

Capture, Handling and Release of Wolves Standard Operating Procedures

Wildlife Care Committee

Government of the Northwest Territories

Marc Cattet

Reviewed by: 2017/2018 NWT Wildlife Care Committee

Table of Contents

1. Purpose	1
2. Application	1
3. Background	1
4. Methods	3
4.1 Noninvasive Sampling	3
4.1.1 Considerations	3
4.2 Live Capture	3
4.2.1 Considerations	3
4.2.2 Chemical Immobilization	5
4.2.3 Techniques for Live Capture:	7
4.2.4 Recommendations for Approaching an Immobilized	Wolf:18
4.2.5 Recommendations for Initial Handling of an Immobili	zed Wolf:19
4.2.6 Recommendations for Monitoring Physiological Fund	ction:21
4.3 Sample Collection and Measurements	22
4.4 Identification, Marking and Telemetry Collars	
4.5 Reversal Drugs and Release of Wolves	26
4.6 Post-Capture Monitoring	27
4.7 Euthanasia	28
4.8 Morbidity and Mortality	29
4.9 Human Safety	30
5. Literature Cited	32
Appendix A – Net Gun Maintenance, Use, and Inspection	38
Appendix B – Drug Volume Table for Capture and Reversal of	Wolves43
Appendix C – Example Wolf Capture Data Form	44
Appendix D – Example Field Necropsy Data Form	46

1. Purpose

- To provide recommendations for the capture, handling, and release of wolves using 'acceptably humane methods' while maintaining minimal risk to staff and allowing appropriate research or management to be conducted.
- The term 'acceptably humane methods' should be recognized as conditional in that some of the methods and techniques recommended in this standard operating procedure (SOP) are neither supported nor contraindicated by scientific evidence. This is because animal-based measures of humaneness and welfare are currently lacking. Consequently, under these circumstances, recommendations are based on other factors, including opinion and intuition. Therefore, personnel involved with the capture and handling of wolves in the Northwest Territories (NWT) **should not** view this SOP as prescriptive, and assume that all recommendations represent best practice. Instead, personnel **should always** strive to integrate animal-based measures (e.g. physiological and behavioural parameters) into their research and/or management activities to provide direct evidence for what wolves are experiencing. Animal-based approaches are required not only to validate some of the current methodologies, but also to facilitate improvements to this SOP.
- See Hampton et al. (2016) for an insightful perspective on the limitations and refinement of procedural documents as they relate to wildlife welfare.

2. Application

This SOP applies to employees of the Government of the NWT (GNWT) and any other
personnel (including contractors) involved with the capture and handling of wolves in
the NWT.

3. Background

- One species of wolf is present in the NWT, the grey wolf (*Canis lupus*). However, this species is subdivided into three groups based largely on their geographical distribution in the NWT. Boreal (timber) wolves live below the tree-line and in the mountains. They maintain regular territories and depend mostly on non-migratory prey like moose, bison, caribou and deer. In contrast, tundra wolves travel above and below the tree-line on the mainland of the NWT, do not maintain regular territories, and depend largely on barren-ground caribou and muskoxen. The third group, Arctic wolves live on the Arctic islands and prey mostly on caribou, muskox and Arctic hare.
- The Species at Risk Committee (SARC) was established under the *Species at Risk* (NWT) (*SARA* (NWT)), which came into effect in February 2010, to assess the biological status of species that may be at risk in the NWT. The *SARA* (NWT) is complementary to the federal *SARA* and addresses concerns at the territorial level.

- As part of the assessment, the SARC identifies threats and positive influences on species and their habitats. They may also recommend conservation actions. The assessments provide the basis for recommending whether a species should be added to the <u>NWT List</u> of Species at Risk.
- Species can be listed under the NWT List of Species at Risk either as
 - o Extinct: no longer exists anywhere.
 - o *Extirpated*: no longer exists in the wild in the NWT.
 - o *Endangered*: facing imminent extirpation or extinction.
 - o *Threatened*: likely to become endangered if nothing is done.
 - o *Special Concern*: may become endangered or threatened because of threats and biological factors.
- Species may also be assessed, but not listed, and categorized instead as
 - *Not at Risk* not currently at risk of extinction.
 - o Data Deficient not enough information to determine status.
- The grey wolf has not been assessed by the NWT SARC. However, it has been assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC).
- Under the federal *SARA*, which came into force in December 2002, COSEWIC is identified as an independent body of experts responsible for identifying and assessing wildlife species considered to be at risk in Canada. COSEWIC reports the results of its assessments to the Canadian government and the public. However, it remains up to the federal Minister of the Environment to decide if wildlife species designated by COSEWIC are to be legally protected.
- Species can be designated either as:
 - o *Extinct*: a wildlife species that no longer exists.
 - o *Extirpated*: a wildlife species that no longer exists in the wild in Canada, but exists elsewhere.
 - o *Endangered*: a wildlife species that is facing imminent extirpation or extinction.
 - o *Threatened*: a wildlife species likely to become an endangered species if nothing is done to reverse the factors leading to its extirpation or extinction.
 - o *Special Concern*: a wildlife species that may become threatened or endangered because of a combination of biological characteristics and identified threats.
 - o *Data Deficient*: A category that applies when the available information is insufficient (a) to resolve a wildlife species' eligibility for assessment or (b) to permit an assessment of the wildlife species' risk of extinction.
 - Not at Risk: A wildlife species that has been evaluated and found to be not at risk
 of extinction given the current circumstances.
- The national status of the grey wolf was assessed by COSEWIC in April 1999 as Not at Risk because at the time of assessment, it had a widespread, large range with no evidence of population declines in the provinces and territories where it resides.

 As with any other wildlife species in the NWT, all investigators must ensure their capture and handling protocols for wolves meet all permitting requirements of the GNWT-Environment and Natural Resources (GNWT-ENR), including the Wildlife Care Committee and others. See www.enr.gov.nt.ca and follow the Research and Data tab for more information.

4. Methods

4.1 Noninvasive Sampling

Noninvasive sampling refers to a suite of techniques that can be used to gather biological information, including certain types of samples (e.g. feces, hair, saliva), without the need to physically capture wolves. Many of these techniques have been validated for other species (e.g. bears), aside wolves, and proven to be as reliable as more conventional approaches involving capture. Noninvasive sampling **should always** be considered as a potential alternative to the capture and handling of wolves in the NWT.

4.1.1 Considerations

- A key reference for noninvasive sampling techniques is the book, edited by Long et al. (2008), and titled *Noninvasive Survey Methods for Carnivores*.
- Noninvasive sampling techniques that have been applied to wolves include camera traps (Šver et al. 2016), hair traps (Stansbury *et al.* 2014), fecal collections (Echegaray and Vilà 2010, Caniglia et al. 2012), urine collection (Nakamura et al. 2017) and saliva collection (Nakamura et al. 2017).

4.2 Live Capture

The techniques used most commonly for the live capture of wolves in the NWT are net gun and remote drug delivery. Both techniques are typically carried out from a helicopter, although the latter technique is sometimes carried out from the ground. Restraining devices, including cage traps, foothold traps, and cable devices (neck and foot snares), may also be used to capture wolves for research and management. However, because these techniques are non-selective, their use may result in the capture of other (non-target) species, instead of wolves. Further, the use of foothold traps is currently ranked as an 'E' on the Categories of Invasiveness scale used by the Canadian Council of Animal Care (CCAC 2003). Procedures ranked at this level are deemed to "cause severe pain near, at, or above the pain tolerance threshold of non-anesthetized, conscious animals", and **must** not be used if the research objectives can be met using less invasive procedures.

4.2.1 Considerations

• The primary focus of all capture events **must** be on the safety of both the personnel and the wolf.

- Investigators **must** be familiar with the advantages and drawbacks of different methods of capture.
- The capture method to be used **must** be selected to minimize trauma and stress to the animal with consideration given to the capture environment and study requirements.
- Capture techniques **must** be applied by experienced individuals only.

Inexperienced persons with appropriate training may also apply capture techniques provided they are under the direct supervision of an experienced person. Although the distinction between "experienced" and "inexperienced" is somewhat subjective, it is the responsibility of the NWT Wildlife Care Committee (NWTWCC) to seek assurance that investigators and personnel have the necessary training and experience to perform procedures required for the capture and handling of wolves.

- Mentoring by experienced persons is strongly recommended as the best approach to become proficient at animal capture.
- Personnel administering drugs for capture or restraint must have recognized and current training in the chemical immobilization of wildlife and must use methods of drug delivery and drugs that are appropriate for wolves.

Current training implies that personnel have completed and passed the Canadian Association of Zoo and Wildlife Veterinarians' wildlife chemical immobilization course, or another recognized course, within the past five years.

• Investigators **should** avoid capturing potentially-pregnant wolves in the period from late February to early May (Hillis and Mallory 1996), because the stress of capture and handling may increase the likelihood of reproductive failure (Wingfield and Sapolsky 2003, Mech et al. 2016). Investigators **should** also avoid capturing wolves and their pups from May to early June to prevent disrupting lactation (Wingfield and Sapolsky 2003, Metz et al. 2012).

Any exceptions to this recommendation must be given careful consideration and must be consistent with the overall study objectives. Wolves may be particularly sensitive to capture stress during pregnancy and lactation. Further, many of the drugs used in wolves are not recommended for use in pregnant or lactating animals because they have not been adequately evaluated under these conditions. These include Telazol®, as well as formulations of ketamine, xylazine, medetomidine, and atipamezole.

• Capture efforts **must** be conducted within a pre-defined safe temperature range and procedures **must** be taken to prevent, or detect and treat, large changes in body temperature during handling or recovery.

Thermal stress (hyper- and hypothermia) may occur in wolves as a consequence of extreme ambient conditions, which include extremes in air temperature, wind, solar radiation, and/or precipitation. The probability of thermal stress may also be exacerbated by body size and condition. A suggested "safe" ambient temperature range for the capture of wolves is from -20° C to $+20^{\circ}$ C but consideration **must** also be given to other weather features, including wind and precipitation. Measures **must** also be taken to monitor body temperature during immobilization, prevent the development of thermal stress during capture, handling, and recovery, including avoidance of prolonged pursuit times, use of a tarp or canopy to minimize solar radiation, and use of natural barriers to prevent wind exposure. Having materials available to mitigate impacts of hypothermia (e.g. solar blankets and heat packs) and hyperthermia (e.g. cool water and alcohol for the foot pads) is strongly recommended.

4.2.2 Chemical Immobilization

Issue: Immobilization of a wolf by administration of one or more drugs to either capture a free-ranging wolf by remote drug delivery or facilitate the safe handling of a wolf that has been captured by other means, e.g. net gun.

Recommendations:

 Ideally, sedative drugs or anesthetic drug combinations whose effects can be counteracted (reversed) by administration of antagonist (reversal) drugs **should** be used instead of drug combinations whose effects cannot be counteracted.

Wolves anesthetized with "reversible" drug combinations can be promptly "reversed" if they respond poorly to anesthesia, and can be reversed following handling to hasten their return to a mobile state (Kreeger and Arnemo 2012). See Appendix B for recommended drug protocols and volumes. These and other drug protocols are described in the wildlife chemical immobilization literature (e.g. Walton et al. 2001, Valerio et al. 2005, Barber-Meyer and Mech 2014, Arnemo and Evans 2017). The dosages and corresponding volumes in Appendix B are intentionally provided as ranges because selection of an appropriate dosage should be based on other factors in addition to the estimated body weight, e.g. age of animal, time of year, method of capture, etc. Investigators should be aware that the use of reversible drug combinations, specifically combinations that include α_2 -adrenoceptor agonist drugs (e.g. xylazine, medetomidine, and dexmedetomidine), cause high blood pressure (marked hypertension) that is sustained until the reversal drug is administered (Sladky et al. 2000). Other carnivores have also been noted to respond similarly to combinations of α_2 -adrenoceptor agonist drugs and ketamine, or Telazol® (Zoletil®), including cheetahs (Deem et al. 1998), otters (Spelman et al. 1994), and bears (Caulkett et al. 1999). Sustained, severe hypertension may put captured carnivores at risk for development of adverse health effects (e.g. cerebral encephalopathy, retinal hemorrhage), although these effects have not been reported in free-ranging carnivores despite the use of these drug combinations over several decades.

