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RESEARCH ARTICLE

Evaluation of the effects of multiple capture methods and immobilization drugs on mountain lion welfare

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Abstract

Using a dataset of 591 capture events between 2001–2019 in California, USA, we examined the impact of capture methods and immobilization drugs on mountain lion (*Puma concolor*) welfare. The 3 methods used to capture mountain lions were cage traps, trained hounds, and cable restraints. The drugs used to immobilize mountain lions were either tiletamine/zolazepam (Telazol®), ketamine/medetomidine, or ketamine/xylazine. Mortality occurred in 1.4% of captures, with only one mortality out of 310 captures occurring since 2012. We used a logistic regression framework to compare morbidity and vital parameters of mountain lions among the different capture methods and immobilization drugs used. Vomiting (a risk factor for developing aspiration pneumonia) was the most common severe risk factor associated with cage trapping and was only seen with the use of ketamine/medetomidine or ketamine/xylazine. Morbidity scores were not well predicted by any of the variables we accounted for. Animals immobilized with Telazol® were more likely to experience abnormal heart and respiratory rates, as well as high body temperatures, than those

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immobilized with the other two combinations. Although there are risks associated with each of the capture methods and drug combinations commonly used in mountain lion captures in California, our analyses demonstrated they are all relatively safe when following appropriate animal welfare practices. Our analyses suggested that unaccounted for factors are equally or more important in explaining injury and physiological abnormality rates, and we urge that agencies train personnel in best practices and conservative decision-making in order to assure that the welfare of the animal takes precedence over collaring. We suggest training on how to choose between several capture techniques, immobilization drugs and monitoring methods and how to reduce both detrimental effects to mountain lions and danger to humans.

KEYWORDS
cable restraint, cage trap, chemical immobilization, hounds, large carnivore, *Puma concolor*, vital parameters, wildlife capture

Wildlife professionals use a variety of methods to safely and effectively answer research questions to aid wildlife conservation and management. Noninvasive research methods can provide substantial information (Gompper et al. 2006, Long et al. 2012), but animal capture is often necessary to investigate research questions that cannot be addressed using noninvasive approaches (Proulx et al. 2012). Captures present the opportunity to collect biological samples, record biometric data, assess age, health and reproductive status, and attach global positioning system (GPS) collars or other tracking devices to study habitat use, mortality, survival, prey selection and other behaviors. Wildlife professionals conducting captures are expected to be trained and use techniques that, in addition to being effective, are safe for both animals and researchers (Powell and Proulx 2003). Advances have been made in devising effective capture techniques and chemical immobilization protocols that provide acceptable margins of safety (Sleeman and Clark 2003, Wobeser 2007, Chinnadurai et al. 2016). Nonetheless, there are inherent risks in every procedure where animals are restrained and chemically immobilized, especially as field conditions can vary and researchers are not afforded previous knowledge of an individual's health status, as would likely be the case in a controlled environment such as a zoo (Caulkett and Arnemo 2015).

Wildlife professionals in charge of carnivore management and conservation are often tasked with capturing and handling potentially dangerous carnivores, such as mountain lions (*Puma concolor*) in North America. The 3 main methods used for capturing mountain lions are trained hounds, cage traps, and cable restraints (also referred to as foot snares; Cougar Management Guidelines Working Group 2005). However, availability of capture methods can vary depending on state or provincial laws. In California, the frequency of use of the 3 capture methods has changed over the last decade; after the use of cable restraints to capture mountain lions was prohibited by state law in 2012, they have only been used by federal employees on lands where wildlife management falls under federal jurisdiction (e.g., certain National Park Service lands). Concomitantly, the use of cage traps increased statewide. The 3 main immobilization drugs used in California, and across North America, over the last 20 years to anesthetize mountain lions are Telazol® (or an equivalent generic tiletamine/zolazepam combination, hereafter referred to as TZ; Lescano et al. 2014), and ketamine in combination with either medetomidine (referred to as KM hereafter) or xylazine (referred to as KX hereafter; Albuquerque et al. 2016). The pros and cons, and ways in which each drug combination work, are important but beyond the scope of this paper (e.g., see West et al. 2007, Kreeger 2012, and Kreeger and Arnemo 2018). In general, the potential for complications from all 3 immobilization drugs is likely to decrease as the familiarity of the wildlife professional increases, both with the animals they are sedating, leading to more accurate weight estimations, and with the immobilization drugs they are using (Arnemo et al. 2006).

Ideally, all capture events should be documented in systematic detail, describing environmental temperatures, pursuit (i.e., after the animal is jumped by hounds) length and distance, how many and which researchers were present, time of capture and length of time captive in traps and cable restraints before processed, drugs used, time between anesthesia and release, injuries, complications, support procedures (e.g., cooling, warming, hydrating) and animal response. Systematic detailed reporting combined with after-capture data such as from GPS collars and analysis of biological samples, can elucidate capture-related stress, long-term behavioral alterations, and shifts in biological parameters (Cattet et al. 2003, Arnemo et al. 2006, Cattet et al. 2008).

Studies examining the effects of capture methods and drugs on the welfare of free-ranging mountain lions are limited to work by Logan et al. (1999) and Elbroch et al. (2013). Logan et al. (1999) suggested improvements to cable restraints to reduce nontarget captures and injuries to mountain lions. Elbroch et al. (2013) compared injury scores to mountain lions captured from the use of hounds versus cable restraints. However, information on how injury scores and vital parameters (i.e., heart and respiratory rates, and body temperature) vary between capture methods and immobilization drugs in captured and anesthetized mountain lions will continue to be useful for supporting safe capture and handling procedures (Lescano et al. 2014, Albuquerque et al. 2016, Andreasen et al. 2018). Further assessment of how capture methods and immobilization drugs influence likelihood of injury and impact physiological responses, through the alteration of vital parameters, is necessary to understand which capture and drug combinations are most effective for conducting capture operations that are as safe as possible (Powell and Proulx 2003).

