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Comparing the Efficacy of Two Immobilization Drug Combinations for the Chemical Restraint of Bobcats (*Lynx rufus*)

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ABSTRACT: Chemical immobilization agents that provide rapid induction time, short duration of action, wide margin of safety, and postreversal recovery are important attributes to the handling process of immobilized animals. We evaluated differences in induction, recovery, and physiologic parameters in 23 (13 female, nine adults and four yearlings; 10 male, nine adults and one yearling) free-ranging bobcats (*Lynx rufus*) chemically immobilized with an intramuscular combination of ketamine (10 mg/kg) and xylazine (KX; 1.5 mg/kg; $n=11$) or a combination of butorphanol (0.8 mg/kg), azaperone (0.27 mg/kg), and medetomidine (BAM; 0.32 mg/kg; $n=12$). Induction parameters, time to first effect, hemoglobin oxygen saturation, and anesthesia between bobcats administered KX and BAM were similar. Pulse rate was significantly higher for KX than for BAM. Time to standing and full recovery after reversal were faster for bobcats administered BAM than KX. Six of 11 (55%) bobcats given KX were effectively immobilized with a single injection, and five required additional drugs to allow adequate time for processing. Of 12 bobcats given BAM, six (50%) were effectively immobilized with a single injection, three (25%) individuals were not completely immobilized and required additional doses to allow adequate time for processing, and three (25%) required additional doses after complete arousal during processing. We found that BAM provided reduced sedation and processing times (<30 min), whereas KX provided extended sedation and processing times beyond 30 min. We suggest that researchers increase initial BAM drug volumes for yearling and adult bobcats at time of processing and consider taking appropriate safety precautions when handling free-ranging bobcats.

Key words: Azaperone, BAM, bobcat, butorphanol, chemical immobilization, ketamine, *Lynx rufus*, medetomidine.

INTRODUCTION

Capturing and handling free-ranging wildlife to investigate behavior, movement, habitat use, disease dynamics, or other objectives present a wide range of challenges to researchers and animals (Ellis et al. 2019). Despite potential safety risks to animals and personnel, physical restraint often is required for studies that involve manipulations, including the recording of morphometric measurements or demographic information (e.g., sex and age), attachment of monitoring devices (e.g., radio collars, ear tags, and vaginal implant transmitters), collection of tissue samples, and subsequent release of the animal to the environment (Rockhill et al. 2011; Ellis et al. 2019). Animal capture and handling in combination with chemical immobilization has been successfully used in wildlife research and management (Harthoorn 1965). Chemical immobilization agents are selected to facilitate the handling of animals in an efficient, predictable, and minimally stressful manner (Nielsen and Woolf 2002a; Rockhill et al. 2011; Kreeger and Arnemo 2012). Selection of the most appropriate immobilization agents should be based on study objectives, immobilization agent availability, external factors (e.g., ambient temperature, environmental conditions, and capture methods), and characteristics of target animals (e.g., sex, age, physical condition, and reproductive status; Ellis et al. 2019). Chemical immobilization agents that provide

rapid induction, short duration of action, wide margin of safety, good sedation, and reversibility are important to the handling process (Kreeger 1996; Rockhill et al. 2011). Immobilizing drugs with prolonged times to full recovery result in postrecovery effects that may influence behavior and survival of animals well beyond the time to full recovery and subsequent release of animals to the environment (Ellis et al. 2019). Assessment of pharmacologic effects of chemical immobilization agents pre-, intra-, and post-immobilization are necessary to achieve study objectives and to ensure that data quality is not compromised (Kreeger 1996).

A drug commonly used to chemically immobilize free-ranging bobcats (*Lynx rufus*) is ketamine hydrochloride (hereafter ketamine; Toweil 1986; Litvaitis et al. 1987; Conner et al. 2001; Chamberlin et al. 2003; Lynch et al. 2008). In addition, combinations of ketamine-xylazine (KX; Rockhill et al. 2011; Broman 2012; Elizalde-Arellano et al. 2012; Zieman et al. 2017), ketamine–promazine hydrochloride (Knick 1990; Blankenship et al. 2006), ketamine–acepromazine hydrochloride (Lovallo and Anderson 1996; Kamler and Gipson 2004), ketamine-tiletamine-zolazepam (Harrison 2010; Thurmond 2014), and ketamine-medetomidine-butorphanol (Rockhill et al. 2011) have been successfully used to chemically restrain bobcats.

