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PhD-thesis

Short- and long-term physiological effects of capture and handling on free-ranging brown bears (*Ursus arctos*)

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Preface

I saw my first brown bear in Somiedo, Asturias, in 2010. It was an early morning of September, and a female with her two cubs of the year appeared on the mountain side just across the valley. We remained seated for a while just watching them eat and play. This was a magical moment. I would like to start by acknowledging the involuntary protagonist of this thesis, the brown bear. I hope that research such as the one conducted in this thesis helps the conservation of the species.

I am indebted to many people for making this thesis to happen and come to an end. I would like to thank my supervisors Jon Arnemo, Andreas Zedrosser and Marc Cattet. Jon gave me the opportunity to collaborate as a wildlife veterinarian in several projects in Scandinavia. I also thank Jon for his help with the Norwegian Sammendrag. I thank Andreas for their always insightful comments on the endless statistical analysis. Marc, I can honestly say that I would not be here today without your guidance and help. In spite of not being based in Norway, you have always been there for me. I have learnt from every email, conversation and meeting we have had. I have been fortunate to work with a great researcher and an even better person. I hope we will continue to collaborate in the future.

Although not officially a part of my PhD supervisory committee, I would like to thank Jon Swenson for his availability, good comments and positivity. Jon, you always made me feel a part of the team.

I am most grateful to all people I have shared the fieldwork with during the PhD. All volunteers, technicians, interns, staff, pilots, rangers, veterinarians, PhD students, etc. that have made this experience unforgettable. I am especially grateful to Sven Brunberg and Andrea Friebe from the Scandinavian Brown Bear Research Project. To me, you are Tackåsen and all the good moments I was lucky to experience there.

I deeply akcnoweldge Gordon Stenhouse and all staff at fRI for warmly welcoming me to the Grizzly Bear Program in Alberta. I am grateful to Amy Stenhouse for kindly giving permission to use a picture of a brown bear family as the thesis cover. I would like to thank Marc and his whole family, Heather, René, Gillian, Liam, Milou, Crosby, and BUC, for the wonderful time I had in Saskatoon. For the meetings in coffe shops with music on the background, for the day at lake Diefenbaker with the boat, for the the nice concert we went to, and the trip to Moose Jaw. I hope you get to come and visit me in Spain. Agnieszka Sergiel was also very much responsible for the amazing Canadian experience. I want to thank Aga for the nights watching Game of Thrones, the quick lunches before running into the lab, the bike rides, the time watching the gophers on campus, etc. You were an amazing companion. I am also grateful for the coffe sessions on Skype and for the talks and laughs.

I want to thank two veterinarians I had a lot of fun working with. Monica Bando, although we only shared one season of fieldwork, I can say without a doubt that it was the best season. Not only it was really easy working with you, but you were so much fun to be around even in not the most favourable circumstances such as during the bear hunt. I will always remember some of your stories! It was a real

También quiero agradecer a Lourdes y Pilar las clases de equitación y los buenos momentos vividos durante el último año. Esos momentos de relax y de diversión en la Finca La Palmosa no tienen precio.

Quiero agradecer a Tasio por ser mi compañero de viaje. No sólo durante esta última etapa de doctorado en Noruega, sino por estar siempre ahí. Por ayudarme cuando has podido y por intentarlo aunque no estuviera en tus sus manos. Por todas las tablas, words con referencias, etc. Y por los estupendos mapas que hay en la tesis. Por estar a mi lado y compartir los momentos felices y los no tan felices. Y porque sigamos compartiéndolos en el futuro.

M'agradria agrair a la meva família el suport que m'ha ofert durant tot aquest temps. Al meu padrí Rafel, a les meves tietes Pilar i Montse, a les meves cosines Sandra i Marta, al meu germà Xavi i als petits de casa, l'Àlex, la Mireia, el Pau i la Mar, perquè sempre he pogut comptar amb vosaltres i perquè després de cada visita he trobat la força i la il.lusió per seguir endavant. Pels dinars tots junts a casa (o a La Simona), per les caminades a la Boca de la Mina, pels esmorzars i tombs al mercat dels dissabtes, pels Ganxets Pintxo, pels Trapezis, per les estones jugant a cartes o al "Rummino", per les estones a Maella, etc. Hi ha moltes coses que he trobat a faltar i que he après a valorar en aquest temps que he estat fora. També m'agardaria recordar la Diana, el Bruno, el Petit, el Tigre, l'Esquitx, la Nina, el Bru i el Melu. La meva vida hagués estat buida sense la vostra companyia i amor incondicional. Però a qui més coses he d'agarair és als mes pares Loli i Enric. Estic agraida perquè em van educar estimant i respectant els animals. Per fer possible que no ens faltés res quan érem petits, per fer possible que estudiés la carrera que volia malgrat les dificultats i per recolzar-me en totes les decisions que he pres des de llavors, tot i que sóc conscient que a vegades no ha estat fàcil. Moltes gràcies!

Contents

Abstract

Brown bears (*Ursus arctos*) are captured and handled for conservation, research or management purposes. However, capture and handling have potential to cause injury and stress, thus, negatively impacting an animal's health. The evaluation of behavioural and physiological effects of capture and handling can provide science-based information to better understand the impact of capture and handling on wildlife health, refine techniques and minimize adverse effects. The main goal of my thesis was to assess the short- and long-term physiological effects of capture and handling on free-ranging brown bears in association with two long-term research projects, one in Scandinavia, and the other in Alberta, Canada. For this, I conducted three studies to: i) evaluate the acute stress response to capture and handling by using a field-based technique called the leukocyte coping capacity (LCC), ii) compare two different anaesthetic protocols based on the behavioural and physiological short-term responses of captured bears and iii) assess the long-term effects of capture, handling and surgery on the body condition of independent male bears. In my first study, I found that LCC values measured in blood samples collected at 30 minutes following capture were significantly lower in solitary bears ($n = 12$) than in bears living in family groups ($n = 12$) which could suggest that mothers and their dependent offspring had greater capacity to cope with capture-induced stress. In addition, LCC values for blood samples collected at approximately 90 minutes following capture were directly correlated with an index used to estimate body condition which suggests the better a bear's body condition, the better its capacity to cope with stress. I also found that the LCC values at 90 minutes following capture did not appear to differ between 19 bears that had abdominal surgery to implant or remove radio transmitters, physiological sensors and/or temperature loggers, and five bears that did no undergo surgery. Although further evaluation of this technique is required, my results from this preliminary study provide support for the use of the LCC technique as a field-based, quantitative measure of stress. In my second study, I found that intramuscular injection of either dexmedetomidine-tiletamine-zolazepam (DTZ), a new anaesthetic protocol, or medetomidine-tiletamine-zolazepam (MTZ), an established anaesthetic protocol, induced anaesthesia of free-ranging brown bears captured by helicopter ($n = 34$) or by culvert trap ($n = 6$) in a smooth and predictable manner with no difference in induction times between the two anaesthetic protocols. Both protocols also caused acidemia (pH of arterial blood < 7.35), hypoxaemia (partial pressure of arterial oxygen < 80 mmHg), and hypercapnia (partial pressure of arterial carbon dioxide ≥ 45 mmHg) to a similar degree. Based on the absence of significant differences in these measurements and in other behavioural and physiological measurements (i.e., the need for supplemental drugs to sustain anaesthesia, serum cortisol, heart and respiratory rates, rectal temperature), I concluded that DTZ offered no advantage over the use of MTZ in the anaesthesia of brown bears. In my third study, I found that the body condition of independent male brown bears ($n = 551$), estimated as a body condition index (BCI) validated for ursids, was associated with the age of the bear, the day the capture occurred, and the area of

study. BCI was positively associated with the age of the bear and the ordinal day of capture. Thus, older bears and bears captured later in the year had higher BCI values. I also found a weak difference in the bear's BCI between study areas. BCI values tended to be higher for bears in Scandinavian than bears in Alberta irrespective of the annual timing of captures, the year of capture, or the age composition of captured animals. However, BCI values did not appear to be influenced by capture, handling, and surgery. Although no measureable long-term effect on BCI was found in independent male brown bears, future studies should be conducted to determine if the same holds true for other sex, age, and reproductive classes. Further, studies assessing long-term effects of capture and handling are needed to determine if research procedures are inadvertently biasing research results.

The findings of this thesis provide scientific evidence that capture and handling caused significant short-term physiological effects on the bears, although no long-term effect on their body condition was detected. I believe that this type of self-assessment of potential effects caused by capture and handling of wildlife is essential to fully understanding the overall impact of anthropogenic activities on wildlife health, and to better interpreting research results. By establishing the extent of the effects of research activities on an animals' physiology, researchers can take measures to reduce their impact on the welfare and health of wildlife, and make better informed-decisions in relation to the use of capture and handling procedures.

Key words: anaesthesia, body condition, brown bear, capture and handling, dexmedetomidine, leukocyte coping capacity, long-term effects, medetomidine, stress, tiletamine- zolazepam*, Ursus arctos*.

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Sammendrag (Norwegian summary)

Brunbjørner (*Ursus arctos*) fanges for ulike forsknings- og forvaltningsformål. Dette kan imidlertid forårsake skader og stress og ha negative innvirkning på dyrenes helse. En vitenskapelig evaluering av konsekvenser av fangst og håndtering vil derfor gi grunnlag for forstå helsemessige effekter, forbedre metoder og minimere uheldig påvirkning. Avhandlingens hovedformål var å vurdere fysiologiske effekter av fangst og håndtering av viltlevende bjørner i to pågående forskningsprosjekter, henholdsvis i Skandinavia og i Alberta, Canada. Jeg utførte tre studier: i) evaluering av den akutte stressresponsen på fangst og håndtering med en feltbasert metode kalt "leukocyte coping capacity" (LCC), ii) sammenligning av to ulike anestesiprotokoller med hensyn på fysiologiske korttidseffekter, iii) vurdering av langtidseffekter av fangst og håndtering på kroppskondisjonen til enslige hannbjørner. I min første studie fant jeg at LCC-verdiene målt i blodprøver tatt 30 minutter etter fangst, var signifikant lavere hos enslige bjørner (n = 12) sammenlignet med bjørner i en en familiegruppe (n = 12), noe som kan indikere at binner og unger var bedre i stand til å håndtere fangst-relatert stress. I tillegg var LCC-verdier målt ca. 90 minutter etter fangst direkte korrelert med en indeks for kroppskondisjon, noe som indikerer at jo bedre kroppskondisjonen er, jo bedre er bjørnen i stand til å håndtere stress. Jeg fant også at det ikke var noen forskjell på LCC-verdiene målt 90 minutter etter fangst hos 19 bjørner som ble operert for å implantere eller fjerne radiosendere eller biologgere sammenlignet med fem bjørner som ikke ble operert. Selv om dette krever flere undersøkelser, støtter mine resultater bruk av LCC-teknikken som en feltbasert, kvantitativ metode for måling av stress. I min andre studie fant jeg ingen forskjeller i induksjonstiden mellom en ny anestesikombinasjon, dexmedetomidine-tiletamine-zolazepam (DTZ), og en velprøvd anestesikombinasjon, medetomidine-tiletamine-zolazepam (MTZ); begge induserte anestesi hos bjørner anestesert fra helikopter (n = 34) eller i tunelfelle (n = 6) som forventet. Begge kombinasjoner forårsaket tilsvarende acidemi (pH i arterielt blod < 7.35), hypoksemi (partialtrykk av oksygen i arterielt blod < 80 mmHg), and hyperkapni (partialtrykk av karbondioksid i arterielt blod ≥ 45 mmHg) hos bjørnene. Basert på fravær av signifikante forskjeller for disse og andre fysiologiske målinger (f. eks. behov for ekstra medikamenter for å opprettholde anestesien, kortisol i serum, hjertfrekvens, rektalteperatur), konkluderte jeg med at DTZ ikke ga noen fordeler sammenlignet med MTZ for anestesi av bjørner. In min tredje studie fant jeg at kroppskondisjonen hos enslige hannbjørner (n = 551),, estimert som en indeks (BCI) validert for bjørner, var korrelert med alder, dato for fangsten og studieområde. BCI økte med alder og forløpet av fangstsesongen. BCI tenderte til å være høyere hos skandinaviske bjørner sammenlignet med bjørner i Alberta, uavhengig av fangstdato, fangstår og alder. BCI var tilsynelatende ikke påvirket av fangst, håndtering eller kirurgi. Selv om det ikke ble funnet noen målbare langtiseffekter på BCI hos enslige hannbjørner, bør det gjennomføres flere studier av andre grupper av bjørner med hensyn på alder, kjønn og

reproduksjonsstatus. I tillegg er det viktig å avklare om mulige langtidseffekter av fangst og håndtering kan påvirke forskningsresultater. Selv om det ikke ble funnet langtidseffekter på kroppskondisjonen, viser resultatene i denne avhandlingen at fangst og håndtering av bjørner forårsaker betydelige fysiologiske korttidseffekter. Jeg mener at denne formen for selvevaluering er essensiell for å forstå konsekvensen av menneskelig påvirkning av viltlevende dyr og for å kunne tolke forskningsresultater. På denne måten kan forskere gjøre kunnskapsbaserte valg når det gjelder metoder for fangst og håndtering.

List of papers

This thesis is based on the following manuscripts. Of these, the first two have been published:

Paper I:

Fandos Esteruelas, N., Huber, N., Evans, A. L., Zedrosser, A., Cattet, M., Palomares, F., Angel, M., Swenson, J. E., and Arnemo, J. M. (2016). Leukocyte coping capacity as a tool to assess capture- and handling-induced stress in Scandinavian brown bears (*Ursus arctos*). *Journal of Wildlife Diseases, 52*(2s), S40-S53.
DOI: http://dx.doi.org/10.7589/52.2S.S40

Paper II:

Fandos Esteruelas, N., Cattet, M., Zedrosser, A., Stenhouse, G. B., Küker, S., Evans, A. L., and Arnemo, J. M. (2017). A double-blinded, randomized comparison of medetomidine-tiletamine-zolazepam and dexmedetomidine-tiletamine-zolazepam anesthesia in free-ranging brown bears (*Ursus arctos*). *PloS ONE, 12*(1), e0170764.
DOI: https://doi.org/10.1371/journal.pone.0170764

Paper III:

Fandos Esteruelas, N., Cattet, M., Zedrosser, A., Stenhouse, G. B., Swenson, J. E., Evans, A. L., Arnemo, J. M.. (2017). Evaluation of the long-term effects of capture, handling, and surgery on body condition in male brown bears (*Ursus arctos*). *Manuscript*.

1. Introduction

1.1. Capture and handling of brown bears

1.1.1. Reasons for capturing bears

In this thesis, I investigated the effects of capture and handling of animals within the context of wildlife research (e.g., Powell and Proulx, 2003; Sikes and Gannon, 2011). However, these procedures are also commonly employed for wildlife management and conservation (Osofsky and Hirsch, 2000). My research was focused on a single species, the brown bear (*Ursus arctos*), but it may also be relevant to other bear species where similar capture and handling procedures are employed.

Although, some information on free-ranging brown bears can be obtained by the use of non-invasive techniques (e.g., faecal samples for DNA analysis and determining hormone concentrations; von der Ohe et al., 2004; Bellemain et al., 2005), capture and handling of brown bears is the sole means of obtaining data on, for example, morphometric measurements, physiology (e.g., body condition) or the age of the individual (Garshelis, 2006). Although useful as a tool in research, capture and handling have the potential to cause significant stress and a negative impact on an animal's health (Cattet et al., 2008a). Thus, evaluating the impact of capture and handling is important for refining capture methods and for ensuring that capture effects do not confound the interpretation of research results.

I used data from two long-term research projects, one in Scandinavia, the Scandinavian Brown Bear Research Project (SBBRP), and the other in Alberta, Canada, the fRI Research Grizzly Bear Program (fRI). In Scandinavia, brown bears are routinely captured and handled for research and management purposes (i.e., from 1984 to 2015, a total of 2,047 captures of 748 individual bears). Data from the SBBRP gave me a unique opportunity to assess the effects caused by research activities in a population of brown bears that is intensively captured and handled. Furthermore, to broaden the scope of my evaluation of the effects of capture and handling, I also used data collected by the fRI where different anaesthetic protocols, capture methods and handling procedures are employed. In addition, I used data collected over almost 30 years (i.e., from 1988 to 2015) which allowed for the evaluation of the long-term effects of capture and handling in brown bears in an objective manner.

In this thesis, I have attempted to identify and/or develop best practices for capturing and handling brown bears to 1) ensure their welfare is maintained during research activities, and 2) assess the potential bias of capture and handling on research results.

1.1.2. Capture as stressor

1.1.2.1. Stress: general concepts and stress responses

Hans Selye defined stress as a generalized physiological mechanism that responds to a threat (also known as General Adaptation Syndrome; Selye, 1946). Since then, several additional definitions and

models of stress have been proposed (Romero and Wingfield, 2016). However, there is consensus that stress involves the perception of a threat (i.e., the stressor) which triggers a physiological and behavioural response, i.e., the stress response. The stress response allows an animal to cope with the current situation, but also to return to a previous state, the homeostasis or dynamic equilibrium, when the threat no longer exists (Creel, 2001).

The two most important physiological responses to stressors are the stimulation of the sympathetic nervous system (SNS) and the activation of the hypothalamic-pituitary-adrenal axis (HPA) (Reeder and Kramer, 2005). The stimulation of the SNS results in the release of catecholamines from the adrenal medulla, while the activation of the HPA results in the secretion of glucocorticoids (GCs). The hypothalamus releases corticotrophin-releasing hormone that stimulates the pituitary gland to release adrenocorticotropic hormone, which in turn, stimulates the cortex of the adrenal gland to release GCs (Sapolsky et al., 2000; Reeder and Kramer, 2005). The response of the SNS to a stressor is almost instantaneous and is known as the "fight-or-flight response". In contrast, the activation of the HPA takes a few minutes. There are studies demonstrating that plasma GCs levels increase significantly after 2-5 minutes from capture and handling in vertebrates (Place and Kenagy, 2000; Boonstra et al., 2001).

Capture and handling procedures are known to increase levels of corticosteroids in wild animals (Arnemo and Caulkett, 2007), including brown bears (Cattet et al., 2003a). Therefore, such procedures are perceived as stressors by the animal. Further, capture likely is one of the most stressful events in a wild animal's life (Wilson and McMahon, 2006; Morellet et al., 2009).

1.1.2.2. Actions of the stress response mediators

The activation of the SNS and the HPA has impacts on the metabolism, metabolic rate, immune system, behaviour, reproductive system, development, growth and visceral activity, osmoregulation and oxygen supply (Romero and Wingfield, 2016). The best documented effects of SNS and HPA are on metabolism and metabolic rate. Catecholamines increase heart rate, arterial blood pressure, and cardiac output, promote glycogenolysis in the liver and muscles and induce lipolysis (Nonogaki, 2000; Reeder and Kramer, 2005). On the other hand, the major metabolic effect of increased secretion of GCs during stress is to increase plasma concentrations of amino acids, glycerol, fatty acids, and glucose (Reeder and Kramer, 2005). During stress and prolonged activation of the HPA axis, GCs could lead to anti-inflammatory effects or inhibition of specific immune responses (Sheriff et al., 2011). Stress in general inhibits reproduction (Sapolsky et al., 2000) and it could be an influential factor during the sensitive period of development *in utero* and early life by negatively affecting growth (Love et al., 2013; Romero and Wingfield, 2016).

1.1.2.3. Stress response: acute vs. chronic

Responses to stress are often divided into two categories: acute and chronic (Arnemo and Caulkett, 2007). Acute responses are those that are triggered by short-term stressors, have a definitive onset, and last for only a few hours. In comparison, chronic stress is defined as either multiple, frequent exposure to stressors and/or long-term constant exposure to stressors. In the short term, or in response to an acute challenge, the stress response is believed to be adaptive (Sapolsky et al., 2000; Wingfield and Romero, 2001). In fact, the adrenocortical response is one of the most conserved physiological mechanisms in vertebrates aimed at avoiding the deleterious effects of stressors (Wingfield et al., 1998; Sapolsky et al., 2000). However, in the long term, frequent activation of the HPA axis may lead to chronic exposure to elevated GCs levels with deleterious consequences on growth and maturity, fitness (i.e., survival and reproduction), brain function, cognitive abilities, and immune system to the point of death (Boonstra et al., 1998; Sapolsky et al., 2000; Blas et al., 2007).

1.2. Capture methods and use of drugs for anaesthesia in brown bears

Although there are numerous techniques and devices available to capture bears, the choice of a technique will depend on the habitat, research goal, project budget, etc. (Powell and Proulx, 2003).

Anaesthetic drugs can be used as a primary method of capture or in combination with restraining capture methods (Proulx et al., 2012). On one hand, the capture of free-ranging bears by remote drug delivery relies on anaesthesia to immobilize an animal and can be done from the ground, from a blind or vehicle, or from the air by helicopter (Arnemo and Evans, 2017). Darting from the ground requires close proximity to the animal and road access, if using a vehicle. From the air, large clear cuts or open areas are required for safe capture from a helicopter. On the other hand, the capture of free-ranging bears by restraining or containing devices does not rely on the use of anaesthesia for capture, and includes the use of foot traps, leg-hold snares, and culvert traps (Cattet et al., 2003a; Powell, 2005; Cattet et al., 2008a).

The most commonly used capture method for brown bears combines a restraining method (i.e., leg-hold snares and culvert traps) and the use of anaesthesia (Caulkett and Fahlman, 2014). Regarding the drugs used for anaesthesia of brown bears, the most common protocols have combined a dissociative agent (e.g., ketamine, tiletamine) with a benzodiazepine (e.g., zolazepam) or an alpha-2 adrenoceptor agonist (e.g., medetomidine). Tiletamine has been routinely used in combination with zolazepam for immobilizing brown bears, especially in North America (Caulkett and Fahlman, 2014). Tiletamine-zolazepam (TZ) produces a reliable anaesthesia in bears and has a wide safety margin (Caulkett et al., 1999; Cattet et al., 2003b). However, the use of TZ requires large drug volumes, provides poor analgesia, and cannot be antagonized, thus resulting in extended recovery times (Taylor et al., 1989; Cattet et al., 1997a; Caulkett and Fahlman, 2014). The incorporation of an alpha-2 adrenoceptor agonist such as medetomidine to TZ counteracts some

of the disadvantages of using TZ alone. Medetomidine-tiletamine-zolazepam (MTZ) can be delivered in smaller volumes (Cattet et al., 2003b), as medetomidine reduces the anaesthetic requirements of other drugs. Additionally, medetomidine improves analgesia (Caulkett et al., 1999), and is specifically antagonized with atipamezole. Currently, TZ combined with xylazine or medetomidine is widely used in the anaesthesia of brown bears, including projects in Scandinavia and in Alberta, Canada.

The capture of brown bears with the above methods has been reported to cause physiological and behavioural short- and long-term effects on the study animals. The effects varied upon the method of captured used and included stress, haemoconcentration, hyperthermia, hypoxaemia, acidemia, injury and muscle damage, and decrease in body condition and movement rates (Cattet et al., 2003a; Cattet et al., 2008a; Fahlman et al., 2011). These effects will be discussed in greater detail below in sub-sections 1.4.2 and 1.4.3.

In this thesis, brown bears were captured by remote drug delivery from a helicopter as a sole method of capture in Scandinavia. In contrast, bears in Alberta were captured by several methods, including remote drug delivery from helicopter, leg-hold snare, or culvert trap.

1.3. Handling procedures in brown bears

Common handling procedures performed with anaesthetized bears include morphometry, weighing, identification or marking, sampling (e.g., blood, faeces, urine, hair, skin, tooth) (Arnemo and Evans, 2017).

