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Field immobilization of Desert Bobcats (*Lynx rufus*) using a Tiletamine-Zolazepam anesthetic combination

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Abstract

Chemical immobilization is used to minimize stress and pain while safely handling wild animals under field conditions. Eight wild bobcats (*Lynx rufus*) were immobilized for radio collar fittings between March 2005 and March 2007, using a commercial tiletamine hydrochloride-zolazepam hydrochloride combination (Zoletil® Virbac, Carros, France). Bobcats were captured using soft catch spring traps (Victor No. 3) in the Sonoran Desert of Baja California Sur, Mexico. A mean ($\pm$ SD) intramuscular total dose of 44.3 ± 2.9 mg (6.0 mg kg^{-1}) produced an induction time of 9 ± 4.9 min, and a duration of cataleptic anesthesia of 46.5 ± 11.4 min. No adverse reactions were observed in animals during handling, and telemetry monitoring indicated that all individuals were alive for at least one year after the procedure. The results of this study indicate that use of tiletamine-zolazepam administered at 6 mg/kg dose is suitable for short-term chemical immobilization of bobcats in the wild.

Keywords: Bobcat; Containment; *Lynx rufus*; Sonoran desert; Zoletil®

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Introduction

Chemical procedures for the immobilization of wildlife minimize stress and pain while animals are being handled. It is a necessary approach in projects that require safety for animals and handlers under field conditions and is frequently used for procedures such as blood drawing and smear sampling to evaluate health status, or telemetry collar fittings for free-ranging population studies.¹ Restraint procedures are especially important in studies attempting to assess the effects of changes in the ecosystems and habitat fragmentation due to human activities. Research in this area is also essential to implement optimal conservation strategies for top-order predators such as carnivorous mammalian species. Wild mammal studies regularly report the effects of different sedatives used for immobilization.²⁻⁹ However, documentation on the efficacy and comfort levels associated with their use is still lacking. This information can be essential since both animal handling and the use of chemicals often have an adverse confounding post-handling impact related to changes in normal animal activity, which may lead to biased research results. Moreover, the post-sedation survival data of individuals has been seldomly reported.¹⁰⁻¹³

For the Felidae carnivore family, the most commonly used sedatives are dissociative anesthetics such as cyclohexamines, which include hydrochlorides of ketamine, phencyclidine and tiletamine.^{7,13,14-16} Cyclohexamines are highly effective in a wide range of animal species, as they have a high therapeutic index, and minimal cardiovascular and respiratory effects.¹⁷ Rarely reported disadvantages of these chemicals are sudden induction and recovery, little muscle relaxation, and occasional prompting of seizures and excessive salivation.¹⁷ To minimize the advent of these negative effects, cyclohexamines are commonly combined with tranquilizers such as xylazine and zolazepam hydrochlorides or acepromazine maleate.¹⁷

A combination of ketamine chloride (100 mg/ml) and xylazine chloride (20 mg/ml) is often used to immobilize felids.^{14,18-21} Nonetheless, the ketamine-xylazine combination has been shown to cause heart and respiratory distress during anesthesia.²² Also, there have been reports of Iberian lynx (*Lynx pardinus*) experiencing seizures.²³

Tiletamine is a dissociative anesthetic agent that falls under the drug category of NMDA receptor antagonists and is chemically related to ketamine. Since it does not provide adequate muscle relaxation by itself, it is generally combined with zolazepam, to achieve sufficient muscle relaxation during deep surgical anesthesia. The cranial and spinal nerve reflexes remain however active (which must not be confused with inadequate anesthesia), and both coughing and swallowing, as well as plantar and palpebral reflexes are also maintained.²⁴

Zoletil[®] (Virbac, Carros, France) is a commercial presentation of a tiletamine-zolazepam combination (containing 250 mg of each anesthetic drug), that once reconstituted with 5 ml sterile water, results in an injectable 100 mg/ml solution. The use of this anesthetic preparation has been reported as safe for many wild species, including lions and leopards,²⁵ polar bears,²⁶ wild ocelots,¹³ American black bears,²⁷ American badgers,²⁸ and South American coatis.²⁹ Induction time was found to be short in all of these species, a good smooth muscle relaxation was achieved during anesthesia, recovery was smooth, and minimum negative effects on breathing and salivation were reported. Information on the use of these chemicals also exists for felids, however particular data for their use during immobilization of wild bobcats (*Lynx rufus*) under extreme weather conditions in desert areas,

such as the Sonoran Desert, are not well known. Desert bobcats are classified as a high stress species, due to the extreme demands imposed on individuals to obtain water and prey. Consequently, handling of these animals can be hazardous since sedation causes further stress. For this work, bobcats were captured and sedated for fitting of telemetry collars as part of a study that evaluates the effects of anthropogenic habitat fragmentation on daily activities of wild individuals. This paper presents information on the use of a tiletamine-zolazepam commercial combination regarding immobilization effects, as well as induction, maintenance, recovery, and inability times during anesthesia, to determine suitability as a protocol for bobcat sedation under field conditions.

