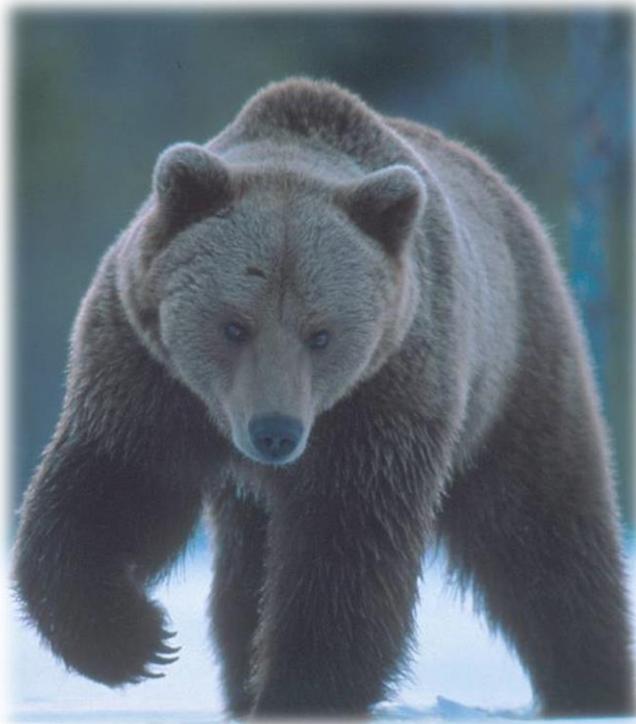


# **Biomedical Protocols for Free-ranging Brown Bears, Wolves, Wolverines and Lynx**

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## PREFACE

Compilation of this document was initiated by the Norwegian Environment Agency in order to establish recommended protocols for capture, chemical immobilization, anesthesia and radiotagging of free-ranging brown bears (*Ursus arctos*), gray wolves (*Canis lupus*), wolverines (*Gulo gulo*) and Eurasian lynx (*Lynx lynx*). In addition, procedures to ensure proper sampling of biological materials for management, research and banking purposes have been included.

The current protocols are based on nearly 4,000 captures of free-ranging brown bears, wolves, wolverines and lynx carried out from 1984 through 2017 in Scandinavia. Some of the results have been published as peer reviewed papers, conference presentations, theses, and reports. However, a large amount of data are still on file and will be published in the future. In addition, comprehensive literature reviews have been carried out in order to include pertinent information from other sources.

Earlier versions of these protocols were approved by all ongoing research projects on brown bears, wolves, wolverines and lynx in Scandinavia. We thank the project leaders, researchers, and field technicians for their cooperative efforts.

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## **INTRODUCTION**

Chemical immobilization of wild animals is a form of veterinary anesthesia conducted under the most difficult of circumstances. Anesthetic drugs are never completely devoid of toxicity and anesthesia invariably carries a risk to the life of even healthy patients. The risk of severe side effects, injuries or death can never be completely eliminated. In addition, all immobilizing drugs are toxic and some are potentially lethal to humans.

Chemical immobilization of free-ranging wildlife should only be considered if it is necessary to accomplish research or management goals, and should be carried out by a team of professionals with proper training, experience and expertise in wildlife capture, veterinary anesthesia, animal handling and basic first aid and CPR techniques. If captures are carried out by darting from a helicopter, the skill of the pilot and the crew members is of paramount importance for a safe and successful outcome.

All captures must be properly planned. Chemical immobilization of brown bears, wolves, wolverines and lynx should preferably be carried out in winter or spring, on snow-covered ground. High ambient temperatures, open water, and bare ground make captures more difficult and increase the risk of morbidity or mortality. The choice of capture method is also important and needs to be adapted to the species in question and the specific landscape. Most large carnivores are captured from helicopters in Scandinavia, although wolverines and lynx are also captured using box-traps.

## **DRUGS AND DOSES FOR CHEMICAL IMMOBILIZATION AND ANESTHESIA**

### **Brown bears**

Brown bears are usually captured in early spring, shortly after emergence from their winter dens. Although brown bears are sometimes chemically immobilized during summer or shortly before denning. Such captures are more difficult due to lack of snow cover, open water, high ambient temperatures, and increased dose requirements due to seasonal changes in physiology and body fat. Bears are sometimes immobilized during wintertime in their dens for hibernation related research.