Morphometry and weighing consist of measuring the size (e. g., body length, head circumference) and body weight of an individual. Morphometric measurements and weighing are easy to perform and provide information on the condition and growth of an individual which are important life-history traits that influence survival and reproduction in brown bears (Dahle et al., 2006; Zedrosser et al., 2007; Zedrosser et al., 2013). Also, body weight allows for an accurate administration of drugs during handling, and for calculation of dosages of drugs used for anaesthesia.

Captured animals are often "marked" with some form of long-term identification to follow them through time. Marked individuals can provided information on population dynamics, movement, behaviour, mortality and density estimates (Silvy et al., 2005). In brown bears, subcutaneous microchips, lip tattooing, ear tags and VHF (Very High Frequency) or GPS (Global Positioning System) radio collars have been used. Sometimes, miniaturized tags (bio-loggers) are also applied to, or implanted in, bears to relay data about their physiological function (Fahlman et al., 2011; Arnemo and Evans, 2017).

Several biological samples are routinely obtained during handling of brown bears (Arnemo and Evans, 2017). For example, blood samples are used for health screening (i.e., blood cell counts, biochemistry) and disease (i.e., serology), measuring stress levels, monitoring oxygenation (i.e., blood gas analysis in arterial blood), genetic studies, and banking. The rudimentary first maxillary or mandibular premolar is extracted

for age determination at first capture in brown bears. Later on, age is estimated by counting cementum annuli (Stoneberg and Jonkel, 1966).

1.4. Impact of research activities: effects of capture and handling
1.4.1. Effects of capture and handling in wildlife

In the past, research requiring the capture and handling of wildlife has been conducted under the premise that these procedures do not adversely affect animals beyond a few days following capture. Nowadays, despite the widespread application of capture and handling techniques in wildlife, and the clear potential for negative consequences, the evaluation of effects of research activities on the health and welfare of animals is still often overlooked (Murray and Fuller, 2000; McMahon et al., 2011; Cattet, 2013). In addition, of the studies assessing the effect of capture and handling on the animal, most report only the short- or intermediate-effects of these procedures, e.g., effects that last from minutes to days after capture, whereas fewer studies report long-term effects, e.g., effects that last in the weeks and months that follow capture. Furthermore, the results of such studies are not consistent. Some studies have reported a negative effect of capture and handling on the animal's survival, reproduction, physiology, behaviour, activity, and/or body condition (Côté et al., 1998; Alibhai et al., 2001; Tuyttens et al., 2002; Cattet et al., 2003a; Moorhouse and MacDonald, 2005; Cattet et al., 2008a; Morellet et al., 2009), whereas others have not found any significant long-term effects of research activities on the study animals (McMahon et al., 2008; Omsjoe et al., 2009; Harcourt et al., 2010; Thiemann et al., 2013; Rode et al., 2014).

1.4.2. Short-term effects on physiology in brown bears

The techniques used for the capture and handling of brown bears can cause short-term physiological effects on the study animals. Several studies have reported patterns of physiologic disturbance resulting from capture and handling that varied with the capture method used (Cattet et al., 2003a; Fahlman et al., 2011).

Capture by leg-hold snares can cause stress, injury, muscle damage and dehydration in brown bears (Cattet et al., 2003a). A "stress leukogram" has been found in brown bears captured with leg-hold snare. This characteristic pattern in the number and proportion of leukocytes (i.e., increase in leukocyte numbers and proportion of neutrophils with a decrease in lymphocytes and eosinophils) is thought to be driven by an increase in cortisol levels in response to capture (Cattet et al., 2003a). In addition to stress, a period of extreme physical exertion can increase serum concentrations of alanine aminotransferase, aspartate aminotransferase (AST) and creatine kinase (CK) suggesting muscle injury (Cattet et al., 2003a; Cattet et al., 2008a) which, in some cases, may be permanent (Cattet et al. 2008b). Serum concentrations of AST, CK and myoglobin were higher in bears captured by leg-hold snare than those captured by remote drug

delivery from helicopter or after being restrained in a culvert trap (Cattet et al., 2003a; Cattet et al., 2008a). Further, bears may develop an electrolyte imbalance as a consequence of capture by leg-hold snare. Cattet et al. (2003a) discovered haemoconcentration, and higher concentrations of total protein, sodium and chloride in the serum of captured bears. These changes were attributed to dehydration resulting from water deprivation and increased water loss related to the struggle to escape.

Main physiologic disturbances in bears captured by remote drug delivery from helicopter include hyperthermia, impairment of pulmonary gas exchange and alteration of acid-base balance (Cattet et al., 2003a; Fahlman et al., 2011). An increase in body temperature, hyperthermia, is common in the first minutes following immobilization as result of strenuous activity by bears fleeing from the helicopter coupled with a decrease in heat loss caused by the catecholamines, ambient temperature, and the effect of anaesthetic drugs (Cattet et al., 2003a; Fahlman et al., 2011). Although bears are not restrained when aerial captures are performed, an increase in lactic acid, potassium, creatinine and calcium concentrations as a result of intense muscle activity during capture can occur (Cattet et al., 2003a; Fahlman et al., 2011).

Effective anaesthesia helps assure safety for capture personnel while reducing anxiety, stress and pain for captured animals (Kreeger and Arnemo, 2012). However, the use of drugs to induce anaesthesia might cause morbidity and even pose a risk to the animal's life (Clarke and Trim, 2014). Anaesthetic combinations commonly used in the anaesthesia of brown bears can cause a variety of physiologic responses in captured bears. For example, xylazine or medetomidine combined with tiletamine-zolazepam caused hyperthermia, bradycardia (a decrease in pulse rate), bradypnoea/hypoventilation (a decrease in respiratory rate), hypercapnia (an increase in partial pressure of arterial carbon dioxide values) and hypoxaemia (low levels of blood oxygen) in free-ranging bears irrespective of whether or not they were previously restraint (Cattet et al., 2003a; Fahlman et al., 2011). Further, capture-related mortality has been directly or indirectly linked to the effects of drug administration in brown bears (Arnemo et al., 2006).

Hyperthermia can be caused by the alteration of thermoregulatory mechanisms driven by the alpha-2 adrenoceptor agonists (Virtanen, 1988). Bradycardia secondary to vasoconstriction and hypertension is a common effect of the administration of alpha-2 adrenoceptor agonists (Jalanka and Roeken, 1990). Also, the use of alpha-2 adrenoceptor agonists can cause hypoventilation or respiratory depression leading to an elevation of partial pressure of arterial carbon dioxide values (Jalanka and Roeken, 1990). In addition, they can produce intrapulmonary changes that may result in low levels of blood oxygen (Read, 2003) which can lead to hypoxia (inadequate oxygen levels in the body). Both hypercapnia and hypoxaemia can have life-threatening consequences, such as myocardial ischemia, brain cell death, narcosis, coma and multi-organ damage (Read, 2003; Fahlman, 2014).

Hypercapnia and hypoxaemia are common physiological alterations found in bears anesthetized with TZ combined with alpha2-adrenergic agonists (Caulkett and Cattet, 1997; Fahlman et al., 2011).

Recently, a study using dexmedetomidine combined with tiletamine-zolazepam in the anaesthesia of brown bears found normal respiratory rates and high oxygen saturations (Teisberg et al., 2014). The authors suggested a potential benefit of dexmedetomidine over medetomidine in bears due to less respiratory depression (i.e., hypoventilation, hypoxaemia). However, this study did not include a comparison of performance or efficacy with equivalent doses of medetomidine.

1.4.3. Intermediate- and long-term effects on behaviour and body condition in brown bears and other bear species

Capture and handling of brown bears can cause alterations in behaviour immediately after capture or in the weeks that follow. Brown bears that were captured during hibernation abandoned their original den and looked for a new one before resuming inactivity (Evans et al., 2012). Cattet et al. (2008a) found that movement rates decreased below normal rates after capture and returned to normal rates in 3-6 weeks. Regarding long-term effects on body condition, the same study by Cattet et al. (2008a) found that repeated captures can have a negative effect on the body condition of the bears. Age-specific body condition of bears captured twice or more often tended to be poorer than that of bears captured only once. In addition, the effect was directly proportional to the number of captures and more evident with age.

Alterations in behaviour during hibernation, such as den abandonment, are likely to affect energy balance by increasing energy use in a critical period when bears do not eat and rely on the energy provided by the fat and lean reserves acquired during autumn. Previous studies have reported weight loss in American black bears (*Ursus americanus*) (Tietje and Ruff, 1980) and a negative impact on reproduction in brown bears due to den abandonment (Swenson et al., 1997). Changes in movement rates for a prolonged period could also affect energy balance (i.e., assimilation and use of stored energy). Cattet et al. (2008a) concluded that a long-term consequence of capture and handling was a reduction in energy storage. The authors attributed this effect to a reduction in energy intake due to alterations in movement rates for a prolonged period of time, an increase in the use of energy (e.g., healing of injured tissue) or a cumulative effect of both. Thus, the physiological and behavioural responses to capture and handling can impose energetic costs (Morellet et al., 2009). According to life history theory, individuals will allocate resources optimally among life-history traits over their lifetime (Stearns, 1992). Therefore, research activities such as capture and handling could impact other vital processes (e.g., growth, reproduction, immune function). If the energetic costs of capture and handling occur in situations when the animal is incapable of overcoming any additional costs imposed by capture stress (i.e. low levels of reserves) or are long-lasting, the body condition of the animal could be reduced. Consequently, a loss of body condition could lead to reduced survival and reproductive rates, as has been reported in ursids (Noyce and Garshelis, 1994; Atkinson and Ramsay, 1995). Therefore, changes in body condition might have an effect at the individual level, but also influence

population dynamics through changes in birth (i.e., reproduction) (Stirling et al., 1999) and death rates (i.e. survival) (Robbins et al., 2012).

However, the results of some studies are not in agreement with a long-term effect of capture and handling on the animal's body condition. A recent study in polar bears (*Ursus maritimus*) concluded that, although activity and movement rates were affected the first days after capture, repeated captures were not related to long-term negative effects on body condition, reproduction or cub growth or survival (Rode et al., 2014). In other studies, a detectable effect of research activities depended upon life-history traits. For example, Ramsay and Stirling (1986) found that recapture had a negative influence on the weight of female polar bears with cubs, but no effect was detected in male bears. In addition, Lunn et al. (2004) reported that capture and handling of adult female polar bears had no effect on either the litter size or the mass of male cubs. However, females captured and handled in the autumn had lighter female cubs than females that were not disturbed.

1.4.4. Animal welfare, research results and the 3Rs principle

As a result of capture and handling, animal welfare can be compromised due to the potential for mortality, injuries, impairment of physiological parameters and alteration of behaviour (Kreeger et al., 1990; Arnemo et al., 2006; Cattet et al., 2008a). The reduction in animal well-being raises issues in animal welfare and research ethics. Also, capture and handling can lead to biased research results if their effects are not evaluated as potentially confounding factors (Powell and Proulx, 2003; Cattet et al., 2008a). For example, in studies evaluating body condition, the effect of capture should be taken into account in the analysis as a predictor variable and/or considered in the interpretation of the results. Otherwise, wrong conclusions can be drawn (Cattet et al., 2008a).

In any study involving the capture of wild animals, researchers should apply the "3R" principle (replacement, reduction, refinement) (Lindsjö et al., 2016). Capture and handling procedures must be in compliance with laws and regulations at different levels (local, state-provincial, federal-national, international). Researchers are also required to follow guidelines for the capture and handling of wildlife by ethical committees and professional associations (e.g., Canadian Council on Animal Care, American Society of Mammalogists, etc.). Also, some scientific journals have developed guidelines that must be followed in order to have work published in their journals (e.g., Animal Behaviour, Journal of Mammalogy).

2. Objectives

The main goal of my thesis was to evaluate the short- and long-term physiological effects of capture and handling on free-ranging brown bears in association with two long-term research projects, one in Scandinavia and the other in Alberta, Canada. For this, I conducted three studies to: i) evaluate the acute stress response to capture and handling by using a field-based technique to measure the leukocyte coping capacity in captured bears, ii) compare two different anaesthetic protocols based on behavioural and physiological short-term responses of captured bears, and iii) assess the long-term effects of capture, handling, and surgery on the body condition of independent male bears.

Stress measurements in wildlife can be used to refine capture and handling protocols and, therefore, reduce negative effects on animal welfare. However, there is presently no "gold standard" technique available for the assessment of stress. In general, the interpretation of stress measurements, irrespective of technique used, is often difficult because of the influence of confounding factors. In paper I, *I aimed to determine if a new technique, the leukocyte coping capacity (LCC), could be used as a practical and reliable method under field research conditions to evaluate the stress response caused by capture and handling of brown bears*. I also evaluated LCC values in relation to life history traits, captured-related variables, and other methods used to measure stress.

Anaesthetic drug combinations are often used to immobilize free-ranging wildlife, either as a primary capture technique (i.e., chemical immobilization) or as an adjunctive procedure to capture by physical restraint. Effective anaesthesia helps assure safety for capture personnel while reducing anxiety, stress and pain for captured animals. In paper II, *I aimed to determine if a new anaesthetic combination, dexmedetomidine-tiletamine-zolazepam, provided better anaesthesia, based on behavioural and physiological responses, than an established protocol, medetomidine-tiletamine-zolazepam, that has been used widely for the anaesthesia of free-ranging brown bears*.

Whereas the short-term (i.e., hours to days) physiological effects of capture and handling in brown bears have been documented in various research reports, fewer studies have addressed the potential long-term (i.e., months to years) effects. In paper III, *I evaluated the body condition of independent male brown bears in association with their capture and handling history to determine if body condition was potentially affected by capture and handling*.

3. Material and methods

3.1. Study areas and brown bear populations

3.1.1. Scandinavian Brown Bear Research Project

The Scandinavian Brown Bear Research Project (SBBRP) was the primary source of support and data for my research. The project started in Sweden in 1984, and then expanded to include Norway in 1987. Its primary goals are to understand the ecology of the Scandinavian brown bear, to provide the scientific basis for the management of the species in Sweden and Norway, and to provide information about brown bears to the general public.

The project's two study areas consist of 13,000 km^2 of intensively managed boreal forest dominated by Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) in the south (61°N, 14°E), and 6,000 km^2 with deep valleys dominated by mountain birch (*Betula pubescens)*, Scots pine, and Norway spruce, glaciers, and high plateaus in the north (67°N, 18°E; Figure 1). Elevations range from 200 m to 2000 m above sea level. The study areas have a continental climate with cold winters (January mean: -7°C in south, -13°C in north) and short, warm summers (July mean: 15°C in south, 13°C in north). Precipitation averages 500–1,000 mm annually. Snow cover lasts from beginning of October-late November until early to late May. The growing season is about 110–180 days (Zedrosser et al., 2006).

In 1930, the Scandinavian brown bear population reached its lowest numbers with only 130 bears in Sweden, and the Norwegian population virtually extinct (Swenson
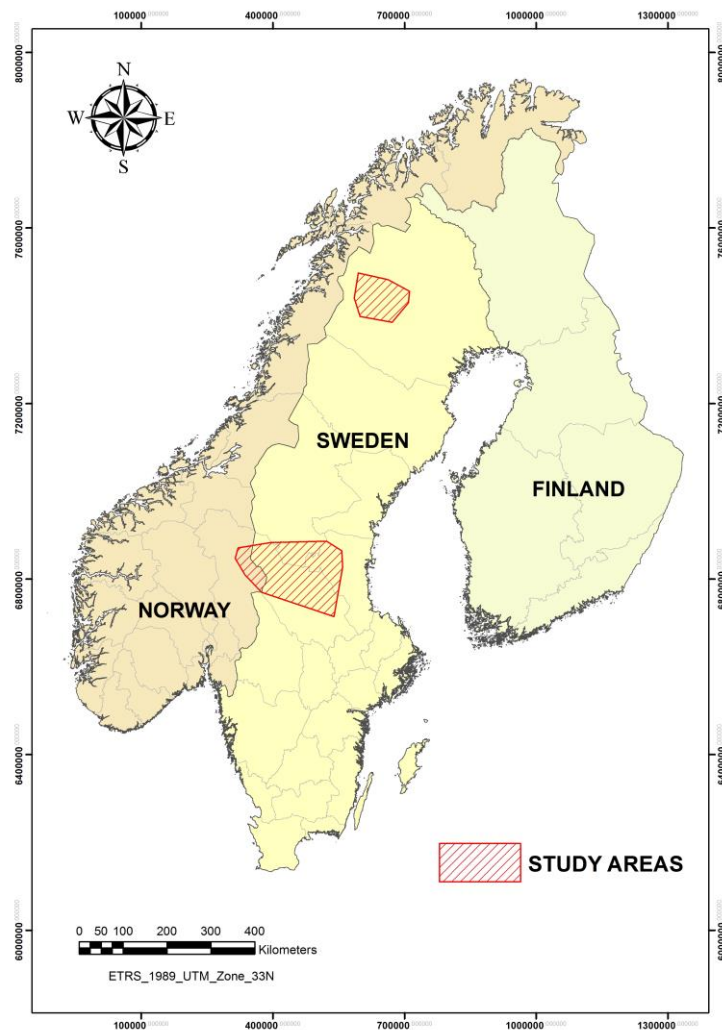
Figure 1. Brown bear study areas in Scandinavia from 1988 to 2014. Research is conducted in two study areas, northern area and southern area, which are about 600km apart.

et al., 1995). However, the conservation measures implemented in the early 20th century proved to be successful and the population recovered in numbers and expanded its distribution (Swenson et al., 1995; Swenson et al., 1998). In 2013, the brown bear population was estimated at 2,782 bears in Sweden (Kindberg and Swenson, 2014), and 150 bears in Norway (Aarnes et al., 2014). Brown bears are protected both in Norway and Sweden. However, hunting is allowed by the government. In addition, an increase in management kills and changes in hunting have been observed in recent years (i.e., increase in the number of specialized bear hunters, increase in the use of dogs by hunters, use of bait for hunting was allowed in 2013, increase in the participation of foreign hunters, etc.) (Swenson et al., 2017).

3.1.2. fRI Research Grizzly Bear Program in Alberta, Canada

My research was also supported by the fRI Research Grizzly Bear Program (fRI). This research project was initiated in 1999 with its primary goal to provide knowledge and planning tools to ensure the long-term conservation of brown bears in Alberta, Canada. The thesis also included data collected by the Eastern Slopes Grizzly Bear Project from 1993 to 2002 (Herrero, 2005).

The projects' study area consists of ~ 300,000 km^2 along the eastern slopes of the Canadian Rocky Mountains (49-58°N, 113-120°W; Figure 2) encompassing mountains and foothills ranging from 200 to 3700 m above sea level. Mountainous land is protected and consists of montane forests, conifer forests, sub-alpine forests, alpine meadows, and high elevation areas of rock, snow, and ice. The adjacent foothills are minimally protected and have a wide range of resource extraction activities (i.e., forestry, oil and gas, and open-pit coal mining). Land cover for the foothills includes conifer, mixed, and deciduous forests, areas of open and treed-bogs, small herbaceous meadows, and areas of regenerating (fire and clear-cut harvesting) forests (Nielsen et al., 2006). The study area is characterized by a continental climate with cold winters (January mean: -5°C in south, -15°C in north) and short, warm summers (July mean: 17°C in south, 15°C in north). Average precipitation is 450-900 mm annually. Snow cover lasts from late October until early May, and the growing season is short <160–185 days (Natural Regions Committee, 2006).

Currently, approximately 700 bears are estimated to occur at low densities throughout their distributional range in Alberta (ASRD and ACA, 2010). Population trends are largely unknown, but likely vary substantially over different parts of the province. In 2010, brown bears were classified as Threatened in Alberta. Since 2006, hunting of brown bears has been prohibited in Alberta (ASRD and ACA, 2010).
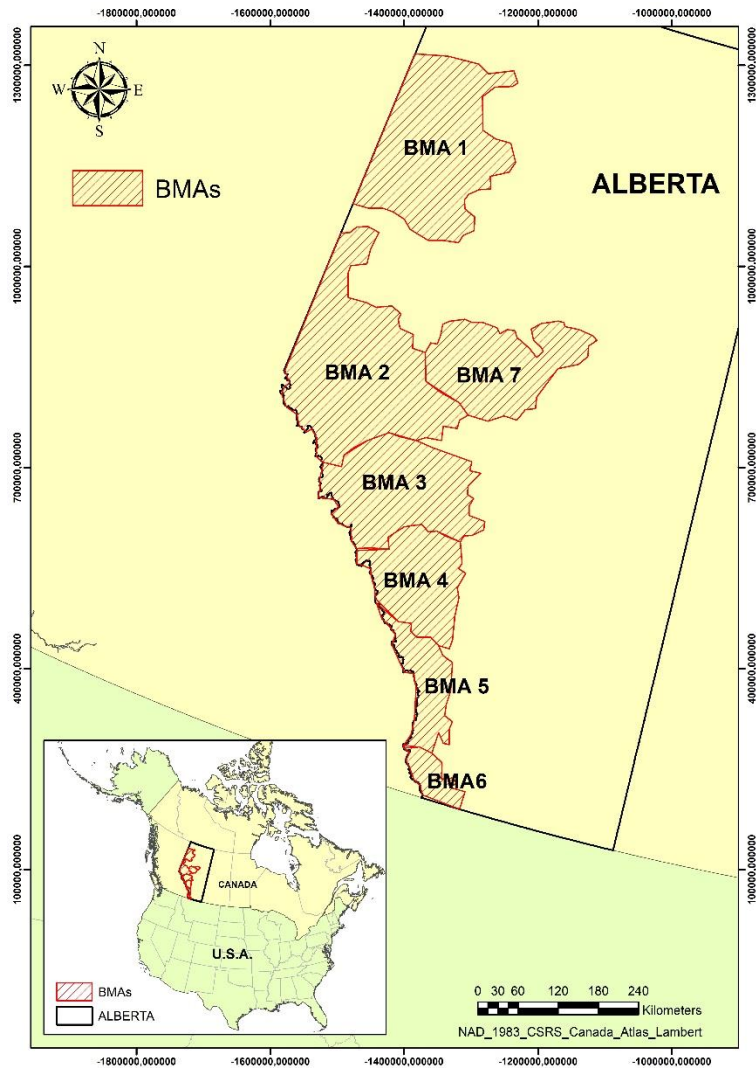
Figure 2. Brown bear study areas in Alberta, Canada from 1993 to 2015, based upon seven bear management areas (BMAs) as defined by the Alberta Department of Environment and Sustainable Resource Development.

3.2. Capture and handling of brown bears

In Scandinavia, captures were carried out by the SBBRP in March-October from 1988 to 2014. All bears were anaesthetized by remote drug delivery (Dan-Inject®, Børkop, Denmark) from a helicopter. Since 1992, brown bears have been anaesthetized for this project using a combination of medetomidine (Domitor® 1 mg/ml or Zalopine® 10 mg/ml, Orion Pharma Animal Health, Turku, Finland) and tiletamine-zolazepam (Zoletil® 500 mg/vial, Virbac, Carros, France). Details on capture methods and drug doses can be found in Arnemo and Evans (2017).

In Alberta, Canada, captures were carried out by the fRI in April-October from 1999 to 2015. Bears were anaesthetized by remote drug delivery (Pneu-Dart Inc., Williamsport, Pennsylvania, Paxarms NZ Ltd., Timaru, New Zealand, or Dan-Inject®, Børkop, Denmark) from a helicopter or were captured first by leg-hold snares (discontinued after 2008) or culvert trap, and then anaesthetized. The two most common anaesthetic protocols were xylazine (Cervizine 300; Wildlife Pharmaceuticals, Inc., Fort Collins, Colorado, U.S.A) or medetomidine (20 mg/ml; Chiron Compounding Pharmacy Inc., Guelph, Ontario, Canada) combined with tiletamine-zolazepam (Telazol®, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, U.S.A.). Details on capture methods and drug doses can be found in Cattet et al. (2003a and 2008a).