Using a dataset of mountain lion capture events between 2001 and 2019 in California, we report summary statistics of vital rates and morbidity scores by capture method and immobilization drugs used. Further, we sought to compare vital rates amongst capture methods and immobilization drugs used, respectively. Lastly, we examined factors influencing prevalence of abnormal vital parameters and capture-related injuries.

STUDY AREA

We conducted captures from 2001 to 2019 within 12 study areas on an assortment of private, county, regional, state, federal, and tribal lands in California, USA, which had an area of 423,970 km^2 (Figure 1; Table 1). Greater details on project areas can be obtained from the individual studies (Table 1). Our study areas represented substantial variability in the level of human use and development with some project areas focused on mountain lions in isolated urban populations while others focused on populations occupying primarily public lands including large contiguous blocks of wilderness (Dellinger et al. 2020; Figure 1). The various ecoregions encompassed large gradients in physical attributes such as elevation (from sea level to ~4,000 m), seasonal precipitation (13.1–140.9 cm), and temperature (−15–45°C).

METHODS

Summary of captures and data management

We analyzed data from 591 capture-events of 355 individual mountain lions that were captured and immobilized in California as part of 9 different research projects conducting captures in 12 different study areas (Table 1). Captures were led by trained and appropriately permitted agency, academic, and nonprofit professionals.

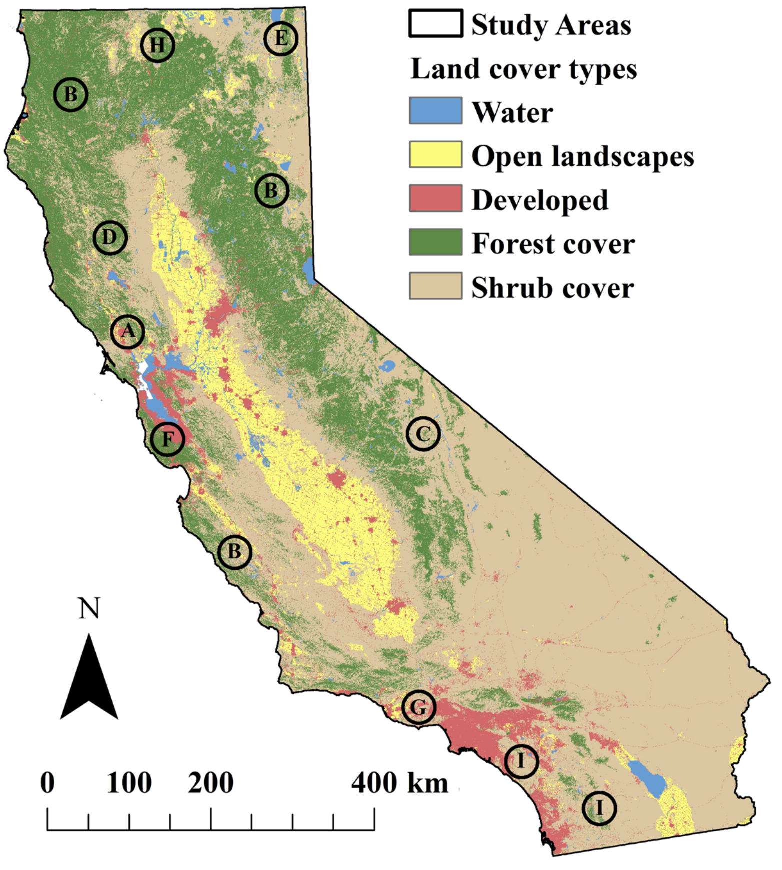

FIGURE 1 Map showing the general areas where the 591 research capture events of free-ranging mountain lions (*Puma concolor*) occurred in California, USA, from 2001 to 2019 to examine the impact of capture methods and immobilization drugs on this species. Note some projects conducted captures in multiple areas. Letters within each project area correspond to Table 1.

Notwithstanding the nuances of each capture method employed, we briefly discuss each method used. Trained hounds follow the scent left by the targeted animal until it is located, typically up a tree or among rocks or boulders, where it can then be safely chemically immobilized with a dart gun (McBride et al. 2008). Cage trapping involves the use of either single or double door box traps that are baited, or of a walk-through design placed on trails. Cable restraints consist of a throwing mechanism that is triggered when the animal steps inside a loop of wire cable, which causes the loop to tighten and restrain the animal's foot. The potential for injuries from all 3 types of captures is likely to increase as ambient temperatures become more extreme and as duration of a chase by hounds or time in cage traps or cable restraints increases (White et al. 2021).

During captures, we sexed and aged (Laundré et al. 2000) immobilized mountain lions and assessed body condition, reproductive status (when possible, based on the situation), and any injuries already present and that occurred during capture (i.e., fresh wounds). Body measurements (e.g., weight, total body length, etc.) and biological samples (e.g., blood, feces, hair, whiskers, small biopsy of ear pinna tissue) were collected when possible. Additionally, 1 or 2 ear tags and a tracking collar (GPS or Very High Frequency [VHF]) were fitted on most animals. We classified animals estimated to be <2 years of age as subadults, and those >2 years of age as adults. We did not evaluate captures of small kittens (<4 months of age) that were able to be physically restrained without anesthesia. Vital parameters were periodically measured during immobilization, including body temperature (°C), and heart and

TABLE 1 Summary of mountain lion (*Puma concolor*) captures, including data recording and morbidity scores, by project in California, USA, from 2001 to 2019. The full suite of temperature, pulse, and respiration (TPR) measurements was not recorded for all captures. Further, there was variation in the amount of note taking between projects which was used in part to inform morbidity scores for each capture. Letters preceding each project citation correspond to letters in Figure 1.