Ketamine-xylazine is the most commonly used drug combination for bobcats and is characterized by stable cardiovascular function and good muscle relaxation (Plumb 2008; Rockhill et al. 2011; Kreeger and Arnemo 2012). Ketamine is a US Drug Enforcement Administration (DEA) schedule III cyclohexamine anesthetic with rapid onset of immobilization (1–4 min) and short duration of action (12–25 min) in mid-sized carnivores when used at the appropriate concentrations and dosages (Plumb 2008; Kreeger and Arnemo 2012). Ketamine is nonreversible, has a high margin of safety when used alone or in combination with other drugs, and is compatible with other agents for synergistic effects (Kreeger and Seal 1986; Spelman et al. 1993). However, important adverse effects of ketamine when used alone may include hyperthermia, increased heart rate and blood pressure, emesis, convulsions, muscle hypertonia and rigidity, and catatonia (Kreeger and Arnemo 2012). Xylazine is an α2-adrenergic agonist providing moderate sedation, analgesia, and muscle relaxation, which is reversible with α2-adrenergic receptor antagonists such as yohimbine or tolazine (Plumb 2008; Kreeger and Arnemo 2012). When combined with cyclohexamines (e.g., ketamine), xylazine works synergistically to improve efficiency and reduce drug volume (Wenker 1997). Important adverse side effects of xylazine include reduced heart rate and cardiac output and vomiting in felids (Plumb 2008; Kreeger and Arnemo 2012).

Butorphanol-azaperone-medetomidine (hereafter BAM) is a DEA schedule IV opioid drug combination that has been used successfully to immobilize carnivores held in captivity such as the cheetah (*Acinonyx jubatus*; Semjonov et al. 2019), black bear (*Ursus americanus*; Wolfe et al. 2008; Williamson et al. 2018), African lion (*Panthera leo*; Semjonov et al. 2017), and in the wild palm civet (*Paradoxurus musangus*; Ahmad et al. 2021) but has not been reported for free-ranging bobcats. Butorphanol is a κ-opioid agonist and a μ-opioid antagonist with analgesic and sedative properties, a wide margin of safety, few adverse effects, and is fully reversible with naltrexone (Fish et al. 2008; Kreeger and Arnemo 2012). In addition, onset of action with peak effect typically occurs 15–30 min after administration (Plumb 2008). Azaperone is a nonreversible butyrophenone tranquilizer that produces anti-anxiety effects but no analgesia. Onset of action is reported as rapid in wild pigs (*Sus scrofa*; 5–10 min), though duration of action is variable (2–4 h; Plumb 2008). Medetomidine is an α2-adrenoreceptor agonist that induces sedation, anxiolysis, and analgesia. However, bradycardia, bradypnea, hypothermia, paradoxical excitation, and apnea have been reported as adverse effects of medetomidine. Medetomidine is rapidly reversible with atipamezole (Plumb 2008; Kreeger and Arnemo 2012).

Our goal was to select a drug combination to minimize capture and handling time of anesthetized bobcats and to facilitate a safe

release, while ensuring researcher safety. Thus, our objective was to assess and compare the efficacy of BAM and KX for chemically restraining free-ranging bobcats.