Except for hibernating animals, brown bears are darted from a helicopter using a remote drug delivery system (Dan-Inject<sup>®</sup>). Currently, the following standard doses of medetomidine (M) and tiletamine-zolazepam (TZ) are used for immobilization of free-ranging bears in April-May: Yearlings (15-45 kg) 1.66 mg M + 83.3 mg TZ; small bears (2-3 years, 45-70 kg) 2.5 mg M + 125 mg TZ; adult females and small males (70-120 kg) 5 mg M + 250 mg TZ; medium-sized adult males (120-200 kg) 10 mg M + 500 mg TZ; large males (>

200 kg) 15 mg M + 750 mg TZ. A fixed M:TZ ratio is used so that doses can be split or combined. The doses and darts are made up as follows:

- 1.66 mg M + 83.3 mg TZ (yearlings): 5 ml of M as 1 mg/ml, 0.5 ml of M as 10 mg/ml, and 1.8 ml of sterile water are used to dissolve 500 mg of TZ powder; split into 6 doses; use 1.5 ml darts with 1.5 x 25 mm barbed needles
- 2.5 mg M + 125 mg TZ (2-3 year old subadults): 5 ml of M as 1 mg/ml, 0.5 ml of M as 10 mg/ml, and 1.8 ml of sterile water are used to dissolve 500 mg of TZ powder; split into 4 doses; use 1.5 ml darts with 2.0 x 30 mm barbed needles
- 5 mg M + 250 mg TZ (adult females and small adult males): 5 ml of M as 1 mg/ml and 0.5 ml of M as 10 mg/ml are used to dissolve 500 mg of TZ powder; split into 2 doses; use 3 ml darts and 2.0 x 40 mm barbed needles
- 10 mg M + 500 mg TZ (medium-sized adult males): 1 ml of M as 10 mg/ml and 1.5 ml of sterile water are used to dissolve 500 mg of TZ powder; one dose; use a 3 ml dart and 2.0 x 40 mm barbed needle
- 15 mg M + 750 mg TZ (large adult males): 1 ml of M as 10 mg/ml and 0.5 ml of sterile water are used to dissolve 500 mg of Zoletil; make up three vials that are split into two doses; use 3 ml darts and 2.0 x 40 mm barbed needles
- Capture of bears late in the fall: Consider increasing the spring dose by 25-50% and using longer needles, but the injection site is probably the most important factor to avoid delayed absorption from subcutaneous fat
- Capture of denning subadult bears in late winter: Ketamine (K) is added to a reduced MTZ spring dose. 4.5 ml sterile water is used to dissolve 500 mg TZ powder (100 mg/ml) and combined with M as 1 mg/ml and K as 100 mg/ml. For 2-year old bears (30-45 kg): 0.625 mg M (0.63 ml) + 31.25 mg TZ (0.31 ml) + 37.5 mg K (0.38 ml); for 3-year old bears (45-60 kg): 1.25 mg M (1.25 ml) + 62.5 mg TZ (0.63 ml) + 75 mg K (0.75 ml). Use 1.5 ml darts for 2-year old bears, 3 ml darts for 3-year old bears, and 2.0 x 30 mm barbed needles for both age groups.

Hypoxemia (low levels of oxygen in the blood) is a common side effect documented by arterial blood gases analyses in both captive and free-ranging brown bears immobilized with different doses of MTZ. Intranasal oxygen supplementation markedly improves the arterial oxygenation and is routinely given throughout anesthesia, as part of the standard field procedures for the Scandinavian Brown Bear Research Project. In relation to the body mass, the following flow rates from oxygen cylinders are adequate during immobilization with MTZ doses used for free-ranging bears in April-May: 0.5 L/min to bears up to 25 kg, 1 L/min to

bears up to 50 kg, 2 L/min to bears up to 200 kg, and 3 L/min to bears > 200 kg. Preliminary studies indicate that in wintertime 0.5 L/min is sufficient for bears up to 55 kg. In addition to oxygen cylinders, a portable battery driven oxygen concentrator (EverGo™) is being used to efficiently provide supplemental oxygen to immobilized bears in Scandinavia.

### **Wolves**

Wolves are immobilized by darting from a helicopter in winter on snow-covered ground. Animals  $\geq$  6 months of age, regardless of sex and body mass, are darted with 250 mg tiletamine-zolazepam per animal using a remote drug delivery system (Dan-Inject®). A 3 ml dart syringe with a 1.5 x 25 mm barbed needle is used. Once recumbent, administration of 0.5 (i.v.) or 1.0 mg (i.m.) medetomidine may be required to induce complete immobilization. Mean (range) body weights in wolves > 18 months old captured in Scandinavia were 48 (36-57) kg for males and 38 (31-46) kg for females. Juveniles 7-10 months old weighed 34 (24-44) kg.

