., drawing head patterns of rattlesnakes) are often effective means of identifying reptiles and amphibians. These methods should be explored before more invasive procedures are approved.

For marking methods that require minor surgery, local anesthesia (e.g., 2% lidocaine) should be used at the surgical site (Wright, 2001b).

6.1.1 Tattoos, paints and dye markers

Tattooing has been used with success on both amphibians and reptiles. 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. Although reptile skin is less permeable, it varies among species and some paints or paint solvents may become absorbed and kill some reptiles. In general, paint should not be applied to the sutures of turtle shells as very tenacious paints applied across shell sutures may severely distort normal shell growth, especially in sub-adults. As well, the use of paint for temporary marking is less suited to turtles that do not tend to bask or shed scutes readily (e.g., snapping turtles) as the paint will not wear off as quickly. If paints are used, they must contain non-toxic pigments, bases and solvents, and should not be brightly colored such that they compromise the animal's camouflage.

The injection of colored 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 (Binckley *et al*., 1998); 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.

6.1.2 Shell marking

In most species of terrestrial turtles, the shell may be marked by notching along the margin or by drilling small holes in the marginal scutes of the carapace. See Ferner (1979) for marking codes.

Living tags (i.e. grafting patches of pale plastron tissue onto the carapace) are acceptable for marking hard-shelled marine turtles, but require time as well as the recapture and careful examination of the animals for subsequent identification (Fontaine & Schexnayder, 1995).

6.1.3 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 behavior. Toe removal should not be performed on chelonians or lizards.

Although toe clipping has been used as a means of permanent marking in the past, with claims of little adverse effect on behavior and survival (Ferner, 1979), Davis & Ovaska (2001) state that the effects of toe clipping on amphibians are largely unknown. Their study indicated some subtle effects of toe clipping on salamanders that might lead to reduced survival. Additionally, Wright (2001b) indicates that the use of toe clipping in field studies has had health implications for some amphibians, including inflammation and necrosis of the site, as well as general infection.

If toe clipping is the only suitable method, a maximum of one toe per foot should be clipped. In addition, specialized toes that are essential for survival activities, such as burrowing, climbing, amplexus, nest excavation, or propulsion, should not be clipped. See Ferner (1979) or Donnelly *et al*. (1994) for a minimal-removal coding method.

Prior to toe clipping, an amphibian should be anesthetized or given a suitable level of analgesia (e.g., 2% lidocaine applied to the site), and the limb to be toe-clipped should be disinfected using a standard surgical protocol (Wright, 2001b). If possible, toes should be amputated at the interphalangeal joint (Wright, 2001b). Sterile stainless steel clips or absorbable monofilament suture may be used to constrict the digit prior to amputation, and tissue glue applied after the procedure (Wright 2001b). Stainless steel clips should not be used on amphibians that are being released because of the risk of entanglement (Wright 2001b).

In salamanders, newts and some frogs, digits can regenerate. Digit regeneration has been prevented by using cauterizing materials, such as phenylmercuric acetate solution, after removal of the toe. However, it should be noted that any mercuric compound is an environmental toxicant.

To maximize data yield, it has been suggested that clipped toes should be kept for determination of age through skeletochronology by preserving them in 10% buffered formalin, or for genetic analysis by preserving them in 70 to 100% ethanol.

6.1.4 Branding and removal of scutes

Both branding and removal of scutes are invasive procedures and are therefore not generally recommended. These techniques provide a permanent marking system for snakes and do not appear to increase mortality or impair locomotion; however, in amphibians, brand marks may not be visible after a few months.

The scute is removed with small surgical scissors or by rapid cauterization according to a standardized numerical code (Ferner, 1979). Healing is usually rapid and infection is rare. A comparable method of marking is the electrocauterization of a number or letter on the skin. In order to be effective, the deep layers of the skin must also be cauterized to prevent regeneration.

The use of a local anesthetic (aerosols containing benzocaine, such as Cetacaine, or an injectable local anesthetic, such as 2% lidocaine) is strongly recommended with branding or electrocauterization. The skin of reptiles, however, is relatively impermeable, reducing the effectiveness of topical anesthetics.

6.2 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 and reptiles, with the exception of turtles. In particular, they are to be avoided for long slender animals such as snakes and many lizards.