 All drug vials must be clearly labeled with drug name(s), concentration, and date of preparation. If a drug combination is prepared by adding one drug to a commercial preparation of another drug (e.g. the addition of medetomidine to a vial of Telazol®), the manufacturer's label **should** be covered with a highly visible adhesive label to avoid confusion between drug preparations.

- Drugs must be protected against prolonged exposure to extreme temperatures, high humidity, or light.
- When not in use at field-sites, drugs should be stored in a labeled, locked, crush-proof, leak-proof container that is lined with absorbent material.
- Empty darts should not be filled with drug while in a moving helicopter or motorized vehicle.
- All used drugs **must** be recorded, including amounts lost in darts that miss the target.
- Drug-filled darts, that are not used, should be emptied at the end of each day, and the drug that is removed should be set aside in a "wasted-drug" vial for disposal.

Many drug solutions are acidic and can cause corrosion of the dart components. Drugs may also leak from darts with time. Drug that has been sitting in a dart for a prolonged period (e.g. several hours) is likely contaminated, and **should not** be injected into an animal.

- If cleaning used darts that require disassembly, the tailpiece (flight) must be removed first in case the drug chamber is still pressurized.
- Adequate steps must be taken to ensure that drugs administered to wolves do not enter the human food chain.

Health Canada's Veterinary Drugs Directorate takes the view that persons administering drugs to wildlife "assume full responsibility for safety in the intended species and any drugresidue-related violations in food derived from treated animals." Although wolf meat is not widely consumed, it is still good practice that wolves **should** be clearly marked to indicate they have been chemically immobilized, or anesthetized, and the individuals or agency performing the capture **must** provide contact information on the collar or ear tag (Cattet 2003). Because the drugs used to immobilize wolves do not have established drug withdrawal times, Health Canada recommends a drug withdrawal time of one year for any of the immobilizing drugs that are used in wolves. The Wildlife Health Committee of the Western Association of Fish and Wildlife Agencies (WAFWA) has also provided recommended drug withdrawal times for various drugs used in wild animals (WAFWA Wildlife Health Committee 2009) in the United States, but these withdrawal times have not been recognized by Health Canada.

4.2.3 Techniques for Live Capture:

4.2.3.1 Net Gun

Issue: A suitable capture technique for short-duration handling, generally less than 15-20 minutes.

- Enables rapid capture and release of target animal.
- Requires use of a helicopter.
- Investigators should be aware that capture by net gun is considered to be a Category of Invasiveness 'D' procedure by CCAC (CCAC 2003). Procedures ranked at this level are believed to "cause moderate to severe distress or discomfort".
- Handling time **should** be minimized in all cases.

Recommendations:

- See Appendix A for detailed information regarding net guns, including additional recommendations.
- Net guns must only be used to capture a single wolf at a time.

Attempts to capture two or more wolves within a single net are likely to result in injury, or possibly death.

• At least two capture guns with loaded nets, or a gun with detachable barrel and multiple nets, **should** be available to the gunner for each capture.

This provides a back-up that can be used to reduce chase duration if the first net misses the target animal or to re-net an animal if the first net does not provide adequate restraint. If a wolf is not netted properly and does not go down shortly after netting, a second net should be deployed.

- Hazing must be done in a controlled manner with the goal of moving animals at a slow pace while gradually separating out target animals.
- Pursuit and capture must occur on smooth, open terrain with good footing, and, whenever possible during the appropriate season, deep soft snow should be used.

This will help prevent injury (e.g. broken limbs, sprains) to the target animal and animals running with it. Deep, soft snow will slow down fleeing wolves. Hummocky ground, wind sculptured snow-drifts, boulder fields, packed snow, open water and glare ice represent different terrains on which the chances of injury are increased.

 Physical trauma is the most common source of injury during net-gunning. Every effort must be made to choose capture locations that minimize risk of injury. The capture crew should evaluate the conditions at the capture site prior to initiating a capture. In the event of physical injury, the crew needs to re-evaluate if the environmental conditions are suitable for continuing captures.

- Chase times (interval from onset of active pursuit to launching of net) must be recorded for each chase attempt and clearly documented.
- Active pursuit **should** be kept short (≤1 min of strenuous running), and **must** always be terminated if the target animal shows signs of exhaustion, e.g. panting, loss of coordination and stumbling.

The risk of injury (including capture myopathy) or death is increased greatly when animals are exhausted (Spraker 1993, Williams and Thorne 1996, Paterson 2014). Because adequate rest periods for exhausted animals are not known, and are likely to be highly variable among individuals, it is strongly recommended that any further attempt at capturing an exhausted animal is not made until the following day at earliest.

• Chases per pack **should** be limited to no more than three chases per pack. Where multiple individuals are to be captured from the same pack, the capture crew **must** terminate chases if animals are showing signs of exhaustion from prolonged hazing.

It is the responsibility of the entire capture crew to ensure that chase times and limits are observed.

- Once a wolf is netted, it must be secured as quickly as possible. Given ample time, wolves are quite capable of finding (including chewing) their way out of nets. Further, when a wolf caught in a net is approached by a handler, it may act aggressively out of fear. Lack of knowledge or hesitation can lead to a handler being severely bitten and/or the wolf escaping (Quicksilver Air Inc. 1996).
- Wolves must be muzzled in a way that does not interfere with respiration, hobbled, blindfolded, handled, and released as quickly as possible following capture. Use of a Y pole (see http://wildliferesources.com/the-y-pole/) to hold the wolf to the ground is extremely helpful to immobilize the animal while attaching muzzle (face cover), blindfold, and hobbles. Care must be taken not to injure the wolf by placing too much pressure on its neck with the Y pole.
- There is considerable risk of being bit when attaching the muzzle. One method to avoid being bitten is to use a one-meter length of cord with a slip noose at one end (Quicksilver Air Inc. 1996). The cord is suspended over the wolf's mouth with the intent of slipping the noose over the wolf's lower jaw (mandible). Once the loop is secure on the lower jaw, proceed to put several half-hitch loops around both jaws pulling them shut in the process. When the cord is tied off with the jaws securely shut, it is safe to place the muzzle and blindfold. Use of muzzles that are effective, but can be easily removed by the handler is recommended.

- When hobbling a wolf, the front and back leg on the top side of the body are first strapped together followed by the front and back leg on the downside of the body, while the animal is still in the net.
- When releasing the wolf, use a Y pole to immobilize its neck and head, place firm weight over its rump, untie the bottom set of legs, remove the muzzle and blindfold, and untie and flick the noose cord from the jaws (Quicksilver Air Inc. 1996). Then, while still keeping pressure on the wolf's neck and rump, untie the topside legs.

4.2.3.2 Remote Drug Delivery

Issue: The administration of anesthetic drugs by use of remote drug delivery systems, which generally include a blowpipe or a projector (i.e., a modified shotgun, rifle, or pistol), and drug-filled darts.

Issue: A suitable capture technique for situations in which a wolf must be physically handled to record measurements, collect biological samples, and/or affix a radio-telemetry device.

Recommendations:

- The method of remote drug delivery should be selected for its effectiveness under the intended capture scenario, and for its safety to the user and target animal.
- Blowpipes or pistols (CO_2 or air-powered) may be used to deliver small volumes (≤ 3 ml) of drug to wolves over short distances (≤ 15 m) under the right conditions e.g. animal can be approached closely by vehicle.
- For larger drug volumes (>3 ml) and/or longer distances (>15 m), CO₂-powered rifles or powder-charged rifles with power adjustment capability **should** be used.
- For wolves restrained by nets, foothold traps, or cable devices (neck and foot snares), or held captive in a cage trap, drug **must** be delivered using the least traumatic method possible without compromising human safety. In these situations, it may be possible to safely control the wolf's head with a Y pole (http://wildliferesources.com/the-y-pole/), and then administer drugs by pole syringe or hand injection instead of using a remote drug delivery technique.
- Slow-injection darts should be used in preference to rapid-injection darts. According to the CCAC, the use of slow-injection darts is regarded as a less invasive procedure than the use of rapid-injection darts (CCAC 2003).

Darts can be described as rapid- or slow-injection depending on the mechanism of injection, and the time required to discharge its content upon impact with the target animal. Rapid-injection darts use an explosive charge that detonates upon impact to advance the plunger and expel the drug quickly, within a fraction of a second. Slow-injection darts use pressurized air or gas to expel drug more slowly (1-3 seconds). The

forceful injection of drug with rapid-injection darts penetrates considerably deeper than the tip of the needle and can cause severe tissue damage (Cattet et al. 2006). Forceful repulsion of the dart during injection can cause tearing of subcutaneous tissue and partial injection of drug into the space created between muscle and skin. Further, rapid-injection darts are often fitted with large bore, end-ported, barbed needles that frequently cause contamination of the wound tract with small portions of tissue and hair, and require expansion of the wound to facilitate removal of the barb. With slow-injection darts, drug injection is less forceful and the resulting wound does not penetrate as deeply (Cattet et al. 2006). In addition, the side-ported needle typically used with this type of dart does not require a large barb to anchor it beneath the skin. However, slow-injection darts that must be pressurized prior to loading into a rifle may leak their contents, so appropriate precautions must be taken to avoid accidental drug exposure, e.g. slide a plastic test-tube or syringe container down over the coupling between needle and drug chamber. Slowinjection darts may also leak air after pressurization resulting in partial or no injection of drug. For these reasons, slow injection darts should only be pressurized immediately before firing.

The dart needle must be long enough to ensure drug injection occurs primarily into muscle, but not too long to cause injury to deeper tissues or too short to result in injection of much of the drug into the subcutaneous connective tissue and fat.

The length of needle should be selected based on the size and body condition (reflected by the amount of subcutaneous fat) of the wolf and the type of dart used. With slow-injection darts, Bergvall et al. (2015) recommended a needle length of 40 mm, in preference to 30 mm, for the chemical immobilization of wild fallow deer (*Dama dama*) on the basis that the longer needle consistently resulted in a shorter induction time. With rapid-injection darts, needle length cannot be selected simply on the basis of estimating fat thickness at the targeted site of injection because the depth of the injection can exceed the needle length by a significant amount, especially when using end-ported needles (Cattet et al. 2006). Until science-based information indicates otherwise, a needle length not exceeding 40 mm (and ideally shorter) **should** be used with rapid-injection darts for capture of wolves by remote drug delivery.

When using barbed dart needles, the position of the barb must be marked on the dart barrel or needle hub (e.g. with a waterproof marker pen) before use.

This ensures the dart needle can be excised from the skin with minimal trauma.

 Darts must be directed into large superficial, thick muscle masses with minimal fat covering to ensure good drug absorption and avoid injury to other tissues.

The gluteal muscles (the rump) are the safest site for injection in wolves. If a rapidinjection dart is used, detonation of the internal charge may cause the needle to be retracted from muscle before the drug is fully expelled from the dart. Consequently, some of the drug will be deposited into the subcutaneous space or fat layer between the skin and muscle. A tell-tale sign of this occurrence is when the dart is seen dangling from the skin of the target animal. Conversely, if the dart needle has remained imbedded in the muscle, as often occurs with slow-injection darts, the dart body will remain firmly positioned and oriented in line with the path of entry.

• Remote drug delivery of free-ranging wolves **should** be employed from a mobile platform, such as a helicopter or snowmobile.

Remote drug delivery is typically employed from a helicopter, but occasionally can be employed on the ground. In either situation, it is important to be able to observe the darted wolf and, if necessary, control its movement away from hazardous terrain during pursuit and induction. The latter term, induction, refers to the time between drug administration and safe immobilization.