### **Wolverines**

Adult wolverines (females 9-11 kg, males 14-16 kg) and juveniles (> 8 months, 8-14 kg) are usually immobilized from a helicopter or in den sites (only secondary dens, never primary natal dens) under boulders or snow. Juveniles of approximately 3 months age (3-8 kg) are captured at den sites with a snare pole followed by drug-injection by hand. Adult wolverines are darted with a dose of 4 mg medetomidine (M) + 100 mg ketamine (K) per animal using a remote drug delivery system (Dan-Inject®) with a 1.5 ml dart syringe and 1.5 x 25 mm barbed needle. For procedures lasting 30-40 min, a dose of 3 mg M + 75 mg K may be sufficient for trapped adults. Juveniles (up to 8 kg) are manually restrained, weighed and immobilized with 0.1 mg/kg M + 5 mg/kg K i.m. (induces 30-40 min of immobilization).

Hypoxemia is a common side effect in wolverines immobilized with medetomidine-ketamine, as documented in an arterial blood gas study. Drug-induced physiological changes and high altitude (500-1,300 m above sea level) contributes to the low levels of oxygen in the blood. Supplemental oxygen is recommended to prevent hypoxemia and improve safety for immobilized wolverines.

## **Lynx**

Adult lynx and juveniles (> 8 months) are either immobilized from a helicopter or captured using box traps or snares set around fresh roe deer kills. Hunting dogs have also been used to chase the lynx into a tree. Adults (males 18-28 kg, females 14-19 kg) are darted with an initial dose of 4 mg medetomidine (M) + 100 mg ketamine (K) per animal using a remote drug delivery system (Dan-Inject<sup>®</sup>). In adults captured in box traps (calm animals) and in juveniles (6-12 months 9-16 kg, yearlings 12-21 kg), the dose can be reduced to 2 mg M + 50 mg (K). A 1.5 ml dart syringe with a 1.5 x 25 mm barbed needle (Dan-Inject<sup>®</sup>) is used. Kittens (4-5 weeks of age; mean body mass 1.5 kg) are captured by hand in their natal lairs, weighed, and immobilized with 0.1 mg/kg M) + 5 mg/kg (K) i.m.

## **Alternative drugs and supplemental doses**

Medetomidine (M) can be substituted by dexmedetomidine (D) at 1 mg D per 2 mg M. Supplemental administration of drugs depends on the situation, species and whether surgical anesthesia is required or not. Animals that are not recumbent 15 minutes after the initial dose, are redarted with a full dose (all species). If the animal is recumbent but incompletely immobilized, administration of additional drugs is usually necessary:

*Brown bears:* In large bears (adult females and adult and subadult males), redarting with either a full dose or half the initial dose is recommended for safety reasons. In yearling and subadult bears 1 mg medetomidine can be given i.m. by hand syringe injection.

*Wolves:* Wolves are usually easy to handle, even if they are not completely immobilized (often the case after darting with tiletamine-zolazepam). To reduce stress and to facilitate sampling, 1 mg medetomidine i.m. is recommended to induce complete immobilization.

*Wolverines and lynx:* If the animal is down but incompletely immobilized, 25-50% of the initial dose can be given i.m. by hand syringe injection.

In case of a prolonged procedure or signs of spontaneous recovery, 0.5-1.0 mg medetomidine i.m. can be given to keep juvenile and adult wolves, wolverines and lynx and yearling bears immobilized for another 15-30 minutes. For larger bears ketamine (1 mg/kg i.v. or 2 mg/kg i.m.) should be used; additional medetomidine should be considered in adult bears for safety reasons. If extra time is needed to finish surgery or other painful procedures, a combination of medetomidine-ketamine should always be administered. Due to the long elimination time, additional tiletamine-zolazepam should not be used, unless for human safety reasons in large bears.