Results from the application of different but similar methods may be highly variable. For example, plastic flipper tags (two part rototags) applied to the fore flippers of some turtle species have a long retention time and do not appear to cause any significant infections or tissue necrosis (Van Dam & Diez, 1997), while similar plastic tags applied to other marine turtles have been reported lost at a very high rate (Eckert & Eckert, 1989). Additionally, plastic tags attached to fore flippers of marine turtles have been implicated in high rates of turtle entanglement in gill nets (Nichols & Seminoff, 1998).

Color tags or beads are being used to mark some amphibians and reptiles. They can be sewn through the head or tail crests of lizards and through the rattles of rattlesnakes. For

amphibians, various colored beads can be added to a stainless steel wire that is passed through the thigh and around the femur, or inserted through the tail of salamanders that do not lose their tail (Wright, 2001b). 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 (Wright, 2001b).

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.

In freshwater turtles, colored mylar ribbon tags that are 2.5 to 5 cm long have been considered an acceptable alternative to Petersen discs which have been associated with mortality in freshwater turtles. Some turtles have been marked with disc-type tags and clamp-on ear-type tags applied to the webs between the toes; however, these could impair their ability to dig for nests.

Some species of turtles (e.g., snapping turtles, *Chelydra serpentina*) have been effectively tagged for long term studies of more than 25 years by individually numbered duraluminum tags attached to the rear of the carapace with stainless steel wire. Metal flipper tags have been attached to marine turtles; however, the attachment of these must allow space for growth, and there is a possibility that they may cause tissue necrosis and become lost (Prince, 1996).

6.2.1 PIT tags

Passive integrated transponders (PIT tags) or microchips have been used to mark amphibians and reptiles permanently, and can be used as an ancillary method of identification. Wright (2001b) notes that these tags have been used successfully on a variety of species, including some newly metamorphosized newts with body mass greater than 2 grams. This method does require that the animals be recaptured, and special equipment is needed to identify and read the tags of marked individuals.

After disinfecting the site where the device will be implanted, using standard surgical protocols, the devices are implanted subcutaneously or intraperitoneally. If possible, tissue glue (cyanomethacrylate) is then applied to close the incision. The use of tissue glue helps to ensure that the PIT tag does not become ejected before the wound heals (Wright, 2001b).

Complications with PIT tags which have been noted include: migration of transponders if applied subcutaneously or internally, which may make them more difficult to read; breakage of the tags; and loss of signals (Wright, 2001b). Marine turtles may be marked by using PIT tags injected into the flippers or shoulder area; however, some tags migrate into deeper tissues and become unreadable.

6.2.2 Binary or analogue coded wire tags

Binary or analogue coded wire tags, widely used for marking individual fish of a variety of species, may be considered for amphibians. They are almost microscopic in size and can be detected electronically. The major limitation is that recovery and reading of the tag is only possible after the death of the animal carrying the tag. However, the low invasiveness of this procedure and the small size and permanence of the tags could be an advantage in some studies.

6.2.3 Harmonic radar tags

Harmonic radar systems have advantages over radio telemetry: the tags do not require a battery and are therefore lighter in weight; and harmonic radar is highly directional and capable of finding tags underground, beneath rocks and behind trees.

7. Radio Transmitters

The attachment of small radio transmitters to amphibians and reptiles has become a routine method for monitoring the location and movement of individuals. In the past, many amphibians and reptiles were considered unsuitable for radio telemetry studies due to their small size or habit of living in confined spaces below ground; however, the application of radio telemetry has been expanded through new technologies. Investigators should therefore review the available methods and choose a transmitter and method of attachment that is suited to the anatomy and behavior of the study animal.

7.1 Externally attached transmitters

Transmitter attachments that will impair reproduction, locomotion, behavioral interactions, thermoregulation, skin shedding or other normal activities should be avoided. The transmitter should be shaped and attached so as to eliminate or minimize the risk of entanglement with vegetation or other obstructions. Transmitters should also be camouflaged for cryptic species such as toads.

Wherever possible, transmitters should be removed upon completion of a study or the transmitter attachments should be designed so that they are ultimately self-detaching. Amphibians and reptiles continue to grow throughout their lives, and therefore, the eventual removal or release of an external transmitter is important to prevent constriction or irritation.