METHODS AND MATERIALS

Animal capture and handling

We captured bobcats from November 2017 to March 2018 and November 2018 to March 2019 using cage traps constructed of galvanized wire mesh (28X43X91 cm; Camtrip Cages, Barstow, California, USA), baited with visual (e.g., bird wings and compact disks) attractants and commercial lures; McDaniel et al. 2000; Nielsen and Woolf 2001, 2002a, b). We checked traps daily to minimize potential injuries and stress on captured animals. In addition, we chemically immobilized and processed bobcats captured by licensed fur trappers throughout the study area. We randomly assigned the first bobcat captured to either 10 mg/kg ketamine (Ketaset, Midwest Veterinary Supply, Sun Prairie, Wisconsin, USA) with 1.5 mg/kg xylazine (Xyla-Ject, Midwest Veterinary Supply; Kreeger and Arnemo 2012) or BAM at 0.03 mL/kg (butorphanol 0.8 mg/kg, azaperone 0.27 mg/kg, and medetomidine 0.32 mg/kg; ZooPharm, Windsor, Colorado, USA) and attempted to use an alternating drug schedule for successive capture events. We obtained KX (100 mg/mL each) individually and premixed BAM (butorphanol, 27.3 mg/mL; azaperone, 9.1 mg/mL; medetomidine, 10.9 mg/mL) from the manufacturer. Drug volumes were calculated based on estimated body weight and manufacturer recommended dosages. Intramuscular injections were administered by hand or using a syringe pole (Tomahawk Live Trap, Hazelhurst, Wisconsin, USA; Kreeger and Arnemo 2012) for foothold-captured individuals and cage-trapped individuals, respectively. Time to sternal recumbency, head down, and full anesthesia when animals were unresponsive to audible or tactile stimuli (e.g., finger snapping and ear pinch) were monitored and recorded. During anesthesia, we monitored respiration rate (breaths per minute), rectal temperature (C), and hemoglobin oxygen saturation (SpO2), and pulse rate (heart beats per minute) with a pulse oximeter (CMS60D-VET, Contec Medical Systems, Quinhaungdao, China) at 5-min intervals. We determined gender and estimated age as yearling (6–18 mo) or adult (>18 mo), based on tooth wear and eruption (Crowe 1975; Rolley 1985; Hughes et al. 2019). We ear-tagged, measured morphometrics, and determined the physical condition and presence of ectoparasites. We fitted bobcats with global positioning system (GPS; 341 g; Survey Globalstar 1C, Vectronic Aerospace, Berlin, Germany) or very high frequency (VHF; 146 g; M2220B, Advanced Telemetry Systems, Isanti, Minnesota, USA) radio collars, depending on body weight: bobcats >6.8 kg with GPS collars and individuals <6.8 kg with VHF collars. All radio collars weighed <5% of an animal's body mass at the time of capture (Hughes et al. 2019). We collected blood samples from the cephalic vein using 22-gauge, 3.81-cm needles and 3-mL syringes and placed in plain (red top) and EDTA whole blood (purple top) tubes (Vacutainer, Becton Dickinson, Franklin Lakes, New Jersey, USA). Blood samples were centrifuged at 2,750 × G for 10 min, and serum samples were frozen prior to further analyses. We collected tissue samples from a 6-mm ear punch for genetic analyses and recorded ambient temperature (C) at bobcat capture sites. We reversed xylazine with an intramuscular injection of 4 mg/kg tolazoline hydrochloride (Tolazine, Midwest Veterinary Supply) and medetomidine with 1.6 mg/kg atipamezole hydrochloride (Antisedan, Midwest Veterinary Supply). We used tolazoline as a reversal agent based on recommendations by Nielsen et al. (Nielsen and Woolf 2001, 2002a) and because it was readily available for purchase from a local vendor.

We recorded time of injection of reversal agents, placed bobcats in a wire cage traps, and recorded times to initial raising of the head (head up), sternal recumbency (laying on stomach with chest and head upright), standing, and time to full recovery. We considered an animal fully recovered when there were no visible signs of sedation (i.e., ability

to stand, ambulate, and walk normally). We released all bobcats at capture locations and monitored movement and survival status of collared individuals daily for 2 wk postcapture and weekly thereafter throughout the study duration. Deaths that occurred within 2 wk postcapture were considered captured related and censored from analyses. All animal handling methods were approved by the Institutional Animal Care and Use Committee at Western Illinois University (approval number 17-01), and strictly adhered to guidelines for the care and use of animals were approved by the American Society of Mammalogists (Sikes and Animal Care and Use Committee of the American Society of Mammalogists 2016).

Our study was conducted in a 9,327-km^2 area throughout Adams, Fulton, Hancock McDonough, and Schuyler Counties of west central Illinois, US. The region was rural and sparsely populated (13.6 persons/km^2; US Census Bureau 2016). The majority (65%) of land across the study site was characterized by row crop (i.e., corn [*Zea mays*] and soybeans [*Glycine max*]) agriculture, whereas remaining acreage constituted forest (25%), development (6%), and open water and wetland (4%; Homer et al. 2015). Elevation across the region ranged from 125 m to 244 m above sea level (Illinois State Geological Survey 2003). Dominant overstory woody vegetation consisted of white oak (*Quercus alba*), post oak (*Quercus stellata*), black oak (*Quercus velutina*), and mockernut hickory (*Carya alba*; Luman et al. 1996). Average summer and winter temperatures were 23.3 and −3.0 C, respectively (Suhl 2006; Walker 2001; Preloger 2006; Tegeler 2002a, b). Total annual precipitation and seasonal snowfall across the region averaged 98.8 and 68.4 cm, respectively (Suhl 2006; Walker 2001; Preloger 2002; Tegeler 2003a, b).