Project	Years	# Captures	Avg. Morbidity Score	# Captures w/all TPR Measurements	Avg. Morbidity Score w/all TPR Measurements	# Captures with Injury/Abnormal TPR Notes	Avg. Morbidity Score Based on Notes	Deaths
A - Dellinger et al. (2020)	2016–2019	23	29.6	21	31.0	6	8.9	0
B - Dellinger et al. (2020)	2016–2019	35	14.6	32	15.2	5	1.4	0
C - Gammons et al. (2021)	2001–2019	150	26.2	49	16.6	18	0.8	5
D - Allen et al. (2015)	2010–2012	15	60.0	10	32.5	3	8.6	1
E - Ewanyk (2020)	2016–2019	36	38.8	35	38.6	18	8.9	0
F - Wilmers et al. (2013)	2010–2019	84	31.9	60	32.3	31	7.3	0
G - Riley et al. (2021)	2002–2019	95	13.7	28	10.9	33	4.9	0
H - Cristescu et al. (2022)	2017–2019	19	58.4	19	58.4	12	17.6	1
I - Vickers et al. (2015)	2001–2106	134	30.2	118	27.7	48	6.7	1
Total		591	33.7	372	29.2	174	7.2	8

respiratory rates in beats/breaths per minute. Pulse oximetry data was collected in approximately 20% of captures. If necessary, interventions (e.g., oxygen supplementation, administration of additional immobilization drugs, or other drugs such as atropine or doxapram to address low heart or respiratory rates) were provided. The frequency of vital rate measurements and physiological parameters that were consistently recorded varied among the teams, with body temperature being the single parameter that was most frequently recorded.

We summarized the data set detailing capture events by capture method, immobilization drugs, number of capture events per individual, age class, and sex of captured mountain lions using descriptive statistics. We did not include reproductive status (i.e., presence of dependent young for adult females captured) given the variability in documentation. We tested the capture data for normality using the Shapiro-Wilk test and then chose the appropriate statistical test for each vital parameter.

Mean vital rates

We investigated the effects of capture method and immobilization drugs on mountain lion vital parameters (i.e., body temperature, heart rate, and respiratory rate). We used a repeated measures ANOVA to compare the mean body temperatures of mountain lions among the 3 capture methods using the first 4 recordings of body temperature after the capture event. We performed similar analyses for heart and respiratory rates. We also used a repeated measures ANOVA to compare the means of vital parameters between the 3 drug combinations: TZ, KM and KX.

Abnormal vital rates

Following the initial statistical comparisons, we examined whether any capture-related (i.e., capture method and immobilization drug) or animal-related (i.e., age class and sex) variables were associated with the occurrence of abnormal vital parameters using individual binomial logistic regressions. Abnormal heart rate (beats/min) was defined as <40 and >200; abnormal respiration (breaths/min) was defined as <15 and >50; and abnormal body temperature (°C) was defined as <37.2° and >40° (Sontakke et al. 2009, Lescano et al. 2014). We differentiated captures in which we recorded at least one abnormal vital parameter occurred from those in which there were no abnormal measurements. We compared individual measurements of each vital parameter and the mean of the first 4 measurements of each vital parameter during a capture to the physiological thresholds. This method allows assessment of the entire capture event as well as any individual vital rate deviations outside normal limits, which both have potential significance to the safety of the animal.

We assigned capture events with ≥1 reported vital parameter values above or below the threshold a 1 (abnormal), whereas captures reporting only values within the thresholds were assigned a 0 (normal). For each of the 3 vital parameters, we created identical sets of models which we then ranked based on Akaike's Information Criterion for small sample sizes (AIC_c) and derived AIC_c weight (w) to choose the most parsimonious model (Burnham and Anderson 2002). We considered models to be supported if $\Delta AIC_c < 2$ and report coefficient estimates (CE) for these top models using a multi-model inference approach. Model fit was assessed by calculating the percentage of deviance explained and by checking the discrimination of the logistic model via the receiver operating characteristic (ROC) and area under the estimated ROC curve (AUC).

Morbidity

To assess the prevalence and severity of injuries, as well as other physiological events and vital parameter alterations, we modified scoring systems introduced by Olsen et al. (1986) and Onderka et al. (1990), to incorporate several capture methods and physiological variations outside of normal limits (Table 2). Our approach resembles that of Elbroch et al. (2013), except we included cage trapping as one of our capture methods. Additionally, our scoring system incorporated specific injuries and physiological events such as vomiting, paddling leg movements, and seizures. Individual injuries, physiological events, and deviations from normal vital parameters were each assigned a score based on severity. Scores reflect risk of negative effects on the animal both during the immobilization procedure and the risk of longer-term effects on fitness, with more serious injuries, events, and deviations assigned higher scores. Morbidity scores for a capture were the total of scores for all injuries or abnormal conditions noted. Each capture received a single cumulative morbidity score. A mortality event received the highest score. Scientific collection permits issued by California Department of Fish and Wildlife require that any study animals dying shortly after capture be necropsied to investigate the cause of mortality which helped determine if mortalities shortly after release were due to injuries suffered during capture.

We performed an ordinal logistic regression using cumulative morbidity score as the response to determine if any capture-related or animal-related variables were associated with increased odds of having a higher morbidity score. We used the same set of variables, candidate models, and model evaluation framework as applied in the logistic regression analysis of mountain lion physiological response to capture and immobilization. We were unable

TABLE 2 Scoring system modified from Olsen et al. (1986) and Onderka et al. (1990) for assessing the severity of injuries and other incidents that might occur during capture events in mountain lions (*Puma concolor*).