- Remote drug delivery of free-ranging wolves **should not** be attempted in areas where the possibility of losing sight of a darted wolf is likely, e.g. where tree cover is heavy.
- Remote drug delivery of free-ranging wolves should not be attempted if the terrain is such that a partially anesthetized animal has a high probability of entering a potentially hazardous location, e.g. close to a steep embankment or waterway.
- Final, close pursuit for the purpose of delivering drug into a free-ranging wolf **should** be kept short (≤1 min of strenuous running), and **must** always be terminated when the target animal shows signs of exhaustion, e.g. panting, loss of coordination and stumbling.

An exhausted wolf could include one that has been hit by one or two darts, but shows no signs of drug effect. Although such an animal may become anesthetized after the capture effort is terminated, and therefore vulnerable to predation, thermal stress, or some other complication, it remains more likely that continued pursuit of the darted wolf would cause serious injury or death.

When more than one dart is required to safely immobilize a wolf, adequate time **should** be given between injections to allow drug effects to occur.

In most situations, allow 10-15 minutes to elapse between the time of injection of the first dart and injection of the second dart (Kreeger and Arnemo 2012). During this time, effort should be taken to avoid unnecessary stimulation of the target animal. For example, if the wolf was darted from a helicopter, the pilot should move the helicopter as far as possible from the animal while still allowing visual monitoring for drug effects. If the animal shows some drug effect, but does not go down, re-administer 50% of the original dose. However, if the animal shows no drug effect within 10 minutes following the first dart, re-administer the entire original dose.

 If a free-ranging wolf does not show any sign of drug effect after two darts, the immobilization effort should be aborted as this strongly suggests a problem with either the drug delivery system (e.g., failed injection, needle too short) or the drug quality (e.g. inappropriate storage or formulation).

Nevertheless, the animal should be visually monitored from a safe distance to determine the extent of drug effects, if any should become apparent. Although there should be no further attempt at capture that day, effort should be made again over the next 24 hours to relocate the animal and assess its status.

• If a free-ranging animal is lost following darting, effort **must** be made to track the animal and assure its safety without compromising human safety.

Reversal drugs may also be administered by remote delivery in situations where capture personnel cannot safely approach an immobilized wolf.

• Whenever possible, effort **should** be made to find darts that miss their target.

This is particularly important in areas frequented by humans, such as near communities, where there is potential for someone to find a "lost" dart.

 Capture of more than one wolf per capture event must be attempted only if there are enough personnel in the capture crew to ensure that each animal can be continuously observed and its physiological function monitored closely throughout the handling period.

Otherwise, unattended wolves are susceptible to physiological complications that might easily be prevented or treated if they were monitored. If more than one capture is planned, the second wolf **should not** be darted until the first wolf has been immobilized and its welfare has been assured. For helicopter-assisted captures, this would require landing the helicopter and assessing the physiologic status of the first wolf prior to capturing the second wolf provided this will not compromise the safety of capture personnel.

 Cargo nets alone, or ropes and hobbles, should not be used to sling a wolf beneath a helicopter.

For movement of wolves over short distances, it is recommended that the anesthetized wolf is transported in the rear cabin of the helicopter. If it is not possible to transport the wolf within the helicopter, it may be secured on a lightweight rigid platform (e.g. portable climbing ledge) and then lifted and carried to a more suitable location. The platform and strapped-down wolf can be further secured within a cargo net. Irrespective of how the wolf is transported, within or beneath the helicopter, it **must** be anesthetized, hobbled, muzzled, and blindfolded for the duration of the transport. The potential for wind chill should also be considered and use of a protective tarp should also be considered.

4.2.3.3 Cage Trap and Box Trap

Issue: A horizontally-positioned, rectangular cage (having wire-mesh walls) or box (having solid walls) that is set to be open at one end, or at both ends, and closes when an animal steps on a treadle or moves a triggering device (Proulx et al. 2012).

- In general, cage traps and box traps appear to cause less trauma than other restraining devices (White et al. 1991).
- When used for canids, including wolves, the capture efficiency of cage traps and box traps is often lower than for other types of restraining traps (Shivik et al. 2005).
- Non-target species may be trapped inadvertently since this method of capture is not selective for wolves.

Recommendations:

- Traps must be inspected and serviced as needed before being used.
- Traps **must** be prepared, set and released by experienced persons only.
- Wolves may break teeth and cut their mouths while biting wire-mesh walls (Proulx et al. 2012). So, if cage traps are used, the wire-mesh holes must be small enough to prevent wolves from gripping the mesh with their teeth. Box traps constructed of solid walls (metal, plastic, or wood) are better from the standpoint of preventing these types of injuries.
- If the tripping force of the trigger/treadle can be adjusted, it **should** be set to match the weight of target animals, e.g. 25-30 kg for wolves.
- Bait should be placed behind the treadle or trigger to attract a wolf, force it to enter the trap, and step on the trigger. Plastic water bottles could also be left in the trap for the wolf.
- Traps must be set in areas that are well-shaded and have minimal wind exposure
- Access routes and trap sites must be clearly marked with signage and flagging tape to prevent persons from inadvertently encountering a trapped wolf.
- Active traps must be inspected routinely at least once daily and potentially more often during periods of extreme heat or cold. Trap alarms that send messages by email or Short Message Service (SMS) may also be used, but these must not be used in place of daily trap-site inspections.

Inspections should be more frequent if traps are set near communities or campgrounds where they may attract the attention of curious people or pets. The likelihood of injury to trapped wolves may increase with the duration of captivity.

 Drugs should be administered to wolves in cage or box traps using the least traumatic method possible without compromising human safety.

Dart pistols or rifles must not be used unless the dart velocity can be precisely controlled to ensure the dart impact occurs at low velocity.

• Wolves **should** not be held captive in cage or box traps longer than 24 hours.

Captivity is extremely stressful to most wild animals. If a wolf must be held captive for a prolonged period (e.g. 6-24 hours) to permit recovery from anesthesia or to transport to a release site, it must be provided water to prevent dehydration. If transport requires a longer time (e.g. several days), captured wolves should be transferred to a larger container designed for translocating large mammals.

• Contingency plans and required materials **must** be in place to release non-target species from cage or box traps.

4.2.3.4 Cable Devices (Leg-Hold Snare, Neck Snare)

Issue: A technique that involves the use of a braided-wire loop to ensnare a wolf by its leg (i.e., leg-hold snare) or neck (i.e., neck snare).

- Leg-hold snares are placed horizontally at ground level and are set to close upon an animal's leg to restrain it. The tightening of the loop around the ensnared leg may be facilitated by the use of a trigger-activated spring, e.g. Aldrich leg-hold snare. The snare cable is equipped with a locking mechanism to prevent the loop from loosening. The end of the snare cable is anchored to a tree with an extension cable and swivel, the latter used to prevent twisting and breaking of the snare cable.
- Neck snares are set vertically above the ground, so the head of the animal enters the wire loop, which then tightens around the neck of the animal. A stop prevents the loop from closing below a certain diameter to prevent strangulation. Although restraining neck snares are used to capture medium-sized canids, such as foxes and coyotes, they are rarely used to capture wolves and are not recommended for use in the NWT (Proulx et al. 2012).
- At this time, the welfare implications for the use of cable devices to live-capture wildlife have not been identified by the CCAC. So, the use of leg-hold snares and neck snares has not been assigned a ranking under the CCAC's Categories of Invasiveness. However, both methods may result in unacceptably high levels of significant injury (Shivik et al. 2000, Powell and Proulx 2003), and should probably be ranked as an 'E' on the Categories of Invasiveness scale, much like foothold traps, until future research suggests otherwise.
- They **must** not be used if the research objectives can be met using less invasive procedures.

- Non-target species may be trapped inadvertently with these methods of capture.

Recommendations:

- Snares should only be considered if the uses of other methods of capture are not possible.
- Snare site selection should always take into account human use and activities in the trapping area with the intent of preventing the public from finding and approaching snare sites.
- Access routes and snare sites must be clearly marked with signage and flagging tape to prevent persons from inadvertently encountering a trapped wolf.
- Snares must be prepared, set, and released by experienced persons only.
- Snare sites must always be set with a clear line of sight to permit visual assessment of a snared wolf from a safe distance.
- When multiple snares are set at a single location, individual snare locations should be marked for inspection from a distance (i.e., flagging tape on anchor trees) and snares should be set far enough apart to prevent captured wolves from making physical contact with each other.
- Cable clamps should be used to ensure the anchor end of the snare is as short as possible.
- Use of tranquilizer tabs **should** be considered as an approach to reducing injuries and more generally improving the humaneness of capture by snares (Pruss et al. 2002).
- Active snare sites **must** be inspected at least once daily and preferably more frequently especially during adverse weather conditions.

The likelihood of serious injury to snared wolves may increase with the duration of restraint by snare. Trap alarms should also be used, but it is still necessary to examine traps visually because alarms may fail. Deployment of electronic timers or trail cameras at snare sites can be used to record the duration of time that a trap has been sprung.

 Drugs should be administered to wolves in snares using the least traumatic method possible without compromising human safety.

Dart pistols or rifles must not be used unless the dart velocity can be precisely controlled to ensure the dart impact occurs at low velocity. A Y pole may be used to gently hold the wolf to the ground to permit safe injection of drug by hand or by pole syringe (see http://wildliferesources.com/the-y-pole/).

- Contingency plans and required materials (e.g. appropriate drugs or catch poles) must be in place to release non-target species from snares.
- Wolves should not be held captive in snares longer than 24 hours
- Snares **must** be set in areas which are well-shaded and have minimal wind exposure.

4.2.3.5 Foothold Trap

Issue: A trap that is characterized by two metal clamping jaws that are activated by coil- or long-springs to snap from an open position, where the jaws are spread apart 180°, to a closed position where the jaws are either completely or partially closed (offset). The closing of the jaws is triggered by an animal stepping on a centrally-positioned pan or trigger, and the entire trap apparatus is anchored with a chain and one or more swivels to a movable (i.e., drag-anchored) or fixed structure (i.e., stake-anchored).

- Although widely used in Canada and the United States, foothold traps are considered inhumane and banned within the European Union, the United Kingdom, and many other countries worldwide (Iossa et al. 2007).
- The use of foothold traps is currently ranked as an 'E' on the Categories of Invasiveness scale used by CCAC (CCAC 2003). Procedures ranked at this level are deemed to "cause severe pain near, at, or above the pain tolerance threshold of non-anesthetized, conscious animals", and **must** not be used if the research objectives can be met using less invasive procedures.
- The skill and experience of a trapper in choosing the appropriate foothold trap type and size, and in making and maintaining proper sets, are important determinants of the potential for injury to captured wolves (Powell and Proulx 2003).
- Non-target species may be trapped inadvertently with this method of capture.

Recommendations:

- Foothold traps **should** only be considered if the use of other methods of capture is not possible.
- Foothold traps **must** be inspected and serviced as needed before being used.
- Foothold traps **must** be prepared, set and released by experienced persons only.
- Padded foothold traps are preferred to unpadded traps.
- Investigators **must** use foothold traps with rubber-padded jaws, offset jaws, or combined rubber-padded offset jaws to minimize the potential for severe injury to the trapped leg, e.g. bone fracture and/or abrasion, joint luxation, skin lacerations.

Wolves captured by foothold traps with wide (1.8 cm) offset jaws have fewer, and less severe, leg injuries than wolves captured by unpadded foothold traps with no offset, or with a narrow (0.7 cm) offset (Kuehn et al. 1986). Similarly, wolves captured by foothold traps with rubber-padded jaws have fewer leg injuries than wolves captured by unpadded foothold traps with no offset (Frame and Meier 2007).

• Freezing injuries **must** be avoided when trapping with foothold traps. It follows that foothold traps **should** only be activated during weather conditions that are unlikely to cause hypothermia and freezing injury (Lossa et al. 2007).

Trapped limbs are prone to freezing because the closed jaws impede the flow of blood within the limb. Complete freezing may result in gangrene and the eventual loss of the limb (Onderka et al. 1990). Partial freezing may result in permanent damage and loss of function (Mowat et al. 1994).