In both projects, capillary refill time, respiratory rate, heart rate, and rectal temperature were recorded to monitor anesthetized bears. Handling procedures common to both projects included morphometric measurements, collection of biological samples (i.e., blood, hair, faeces, anal glands secretion, ear plugs, skin, and a tooth), subcutaneous implantation of a microchip and fitting of a radio collar. For my studies I and II, I carried out several additional handling procedures as described below.

In Scandinavia, different types of surgeries have been performed on brown bears since 1997. These have included the implantation or removal of intraperitoneal and subcutaneous devices such as radio transmitters (Telonics®, Telonics Inc., Mesa, Arizona, USA), physiological sensors (Vectronic Aerospace®, Berlin, Germany), temperature loggers (Star-Oddi®, Gardabaer, Iceland) and ECG monitors (Reveal® XT, Medtronic Inc., Minneapolis, Minnesota, USA). Muscle biopsies have also been collected by the SBBRP. To provide analgesia for painful procedures, project veterinarians have administered bupivacaine (Marcain®, AstraZeneca, Cambridge, UK), carprofen (Rimadyl® vet. 50 mg/ml, Orion Pharma Animal Health, Espoo, Finland) and/or meloxicam (Metacam® 5mg/ml, Boehringer Ingelheim, Reihn, Germany) to anaesthetized bears.

After completion of procedures in both projects, atipamezole (Antisedan® 5 mg/ml, Orion Pharma Animal Health) was administered as a "reversal drug" to counteract the anaesthetic effects (Cattet et al., 2003a and 2008a; Arnemo and Evans, 2017).

3.3. Leukocyte coping capacity technique (Paper I)

The leukocyte coping capacity (LCC) technique was applied to 24 male and female bears, from one to 20 years old, solitary or in a family group, in south-central Sweden in April-May 2012 and 2013.

Leukocytes circulating in the blood have receptors that are sensitive to biochemical alterations linked to stress (Mian et al., 2005). In response to external stimuli, e.g. stressful situations, leukocytes are activated and release reactive oxygen species (ROS) via a process called respiratory burst (Ellard et al., 2001; Montes et al., 2004). Also, leukocytes produce ROS in response to the activation of protein kinase C with phorbol myristate acetate (PMA; Hu et al., 1999). After a stressful event, there is a latent period when the leukocytes' capacity to respond to a secondary external stimulus (e.g., bacterial challenge, PMA) is reduced (McLaren et al., 2003). The respiratory burst activity of leukocytes decreased in individuals of several animal species in association with stress caused by transport (McLaren et al., 2003), trapping and handling (Moorhouse et al., 2007; Gelling et al., 2009), and housing conditions (Honess et al., 2005; Moorhouse et al., 2007). By quantifying the reduction in the amount of ROS released by leukocytes in response to a secondary stimulus, one can assess the effect of the known or suspected stressor (Mian et al., 2005). The response of leukocytes to PMA challenge after a stressful event is defined as the individual's leukocyte coping capacity.

I performed the technique twice for each bear with the first occurrence as soon as possible after the bear was safely anaesthetized, and the second occurrence at approximately 90 minutes following the onset of anaesthesia. The purpose of the first LCC measurement was to evaluate the bear's stress response to capture whereas the second measurement was to assess the bear's stress response to surgery. To perform the LCC technique, I first collected a venous blood sample from the jugular vein of each bear using a vacutainer system (BD Vacutainer®, BD Diagnostics, Preanalytical Systems, Franklin Lakes, NJ, USA). I then transferred 10 μl of heparinized whole blood into a silicon anti-reflective tube (Lumivial, EG & G Berthold, Germany), to which I also added 90 μl of luminol (5-amino-2.3-dihydrophthalzine; Sigma A8511, Sigma-Aldrich, Oslo, Norway) at a concentration of 10^{-4} mol per litre diluted in phosphate buffered saline (PBS), and 10 μl of phorbol 12- myristate 13-acetate (PMA; Sigma P8139, Sigma-Aldrich, Oslo, Norway) at a concentration of 10^{-5} mol per litre. PMA activates leukocytes and the release of reactive oxygen species (Hu et al., 1999). Luminol chemiluminescenses when combined with an oxidizing agent (i.e., reactive oxygen species produced by leukocytes) to produce a low-intensity light reaction (Whitehead et al., 1992). I also transferred another 10 μl of the same heparinized whole blood sample into a tube containing luminol, but not the PMA challenging solution to measure the unstimulated blood chemiluminescence to provide a baseline against which to measure an individual's LCC response. For each tube, I measured chemiluminescence in relative light units using a portable chemiluminometer (Junior LB 9509, E G & G Berthold, Germany) every 5 min for a total of 30 min. The measurements were carried out in the field

immediately after the blood sample collection. To summarize the LCC measurements over a 30-min period, I calculated the area under the response curve (AUC) (Fekedulegn et al., 2007). I also noted the maximum LCC value over the 30-min period (LCC peak). To ensure that there was no bias in the LCC results due to individual differences, I subtracted the PMA-unstimulated from the PMA-stimulated values for each animal and used these values for the AUC calculation.

In addition to measuring the LCC, I also used the first venous blood sample collected as soon as possible after the bear was safely anaesthetized to determine total leukocyte counts, percentage of neutrophils and lymphocytes, and neutrophil-to-lymphocyte (N:L) ratio of each bear. I assessed these response variables in relation to pursuit time, medetomidine dose, number of times the bear had been captured, the occurrence of surgery, social status and body condition with generalized linear models (GLMs). The social status of a bear was defined as solitary or as member of a family group. I performed separate GLMs for measurements of the first and the second blood samples. I used parametric statistics (Pearson's correlation) to evaluate associations between LCC values and other methods that have been used to quantify acute stress (heart rate, N:L ratio, serum glucose concentration and serum cortisol concentration).

3.4. Comparison of medetomidine-tiletamine-zolazepam and dexmedetomidine-tiletamine-zolazepam (Paper II)

I compared a new anaesthetic protocol for brown bears, dexmedetomidine-tiletamine-zolazepam (DTZ), against an anaesthetic protocol, medetomidine-tiletamine-zolazepam (MTZ), that has been used for many years to capture brown bears in Scandinavia and in Alberta, Canada. I administered the anaesthetic combinations to bears using a randomized design and I compared the bear's responses to the different protocols based on a suite of immobilization characteristics and physiological measurements. My tests subjects were 37 free-ranging brown bears that were captured on 40 occasions either by helicopter in Sweden or by culvert trap in Alberta, Canada, in the spring of 2014 and 2015.

In Sweden, study bears were limited to yearlings (22 captures) and two-year-old bears (12 captures) because I only had access to a low concentration (0.5 mg/ml) of D and because I did not want to use dart volumes that exceeded 3 ml. For yearlings, each dart contained 1.66 mg of M or 0.415 mg of D and 83.3 mg of TZ. In two-year-old bears, each dart contained 2.5 mg M or 1.25 mg D and 125 mg TZ. Three bears were captured at both ages, as yearlings and as two-year olds. In Alberta, my test subjects were six adult males. Each animal was administered a combination of 50µg/kg estimated body weight of M, or 25µg/kg of D, and 2.45 mg/kg of TZ.

In both study areas, I collected two anaerobic arterial blood samples from the femoral artery of each bear in pre-heparinized syringes (PICO™70, Radiometer Copenhagen, DK-2700 Brønshøj, Denmark), the first at 30 min and the second at 60 min after the bear was darted. With each sample, I immediately

measured blood gases, acid-base status and selected hematologic and biochemical variables using a portable analyser (iSTAT 1®Portable Clinical Analyser and i-STAT® cartridges CG4+ and 6+ , Abbott Laboratories, Abbott Park IL, 60064-6048, USA). I administered medical-grade oxygen by intranasal cannula to any bears with low levels of blood oxygen (≤ 80 mmHg) based on measurements of partial pressure of arterial oxygen.

Due to differences in age composition of the bears evaluated in Sweden (yearlings and two-year olds) and in Alberta (adults), I analysed the data in two ways. For the first analysis, I used data collected in Sweden only and, in the second analysis, I combined the data from both study areas. I analysed immobilization characteristics (induction time and need for supplemental drugs to sustain anaesthesia) and serum cortisol concentrations using generalized linear models (Table 1). For response variables involving repeated measurements, which included arterial blood gases, acid-base status, heart rate, respiratory rate, and rectal temperature, I used linear mixed models for the analyses, with bear identification as a random effect (Table 1).

Table 1. Response variables, predictor variables (interactions not shown), and models used to compare anaesthetic events with either medetomidine-tiletamine-zolazepam (MTZ) or dexmedetomidine-tiletamine-zolazepam (DTZ) in brown bears captured in Sweden and Alberta, Canada in 2014-2015.

Response variable[a]	Predictor variable[b] combinations	Random effects[c]	Model type[d]
Induction time	Age + Sex + Drug + TZ + CD time + ODC[e]	NA	GLM Gamma link inverse
Supplemental drugs	Age + Sex + Drug + Weight + CD time + ODC + Induction time + Surgery + Handling time[e]	NA	GLM binomial
Cortisol	Age + Sex + Drug + Weight + CD time + ODC + Induction time[e,f]	NA	GLM Gaussian
pH	Time + Age + Drug + $PaCO_2$ + BE + Lactate	Bear ID	LMM
PaO_2	Age + Drug + Length + RT + RR + Oxygen	Bear ID	LMM
$PaCO_2$	Age + Drug + Weight + RT + RR + PaO_2	Bear ID	LMM
Heart rate	Time + Age + Sex + Drug + Length + CD time + ODC + Induction time + Surgery + Ket + RT + RR[e]	Bear ID	LMM
Respiratory rate	Time + Age + Sex + Drug + Length + CD time + ODC + Induction time + Surgery + Ket + RT + HR[e,f]	Bear ID	LMM
Rectal temperature	Time + Age + Sex + Drug + Weight + CD time + ODC + Induction time + Surgery + Ket + HR + RR[e]	Bear ID	LMM

[a] Response variables – (i) Induction time: time interval in minutes from a bear was darted to when it was fully immobilized; (ii) Supplemental drugs: yes, no; (iii) Cortisol: serum concentration in nmol/L; (iv) pH: arterial blood acid-base status; (v) PaO_2: partial pressure of arterial oxygen in mmHg; (vi) $PaCO_2$: partial pressure of arterial carbon dioxide in mmHg; (vii) Heart rate (HR): beats per minute; (viii) Respiratory rate (RR): breaths per minute (log-transformed); and (ix) Rectal temperature (RT): °C

[b] Predictor variables – (i) Age: yearlings, two year olds, adults (≥ 5 yr); (ii) Sex: male, female; (iii) Drug: MTZ or DTZ in mg/kg body weight; (iv) TZ: tiletamine-zolazepam in mg/kg body weight; (v) CD time: time interval in minutes from when active pursuit began to when the bear was darted; (vi) ODC: ordinal day of capture; (vii) Weight: body weight in kg; (viii) Surgery: yes or no; (ix) Handling time: time interval in minutes from immobilization to atipamezole administration; (x) Area: Sweden, Alberta; (xi) $PaCO_2$; (xii) Time: sampling and/or measurements recorded at 15; 30; 45; 60; 75; 90; 105; 120; 135 minutes after darting in Sweden, and at 15; 30; 45; 60; 75 minutes after darting in Sweden+Alberta; (xiii) BE: base excess in mmol/L; (xiv) Lactate: blood concentration in mmol/L; (xv) Length: contour body length in cm; (xvi) RR: respiratory rate; (xvii) RT: rectal temperature; (xviii) Oxygen: yes or no; (xiv) PaO_2; (xx); Ket: ketamine dose level in mg/kg body weight; (xxi) HR: heart rate; (xxii) RR: respiratory rate; (xxii) RT: rectal temperature

[c] NA: not applicable

[d] GLM: generalized linear model; LMM: linear mixed model

[e] CD time was excluded as explanatory variable for the analysis of the Sweden+Alberta dataset

[f] Area (Sweden; Alberta) substituted age as explanatory variable for the analysis of the Sweden+Alberta dataset

3.5. Effects of capture on body condition index (Paper III)

I evaluated the potential long-term effect of capture, handling, and surgery on the body condition index values of free-ranging independent male brown bears captured in Scandinavia and in Alberta, Canada, from 1988 to 2015. I defined "independent males" as those that were unaccompanied by their mother at the time of capture. I collated data from 302 individual bears (157 in Scandinavia and 145 in Alberta) captured by using Aldrich leg-hold snares (Aldrich Snare Co., Clallam Bay, Washington), culvert traps, or remote drug delivery from a helicopter. Additional details on capture and handling procedures can be found in Arnemo and Evans (2017) and Cattet et al. (2003a and 2008a). As the response variable, I used a body condition index (BCI) that has been validated for ursids and is based on standardized residuals derived from the regression of body weight against body length (Cattet et al., 2002). Body weight was obtained by suspending the bears from a spring-loaded or an electronic scale, and body length by measuring the contour from tip of nose to end of last tail vertebra with the bear in sternal or lateral recumbency. I focused on a single subset of bears (i.e., independent males) because demands on body condition, and the influence of different factors on body condition, might vary among sex, age and reproductive classes (Coulson et al., 2001; Bonenfant et al., 2009; Nielsen et al., 2013).

I used generalized linear mixed models (Zuur et al., 2009) to evaluate the potential effect of method of capture, number of times a bear was captured, time interval between capture events, and if a bear had undergone previous surgeries on the body condition of male bears. Concurrently, I also evaluated the effect of several known determinants of body condition. These included age of the bear, ordinal day of capture, and study area. Data on more specific potential factors associated with body condition (e.g., bear density) were not readily available to be included in the analysis. I assigned the identification of each individual bear and the year of capture as random effects in all models.

4. Results and discussion

4.1. Social status and body condition drive leukocyte coping capacity in brown bears (Paper I)

For the first blood sample, which was collected as soon as possible after a bear was safely anaesthetized, the most supported model ($\Delta AIC_C = 0.00$) suggested that the area under the curve (AUC) differed by social status. Members of family groups had a higher AUC than solitary bears (Figure 3). For the second blood sample, which was collected following surgery, the most supported model ($\Delta AIC_C = 0.00$) suggested that body condition was positively associated with AUC. However, there was also some support ($\Delta AIC_C \leq 2.00$) for a model that included the occurrence of surgery, in addition to body condition. Further, the intercept only (null) model also was supported.
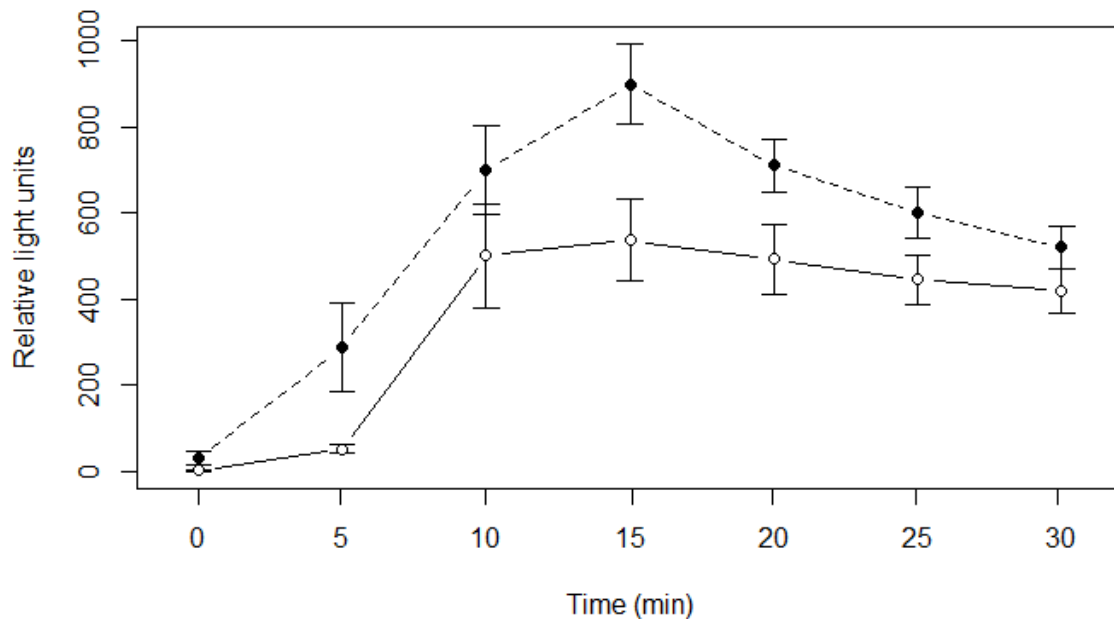
Figure 3. Leukocyte coping capacity measured every 5 minutes over a 30-minute period in 24 brown bears (*Ursus arctos*) captured in Dalarna and Gävleborg counties, Sweden, in April and May 2012 and 2013. The measurements represent the mean leukocyte coping capacity values (in relative light units) by social status (solitary bear or bear within a family group) for a blood sample collected as soon as possible after the bear was safely anaesthetized. The black dots connected by the dashed line represent values for bears in family groups; the white dots connected by the solid line represent solitary bears. Standard error bars are represented for each time point.

When using LCC peak values, instead of AUC, I found that the most supported model ($\Delta AIC_C = 0.00$) for the first blood sample suggested that LCC peak values also differed by social status with bears in family groups having higher values than solitary bears (Figure 3). However, the intercept-only (null) was also supported ($\Delta AIC_C \leq 2.00$). Due to the low sample size of the study, I assessed capture-related variables, such as medetomidine dose, pursuit time, and number of captures on the LCC peak separately, trying to identify a potential influence on the maximum capacity of ROS production by leukocytes. Capture-related variables did not explain the variation in LCC values. For the second blood sample, the results using LCC peak values were nearly identical to what I found when using AUC.

The total leukocyte count (5.3 ± 1.2 x10^9/litre) in the first blood sample was neither associated with body condition nor differed between solitary bears and family members. However, members of family groups had a higher proportion of neutrophils (family groups: 71.9 ± 7.2 %; solitary bears: 63.1 ± 9.3 %), a lower proportion of lymphocytes (family groups: 17.6 ± 7.9 %; solitary bears: 25.3 ± 10.6 %) and, therefore, a higher N:L ratio (family groups: 5.0 ± 2.2 x10^9/litre; solitary bears: 2.7 ± 1.8 x10^9/litre) than solitary bears. There was also support ($\Delta AIC_c \leq 2.00$) for a "body condition only" model, and the intercept-only (null) model, with the N:L ratio as the response variable.

AUC and LCC peak values were not significantly correlated with heart rate, N:L ratio, serum glucose concentration or serum cortisol concentrations, in either the first or second blood samples.

Research evaluating the effects of capture and handling can have the drawback of lacking a control group of uncaptured or unmarked individuals (Coté et al., 1998). In this study, it was not possible to measure ROS production and leukocyte composition prior to capture. No control group, i.e., uncaptured bears, was available as capture was necessary to obtain blood for the LCC and other measurements. Also, the first blood sample was obtained 30 ± 12 minutes after the bears were immobilized and, therefore, couldn't be used as a baseline (i.e., the activation of the HPA only takes 2-5 minutes, Boonstra et al., 2001) to evaluate the magnitude of change in AUC and peak LCC values in comparison with post-capture samplings. Although there is evidence that repeated capture, anaesthesia and handling can reduce LCC values (Moorhouse et al., 2007), I can only hypothesise that bears subjected to capture, a known stressor, reduced their LCC capacity and/or the differences in LCC measurements were the result of the stress of capture. Alternatively, the differences I found in LCC values in the first blood sample between groups could be due to pre-existing differences (i.e., higher LCC values in members of family groups than solitary bears in the first sampling could reflect higher values prior to capture) or a combination of both. Differences in neuroendocrine and immune system function can be attributed to life history traits such as sex (Gelling et al., 2009). Members of a family group had higher AUC and LCC peak values than solitary bears at the first blood sampling following capture. These results suggest that mothers and their dependent offspring had higher baseline LCC levels or a greater capacity to cope with capture-induced stress. In either case, higher

LCC levels might indicate a bear better able to respond to a bacterial challenge after stress (McLaren et al., 2003). Previous studies suggest that social interactions in humans (Kirschbaum et al., 1995) and affiliative behaviours in animals (Giralt and Armario, 1989; Smith and French, 1997) could provide a buffer against stress by dampening the hypothalamic-pituitary-adrenal (HPA) axis response (Carter, 1998). Studies with rats (Windle et al., 1997) and sheep (Cook, 1997), suggest a mechanism involving oxytocin, which is implicated in both the modulation of the HPA axis and prosocial behaviours (DeVries et al., 2003). I also discovered a higher proportion of neutrophils and N:L ratio, and a lower proportion of lymphocytes in members of family groups compared to solitary animals. In domestic species, a "stress leukogram" characterized by a leucocytosis, neutrophilia, lymphopenia, and eosinopenia typically occurs following adrenal stimulation, which leads to an increased N:L ratio (Feldman et al., 2000). The N:L ratio increases after restraint stress in rhesus monkeys (*Macaca mulatta*) (Morrow-Tesch et al., 1993) and after transport in Southern chamois (*Rupicapra pyrenaica*) (López-Olvera et al., 2006). However, age could also have contributed to differences in the percentage of neutrophils and lymphocytes between groups as family groups were composed by adult females and yearlings or two-year-old bears and solitary animals included sub adult and adult males and females (Græsli et al., 2014).

On the contrary, capture-related variables did not influence LCC values. I suggest that these results could be due to LCC values not reflecting the stress of capture. Further, inaccurate estimates of induction times and medetomidine doses might also provide a plausible explanation. Although it has been suggested that leukocyte reactivity exhibits habituation (Shelton-Rayner et al., 2010), I found no effect of the number of captures on LCC levels and concluded that there was no habituation to capture. One could argue that capture is a strong negative stimulus, therefore not causing habituation in the bears.

Body condition was an influential factor in the ROS production by leukocytes after capture and surgery in the bears. Bears in better body condition had higher overall LCC and peak levels, indicating that they coped better with handling stress. These results agree with studies in birds and mammals that have concluded that animals in better body condition show an enhanced immune response (Alonso-Alvarez and Tella, 2001; Bachman, 2003). I found no difference in LCC levels related to surgery. I suggest that the administration of anaesthetic and analgesic drugs to the bears, and the low sample size of the study should be taken into consideration when interpreting these results.

AUC and LCC peak values did not correlate with any of the commonly used stress indicators, e.g. heart rate, N:L ratio, or glucose and cortisol concentrations. Shelton-Rayner et al. (2012) did not find a correlation between LCC and heart rate, blood pressure, body temperature, or cortisol levels in humans. They attributed this to physiological variables and hormones being influenced by a range of factors in addition to stress, which may also be a plausible explanation for my findings in this study.

4.2. No benefit of using dexmedetomidine-tiletamine-zolazepam instead of medetomidine-tiletamine-zolazepam in the anaesthesia of brown bears (Paper II)

In Sweden, bears allocated to the MTZ group received an average dose level of 93.62 ± 36.96 µg/kg M and 4.69 ± 1.85 mg/kg TZ. Bears in the DTZ group received an average dose level of 57.51 ± 38.37 µg/kg D and 4.87 ± 2.49 mg/kg TZ. In Alberta, bears allocated to the MTZ group received an average dose level of 52.23 ± 18.55 µg/kg M and 2.5 ± 0.88 mg/kg TZ. Bears in the DTZ group received an average dose level of 21.97 ± 10.12 µg/kg D and 1.6 ± 0.78 mg/kg TZ. The difference in drug dose levels between study areas was due to the different capture method used. In bears captured by remote drug delivery from helicopter, it is important that induction time (time from darting to a bear fully immobilized) is short to minimize capture-related stress, the risk of injury and physiological disturbances resulting from physical exertion, such as hyperthermia and lactic academia (Fahlman et al., 2011). As induction time is dose-dependent, higher doses of anaesthetic drugs are used (Painer et al., 2012). Further, sensitivity of bears to the anaesthetic drug and, therefore, doses required, might differ depending on the capture method (Cattet et al., 2003a).