Condition	Points	Justification
Apparently normal	0	
Paddling or light anesthesia	5	Increased stress, risk of myopathy, possibility of injuries, puts researchers at risk, higher risk of hyperthermia
Broken claw	5	Minor restriction gripping prey
Abrasion	5	Pain
Prolonged recovery	5	Risk of vital parameter alteration, increased stress
Limb swelling	10–Mild	Pain, decreased mobility, skin damage, possible ligament, muscle, and nerve damage
	15–Moderate	
	20–Severe	
Cutaneous laceration <2 cm	10	Pain, risk of infection
Chipped canine	10	Pain, potential decreased hunting effectiveness
Light hound bite punctures	10	Pain, risk of infection
Cutaneous laceration >2 cm	15	Pain, risk of infection
Anesthesia too light procedure was not complete	15	Increased stress, risk of myopathy, puts researchers at risk, higher risk of hyperthermia
Digit fracture	20	Pain, decreased mobility, potential decreased hunting effectiveness
Severe hound bite punctures	30	Pain, risk of infection
Hypo/Hyperthermia	30	Risk of death under anesthesia or secondary effects on body systems
Tachycardia/Bradycardia	30	Risk related to inadequate blood flow
Tachypneic/Apneustic breathing	30	Risk related to inadequate oxygenation/ventilation
Broken canine	30	Pain, risk of infection and tooth decay, potential decreased hunting effectiveness
Amputation of digit	40	Pain, decreased mobility, potential decreased hunting effectiveness
Joint luxation	50	Pain, decreased mobility, potential decreased hunting effectiveness
Seizure	60	Pain, increased stress, risk of myopathy, puts researchers at risk, higher risk of hyperthermia
Vomit/Regurgitation	80	Choking, risk of aspiration pneumonia
Other fractures	100	Pain, decreased mobility, potential decreased hunting effectiveness, may lead to life-threatening injury
Apnea/Cessation of breathing/Oxygen support/Doxapram administration	100	Risk of immobilization-related death, brain or other tissue injury secondary to oxygen deprivation
Death	500	

to assess the impact of likely influential variables including, but not limited to, pursuit time, time in trap, handling and processing time, and weather on vital parameters and injuries because such observations were rarely collected. We used Program R version 4.1.1 (R Core Team 2021) for data management and all statistical analyses.

RESULTS

Summary of captures and data management

Of the 355 individual mountain lions captured at least once, 193 were males and 162 were females. Of the 236 recaptures, 119 were males and 117 were females. The average measured (by scale in over half of captures) or estimated (by experienced biologists) weight of all animals captured was 43.7 kg (SD = 13.2 kg; min–max = 9.5–80 kg). The average estimated age of all animals captured was 48 months (SD = 34; range = 5–180). Most animals were only captured once (n = 355; 60%) or twice (n = 151; 25.5%) but one female was captured 9 times and was estimated to be 180 months old at her last capture. Cage traps (n = 270) and trailing hounds (n = 264) were the most frequently used capture methods; cable restraints were less commonly employed, especially since 2012 (n = 57; Figure 2A).

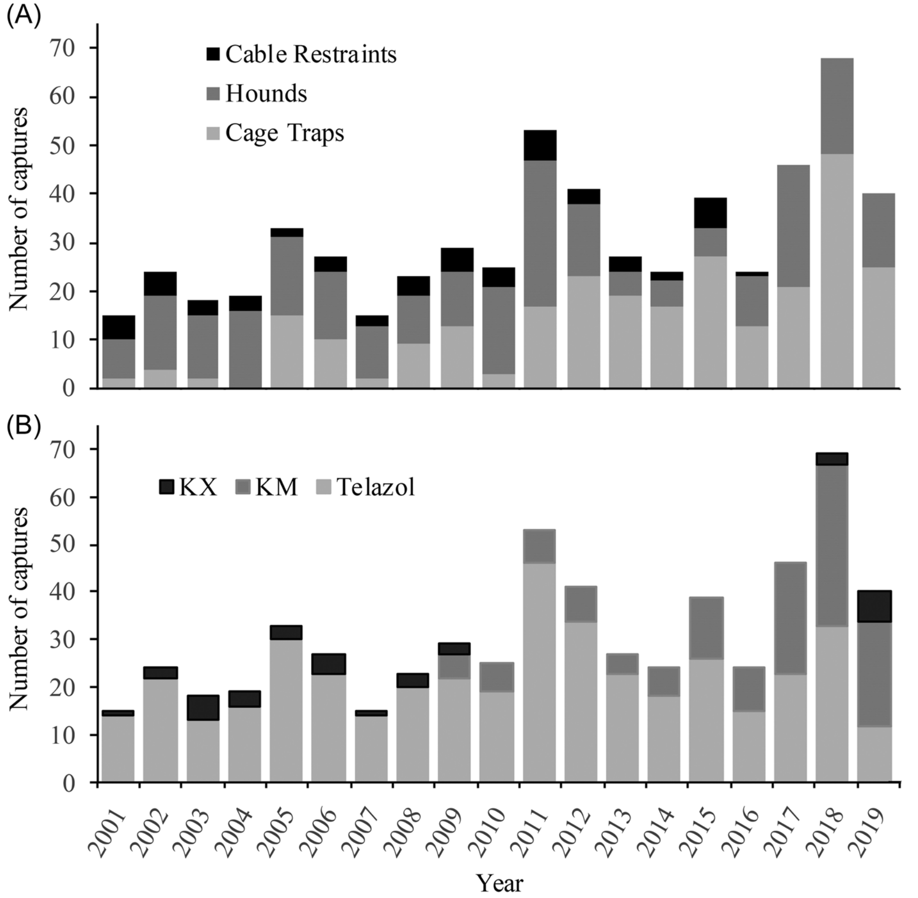

FIGURE 2 Variation in use of the (A) 3 capture methods (cage trap, cable restraint and hounds) and (B) 3 immobilization drugs (Telazol, Ketamine/Medetomidine, and Ketamine/Xylazine) used in 591 research captures of free-ranging mountain lions (*Puma concolor*) in California, USA, from 2001 to 2019.