### **CHASING, TRAPPING, DARTING, AND STRESS**

Animals that have not been previously captured from or chased by a helicopter are usually naïve when approached and darting can be performed within a few minutes of initial observation if the snow conditions and the landscape are optimal (proximity to ice-covered lakes, clear-cuts, open terrain etc.). Animals that have been captured previously, especially wolves, will usually run for cover when they hear the helicopter and are more difficult to approach. To avoid stress and physiological side effects (hyperthermia, lactic acidemia) during immobilization, intensive chasing should be kept to a minimum (< 3 min of strenuous running), and the total time of pursuit (the time from initial observation, including alternating periods of intensive and extensive pursuit) should never exceed 30 minutes.

To avoid impact trauma, animals should be darted with the lowest pressure setting of the dart gun that still ensures an adequate ballistic trajectory of the dart and proper injection of drugs. This is especially important for small animals such as lynx, wolverine, and yearling bears. In general, animals should be darted in body parts with large muscle groups, preferably in the lumbar region. Misplaced darts or darts with high velocity and impact energy can easily fracture bones, penetrate the thorax, or cause massive soft tissue trauma.

Lynx and wolverines are sometimes immobilized after being captured in walk through wooden box-traps (lynx), baited wooden box-traps (wolverines), spring-loaded foot-snares (lynx), or after being chased into a tree by hunting dogs (lynx). Traps can be monitored using radio-alarms (VHF or SMS) or cameras (MMS). For spring-loaded foot-snares placed at lynx-killed prey should be continuously monitored and the reaction time upon capture should never exceed 20 minutes. Reaction time upon captures in box-traps should not exceed 12 hours. Intensive chasing with dogs should be kept to a minimum, and should never exceed 30 minutes. Special care should be given to avoid accidents or physiological side effects due to prolongation of the capture attempt.

### **HANDLING AND MONITORING OF IMMOBILIZED ANIMALS**

Immobilized animals should be monitored and clinically examined by professionals with experience in wildlife medicine and anesthesia. Possible side effects include hypoxemia (inadequate amount of oxygen in the blood), respiratory depression (hypoventilation; increased carbon dioxide levels in the blood) and thermoregulatory dysfunction (hyperthermia or hypothermia). Drug overdose in individuals with poor body condition, aspiration of vomitus/saliva, pneumothorax due to misplaced dart, and vomiting (in wolves) are other

possible complications. If several animals are being captured at the same time, e.g.: members of a pack or family group, they should be brought together for monitoring and processing.

To prevent aspiration of saliva or vomitus, immobilized animals should be kept in lateral recumbency with the mouth and head low relative to the body. An eye gel should be applied to the cornea to prevent drying. Animals should be protected from direct sunlight into the eyes. A blind-fold and ear plugs should be used.

Thermoregulation should be monitored by frequent measurements of the rectal temperature (RT). “Normal” body temperature in brown bears is 37-37.5 C. Typical and acceptable RT during immobilization of bears, wolves, wolverines and lynx is thought to be 38.0-39.5 C. Hyperthermic animals (RT > 39.0 in bears and > 40.0 C in the other species) should be cooled by applying snow (or water in summertime) to the axilla, groin, and/or tongue. If the hyperthermia is not resolving within 15 minutes, i.v. fluid therapy should be initiated (10-15 ml/kg) of cool (20 C) Ringer<sup>®</sup>-acetat. Oxygen supplementation is recommended for hyperthermic animals because of increased oxygen demand. Hypothermic animals (RT < 36.0 C in bears and < 37.0 C in the other species) should be protected from wind and cold surfaces to avoid further cooling using a waterproof, insulated cover. Hot water bottles can be placed in the groin and axilla as an external heat source in the field. In case of prolonged immobilization and recovery, hypothermic animals should be warmed, and body warm (38 C) Ringer<sup>®</sup>-acetat should be administered i.v.

The color of the mucous membranes in the mouth (gums) can be used to assess blood oxygenation. A pink or red color is normal; bluish membranes indicate hypoxemia. The capillary refill time (CRT) can be used to assess peripheral circulation by pressing a finger on the gums, releasing and counting the seconds it takes for the blood to return. Normal CRT is < 2 sec. Cardiorespiratory function should be monitored using a pulse oximeter with the sensor applied to the tongue. Hemoglobin oxygen saturation (SpO<sub>2</sub>) should be above 95%. A decreasing trend indicates hypoxemia and supplementation with intranasal oxygen should be given to improve oxygenation and the safety for the immobilized animal. A portable oxygen cylinder or oxygen concentrator should be part of the standard field equipment, as well as a laryngoscope, endotracheal tubes, and a ventilation bag. In case of apnea (absence of breathing), intubation and ventilation with a bag should be initiated. Doxapram at 5-10 mg/kg i.v. can be given to stimulate respiration. The effect of doxapram is short-acting and, as a general central nervous system stimulant, it may lead to the animal waking up from anesthesia. Field personnel should be able to intubate (place an endotracheal tube in the wind pipe) and provide assisted ventilation to an animal with apnea.