Induction of anaesthesia was predictable and smooth in all bears in both study areas irrespective of anaesthetic protocol. The induction time for bears captured in Sweden was significantly influenced by TZ dose level, sex and age. It was positively associated with TZ dose, greater in males than in females, and greater in two-year-old bears than yearlings. For the combined dataset, induction was faster in yearlings than in adult bears. Mean induction time did not differ between drug combinations.

In both study areas, it was necessary to administer ketamine to some bears to extend anaesthesia. Handling time was the only variable that significantly influenced the need to administer ketamine; the longer the handling time, the more likely that ketamine was required. The need to administer ketamine did not differ between DTZ and MTZ protocols.

Among brown bears in Sweden, blood cortisol concentrations were inversely associated with body weight, greater in males than in females and positively associated with induction time. I found differences between the study areas for the combined dataset. Mean cortisol concentrations were significantly higher in Alberta bears than in Sweden bears. This may have been an effect of capture method because all bears in Sweden were captured by remote drug delivery from helicopter whereas bears in Alberta were captured by culvert trap only. In this regard, Cattet et al. (2003a) found serum cortisol concentrations in brown bears captured by leg-hold snare to be significantly higher than values recorded for bears captured by remote drug delivery from helicopter. I found cortisol levels to be similar between MTZ and DTZ protocols.

I documented acidemia (pH of arterial blood < 7.35), hypoxaemia (partial pressure of arterial oxygen < 80 mmHg), and hypercapnia (partial pressure of arterial carbon dioxide ≥ 45 mmHg) in both study areas with both anaesthetic protocols.

In Sweden, I observed acidemia in 28 bears (13 of 14 bears in the MTZ group, 15 of 16 bears in the DTZ group) in the first arterial blood sample collected at 30 min after darting. In the second arterial blood sample collected at 60 minutes after the bear was darted, 27 bears (13 of 16 bears in the MTZ group, 14 of 18 bears in the DTZ group) had acidemia. In Alberta, I reported acidemia in two bears (one of three bears in each group) only at 30 minutes after darting. Arterial blood pH decreased with partial pressure of arterial carbon dioxide ($PaCO_2$) values and increased with base excess values in both datasets (Table 2). However, pH was not affected by drug protocol.

Table 2. Regression coefficients (β) and significance (p) of the predictor variables in the best model explaining variation in acid-base status arterial blood gases in brown bears anesthetized with either medetomidine-tiletamine-zolazepam (MTZ) or dexmedetomidine-tiletamine-zolazepam (DTZ) in Sweden and Alberta, Canada in 2014-2015.

	pH				PaO_2				$PaCO_2$			
	Sweden		Sweden + Alberta		Sweden		Sweden + Alberta		Sweden		Sweden + Alberta	
Predictors[a]	β	p	β	p	β	p	β	p	β	p	β	p
Age (Yearling)							-34.177	0.106				
Age (Two year old)					18.560	0.029	-19.3013	0.242	6.597	0.004		
Sex (Male)												
Drug (MTZ)					1.628	0.704	2.903	0.449	0.926	0.363	0.398	0.730
Weight									-2.584	0.018		
Length					-8.181	0.044	-16.892	0.026				
Rectal temperature					-7.957	0.005	-6.478	0.004	-1.423	0.015	-0.715	0.231
Rectal temperature*MTZ					3.265	0.460			1.359	0.108	1.691	0.058
Respiratory rate					0.945	0.645	0.892	0.764	-1.867	0.001	-1.756	0.002
Respiratory rate*MTZ							0.326	0.928	2.078	0.004	0.662	0.006
$PaCO_2$	-0.029	<0.001	-0.031	<0.001								
BE	0.058	<0.001	0.058	<0.001								
PaO_2									1.755	<0.001	1.964	<0.001
Oxygen (Yes)					62.134	<0.001	62.288	<0.001				

[a] Predictor variables – (i) Age: yearlings, two year olds, adults (≥5 yr); (ii) Sex: male, female; (iii) Drug: MTZ or DTZ in mg/kg body weight; (iv) Weight: body weight in kg; (v) Length: contour body length in cm; (vi) $PaCO_2$: partial pressure of arterial carbon dioxide in mmHg; (vii) BE: base excess in mmol/L; (viii) Oxygen: supplementation with oxygen, yes, no. Regression coefficients for factors are relative coefficients such that: (i) β for Age (Two year old) was determined with β for Age (Yearling) set to 0 for the Sweden dataset; (ii) β for Age (Yearling) and for Age (Two year old) were determined with β for Age (Adult) set to 0 for the Sweden + Alberta dataset; (iii) β for Sex (Male) was determined with β for Sex (Female) set to 0; (iv) β for Drug (MTZ) was determined with β for Drug (DZT) set to 0; and (v) β for Oxygen (Yes) was determined with β for Oxygen (No) set to

I discovered hypoxaemia in 27 Sweden bears (13 of 14 bears in the MTZ group, 14 of 16 bears in the DTZ) at 30 min after drug administration. All hypoxaemic bears at the first sampling received oxygen supplementation. At the second sampling time, only 4 bears (two bears in each anaesthetic protocol) were hypoxaemic. I documented hypoxaemia in all Alberta bears at both sampling times. Arterial oxygen partial pressures (PaO_2) were significantly correlated with the time interval from darting to sampling time ($r = 0.75$ in Sweden, $r = 0.68$ in the combined dataset, $p < 0.001$). The PaO_2 values were higher in two-year-old bears in the Swedish dataset, but age class was not significant in the combined dataset (Table 2). Arterial oxygen partial pressures were inversely correlated with body length and rectal temperature in both datasets. However, PaO_2 values were not affected by anaesthetic protocol. Arterial oxygen partial pressures also consistently increased in response to the provision of supplemental oxygen.

In Sweden, I found hypercapnia in three bears 30 min after darting (one of 14 bears in the MZT, two of 16 bears in the DTZ group), and in 10 bears (six of 16 bears in the MTZ group, four of 18 bears in the DTZ group) at one hour following drug administration. In Alberta, one bear in the MTZ showed hypercapnia at both sampling times. Arterial carbon dioxide partial pressures were higher in two-year-old bears than yearlings and inversely correlated with body weight and rectal temperature in bears from Sweden (Table 2). Arterial carbon dioxide partial pressures were positively correlated with PaO_2 values and inversely correlated with respiratory rates in both datasets. The association with respiratory rates was also modulated by anaesthetic protocol in both datasets; $PaCO_2$ values decreased as respiratory rate increased in the DTZ group, but remained relatively constant with changes in respiratory rate in the MTZ group (Table 2, Figure 4).

I detected bradycardia (< 50 beats per minute) in bears from both study areas. Three Sweden bears (one of 16 bears in the MTZ group, two of 18 bears in the DTZ group) had bradycardia at 75 min following drug administration. In Alberta, I detected bradycardia in four bears (one of three bears in the MTZ group, all three bears in the DTZ group) as early as 15 min after drug administration, and sustained until the end of the anaesthesia. Mean heart rate was lower in two-year-old bears than in yearlings among the Swedish bears, but this age class difference was not apparent in the model derived from the combined dataset. Heart rate was positively associated with ordinal day of capture, rectal temperature and respiratory rate in both datasets. However, its association with respiratory rate was not statistically significant in the combined dataset. Relative to heart rates recorded at 15 min following drug administration, heart rates in both datasets were generally lower at subsequent time points. Heart rate was not differentially affected by anaesthetic protocol.
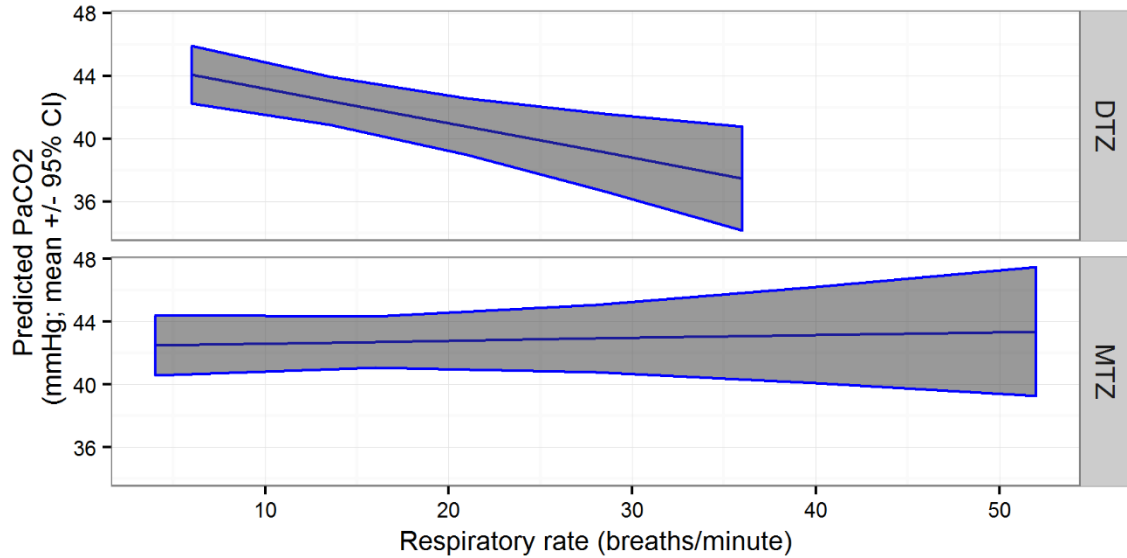
Figure 4. Partial pressure of arterial carbon dioxide ($PaCO_2$, mmHg) in association with respiratory rate (breaths/minute) by drug combination (MTZ: medetomidine-tiletamine-zolazepam; DTZ: dexmedetomidine-tiletamine-zolazepam) in 40 anaesthetic events of free-ranging brown bears captured in Sweden and Alberta, Canada in 2014-2015.

I detected bradypnoea (< 5 breaths per min) and tachypnoea (> 30 breaths per min) in the Swedish bears. Two of 16 bears in the MTZ group had bradypnoea at various times following drug administration. Tachypnoea occurred in eight bears (five of 16 in the MTZ group, three of 18 in DTZ group) during anaesthesia. However, respiratory rates remained within normal range (5-30 breaths per minute) throughout anaesthesia for bears in Alberta. Mean respiratory rate was significantly higher in bears captured in Sweden than in bears captured in Alberta. This was likely because all bears in Sweden were captured by remote drug delivery from helicopter whereas bears in Alberta were captured by culvert trap. Respiratory rates were also affected by an interaction between rectal temperature and age in bears from Sweden, but this effect was not evident in the model derived from the combined dataset. Respiratory rates in bears from Sweden were significantly lower at 45 min than the first recording at 15 min following drug administration, and significantly higher at all time points from 90 to 135 min after drug administration. Respiratory rate was not differentially affected by anaesthetic protocol (Figure 5).
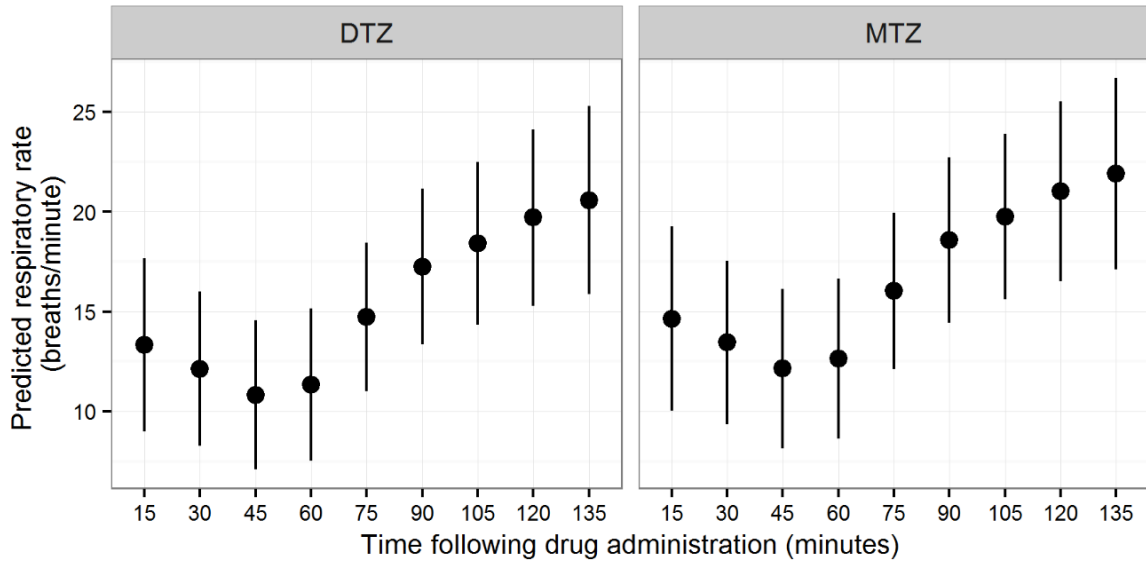
Figure 5. Respiratory rate (breaths/minute) at 15-minute intervals following drug administration (MTZ: medetomidine-tiletamine-zolazepam; DTZ: dexmedetomidine-tiletamine-zolazepam) in 34 anaesthetic events of free-ranging brown bears captured in Sweden in 2014-2015.

Hyperthermia (T $\geq$ 40°C) was recorded in bears receiving both drug combinations in Sweden. Five bears within each drug group were hyperthermic at 30 min after darting, and two bears within each drug group were still hyperthermic at 60 min. Conversely, rectal temperature remained < 40°C throughout anaesthesia for bears captured in Alberta. Again, the use of different methods of capture between the two study areas likely accounts for this difference. Rectal temperature was also positively associated with heart rate and inversely associated with the time following drug administration. For the combined dataset, two-year-old bears had significantly higher rectal temperatures than adult bears. Rectal temperature was not differentially affected by anaesthetic protocol.

Studies using dexmedetomidine for the anaesthesia of bears found normal respiratory rates and high oxygen saturations (Teisberg et al., 2014; Coltrane et al., 2015). The authors suggested a potential benefit of dexmedetomidine over medetomidine in bears due to less respiratory depression, i.e., little or no hypoventilation or hypoxaemia. However, these studies did not include a comparison of performance or efficacy with equivalent doses of medetomidine. Contrary to Teisberg et al. (2014), I found that both MTZ and DTZ caused hypoventilation and hypoxaemia (PaO$_2$ < 80 mm Hg). Hypoxaemia (inadequate oxygen levels in the blood) is a common physiological alteration found during the anaesthesia of ursids (Caulkett and Cattet, 1997; Caulkett et al., 1999; Fahlman et al., 2010 and 2011). The use of alpha-2 adrenoceptor agonists can cause respiratory depression and produce intrapulmonary changes that may result in

hypoxaemia (Jalanka and Roeken, 1990; Read, 2003; Fahlman et al., 2008 and 2011). It is widely documented that effects of alpha-2 adrenoceptor agonists (e.g., sedation, analgesia, cardiovascular function) are dose-dependent (Jalanka and Roeken, 1990; Painer et al., 2012; West et al., 2014). The alteration of the central and peripheral response to CO_2 and oxygen is also dose-dependent (McDonell and Kerr, 2015). A previous study in brown bears suggested that the hypoxaemia caused by medetomidine could be dose-dependent (Fahlman et al., 2011). Moreover, significantly lower PaO_2 values were found when high doses of medetomidine and dexmedetomidine were administered to dogs compared to lower doses (Kuusela et al., 2001). In my study, the PaO_2 values of bears decreased with an increasing body length. Body length was significantly correlated to the dose level of alpha-2 adrenoceptor agonist. Therefore, I hypothesise that the different findings between Teisberg et al. (2014) and our study are due to the dose-dependent effect of alpha-2 adrenoceptor agonists on PaO_2. The mean dexmedetomidine dose level used in our study (21.97 $\pm$ 10.12 µg/kg in Alberta, 57.51 $\pm$ 38.37 µg/kg in Sweden) was two to five times higher than in Teisberg et al. (2014) (10.11 $\pm$ 1.04 µg/kg). Hypoxaemia can be effectively treated with oxygen supplementation as reported in our study and other studies in brown bears (Fahlman et al., 2010 and 2014).

Among the response variables assessed in the study, $PaCO_2$ was the only one affected by the anaesthetic protocol used. Hypercapnia was a common physiological alteration documented in the study. $PaCO_2$ values in our study were similar to previously reported values in brown bears anesthetized with MTZ in Scandinavia (Fahlman et al., 2011). The elevation of $PaCO_2$ values usually indicates low respiratory rates (hypoventilation) that, in the study bears, were probably caused by the alpha-2 adrenoceptor agonists (Jalanka and Roeken, 1990; Fahlman et al., 2011). In relation to $PaCO_2$ values, I observed a differential effect of the anaesthetic protocol. In the DTZ group, $PaCO_2$ values decreased with increasing respiratory rates due to increased elimination of CO_2. In contrast, $PaCO_2$ values remained constant with increasing respiratory rates in the MZT group. Surprisingly, these findings were not supported by significantly different respiratory rates between anaesthetic protocols, i.e., higher respiratory rate in the DTZ group. Thus, I suggest that the results regarding $PaCO_2$ values may have been caused by a different drug effect on the tidal volume (i.e., alveolar volume) and ventilation. The use of DTZ in the anaesthesia of giant pandas (*Ailuropoda melanoleuca*) revealed changes in haemoglobin oxygen saturation with constant respiratory rates (Jin et al., 2015), supporting the fact that changes in ventilation might occur independently of respiratory rates. Anaesthetic drugs can influence tidal volume by causing ventilation-perfusion problems (McDonell and Kerr, 2015). Ventilation-perfusion problems lead to a decrease in PaO_2 levels before any changes in $PaCO_2$ levels. The administration of supplemental oxygen during anaesthesia prevented us from detecting this effect. I believe that D resulted in better ventilation than M, but only when respiratory rates increased. If this is true, D could prove more beneficial than M in situations when respiratory rates are anticipated to increase as in captures involving pursuit with a helicopter, captures with high ambient

temperatures, or in later stages of anaesthesia and during recovery. Nevertheless, I acknowledge that other comparative studies have not revealed differences between the use of M and the use of D on arterial blood gases and acid-base status (Kuusela et al., 2001; Bouts et al., 2010 and 2011).

4.3. BCI depends upon age, day of capture and study area (Paper III)

The mean, standard deviation and range in body weight and body length for the bears used in this study were 138.9 ± 61.89 kg (22-311) and 177.9 ± 22.96 cm (96-229), respectively. Mean BCI was 0.0 ± 1.0, and ranged from -3.08 (poor) to + 3.83 (excellent).

The highest-ranked candidate model indicated that age of the bear, ordinal day of capture and study area were the main factors associated with BCI values in the study bears. The fixed effects in our best model explained 46% of variation in BCI among bears. Age of the bear had a significant positive curvilinear association with BCI (Figure 6). The mean BCI of bears increased with age until they reached 15.7 years old. From 15.7 to approximately 23 years old, mean BCI was positive and stable, but decreased significantly in bears > 23 years. However, the data set included only three bears that were >23 years. The ordinal day of capture also had a positive curvilinear association with BCI values (Figure 7). Bears were in better body condition with increasing ordinal day of capture (i.e., bears captured later in the year). The increase in BCI was slow from den emergence in spring until the beginning of summer. Mean BCI values turned positive over summer, and markedly increased in fall before the bears began hibernation. There was also a weak effect of study area on BCI, i.e., bears in Scandinavia were likely to be in better body condition than bears in Alberta, Canada. On the contrary, models that included capture-related variables (i.e., method of capture, number of previous captures, capture interval, abdominal surgery) were not supported ($\Delta AIC_c > 2$).
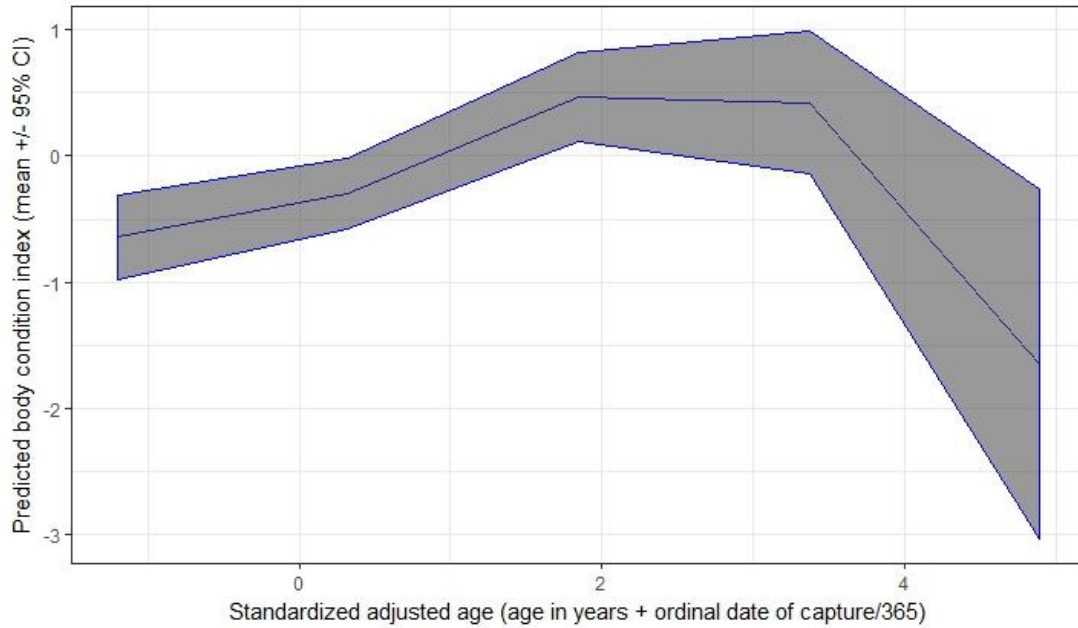
Figure 6. Body condition index by adjusted age in free-ranging independent male brown bears captured either in Scandinavia (N=371) or Alberta, Canada (N=180) between 1988 and 2015.
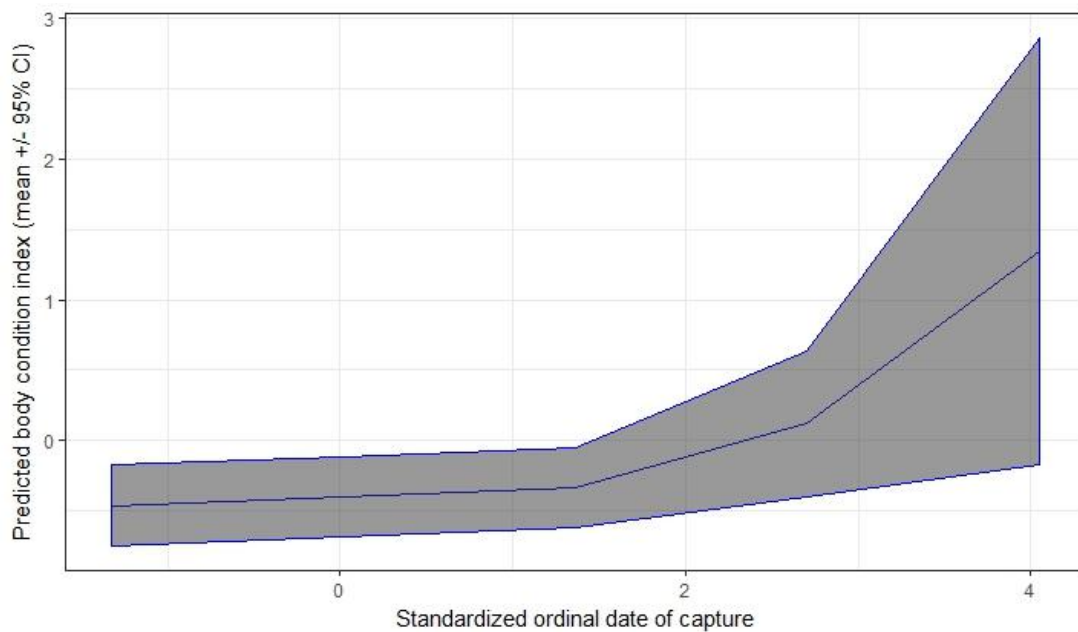

Figure 7. Body condition index by ordinal day of capture in free-ranging independent male brown bears captured either in Scandinavia (N=371) or Alberta, Canada (N=180) between 1988 and 2015

The results of this study showed that variation in BCI values of independent male brown bears was associated with the age of the bears, the day they were captured, and the area of study. Conversely, I did not find any associations between capture-related variables and the bears' BCI.