Frequency of immobilization drug use changed over the 19-year time period. Except for the last 3 years of data collection (2017–2019), TZ was the most frequently used drug combination ($n = 423$), with KM ($n = 136$) increasingly used from 2009 onward. Use of KX ($n = 32$) was infrequent and largely unused from 2009 onward (Figure 2B). For some captures, immobilization drugs were premixed, while for others the capture lead mixed the drugs based on the visually-estimated weight of the animal. Thus, the dosage rates and ratios (e.g., ratio of ketamine to medetomidine) of immobilization drugs were not consistent across projects. Average dosage of TZ administered was 5.2 mg/kg (SD = 1.76). In contrast, average dosage in the KM combination was 4.07 mg/kg (SD = 2.91) and 0.08 mg/kg (SD = 0.03), respectively. Lastly, average dosage in the KX combination was 7.56 mg/kg (SD = 2.11) and 0.45 mg/kg (SD = 0.37), respectively.

Mean vital rates

The mean of each of the 3 vital parameters varied across all capture methods and drugs used. Although not all vital parameters were measured during all captures, the mean of vital rates that were recorded remained within normal limits in 59% of captures. All 3 vital rates were recorded in 63% (372 of 591) of captures. For the aforementioned 63% of captures, mean vital rates remained within normal limits for 50% (186 of 372) of those captures. Mean heart rates were highest in cage trap captures ($\bar{x} = 142$ beats/min.) and mean body temperatures highest in hound captures ($\bar{x} = 39.7$°C) but mean respiratory rates did not vary significantly by capture method. Means of all 3 vital parameters were highest with TZ ($\bar{x} = 148$ beats/min., 34 breaths/min., and 39.3°C), compared to KM ($\bar{x} = 102$ beats/min., 23 breaths/min., and 39.0°C) and KX ($\bar{x} = 70$ beats/min., 24 breaths/min., and 39.2°C).

We observed that mean heart rate of mountain lions was significantly higher for animals captured with cage traps ($\bar{x} = 142$ beats/min.; 95% CI = 136–148 beats/min.) than those we captured using hounds or cable restraints (Table 3). However, mean heart rates were not significantly different between hound and cable restraint captures. Mean body temperature of mountain lions was significantly higher ($\bar{x} = 39.7$°C; 95% CI = 39.5–39.9°C) for animals captured with hounds than those we captured using cage traps or cable restraints. However, mean body temperatures were not significantly different between cage trap and cable restraint captures. We detected no difference in mean respiratory rate of mountain lions when they were captured by our 3 methods (Table 3).

We observed that mean heart rate of mountain lions was significantly higher for animals immobilized with TZ compared to those immobilized with KM and KX. Further, mean heart rates were also significantly higher for captures that used KM compared to KX (Table 3). Mean respiratory rate of mountain lions was significantly higher for animals immobilized with TZ ($\bar{x} = 34$ breaths/min.; 95% CI = 31–37 breaths/min.) compared to those immobilized with KM ($\bar{x} = 23$ breaths/min.; 95% CI = 17–29 breaths/min.). However, mean respiratory rate of animals immobilized with KX were not significantly different from either TZ or KM (Table 3). Mean body temperature of mountain lions was significantly higher for animals immobilized with TZ ($\bar{x} = 39.3$°C; 95% CI = 39.2–39.4°C) compared to those immobilized with KM ($\bar{x} = 39.0$°C; 95% CI = 38.9–39.1°C). However, mean body temperature of animals immobilized with KX were not significantly different from either TZ or KM.

Abnormal vital rates

In 89% (524 of 591) of captures, at least one vital parameter was recorded with multiple measurements over time. Of these captures, a single measurement of a vital parameter outside the normal range occurred in 45.8% ($n = 240$) of captures, with 19.8% ($n = 104$) of captures having abnormal values for two or more vital parameters during the same capture. Given that the only abnormal heart rates documented were associated with TZ, which means there was quasi-complete separation in the dataset, we did not include drug combination in modeling of abnormal heart rates as this would have influenced coefficient estimates and inflated standard errors. Thus, we identified two

TABLE 3 Mean, standard deviation (SD), and results of statistical comparisons of vital parameters in free-ranging mountain lions (*Puma concolor*) captured in California, USA, from 2001 to 2019. Values are shown by capture method and immobilization drug, respectively. Matching letters indicate no significant difference between capture methods or immobilization drug at $P = 0.05$. Mismatched letters indicate significant difference between capture methods or immobilization drugs ($P \leq 0.05$).

	Heart rate (beats/min)				Heart rate (beats/min)		
Method	**Mean**	**SD**	***P***	**Drug**	**Mean**	**SD**	***P***
Hounds	114	65.9	A	TZ	148	45.3	A
Cable Restraint	112	61.6	A	KX	70.0	55.8	B
Cage Trap	142	46.2	B	KM	102	34.5	C
	Body temperature (°C)				Body temperature (°C)		
Hounds	39.7	1.66	A	TZ	39.3	1.05	AB
Cable Restraint	39.2	0.77	B	KX	39.2	1.15	B
Cage Trap	39.0	0.84	B	KM	39.0	0.59	BC
	Respiration (breaths/min)				Respiration (breaths/min)		
Hounds	32.0	39.2	A	TZ	34.0	30.4	AB
Cable Restraint	41.0	116.3	A	KX	24.0	28.6	B
Cage Trap	31.0	25.6	A	KM	23.0	33.9	BC

competing models (ΔAIC_c < 2) to explain the variation in abnormal heart rates of mountain lions during a capture. The null model was the top model and sex (CE = −0.22; SE = 0.35), though not significant ($P \leq 0.05$), was the only variable included in the other model (Table 4).

