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Captive Management Recommendations for the Eastern Massasauga Rattlesnake

Version 00.10

This document is intended as a guide for researchers and others working with this species in a captive environment and will be updated periodically as circumstances warrant.

1. MEDICAL CONSIDERATIONS FOR MASSASAUGA RATTLESNAKES

1.1. Introduction:

Currently very little is known about the health status of wild populations of Massasauga rattlesnakes. Most information comes from captive animals where the incidence of disease may seem high. This may be due to the fact that captive snakes are monitored and screened for disease on an ongoing basis from the time they are first acquired (by birth or capture) until a final post mortem exam. In wild populations, disease has generally gone undetected. Whether the health status of captive populations mirrors that of wild populations is not known. Whenever wild Massasaugas are handled for identification, measurements, radiotransmitter placement, etc., valuable medical information can be gained. Recognising the presence of disease is the first step toward understanding the role of diseases in wild populations, and the potential effect of diseases in translocation and reintroduction projects.

Whenever free-ranging animals are being handled, it is recommended that blood samples and/or faecal samples be collected for examination (see below). Apart from testing for genetic purposes, blood samples can provide normal physiological values (and detect unhealthy animals), and serum can be saved for reference for future surveys of disease incidence. Fresh faecal samples can be checked for parasites, and an acid-fast stain can be performed to check for the presence of *Cryptosporidium* and acid-fast bacteria. Faecal cultures for Salmonella and other gastrointestinal pathogens can also be performed.

Any very sick or dead Massasaugas found in the wild should be submitted (fresh or preserved) for thorough post-mortem examination and diagnosis by an experienced wildlife pathologist (see below). All results should be catalogued to add to the data base of disease incidence.

1.2. Diseases of concern:

Ophidian paramyxovirus (OPMV)

OPMV is a serious viral infection of captive snakes, particularly viperids, but it has not been reported in Massasaugas. OPMV is contagious, and is characterised by signs of inappetence, regurgitation, incoordination, and pneumonia, with death in several days to weeks after clinical signs develop. A more chronic form of OPMV shows poor appetite, wasting, inactivity, and laboured respiration. Diagnosis is based on histopathology, and on viral testing and isolation. Only a few labs (in the US) are able to perform serological testing for OPMV. It is strongly recommended that the status of Massasaugas in the wild and in captivity be evaluated, and any snakes considered for release from captivity should be tested. No Massasaugas in captive breeding programs should come in contact with OPMV positive snakes.

Mycobacteriosis (Tuberculosis)

Mycobacteriosis has caused the death of several Massasaugas in captivity. The *Mycobacterium* species involved has not been identified in most of these cases. Control of the disease is limited by the ubiquitous nature of the organism, and by the lack of knowledge of its transmission and pathogenesis in reptiles. There are many things that need to be learned about mycobacteriosis in wild and captive reptiles. The clinical picture of mycobacteriosis is of an animal demonstrating poor appetite and progressive weight loss. Death occurs after months of progressive loss of general condition. Diagnosis in the living animal is difficult. Gross post-mortem lesions are usually limited to abscesses in a variety of internal organs, with recognition of the bacteria in acid-fast stain preparations of abscess smears, and in histological sections of affected tissues.

Cryptosporidiosis

Cryptosporidiosis is an insidious parasitic disease of snakes, and occasionally of lizards and turtles. *Cryptosporidium serpentis* is a small protozoan parasite (coccidian) which inhabits the surface of the gastro-intestinal (GI) tract, causing massive thickening of the wall of the stomach in snakes. Clinical signs include regurgitation, weight loss, and palpable swelling in the mid-body. Some snakes may show little or no sign of disease, but may shed the organisms during periods of stress. In other cases, snakes may show signs for many months before death, and persistently shed *Cryptosporidium* in the faeces. Infected Massasaugas have been severely affected. *Cryptosporidium* is highly resistant to drugs and chemicals, and there is no definitive cure for the disease. Control in the captive environment depends on adequate screening of new animals during quarantine, using special staining techniques on weekly faecal samples. An animal which is positive should not be introduced to an established group. Cryptosporidiosis has been recognised in a roadkill Massasauga.