External attachment of transmitters on a number of amphibian and reptile species is possible by using various attachment harnesses (such as small-diameter, hollow PVC tubing for amphibians) and other techniques; however, the device may alter the appearance of an individual enough to affect its behavior and interactions with

conspecifics and predators. The attachment method should be suited to the particular animal of interest. As well, in a study on spotted frogs, Bull (2000) notes that the sex of a frog and the time of year are important factors in determining the most appropriate technique. In comparing two types of attachments, waist-band and arm-band, Bull (2000) states that abrasions can occur rapidly, and that frequent inspection of the animal is recommended for both attachment methods, but particularly for arm-band attachments.

Radio transmitters may be attached to the dorsal surface of the shell of terrestrial and freshwater turtles by screws and clamps over the edges of the carapace. They can also be sutured to the dorsum in large or flat-bodied lizards.

Transmitters that are attached to the scutes of hard-shelled marine turtles with epoxy may result in the scutes detaching (M. James, pers. comm.). In addition, burn injuries may result when using epoxy hardeners (two-stage epoxies); however, the problem of heat can be avoided by using two-part epoxies that cure underwater (Hickerson, 1999). Cold-curing compounds, such as silicon elastomers, may be used in combination with polyester resin and applied in layers for attaching transmitters to the carapace (Hickerson, 1999).

Harnesses for the attachment of transmitters to marine turtles should be of a soft elastic material to fit around the limbs, with soft PVC tubing to prevent skin chafing and corrodible links that will eventually permit the transmitters to fall off the animal (M. James, pers. comm.). Transmitters tethered to hard-shelled marine turtles with monofilament looped through a hole drilled in the posterior point of the carapace have been used for tracking these animals (M. James, pers. comm.). Investigators working with marine turtles should be aware of the adverse hydrodynamic effects of older rectangular transmitters and should attempt to reduce transmitter size and use tear-drop shaped housing with an internal antenna to minimize such effects (Watson & Granger, 1998).

7.2 Implanted and force-fed transmitters

Madison (1997) notes that implanted or force-fed transmitters are more appropriate than external transmitters for long-term studies of burrowing organisms, such as salamanders.

Transmitters may be surgically implanted inside or outside of the body cavities of large snakes, lizards and some large salamanders and frogs. The transmitters should be of a suitable size and mass, and be covered with an impervious, biologically inert coating. Additionally, investigators need to be aware of possible effects of the implanted transmitters on the physiology and behavior of study animals. For example, Graves and Duvall (1993) found that if transmitters were implanted in snakes early in the season, ovarian follicles could be reabsorbed, thus interfering with reproduction.

Implanted transmitters should not interfere with the function of the organs surrounding them. In coelomic and subcutaneous implants, it may be necessary to suture the transmitter package in place to prevent movement and interference with vital organs. The

implantation of transmitters should only be performed by properly trained and experienced investigators or supervised by a veterinarian (see Reinert & Cundall, 1982 for an acceptable technique).

Force-fed transmitters may yield less reliable data because the packages can alter the animal's behavior by mimicking the effect of food in the gut and thereby influence thermoregulatory behavior. Force-fed packages must be small enough to pass through the gut without obstructing the passage of food or be removable by forced regurgitation. This further supports the use of the smallest transmitters possible, and investigators should strive to use transmitters which are no more than 1% of the animal's body mass. Gut residence time of up to several days has been long enough to provide useful information on movement and body temperature (Sato *et al*., 1994; 1995).

The minimum size/weight of a snake that can receive an implant depends on the size of the transmitter and the skills of the surgeon. Generally, the transmitter should be no more than 50% of the width of the snake at the surgery site, and the weight of the transmitter should be no more than 5% of the snake's body weight. With large-bodied snakes, such as the Massasauga rattlesnake, animals as small as 30 to 40 cm could be implanted with transmitters.

For gravid female snakes (which lose up to 50% of their body weight when giving birth), the transmitter should be no more than 2.5% of the snake's body weight. Unless the availability of study animals is a limiting factor, strict adherence to this standard is particularly important because the stress of gestation and parturition apparently results in increased mortality among post-parturition females, and transmitter implantation could compound that stress. Implantation of transmitters from the time that females are heavily gravid to the time of parturition could result in an increased incidence of complications. The muscular activity of parturition could also result in dehiscence of an incompletely healed implant incision. The latest date for implant surgery should be four weeks prior to expected parturition and no surgery should occur after mid-June. It is important to note that parturition dates can vary considerably between years within a given population.