- Although less frequent than limb injuries, oral injuries (chipped or broken teeth, tongue and/or gum lacerations) can occur when trapped wolves attempt to free themselves by biting through the anchor chain and swivel (Kuehn et al. 1986, Onderka et al. 1990).
- Use of tranquilizer trap devices (TTDs) **should** be considered as an approach to reducing injuries, and more generally improving the humaneness of capture by foothold traps (Sahr and Knowlton 2000, Savarie et al. 2004).
- Trap site selection should always take into account human use and activities in the trapping area with the intent of preventing the public from finding and approaching trap sites.
- Traps **must** be set in areas that are well-shaded and have minimal wind exposure.
- Access routes and trap sites must be clearly marked with signage and flagging tape to prevent persons from inadvertently encountering a trapped wolf.
- Investigators should use a secure, fixed anchor for foothold traps, e.g. stake or hookanchored

The anchoring system should be sufficient to hold the largest animal that might be captured. Limb injuries are more likely when using a drag-anchored system or when stake anchors are pulled loose (Turnbull et al. 2013).

- If the tension of the pan (trigger) can be adjusted, it **should** be set to match the weight of target animals, e.g. 25-30 kg for wolves.
- Active traps must be inspected routinely at least once daily. Trap alarms that send
 messages by email or Short Message Service (SMS) may also be used, but these must
 not be used in place of daily trap-site inspections.

Inspections should be more frequent if traps are set near communities or campgrounds where they may attract the attention of curious people or pets. The likelihood of injury may be reduced if traps are checked frequently (Lossa et al. 2007, Turnbull et al. 2013).

- Wolves **should** not be held captive in traps longer than 24 hours.
- Drugs should be administered to wolves in foothold traps using the least traumatic method possible without compromising human safety.

Dart pistols or rifles must not be used unless the dart velocity can be precisely controlled to ensure the dart impact occurs at low velocity. A Y pole may you used to gently hold the wolf to the ground to permit safe injection of drug by hand or by pole syringe (see http://wildliferesources.com/the-y-pole/).

 Contingency plans and required materials (e.g. appropriate drugs or catch poles) must be in place to release non-target species from traps.

4.2.4 Recommendations for Approaching an Immobilized Wolf:

• Wolves immobilized by remote drug delivery **must** be approached quietly and slowly from the rump to assess their response to noise (e.g. snapping fingers) and touch (e.g. gentle pole with a stick).

Minimal stimulation of a wolf immediately following immobilization is critical because peak drug effects generally occur sometime (10-15 minutes) after the onset of immobilization. Excessive stimulation of a lightly anesthetized wolf may be sufficient to cause it to regain mobility.

• If a helicopter is used, the pilot **must** be instructed to land a safe distance from the animal while maintaining clear view of the downed animal.

Although the distance between helicopter and wolf will be variable depending on terrain, the goal is to reduce stimulation of the immobilized animal while maintaining the safety of the capture crew.

• Only two people **should** approach the immobilized wolf initially. One person is responsible for assessing the animal while the other person carries a firearm to provide emergency support for the approach person. The immobilized animal should be approached from behind.

The person carrying the firearm must be familiar with the "normal" behaviour of a drugged wolf, in particular, what movements are acceptable, e.g. slight twitching of limbs, movement of tongue, etc.

• During the approach, the helicopter **should** remain idling and other members of the handling crew **must** remain at the helicopter to await instruction from the approach person.

Once the animal is determined to be safely immobilized, the approach person signals the pilot to shut down the helicopter and other members of the handling crew to approach.

- Anesthetic depth should be determined by assessing reflex activity (e.g. palpebral, ear twitch, and tongue withdrawal reflexes) before signaling other crew members to approach and/or initiating handling procedures.
- Wolves captured by cable devices (neck- or leg-hold snare) or foothold trap **should** be approached in the same manner as wolves captured by remote drug delivery.

However, the initial approach should only be close enough to allow evaluation of the security of the restraint and the approximate size of the captured animal.

4.2.5 Recommendations for Initial Handling of an Immobilized Wolf:

- Noise and touching of the immobilized wolf **must** be kept to a minimum.
- All personnel involved with handling immobilized wolves **should** wear gloves (latex, nitrile or heavier material) to protect themselves from exposure to drugs and reduce risk of disease transmission.

Although latex or nitrile gloves may be impractical in some situations, such as in frigid temperatures, it is important to protect your hands with an impermeable barrier because wildlife drugs can be absorbed across the skin and, therefore, represent a serious health hazard (Kreeger and Arnemo 2012). Although disease transmission is probably less frequent than accidental drug exposure, it is important to be aware that wolves may potentially transmit some diseases (zoonoses) to humans, including rabies, *Echinococcus*, sp. and sarcoptic mange.

• All personnel involved with direct handling of wolves **must** be immunized against rabies (CDC 2009)

Rabies antibody titers should be checked every two years, and records of titer levels should be maintained (CDC 2008). In the case of a bite that breaks the skin, personnel must contact the regional health authority as soon as possible to be evaluated for possible post-exposure prophylaxis administration. Immediately wash out any wounds with ample amounts of soap and water.

• The eyes of anesthetized wolves **must** be lubricated and covered.

Apply a non-medicated eye lubricant (e.g. methylcellulose) to the cornea to prevent drying and apply a blindfold to protect the eyes and prevent visual stimulation.

• The dart(s) **must** be removed and safely stored in an appropriate container from anesthetized wolves at the onset of handling.

If using darts that require some assembly (e.g. Dan-Inject darts, blow darts), slowly unscrew the tailpiece to vent the rear chamber before removing the dart. This will eliminate any possibility of drug spraying from the dart during removal if the needle port was occluded by tissue during injection. In most cases, treatment of the dart wound should require no more than wiping away excess blood, removing imbedded hair, clipping surrounding hair, and flushing the area with liberal amounts of sterile water to clean the wound. Antiseptic ointments, such as Hibitane® veterinary ointment, may also be applied although the effectiveness of these preparations in preventing infection in wild animals is unknown. Only a qualified veterinarian should treat more serious dart wounds, e.g. wounds that require sutures, restoration of intra-thoracic pressure, excision of darts that have fully penetrated the skin, etc.

• Anesthetized wolves **must** be positioned so the animal's breathing is not impinged, i.e., keep neck straight and ensure nostrils and mouth are not blocked.

Position the anesthetized wolf on its chest and abdomen (ventrally recumbent) with its head held higher than its thorax and its nose pointing down to avoid aspiration of fluids. In some cases, canids may be placed in lateral recumbency for quick procedures. Ensure the ground under the wolf is flat with no protruding surfaces, e.g. rocks. Use of a reflective and/or insulated ground sheet could be considered. Should the animal need to be rolled, it is preferable to roll across the sternum as opposed to across the back. When rolling, two or more persons must work together to ensure the head and tail ends of the wolf are rolled in parallel to avoid twisting the animal along its spinal axis. Anesthetized wolves should never be moved (rolled or picked up) by grasping or pulling their skin and hair.

- The physiologic response to chemical immobilization (anesthesia) **must** be assessed, and the assessment **should** include the following measures:
 - <u>Reflex activity</u>: the presence and strength of reflexes (e.g. palpebral, ear twitch, and tongue withdrawal reflexes) is used to evaluate the level of immobilization (deep vs. light) and need for additional drug or reversal.
 - Respiratory function: evaluated by respiratory rate, depth, and sound. Although respiratory rate is affected by many factors (age, activity, drugs, etc.), it should remain ≥4 breaths per minute in an anesthetized wolf. Each breath should be quiet and characterized by full expansion and relaxation of the rib cage. If the respiratory rate is less than four breaths per minute, artificial ventilation (chest compressions, ventilation via endotracheal tube and resuscitation bag) and/or administration of a reversal drug may be required, if other signs point toward respiratory depression,

i.e., blue or gray mucous membranes, oxygen saturation trend is continually downwards.

- Cardiovascular function: evaluated by pulse or heart rate, mucous membrane colour, and capillary refill time. Although pulse or heart rate is affected by many factors (age, activity, drugs, etc.), it should remain between 40 and 120 beats per minute in an anesthetized wolf. In addition, mucous membranes (i.e., gums, anus, vulva) should be pink and the capillary refill time should be <3 seconds. If the pulse or heart rate increases or decreases outside of the recommended range, respiratory function should be re-assessed immediately and corrected, if necessary. Reversal drug should also be available to administer, but should not be given unless other signs point toward cardiovascular distress or collapse, i.e., blue or gray mucous membranes, prolonged capillary refill time (>2 seconds), dilated pupils.
- Body temperature: evaluated rectally using an electronic digital thermometer. A spare thermometer should always be carried in case the active thermometer malfunctions. The rectal temperature should range between 36°C and 40°C. Administration of a reversal drug is the most effective treatment if hyperthermia (>40°C) develops, because it enables the wolf to use its normal cooling mechanisms, e.g. panting. Other cooling methods, including dousing with cold water and coldwater enemas, may or may not be effective depending on the size of the wolf, the thickness of its hair coat, and the rate of temperature increase. Hypothermia (<36°C) may also develop in some wolves, especially when captured by cage/box trap, snare, or foothold trap under cold ambient conditions. Treatment should be directed toward active warming of the animal first (heating pads, place in sleeping bag, drying wet hair coat), and not administering a reversal drug until the body temperature has returned to within the recommended range.

4.2.6 Recommendations for Monitoring Physiological Function:

• The physiologic response to anesthesia **must** be monitored throughout handling until the wolf is administered the reversal (antagonist) drug or left to recover (if using Telazol® or Zoletil® alone). Assessments **should** be made and recorded every 10-15 minutes.

Attention to physiologic function can provide advanced warning of developing complications (e.g. hyperthermia) and provide opportunity for preventative measures. Further, detailed records of physiologic function are invaluable for the investigation of any post-handling mortality.

• A pulse oximeter **should** be included as a standard component of the capture equipment.

It provides a useful means of evaluating respiratory and cardiovascular functions by measuring the pulse rate and trends in the hemoglobin oxygen saturation (in %) of blood

(Kreeger and Arnemo 2012). Small, battery-powered, portable pulse oximeters are available commercially for use in the field. The recorded oxygen saturation values may be inaccurate for various reasons including calibration of the instrument for use in domestic species, decreased blood perfusion of peripheral tissues, variation in skin colour, and variation in probe placement (Sladky et al. 2000, Arnemo et al. 2013). However, monitoring for trends in oxygen saturation is valuable: if readings steadily decrease, it is likely the animal is in some sort of physiological crisis. When using a pulse oximeter, the probe should be applied at a consistent location (e.g. the tongue) and left in place until a stable signal is obtained before recording the oxygen saturation and pulse values. Concurrent evaluation of mucous membrane colour will enable detection of hypoxemia, i.e., oxygen saturation is <85%, pulse rate increasing, and mucous membranes are becoming blue. Hypoxemia refers to low oxygenation of blood and, if prolonged, eventually leads to hypoxia, which is the diminished availability of oxygen in body tissues. Although the most obvious cause of hypoxemia is respiratory depression, it also occurs in captured wildlife as a consequence of elevated body temperature increasing the oxygen demand of body tissues, particularly skeletal muscle (Arnemo and Evans 2017).

• Supplementary oxygen **should** be available to treat hypoxemia and prevent hypoxia.

Oxygen therapy is the most effective treatment for hypoxemia (Read et al. 2001, Larsen et al. 2002). Supplementary oxygen can be carried readily in the field in pressurized "D" cylinders (weigh approximately 6 kg when full and are safe to carry aboard a helicopter) and administered to animals by use of a mini-regulator and nasal cannula. A flow rate of 0.5-1.5 liters per minute is required for most wolves and the efficacy of treatment should be monitored with a pulse oximeter (Arnemo and Evans 2017).

• All capture data, including drug doses and measures of physiological function, **must** be recorded on data forms at the time of capture and handling. Additional data recorded may include time of darting, number of darts used, start and end times of handling.

These data are invaluable in investigating health complications or the death of a wolf during or following handling. A template for a capture data form is provided in Appendix C.

4.3 Sample Collection and Measurements

Recommendations:

- All handling, including sample collection and measurements, must be completed
 quickly and quietly with the objective of minimizing the handling time, and releasing
 the animal as soon as possible.
- Samples and measurements **must** be consistent with the experimental design and details provided in the animal handling protocol.
- Extraction of a premolar for the purpose of aging **must** only be done if 'age in years' is data critical to the study objectives.