Coccidiosis

Coccidians are common protozoan parasites of wild lizards and snakes, which undergo development in the lining of the GI tract. Some species have a direct life cycle: infective sporulated oocysts are ingested during feeding. Other species, including *Sarcocystis*, have an indirect life cycle involving both sexual development in the intestine of the reptile and asexual stages in a prey species. Cysts in the muscle of the prey are released upon digestion. Coccidian infections usually cause no clinical signs, except for the presence of oocysts in faeces. However, a very heavy infection may produce weight loss, enteritis, anaemia, and death. At this time, there are no drugs effective at reducing the excretion of oocysts. Most wild caught Massasaugas checked at the Toronto Zoo have been excreting coccidian oocysts, identified as *Sarcocystis* and *Caryospora*—further development of the organism takes place in meadow voles. The snakes appear to be unaffected by the infection.

1.3. Stress

Disturbance, handling, and translocations can produce degrees of stress. Long-term stress can be more insidious, and can have a detrimental effect on the functioning of the immune system. As in other reptiles, many aspects of Massasauga metabolism, including immune function, are controlled by external influences. Whether in the wild or in captivity, confinement, handling, environmental, social, and nutritional stresses can affect the snake's ability to fend off infections and parasites.

2. QUARANTINE

2.1. Facilities

Isolation of any new Massasauga is an essential aspect of preventive medicine, whether the snake is confined for a few days or intended for long term captive breeding. Strict isolation and hygiene will prevent transmission of any diseases between the new animal and others. Quarantined Massasaugas should be housed separately from established animals. In order to reduce the possibility of cross contamination, a separate room for quarantine is preferable. It is strongly recommended that Massasaugas brought into captivity for a short period, and designated for eventual release, be kept in a separate facility from long term (three months or more) captives.

2.2. Procedures

For any snakes that are to be long-term captives, the quarantine period should last at least 90 days. In this time period any viral or bacterial diseases should express themselves. At least three faecal checks should be performed during the quarantine and the snakes treated for any parasite infection. Animal handlers must be diligent in their hygiene practices. Newly quarantined animals should be cared for after established and longer-term quarantine animals. Between animals, a handler should either wash hands or change latex gloves. Separate cleaning and handling equipment should be used for each animal's container—if this is not possible, then equipment should be thoroughly cleaned and disinfected before use for another animal. A 1.0% sodium hypochlorite solution may be used to disinfect holding cage, props and some tools. Household chlorine bleach ranges from 3% to 5% sodium hypochlorite and commercially available bulk solutions can be of much higher concentration (up to 12%). It is therefore vital to confirm the concentration with the manufacturer or supplier before preparing a dilute working solution. Since chlorine bleach is corrosive to metal hooks and tools, contact should be minimal or an Iodine based disinfectant may be used. Hygiene considerations are also important for staff safety, to avoid possible human infection (zoonoses) as well as avoiding the transmission of disease between animals.

3. FAECAL SAMPLING FOR DETECTION OF PARASITES

This is done to assess the prevalence of oocysts of coccidia and eggs of helminth worms. Relatively fresh (up to 2 days) faecal material from Massasaugas should be collected. Each sample should be divided in two subsamples. At least 2 g of faeces should compose each of these subsamples. One subsample (in a faecal cup or plastic ziplock type bag) should be kept frozen at -20°C (in a regular freezer). The other subsample should be mixed with a solution of 2.5% Potassium Dichromate in a 1/10 volume ratio. To prepare a 2.5 % solution, dissolve 2.5 grams of reagent grade 100% potassium dichromate crystals in 100 mls distilled water. The faecal sample mixed with this solution should then be kept refrigerated (approximately at 4°C) in a closed container until forwarded to the lab. If shipping is delayed, these samples should be shaken once a week in order to induce sporulation of oocysts. These samples can be kept for several weeks, and sent to the Clinical Laboratory of the Toronto Zoo when convenient (361A Old Finch Avenue, Scarborough, Ontario M1B 5K7; 416-392-5975). Each sample should be identified with capture location, capture date, sampling date, sex, weight, and length of the animal. Toronto Zoo can provide bags and containers of Potassium Dichromate for sample collection.

4. EUTHANASIA

Any method used to euthanise snakes must render the animal insensitive to pain until it is dead. The recommended euthanasia method is the administration of T-61 or pentobarbitone by a veterinarian or trained technician (injected either i.v. or i.p).

Methods that will instantaneously destroy the brain (thus rendering the animal insensitive to pain) also include concussion by a forceful crushing blow to the head in order to destroy the brain. This method although not recommended is considered humane. If used, extreme care and caution should be used to prevent injury to humans and undue pain to the snakes.