In snakes, the effect of local inflammation from a newly implanted transmitter on the process of ovulation and uptake of follicles by the oviduct is unknown but may also result in complications. The standard location for transmitter implantation is two-thirds of the distance from the snout to the vent, which places the transmitter immediately adjacent or anterior to the ovaries. If a female has large preovulatory follicles, insertion of a transmitter could be traumatic. Debilitation at ovulation could result in regurgitation of ova from the oviduct into the coelom, resulting in yolk peritonitis. Implanting gravid snakes early in gestation may also result in follicle re-absorption. Parturition may cause a degree of stress and debilitation in female snakes, which would be exacerbated by surgery at this time. It is recommended that female snakes be allowed two weeks to recover from parturition prior to implant surgery.

Following surgery, the snake should be kept in a warm (28 to 30°C) holding area for a minimum of 24 hours. If no feeding or drinking is observed, intracoelomic fluids (sterile

normal saline) of a volume equal to 2% of the snakes body weight could be administered intraperitoneally (i.p.) to ensure the snake is well hydrated when released. Antibiotics may be advised and investigators experienced with this technique should be consulted prior to performing procedures to ensure proper post surgical care. An extended recovery time of 3 to 6 days can allow the snake to reach and maintain its preferred body temperature. However, some suggest that to minimize stress experienced by the snake, it should be released as soon as possible, as long as it has regained full locomotor abilities.

Snakes require at least four weeks post-operatively to heal completely. While females should not be implanted after mid-June, no snake should be implanted after the end of August as they may not completely heal before hibernation (in Ontario, the Snake and Lizard Advisory Group recommend not to implant transmitters after August 1st [Willson, in prep.]). However, if snakes have to be implanted later than August, they should be kept in captivity until the incision has healed and consideration must be given to the fact that delaying release may affect the snake's ability to reach a hibernation site. More information is available from the Snake and Lizard Advisory Group of the Ontario Ministry of Natural Resources (contact Roxanne St-Martin, 705-755-3201; roxanne.stmartin@mnr.gov.on.ca).

8. 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 anesthesia.

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.

Currently, there is a lack of information regarding the effects of analgesics on amphibians (Wright, 2001b) and reptiles (Bennett, 1996). Bennett (1996) notes that nonsteroidal anti-inflammatory drugs, such as flunixin meglumine, may provide pain relief in reptiles; however, in many cases an analgesic or antiseptic can be more lethal to an amphibian than simply leaving it untreated.

8.1 Blood collection

For frogs and toads, blood may be taken from the femoral vein, the ventral abdominal vein or the lingual vein (Whitaker & Wright, 2001). For most salamanders, the ventral

tail vein is the easiest site for blood collection (Whitaker & Wright, 2001). Blood may also be collected using cardiac puncture; however, this is an invasive procedure and should be carried out under anesthesia unless the animal is adequately restrained. It has been reported that anesthesia may be more stressful to the amphibian or reptile than the cardiac puncture. There are concomitant risks in using anesthetics if the animal is not carefully monitored, but research is needed to determine the relative levels of stress for cardiac puncture, with or without anesthesia (Busk *et al*., 2000; B. Pauli, pers. comm.).

In a review of blood collection in reptiles, Campbell (1996) notes that for lizards, blood is commonly collected from the ventral coccygeal vein; while in Chelonians, it is collected from a number of sites including the jugular vein, dorsal coccygeal venous sinus, heart, scapular vein and brachial vein or artery.

In snakes, blood may be collected from the caudal vein (ventrally). Once the snake is restrained, the ventral surface of the tail should be cleaned with an alcohol swab. The snake's body should be elevated vertically to ensure blood flow toward the tail. The needle should be gently inserted at an angle of 45° between the ventral scutes of the tail at a location between one-half and two-thirds of the distance from the cloaca to the tip of the tail. Inserting the needle as close as possible to the mid-line of the ventral surface of the tail increases the chance of hitting the centrally-located caudal vein or artery. The needle should be inserted slowly until a slight resistance from the caudal vertebrae is felt; the needle should then be withdrawn slightly (i.e. 0.5 to 2.0 mm), and then the plunger of the syringe gently drawn back.