Data analyses

Before analyses, we screened physiologic parameters and timing to stages of immobilization variables for normality using quantile plots and a Shapiro-Wilk test (Shapiro and Wilk 1965). We used a two-sample *t*-test for parameters that were normally distributed and used the appropriate *P* value for equal or unequal variance (Williamson et al. 2018). We used a Wilcoxon two-sample test for parameters that were not normally distributed (Wilcoxon 1945). We used analysis of variance (ANOVA) with all possible two-way interactions and main effects to evaluate potential effects of ambient temperature and drug type on time to anesthesia and full recovery of chemically restrained bobcats. We used one-way ANOVA to evaluate potential differences in ambient temperature at processing sites for KX- and BAM-treated bobcats. We conducted statistical tests using Program R (version 3.3.3; R Core Team 2017); statistical tests were conducted at $\alpha=0.05$.

RESULTS

We captured and processed 23 bobcats (21 by licensed fur trappers and two cage trapped) from November 2017 to March 2018 and November 2018 to March 2019. Median body mass (kilograms) of male and female bobcats was 10.78 (interquartile range [IQR]=10.21–11.97) and 7.48 (IQR=6.58–8.17), respectively. We used BAM on 12 bobcats (five adult males, five adult females, one yearling male, and one yearling female) and KX on 11 bobcats (four adult males, four adult females, and three yearling females). The mean initial dosage for KX was 11.88 mg/kg ketamine (range=7.73–14.32 mg/kg) and 1.93 mg/kg xylazine (range=1.53–2.27 mg/kg). The mean initial dosage for BAM was 1.04 mg/kg butorphanol (range=0.724–1.12 mg/kg), 0.35 mg/kg azaperone (0.22–0.39 mg/kg), and 0.41 mg/kg medetomidine (range=0.37–0.46 mg/kg). Six of 11 (55%) bobcats given KX were effectively immobilized with a single injection. Four (36%) individuals required additional drugs (one-half of the original dosage) to allow adequate anesthesia for processing (one of which had a weight that was underestimated). In addition, one individual injected with a pole syringe and initially immobilized required an additional dose of ketamine after partial arousal (~15 min after

TABLE 1. Comparisons of physiologic parameters and time to stages of immobilization for bobcats (*Lynx rufus*) captured and immobilized across west central Illinois, USA, November 2017 to March 2018 and November 2018 to March 2019. Bobcats were administered an intramuscular injection of ketamine-xylazine (i.e., 10 mg/kg ketamine and 1.5 mg/kg xylazine) reversed with 4 mg/kg tolazoline or BAM (i.e., 0.8 mg/kg butorphanol, 0.27 mg/kg azaperone, and 0.32 mg/kg medetomidine) reversed with 1.6 mg/kg atipamezole. Six of 11 (55%) bobcats administered KX and six of 12 (50%) bobcats administered BAM were successfully immobilized with a single injection.