In brown bears, age-specific growth curves have been described for males of different populations (Zedrosser et al., 2007; Bartareau et al., 2011). These curves show an increase in body weight and body length with age. Previous research on brown bears supports my findings of a curvilinear relationship of age with body condition (Nielsen et al., 2013). A biological explanation for this result is that juvenile animals are poor at acquiring food and may not survive. Consequently, animals that get older are those animals that were successful at acquiring food and are, therefore, in better body condition. In American black bears, Schroeder (1987) concluded that differences in haematological patterns and the ratio body weight/body length reflected the competitive ability of bears to successfully forage on limited food resources, and produced a ranking of condition within a sex and age class (i.e., highest to lowest: adult males, adult females, sub adult males, sub adult females). The drop off in BCI in bears > 23 years could reflect senescence, where animals of advanced age have reduced the ability to acquire food. Senescence could be defined as a biological deterioration in physiological functions which predicts that older individuals will show an age-specific increase in mortality and a decline in somatic and reproductive investment (Broussard et al., 2003). Thus, body condition would initially increase with age, reach a maximum at intermediate age, and decline at the oldest ages.

The brown bear is an omnivorous mammal that inhabits highly variable environments (Ferguson and McLoughin, 2000; Munro et al., 2006), and has developed a strategy to cope with seasonal food scarcity. Brown bears are active from spring to autumn and during this period they need to consume large amounts of high-energy food to accumulate fat for hibernation (Swenson et al., 2000). During hibernation they rely on the energy provided by the fat and lean reserves acquired during autumn (Farley and Robins, 1995; Robbins et al., 2012). Not surprisingly, I found an increase in the bears' BCI values with increasing ordinal day of capture.

In this study, I used data collected from two independent brown bear populations that inhabit boreal forest ecosystems in Europe and North America. Both areas are similar in that they are characterized by a continental climate with cold winters and short, warm summers, and have similar values of average precipitation, snow cover and growing season (Natural Regions Committee, 2006; Zedrosser et al., 2006). In addition, both bear populations are interior and have similar diets with no access to spawning salmon (Munro et al., 2006; Stenset et al., 2016). However, there is no reason to believe that the same species living in different areas will respond in the same way to climate, as the forms of regulation may differ among populations or populations may experience limiting factors at different times of the year (Martínez-Jauregui et al., 2009). In fact, my findings suggest a difference in body condition in brown bears due to study area,

i.e., brown bears in Scandinavia were likely to be in better body condition than bears in Alberta. While their respective habitats and weather exposure may be similar, brown bear populations in Scandinavia and Alberta differ in a wide range of factors such as genetics (Taberlet and Bouvet 1994, Waits et al., 1998), temporal trends in population numbers and current population status (ASRD and ACA, 2010; Swenson et al., 2017), and human-pressure activities (Nielsen et al., 2006; Zedrosser et al., 2006). These factors likely also contribute to our findings. However, without the findings from comparative studies, I cannot be certain of the specific factor or combination of factors that explain the study area difference in mean BCI values.

My results indicated that capture, handling, and surgery of independent male brown bears did not influence the variation in their body condition estimated as a BCI. These results are in agreement with Rode et al. (2014), who concluded that repeated captures were not related to long-term negative effects on body condition in polar bears. Conversely, there are a few studies demonstrating a negative effect of capture and handling on body condition in mammals. Tuyttens et al. (2002) showed that European badgers (*Meles meles*) that had been carrying a radio-collar for up to 100 days were more likely to have a low body condition score compared to control badgers that had never been fitted with a collar. In water voles (*Arvicola amphibius*), the attachment of radio-collars to females caused a male-skewed sex ratio of the offspring (Moorhouse and MacDonald, 2005). The authors attributed this finding to a deterioration in maternal condition in response to radio-collaring.

In brown bears, Cattet et al. (2008a), found that age-specific body condition of bears captured twice or more tended to be poorer than that of bears captured only once. Further, the authors found that the negative effect of capture and handling was proportional to the number of times a bear was captured, and this effect was more apparent with age. The fact that Cattet et al. (2008a) identified significant capture effects, not only in the same species, the brown bear, but also within the same population (i.e., Alberta) of bears used within my study brings to question the apparent disparity in findings between this study and my study. I hypothesized that this might be due to 1) the calculation of BCI based on different measurements of body length, and/or 2) the focus of the study on different sex-reproductive classes. First, Cattet et al. (2008a) calculated the bears' BCI values based on straight-line body length, which is measured as the straight-line distance, from the tip to the nose to the end of the last tail vertebra, using a measuring tape extended above the bear in sternal recumbency. This follows from the procedure recommended by Cattet et al. (2002) in their validation study of the BCI. In my study, body length was measured along the curvature of the dorsum with the bear in either sternal or lateral recumbency. To compare both measures of body length, I used 294 records from the fRI Research Grizzly Bear Program in Alberta, Canada, where both straight-line and curvature body length were measured in the same bear. The regression of body weight against straight-line body length showed a lower coefficient of variation in comparison to curvature length. This, the lack of precision would be higher when measuring the curvature of the dorsum. Although, straight-line body length

seems to be a slightly more precise method to measure body length, poor repeatability is found with both methods. The BCI method has been validated for ursids, and has been demonstrated to reflect true body condition (Cattet et al., 2002). However, we should take into consideration that body length measurements have poor repeatability (i.e., inter- and intra-individual errors in the measurement of body length), and/or that the presence/absence of food in the digestive tract may lead to wrong estimates of body mass, and thus, body condition (Cattet et al., 1997b). Second, I focused on a single group of animals in the population, the independent males, whereas Cattet et al. (2008a) and Nielsen et al. (2013) included both sexes, and several reproductive classes (i.e., male, female, and female with dependent offspring). Both studies concluded that BCI values varied as a function of sex and reproductive class. Nielsen et al. (2013) found that adult females were more likely to have a lower BCI than sub adult or adult male bears, and this association was more pronounced with the presence of dependent young. However, in these studies, potential interactions between sex-reproductive class and capture variables were not evaluated. Nevertheless, given the different energetic demands among sex-reproductive classes, it is possible that the capture effects identified in these studies were not the same for all groups. In polar bears, Ramsay and Stirling (1986) only found a detectable negative effect of capture and handling on the weight of females with cubs, and suggested that the additional energetics costs of capture to a pregnant female might reduce their weight, and could potentially reduce the weight and size of her offspring. Thus, the cumulative cost of reproduction and provisioning offspring (i.e., lactation and maternal care) in female bears might result in the energetic response to capture and handling having a measurable effect on their body condition. In contrast, the energetic response to capture and handling may have a negligible effect on the body condition of males, as was found in this study, because they are not additionally burdened by the energetic demands of pregnancy and lactation.

5. Conclusions

I conclude that the leukocyte coping capacity (LCC) technique has potential to be used as a quick, practical and reliable method under field research conditions to quantitatively measure the stress response caused by capture and handling of free-ranging brown bears. I documented that life history traits are important factors driving stress responses to capture and handling in brown bears, and should be taken into consideration by researchers in their study designs. Nevertheless, the measurement and interpretation of LCC values may be confounded by various factors that are likely more prevalent during field research where conditions can be unpredictable and difficult to control. These challenges, however, are not unique to the LCC technique, and are also encountered with more conventional measures of stress (e.g., serum cortisol concentration) that are used to assess wildlife welfare. Thus, I recommend further evaluation of the LCC technique under field research conditions in order to clarify stress responses to capture and handling and coping mechanisms in mammals. The response to a stressor is an extremely complex phenomenon that can vary depending on the nature, severity, and context the stressor, as well as the attributes of the individual, including age, sex, life history stage, and personality (Romero and Wingfield, 2016). The choice of the technique used to measure and/or quantify stress should be based on the nature of the study, the study species, and the type of response we aim to evaluate (i.e., short- vs long-term evaluation) (Sheriff et al., 2011; Romero and Wingfield, 2016). However, given the complexity of measuring and interpreting the stress response in wildlife, a combined approach using multiple stress parameters representing different physiological systems is recommended (Gelling et al., 2009). The LCC technique could be used in combination with traditional techniques to provide a more comprehensive approach to evaluating stress in wildlife and its potential impact on their welfare.

I assessed the bears' behavioural and physiological responses to capture and handling using two different anaesthetic protocols (i.e., dexmedetomidine-tiletamine-zolazepam (DTZ) vs medetomidine-tiletamine-zolazepam (MTZ)). I discovered numerous, short-term, physiological effects (e.g., acidemia, hypoxaemia) with both protocols. However, the monitoring of bears while under anaesthesia allowed for the early detection of such alterations and the application of corrective measures was successful. For example, oxygenation improved after supplementing the bears with oxygen, and hyperthermia was resolved by applying snow to the bear's paws, groin and axillae, and by administering intravenous fluids. Both MTZ and DTZ proved to be safe and reliable anaesthetic combinations for anesthetizing free-ranging brown bears captured by remote drug delivery from helicopter, or by culvert trap. Both protocols produced a rapid onset of anaesthesia, smooth induction, good analgesia and muscle relaxation, and smooth predictable recovery. However, I found no detectable differences in induction time, the need for supplemental drugs to sustain anaesthesia, capture-related stress, acid-base status, partial pressure of arterial oxygen, heart rate, respiratory rate and rectal temperature in the bears. I conclude that dexmedetomidine, when combined with

tiletamine-zolazepam, offers no advantage over the use of MTZ in the anaesthesia of free-ranging brown bears. I also recommend the use of supplemental oxygen to counteract hypoxaemia at the dose levels of alpha-2 adrenoceptor agonists used in the study.

I found that the body condition of independent male brown bears, as estimated by a body condition index (BCI), was associated with age of bear, ordinal day of capture, and study area. Both age of bear, and ordinal day of capture had a positive curvilinear association with BCI. Also, there was evidence of a weak difference in mean BCI values between study areas with bears captured in Scandinavia tending to be in better condition than bears captured in Alberta irrespective of the annual timing of captures, the year of capture, or the age composition of captured animals. However, without the findings from comparative studies, I cannot be certain of the specific factor or combination of factors that explain the study area difference in mean BCI values. In the future, I could compare specific temporally and spatially-related environmental variables that have been previously found to be significantly associated with body condition in brown bears, such as weather conditions, and population density. Conversely, capture-related variables, including method of capture, number of captures, capture interval, and abdominal surgery, did not have a significant impact on BCI values. I considered the limitations of the index used to estimate the bears' body condition. More studies like this are needed to determine if capture and handling procedures are inadvertently biasing research results. Although I did not identify any capture-induced biases in this study of body condition in independent male brown bears, future studies should be conducted to determine if the same holds true for other sex, age, and reproductive classes.

In order to achieve best practices for capturing and handling wildlife, the effects of such procedures should be minimized by carefully designing the study, and choosing the capture technique and deployment device (Casper, 2009). Through the refinement of the techniques such as species-specific anaesthetic protocols, standardization of doses, improvement of capture methods or species-specific capture methods, researchers can reduce mortality rates and counteract some negative effects caused by capture and handling (Arnemo et al., 2006).

Selection of drugs should be based on the species, drug availability, drug effectiveness, and safety in the target species (Kreeger and Arnemo, 2012; Proulx et al., 2012). Researchers working with free-ranging wildlife should consider that health assessment prior to capture is not possible, accurate dosing might be difficult, and monitoring systems and emergency drugs or equipment may be lacking. More importantly, when using anesthetic drugs, supporting care and close monitoring of the vital signs should be priovided to minimize the risk of morbidity and mortality (CCAC, 2003; Proulx et al., 2012).

Capture methods should continuously be assessed and improved to work more efficiently and more safely for both animals and people (Powell and Proulx, 2003). The most updated techniques and those that optimize animal welfare should be used (CCAC, 2003; Proulx et al., 2012). In the capture and handling of

bears by remote injection from a helicopter, or by live-trap, pursuit, restraint and induction times should be kept to a minimum to minimize stress and physiological alterations such as hyperthermia or acid-base imbalance (Cattet et al., 2003a). The use of leg-hold snares is not advised due to its high potential to cause irreversible muscle injury (Cattet et al., 2003a and 2008a). The time a bear spends constrained in a culvert trap can be minimized by using trap-monitoring devices which may help reduce capture-related stress and injury, and will enable researchers to record the duration of constraint (Cattet et al., 2008a).

In any study involving wildlife, the benefits of the study should be balanced against the potential negative effects on the animal's health, while taking into consideration 1) all potential negative effects of capture and handing on the animal's behaviour and ecology, e.g., change in space use as a result of capture may add nutritional stress to the stress of capture (Morellet et al., 2009), 2) other stressors the animals may be experiencing at the time the study is conducted, e.g., food scarcity, provisioning offspring (Romero and Wingfield, 2016), and 3) the cumulative impact of capture effects and environmental stressors, e.g., capture effects might have a greater impact on an animal with offspring in a year with food scarcity.

5. Future perspectives and work

The assessment of the potential effects caused by research activities, such as capture and handling, is of paramount importance to fully understand the overall impact of anthropogenic activities on wildlife health. Thus, short- and long-term capture and handling effects should be taken into consideration in studies involving wildlife.

The measurement of the leukocyte coping capacity (LCC) is a promising technique to quantitatively measure stress responses in free-ranging wildlife. However, further evaluation of the LCC technique is needed in order to disentangle the animal's stress response to capture and handling from confounding factors such as sex, age or season. Therefore, attention should be paid to the study design, with emphasis toward larger sample size, and broader representation of different age-sex classes, than used in my preliminary study. Also, the evaluation of surgery's impact on the LCC response warrants further investigation. In my study, no influence of surgery on LCC values was detected. However, the sample size of the study was low, and the number of bears undergoing surgery vs. those not having surgery was unbalanced. In addition, it would be intresting to assess the response to different capture methods by using the LCC technique. LCC values could be compared to results from studies assessing stress responses to capture and handling based on more traditional parameters (Cattet et al., 2003a; Fahlman et al., 2011). In this thesis, I accounted for the response to a single capture method, remote dug delivery from a helicopter. I strongly encourage researchers to use the LCC technique in combination with traditional techniques to obtain a more comprehensive approach on the different pathways of the stress response.

The decision to use physical or chemical restraint in wildlife, should be based upon the complexity and duration of the handling procedures, the invasiveness of the procedures, the need for anaesthesia, the degree of stress involved in the capture and restraint of a particular species, and the safety of the investigator (CACC, 2003). For some species, physical restraint can be accomplished faster with fewer complications (i.e., lower mortality) (Peterson et al., 2003). For others, the use of anaesthetic drugs helps to reduce the stress and pain caused by capture and handling, while providing safety for capture personnel (Cattet et al., 2004). When the use of drugs is needed to accomplish research goals, such as in the brown bear, researchers should be aware that drugs have the potential to alter physiological parameters, and animal behaviour, or even cause death (Arnemo et al., 2006; Cattet et al., 2008a). Thus, close monitoring and supportive care should be provided to detect and correct any physiological disturbances. The ongoing development of safer and more effective anaesthetic protocols for wildlife is essential for research and management purposes.

In my PhD study, I limited the assessment of the long-term effects of capture and handling on the bears' body condition to a subset of bears, the independent males. Future work should be conducted to also evaluate potential effects on other sex-age-reproductive classes including independent females (with and without offspring) and dependent bears. From previous reports, it appears that the effects of capturing and

handling may depend upon the individual's physiological state (e.g., reproductive status), the equipment and techniques used, and environmental conditions at the time of capture (Moorhouse and MacDonald, 2005; Proulx et al., 2012; Nielsen et al., 2013). In species with sexual size dimorphism, such as the brown bear, males maximize growth rate, whereas females have to trade-off between growth and reproduction (Isaac, 2005). Thus, the cumulative cost of reproduction and provisioning offspring (i.e., lactation and maternal care) in independent female bears could result in capture and handling having a significant effect on body condition. Nielsen et al. (2013) found that adult females were more likely to have a lower body condition than male brown bears, and this association was even more pronounced when the females had cubs. In polar bears, Ramsay and Stirling (1986) only found a detectable negative effect of capture and handling on the weight of females with cubs, and suggested that the additional energetics costs of capture to a pregnant female might reduce their weight, and could potentially reduce the weight and size of her offspring. As for dependent bears, which I defined as young bears that were with their mothers at the time of capture, I have carried out preliminary analysis of data from 476 captures conducted by the Scandinavian Brown Bear Research Project from 1988 to 2014. Preliminary results suggest that biological (i.e., age, maternal age) and capture-related variables (i.e., number of captures, capture interval) are the main factors associated with body condition index values in young bears. These results also suggest that maternal capture history (e.g., whether or not the mother was captured the year of pregnancy) may affect the offspring's body condition. However, data on other potential sources of variation in body condition (e.g., bear density) were not readily available to be included in the analysis. Thus, there is need to follow-up on this preliminary analyses to get a better sense of whether or not capture and handling is likely to have long-term effects on the body condition of brown bears.

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Paper I

Fandos Esteruelas, N., Huber, N., Evans, A. L., Zedrosser, A., Cattet, M., Palomares, F., Angel, M., Swenson, J. E., and Arnemo, J. M. (2016). Leukocyte coping capacity as a tool to assess capture- and handling-induced stress in Scandinavian brown bears (*Ursus arctos*). *Journal of Wildlife Diseases, 52*(2s), S40-S53.

LEUKOCYTE COPING CAPACITY AS A TOOL TO ASSESS CAPTURE- AND HANDLING-INDUCED STRESS IN SCANDINAVIAN BROWN BEARS (*URSUS ARCTOS*)

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ABSTRACT: Brown bears (*Ursus arctos*) are often captured and handled for research and management purposes. Although the techniques used are potentially stressful for the animals and might have detrimental and long-lasting consequences, it is difficult to assess their physiological impact. Here we report the use of the leukocyte coping capacity (LCC) technique to quantify the acute stress of capture and handling in brown bears in Scandinavia. In April and May 2012 and 2013, we collected venous blood samples and recorded a range of physiological variables to evaluate the effects of capture and the added impact of surgical implantation or removal of transmitters and sensors. We studied 24 brown bears, including 19 that had abdominal surgery. We found 1) LCC values following capture were lower in solitary bears than in bears in family groups suggesting capture caused relatively more stress in solitary bears, 2) ability to cope with handling stress was better (greater LCC values) in bears with good body condition, and 3) LCC values did not appear to be influenced by surgery. Although further evaluation of this technique is required, our preliminary results support the use of the LCC technique as a quantitative measure of stress.

Key words: Animal welfare, brown bear, capture, chemical immobilization, leukocyte coping capacity, stress, surgery, *Ursus arctos*.

INTRODUCTION

Effective wildlife research and management often require the capture and handling of animals. However, the evaluation of capture and handling effects on target animals is often overlooked, despite the high potential for significant stress (Cattet 2013). For example, data loggers are increasingly used in research to enable remote collection of physiological information. This often involves surgical implantation, which can cause pain and distress (Hawkins 2004) or can lead to mortality (Quinn et al. 2010; Léchenne et al. 2012). Studying changes in physiological parameters due to capture is important because morbidity can cause subtle but harmful effects that might go undetected (Cattet et al. 2003) and bias research data (Powell and Proulx 2003; Cattet et al. 2008).

For animal welfare, objective and quantitative measures of stress are central (McLaren et al. 2007). Several techniques can be used to measure the stress response

in animals (Palme and Möstl 1997; Windle et al. 1997b; Millspaugh et al. 2000), but to date, blood concentrations of glucocorticoids (GCs) has been the most widely used parameter to assess the acute stress of capture in free-ranging wild animals (Creel et al. 1997; Arnemo and Caulkett 2007; Delehanty and Boonstra 2009). However, GC levels alone may not equate to stress levels (Sheriff et al. 2011). Using GC levels to measure stress can be complicated, as they are affected by multiple factors, including time of day, season, handling, and anesthetic drugs (Boonstra et al. 2001; Owen et al. 2005; Arnemo and Caulkett 2007). Consequently, using cortisol measurements alone to accurately measure stress in an individual can be challenging, and results should be interpreted with caution.

Recently the interaction between stress and the immune system has received attention. Stress affects the immune system by altering the quantity, composition, activity, and responsiveness of circulating immune cells (Dhabhar et al. 1995; Ellard et al. 2001). Leukocytes circulating in the blood have receptors that are sensitive to biochemical alterations linked to stress (Mian et al. 2005). In response to external stimuli, e.g., stressful situations, leukocytes (particularly neutrophils) are activated and release reactive oxygen species (ROS) via a process called respiratory burst (Ellard et al. 2001; Montes et al. 2004). During respiratory burst, oxygen uptake by leukocytes accelerates to produce ROS that destroy bacteria and other pathogens (Halliwell and Gutteridge 2007). However, the respiratory burst activity of leukocytes decreases in individuals of several animal species in association with stress caused by transport (McLaren et al. 2003), trapping and handling (Gelling et al. 2009), and housing conditions (Honess et al. 2005; Moorhouse et al. 2007) and by psychological stress in humans (Ellard et al. 2001; Shelton-Rayner et al. 2010). Also, leukocytes produce ROS in response to agonists such as bacterial peptides and the activation of protein kinase C with phorbol myristate acetate (PMA; Hu et al. 1999). After a stressful event, there is a latent period when the neutrophils' capacity to respond to a secondary external stimulus (e.g., bacterial challenge, PMA) is reduced (McLaren et al. 2003). As a result, an animal can be immunocompromised. By quantifying the reduction in the amount of ROS released by leukocytes in response to a secondary stimulus, one can assess the effect of the known or suspected stressor (Mian et al. 2005). The response of leukocytes to PMA challenge after a stressful event is defined as the individual's leukocyte coping capacity (LCC). Therefore, animals with a higher LCC will have greater potential to produce a respiratory burst and will be better able to respond to bacterial challenge after stress. Hence, LCC is an *in vitro* assessment of the animal's current physiological status and its overall ability to cope with stress (McLaren et al. 2003).

In this study, we used the LCC technique to investigate the stress response caused by capture and subsequent abdominal surgery of free-ranging brown bears (*Ursus arctos*). Our primary goal was to evaluate LCC values in relation to life history traits (social status, body condition), capture-related variables (pursuit time, medetomidine dose, number of times the bear had been captured), and intensity of handling (surgery, no surgery). We also aimed to compare LCC results with established methods to measure and quantify acute stress: heart rate, neutrophil-to-lymphocyte (N:L) ratio, and blood glucose and cortisol concentrations. We hypothesized that 1) bears within family groups would have higher LCC values than solitary bears, 2) bears in better body condition would have higher LCC values, 3) bears with longer pursuit times during capture would have lower LCC values, 4) bears undergoing surgery would have lower LCC values, and 5) there would be a negative correlation between LCC and other physiological measures of stress.