A small surgical kit for treating wounds and an electrical clipper should be part of the standard equipment.

### **TAGGING, SAMPLING AND DOCUMENTATION**

Most animals are captured for tagging or sampling purposes and should be processed according to the aim of the project. Capture data should be recorded according to an established animal capture form and photos should be taken.

Radiocollars should be fitted according to the size, age and sex of the animal. The weight of the radiocollar should not exceed 2% of the animal's body mass. When changing the collar the neck should be examined for hair loss and possible skin irritation. Depending on animal species, sex and/or age, authorities may require that collars should have a break-away zone or a drop-off mechanism.

*Brown bears:* The collar should be fitted so that it can be pulled on and off over the head. Drop-off collars or a break-away zone (double webbing in males, single in females) should be used on all growing bears and on bears of unknown age. For adult males, which may have a greater circumference of the neck than the head, consider clipping hair on the neck to avoid losing the collar. Ensure that it is possible to pass a flat hand between the collar and the neck. Collars should have a break-away zone with double or tripple webbing.

*Wolves:* Minimum collar circumference should be 44.5 cm for females and 48.0 cm for males. Ensure that there is enough space for two fingers (4 cm) between the collar and the neck.

*Wolverines:* The circumference of the animal's head and neck should be measured before fitting the collar. The circumference of the collar should be adjusted so it is slightly less than the circumference of the head, but larger than the circumference of the neck. Ensure that the collar is not too tight (make room for one finger between the neck and the collar) or that it can be pulled over the head of the animal. In some cases the difference in circumference of the head and neck is very small (especially in males) and fitting the collar can be difficult.

*Lynx:* The minimum collar circumference should be 26 cm for females and 30 cm for males. Ensure that at least one finger can be passed between the collar and the neck. Collars for juvenile males should have a break-away zone or an implant should be used if 30 cm is too great to be retained by the animal.

*All species:* The transmitter (VHF) and GPS unit should be activated by removing the magnet(s) and should be tested with the receiver before the animal is released. Make sure that the GPS unit is working properly before any capture is initiated.

A microchip should be implanted s.c. at the base of the nose of brown bears and the insertion hole sealed with a drop of tissue or super glue. In all the other species the microchip is implanted s.c. at the base of the right ear. The microchip should be tested with the scanner after implantation. Application of ear tags and tattooing depend on the species, age of the animal, and aim of the project. The area around previously applied ear tags should be inspected for signs of infection or irritation (redness, swelling or discharge) and if found, the ear tag should be removed.

*All species:* Body measurements should be recorded according to the animal capture form.

Blood can be sampled from the jugular vein (all species), cephalic vein (wolves, lynx), or the femoral vein (all species) using evacuated plastic tubes and multisample needles. Blood for genetic studies (EDTA) should be stored at  $-20^{\circ}\text{C}$  until shipment to the laboratory. Tubes without anticoagulant for serum biochemistry and serology should be kept at room temperature for 1-2 hours to ensure complete coagulation. Serum should then be separated by centrifugation (1500 g for at least 15 minutes) and transferred to 2 ml cryogenic vials. Serum for banking (serology and back-up) is stored at  $-20^{\circ}\text{C}$  until shipment to the laboratory. Blood samples are taken from all captured carnivores for ongoing and future studies regarding health, disease and stress, and are available for investigations of marked animals submitted for necropsy.

In brown bears and wolverines, the rudimentary first maxillary premolar is extracted for age determination. At least five minutes before tooth extraction, local anesthetics should be injected under the mucus membrane above the second premolar (over the maxillary foramen) and carprofen or meloxicam administered i.m. The tooth is preserved in 96% alcohol in a 2 ml cryogenic vial.

Hair should be collected with pliers and transferred to 15 ml sterile plastic tubes (brown bears and wolves) or 5 ml sterile cryogenic vials (wolverines and lynx). Hair samples can be preserved by drying in paper envelopes at room temperature. Skin biopsies are taken from the inside of the ear, after clipping the hair and cleaning the skin with chlorhexidine alcohol, by the use of a 4 or 6 mm sterile dermal biopsy punch. The skin biopsy is transferred to a 2 ml cryogenic vial and preserved by adding 96% ethanol. To stop bleeding, pressure is

applied to the area where the biopsy was taken, using a piece of gauze held in place with a clothespin.