In mammals electrical activity in the brain continues for 13-14 seconds following decapitation or cervical dislocation. Both these methods of euthanasia are considered humane for mammals and birds. However in reptiles, brain activity continues for several minutes following decapitation because a reptile brain can survive low oxygen levels and lack of blood for a relatively long period of time. Freezing of reptiles is not considered a humane method of euthanasia by the American Veterinary Medical Association. There is no evidence that death by hypothermia is any less painful for reptiles than it is for mammals. Decapitation and freezing alone cannot be used to euthanise snakes. These methods are however acceptable following the administration of an anaesthetic. At surgical levels of anaesthesia, decapitation and freezing may be employed.

5. PATHOLOGY

Dead rattlesnakes still pose a snakebite threat. In a study of reported snakebites at Good Samaritan Regional Medical Center in Phoenix, 14.7 percent of cases were bitten by snakes that had been fatally injured and were presumed to be dead. It is therefore recommended that dead rattlesnakes be handled with caution. Any *Massasauga* which is found dead, whether in the wild or in captivity, should be submitted for a thorough post-mortem examination. Information gleaned from the examination will add to the database of disease incidence and the level of susceptibility of *Massasaugas*. The whole animal should be submitted as soon as possible to the Canadian Co-operative Wildlife Health Centre (CCWHC) at the University of Guelph (CCWHC Ontario Region, Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1. Tel: 519-824-4120 ext. 4616 or 4556; Fax: 519-824-5930).

Snakes should be placed in a clearly labelled plastic bag, refrigerated and transported on ice as soon as possible. If unable to submit the snake to the laboratory within 2 days it should be preserved in 10% buffered formalin. If formalin is not available, then it can be frozen and shipped frozen. Freezing is the least desirable method of preservation since freezing damages cells and makes histopathology less rewarding.

6. BLOOD SAMPLING

In order to secure blood for clinical analysis, relatively small samples of blood are required (0.2– 0.3 mls of blood). The maximum volume that can be safely drawn from a snake is 7%- 10% of the total blood volume. This is roughly 1% of the snake's total weight. Blood sampling is appropriate for all age-classes (neonates to adults), but is more difficult in smaller snakes.

6.1. Technique

Insulin syringes are appropriate for sampling blood and are widely available at pharmacies. Once collected, two blood smears should be prepared and the remaining sample should be transferred to a Microtainer™ blood collection vial. Details of the sampling procedure, smear preparation, and storage and shipping instructions are outlined below. Please read through the procedures before you attempt to obtain a sample.

- Pull back and forth on the syringe plunger several times to ensure smooth action.
- With an assistant holding the snake (safely restrained), clean the ventral surface of the tail with an alcohol swab.
- Elevate the snake's body vertically so as to ensure blood flow toward the tail.
- Hold the tail in one hand (with the snake's head facing away from you) and the syringe in your other hand - see attached photo print.
- Gently insert the needle an angle of ca. 45° between the ventral scutes of the tail at a location between ½ and 2/3 from the cloaca and the tip of the tail. The closer to the mid-line of the ventral surface of the tail that you insert the needle the better your odds of hitting the centrally located caudal vein/artery. (Be prepared for the snake to flinch in your hand at this point).
- Continue to slowly insert the needle until you feel a slight resistance from the caudal vertebrae, withdraw the needle slightly (i.e., 0.5-2.0 mm), and then gently draw back on the syringe plunger.