	BAM				Ketamine-xylazine				
Measurement	$\overline{X}$ (n)	SE	Median (n)	Interquartile range	$\overline{X}$ (n)	SE	Median (n)	Interquartile range	P
Time to stages of immobilization (min)									
Sternal recumbency	3.14 (10)	0.27	7.00	4.25–8.00	6.71 (10)	0.74	7.5	4.50–10.75	0.19[a]
Head down	5.60 (9)	0.30	5.00	5.00–5.75	5.83 (9)	0.77	6	3.00–6.00	0.91[a]
Full anesthesia	14.50 (11)	1.13	7.50	6.00–12.00	11.40 (11)	0.67	8	7.00–13.50	0.82[a]
Head up after reversal	4.00 (10)	0.20	4.00	3.00–5.63	4.43 (9)	0.58	2	2.00–3.00	0.73[a]
Sternal recumbency after reversal	5.42 (12)	0.18	6.00	3.00–7.00	7.33 (10)	0.8	6.5	4.63–8.50	0.89[a]
Standing after reversal	10.20 (12)	0.60	9.00	6.00–10.50	47.40 (10)	3.21	44	31.00–63.75	0.02[a]
Total recovery time after reversal	15.40 (12)	1.32	12	8.88–15.25	69.80 (10)	3.28	69	51.00–76.00	0.002[a]
Physiologic parameters									
SpO_2 (%)[b]	91.11 (10)	0.72	90.00	87.00–96.25	93.13 (10)	0.78	92.50	91.25–97.50	0.52[c]
Rectal temperature (C)	38.53 (10)	0.14	38.26	36.27–38.83	39.13 (10)	0.12	38.71	37.97–39.43	0.29[c]
Pulse rate (heart beats/min)	83.44 (10)	1.20	88.00	75.50–89.00	134.88 (10)	1.77	132.00	121.75–142.25	<0.001[c]
Respiration rate (breaths/min)	27.89 (10)	0.61	28.00	24.00–31.50	28.13 (10)	0.81	28.00	21.25–30.75	0.94[c]

[a] P value from Wilcoxon two-sample test.
[b] SpO_2 = hemoglobin oxygen saturation; measure (%) of how much hemoglobin is bound to oxygen relative to how much hemoglobin remains unbound.
[c] P value from two-sample t-test.

initial injection) during processing. Of 12 bobcats given BAM, six (50%) were effectively immobilized with a single injection, and three (25%) individuals were not completely immobilized and required additional drugs to allow adequate time for processing. In addition, three (25%) of 12 bobcats treated with BAM required additional doses after complete arousal during processing (including a pregnant female that had a seizure).

Our analyses revealed no significant two-way interactions between drug type or ambient temperature on time to anesthesia ($F_{1,19}$=0.44; P=0.51) or full recovery since reversal ($F_{1,19}$=0.50; P=0.49); thus, we report results for main effects. Induction parameters, times to first effect (i.e., sternal recumbency and head down), and full anesthesia did not differ between bobcats treated with KX and BAM (Table 1). Median pulse rate was higher ($t_{18.45}$=−10.15; P<0.001) for bobcats given KX (median=132.00; IQR=121.75–142.25) than bobcats given BAM (median=88.00; IQR=75.50–89.00; Table 1). We did not detect differences in hemoglobin oxygen saturation, rectal temperature, or respiration rates between BAM or KX (Table 1). Similarly, times to head up and sternal recumbency after reversal were similar (P≥0.64) between bobcats given BAM and KX (Table 1). However, times to standing and full recovery after reversal were faster for bobcats given BAM compared with bobcats given KX (Table 1). Median ambient temperatures at processing sites were similar ($F_{1,21}$=0.02; P=0.89) among BAM (median=6.00; IQR=1.05–6.55) and KX treatments (median=6.00; IQR=0.85–7.50).

DISCUSSION

Our results support the use of KX as an effective immobilization agent for free-ranging bobcats. A mixture of ketamine (10–15 mg/kg) and xylazine hydrochloride (1–1.5 mg/kg) is an effective dose to safely immobilize bobcats for radio tagging, blood sampling, and other basic procedures under field conditions (Beltrán and Tewes 1995; Rockhill et al. 2011). The actual dose for KX we used (11.88 mg/kg KX and 1.93 mg/kg xylazine) is within the published range of dosages for immobilizing free-ranging medium-sized felids (Fuller et al. 1985; Crawshaw and Quigley 1989; Rockhill et al. 2011), though lower doses of ketamine may be used if the dose of xylazine is increased (Beltrán and Tewes 1995). Bobcats were largely unresponsive to external stimuli (i.e., noises and motion) during processing; thus, safe handling was achieved using KX. Individuals that required additional drug volume were attributed to underestimation of body weight or partial loss of drug at the time of initial injection. Our results indicated that KX provided relatively fast anesthesia (i.e., <11 min) and acceptable heart and respiratory rates, rectal temperature, and oxygen saturation. However, consistent with Williamson et al. (2018), time to full recovery was long and variable (range=41–116 min), and bobcats given KX commonly exhibited a lack of coordination while attempting to stand during recovery.