Materials and methods

Study area

The study was conducted in the southern portion of the Baja California peninsula in the Santo Domingo valley, Mexico (Valle de Santo Domingo, 24° 55' 49.64" N, 111° 37' 40.28" W and 25° 23' 46.82" N, 111° 50' 34.11" W). The climate is dry, with an average annual temperature of 22.7 °C³⁰ and an average rainfall of 165 mm/year.³¹ Vegetation is dominated by relatively dense xerophytic shrubland, with typical desert plants such as columnar cacti (*Pachycereus pringlei*), creosote bush (*Larrea tridentata*), Adam's tree (*Fouquieria diguetii*), and mesquite (*Prosopis* sp.), among other species.³²

Animals and immobilization procedure

Eight adult bobcats were captured (4 females and 4 males) using snare traps with plastic protection (Victor No. 3, soft catch spring trap) to minimize harm.^{33,34} Hare and feathered chicken carcasses and sardines were used as baits for capture. Traps were prepared in the afternoon and checked every morning for caught individuals.

Once caught, bobcats were immobilized while inside their holding crates, using a pole syringe that allowed for distance intramuscular injection in the hindquarters of the of tiletamine zolazepam (1:1) commercial combination.

Before injection of the sedative, health status of each individual was assessed by observation of external and behavioral characteristics, and body weight was estimated by an experienced researcher.^{5,12,35} Average weight of all captured bobcats was estimated at 6.8 kg. Animals were then given the recommended dose of 6 mg/kg of the tiletamine-zolazepam combination, taking extreme care to avoid potential overdosing (i.e., a total initial injection of 40 mg or 0.4 ml of the solution per individual was administered IM). For bobcats that exhibited light sedation signs and were still responsive to stimuli after the first injection, a second dose (or at occasion a third dose) of 1 mg/kg was administered to allow appropriate immobilization and safe handling.

The purpose of the immobilization procedure was to fit animals with a VHF radio collar (HLPM-3180M Wildlife Materials, Murphysboro, IL), for tracking of daily movements and monitoring of behavioral activities within their habitats. Total time for handling and collar fitting was between 20 and 40 minutes in all animals. As

the procedure was not painful, animals were not put under deep anesthesia, which also facilitates recovery. Animals were followed by telemetry every 6 hours for 15 days per month, over the course of one year.

Age, sex and actual individual weight were ascertained once animals were anesthetized. Age was determined from mass and body size, and tooth wear.³⁶ During handling, eyes of bobcats were protected from UV light and dust entry by a cotton cloth. A sterile ointment was also applied to avoid drying of the corneal surface. Sedation was monitored by recording of physiological parameters every 15 min (heart and respiratory rates, body temperature, Table 1), and by tracking the duration of the different stages of anesthesia. Induction time was considered from injection of the tiletamine - zolazepam solution until the onset of cataleptic anesthesia; Cataleptic anesthesia was the period where the animal was insensitive to external stimuli, which ended when individuals raised their heads. Finally, the recovery phase, was considered to start when bobcats were able to lift and hold up their heads, and to end when they could remain standing without showing any signs of drowsiness or lack of coordination. The sum of time taken to complete these three stages yielded the inability time (in minutes):³⁷

IN (Induction time) + CA (Cataleptic Anesthesia time) + R (Recovery time) = IT (Inability time).

Physiological parameters were recorded until the animals became responsive and alert (an average of 3 records per individual per parameter were taken). Bobcats were kept under observation for as long as needed to ascertain complete recovery from the anesthesia. Once animals exhibited normal posture and reflexes, they were released from holding crates, and naturally returned to their habitat. All bobcats recovered from immobilization without the assistance of an antagonist.

Data analysis

Student's t- tests were used to compare physiological parameters between sexes during anesthesia.

Results and discussion

The commercial analgesic solution Zoletil® was administered according to manufacturer instructions (Virbac S.A., Carros, France 2003), using the recommended dose for bobcats (6.0 mg/kg). All animals survived the handling procedure, and no signs of sedation or post-sedation distress (dyspnea, seizures) were observed. Typical behavior in terms of stress and resistance to capture was presented by the eight individuals before anesthetic administration. According to the average estimated weight of individuals at 6.8 kg, an initial dose of 40 mg was administered to every bobcat. There were differences in induction time after anesthetic injection between animals (Table 1). Two bobcats (one female and one male) had a rapid induction time (2 min), going almost directly into a cataleptic state after sedative injection. The female bobcat remained in the cataleptic stage for 64 minutes, while the male stayed in this phase for 50 min (Table 1).

Table 1. Weight, physiological parameters, and duration of anesthesia stages of captured bobcats immobilized with a tiletamine-zolazepam solution.