Photo by Rob Tervo

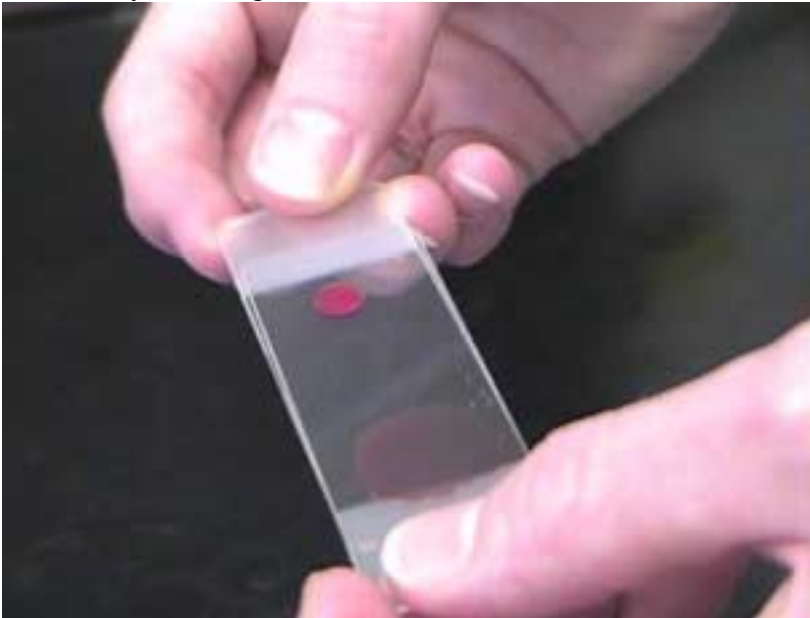
- If you have hit the caudal vein/artery directly, the syringe tip should quickly fill with blood. Slowly draw back on the plunger until you secure an adequate blood sample. Do not pull back too hard on the plunger as too much vacuum pressure can cause hemolysis of the blood (depending on how the syringe is oriented in your hand, you should be able to draw back on the plunger with either your thumb or pinkie finger of the same hand, leaving your other hand free to secure the tail of the snake).
- In the event that you do not get adequate blood flow immediately or the flow of blood stops you may have to either move the needle in and out slightly (i.e., a few mm) or rotate the needle until blood flow resumes. Alternatively, you may have to completely withdraw the needle and reinsert a new one at a new location before you hit the vein/artery dead-centre and get sufficient blood flow.
- Once an adequate blood sample has been collected, withdraw the needle and prepare two blood smears (see 5.2. SMEARS) on clean microscope slides. Then slowly deliver the blood into the microtainer by removing the needle from the syringe and gently expelling the blood. If the needle cannot be removed, gently expel the sample through the needle, do not force the blood through the needle, as this will cause hemolysis. Cap the tube securely and then gently invert the tube to mix the blood sample with the heparin. It is very important to ensure the blood is well mixed to prevent clotting. **DO NOT SHAKE THE TUBE**. Dispose of the syringe in a bio-hazard sharps container. Use a new syringe for each individual snake sampled.
- Apply light pressure to the point on the tail from which blood was drawn in order to stem further blood flow.
- Label tubes containing blood samples with a unique identification number and record the following information on an accompanying data sheet: sample identification number, species name, collection date, collection locality (e.g., direction and distance from nearest town or village), sex, weight, and SVL.

- Store labelled blood samples upright in the storage box, in a cool dark place (preferably in a refrigerator) until you can spin the blood down (see 5.3. Plasma Storage). **The maximum time between collection and spinning down should be no more than 24 hours.** The longer the blood cells are in contact with the plasma portion of the blood, the more the clinical results will be affected.

6.2. Smears

The squash technique is used to prepare reptilian blood differential smears. This method is gentle on the delicate cells, and gives a good distribution of the cells on the slide. What is needed is an area of the slide where the cells are one cell layer thick. (If the cells are piled on top of one another, it becomes difficult to differentiate the cells accurately.)

1. Always prepare two smears, one for reading and one for future reference. Place two clean glass microscope slides on a clean surface.
2. After drawing a blood sample with an Insulin syringe, place one small drop on each slide near the frosted end (but not touching), by allowing ONE drop from the needle tip to drop onto the slide surface. After placing the drop, place the rest of the blood gently into a Microtainer™. (Do not force the blood through the needle, as this will cause hemolysis). To prevent the blood drops from drying on the slide, this needs to be done quickly. If a second person can place the blood in the Microtainer™, the first person can smear the blood without delay.
3. To smear the blood, gently place a clean slide on top of the drop of blood. Allow the blood to spread out a little between the slides, without pressing down at all, for a count of 1-2 seconds. You will be trying to achieve a monolayer of cells on the smear (If the slides are together too long the WBC's (white blood cells) will move to the periphery of the smear thereby affecting the count).



4. Fairly quickly, but gently, slide the slides apart. Again, do not press down on the slides as you pull apart (this will crush the delicate cells).



5. Repeat steps 3 and 4 for the second smear. It is important that the smears be made quickly to avoid drying and clotting of the blood drop.
6. Dry the completed smears by gently waving them back and forth in the air. Do not blow on the slides.
7. With a pencil or a permanent marker-pen, label the frosted end of the slides with the ID of the animal, the date and the species.