Animal welfare is relevant for conservation biology (McLaren et al. 2007). Stress

measurements allow for the refinement of capture and handling protocols and, therefore, improvements in animal welfare. From the perspective of evaluating wildlife welfare, our broader goal with this study was to determine if the LCC technique could be used as a practical and reliable method under field research conditions to evaluate the stress response of captured brown bears. If this technique proved to be dependable, it could have future application as a basis for improving techniques of capture and handling free-ranging brown bears.

MATERIALS AND METHODS

Study area and animals

Field work was conducted in south-central Sweden (61°N, 15°E). Animals were captured in April–May 2012 and 2013, shortly after they exited the dens after hibernation. Ambient temperatures ranged from 2 to 5 C. Brown bears were anesthetized for GPS collaring and sampling for ecological studies within the Scandinavian Brown Bear Research Project.

Capture methods and handling procedures

Bears were immobilized according to the biomedical protocol used for captures of free-ranging brown bears in Scandinavia (Arnemo et al. 2012). All captures were approved by the Swedish Ethical Committee on Animal Research (application number C 7/12) and the Swedish Environmental Protection Agency.

Anesthetic agents were administered by remote darting from a helicopter with a CO_2-powered rifle (Dan-Inject®, Børkop, Denmark). We used a combination of medetomidine (Domitor® 1 mg/mL or Zalopine® 10 mg/mL, Orion Pharma Animal Health, Turku, Finland) and tiletamine-zolazepam (Zoletil® 500 mg/vial, Virbac, Carros, France) at standard doses depending on the estimated weight of the animal. Ketamine (Narketan 10®, 100 mg/mL, Chassot, Dublin, Ireland) was used to extend immobilization when needed based on monitoring anesthetic depth. The movement of bears with the helicopter was kept to less than 3 min, with active pursuit lasting no more than 30 s. We recorded time of pursuit, defined as the time between first observation and when the bear was immobilized on the ground (recumbency). All yearlings were naïve to capture, whereas the other bears had been captured 1–12 times previously.

Once anesthetized, we recorded the bear's capillary refill time, respiratory rate, heart rate, and rectal temperature and assessed these parameters every 15 min throughout anesthesia. We collected two heparinized blood samples from the jugular vein from each bear using a vacutainer system (BD Vacutainer®, BD Diagnostics, Preanalytical Systems, Franklin Lakes, NJ, USA). We collected the first sample as early as possible after recumbency to assess the stress of capture. We performed complete blood counts, serum biochemistry, cortisol, and LCC determination from this sample. Hematology and chemistry analysis followed standard procedures; see Græsli et al. (2014). We collected the second sample 90 min after recumbency, during or after surgery, and measured LCC to assess the stress of surgery. Our study focused on stress caused by surgical implantation or removal of radio transmitters, physiological sensors, and temperature loggers in the peritoneal cavity. For analgesia, we administered 4 mg/kg carprofen (Rimadyl® vet. 50 mg/mL, Orion Pharma Animal Health, FI-02200 Espoo, Finland) or 0.2 mg/kg meloxicam (Metacam® 5 mg/mL, Boehringer Ingelheim, Reihn, Germany) subcutaneously before the surgery started. After completing all procedures, we administered 5 mg of atipamezole (Antisedan® 5 mg/mL, Orion Pharma Animal Health, Turku, Finland) per mg of medetomidine intramuscularly and left the bears to recover undisturbed at the capture site.

Leukocyte Coping Capacity (LCC) measurement

To measure the unstimulated blood chemiluminescence levels and provide a baseline with which to measure an individual's LCC response, we immediately transferred 10 μL of heparinized whole blood into a silicon antireflective tube (Lumivial, EG & G Berthold, Germany) and added 90 μL of 10^{-4} mol L^{-1} luminol (5-amino-2.3-dihydrophthalzine; Sigma A8511, Sigma-Aldrich, Oslo, Norway) diluted in phosphate buffered saline (PBS). We shook the tube gently for mixing. Luminol chemiluminesces when combined with an oxidizing agent to produce a low-intensity light reaction (Whitehead et al. 1992). To measure the chemiluminescence produced in response to challenge, we prepared another tube as above but added 10 μL of phorbol 12-myristate 13-acetate (PMA; Sigma P8139, Sigma-Aldrich, Oslo, Norway) at a concentration of 10^{-5} mol L^{-1}. The PMA solution had been prepared in advance by diluting 5 mg of PMA in 500 μl of dimethyl sulfoxide (Sigma D 5879, Sigma-Aldrich, Oslo, Norway), which was then diluted to a concentration of 10^{-5} mol L^{-1} in PBS buffer (Shelton-Rayner et al.

2012). Individual aliquots were kept in the dark at −20 C until required. For each tube, we measured chemiluminescence in relative light units using a portable chemiluminometer (Junior LB 9509, E G & G Berthold, Germany) every 5 min for a total of 30 min. The measurements were done in the field immediately after the blood sample was collected. When not in the chemiluminometer, tubes were incubated at 37 C in a lightproof water bath.

Statistical analysis

We categorized the bears according to the following criteria; social status: solitary (single animals: males, females without dependent offspring) or family groups (mothers with dependent offspring) and whether or not surgery was performed. We also estimated a sex-specific body condition index by standardizing the residuals of the regression of body mass against body length for males and females separately (Cattet et al. 2002).

To summarize the LCC measurements over a 30-min period, we calculated the area under the response curve (AUC) (Fekedulegn et al. 2007). To ensure that there was no bias in the LCC results due to individual differences, we subtracted the PMA-unstimulated from the PMA-stimulated values for each animal and used these values for the AUC calculation. We also assessed the LCC per 10^9 neutrophils L^{-1} to examine the effect of the number of circulating neutrophils on ROS production.

We applied generalized linear models (GLMs) to evaluate the effects of life history traits, variables of capture and surgery on LCC, leukocyte counts and composition, and N:L ratio. We performed separate GLMs for measurements of the first and the second blood samples. The response variables for the first blood sample were AUC1, LCC1, total leukocyte counts, percentage of neutrophils and lymphocytes, and N:L ratio. AUC1 was defined as the area under the response curve for the first blood sample. LCC1 was defined as the LCC peak value (mean of the maximum LCC measurements, regardless of when they occurred during the 30-min period) for the first blood sample. We used two different sets of explanatory variables for analysis relating to the first blood sample. The first set contained the variables "social status" and "body condition." The second set contained the variables "pursuit time," "medetomidine dose," and the lifetime "number of captures." We constructed four candidate models for the first set and eight models for the second set of explanatory variables a priori, based on our hypotheses. The candidate models contained all possible combinations of variables.

For the second blood sample, the response variables were AUC2 and LCC2 (area under the response curve and LCC peak value for the second blood sample, respectively). The explanatory variables were "social status," "body condition," and whether a "surgery was performed or not." We also constructed eight a priori models for all possible combinations of variables for the second blood sample.

We did not include interactions among variables into the models, due to low sample size. We selected the most parsimonious model, based on Akaike's Information Criterion corrected for small sample sizes (AIC_c) (Burnham and Anderson 2002; Burnham et al. 2011). For model selection we used $\Delta AIC_c \leq 2$ and Akaike model weights (AIC_{cWt}) (Burnham and Anderson 2004). Due to model selection uncertainty, we also applied a full-model averaging approach and used the relative importance of the predictor variables (Symonds and Moussali 2011).

We used parametric statistics (Pearson's correlation) to investigate correlations among variables and present the mean ± standard deviation for all variables. Differences were considered significant when $P \leq 0.05$. For statistical analysis we used the software R 3.0.2 (R Development Core Team 2012).

RESULTS

Study animals

We used 24 bears in the study: six yearlings, five subadults, and 13 adults; 10 males and 14 females; and 12 were solitary and 12 were part of a family group. We conducted surgery on 19 bears (Table 1). No mortalities occurred during anesthesia or within 30 days postcapture.

Leukocyte coping capacity (LCC)

We obtained the first and second blood samples 30 ± 12 min and 93 ± 8 min after recumbency, respectively. For the first sample, the AUC1 was mainly affected by the social status of a bear. Members of a family group had a higher AUC1 than solitary bears at capture (Tables 2–3; Fig. 1). For the second sample, body condition had a positive effect on AUC2 values; bears in better body condition had a higher AUC2. We also used the LCC per 10^9 neutrophils L^{-1} as response variable and obtained the same results.

TABLE 1.   Sex, age, social status, type of handling (surgery vs. no surgery), and leukocyte coping capacity measured in 24 brown bears anesthetized in Sweden in April–May 2012 and 2013.

Bear ID	Sex[a]	Age[b]	Social status[c]	Surgery[d]	AUC1[e]	LCC1[f]	AUC2[g]	LCC2[h]
W0806	F	5	S	Y	11,015.5	556	15,070	801
W0904	F	4	S	Y	11,618	594	10,362	502
W1019	M	8	S	Y	9,635.5	605	15,576.5	860
W0820	F	5	S	Y	4,724	308	8,086	549
W0818	F	5	S	Y	3,391.5	239	4,244	362
W0716	F	11	F	Y	23,483	1,071	21,901	1,255
W1204	M	1	F	Y	31,557	1,630	24,314	1,681
W0104	F	12	F	Y	13,411	557	21,863	1,365
W0620	F	7	F	Y	16,477.5	789	21,469	1,172
W1207	M	1	F	N	12,490	704	13,375	753
W1103	M	3	S	Y	7,840.5	423	9,542	521
W0812	M	6	S	Y	9,148.5	555	13,289.5	798
W0811	M	6	S	N	16,975	854	41,329.5	2,317
W1210	M	4	S	Y	25,042	1,635	29,607	1,532
W0625	M	10	S	Y	17,572	1,103	27,321.5	1,849
W0825	F	6	S	N	9,090.5	510	25,459	1,443
W0610	F	8	S	Y	8,700.5	545	24,782.5	1,248
W1301	M	1	F	Y	15,140	978	28,450	1,619
W1302	M	1	F	Y	16,487	999	26,995	1,655
W9403	F	20	F	Y	21,507.5	1,135	29,508	2,312
W1303	F	1	F	Y	6,347	340	13,891.5	895
W1304	F	1	F	Y	15,272	861	16,346.5	1,005
W1206	F	2	F	N	17,708.5	1,123	21,728.5	1,264
W1205	F	2	F	N	17,232	909	16,068	910
Mean ± SD		5±5			14,244.4 ±6,734.58	792 ±372	20,024.1 ±8,562.95	1,195 ±538

[a] F = female; M = male.

[b] In years.

[c] S = solitary (no other bears observed during the capture); F = family (mothers with cubs).

[d] Y = yes; N = no.

[e] Area under the response curve (in relative light units) for leukocyte coping capacity measurements obtained as soon as the animal was immobilized.

[f] Maximum leukocyte coping capacity value (in relative light units) obtained as soon as the animal was immobilized.

[g] Area under the response curve (in relative light units) for leukocyte coping capacity measurements obtained during or after surgery.

[h] Maximum leukocyte coping capacity value (in relative light units) obtained during or after surgery.

From LCC peaks, we found that LCC1 was produced at 15 min in 55% of bears, with other peaks produced at 5 (4%), 10 (29%), 20 (8%), and 30 (4%) min. Social status was an important variable affecting LCC1 values (Tables 2–3; Fig. 1). Bears in family groups had higher LCC1 values than solitary bears. Capture-related variables, such as medetomidine dose, pursuit time, and number of captures, did not explain the variation in LCC1 values (Tables 2–3). For the second sample, LCC2 values were produced at 15 min in 70% of cases, with other peaks produced at 10 (13%), 20 (13%), and 25 min. (4%). Body condition also influenced LCC2 values; bears in

TABLE 2.   Candidate models for the stress response to capture (measured by AUC1 and LCC1) and surgery (measured by AUC2 and LCC2) of 24 brown bears anaesthetized in Sweden in April–May 2012 and 2013. The four or five models with the lowest AIC$_c$ for each response variable are presented.

Response variable	Candidate models	k[a]	AIC$_c$[b]	ΔAIC$_c$[c]	AIC$_c$Wt[d]
AUC1[e]	Social status	3	491.78	0.00	0.64
	Body condition+Social status	4	494.66	2.88	0.15
	Null	2	494.78	3.00	0.14
	Body condition	3	497.56	4.78	0.06
LCC1[f]	Social status	3	355.03	0.00	0.47
	Null	2	355.78	0.75	0.32
	Body condition+Social status	4	357.93	2.90	0.11
	Body condition	3	358.08	3.05	0.10
LCC1[f]	Null	2	355.78	0.00	0.39
	Number of captures	3	357.26	1.49	0.19
	Pursuit time	3	357.87	2.09	0.14
	Medetomidine dose	3	358.10	2.32	0.12
	Number of captures+Pursuit time	4	359.66	3.88	0.06
AUC2[g]	Body condition	3	505.45	0.00	0.28
	Body condition+Surgery	4	505.75	0.30	0.24
	Null	2	506.31	0.86	0.18
	Surgery	3	507.76	2.31	0.09
	Body condition+Social status	4	508.33	2.88	0.07
LCC2[h]	Body condition	3	371.57	0.00	0.37
	Body condition+Surgery	4	372.94	1.36	0.19
	Null	2	373.45	1.88	0.14
	Body condition+Social status	4	374.15	2.57	0.10
	Social status	3	374.59	3.01	0.08

[a] Number of estimated parameters.

[b] Akaike's Information Criterion corrected for small sample sizes.

[c] Differences in AIC$_c$ values between the best model (lowest AIC$_c$) and each candidate model.

[d] AIC weights.

[e] Area under the response curve for leukocyte coping capacity measurements obtained as soon as the animal was immobilized.

[f] Maximum leukocyte coping capacity value obtained as soon as the animal was immobilized.

[g] Area under the response curve for leukocyte coping capacity measurements obtained during or after surgery.

[h] Maximum leukocyte coping capacity value obtained during or after surgery.

better body condition had higher LCC2 values. The relative importance of social status and surgery was low and neither influenced LCC2 values.

Physiological variables, complete blood counts, and biochemistry

Mean values for complete blood counts and biochemistry parameters were within the reference range for the species (Græsli et al. 2014). All animals were considered to be in good health status.

Life history traits did not affect total leukocyte numbers but did affect leukocyte composition and N:L ratio (Tables 4–5). Members of family groups had a higher proportion of neutrophils, a lower proportion of lymphocytes and, therefore, a higher N:L ratio than solitary bears.

AUC and LCC peak values in both samples did not correlate with any of the other parameters used as stress indicators, such as heart rate, N:L ratio, or glucose and cortisol concentrations (Table 6).

TABLE 3. Model averaging for the stress response to capture (measured by AUC1 and LCC1) and surgery (measured by AUC2 and LCC2) of 24 brown bears anaesthetized in Sweden in April–May 2012 and 2013.

Response variable	Predictor variable	β[a]	2.5% CI[b]	97.5% CI[b]	SE[c]	Variable importance[d]
AUC1[e]	Intercept	16,634.4	12,614.15	20,959.78	2,099.9	
	Social status (solitary)	−6,004.2	−11,288.57	−719.81	2,546.6	0.80
	Body condition	475.9	−2,626.34	3,005.48	1,434.2	0.21
LCC1[f]	Intercept	868.90	645.25	1,110.15	113.87	
	Social status (solitary)	−264.71	−570.63	41.21	147.42	0.58
	Body condition	17.77	−167.75	158.48	80.91	0.21
LCC1[f]	Intercept	875.52	529.7	1,206.15	175.17	
	Number of captures	−23.69	−71.16	23.81	22.91	0.31
	Pursuit time	−6.45	−25.89	12.86	9.38	0.24
	Medetomidine dose	−1,379.25	−6,574.64	3,766.76	2,500.34	0.23
AUC2[g]	Intercept	21,918	14,739.12	28,660.04	3,553	
	Body condition	3,287	−226.68	6,737.06	1,692	0.64
	Surgery (yes)	−5,783	−14,505.34	2,879.92	4,232	0.40
	Social status (solitary)	−1,144	−9,266.57	6,175.39	3,801	0.21
LCC2[h]	Intercept	1,273.3	891.83	1,631.91	191.0	
	Body condition	219.9	3.60	435.49	104.6	0.70
	Surgery (yes)	−267.2	−821.62	270.34	266.4	0.30
	Social status (solitary)	−170.5	−658.26	294.46	233.4	0.25

[a] Model averaged coefficients.

[b] Confident intervals.

[c] Standard error.

[d] Relative importance of the predictor variables.

[e] Area under the response curve for leukocyte coping capacity measurements obtained as soon as the animal was immobilized.

[f] Maximum leukocyte coping capacity value obtained as soon as the animal was immobilized.

[g] Area under the response curve for leukocyte coping capacity measurements obtained during or after surgery.

[h] Maximum leukocyte coping capacity value obtained during or after surgery.

DISCUSSION

We determined in this study that LCC values in captured brown bears were primarily influenced by their social status and body condition, but surgical effects appeared to be minimal to inconsequential. Further, LCC values did not correlate with more conventional measures of physiological stress, including serum cortisol concentrations.

Stress of capture

Stress affects the number and distribution of circulating leukocytes rapidly and reversibly (Dhabhar et al. 1995). In our study, LCC was not affected by the number of circulating neutrophils, as shown in McLaren et al. (2003). However, the stress of capture influenced ROS production and leukocyte composition. The bear's social status was the main evaluated factor shaping the stress response to capture in Scandinavian brown bears. Members of a family group had higher overall LCC levels (calculated as the increase of the area under the curve), as well as LCC peak levels, than solitary bears. This confirmed our first hypothesis, suggesting that mothers with dependent offspring had greater capacity to cope with capture-induced stress and might have a higher ability to combat infection after the capture event. Studies suggest that social interactions in humans (Kirschbaum et al. 1995) and affiliative
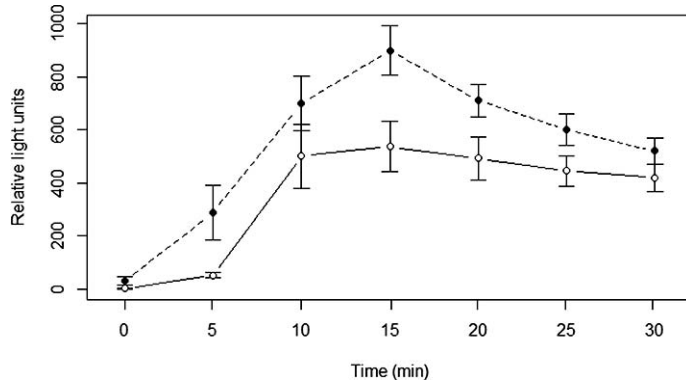
FIGURE 1.   Leukocyte coping capacity measured every 5 min over 30 min time in 24 brown bears (*Ursus arctos*) captured in Sweden in April–May 2012 and 2013. The measurements represent the mean leukocyte coping capacity values (in relative light units) by social status (solitary bear or bear within a family group) for the blood sample collected as soon as possible after recumbency. The black dots connected by the dashed line represent values for bears in family groups; the white dots connected by the solid line represent solitary bears. Error bars are represented for each time point.

behaviors in animals (Giralt and Armario 1989; Smith and French 1997) could provide a buffer against stress by dampening the hypothalamic-pituitary-adrenal (HPA) axis response (Carter 1998). However, little is known about how positive social interactions suppress corticosteroids. Some studies suggest a mechanism involving oxytocin (Cook 1997; Windle et al. 1997a), which is implicated in both the modulation of the

TABLE 4.   Candidate models for the stress response to capture (measured by leukocyte counts, leukocyte composition, and neutrophil-to-lymphocyte ratio) of 24 brown bears anaesthetized in Sweden in April–May 2012 and 2013. The four models with the lowest AICc for each response variable are presented.

Response variable	Candidate models	k[a]	AICc[b]	ΔAICc[c]	AICcWt[d]
Total leukocyte counts	Null	2	56.09	0.00	0.68
	Body condition	3	59.09	3.00	0.15
	Social status	3	59.12	3.03	0.15
	Body condition+Social status	4	62.72	6.63	0.02
% Neutrophils	Social status	3	119.60	0.00	0.62
	Null	2	122.56	2.96	0.14
	Body condition	3	122.73	3.14	0.13
	Body condition+ Social status	4	123.07	3.47	0.11
% Lymphocytes	Social status	3	122.42	0.00	0.61
	Null	2	124.68	2.26	0.20
	Body condition+Social status	4	126.03	3.61	0.10
	Body condition	3	126.30	3.87	0.09
Neutrophil-to-lymphocyte ratio	Social status	3	74.05	0.00	0.38
	Body condition	3	74.29	0.24	0.34
	Null	2	75.80	1.76	0.16
	Body condition+Social status	4	76.40	2.35	0.12

[a] Number of estimated parameters.

[b] Akaike's Information Criterion corrected for small sample sizes.

[c] Differences in AICc values between the best model (lowest AICc) and each candidate model.

[d] AIC weights.

TABLE 5.   Model averaging for the stress response to capture (measured by leukocyte counts, leukocyte composition, and neutrophil-to-lymphocyte ratio) of 24 brown bears anaesthetized in Sweden in April–May 2012 and 2013.

Response variable	Predictor variable	β[a]	2.5% CI[b]	97.5% CI[b]	SE[c]	Variable importance[d]
Total leukocyte counts	Intercept	5.26	4.60	5.93	0.33	
	Body condition	−0.09	−0.84	0.66	0.37	0.18
	Social status (solitary)	0.12			0.69	0.17
% Neutrophils	Intercept	70.75	64.01	77.33	3.19	
	Social status (solitary)	−10.88	−20.44	−1.70	4.61	0.73
	Body condition	2.82	−1.22	9.75	3.19	0.24
% Lymphocytes	Intercept	18.78	11.38	25.61	3.45	
	Social status (solitary)	11.31	0.56	22.07	5.01	0.71
	Body condition	−1.28	−6.65	7.63	3.64	0.19
Neutrophil-to-lymphocyte ratio	Intercept	4.55	3.03	6.02	0.72	
	Social status (solitary)	−2.14	−4.60	−0.08	1.18	0.50
	Body condition	1.09	0.01	2.42	0.64	0.46

[a] Model averaged coefficients.

[b] Confident intervals.

[c] Standard error.

[d] Relative importance of the predictor variables.

HPA axis and prosocial behaviors (DeVries et al. 2003).

Stress of surgery

Body condition was an influential factor in the ROS production by leukocytes after capture and surgery in our study animals. Bears in better body condition had higher overall LCC and peak levels, indicating that they coped better with handling stress. This confirmed our second hypothesis, agreeing with studies in birds and mammals that have concluded that animals in better body condition show an enhanced immune response (Alonso-Álvarez and Tella 2001; Bachman 2003).

We found no difference in LCC levels related to surgery. Therefore, we rejected our fourth hypothesis that bears undergoing surgery would have lower values of LCC. However, the conclusion that surgery was not an additional stressor at the time of sampling must be interpreted cautiously. The low sample size of the study

TABLE 6.   Association among heart rate, neutrophil-to-lymphocyte ratio, glucose and cortisol concentrations, and LCC measurements in 24 brown bears anaesthetized in Sweden in April–May 2012 and 2013. Pearson correlation coefficients (r) and P values (in parentheses) are shown.