Otherwise, age or age class should be estimated based on body size and appearance. Photographs of tooth wear can also assist with age estimation.

• Local or regional anesthesia **should** be provided to manage pain resulting from premolar extraction. However, investigators **must** receive training by a licensed veterinarian before applying either of these procedures.

Extraction of a tooth produces strong, long-lasting, painful stimuli associated with tearing of the periodontal ligament and sensory nerve supply. Where premolar extraction is justified, local or regional anesthesia should be provided to manage pain during the procedure and in the hours following handling (Holstrom et al. 2004). Although different local anesthetics are available, 0.5% bupivacaine is recommended for the long duration of analgesia it provides, i.e., 6-10 hours. Local anesthesia is accomplished by injecting anesthetic (e.g. 1 ml of bupivacaine) into the periodontal space and gingiva surrounding the premolar approximately ten minutes prior to extraction. Regional anesthesia is accomplished by using either an infraorbital nerve block for an upper premolar, or a mental nerve block for a lower premolar, approximately ten minutes prior to extraction. Of the two techniques, regional anesthesia is technically more difficult but provides more complete pain relief. Both techniques require prior instruction and training by a licensed veterinarian, but can be perfected easily with practice.

• Local anesthesia **should** be considered for other sampling procedures (aside premolar extraction) that are likely to elicit pain, e.g. tissue biopsy.

Local (or regional) anesthesia is generally not required for most routine sampling procedures (blood collection, ear punch, skin/fat biopsy) because the pain elicited by these procedures is of short duration (when compared to tooth extraction) and adequately controlled by the immobilizing drug. However, where zolazepam-tiletamine (Telazol® or Zoletil®) is used alone as the immobilizing drug, local anesthesia may be required because of the poor analgesic effect of this drug (Kreeger et al. 1990, Caulkett et al. 1999). In such case, infiltration of the biopsy/punch site with 2-3 ml of lidocaine (with 2% epinephrine) approximately five minutes before tissue collection will provide sufficient pain control and minimize bleeding.

• Least invasive procedures **should** be used for genetic (DNA) sampling, e.g. hair follicle extraction, oral swab.

If more invasive procedures for tissue collection are used (e.g. biopsy) use of the materials for multiple analyses (e.g. DNA analysis, contaminants, and stress indicators) is preferred to only one analysis.

• If an ear tag is applied, the tagging area of the wolf's ear **should** be cleaned (e.g. wiped with isopropyl alcohol), the hole **should** be made with a sterile biopsy punch (6 mm diameter), and the tissue plug **should** be preserved for any analyses that require tissue

samples. The tags should be placed at a location on the ear that should not be ripped out. Major blood vessels should be avoided in ear tag placement.

The ear tag stud is manually directed through the biopsy hole prior to securing the tag with the applicator.

• If a commercial ear-tag applicator is used instead of a biopsy punch, the jaw and pin portion of the applicator **must** be sanitized (e.g. isopropyl alcohol) before and after each use. You **should** also clean the tagging area of the animal's ear.

Sampling of blood and tissue **should** be performed only after appropriate training and supervised experience. Proper collection, handling, and preservation protocols **must** be followed in order to obtain useful field data.

• If the handling protocol requires weighing captured wolves, the weighing **must** be done in the least stressful manner possible.

Wolves should not be suspended in a cargo net, or by "cuffs" attached to their four limbs, because of the potential for adverse health effects, e.g. regurgitation and aspiration (Kreeger and Arnemo 2012). Instead, wolves should be weighed by first positioning them on a portable climbing ledge, or on a weighing stretcher supported by lateral poles, and then suspending the platform or stretcher from a pole, bipod, or tripod.

 At some point during handling, the anesthetized wolf must be checked for wounds, injuries, and general condition and this information must be recorded on the field data sheet.

This should include a thorough examination for injuries caused by the capture procedure.

- Antibiotics **must** not be administered routinely to captured wolves. There are a growing number of reports of antibiotic resistance in bacteria living in wildlife (Cristóbal-Azkarate et al. 2014). This is a cause for concern as antibiotic resistance in wildlife represents a potential public health threat.
- Antibiotics **must** only be administered under the advice or direct supervision of a veterinarian.

The effectiveness of these drugs in free-ranging wildlife is often unproven and largely unknown (Pietsch et al. 1999).

4.4 Identification, Marking and Telemetry Collars

Recommendations:

• Investigators **must** aim to minimize any adverse effects of identification or marking procedures on the physiology and behaviour of individual study animals.

• Primary consideration **must** be given to identification or marking techniques that are not invasive, do not require recapture for identification, will remain visible for the duration of the study, and will not compromise the animal's welfare.

Ideally, techniques used should comply with the following criteria:

- Should be quick and easy to apply;
- Should be readily visible and distinguishable;
- Should be persistent, remaining for the duration of the research;
- Must not cause long-term adverse health effects;
- Must be recorded accurately on field data sheets; and
- Must allow for seasonal changes in size and growth.
- Tattoo dyes **must** be cleaned well (e.g. rinsed in isopropyl alcohol) between uses to prevent transmission of disease between wolves.

Tattooing is a common method of permanent identification in wolves, although the legibility of tattoos is variable over long periods. Tattoos are applied either to the inside of the lip or to the inside of the thigh at the groin where hair cover is sparse.

- Telemetry collars **should** be as light in weight as possible and **should** be selected for long duration battery life and remote drop capability to minimize re-capturing of collared wolves. Although some researchers recommend the telemetry collar weigh ≤2% of bodyweight (Arnemo and Evans 2017), there are no evidence-based rules for what a collar should weigh, relative to a wolf's bodyweight. However, a few studies in other species have reported negative effects on movements, behaviour, and survival when comparing use of heavier collars versus lighter collars (Brooks et al. 2008, Rasiulis et al. 2014).
- All collars **should** incorporate connecting material that will eventually rot off, allowing the collar to drop from the animal.

Collars with self-removing or breakaway devices should also incorporate "rot off" material (e.g. cotton spacers), in case the devices fail to function.

- The shape and flexibility of the collar **must** be selected to avoid causing debilitating injuries to study animals (Krausman et al. 2004).
- Collars **must** be fitted tightly enough that they are not easily shed, but loose enough to allow for weight gain without impeding normal neck movement or causing abrasion.

The best fit is usually achieved when the collar is as tight as possible, but still able to be pulled over the head (Arnemo and Evans 2017). A collar that is snug but not too tight at the width of the zygomatic arch (i.e., finger's width between the collar and zygomatic arch on either side) will generally ensure that the collar will be loose enough to accommodate seasonal changes in body mass. Based upon some preliminary data from the south slave region some general estimates for minimum circumference for collars are~44cm for adult females and ~48cm for adult males.

• Conventional VHF collars **should** not be deployed unless funds have been procured to monitor the collars for the length of the battery life.

Conventional VHF collars should not be fitted on wolves if funding is not sufficient to ensure that radio locations will be recorded at a rate (i.e., number of locations per unit time) that will adequately meet the study objectives.

• If ear tag transmitters must be used to meet the study objectives, the transmitter weight **should** be as light as possible (<30 g) and only one transmitter should be applied per wolf.

Larger ear tag transmitters are likely to cause chronic irritation and abrasion of the "tagged ear". Ear tag transmitters should not be applied to both ears of a wolf for the purpose of attempting to ensure the transmission life is of adequate length. Instead, investigators should purchase high quality transmitters and perform controlled outdoor testing prior to deploying the transmitters on wolves.

- The implantation of radio transmitters beneath the skin or in the abdominal cavity (peritoneum) **should** be avoided because of the invasiveness of these procedures, and the risk of adverse health effects as a consequence of the implantation procedure, the integrity of the radio transmitter implant, and/or migration of the (intraperitoneal) implant within the abdominal cavity (Arnemo et al. 2007, Quinn et al. 2010, Léchenne et al. 2012, Blundell et al. 2014).
- If implantation of radio transmitters is deemed necessary, it **must** be performed by an experienced veterinarian using proper surgical technique (Mulcahy 2013, Arnemo and Evans 2017, Horning et al. 2017).
- Any deficiencies in collar performance **must** be well documented and reported to ENR.

4.5 Reversal Drugs and Release of Wolves

Recommendations:

• Antagonist (also called "reversal") drugs **must** not be administered until all equipment has been repacked and removed, and all personnel except the person administering the drug and the person(s) providing firearm cover have cleared the area.

• Once the reversal drug is administered, capture personnel **must** retreat to a safe location to monitor the recovery.

Every effort should be made to observe the animal until it is ambulatory and coordinated in its movements.

- When using non-reversible drug combinations (e.g. Telazol®), every effort should be made to observe the recovering wolf until it is ambulatory and coordinated in its movements.
- Where a wolf has been captured by a trap (e.g. cage trap, foothold trap, etc.), all other active traps in the area **should** be de-activated or removed before the anesthetized wolf is administered reversal drug or left to recover.
- When releasing a wolf captured by remote drug delivery from helicopter, capture
 personnel should not depart from the capture area until the wolf is standing and stable
 on its feet.

4.6 Post-Capture Monitoring

Recommendations:

 Wolves fitted with radio collars should be observed visually at least once within the three days immediately following capture and handling, and ideally within 24 hours of release.

Although animals sometimes die during capture and handling, death may also occur within hours to days following release (Spraker 1993, Fowler 2008). If a wolf dies following its release, the determination of cause of death is important for two reasons (Nielsen 1999). First, if the wolf died as a direct result of the procedures used during capture and handling, then a detailed necropsy should be followed by a review of the capture event and, if required, a revision of the methodology used. Second, if the wolf died as a result of a pre-existing illness or disease exacerbated by the stress of capture and handling, then a detailed necropsy will help to assure continued confidence in the capture methodology used and may also provide new information regarding the health of the species.

• Ideally, wolves **should** be visualized from high altitude to minimize stress associated with the noise and proximity of the aircraft. However, sighting a wolf where tree cover is extensive may be difficult, if not impossible. In such case, movement of the animal **should** be confirmed by detecting changes in its radiolocations.

Although most telemetric devices are equipped with motion-sensitive mortality sensors, these alone are not adequate for confirming movement of the released wolf and should not be used as a substitute for visual observation or radiolocation in the immediate period following capture. Activation of the sensor may not always occur within the programmed time because of intermittent movement of the collar following death caused by animals

feeding on the carcass. Detailed examination of a carcass that has been scavenged extensively is unlikely to provide any insight into the cause of death.

- The "drop-off schedule" **must** be used to monitor and confirm that collars have indeed dropped off from the animals that once wore them.
- As collaring wildlife has responsibilities to the animals, investigation and documentation of mortalities with collar retrieval **should** be attempted in all cases within a reasonable time frame with appropriate financial resources allocated for this purpose in the project proposal.

4.7 Euthanasia

Recommendations:

- The investigator must be prepared to kill any wolf that has been severely injured, is suffering, and its death appears imminent, as a result of the capture or handling procedures.
- The technique(s) used to kill a wolf **must** be humane, in that they **must** reduce pain and distress to the greatest extent possible.
- A detailed report of the euthanasia **must** be written and every effort **must** be made to salvage the carcass, hide, skull, and other parts legally required, and submit these with the report to a wildlife officer as soon as possible.
- Detailed information on acceptable and unacceptable methods of humane killing have been developed and compiled by the American Veterinary Medical Association (AVMA 2013)
- Acceptable methods for humanely killing wolves are as follows:

a) Gunshot

- A shot to the brainstem of an animal produces a quick and humane death, but is best attempted when the animal is fully immobilized by injury or chemical immobilization.
- In free-ranging situations, or where the intact brain is required for diagnostic testing (e.g. rabies), gunshot to the heart and lung area may be more appropriate.

b) Exsanguination (bleeding)

- This method is considered humane if performed on a deeply anesthetized animal.