If the caudal vein or artery is hit directly, the syringe tip should quickly fill with blood, in which case the plunger should be slowly drawn back until an adequate blood sample is secured. In the event that an adequate blood flow is not immediately obtained, or the flow of blood stops, the needle may be either moved in and out slightly (i.e. a few millimetres) or rotated until blood flow resumes. Alternatively, the needle may need to be completely withdrawn and a new one inserted at a new location before the vein or artery is hit in the centre and a sufficient blood flow is obtained.

Once an adequate blood sample has been collected, the needle should be withdrawn and light pressure applied to the point on the tail from which blood was drawn in order to stop further blood flow.

Blood can be obtained from larger snakes and even turtles fairly easily by cardiac puncture; however, this method does impose greater potential risk. If cardiac puncture is to be used to obtain blood, the operator must be very familiar with the species anatomy (Tyler, 1999).

8.2 DNA analysis

DNA suitable for some types of analysis can be obtained from some freshly shed snake skin (Clark, 1998) and also from scute shavings.

Toe clips obtained during marking procedures can be retained for biopsy samples (see Section 6.1.3 Toe clipping for preservation techniques).

Skin tissue biopsies may be obtained from the dorsal axial region of the hind flipper of captured marine turtles with little or no bleeding if the procedure is done carefully with the appropriately sized biopsy instrument (Dutton & Balazs, 1995). Biopsy punches are also used to extract samples from the margins of the front flippers of marine turtles for this purpose.

9. 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 and reptiles should be placed in containers that are closed, adequately ventilated, constructed of non-toxic materials, and insulated to protect the animals against temperature variations (IATA, 2001). 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 (IATA, 2001).

For short periods of time, most species 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 specimens can overheat quickly.

9.1 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.

9.2 Reptiles

Freshwater turtles should travel in wet bags, while lizards and snakes can travel in dry bags providing they are sprayed with water when transported over more than one day.

Venomous snakes travel well in bags but must be further enclosed in wooden or metal boxes to prevent escape and to prevent handlers from being bitten through the bag. The container used should be insulated (e.g., a plywood crate lined with rigid foam insulation). Venomous snakes should be double-boxed: each snake is confined in a cloth bag with a secure knot and placed into a smaller container/box with a secure lid, which is

then placed in a larger insulated box for shipping. Crumpled newspaper should be packed around the inner box to prevent it from bouncing during transit.

10. Housing and Husbandry

The living conditions of animals held in captivity at field sites 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 behavioral needs of the animal (CCAC, 1993, vol. 1, chap. VI)

Amphibians and reptiles 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). Other useful information can be found in Tyler (1999).

10.1 Housing

Captive amphibians and reptiles 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 and reptile 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. For example, cover is an important natural element for snakes and can often be provided in the form of inverted plastic containers or sheets of paper, without compromising maintenance and cleanliness. Fossorial species require suitable material in which to burrow and toads require dirt. Other species of amphibians and reptiles may benefit from other forms of habitat enrichment, such as manipulations to accommodate turtles that eat underwater. Although most commonly maintained species of snakes have fairly simple needs, in some cases the requirements are species-specific.

Opportunities for thermoregulation should be provided in the form of a thermal gradient. Many lizards need access to full spectrum light, including UV wavelengths, to maintain healthy skin (Barten, 1996).

Reptiles and amphibians should always have access to fresh water; however, the form in which water is supplied should depend on the species. For example, some animals will drink from a bowl or soak in it as a normal behavior which keeps them well-hydrated and healthy. Other animals, particularly arboreal snakes and lizards, are more likely to drink from water droplets collecting on their skin or in their surroundings. These animals require daily misting (some for extended periods) to maintain hydration. While overly wet and humid conditions (especially if accompanied by poor hygiene and sub-optimal temperatures) can lead to bacterial and fungal skin infections, the danger in not offering access to a shallow water dish and/or daily misting is dehydration and the accompanying disease processes (e.g., gout, inanition, chronic stress, etc.).

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 or reptiles are being held. After use, however, appropriate disinfectants should be used on all containers, tools, and non-porous furniture. All substrates and porous furniture (e.g., perches and wooden hides) should be discarded and replaced between uses.

10.2 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 or reptiles are to remain in captivity, efforts should be made to adapt them to accept previously killed food items. Many insectivore/predator species of amphibians and reptiles (particularly amphibians) prefer live, moving food and do not readily accept dead food items or pellets. With most snakes, lizards and turtles, however, dead food items are accepted.