We identified two competing models to explain the variation in abnormal respiratory rates of mountain lions during a capture (Table 4). The top model contained drug combination and age and the next best model only contained drug combination. Only KM (CE = −0.71; SE = 0.30) was significant in the multi-model inference approach suggesting animals immobilized with KM were 2.03 times less likely to exhibit abnormal respiration rates than animals immobilized with TZ (CE = −0.45; SE = 0.26), which typically exhibited higher respiration rates. There was no difference in prevalence of abnormal respiration rates in animals immobilized with KM and KX (CE = −0.74; SE = 0.58).

We identified two competing models to explain the variation in abnormal body temperatures of mountain lions during a capture (Table 4). The top model included capture method and sex and the next best model only contained capture method. Hounds (CE = 0.80; SE = 0.21) and cage traps (CE = −0.99; SE = 0.19) were significant in the multi-model inference approach such that animals captured using hounds were 2.22 times more likely to exhibit abnormally high body temperatures than animals captured using cage traps. Cable restraints (CE = 0.53; SE = 0.32) and sex (CE = −0.28, SE = 0.24) were not significant in the two competing models. None of the top models for predicting abnormal vital parameters overall had good predictive ability based on percent deviance explained and AUC scores (Table 4).

Morbidity

Injuries (of any severity), variations in vital parameters outside normal levels, or complications, such as seizures, vomiting, or death were documented in 58.5% (346/591) of all captures. Superficial injuries and minor incidents

TABLE 4 Diagnostics for the most competitive models ($\Delta AIC_c \leq 2$) examining variables influencing abnormal vital parameters and morbidity scores for captured free-ranging mountain lions (*Puma concolor*) in California, USA, between 2001 and 2019. In addition to ΔAIC_c, diagnostics detailed below include AIC_c weight (w), percent (%) deviance, area under the curve (AUC), and number of parameters (K).

Model	ΔAIC_c	w	% Deviance	AUC	K
Heart rate					
Null	0.00	0.58	0.00	0.50	1
Sex	0.66	0.42	0.81	0.56	2
Respiration rate					
Drug + age	0.00	0.70	1.97	0.58	3
Drug	1.68	0.30	1.21	0.56	2
Body temperature					
Method + sex	0.00	0.72	2.41	0.60	3
Method	1.87	0.28	2.69	0.61	2
Morbidity scores[a]					
Null	0.00	0.30	0.00		1
Method	1.14	0.17	0.12		2
Age	1.21	0.16	0.12		2
Drug	1.74	0.13	0.10		2
Sex	1.80	0.12	0.01		2
Method + age	1.92	0.12	0.26		3

[a]AUC values not calculated for these models.

(scores ≤30) such as abrasions, light limb swelling, or light anesthesia constituted 63.6% (220/346) of the captures in which injuries or abnormal vital parameters were reported. Moderate injuries or incidents (scores 31–50) such as lacerations, broken canines, or subcutaneous wounds, were reported for 11.6% (40/346) of these captures. More significant injuries or complications (scores 51–499) were reported for 22.5% (78/346) of these captures. Mortalities (score of 500) occurred in 2.3% (8/346) of this subset of captures which was 1.4% (8/591) of all captures. Six mortalities occurred with hound captures, whereas one death each occurred at a cage and cable restraint capture. All of these captures occurred with TZ as the immobilization drug, the primary drug being used by all the projects at that time. Seven of the eight capture-related deaths occurred prior to 2012 (i.e., 2.5 deaths/100 capture events); one occurred during 2012 – 2019 (i.e., 0.3 deaths/100 capture events; Table 5). We did not detect any animals dying shortly after being captured due to capture-related injuries. Overall, morbidity scores were low ($\bar{x}$ = 28; SD = 61.2), and there was no statistical difference in injury severity between capture methods: cage traps ($\bar{x}$ = 24; SD = 40.6); cable restraints ($\bar{x}$ = 32.8; 67.5); and hounds ($\bar{x}$ = 31.1; SD = 75.6). Additionally, there was no statistical difference in morbidity scores between immobilization drugs: TZ ($\bar{x}$ = 30.7; SD = 70); KM ($\bar{x}$ = 20; SD = 27); and KX ($\bar{x}$ = 26; SD = 28).

Our ordinal logistic regression modeling morbidity scores yielded six competing models (Table 4). The top model was the null model. None of the variables were significant in any of the other competing models and overall, no variables were significant from a multi-model inference framework. Thus, as with the models concerning vital parameters, none of the top models for predicting morbidity scores had good predictive ability based on nonsignificant coefficient estimates, percent deviance each model explained, and low AIC_c weights.

TABLE 5 Detailed description of eight capture-related mortalities reported from a total of 565 capture events of free-ranging mountain lions (*Puma concolor*) in California, USA, between 2001 and 2019. The year, estimated animal age, sex, capture method, drug combination, capture number and description of the event are shown.