- The technique requires bleeding the anesthetized wolf by slicing deeply across the throat to sever the carotid arteries.
- c) Intravenous Administration of Potassium Chloride
 - The rapid injection of a potassium chloride (KCl) solution into the jugular vein, or directly into the heart, will cause a fatal heart attack.
 - Can be pre-made as a stock solution by adding 5 mg of KCI per 25 ml water.
 - Use of this method requires prior training by a veterinarian or experienced person.
 - This method is considered humane when performed on a deeply anesthetized animal.

4.8 Morbidity and Mortality

Recommendations:

- Any injury, disease, or abnormality observed during or following capture or handling
 must be documented and reported to the GNWT-ENR Wildlife Veterinarian or delegate
 as soon as possible. Depending on the significance of the finding, it may be helpful to
 consult with the Wildlife Veterinarian by satellite phone at the time of the incident to
 identify appropriate action plans (e.g. euthanasia), samples, measurements, and/or
 images to collect.
- Dead wolves must receive a detailed necropsy to determine the cause of death and must be reported to the GNWT-ENR Wildlife Veterinarian or delegate as soon as possible. Ideally recovery of the entire carcass from the field is desired.

If the necropsy is performed in the field, appropriate tissue samples should be collected and frozen for submission to a veterinary pathology facility. Appropriate tissue samples should include brain, lung, heart, liver, kidney, spleen, lymph nodes, and muscle. Investigators should refer to a wildlife necropsy manual for details regarding required equipment, techniques, and sampling procedures (see for example, Munson 2006). A template for a field necropsy data form is provided in Appendix D. Documentation should also include a detailed history and digital images of the field necropsy to assist the veterinary pathologist diagnosing the cause of death. Alternatively, under some circumstances, it may be better to arrange shipment of the entire carcass to a veterinary pathology facility for detailed necropsy.

- A detailed report of the death **must** be written and every effort **must** be made to salvage the hide, skull, and other parts legally required, and submit these with the report to a conservation officer as soon as possible.
- In situations where a wolf is seriously injured, or dies, as a consequence of the capture or handling procedures, further captures must be suspended until the incident has been

reviewed by the GNWT-ENR Wildlife Veterinarian or delegate, and continuation of capture operations has been approved.

4.9 Human Safety

Recommendations:

 Appropriate handling and restraint techniques must be used for wolves, and personnel must have appropriate training and experience in their use to avoid injury.

Wolves are capable of inflicting serious injury and transmitting disease to persons handling them.

- The risks involved in using drugs for the capture and immobilization of wolves **must** be identified and communicated to all capture personnel, including the helicopter pilot when using remote drug delivery by helicopter as the capture technique.
- The investigator **must** ensure that an emergency action plan is in place.

The emergency action plan provides step-by-step details on what to do in the event of an accident or emergency (e.g. human drug exposure, downed aircraft) and, if well designed and implemented, can reduce the severity of emergencies and save lives.

- At least two people on the handling team must be trained in first aid and cardiopulmonary resuscitation (CPR), local medical authorities should be informed of the potential hazards (accidental drug injection, animal bite), and an evacuation plan to medical facilities must be discussed prior to fieldwork.
- Emergency numbers and information should be available to the capture crew ahead of any capture activities.
- Personnel handling drugs must have current training (within five years) in the chemical immobilization of wildlife and inform other members of the team of the risks of human exposure and procedures for addressing drug exposure.
- When contracting helicopter services, project leaders **should** be able to insist on only using the most experienced pilots.

An experienced pilot is not only essential for the safety of the capture crew, but also to ensure that captures can be performed quickly and efficiently.

- Helicopter pilots assisting with wildlife capture operations must have demonstrated skills in their ability to pursue target animals and, when required, control animal movements in a gradual manner that imposes as little stress as possible on the target animal.
- The investigator **must** ensure that potentially hazardous conditions involved in fieldwork are identified to the personnel involved.

Some situations may require particular experience or training, such as working around aircraft or firearms, or in extreme cold temperatures.

- Helicopter egress training **should** be encouraged for all capture crew participants. The use of appropriate safety clothing (i.e., Nomax coveralls, flight helmet) **should** be used.
- Personnel involved in capture and restraint **must** have current training and proficiency in the use of pertinent equipment, e.g. firearms, dart rifles, etc.
- Personnel carrying firearms **must** have the legal authorization to use a firearm, e.g. Possession and Acquisition License, an Authorization to Carry a Restricted Weapon is required by a person carrying a handgun.
- Appropriate training and preparation for working in bear country is recommended including having at least one member of the team trained as a bear monitor during the appropriate season.
- Following completion of a training course, inexperienced personnel **should** develop and refine their skills by working with a mentor.

Although training courses can provide basic safety information, they cannot provide the breadth of knowledge acquired through field experience.

5. Literature Cited

- American Veterinary Medical Association (AVMA). 2013. AVMA Guidelines for the Euthanasia of Animals: 2013 Edition. American Veterinary Medical Association, Schaumburg, IL. 102pp.
 - (Last accessed at www.avma.org/KB/Policies/Documents/euthanasia.pdf on January 1, 2018)
- Arnemo, J.M. and A.L. Evans. 2017. Biomedical Protocols for Free-ranging Brown Bears, Gray Wolves, Wolverines and Lynx.

 Available at: https://brage.bibsys.no/xmlui/handle/11250/2444409.
- Arnemo, J.M., Evans, A.L., Ahlqvist, P., Segerström, P., and O. Liberg. 2013. Evaluation of medetomidine-ketamine and atipamezole for reversible anesthesia of free-ranging gray wolves (*Canis lupus*). Journal of Wildlife Diseases 49:403-407.
- Arnemo, J.M., Å. Fahlman, K. Madslien, S. Brunberg, B. Ytrehus and J.E. Swenson. 2007. Long-term Evaluation of Telonics Intraperitoneal Radiotransmitters in Free-ranging Brown Bears (*Ursus arctos*). Proc AAZV, AAWV, AYA/NAG Joint Conference: 96
- Ballard, W.B., L.A. Ayres, K.E. Roney and T.H. Spraker. 1991. Immobilization of Gray Wolves with a Combination of Tiletamine Hydrochloride and Zolazepam Hydrochloride. The Journal of Wildlife Management 55:71-74.
- Barber-Meyer, S.M. and L.D. Mech. 2014. How Hot is Too Hot? Live-trapped Gray Wolf Rectal Temperatures and 1-year Survival. Wildlife Society Bulletin 38:767-772.
- Bergvall, U.A., P. Kjellander, P. Ahlqvist, Ö. Johansson, K. Sköld and J.M. Arnemo. 2015. Chemical Immobilization of Free-ranging Fallow Deer (*Dama dama*): Effect of Needle Length on Induction Time. Journal of Wildlife Diseases 51:484-487.
- Blundell, G.M., A.A. Hoover-Miller, C.A. Schmale, R.K. Berngartt and S.A. Karpovich. 2014. Efficacy of Subcutaneous VHF Implants and Remote Telemetry Monitoring to Assess Survival Rates in Harbor Seals. Journal of Mammalogy 95:707-721.
- Brooks, C., C. Bonyongo and S. Harris. 2008. Effects of Global Positioning System Collar Weight on Zebra Behaviour and Location Error. Journal of Wildlife Management 72:527-534.
- Canadian Council on Animal Care (CCAC). 2003. Guidelines on: The Care and Use of Wildlife. CCAC, Ottawa, ON. 66pp.

 (Available online at www.ccac.ca/Documents/Standards/Guidelines/Wildlife.pdf)

- Caniglia, R., E. Fabbri, S. Cubaynes, O. Gimenez, J-D. Lebreton and E. Randi. 2012. An Improved Procedure to Estimate Wolf Abundance using Non-invasive Genetic Sampling and Capture–recapture Mixture Models. Conservation Genetics 13:53-64.
- Cattet, M.R.L. 2003. A CCWHC Technical Bulletin: Drug Residues in Wild Meat Addressing A Public Health Concern.

 (Available online at http://digitalcommons.unl.edu/icwdmccwhcnews/46/)
- Cattet, M.R.L., A. Bourque, B.T. Elkin, K.D. Powley, D.B. Dahlstrom and N.A. Caulkett. 2006. Evaluation of the Potential for Injury with Remote Drug Delivery Systems. Wildlife Society Bulletin 34:741-749.
- Caulkett, N.A., M.R.L. Cattet, J.M. Caulkett and S.C. Polischuk. 1999. Comparative Physiologic Effects of Telazol[®], Medetomidine-ketamine and Medetomidine-Telazol[®] in Captive Polar Bears (*Ursus maritimus*). Journal of Zoo and Wildlife Medicine 30: 504-509.
- Centers for Disease Control (CDC). Human Rabies Prevention United States, 2008. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 2008 / 57(RR-03); 1-26, 28.
- Centers for Disease Control (CDC) and Prevention. 2009. Rabies Vaccine: What You Need To Know. US Department of Health and Human Services Centers for Disease Control and Prevention, Vaccine Information Statement. 2pp.

 (Available online at: www.cdc.gov/vaccines/hcp/vis/vis-statements/rabies.pdf)
- Cristóbal-Azkarate, J., J.C. Dunn, J.M.W. Day and C.F. Amábile-Cuevas. 2014. Resistance to Antibiotics of Clinical Relevance in the Fecal Microbiota of Mexican Wildlife. PLoS ONE 9(9): e107719.
- Deem, S.L., J.C.H. Ko and S.B. Citino. 1998. Anesthetic and Cardiorespiratory Effects of Tiletamine-zolazepam-medetomidine in Cheetahs. Journal of the American Veterinary Medical Association 213:1,022-1,026.
- Echegaray, J. and C. Vilà. 2010. Noninvasive Monitoring of Wolves at the Edge of Their Distribution and the Cost of Their Conservation. Animal Conservation 13:157-161.
- Fowler, M.E. 2008. Restraint and Handling of Wild and Domestic Animals (3rd Edition). Wiley-Blackwell, Ames, IA. 470pp.
- Frame, P.F. and T.J. Meier. 2007. Field-assessed Injury to Wolves Captured in Rubber-padded Traps. Journal of Wildlife Management 71:2,074-2,076.
- Hampton, J.O., T.H. Hyndman, M. Laurence, A.L. Perry, P. Adams and T. Collins. 2016. Animal Welfare and the Use of Procedures Documents: Limitations and Refinement. Wildlife Research 43:599-603.

- Hillis, T.L. and F.F. Mallory. 1996. Fetal Development in Wolves, *Canis lupus*, of the Keewatin District, Northwest Territories, Canada. Canadian Journal of Zoology 74:2,211-2,218.
- Holstrom, S.E., P. Frost-Fitch and E.R. Eisner. 2004. Veterinary Dental Techniques for the Small Animal Practitioner (3rd Edition). W.B. Saunders Company, PHL. 689pp.
- Horning, M., M. Haulena, P.A. Tuomi, J.E. Mellish, C.E. Goertz, K. Woodie, R.K. Berngartt, S. Johnson, C.R. Shuert, K.A. Walker, J.P. Skinner and P.L. Boveng. 2017. Best Practice Recommendations for the Use of Fully Implanted Telemetry Devices in Pinnipeds. Animal Biotelemetry 5:1-15.
- Iossa, G., C.D. Soulsbury and S. Harris. 2007. Mammal Trapping: A Review of Animal Welfare Standards of Killing and Restraining Traps. Animal Welfare 16:335-352.
- Krausman, P.J., V.C. Bleich, J.W. Cain III, T.R. Stephenson, D.W. DeYoung, P.W. McGrath, P.K. Swift, B.M. Pierce and B.D. Jansen. 2004. From the Field: Neck Lesions in Ungulates from Collars Incorporating Satellite Technology. Wildlife Society Bulletin 32:987-991.
- Kreeger, T.J. and J.M. Arnemo. 2012. Handbook of Wildlife Chemical Immobilization Fourth Edition. Published by authors. 448pp.
- Kreeger, T.J., U.S. Seal, M. Callahan and M. Beckel. 1990. Physiological and Behavioural Responses of Gray Wolves (*Canis lupus*) to Immobilization with Tiletamine and Zolazepam. Journal of Wildlife Diseases 26:90-94.
- Kuehn, D.W., T.K. Fuller, L.D. Mech, W.J. Paul, S.H. Fritts and W.E. Berg. 1986. Trap-related Injuries to Gray Wolves in Minnesota. Journal of Wildlife Management 50:90-91.
- Larsen, R.S., M.R. Loomis, B.T. Kelly, K.K. Sladky, M.K. Stoskopf and W.A. Horne. 2002. Cardiorespiratory Effects of Medetomidine-butorphanol, Medetomidine-butorphanol-diazepam and Medetomidine-butorphanol-ketamine in Captive Red Wolves (*Canis rufus*). Journal of Zoo and Wildlife Medicine 33:101-107.
- Léchenne, M.S., J.M. Arnemo, C. Bröjer, H. Andrén and E.O. Ågren. 2012. Mortalities Due to Constipation and Dystocia Caused by Intraperitoneal Radio-transmitters in Eurasian Lynx (*Lynx lynx*). European Journal of Wildlife Research 58:503-506.
- Long, A.R., P. MacKay, J. Ray and W. Zielinski (Editors). 2008. Noninvasive Survey Methods for Carnivores. Island Press, Washington DC, MD. 385pp.
- Mech, L.D., S.M. Barber-Meyer and J. Erb. 2016. Wolf (*Canis lupus*) Generation Time and Proportion of Current Breeding Females by Age. PLoS ONE 11(6): e0156682. doi:10.1371/journal.pone.0156682.