6.3. Plasma storage

To store the remaining blood, it is necessary to separate the plasma from the cellular portion of the blood by centrifuge. Most veterinarians and Universities have a blood centrifuge, and it may be possible to have them spin down the samples. The blood should have been collected and carefully placed in heparin Microtainer™. A feature of these tubes is the addition of a separating jelly which, when spun down, forms a layer between the plasma and the cells. Place the Microtainer™ in a blood centrifuge and spin it for ten minutes. Then place the Microtainer™ upright in a freezer for storage. Ensure that each sample has been clearly labelled. The dry smears and frozen plasma/blood should be sent to the Clinical Laboratory of the Toronto Zoo when convenient (361A Old Finch Avenue, Scarborough, Ontario M1B 5K7; 416-392-5975).

7. HANDLING AND HUSBANDRY

Whether Massasaugas are being housed temporarily as part of a field study, or are being established as a captive breeding group, several basic recommendations can be made to provide optimal conditions and minimise stress in order to safely maintain the health of the snakes.

7.1. Safety considerations

Under the Occupational Health and Safety Act those housing snakes may have responsibility for ensuring the safety of staff/employees/volunteers working with snakes. In developing safety measures, the first step is to minimise the possibility of snakebite. This can be achieved by:

- Providing appropriate training in handling and working around venomous snakes.
- Providing adequate handling equipment such as snake hooks, capture tongs, catch boxes, transport boxes, restraint tubes and holding cages.
- Ensuring that backup staff is available during hands on restraint or handling.
- Restricting access to venomous snake areas.



Lockable and labeled transport box.

It is recommended that all holding containers and areas be kept locked with restricted access. These containers and areas must be labelled as having venomous animals in them. Labels should also list the species (scientific and common name) and number of animals present. Procedures should be established to deal with snakebite. These include a reliable alarm system and written response procedure that includes contact phone numbers for supervisory staff and emergency services. The emergency response system should be tested. The hospital to which a snake bite victim would be transported should be contacted and informed that work with

venomous snakes is being done in the area. An adequate supply of antivenom should be available and clearly labelled.

7.2. Transport

Snakes should be transported in a clearly labelled and locked container. This should be ventilated, and if exposed to temperature extremes (direct sun or winter use) it should be insulated. Ventilation holes should be small enough to prevent the snake from biting through the hole and to prevent the escape of any new-born snakes. Since snakes can deliver a bite through a cloth bag, transporting venomous snakes in cloth bags alone is not recommended. Snakes should not be transported in cages with heavy items (e.g. decorative rocks) that might shift and cause injury.



Placing a snake into a bag.

Snake bags should be as large as possible (King Size pillow cases work well). Snake bags should be carefully inspected for wear and tear (holes) prior to use. When placing a snake in a snake bag, the bag should not be held open by hand. The bag should be held open using Pilstrom tongs (long handled animal tongs) or a specially designed solid metal frame at the end of a pole (Killbear Rattlesnake Research Team calls these “triangles” and orders them from Reptilia in Toronto), or by draping the bag over a bucket. Once the snake has been transferred to the bag with a snake hook or tong, the snake bag should be knotted shut (rather than tied shut with a cord or drawstring). This can be done safely by placing the bag on a flat surface and

isolating the snake at the bottom of the bag.



Isolating a snake in a bag (using a snake hook) in order to tie it.

This is accomplished by laying a long solid object (broom handle, snake hook etc.) across the bag so that the snake cannot crawl beneath this barrier to the open end of the bag. Slide the barrier towards the rear of the bag so that the snake is isolated as far as possible from the end where hands are tying a knot. If using a bucket, the snake can be isolated at the bottom of the bag by pulling the open end of the bag across the bucket lip and placing a lid on the bucket over the bag creating a barrier that the snake cannot pass. The open end of the bag will be outside the bucket where it can be knotted.



Isolating a snake in a bag (using a bucket) in order to tie it.

To untie the bag, these procedures should be reversed so that the snake will be isolated at one end of the bag while the knot is untied. To facilitate the release of a snake from a bag, the

corners can be sewn to create “hot corners”. This provides a rounded bottom to the bag and leaves material at the corners that can be grasped with tongs when releasing a snake.