Year	Estimated age (yrs.)	Sex	Method	Immobilization drug	Event
2001	3	Male	Hounds	TZ	Drowned after being darted and trying to escape
2002	0.5	Male	Hounds	TZ	Hyperthermia
2005	8	Male	Hounds	TZ	Drowned after being darted and trying to escape
2005	1.2	Male	Cage trap	TZ	Asphyxiated with the collar while still inside cage
2011	4	Female	Hounds	TZ	Drowned after being darted and trying to escape
2011	8	Male	Hounds	TZ	Drowned after being processed and waking up from anesthesia
2011	12	Female	Cable restraint	TZ	Capture related mortality, no details recorded but broke teeth in cable restraint
2018	6	Female	Hounds	TZ	Fell from tree after being immobilized and broke its spine

DISCUSSION

In our analysis of a 19-year capture dataset for mountain lions, we found that despite many risks inherent with capturing a large carnivore, the occurrence of serious to severe injuries or complications was uncommon. Further, mortality rates decreased over time and occurred in few captures. In general, while the factors we examined in our analysis do influence mountain lion vital rates and likelihood of injury, our results suggest the effect of these factors is small relative to other factors unaccounted for herein. Personnel training, experience, and judgement (choice of acceptable environmental and capture-related variables) are likely crucial aspects of helping avoid negative outcomes. The low number of mortalities and severe injuries show that in general, researchers in California were following protocols and regulations that help assure animal safety and welfare, especially considering the difficulties of capturing large carnivores. Our results suggest that the immobilization drugs and capture methods currently used to capture mountain lions in California for research and management purposes are generally safe.

Although 41% of captures had at least one abnormal vital parameter, results between our ANOVAs and logistic regression models examining abnormal vital parameters were in agreement. For example, in both the logistic regression models and ANOVAs we observed a relationship between the immobilization drugs used and mountain lion respiration rates. However, the diagnostics associated with the logistic regressions (e.g., P values and ROC values) indicated that our variables accounted for a small amount of variation observed in the vital parameters and injuries that occurred during captures. This indicates that the capture methods and immobilization drugs examined herein are not consistently associated with instances of abnormal vital parameters of captured mountain lions. This also demonstrates that while there was statistical significance associated with certain variables, that significance is likely not biologically meaningful. Thus, in addition to the variables examined in our analyses, unrecorded variables (e.g., weather, chase or confinement time) or hard to quantify variables (e.g., experience of the capture lead) likely were more important factors influencing vital parameters of captured animals.

Though we did not include immobilization drug in assessing influences on heart rate due to quasi-complete separation in the data, our raw data indicate that only TZ was associated with abnormal heart rates. Further, animals immobilized with KM were less likely to have abnormal respiratory rates compared to animals immobilized with other immobilization drugs. However, the mean heart and respiratory rates, and associated standard deviations, for animals immobilized using any of the 3 immobilization drugs indicated abnormal heart and respiratory rates did not occur regularly. Nevertheless, researchers immobilizing mountain lions could try and anticipate these abnormal vital parameters and carry supplemental drugs (e.g., midazolam for abnormally high heart rates).

The higher body temperatures in animals captured using hounds, compared to animals captured using cage traps or cable restraints, is likely due to 1) being pursued and the animal attempting to flee rather than immediately seeking refuge (e.g., up a tree) and 2) this method being employed during the daytime when ambient temperatures are higher. However, while body temperatures were higher when hounds were employed, this difference is probably not biologically meaningful because, on average, mean body temperatures, and the associated standard deviation for animals captured using hounds indicates these rates were not abnormal. Nonetheless, researchers conducting mountain lion captures should always carry supplies to cool animals that may overheat. Further, researchers should develop specific criteria for considering when to suspend capture efforts. For example, setting limits on ambient temperatures (e.g., 25°C when using hounds) and response time (e.g., <1 hour response time for animals caught in cage traps, cable restraints, or treed by hounds) under which mountain lion captures can occur could reduce likelihood of abnormal body temperatures.

None of the variables we examined were highly associated with increased morbidity. Although capture method and animal age were each in two of the top five models, those sets of models were not very well supported. Thus, method and age does not increase mountain lion morbidity. Captures with hounds resulted in a lower overall risk of injury relative to cage traps and cable restraints, but a higher risk of severe injury. We found that few hound captures involved injuries to mountain lions due to fighting with hounds. Training hounds to minimize severe injuries is of utmost importance. Misplaced darts, falls, injuries by dogs, and drowning due to onset of anesthesia outside the sight or control of the researchers are hazards inherent in this technique that are not present in the other two capture methods.

Additionally, capture leads should avoid pursuing or immobilizing mountain lions when using hounds if the animals are 1) high enough in trees that they cannot be retrieved quickly (<5 minutes) after being immobilized and thus a possible subsequent fall would result in significant injury or 2) treed/bayed close to water such that they might run away after being darted and chance drowning. To reduce the risk of an animal falling, we encourage researchers using hounds to have fall nets set up and be prepared to climb the tree to secure and lower the animal. Decision making related to such scenarios should occur before any pursuits/captures are attempted.

While cage traps and cable restraints caused injuries more frequently than hounds, they were generally not as severe as injuries caused by hounds. The less severe injuries included broken canines associated with biting the cage traps or lacerations associated with pulling against the cable restraints. Other studies report that time spent in a trap is a main source of injury to study animals, which highlights the necessity to monitor and check traps often (Quinn et al. 2012, Andreasen et al. 2018). Monitoring (e.g., every 30 minutes) of a trap using a satellite GPS or VHF trap transmitter, cell or wireless camera, or similar device, combined with a quick response time (e.g., <60 minutes) may reduce the likelihood of injuries associated with these methods. With both cage traps and cable restraints, the skill of personnel setting and assembling the trap, safety measures built into the trap, how the trap is anchored, and placement in relation to vegetation and topography are all factors affecting the likelihood of injury (Logan et al. 1999, Flaa et al. 2009). Selecting the most appropriate capture method may help to reduce the likelihood of injury. For example, cable restraints may be more appropriate than hounds in areas without suitable trees and more appropriate than cage traps in areas without roads where carrying a cage trap for long distances may be impractical. For long-term research where recaptures are required, multiple methods can help ensure that an animal does not become wary of some capture methods (e.g., hesitant to walk inside a cage trap; Frank et al. 2003).