- Metz, M.C., D.W. Smith, J.A. Vucetich, D.R. Stahler and R.O. Peterson. 2012. Seasonal Patterns of Predation for Gray Wolves in the Multi-prey System of Yellowstone National Park. Journal of Animal Ecology 81:553-563.
- Mowat, G., B.G. Slough and R. Rivard. 1994. A Comparison of Three Live Capturing Devices for Lynx: Capture Efficiency and Injuries. Wildlife Society Bulletin 22:644-650.
- Mulcahy, D.M. 2013. Legal, Ethical and Procedural Bases for the Use of Aseptic Techniques to Implant Electronic Devices. Journal of Fish and Wildlife Management 4:211-219.
- Munson, L. 2006. Necropsy of Wild Animals. University of California, Davis. Wildlife Health Center, Wildlife Conservation Society, New York, NY. 56pp.
- Nakamura, M., R. Godinho, H. Rio-Maior, S. Roque, A. Kaliontzopoulou, J. Bernardo, D. Castro, S. Lopes, F. Petrucci-Fonseca and F. Álvares. 2017. Evaluating the Predictive Power of Field Variables for Species and Individual Molecular Identification on Wolf Noninvasive Samples. European Journal of Wildlife Research 63: 53. https://doi.org/10.1007/s10344-017-1112-7.
- Nielsen, L. 1999. Chemical Immobilization of Wild and Exotic Animals. Iowa State University Press, Ames, IA. 341pp.
- Onderka, D.K., D.L. Skinner and A.W. Todd. 1990. Injuries to Coyotes and Other Species Caused by Four Models of Footholding Devices. Wildlife Society Bulletin 18:175-182.
- Paterson, J. 2014. Capture Myopathy. *In:* West, G., D. Heard and N. Caulkett (eds). Zoo Animal and Wildlife Immobilization and Anesthesia, Second Edition. John Wiley & Sons, Inc., Hoboken, NJ. Pp. 171-179.
- Pietsch, G., G. Finstad, J. Bevins and A.K. Prichard. 1999. The Effect of Antibiotic Treatment on Post-handling Stress and Survival of Reindeer Calves. Journal of Wildlife Diseases 35:735-740.
- Powell, R.A. and G. Proulx. 2003. Trapping and Marking Terrestrial Mammals for Research: Integrating Ethics, Performance Criteria, Techniques and Common Sense. ILAR Journal 44:259-276.
- Proulx, G., M.R.L. Cattet and R.A. Powell. 2012. Humane and Efficient Capture and Handling Methods for Carnivores. *In*: Boitani, L. and R.A. Powell (eds). Carnivore Ecology and Conservation: A Handbook of Techniques. Oxford University Press, Oxford, United Kingdom. Pp. 70-129.
- Pruss, S.D., N.L. Cool, R.J. Hudson and A.R. Gaboury. 2002. Evaluation of a Modified Neck Snare to Live-capture Coyotes. Wildlife Society Bulletin 30:508-516.

- Quicksilver Air Inc. 1996. Animal Handling & Safety Manual. Fairbanks, Alaska and Peyton, CO. 45pp. (Available at www2.nrel.colostate.edu/projects/modelingCWD/Animal Handling Safety Manual.pdf last accessed on Jan. 9, 2018)
- Quinn, J.H., P.M. Gaffney, K. Gilardi, M. Murray, D.A> Jessup and C.K. Johnson. 2010. Complication Associated with Abdominal Surgical Implantation of a Radio Transmitter in an American Badger (*Taxidea taxus*). Journal of Zoo and Wildlife Medicine 41:174-177.
- Rasiulis, A.L., M. Festa-Bianchet, S. Couturier and S.D. Côté. 2014. The Effect of Radio-collar Weight on Survival of Migratory Caribou. Journal of Wildlife Management 78:953-956.
- Read, M.R., N.A. Caulkett, A. Symington and T.K. Shury. 2001. Treatment of Hypoxemia during Xylazine-tiletamine-zolazepam Immobilization of Wapiti. Canadian Veterinary Journal 42:861-864.
- Sahr, D.P. and F.F. Knowlton. 2000. Evaluation of Tranquilizer Trap Devices (TTDs) for Foothold Traps used to Capture Gray Wolves. Wildlife Society Bulletin 28:597-605.
- Savarie, P.J., D.S. Vice, L. Bangerter, K. Dustin, W.J. Paul, T.M. Primus and F.S. Blom. 2004. Operational Field Evaluation of a Plastic Bulb Reservoir as a Tranquilizer Trap Device for Delivering Propiopromazine Hydrochloride to Feral Dogs, Coyotes, and Gray Wolves. USDA National Wildlife Research Center Staff Publications. 383. http://digitalcommons.unl.edu/icwdm usdanwrc/383
- Shivik, J.A., K.S. Gruver and T.J. DeLiberto. 2000. Preliminary Evaluation of New Cable Restraints to Capture Coyotes. Wildlife Society Bulletin 28:606-613.
- Shivik, J.A., D.J. Martin, M.J. Pipas, J. Turnam and T.J. DeLiberto. 2005. Initial Comparison: Jaws, Cables and Cage-traps to Capture Coyotes. Wildlife Society Bulletin 33:1,375-1,383.
- Sladky, K.K., B.T. Kelly, M.R. Loomis, M.K. Stoskopf and W.A. Horne. 2000. Cardiorespiratory Effects of Four α_2 -adrenoceptor Agonist-ketamine Combinations in Captive Red Wolves. Journal of the American Veterinary Medical Association 217:1,366-1,371.
- Spelman, L.H., P.W. Sumner, J.F. Levine and M.K. Stoskopf. 1994. Anesthesia of North American River Otters (*Lutra canadensis*) with Medetomidine-ketamine and Reversal by Atipamezole. Journal of Zoo and Wildlife Medicine 25:214-223.
- Spraker, T.R. 1993. Stress and Capture Myopathy in Artiodactylids. *In:* Fowler, M.E. (ed). Zoo and Wild Animal Medicine (Current Therapy 3). W.B. Saunders Company, Philadelphia, PA. Pp. 481-488.

- Stansbury, C.R., D.E. Ausband, P. Zager, C.M. Mack, C.R. Miller, M.W. Pennell and L.P. Waits. 2014. A Long-term Population Monitoring Approach for a Wide-ranging Carnivore: Noninvasive Genetic Sampling of Gray Wolf Rendezvous Sites in Idaho, USA. Journal of Wildlife Management 78:1,040-1,049.
- Šver, L., A. Bielen, J. Križan and G. Gužvica. 2016. Camera Traps on Wildlife Crossing Structures as a Tool in Gray Wolf (*Canis lupus*) Management Five Years Monitoring of Wolf Abundance Trends in Croatia. PLoS ONE 11(6): e0156748. doi:10.1371/journal.pone.0156748.
- Turnbull, T.T., J.W. Cain III and G.W. Roemer. 2013. Anthropogenic Impacts to the Recovery of the Mexican Gray Wolf with a Focus on Trapping-related Incidents. Wildlife Society Bulletin 37:311-318.
- Valerio, F., L. Brugnola, F. Rocconi, V. Varasano, C. Civitella and C. Guglielmini. 2005. Evaluation of the Cardiovascular Effects of an Anaesthetic Protocol for Immobilization and Anaesthesia in Grey Wolves (*Canis lupus* L, 1758). Veterinary Research Communications 29(Suppl. 2):315-318.
- Walton, L.R., H.D. Cluff, P.C. Paquet and M.A. Ramsay. 2001. Movement Patterns of Barrenground Wolves in the Central Canadian Arctic. Journal of Mammalogy 82:867-876.
- Western Association of Fish and Wildlife Agencies' (WAFWA) Wildlife Health Committee. 2009. A Model Protocol for Purchase, Distribution and Use of Pharmaceuticals in Wildlife. Western Association of Fish and Wildlife Agencies. 10pp.
- White, P.J., T.J. Kreeger, U.S. Seal and J.R. Tester. 1991. Pathological Response of Red Foxes to Capture in Box Traps. Journal of Wildlife Management 55:75-80.
- Williams, E.S. and E.T. Thorne. 1996. Exertional Myopathy (Capture Myopathy). *In*: Fairbrother, A., L.N. Locke and G.L. Hoff (eds). Noninfectious Diseases of Wildlife. Manson Publishing, Ames, IA. Pp. 181-193.
- Wingfield, J.C. and R.M. Sapolsky. 2003. Reproduction and Resistance to Stress: When and How. Journal of Neuroendocrinology 15:711-724.

Appendix A – Net Gun Maintenance, Use, and Inspection

(Contributed by Ian Ellsworth, Trinity Tactical Consulting Ltd.)

1.0 Net Gun Standards:

Various makes and models of net guns are currently used for wildlife capture operations. However, regardless of differences among types of net guns, existing or new, all net guns being used in the Northwest Territories **must** meet the following minimum standards:

- 1. Have removable barrels that contain net canisters for quick reloading;
- 2. Be equipped with a minimum of three barrel/canister units and appropriate-sized nets;
- 3. Have a minimum of four spare weights complete with 0-rings and tethers;
- 4. All weight tethers must utilize rubber tubing (capable of withstanding cold temperatures) at the junction of the weight eyelet; and
- 5. Have a safety mechanism that prevents the gun from being fired.

2.0 Maintenance:

2.1 Pre-Capture:

Net guns and accessory equipment **must** be inspected by the gunner prior to capture operations to ensure everything is fully functional and reliable.

Gun

- Inspect for cracks or loose parts
- Ensure action is clean and free of oil or grease
- Ensure breech is clean and free of obstructions
- Ensure action operates smoothly and firing pin engages
- Check safety mechanism for proper operation
- Check trigger for proper operation

Canister Units

- Ensure barrels are clean and free of cracks and obstructions
- Check retaining bolts for tightness
- Inspect canister for cracks or defects
- Inspect flaps and Velcro fasteners

Nets and Weights

- Check for holes or tears in nets, and repair or replace as needed
- Ensure weights are not damaged and file burrs, if required
- Check 0-rings for wear
- Check weight tethers for fraying or other damage
- Check rubber sleeves and fasteners for wear

2.2 Capture:

As you use the net gun, you **must** regularly check nuts and bolts that may loosen with continued use. Similarly, you **must** continually check canisters and weights after each capture. Fiberglass canisters can break, rivets may tear away from closure flaps, and Velcro may lose its effectiveness. It is also necessary to inspect weights as O-rings will wear and weight tethers will fray and break with repeated use and may need to be replaced. Nets **must** be dried out at the end of each capture day.