	AUC1[a]	AUC2[b]	LCC1[c]	LCC2[d]
Heart rate	−0.47 (0.07)	0.08 (0.76)	−0.31 (0.24)	−0.004 (0.99)
Neutrophil-to-lymphocyte ratio	0.43 (0.10)	0.03 (0.89)	0.27 (0.31)	0.17 (0.52)
Glucose	0.16 (0.45)	0.11 (0.61)	0.29 (0.17)	0.10 (0.65)
Cortisol	−0.30 (0.15)	−0.04 (0.85)	−0.25 (0.24)	−0.02 (0.93)

[a] Area under the response curve for leukocyte coping capacity measurements obtained as soon as the animal was immobilized.

[b] Area under the response curve for leukocyte coping capacity measurements obtained during or after surgery.

[c] Maximum leukocyte coping capacity value obtained as soon as the animal was immobilized.

[d] Maximum leukocyte coping capacity value obtained during or after surgery.

($n=24$) and the control group ($n=5$), and the time the blood sample was obtained, could have influenced the results. Moreover, the administration of additional analgesic drugs to bears undergoing surgery could help explain the results.

Our second blood sample was collected 49 ± 14 min after the surgery started. Although the production of ROS increases after surgical injury (Wakefield et al. 1993), the exact time at which this increase occurs is not known. Shelton-Rayner (2009) stated that neutrophils react within an hour of tissue injury during an acute inflammatory response. In studies in humans and animals, leukocytes counts increased from hours to days postoperatively (Kreeger et al. 1990; Yokoyama et al. 2005). Other parameters, such as cortisol and IL-6, a cytokine that has a major role in the early inflammatory response to surgery, also increased their levels within minutes after surgery, but the increase was not significant before 2–6 h (Desborough 2000). Therefore, time of sampling would be an important factor to account for in future studies aiming to quantify the stress response.

Analgesic drugs, which were only administered to bears undergoing surgery, can attenuate the stress response to surgery (Rademaker et al. 1992; Kehlte and Holte 2001). However, nonsteroidal anti-inflammatory drugs, such as meloxicam and carprofen, are analgesics with little effect on surgical stress responses (Kehlte and Holte 2001). In our case they provided postoperative analgesia rather than reduced the stress response to surgery.

In addition, anesthetics drugs (medetomidine+tiletamine-zolazepam), that were used in all bears, can modify the stress response by affecting the HPA axis (Desborough 2000; Ko et al. 2000; Bentson et al. 2003; Champagne et al. 2012). Nonetheless, we believe that the LCC measurements after capture were representative of the stress experienced by the bears. This is because the stressor, the capture event, occurred before the administration of the anesthetic drugs, presumably allowing complete activation of the stress response. Thus, the effect of the anesthetic drugs, which was not immediate, was probably minimal on an already-established endocrine response. On the other hand, for the LCC measurements 90 min after the bears were recumbent, the stress response to surgery was probably blocked or diminished by the use of anesthetics $\pm$ analgesics and were therefore not representative of the stress experienced by the bears.

LCC peaks and variables of capture

Capture variables affect an animal's physiological parameters, including body temperature and cortisol levels (Arnemo and Ranheim 1999; Cattet et al. 2003). We rejected our third hypothesis that bears with longer pursuit time during capture would have lower LCC values; neither pursuit time nor medetomidine dose had a significant effect on the LCC response. Bears probably became aware of the helicopter before being observed from the air, which perhaps resulted in an inaccurate estimate of pursuit time. Additionally, the dose of medetomidine administered was estimated, as a few darts were not retrieved.

We also assessed the number of captures an animal had experienced. Shelton-Rayner et al. (2010) suggested that leukocyte reactivity exhibits habituation in humans. However, we found no effect of the number of captures on LCC levels and concluded that there was no habituation to capture. We could argue that capture is a strong negative stimulus, therefore not causing habituation in this species. A more complex analysis of the data would be necessary to properly evaluate this variable.

Leukocyte number and composition

Differences in leukocyte composition and the N:L ratio were mainly due to social status. We discovered a higher proportion of neutrophils and N:L ratio and a lower proportion of lymphocytes in members of family groups compared to solitary animals. In domestic species, a "stress leukogram"

characterized by a leukocytosis, neutrophilia, lymphopenia, and eosinopenia typically occurs following adrenal stimulation, which leads to an increased N:L ratio (Feldman et al. 2000). The N:L ratio increases after restraint in rhesus monkeys (*Macaca mulatta*; Morrow-Tesch et al. 1993) and after transport in Southern chamois (*Rupicapra pyrenaica*; López-Olvera et al. 2006). However, leukocyte profiles provide information about the number of circulating cells rather than an individual's ability to mount an immune response. Based on our results and other studies (Dufva and Allander 1995; Bachman 2003), we suggest that the observed neutrophilia exhibited by the bears occurred as preparation of the body for injury and potential bacterial infection.

Correlation between LCC measurements and other stress indicators

AUC and LCC peak values did not correlate with any of the commonly used stress indicators, e.g., heart rate, N:L ratio, or glucose and cortisol concentrations. Therefore, we rejected our fifth hypothesis that there would a negative correlation between LCC and other variables used as stress indicators. Shelton-Rayner et al. (2012) did not find a correlation between LCC and heart rate, blood pressure, body temperature, or cortisol levels in humans. They attributed this to physiological variables and hormones being influenced by a range of factors in addition to stress, which is a plausible explanation for our findings.

The effectiveness of the LCC technique to evaluate the stress of capture and handling

Leukocytes are recognized as ideal indicators of stress because they are constantly exposed to multiple factors such as endocrine factors in plasma, changes in blood biochemistry parameters, changes in the HPA axis, etc. (Mian et al. 2003). LCC has been shown to be rapidly affected by stress and has proven to be a quick and reliable method to quantitatively measure stress in both animals and humans (McLaren et al. 2003; Honess et al. 2005; Moorhouse et al.

2007; Gelling et al. 2009; Shelton-Rayner et al. 2010). LCC measurements can be taken during or immediately after a stressful event, and the results can be obtained while the animal is still under anesthesia. Thus, the technique allows a rapid assessment of the physiological status of an animal *in situ* (McLaren et al. 2003).

Animal welfare, stress, and conservation

There are several methods to assess stress and welfare (e.g., blood parameters or behavior). Moberg (2000) stated that the biological cost of mounting a stress response is the key to determine the welfare implications of a stressor and might be more relevant than other measures of stress such as physiological or behavioral changes. The LCC technique measures the biological costs associated with the release of ROS after a stressful event (McLaren et al. 2003). Therefore, it provides a relevant measure to assess welfare. However, a combined approach using two or more stress parameters is recommended. The LCC technique can be used in combination with traditional techniques to provide a more comprehensive approach on stress and wildlife welfare.

Disentangling the stressful components of trapping and handling procedures is important as shown by previous studies (Bonacic and Mc Donald 2003; McLaren et al. 2003). The results obtained by McLaren et al. (2003) using the LCC technique indicated that the transport of badgers before capture was an additional stressor. These results led to a refinement in the capture protocol of badgers.

Given the implications that welfare has on conservation, information provided by new techniques, such as LCC, will allow researchers to better evaluate the impact of their work and plan conservation actions consequently.

LITERATURE CITED

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Paper II

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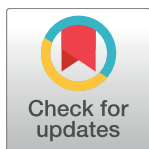
A Double-Blinded, Randomized Comparison of Medetomidine-Tiletamine-Zolazepam and Dexmedetomidine-Tiletamine-Zolazepam Anesthesia in Free-Ranging Brown Bears (*Ursus Arctos*)

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Abstract

We compared anesthetic features, blood parameters, and physiological responses to either medetomidine-tiletamine-zolazepam or dexmedetomidine-tiletamine-zolazepam using a double-blinded, randomized experimental design during 40 anesthetic events of free-ranging brown bears (*Ursus arctos*) either captured by helicopter in Sweden or by culvert trap in Canada. Induction was smooth and predictable with both anesthetic protocols. Induction time, the need for supplemental drugs to sustain anesthesia, and capture-related stress were analyzed using generalized linear models, but anesthetic protocol did not differentially affect these variables. Arterial blood gases and acid-base status, and physiological responses were examined using linear mixed models. We documented acidemia (pH of arterial blood < 7.35), hypoxemia (partial pressure of arterial oxygen < 80 mmHg), and hypercapnia (partial pressure of arterial carbon dioxide ≥ 45 mmHg) with both protocols. Arterial pH and oxygen partial pressure were similar between groups with the latter improving markedly after oxygen supplementation (p < 0.001). We documented dose-dependent effects of both anesthetic protocols on induction time and arterial oxygen partial pressure. The partial pressure of arterial carbon dioxide increased as respiratory rate increased with medetomidine-tiletamine-zolazepam, but not with dexmedetomidine-tiletamine-zolazepam, demonstrating a differential drug effect. Differences in heart rate, respiratory rate, and rectal temperature among bears could not be attributed to the anesthetic protocol. Heart rate increased with increasing rectal temperature (p < 0.001) and ordinal day of capture (p = 0.002). Respiratory rate was significantly higher in bears captured by helicopter in Sweden than in bears captured by culvert trap in Canada (p < 0.001). Rectal temperature

significantly decreased over time (p ≤ 0.05). Overall, we did not find any benefit of using dexmedetomidine-tiletamine-zolazepam instead of medetomidine-tiletamine-zolazepam in the anesthesia of brown bears. Both drug combinations appeared to be safe and reliable for the anesthesia of free-ranging brown bears captured by helicopter or by culvert trap.

Introduction

Capture, and anesthesia of wild mammals are required for conservation, research and management purposes [1–3]. The use of anesthetic drugs helps to reduce the stress and pain caused by capture and handling, while providing safety for capture personnel [4]. Brown bears (*Ursus arctos*) have been anesthetized for management and conservation throughout their global range using a variety of anesthetic agents. The most common protocols have combined a dissociative agent with a benzodiazepine or an alpha-2 adrenoceptor agonist [5, 6].

Tiletamine, a dissociative anesthetic, combined in equal parts by weight with zolazepam, a benzodiazepine agonist, has been used for many years in the anesthesia of brown bears, especially in North America [6]. Tiletamine-zolazepam (TZ) produces reliable anesthesia in bears, has a wide safety margin, and causes minimal depression of the cardiovascular and respiratory systems [7, 8]. However, use of TZ requires large drug volumes, provides poor visceral analgesia, and cannot be antagonized [6]. Another concern is extended recovery times, especially when additional (top-up) doses of TZ are administered, exposing anesthetized bears to the risks of inclement weather and predation [9, 10].

Combining TZ with medetomidine (M), an alpha-2 adrenoceptor agonist, counteracts some of the undesired effects of TZ. Medetomidine-tiletamine-zolazepam (MTZ) can be delivered at approximately 25% of the volume of TZ alone [8]. Additionally, M improves analgesia and reduces the effective TZ dose level (mg/kg) required by 75%. The effects of M can be specifically antagonized by atipamezole, an alpha-2 adrenoceptor antagonist [7], making MTZ a "partially reversible" anesthetic protocol.

Medetomidine is a potent, selective, and specific alpha-2 adrenoceptor agonist composed by equal parts of two optical enantiomers, dexmedetomidine and levomedetomidine [11]. The pharmacological effects of M are due almost exclusively to dexmedetomidine [12, 13]. Levomedetomidine is considered an inactive ingredient [12], but may act as a weak partial alpha-2 adrenoceptor agonist or as an inverse alpha-2 adrenoceptor agonist [14], producing opposite sedative and analgesic effects [13, 15].

Dexmedetomidine (D), the dextrorotatory enantiomer, has been used in recent years in the anesthesia of a few wildlife species, including bears [16–20]. Dexmedetomidine combined with TZ (DTZ) has been suggested to cause less respiratory depression than MTZ in bears potentially offering a benefit of using D instead of M [21, 22].

Our study goal was to determine whether DTZ offers any advantage over MTZ in the anesthesia of free-ranging brown bears by comparing induction times, the need for supplemental drugs to sustain anesthesia, stress as quantified by serum cortisol concentrations, arterial blood gases, acid-base status, and physiological responses between anesthetic protocols. To our knowledge, this is the first double-blinded, randomized comparison of the effects of DTZ and MTZ in ursids. We hypothesized that:

1. Induction time—The induction of anesthesia occurs faster with DTZ than with MTZ. Quick inductions reduce the potential for physical injury and physiological stress. Shorter

induction times have been reported in golden-headed lion tamarins (*Leontopithecus chrysomelas*) anesthetized with D-ketamine compared to M-ketamine [16].

2. Duration of anesthesia—The need for supplemental drugs to sustain anesthesia is lower with DTZ than MTZ.
Drugs used in wildlife anesthesia should provide enough depth and duration of anesthesia to perform all planned handling procedures without the administration of supplemental (also referred to as top-up) drugs. Further, the supplemental administration of TZ may result in prolonged recoveries [9, 10]. Studies have discovered a longer lasting anesthetic effect of D over M [16].

3. Stress—Stress in response to capture and handling is lower with DTZ than MTZ.
Blood concentrations of cortisol, and glucose to a lesser extent, are widely-used parameters to assess the stress response to capture and handling in free-ranging wild animals [23, 24]. Medetomidine has been shown to cause greater increases in serum glucose concentration than D [25]. Although the effects of alpha-2 adrenoceptor agonists on cortisol concentrations are controversial [26, 27], we hypothesized that serum concentrations of cortisol, as an indicator of stress, would be less with DTZ.

4. Arterial blood gases and acid-base status—Bears anesthetized with DTZ have higher pH and partial pressure of arterial oxygen (PaO_2), and lower partial pressure of arterial carbon dioxide ($PaCO_2$) than bears anesthetized with MTZ.
Hypoxemia ($PaO_2 < 80$ mmHg) is a common finding in bears anesthetized with MTZ [28, 29]. DTZ, however, was reported to not cause hypoxemia in a study of brown bears [21]. Although pH and blood gases are not routinely recorded in wildlife studies, they provide a valuable physiological assessment of an animal's response to capture and anesthesia.

5. Physiological responses—DTZ produces less cardio-respiratory depression and quicker recovery of normal body temperature than MTZ.
Ideally, anesthetic drugs should cause minimal depression of the cardiovascular and respiratory systems, and should not suppress the dissipation of excess body heat caused by physical exertion and stress. Several studies have suggested that D has minimal effects on these physiological variables [18, 21].

Material and Methods

Scandinavian Brown Bear Research Project (SBBRP)

We captured 31 individual free-ranging brown bears on 34 occasions in Dalarna County, Sweden (61.219756–61.579688 N, 13.019778–15.416586 E) in April-July 2014 and April-May 2015. We applied a randomized, double-blinded design in which 15 individuals were allocated to the MTZ group and 16 to the DTZ group. Three bears were captured twice, once per year, with one bear receiving MTZ followed by DTZ, another receiving DTZ followed by MTZ, and the third receiving DTZ both years. Consequently, the MTZ group comprised 16 anesthetic events and the DTZ group comprised 18 anesthetic events. When two or more bears were together at the time of capture (i.e., family groups), we randomly used one of the study drug combinations for the first bear and alternated the drug for the accompanying bear(s). Captured bears in this study were composed of 16 males and 15 females with 19 bears captured as yearlings, nine bears captured as two year olds, and three captured at both ages. We did not capture larger bears because our dart volumes were limited to ≤3 ml and because access to D in Sweden was limited to a low concentration (0.5 mg/ml) drug solution.

For yearlings, we prepared MTZ by adding 5 mg of M (Domitor® 1 mg/ml per 10 ml per vial, Orion Pharma Animal Health, Turku, Finland) to a vial of TZ (Zoletil® 500 mg/vial, Virbac, Carros, France). We split the solution into six 1.5 ml darts, each dart containing 0.83 mg of M and 83.3 mg of TZ. The remaining 5 mg of M were equally divided and added to each dart (0.83 mg of M per dart). The final solution contained 1.66 mg of M and 83.3 mg of TZ in each dart, with a M:TZ ratio of 1:50. We prepared DTZ in the same way as described above adding 2.5 mg of D (Dexdomitor® 0.5 mg/ml per 10 ml per vial, Orion Pharma Animal Health) to a vial of TZ. We split the solution into six darts, each dart containing 0.415 mg of D and 83.3 mg of TZ. The remaining 2.5 mg of D were equally divided and added to each dart (0.415 mg of D per dart). The final solution contained 0.83 mg of D and 83.3 mg of TZ in each dart, with a D:TZ ratio of 1:100. For two-year-old bears, we prepared both drug combinations as described for yearlings, but divided the initial solution of M or D and TZ, and the remaining M or D into four 3 ml darts. The final solution contained 2.5 mg M or 1.25 mg D and 125 mg TZ in each dart, again with a M:TZ ratio of 1:50, and a D:TZ ratio of 1:100. The dose for each age class remained unchanged throughout the study.

We administered the anesthetic combination by remote delivery from a CO_2-powered rifle (Dan-Inject®, Børkop, Denmark) at a distance of 3–7 meters from a helicopter. Darts used in the study consisted of 1.5 ml syringes with 1.5x25mm barbed needles with side ports (Dan-Inject®) in yearlings, and 3 ml syringes with 2.0x30mm needles in two-year-old bears. When needed, 1–2 mg/kg of ketamine (Narketan® 100 mg/ml, Chassot, Dublin, Ireland) was administered intravenously or intramuscularly by syringe and needle to extend the duration of anesthesia.

The time intervals in minutes (min) from when a bear was first observed to when a bear was hit by a drug-filled dart (observed-darted time), from when active pursuit with the helicopter began to when the bear was darted (chased-darted time), and from when a bear was darted to recumbency (induction time) were recorded. We recorded capillary refill time (seconds), respiratory rate (breaths per min), heart rate (beats per min) and rectal temperature (˚C) of anesthetized bears immediately after induction and every 15 min throughout the duration of anesthesia. Respiratory rate was monitored by observation of thoracic movements and heart rate by auscultation of the heart. Rectal temperature was measured with a digital thermometer (Accutemp®, Jahpron Medical Int., Jensvoll, Norway). When hyperthermia ($\geq$ 40˚C) occurred, we applied snow to the paws, groin and axillae, and administered intravenous fluids to reduce body temperature.

We collected one venous blood sample (8 ml) from the jugular vein of each bear as early as possible following induction using a vacutainer system (BD Vacutainer®, BD Diagnostics, Preanalytical Systems, Franklin Lakes, NJ, USA). We measured serum cortisol concentration (nmol/L) with this sample [30]. We also collected two anaerobic arterial blood samples (3 ml each) from the femoral artery of each bear in pre-heparinized syringes (PICOTM70, Radiometer Copenhagen, DK-2700 Brønshøj, Denmark), the first at 30 min, and the second at 60 min, after the bear was darted. We measured blood gases, acid-base status and selected hematologic and biochemical variables on site using a portable analyzer (iSTAT 1® Portable Clinical Analyzer and i-STAT® cartridges CG4+ and 6+, Abbott Laboratories, Abbott Park IL, 60064–6048, USA). The parameters included pH, partial pressure of arterial carbon dioxide ($PaCO_2$; mmHg), partial pressure of arterial oxygen (PaO_2; mmHg), base excess (BE; mmol/L), bicarbonate (HCO_3; mmol/L), total carbon dioxide (TCO_2; mmol/L), arterial oxygen saturation (SaO_2; %), lactate (mmol/L), sodium (mmol/L), chloride (mmol/L), potassium (mmol/L), blood urea nitrogen (BUN; mg/dL), glucose (mmol/L), hematocrit (% packed cell volume), and hemoglobin (g/dL). Blood gas values and pH were corrected to the rectal temperature.

Bears are routinely supplemented with intranasal oxygen throughout anesthesia, as part of the standard field procedure of the SBBRP [31]. However, for this study, we only administered oxygen to bears with low levels of blood oxygen (hypoxemia) based on PaO_2 measurements. Below 80 mmHg, we considered bears to be hypoxemic and administered oxygen at a flow rate of 0.5 L/min in yearlings and 1L/min in two-year-old bears [29].

We performed different types of surgery (i.e., abdominal, muscle biopsy) on selected bears to meet the research objectives of other studies. In bears undergoing surgery, we preemptively administered 0.2 mg/kg of meloxicam (Metacam® 5 mg/ml, Boehringer Ingelheim, Reihn, Germany) subcutaneously to reduce pain and inflammation caused by the surgery. We followed a standard protocol for other sampling and handling procedures [31]. Body weight was obtained by suspending bears from a spring-loaded scale to accurately determine drug dose levels (mg/kg of body weight).

After completion of all procedures, we administered 5 mg of atipamezole (Antisedan® 5 mg/ml, Orion Pharma Animal Health) per mg of M or 10 mg of atipamezole per mg of D intramuscularly to reverse anesthesia. We recorded the time interval in min from recumbency to atipamezole administration (handling time), and left bears to recover undisturbed at the site of capture.

Brown bear captures occurred both on private and public lands. Captures were approved by the Swedish Ethical Committee on Animal Research (application numbers C 7/12 and C 18/15) and the Swedish Environmental Protection Agency (NV-0758-14).

fRI Research Grizzly Bear Program (fRI)

We captured six free-ranging adult (6–15 years) male brown bears in western Alberta, Canada (52.865360–54.368277 N, 117.865738–119.017687 E) in May 2014–2015 by barrel (culvert) trap [32]. We applied a randomized, double-blinded study in which three bears were allocated to the MTZ group and three to the DTZ group. We prepared MZT by adding 12 mg of M (20 mg/ml; Chiron Compounding Pharmacy Inc., Guelph, Ontario, Canada) and 0.9 ml of sterile water for injection (Hospira 10 ml per vial, Montreal, Quebec, Canada) per vial of TZ (Telazol®, 286 mg tiletamine + 286 mg zolazepam; Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, U.S.A.). DZT was prepared in 2014 by adding 5.7 mg of D (3 mg/ml; Chiron Compounding Pharmacy Inc.) and 0.2 ml of sterile water for injection per vial of Telazol®. In 2015, we used 6 mg of a higher concentration of D (5 mg/ml), plus 0.9 ml of sterile water for injection, per vial of Telazol®. All formulations resulted in 2.5 ml of drug solution per vial with concentrations of 234 mg/ml for MTZ and 231 mg/ml for DTZ, and ratios of 1:48 for M:TZ and 1:95 for D:TZ.

We used a remote drug delivery system (Dan-Inject®) to administer a combination of 50µg/kg estimated body weight of M, or 25µg/kg of D, and 2.45 mg/kg of TZ intramuscularly. Darts used in the study consisted of 3 ml syringes with 2.0x40mm barbed needles (Dan-Inject®). When necessary, we administered ketamine at 2 mg/kg (200 mg/ml; Chiron Compounding Pharmacy Inc.) intramuscularly by syringe and needle to extend the duration of anesthesia.

We recorded the induction time for each bear. Capillary refill time, respiratory rate, heart rate, and rectal temperature of anesthetized bears were obtained immediately after induction and every 15 min throughout anesthesia. Respiratory rate was monitored by observation of thoracic movements. We recorded pulse rate and hemoglobin oxygen saturation (SpO_2; %) with a pulse oximeter (Nellcor NPB-40, Nellcor, Pleasanton, California, U.S.A). Rectal temperature was measured with a digital thermometer (Adtemp V Fast Read Pen Type Digital Thermometer, American Diagnostic Corporation, New York, U.S.A).

We collected one venous blood sample (4 ml) from the femoral vein of each bear to measure cortisol concentrations (nmol/L; Immulite 1000; Siemens Medical Solutions Diagnostics, California, U.S.A). We also collected two anaerobic arterial blood samples (3ml each) from the femoral artery of each bear in pre-heparinized syringes 30 and 60 min after the bear was darted. We used the same equipment and measured the same parameters as previously described. Blood gas values and pH were corrected to the rectal temperature. Although oxygen was available, we did not administer it to any of the bears captured in Alberta, Canada.