Immobilization drug occurred in one of the five top models of morbidity scores wherein TZ had the highest likelihood of injury, followed by KX and then KM. It is likely that a capture lead's level of experience with an immobilization drug could mediate likelihood of injury stemming from the immobilization drug used. A drug combination that is reversible and thus allows for a quicker/shorter recovery time such as KM has a lot of advantages, but awareness of the potentially dangerous effects of more potent drugs like medetomidine should always be considered (West et al. 2007). As noted previously, animals anesthetized with KM were more likely to exhibit some of the more serious or life-threatening complications (i.e., vomiting, low oxygen saturation, cyanosis, and low respiratory rates). Given that most of the projects would not have detected some of these complications of KM, and its use is becoming more common than TZ, all individuals using this drug combination should be diligent in monitoring vital parameters. For example, projects using KM should monitor SpO2 and utilize supplemental oxygen as needed to address low oxygen saturation. Further, being prepared to use supplemental drugs such as doxapram hydrochloride can help mitigate low respiratory rates. We recommend researchers use a cutoff of ≥95% SpO2 to help avoid some of these additional complications. Further, xylazine and medetomidine can induce vomiting in an immobilized animal. All five instances of vomiting in the dataset occurred when either KM (n = 3) or KX (n = 2) were used to immobilize an animal. Thus, if an animal is caught in a baited cage trap such that it could have fed before being immobilized, TZ may then be preferred to either KM or KX, or immediate administration of an antinausea drug if a large amount of feeding has been suspected to occur and KM or KX are used. Lastly, the way immobilization drugs are administered can reduce likelihood of injury as well. When using hounds, it may be desirable to not dart the animal unless it is likely that the animal can be retrieved within <5 minutes to reduce the chance of falling out of the tree. If the mountain lion is treed in a dangerous position, researchers can take measures to encourage mountain lions to jump from where it is bayed and tree it again, ideally in a safer location. Additionally, depending on the situation, the capture lead might want to encourage the animal to jump from where it is bayed immediately after being darted (e.g., hitting the trunk of the tree with a stick, hollering loudly, throwing sticks in the vicinity of the animal) such that the animal goes to sleep on the ground. However, such decision-making should consider various important details (e.g., being able to track down the animal if it runs off a short distance before going down and immediate surroundings which include any bodies of water the animal could encounter). These approaches demonstrate that it is important for the capture lead to have a high level of knowledge and experience with both the capture methods and immobilization drugs.

In our study, there were significant differences in the observations collected depending on the project which limited our ability to conduct additional analyses. Most projects recorded rectal temperature every 10 minutes as suggested by literature and as protocol demands, but the number of recorded observations for respiratory and heart rates was often much lower. While our results suggest the capture and drug combinations we evaluated can be considered relatively safe for mountain lions during the capture episode, this inference is based on monitoring coarse variables (i.e., temperature, pulse, and respiration and injuries) that (1) were not monitored continuously, (2) were measured somewhat subjectively, and (3) may not adequately describe all negative physiological effects of the capture process. Some of the capture teams monitored and recorded other objective data on physiological parameters, such as SpO2 via pulse oximetry and electrocardiogram (ECG) character, which can be obtained by using widely available inexpensive anesthetic monitors appropriate for field use and may be more appropriate measures of physiological states. These devices would also aid in informing research teams during processing if remedial action to correct abnormal conditions is warranted prior to this need becoming apparent by monitoring temperature, pulse, and respiration (TPRs) alone (Ramsay 2014). We stress that it is essential for the professionals responsible for wildlife capture to 1) monitor all vital parameters during any procedure where an animal is under anesthesia, 2) train their team of field technicians to closely monitor, at a minimum, the parameters discussed herein, and 3) be trained to take corrective action when indicated. The practices outlined above should be standard practice for all agencies that permit/oversee wildlife capture operations to assure that animal welfare takes priority over deploying collars. This is especially true for areas where large carnivores are recolonizing parts of their historic range and state wildlife agencies have little to no experience regulating capture of these species. Hopefully, this manuscript can offer important insight to guide any capture efforts such agencies would undertake.

RESEARCH IMPLICATIONS

While our assessment of the safety of the capture methods and drug combinations we considered demonstrates these tools are generally safe and effective for conducting mountain lion research, our evaluation is limited to the capture episode and the few days (e.g., ≤14 days) after the capture event. We cannot speak to longer-term consequences of capture methods and immobilization drugs on animal welfare (e.g., abortion, capture myopathy, internal organ damage, increased disease susceptibility). However, we did not document any mortalities that occurred shortly after capture that could be attributed to the capture itself. While it may be difficult to directly collect data on these issues, it is easier to obtain data such as survival and reproductive rates that could serve as useful surrogates. To further understand the potential long-term consequences of capture and drug combinations on mountain lion welfare, a similar analysis to the one we performed comparing such rates between capture methods and drug combinations is warranted.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ETHICS STATEMENT

Captures were conducted under authorization of California Department of Fish and Wildlife (CDFW). CDFW staff operated under CDFW approved capture plans and entities outside CDFW operated under CDFW issued scientific collection permits (Nos. SC-013416, SC-007303, SC-005636, SC-191710009) and associated IACUC approval (Protocol Nos. 22408, 15341, 16886; Wilmc1509).

DATA AVAILABILITY STATEMENT

Data is available upon request from the corresponding author.

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