2.3 Post-Capture:

Use the pre-capture list as your guide. In addition, you **should** thoroughly clean the gun and weights. Lubricate metal parts (except the bolt/firing pin) to inhibit rust. If you wish to lubricate the action use graphite or an equivalent product that will not freeze in the cold. Dry out all nets prior to storage.

2.4 Equipment Inspection:

All net-gunning equipment to be used during a capture season **must** be inspected by an experienced gunner prior to use. If any issues arise, the equipment **must** be repaired by a qualified technician. All inspections **must** be recorded, problems noted, and corrective actions listed on the Inspection Checklist.

3.0 Safety Equipment:

The safety of all personnel during capture operations is paramount. So, all team members **must** receive a safety briefing prior to departure. Further, all personnel **should** wear an approved helicopter helmet and must wear clothing appropriate to the weather and situation (e.g. fire-resistant material) during capture operations. Net gunners **must** wear a harness or safety belt with a minimum of two lanyards with safety clips attached to the harness or belt and secured to fixed anchoring points in the aircraft. Lanyard tension **must** be assessed and, if necessary, altered prior to each capture. Harnesses or safety belts **must** also be adjusted to the appropriate tension to limit forward and side-to-side motion. Harnesses, safety belts and lanyards **must** also be inspected daily and replaced at the first sight of any fraying or wear. Safety equipment that has passed an expiration date **should not** be used.

4.0 Ammunition:

All net-gun ammunition **must** be hand loaded by competent personnel or purchased from a commercial distributor if available. All ammunition should be used in accordance to helicopter company and other appropriate transportation regulations. Used brass **should not** to be used for reloading of net-gun ammunition.

5.0 Net Gunning Sequence and Considerations:

5.1 Pre-Capture:

The gunner will ensure:

- All equipment is ready
- Net weights are plunged into barrels and properly seated (0-rings help to secure the weights in the barrels)
- Spare canister units are accessible if a second shot is required
- Harness/safety belt and lanyards are properly adjusted
- Door is removed from the helicopter, unless it is a sliding door

5.2 Capture:

The gunner will:

- Communicate with pilot to see if airspeed is sufficiently slowed to open the sliding door
- With the door open (or removed), place a cartridge into the chamber but **do not** close the action
- Get into shooting position and select a target animal, while controlling the muzzle direction at all times to ensure it is pointing outward from the cabin, but never directed toward the rotor blades or skid
- Once chase begins, close the action, disengage the safety, and keep finger outside of the trigger guard until ready to fire
- When animal is safely within range, deploy the net
- If a second shot is required, re-engage safety, open action, change canister, reload and follow preceding steps.
- If unable to shoot during the first pass and extended time is required to get into range, re-engage safety and open action to prevent accidental discharge. Keep muzzle pointed in safe direction and finger outside the trigger guard.
- If a misfire occurs, wait a minimum of 30 seconds before ejecting the cartridge. Always ensure the gun is pointed in a safe direction in case of delayed detonation (also called hang fire).
- Use of a GoPro or other device to record capture sequences is helpful to review capture techniques and assess any morbidity and mortality events.

5.3 Post Capture:

The gunner **must** inspect the net, weights and O-rings prior to reuse. Any worn parts **must** be replaced. It is quite common for O-rings to compress and fail to hold the weight in the barrel. This can result in a net unexpectedly falling out of the canister during a chase, resulting in a dangerous situation. If this should occur, the gunner **must** immediately release the canister unit or drop the entire net gun unit to the ground. Failure to do so may result in the net becoming entangled in the tail rotor.

Worn weight tethers are also a concern as they may break during a shot and end up hitting a rotor or other undesirable places if the muzzle is not pointed in a safe direction.

When reloading nets, ensure the net gun is unloaded, the action is open, and the safety is engaged. Also, ensure the action and barrels are kept out of the snow.

Net Gun Inspection and Verification Checklist

	•	COMPANY INFORMATION	ON
Inspe	ectors Name:		
Address:			
Telep	hone Number:		
Unit	ID (of Gun)		
Date	of Inspection:		
CHE	CKLIST		Comments/ Deficiencies
	Gun unit free or damage	f cracks, loose parts or other	
	Action/Breech, and operable	/Trigger clean, free of obstruction	
	Safety mechani	sm in working order	
	Barrels on all cand obstruction	anister units clean, free of cracks ns	
	Canister releas all canisters	e mechanism operates smoothly on	
	Canisters free (inside/outside	of cracks or other damage	
	Canister flaps s	secure/adequate Velcro to hold net	
	All nuts/bolts o	on canisters tight	
	Weights undan	naged and fit smoothly into barrels	
	O-rings capable	e of holding weights into barrels	
	Weight tethers	free of fraying or wear	
	Nets free of hol	es/tears	
			Signature of Inspector
	I hereby declar serviceable	e that the above listed unit is	
	unserviceable l	e that the above listed unit is based on the noted deficiencies and ed until rectified and re-inspected	

Supplemental Notes:

Appendix B – Drug Volume Table for Capture and Reversal of Wolves

Drug Protocol		Telazol ^a	XKb	XK Reversal	MKb	MK Reversal				
Drugs		Zolazepam + tiletamine	Xylazine (X) + ketamine (K) [1:5 ratio]	Yohimbine	Medetomidine (M) + K [1:50 ratio]	Atipamezole				
Formulation		Add 1.8 ml sterile water per vial of ZT	Add 0.4 ml X (100 mg/ml) per 1.0 ml K (200 mg/ml)	Prepared for injection	Add 0.4 ml M (10 mg/ml) per 1.0 ml K (200 mg/ml)	Prepared for injection				
Concen	tration	260 mg/ml	171 mg/ml	10 mg/ml	146 mg/ml	20 mg/ml				
Volume Vial	per	2.2 ml	Not applicable	30 ml	Not applicable	10 ml				
Dosage (mg/kg		10	2 X + 10 K	0.15	0.08 M + 4 K	0.4				
Body M	ass		Volume (ml)							
kg	Lb.			voiume (iiii)						
15	33	0.6	0.3 + 0.8	0.2	0.12 + 0.3	0.3				
20	44	0.8	0.4 + 1.0	0.3	0.16 + 0.4	0.4				
25	55	1.0	0.5 + 1.3	0.4	0.20 + 0.5	0.5				
30	66	1.2	0.6 + 1.5	0.5	0.24 + 0.6	0.6				
35	77	1.3	0.7 + 1.8	0.5	0.28 + 0.7	0.7				
40	88	1.5	0.8 + 2.0	0.6	0.32 + 0.8	0.8				
45	99	1.7	0.9 + 2.3	0.7	0.36 + 0.9	0.9				
50	110	1.9	1.0 + 2.5	0.8	0.40 + 1.0	1.0				
55	121	2.1	1.1 + 2.8	0.8	0.44 + 1.1	1.1				
60	132	2.3	1.2 + 3.0	0.9	0.48 + 1.2	1.2				
65	143	2.5	1.3 + 3.3	1.0	0.52 + 1.3	1.3				

^a Adapted from Ballard et al. (1991). **Note:** The immobilizing effects of Telazol® cannot be counteracted by administration of reversal drugs. Therefore, this protocol should only be used if the drugs required for reversible immobilization (e.g. XK, MK) are not available.

^b Adapted from Kreeger and Arnemo (2012). Volumes presented as X+K and M+K, respectively.

Appendix C – Example Wolf Capture Data Form PERSONNEL:												
PERSUNNI	L:	i o O1 M	[av. 10]									
DATE: (dd-mm-yy, i.e. 01-May-18)ANIMAL DATA:												
	Recapture? Sex:					GNWT ID# Other ID#						
☐ Yes ☐ N			\square M \square F \square Unk.					s at si		101 12	_	
Relocated			Age class:			<u>- ·</u>		9 010 01	Age	c]	ass	
□ Yes □ N	lo	_	ıp 🗆 Yea	arling						1 - 8 -		
UTM:		_	•	Ü								
Location:		Lact	ating?							l		
-		_ 🗆 Ye	es 🗆 No									
CAPTURE	INFORM	ATION:										
Capture: 🗆			Delive	ery: 🗆 po	le syrin	ıge	Da	rt: 🗆 s	slow i	njectio	n	
Method: □	l Heli Da	rt	Systen	n:				tem:				
☐ Trap: Ty				wpipe 🗆	pistol					on □ b		
Site name _			□ rifle	!				other:				
LOCATION	DATA:											
General:												
Please use	Datum	NAD83	UTM e	ast:				UTM	nort	h:		
MARKING	S: Circle	Y/N cho	ices									
Collar: bra	nd:	fr	eq:		Ea	ar '	Trans	s: freq				
rotoff? [Y/I	N]				In	itia	al. Tin	ne:				
Drop-off: b Initial. time			lays:	Re-ca	apture?	[Y	/N] S	erial#	:			
Transpond	or: [V/N]	lifyoc	brandi		Т	D#	.					
Implant loc							r		 loca	tion:		
					• п				100	tion.		
Tattoo # Tattoo Location:												
Quality & location of previous tattoo(s):												
DRUG INFORMATION: VITAL STATISTICS:												
	Inject	Inject	Inject	Inject	Reversa	al					C	SpO ₂ (O ₂
	#1	#2	#3	#4	revers.	a.	111110	i disc	псор	Temp		flow)
Drug				-								
Amount												
Volume												
Time							-					
Inj. site/												

Miss

Induction Sequence (record approx. times) **Recovery Sequence** (record approx. times) Head Immobilized Head Standing Staggering Staggering Rump Running down down up BODY MEASUREMENTS: Indicate the units used, if different Straight-line length: **Testicle size:** Weight: ____lb. or kg (nose to tip of tail) ____cm Left (L) ____cm Zoological length: (W) ____cm (body contours) ____cm Estimate or Actual Right (L) ____cm Chest girth: (behind shoulders) cm (W) ____cm **SAMPLES COLLECTED: CHECK LIST: RECORDED:** ☐ Collar/Ear Tag freq. Tooth ☐ Ear plug ☐ Dart recovered ☐ Animal data (circle): ☐ Hair ☐ Hibitane ☐ Transponder # ☐ Capture data ☐ Feces ☐ Tattoo # & location ☐ Location data LLPm1 \square Eye ointment LRPm1 ☐ Claw tip ☐ Transmitter ☐ Body measurements □ Body weight ☐ Testicle size ULPm1 magnets off URPm1 **Blood:** □ red

□ purple

COMMENTS: _

Appendix D – Example Field Necropsy Data Form

GENERAL INFORMATION:

Date:	Location:	Personnel:							
Species:	Sex: □ M □ F □ Unk.	. Age Class: □ Pup □ Yearling □ Adult							
Euthanasia: □ Yes □	Euthanasia: □ Yes □ No If yes, method of euthanasia:								
CARCASS INFORMATI	CARCACC ANTORNATION								
_	State of preservation: \square fresh \square frozen \square decomposed \square whole carcass								
☐ partial carcass	□ partial carcass								
Nutritional condition	:	Pregnant: □ yes □ no							
F-44-6		Identifi	cation number (PTT ID or ear						

tag):

NECROPSY OBSERVATIONS:

External Assessment:

Extent of scavenging:

Photo numbers

Location	Description	Photos [Y/N]
Skin		
Analysis of any potential damage from the dart wound		
Orifices (mouth, ears, etc.)		
Evidence of tattoos in mouth or groin?		
Other (eyes, limbs, etc.)		

Internal Assessment:

Location	Description	Photo [Y/N]
Muscle		
Bones and Joints		
Lungs and Trachea		
Liver		
Heart		
Spleen and Lymph Nodes		
Stomach(s)		
Intestines		
Urinary		
Reproductive System		
Brain		
Other Observations		

TISSUE COLLECTION:

	Muscle	Lung	Liver	Heart	Stomach	Intestine	Kidney	Brain	Other
Formalin?									
Frozen?									

Additional tissues to collect:	
☐ Premolar or canine tooth	
□ Hair	
☐ Baculum	
☐ External Fat	
☐ Internal Fat (if present)	
☐ Skin biopsy	
☐ Ear tip	
Specimens collected for other tests:	