Any captive snake or other reptile that must be fed live, fully conscious small mammals or birds presents an ethical problem. In such cases, the research or teaching value of maintaining the captives must be weighed against the exposure of conscious higher vertebrates to attack by a predator. The only alternative is to force-feed the animals, which risks injury to the animal being fed and possible nutritional defficiencies. In the case of venomous snakes, it also places the handler at risk.

Live foods, such as rodents and crickets, are also capable of injuring captive animals. If an animal is ill, injured or even stressed from being in captivity, it may not feed and the food animals provided could inflict bite wounds. Rats and mice are capable of killing compromised snakes, while crickets can inflict wounds that could become infected.

Amphibians and reptiles 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 (Wright, 2001c). 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 (Wright, 2001c).

Providing a small amount of fruit (20%) for herbivores and omnivores has been found to increase the palatability of the diet offered and encourage the animals to feed. To prevent the animal from only eating the fruit and leaving the rest, food items should be chopped into small pieces and mixed well.

There are commercial diets available for many lizards, snakes and turtles; these can be used to compliment a fresh diet (50/50). Commercial diets vary in quality and should be reviewed before use.

Long-term lab specimens require vitamin/mineral supplementation on (dusting) or in (gut loading) food items.

11. Euthanasia

The main reference to be used with regard to euthanasia is the *Report of the AVMA Panel on Euthanasia* (AVMA, 2000). Euthanasia is a rapid loss of consciousness and death, with no pain or distress accompanying the procedure. For ectothermic animals, euthanasia must take into account differences in metabolism, respiration and tolerance to cerebral hypoxia.

Adult amphibians and reptiles may be humanely killed through an overdose of anesthetics such as injectable sodium pentobarbital for reptiles or solutions of buffered TMS (MS-222®) for amphibians. Anesthetics 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.

Many reptiles and amphibians can hold their breath and survive long periods of anoxia (up to 27 hours for some species) (Bennett, 1991). Therefore, euthanasia of some amphibians and reptiles using inhalation agents, such as CO_2, is difficult. Snakes will

become paralysed by anesthetic and stop breathing, and thus cannot be overdosed by inhaling a gas anesthetic.

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 anesthesia 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 anesthetized animals is acceptable.

12. Human Safety Considerations

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

The need to handle venomous snakes with care cannot be overemphasized. Investigators handling such animals, and those working under their direction, should know and be experienced with the proper procedures for restraining the animals. Additionally, they should be familiar with the emergency procedures that are to be initiated in the event of an accidental bite, and have the requisite equipment, such as a supply of antivenin, on hand. A second person, knowledgeable of capture and handling techniques of venomous snakes and emergency treatment measures, should always be present. However, antivenin should be administered only by a physician in a suitably equipped facility as some people are allergic to antivenin and could suffer an adverse reaction.

Although not generally recommended, venomous snakes that are to be permanent captives can be surgically rendered non-venomous in some circumstances. This may be a particularly important issue where effective antivenin is not available and affordable.

Medical personnel with knowledge of treatment of snake envenomation should be identified in advance of any study in which this might be a concern. Additionally, a medical facility should be made aware of the nature of the studies being undertaken so that proper arrangements can be made for emergency care and examination of personnel. In the event that someone is bitten by a venomous snake, it is recommended that a co-worker remain with the affected party at all times, especially if he or she is transferred to a medical facility, to ensure that proper procedures for the care of the individual are followed.

Prior consultation with other researchers experienced with venomous species, in addition to review of the relevant literature, is of particular importance since some information on handling dangerous species is not often published, but passed from one investigator to another.

The Ontario Ministry of Natural Resources has formed an expert panel called the Snake and Lizard Advisory Group (SLAG). SLAG is in the process of compiling field research protocols and may be an additional source of information on many of the issues dealt with in these guidelines. For more information, contact Roxanne St-Martin (705-755-3201; roxanne.stmartin@mnr.gov.on.ca).

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15. Glossary

Amplexus—The position that frogs take during mating in which the male grasps the female from behind

Neotenic—salamanders that become sexually mature but do not metamorphosize, such that they remain as larvae and breath with gills

16. Abbreviations

ASIH—American Society of Ichthyologists and Herpetologists

DAPTF—Declining Amphibian Population Task Force