7.3. Housing and husbandry

The handling or holding of threatened species requires appropriate Ministry of Natural Resources permits. Facilities housing Massasaugas may require the approval of an Animal Care Committee. All holding containers and areas should be kept locked with restricted access. It is recommended that short term holding enclosures used to house a snake should be at least 30 cm x 60cm x 30 cm high. A temperature gradient (22-32° C) should be offered, with a specific hot spot (30-32° C) if the snake is housed for more than a few days. Each holding area should have a thermometer to monitor changes in temperature. A humidity of 50% to 70% is desirable. A simple shelter should be available as a secure hiding place. Fresh water should be offered daily. A simple, easily removable substrate (e.g., newspaper) is suitable for temporary holding. If maintained in captivity for more than a week, then appropriate nutrition must be considered. Healthy prey animals of appropriate size (fresh or previously frozen), from known sources, free from contaminants, should be offered. Monthly weights for each individual should be recorded in order to monitor an animal’s condition. Snakes, which will be kept in captivity indefinitely, should have access to a larger space in which a normal array of behaviour may occur. Ideally it should be possible to separate the snake from the main compartment without handling it, in order to clean, water, and feed with minimal disturbance to the snake, and maximum safety for the handler. This can be accomplished by using a shelter or shift box that can be securely closed while the snake is in it.

7.4. Identification and records

Permanent identification is essential for following the life history of individual animals, whether in the field or in captivity. Non-invasive ID methods include paint or nail polish spots (temporary until the next skin shed or rattle breakage), or recording the individual’s dorsal patterns of the entire body by diagram, photo or shed skin. Mildly invasive ID methods include scute clips, rattle tags, or PIT tags (Passive Integrated Transponder e.g., Trovan or AVID), under the skin. PIT tags are injected with a specific applicator needle sub-cutaneously on the right side just cranial to (ahead of) the cloaca (vent). Some investigators have found that PIT tags injected with the application needle directed to the head of the snake risk migrating down and escaping through the injection site. Ensuring that the PIT tags are injected well past the entry hole of the applicator needle or injecting PIT tags with the needle directed towards the tail, and sealing the injection site with surgical glue should prevent this. For free-ranging snakes included in special field studies, a surgically implanted radiotelemetry transmitter is appropriate, but these devices should only be implanted in larger Massasaugas, while applying techniques of surgical anaesthesia and sterility. A veterinarian who has experience with reptiles and is familiar with the surgical procedure should be contacted for this surgery. Regular records of field data, behavioural observations, food intake, weight, handling, diagnostic procedures, treatments, and other observations should be maintained throughout the animal’s life.

8. MEDICAL ASPECTS OF TRANSMITTER IMPLANTS

The minimum size/weight of a snake that can receive an implant depends on how small the transmitter is, 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 a large-bodied snake such as a Massasauga, animals as small as 30-40 cm SVL could be implanted with transmitters. For gravid females (which lose up to 50% of their body weight when they gave birth) the transmitter should be no more than 2.5% of the snakes 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 and is less than ideal. The muscular activity of parturition could also result in dehiscence of an incompletely healed implant incision. Four weeks prior to expected parturition should be the latest date for implant surgery (**no surgery after mid June**). It is important to note that parturition dates can vary considerably between years within a given population. 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 2/3 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, both resulting in yolk peritonitis. Implanting gravid snakes early in gestation may also results in follicle re-absorption. Parturition likely causes some degree of stress and debilitation in female snakes, which immediate surgery would compound. It is recommended that female snakes should be allowed two weeks to recover from parturition prior to implant surgery.

Following surgery the snake should be kept in a warm (28-30C) holding for a minimum of 24 hours. To ensure the snake is released well hydrated, intracoelomic fluids (sterile normal saline) of a volume equal to 2% of the snakes body weight should be administered intraperitoneally (i.p.). Ideally a 3-6 day stay would allow for antibiotic injections at 3 and 6 days post-surgery. This extended recovery time would allow the snake to maintain it's preferred body temperature for the first few days post-operatively with minimal effort. Some suggest that to minimise the stress experienced by the snake it should be released as soon as possible, as long as the snake has regained full locomotor abilities. In this case antibiotics can be injected on the day of release.

A snake requires at least 4 weeks post-operatively during which it can achieve its preferred body temperature to heal completely. A snake will not likely heal at all during hibernation. Therefore, any snakes implanted after the end of August may run the risk of not completely healing before hibernation. Snakes implanted later than August should be kept in captivity until the incision has healed to avoid releasing a snake into sub-optimal weather conditions. However, consideration must be given to the fact that late in the season snakes may be migrating to a hibernation site and delaying release may affect their ability to reach a hibernation site.

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Prepared by:

Andrew Lentini, Curatorial Keeper-Amphibians and Reptiles
Toronto Zoo
361A Old finch Ave.
Toronto, Ontario
M1B 5K7