Average (rate)									
Bobcat	Weight (kg)	IN (min)	CA (min)	R (min)	IT (min)	RT (°C)	Heartbeat /min	Respiration /min	Dose (mg)
Female	6.0	2	64	25	89	38	156	40	40
Female	8.0	5	66	19	85	38.05	184	36	45
Female	8.0	15	35	55	90	38.6	180	40	50
Female	7.5	15	55	35	90	38	180	40	40
Average	7.8	9.25	55.00	33.50	88.50	38.16	175.00	39.00	43.75
Standard deviation	0.95	6.75	14.17	15.78	2.38	0.29	12.81	2.00	4.79
Male	7.6	5	52	68	120	38.9	180	30	45
Male	7.0	15	30	40	70	39.3	153	55	50
Male	9.5	20	20	60	80	38	200	40	45
Male	7.2	2	50	55	105	37.85	180	28	40
Average	7.83	10.50	38.00	55.75	93.75	38.51	178.25	38.25	45.00
Standard deviation	1.14	8.43	15.58	11.79	22.87	0.70	19.29	12.34	4.08
Average ^t	7.60	9.88	46.50	44.63	91.13	38.34	176.63	38.63	44.38
Standard deviation ^t	1.00	7.10	16.51	17.54	15.31	0.53	15.26	8.19	4.17

CA: cataleptic anesthesia; IN: induction time; IT: inability time; R: recovery; RT: rectal temperature.

^t Total.

Induction

The average induction time was 9.8 minutes and the cataleptic state lasted for 46.5 minutes. Awakening was gradual and peaceful in all bobcats and occurred at an average of 44.6 minutes after catalepsy (Table 1).

There were no differences between sexes regarding physiological parameter data: rectal temperature ($t = -0.806$, $P = 0.479$), respiratory rate ($t = 106$, $P = 0.922$), or heart rate ($t = -.259$, $p = .813$) (Table 1).

Dosage, sedation, and duration of stages of anesthesia are all critical to eliminate or decrease mortality and adverse effects of wild individuals during handling. Assessment of chemicals used for this practice is therefore paramount to understand potential impact on different animal species. Information on putative effects is of value for reliability and safety when handling wildlife.

The effects of a commercial tiletamine-zolazepam solution were evaluated at each stage of sedation in desert bobcats captured in the wild. Standard doses of 40 mg (6.0 mg/kg) were given to both males and females since visual weight estimations were similar for both sexes. Estimated weight was subsequently ascertained by using a scale in sedated animals: females = 7.37 kg, males = 7.82 kg. The administered anesthetic dose allowed for safe animal handling, permitting to complete the collar fitting procedure within 20 to 40 minutes. The average inability time for the captured cats was 91 minutes. This period is relatively short compared

to other studies conducted with the same sedative in wild felids, where IT for nine ocelots (*Leopardus pardalis*) in southern Texas using a dose of 5.05 mg/kg, was reported to last 250 minutes, even if ocelot average weight was greater (9.2 kg) to that found for the bobcats in this study.¹³ Also, an IT of 104 minutes was reported for leaner leopard cats (*Prionailurus bengalensis*, 2.7 kg) in Thailand.³⁸ For the purposes of this study, the 91 minute IT period was also deemed adequate as to avoid stress or harm for the bobcats. Shorter IT periods had been reported when using a combination of ketamine hydrochloride and xylazine: 64 minutes in North Carolina bobcats,¹¹ 59.1 min in Texan bobcats,²⁰ and 69 min in Iberian lynxes (*Lynx pardinus*).²³ However, 9% of the 31 wild Iberian lynxes in this latter study presented chemical induced seizures,²³ which were not observed in any of the bobcats sedated with tiletamine-zolazepam combination in this study.

Telemetry data taken through fitted radio collars (with at least 60 detections throughout the one-year monitoring period) showed that all handled bobcats in this study displayed normal behavior in terms of movement, resting and feeding within their habitat area. This suggest that there were no apparent ulterior adverse effects of sedation and handling.

Conclusions

An adequate inability time that allowed completion of collar fitting procedures in wild desert bobcats without the associated risk of extended anesthetic states, lack of complications during anesthesia, and a return to stable body conditions was achieved using a commercial tiletamine hydrochloride and zolazepam hydrochloride solution at a 6 mg/kg dose. Sedated animals were returned to the wild after handling and monitored by telemetry for at least 60 times over the course of one year in their natural habitats. All animals were alive and displaying apparent normal behavior by the completion of the observation year, tracked movements indicating thriving individual performance. Hence, the anesthetic protocol and handling procedures used in this study is safe and suitable for short-term immobilization of Sonoran Desert bobcats.

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Conflicts of interest

The authors declare no conflict of interest related to this report.

Author contributions

R. R. E.: logistic support and manuscript revision.

L. A. T. C.: field animal support.

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