In brown bears and wolves, feces is collected with the index finger from the rectum using examination gloves. The feces is transferred to 50 ml sterile plastic tubes. In wolverines and lynx, feces is sampled by inserting a sterile cotton into the rectum. Fecal samples are stored either at  $-20$  or  $-80$  C, depending on the purpose of sampling

Urine is collected when relevant for research purposes using a 0.6 x 40 mm needle with a 20 ml syringe. The animal is placed in dorsal recumbency and the location of the bladder is determined by palpation or ultrasound. The skin can be wiped with alcohol for disinfection and to improve contact of the ultrasound probe with the skin. The needle is inserted into the bladder through the midline of the abdomen and urine is withdrawn using the syringe. Urine can also be sampled by manual pressure on the bladder, collected in a sterile container and stored at  $-20$  or  $-80$  C, depending on the purpose of sampling.

If muscle biopsies are required, they are taken from the biceps femoris. Routine surgical preparation of the skin overlying the biopsy site is done prior to the biopsy. A short incision is made through the skin, fat, and fascia overlying the biopsy site and the required amount of muscle tissue is excised. Muscle tissue is washed with sterile saline before placement in sterile cryovials. Fat samples are taken from the same surgery site before closing in two layers (muscle fascia and skin) using 2-0 absorbable sutures. If there is significant subcutaneous fat, it can also be opposed using similar sutures.

Depending on the situation and the study protocol, other biological materials should be sampled according to current standards in veterinary medicine or specific instructions from the laboratory.

#### **ANALGESIA AND ANESTHESIA FOR SURGERY**

In brown bears, wolverines and lynx, surgical anesthesia is induced by the recommended immobilizing drugs and doses. In wolves, supplemental medetomidine is needed. For post operative analgesia, 4 mg/kg carprofen or 0.2 mg/kg meloxicam is administered s.c. as soon as possible after immobilization is induced and before surgery is initiated (all species). The carprofen dose is reduced to 1 mg/kg in hibernating bears (due to decreased metabolic rate and drug excretion).

## **SURGICAL PROCEDURES FOR IMPLANTATION**

### *Intraperitoneal transmitters*

For surgery, the animal is kept in dorsal recumbency. An appropriate area caudal to the umbilicus is clipped and swabbed with chlorhexidine in 60% ethyl alcohol (Klorhexidin<sup>®</sup>). To avoid frostbite and excessive heat loss at low ambient temperatures, clipping should not be done in the fall or winter. Instead an antiseptic cream can be rubbed into the fur along the midline and the hair is parted to expose the skin. For access to the peritoneal cavity, a ventral midline incision is made using standard surgical procedures. The weight of the implant should not exceed 2% of the body mass of the animal [note: 2% of body mass is the maximum weight of the collar *and* implant]. The radio transmitter should be tested with the receiver before implantation. Implants need to be gas sterilized. They should be prewarmed prior to insertion into the peritoneal cavity. The incision is closed in two layers with absorbable sutures, using a simple interrupted pattern for the *Linea alba* (US 1 in all bears except yearlings, US 0 in juvenile and adult wolves, wolverines, lynx and yearling bears and US 2-0 in wolf pups, wolverine cubs and lynx kittens; use a round needle) and an intradermal or interrupted horizontal mattress pattern for the skin (US 0 in all bears except yearlings and US 2-0 in all other animals; use a cutting needle). The skin wound is covered with a spraydressing.

### *Temperature loggers, ECG monitors and physiological sensors*

Surgery should be carried out according to accepted standards in veterinary medicine. For temperature loggers and physiological sensors, surgery is carried out as described for intraperitoneal transmitters with the incision length appropriate for the size of the logger or sensor. Temperature loggers, ECG monitors and physiological sensors should be gas sterilized. Sensors are placed into the peritoneal cavity (free-floating), whereas temperature loggers are placed in the peritoneal cavity but sutured to the body wall under the umbilicus using a non-absorbable suture. ECG monitors are placed subcutaneously on the left thorax at the level of the heart. Biologgers should be programmed and tested before implantation. Heart monitors are usually programmed on site (after implantation), depending on the model.