The time to standing and full recovery of bobcats given BAM was consistently smooth and rapid relative to bobcats given KX, with no visible loss of coordination or ataxia upon release. Rapid recovery associated with antagonism with atipamezole is a primary advantage of the use of BAM in the capture and restraint of carnivores (Wolfe et al. 2008). During our study, bobcats given BAM and reversed with naltrexone and atipamezole immediately ran away from the capture site upon release. Although induction appeared complete in bobcats given BAM, most individuals responded to tactile stimuli and mild to moderate noise levels after injection; 11 bobcats (six BAM treated and five KX treated) required boosters (50% of initial drug volume) to complete chemical restraint after partial or full arousal during processing. The need for additional doses may have been attributed to the drug volume (i.e., partial vs. full), injection location (i.e., subcutaneous and adipose tissue), the drug coming out of solution in cold temperatures, variation in stress level of captured bobcats, or inadequate drug dosages (Williamson et al. 2018).

Reproductive status may play a role in the overall efficiency of immobilization; Rockhill et al. (2011) and this study noted seizures in bobcats postimmobilization or upon reversal. Pregnant females may process drugs differently, thereby contributing to greater uncertainty in the physiologic responses to immobilization drug. Medetomidine has been linked to seizures in domestic dogs (*Canis familiaris*; Rainger et al. 2009). Although pregnancy status is typically not confirmed until after immobilization, researchers should be prepared to mitigate seizures.

Decreases in heart rate with BAM were consistent with previous research and likely due to peripheral vasoconstriction and lack of a dissociative to stimulate cardiac activity associated with the individual constituents in BAM (Lamont et al. 2001; Wolfe et al. 2008; Williamson et al. 2018). Consequently, nearly half ($n=4$) of bobcats given BAM exhibited mild hypoxemia. In contrast, all but one bobcat given KX had initial SpO_2 values $\geq$90%; the lowest SpO2 for BAM-treated bobcats was 83%. Our BAM dosages were more than double the recommended starting dosages and exceeded the upper limit of published dosages used for immobilizing captive black bears and African lions (Wolfe et al. 2008; Semjonov et al. 2017), indicating that doses of butorphanol tartrate, azaperone, and medetomidine used in our study may require additional adjustments for more desirable sedation outcomes for free-ranging bobcats or that small animals with high metabolic rates need higher dosages per unit of body weight (Sontakke et al. 2017). Nevertheless, our results revealed no evidence that recovery or postrelease survival was negatively affected for bobcats given BAM, as was observed with other immobilizing agents that incorporate α-2 agonists (Wolfe et al. 2008). Further evaluation of BAM for use in anesthetizing free-ranging bobcats appears warranted. Optimizing the individual constituents in BAM might result in anesthesia levels that reduce potential stress. Although reducing crew size and noise is a common practice, extra caution may need to be taken with BAM, especially during the initial immobilization. Bobcats were easily aroused with BAM, and minimizing disturbance helps ensure full immobilization and increased safety to animals and field personnel.

Primary goals of field-based research include the safety of animals and human operators and handling of research subjects that ensures postrelease survival (Ellis et al. 2019). Although the incidence of partial or full arousal in BAM-treated bobcats was relatively high (i.e., 50%), researchers should consider potential trade-offs between taking appropriate safety precautions when further evaluating and optimizing BAM dosages and prolonged recovery periods with KX. Long recovery times associated with KX may impede the ability of immobilized bobcats to thermoregulate during extreme weather conditions and, in turn, contribute to prolonged stress. If processing times extend beyond 30 min, the KX combination may be necessary, in which case researchers must be prepared to mitigate potentially undesirable side effects or increased vulnerability of individuals to natural (e.g., predation) or anthropogenic (e.g., vehicle collisions or harvest) mortality factors to obtain the most relevant research data as possible for free-ranging bobcats (Rockhill et al. 2011, Ellis et al. 2019). In contrast, BAM provided minimal sedation and reduced processing times (<30 min). Hence, we suggest that researchers increase initial BAM drug volumes for yearling and adult bobcats at the time of processing and take appropriate safety precautions (e.g., secure individuals using hobble straps, muzzles, or in capture bags to minimize risk of injury, particularly during instances of partial or full arousal) when handling free-ranging bobcats.

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