## **REVERSAL OF IMMOBILIZATION**

For reversal of immobilization in animals that have received medetomidine, 5 mg of atipamezole per mg of the total medetomidine dose is administered i.m. Due to the long elimination time of tiletamine-zolazepam, atipamezole should not be given until earliest 50-60

min after darting. In an emergency, atipamezole can be given at any time, but recovery may then be rough with possible incoordination, excitation and convulsions. Such an animal can be calmed by administration of midazolam i.m. (suggested dose 0.1-0.2 mg/kg).

Immobilized animals can usually be left to recover undisturbed at the site of capture. Possible side effects and dangers during and immediately after recovery include vomiting (wolves), hypothermia (especially in animals with a small body mass relative to body surface or in case of extended procedures), hyperthermia (due to extensive chasing prior to capture, sun and/or high ambient temperatures), intraspecific strife (attack by pack members, males attacking other males, males trying to mount immobilized females in estrus, males attacking dependent young), open water, lack of fear, traffic, and poaching. Wolves should be observed by trained personnel until full recovery is evident. This may take several hours in wolves immobilized with tiletamine-zolazepam. All GPS and radio-instrumented animals should be checked the day after capture.

#### **OTHER TREATMENT**

Captured animals with health-threatening diseases should be treated according to accepted standards in veterinary medicine. In animals with severe or terminal illness, euthanasia should be considered. For immigrant wolves, additional sampling and treatment may be needed depending on requirements from authorities.

#### **TRANSLOCATIONS**

In some cases, genetically valuable wolves in problem areas are moved to places where they are less likely to cause conflict and have a higher likelihood of survival. In these cases, wolves are darted with the standard dose of tiletamine-zolazepam and 0.3 mg/kg midazolam is given after the wolf is captured, and repeated immediately before the wolf is loaded into the transport crate in order to prolong the effect of the midazolam. All wolves to be translocated over 1 hour are given 500 ml of Ringer®-acetate i.v. (1L if hyperthermic due to ambient temperature or extended chasing). The wolf must be capable of sitting with its head elevated and not showing any signs of vomiting before transport begins, this is usually at least one hour from darting.

#### **NECROPSY PROCEDURES**

In case of a capture-related mortality, the carcass should be sent to a diagnostic laboratory for necropsy. To ensure rapid cooling, skinning and evisceration should be considered. If

transportation to the laboratory is not possible within 24-48 hours, the carcass should be frozen. As an alternative, a field necropsy and proper tissue sampling can be carried out by a veterinarian after consultation with the laboratory.

### **LEGAL ASPECTS**

All captures must be approved by the appropriate authorities. The use of motor vehicles, landing with a helicopter, and setting up traps may require special permits from local, regional and/or national authorities as well as landowners. Prior to starting capture activities, the police, animal welfare and local wildlife authorities should be informed according to the permit. The use of radiotelemetry equipment requires a permit. Note that additional permits may be needed from county or national authorities when working in protected areas.

Immobilizing agents are prescription drugs and must be used by or on the order of a licensed veterinarian. Some of these drugs are also controlled substances, i.e. drugs that can be abused, for which specific regulations apply. In Norway, a veterinarian has to be on site when wildlife are chemically immobilized. In Sweden a special permit is required for non-veterinarians and the veterinarian must be available.

Regulations in Sweden require that the person doing the darting has an immobilization course valid in Sweden and a hunting license (Class one license, either Swedish or valid in Sweden), that a veterinarian experienced with wildlife be readily available, that all missed darts be retrieved, that darts are clearly labeled "WARNING! Do not touch the needle. May contain poison. Call (phone number) or police (police phone number) ", that a tracking dog is available, and that in case of a mortality, the carcass has to be sent for necropsy at the National Veterinary Institute.

Withdrawal times (general considerations): Very few of the drugs currently used for wildlife immobilization have been authorized for use in food producing animals in the European Union (applies in Norway as well) and most drugs are used off-label. A withdrawal period is set within the procedure of granting a marketing authorization, i.e. either by the national authority concerned (Norway: Statens legemiddelverk; Sweden: Läkemedelsverket) or, in case of a centrally authorized product, by the European Medicines Agency .

Withdrawal times (brown bears in Sweden): Permission to use medetomidine-tiletamine-zolazepam and atipamezole has been granted by Läkemedelsverket, but the drugs should not be used less than 3 months before the opening of the hunting season.

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