We extracted a premolar tooth for age estimation by counting cementum annuli [33]. We administered 0.1 mg/kg of meloxicam (Metacam®, 5mg/ml solution for injection; Boehringer Ingelheim Vetmedica Inc., Missouri, U.S.A) subcutaneously to provide analgesia. We weighed all bears with an electronic load-cell scale.

After completion of measurements and sampling, we administered 5 mg of atipamezole (20 mg/ml; Chiron Compounding Pharmacy Inc.) per mg of M or 10 mg of atipamezole per mg of D intramuscularly for anesthetic reversal. Bears were left to recover from anesthesia undisturbed at the site of capture. We recorded the handling time, and the time interval from atipamezole administration until the bear showed the first signs of recovery (recovery time, in min).

Brown bear captures were authorized under the permitting authority of the Alberta Department of Environment and Sustainable Resource Development (provincial jurisdiction lands), Alberta Tourism and Parks (provincial parks and protected areas jurisdiction lands), and Parks Canada (federal jurisdiction lands). Captures were approved by the University of Saskatchewan's Committee on Animal Care and Supply (Animal Use Protocol # 20010016) and were in accordance with guidelines provided by the American Society of Mammalogists' Animal Care and Use Committee [3] and the Canadian Council on Animal Care for the safe handling of wildlife [34].

Statistical analysis

We approached the statistical analyses in three sequential phases, data exploration, model development, and model validation, using the software R 3.1.0 [35]. For data exploration, we evaluated the raw data for (i) missing values, (ii) presence of outliers, (iii) collinearity among potential predictor (independent) variables, and (iv) relationships or associations between response (dependent) and predictor variables [36]. We used mean values to substitute for missing values (i.e., we substituted two missing induction times when used as predictors with the mean value). Collinearity among predictor variables was evaluated by variance inflation factors (VIF $\geq$ 3.0) and pairwise correlations (r $\geq$ 0.7). Collinear variables were not used together in the same model. We standardized continuous predictor variables (covariates) prior to model development to facilitate comparisons among different models [37].

For model development, we worked with two different data sets. The first, containing data collected in Sweden only, and the second, combined datasets containing data collected both in Sweden and Alberta. We carried out different analyses for each of the hypotheses to be tested (Table 1). For the induction time, the need for supplemental drugs and stress hypotheses (i.e. Hypotheses 1–3), we used the 'dredge' function in package *MuMIn* [38] to build all possible models containing a maximum of 3 (Swedish dataset) or 4 (combined datasets) predictor variables to avoid model overfitting. With the same goal, we also did not evaluate possible interactions. Model selection was based on the Akaike's Information Criterion (AIC) [39]. For evaluation of the arterial blood gases and acid-base status, and physiological responses hypotheses (i.e. Hypothesis 4 and 5), we build multiple global models for each response variable to avoid collinearity (i.e., predictor collinear variables were not used together in the same model). We selected the most parsimonious (based on AIC) of these models for further analysis. Then

Table 1. Response and predictor variables (interactions not shown), model types, and sample sizes (N) used to test hypotheses in brown bears anesthetized with either medetomidine-tiletamine-zolazepam (MTZ) or dexmedetomidine-tiletamine-zolazepam (DTZ) in Sweden (S, N = 34) and Alberta, Canada (A, N = 6) in 2014–2015.

Hypotheses	Response variable[a]	Predictor variable[b] combinations	Random effects[c]	Model type[d]	N
1	Induction time	Age + Sex + Drug + TZ + CD time + ODC[e]	NA	GLM Gamma link inverse	S = 34, S+A = 38
2	Supplemental drugs	Age + Sex + Drug + Weight + CD time + ODC + Induction time + Surgery + Handling time[e]	NA	GLM binomial	S = 34, S+A = 40
3	Cortisol	Age + Sex + Drug + Weight + CD time + ODC + Induction time[e,f]	NA	GLM Gaussian	S = 34, S+A = 39
4	pH	Time + Age + Drug + $PaCO_2$ + BE + Lactate	Bear ID	LMM	S = 64, S+A = 76
4	PaO_2	Age + Drug + Length + RT + RR + Oxygen	Bear ID	LMM	S = 64, S+A = 76
4	$PaCO_2$	Age + Drug + Weight + RT + RR + PaO_2	Bear ID	LMM	S = 64, S+A = 76
5	Heart rate	Time + Age + Sex + Drug + Length + CD time + ODC + Induction time + Surgery + Ket + RT + RR[e]	Bear ID	LMM	S = 223, S+A = 165
5	Respiratory rate	Time + Age + Sex + Drug + Length + CD time + ODC + Induction time + Surgery + Ket + RT + HR[e,f]	Bear ID	LMM	S = 224, S+A = 167
5	Rectal temperature	Time + Age + Sex + Drug + Weight + CD time + ODC + Induction time + Surgery + Ket + HR + RR[e]	Bear ID	LMM	S = 223, S+A = 165

[a] Response variables—(i) Induction time: time interval in minutes from when a bear was darted to recumbency; (ii) Supplemental drugs: yes, no; (iii) Cortisol: serum concentration in nmol/L; (iv) pH: arterial blood acid-base status; (v) PaO_2: partial pressure of arterial oxygen in mmHg; (vi) $PaCO_2$: partial pressure of arterial carbon dioxide in mmHg; (vii) Heart rate (HR): beats per minute; (viii) Respiratory rate (RR): breaths per minute (log-transformed); and (ix) Rectal temperature (RT): ˚C.

[b] Predictor variables—(i) Age: yearlings, two year olds, adults ($\geq$5 yr); (ii) Sex: male, female; (iii) Drug: MTZ or DTZ in mg/kg body weight; (iv) TZ: tiletamine-zolazepam in mg/kg body weight; (v) CD time: time interval in minutes from when active pursuit began to when the bear was darted; (vi) ODC: ordinal day of capture; (vii) Weight: body weight in kg; (viii) Surgery: yes or no; (ix) Handling time: time interval in minutes from recumbency to atipamezole administration; (x) Area: Sweden, Alberta; (xi) $PaCO_2$; (xii) Time: sampling and/or measurements recorded at 15; 30; 45; 60; 75; 90; 105; 120; 135 minutes after darting in Sweden, and at 15; 30; 45; 60; 75 minutes after darting in Sweden+Alberta; (xiii) BE: base excess in mmol/L; (xiv) Lactate: blood concentration in mmol/L; (xv) Length: contour body length in cm; (xvi) RR: respiratory rate; (xvii) RT: rectal temperature; (xviii) Oxygen: yes or no; (xiv) PaO_2; (xx); Ket: ketamine dose level in mg/kg body weight; (xxi) HR: heart rate; (xxii) RR: respiratory rate; (xxii) RT: rectal temperature

[c] NA: not applicable.

[d] GLM: generalized linear model; LMM: linear mixed model.

[e] CD time was excluded as explanatory variable for the analysis of the Sweden+Alberta dataset.

[f] Area (Sweden; Alberta) substituted age as explanatory variable for the analysis of the Sweden+Alberta dataset

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we applied the 'drop 1' function [40] to obtain the final model. However, before dropping a predictor variable, we also evaluated it for any two-way interactions of potential physiological significance, e.g., drug combination x respiratory rate.

For model validation, we plotted the standardized residuals of the best model against the fitted values to assess homogeneity. If a pattern was observed in the spread, we applied a transformation to the response variable.

We present the mean ± standard deviation for all variables, unless otherwise stated. Differences were considered significant when p $\leq$ 0.05.

Results

Hypothesis 1: The induction of anesthesia occurs faster with DTZ than MTZ

We used a single dart in the anesthesia of 30 bears (88%) in Sweden. Four bears (12%, two bears in each drug group) required an additional dart to achieve anesthesia. Bears allocated to

Table 2. Regression coefficients (β) and significance (p) of the predictor variables in the best model explaining variation in the response variables for hypotheses (H) 1, 2, 3 in brown bears anesthetized with either medetomidine-tiletamine-zolazepam (MTZ) or dexmedetomidine-tiletamine-zola-zepam (DTZ) in Sweden (n = 34) and Alberta, Canada (n = 6) in 2014–2015.

Predictors[a]	H1: Induction time Sweden β	p	Sweden + Alberta β	p	H2: Supplemental drugs Sweden β	p	Sweden + Alberta β	p	H3: Cortisol Sweden β	p	Sweden + Alberta β	p
Area (Sweden)											-369.59	0.034
Age (Yearlings)			0.286	<0.001			14.081	0.147				
Age (Two year olds)	-0.199	0.002	0.094	0.090			18.850	0.102				
Sex (Male)	-0.150	0.012	-0.145	0.012					134.03	0.007	104.99	0.037
TZ dose level	-0.051	<0.001	-0.049	<0.001								
Weight					4.947	0.054			-86.07	<0.001	-163.84	0.009
Ordinal day of capture					36.267	0.093	18.695	0.088			-43.17	0.071
Induction time									46.54	0.045		
Handling time					4.107	0.034	2.124	0.008				

[a] Predictor variables–(i) Area: Sweden, Alberta; (ii) Age: yearlings, two year olds, adults ($\geq$5 yr); (iii) Sex: male, female; (iv) TZ: tiletamine-zolazepam in mg/kg body weight; (v) Weight: body weight in kg; (vi) Induction time: time interval in minutes from when a bear was darted to recumbency; (vii) Handling time: time interval in minutes from recumbency to atipamezole administration. Regression coefficients for factors are relative coefficients such that: (i) β for Area (Sweden) was determined with β for Area (Alberta) set to 0; β for Age (Two year olds) was determined with β for Age (Yearlings) set to 0 for the Sweden dataset; (iii) β for Age (Yearlings) and for Age (Two year olds) were determined with β for Age (Adults) set to 0 for the Sweden + Alberta dataset; and (iv) β for Sex (Male) was determined with β for Sex (Female) set to 0.

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the MTZ group (N = 16) received an average dose level of 93.62 ± 36.96 μg/kg M and 4.69 ± 1.85 mg/kg TZ. Bears in the DTZ group (N = 18) received an average dose level of 57.51 ± 38.37 μg/kg D and 4.87 ± 2.49 mg/kg TZ. Induction of anesthesia was quick (3.73 ± 2.81 min), predictable, and smooth in all bears irrespective of anesthetic protocol.

We used a single dart in the anesthesia of all bears captured by culvert trap in Alberta. Bears allocated to the MTZ group (N = 3) received an average dose level of 52.23 ± 18.55 μg/kg M and 2.5 ± 0.88 mg/kg TZ. Bears in the DTZ group (N = 3) received an average dose level of 21.97 ± 10.12 μg/kg D and 1.6 ± 0.78 mg/kg TZ. Induction of anesthesia was predictable and smooth in all bears irrespective of anesthetic protocol, but mean induction time was longer (6.25 ± 1.89 min) than recorded for bears in Sweden.

The induction time was significantly affected by TZ dose level, age, and sex (i.e., longer induction with increasing TZ dose level, in two-year-old bears, and in males) (Table 2). For the combined datasets, induction was faster in yearlings than in adult bears (Fig 1). Drug combination did not have a significant effect on induction time, and was not included in the best model. Thus, hypothesis 1 was not supported.

Hypothesis 2: The need for supplemental drugs to sustain anesthesia is lower with DTZ than MTZ

We administered supplemental drugs to extend anesthesia in 21 (62%) bears in Sweden. Of these, 11 bears belonged to the MTZ group, and 10 to the DTZ group. All bears but two received ketamine (1.81 ± 0.5 mg/kg) as the supplemental drug. Of these two bears, one showed signs of recovery 28 min after darting and received 2.55 mg/kg TZ. The other bear only received 2/3 of the DTZ dose when darted. So, the remaining 1/3 (15.22 μg/kg D and 1.49 mg/kg TZ) was administered when it showed signs of recovery 45 min after darting. We administered an average dose level of 2.22 mg/kg ketamine to extend anesthesia in two bears from the DTZ group in Alberta.
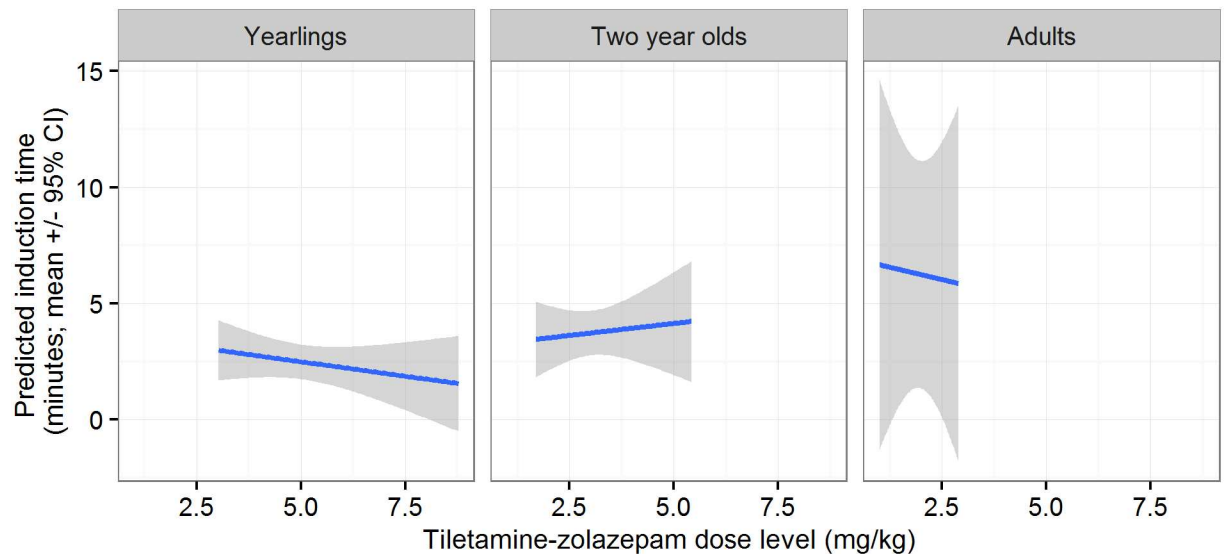
Fig 1. Induction time (time interval from when a bear was darted to recumbency, in minutes) by tiletamine-zolazepam dose level (in mg/kg body weight) and age class in 34 anesthetic events of free-ranging brown bears using a single dart of either medetomidine-tiletamine-zolazepam or dexmedetomidine-tiletamine-zolazepam in Sweden and Alberta, Canada in 2014–2015.

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Handling time was the only variable that significantly influenced the need to administer additional drugs such that the longer the handling time, the greater the likelihood of using supplemental drugs to sustain anesthesia (Table 2). Because the need to administer supplemental drugs did not differ between DTZ and MTZ protocols, we did not find support for hypothesis 2.

Hypothesis 3: Stress in response to capture and handling is lower with DTZ than MTZ

Among brown bears in Sweden, blood cortisol concentrations were significantly higher in bears that weighed less, in males, and in bears with longer inductions (Table 2). For the combined datasets, study area was also a determining factor. Cortisol concentrations were significantly higher in bears captured by culvert trap in Alberta than in bears captured by helicopter in Sweden (Table 2). Anesthetic protocol did not have a significant effect on cortisol levels. Therefore, hypothesis 3 was not supported.

Hypothesis 4: Bears anesthetized with DTZ have higher pH and partial pressure of arterial oxygen (PaO$_2$), and lower partial pressure of arterial carbon dioxide (PaCO$_2$) than bears anesthetized with MTZ

We documented acidemia (pH < 7.35), hypoxemia (PaO$_2$ < 80 mmHg), and hypercapnia (PaCO$_2$ > 45 mmHg) as the main alterations in arterial blood gases and acid-base status using both anesthetic protocols and in both study areas (S1 Text).

Arterial blood pH decreased with PaCO$_2$ values and increased with BE values in both datasets (Table 3). However, pH was not affected by drug protocol in either dataset. Thus, hypothesis 4 was not supported from the standpoint of our prediction that bears anesthetized with DTZ would have higher pH values than bears anesthetized with MTZ.

Table 3. Regression coefficients (β) and significance (p) of the predictor variables in the best model explaining variation in the response variables for hypothesis (H) 4 in brown bears anesthetized with either medetomidine-tiletamine-zolazepam (MTZ) or dexmedetomidine-tiletamine-zolaze-pam (DTZ) in Sweden (n = 34) and Alberta, Canada (n = 6) in 2014–2015.

| | H4: pH | | | | H4: PaO_2 | | | | H4: $PaCO_2$ | | | |
| | Sweden | | Sweden + Alberta | | Sweden | | Sweden + Alberta | | Sweden | | Sweden + Alberta | |
Predictors[a]	β	p	β	p	β	p	β	p	β	p	β	p
Age (Yearlings)							-34.177	0.106				
Age (Two year olds)					18.560	0.029	-19.3013	0.242	6.597	0.004		
Sex (Male)												
Drug (MTZ)					1.628	0.704	2.903	0.449	0.926	0.363	0.398	0.730
Weight									-2.584	0.018		
Length					-8.181	0.044	-16.892	0.026				
Rectal temperature					-7.957	0.005	-6.478	0.004	-1.423	0.015	-0.715	0.231
Rectal temperature*MTZ					3.265	0.460			1.359	0.108	1.691	0.058
Respiratory rate					0.945	0.645	0.892	0.764	-1.867	0.001	-1.756	0.002
Respiratory rate*MTZ							0.326	0.928	2.078	0.004	0.662	0.006
$PaCO_2$	-0.029	<0.001	-0.031	<0.001								
BE	0.058	<0.001	0.058	<0.001								
PaO_2									1.755	<0.001	1.964	<0.001
Oxygen (Yes)					62.134	<0.001	62.288	<0.001				

[a] Predictor variables–(i) Age: yearlings, two year olds, adults ($\geq$5 yr); (ii) Sex: male, female; (iii) Drug: MTZ or DTZ in mg/kg body weight; (iv) Weight: body weight in kg; (v) Length: contour body length in cm; (vi) $PaCO_2$: partial pressure of arterial carbon dioxide in mmHg; (vii) BE: base excess in mmol/L; (viii) Oxygen: supplementation with oxygen, yes, no. Regression coefficients for factors are relative coefficients such that: (i) β for Age (Two year olds) was determined with β for Age (Yearlings) set to 0 for the Sweden dataset; (ii) β for Age (Yearlings) and for Age (Two year olds) were determined with β for Age (Adults) set to 0 for the Sweden + Alberta dataset; (iii) β for Sex (Male) was determined with β for Sex (Female) set to 0; (iv) β for Drug (MTZ) was determined with β for Drug (DZT) set to 0; and (v) β for Oxygen (Yes) was determined with β for Oxygen (No) set to 0.

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Arterial oxygen partial pressures (PaO_2) were significantly correlated to the time interval from darting to sampling time (r = 0.75 in Sweden, r = 0.68 in the combined datasets, p < 0.001). The PaO_2 values were higher in two-year-old bears in the Swedish dataset, but age class was not significant in the combined datasets (Table 3). Oxygen supplementation increased PaO_2 values in the Sweden bears (Table 3). Although oxygen supplementation was also significant in the model describing the combined datasets, oxygen was not administered to bears in Alberta. Arterial oxygen partial pressures decreased with increasing body length and increasing rectal temperature in both datasets. However, PaO_2 values were not affected by anesthetic protocol in either dataset (Table 3). Thus, hypothesis 4 was not supported from the standpoint of our prediction that bears anesthetized with DTZ would have higher PaO_2 values than bears anesthetized with MTZ.

Arterial carbon dioxide partial pressures ($PaCO_2$) were higher in two-year-old bears than yearlings, and decreased with increasing body weight and rectal temperature, in bears from Sweden, but these associations were not evident in the combined datasets (Table 3). There was a positive association between $PaCO_2$ and PaO_2 values, and a negative association between $PaCO_2$ values and respiratory rates, in both datasets. The latter association was also significantly affected by anesthetic protocol in both datasets; $PaCO_2$ values decreased as respiratory rate increased in the DTZ group, but remained relatively constant with changes in respiratory rate in the MTZ group (Table 3, Fig 2). Although not significant, there was a trend towards increasing PCO_2 values with increasing rectal temperatures in the MTZ group in the combined datasets. These findings provide partial support for our prediction that
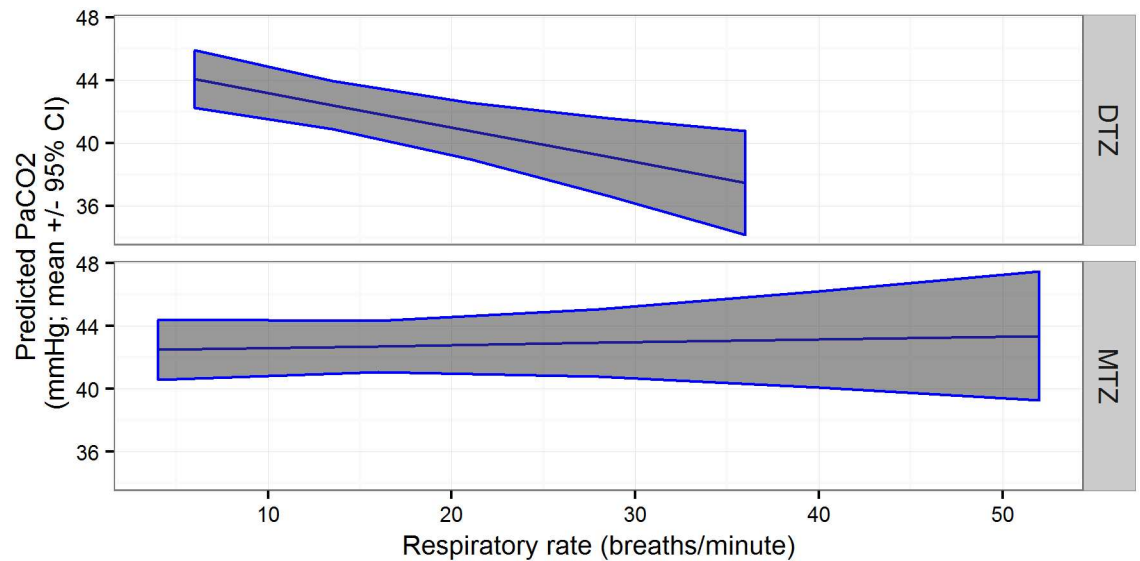
Fig 2. Partial pressure of arterial carbon dioxide (PaCO$_2$, mmHg) by respiratory rate (breaths/minute) and drug combination (MTZ: medetomidine-tiletamine-zolazepam; DTZ: dexmedetomidine-tiletamine-zolazepam) in 40 anesthetic events of free-ranging brown bears captured in Sweden and Alberta, Canada in 2014–2015.

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bears anesthetized with DTZ would have lower PaCO$_2$ values than bears anesthetized with MTZ, but this association was dependent on concurrent changes in respiratory rate. Overall, we found very little support for hypothesis 4.

Hypothesis 5: DTZ produces less cardio-respiratory depression and quicker recovery of normal body temperature than MTZ

We detected bradycardia (< 50 beats per min), bradypnea (< 5 breaths per min), and hyperthermia (T ≥ 40°C) as the main physiological alterations during the anesthesia of bears with both anesthetic protocols. However, we observed differences between study areas (S2 Text).

Mean heart rate was lower in two-year-old bears than in yearlings among the Swedish bears, but this age class difference was not apparent in the model derived from the combined datasets (Table 4). Heart rate was positively associated with ordinal day of capture and with rectal temperature in both datasets. It was also positively associated with respiratory rate in both datasets, albeit non-significantly in the combined datasets (Table 4). Relative to heart rates recorded at 15 min following drug administration, heart rates in both datasets were generally lower at subsequent time points. Heart rate was not differentially affected by anesthetic protocol. Therefore, our prediction that DTZ would depress cardiovascular function (heart rate) less than MTZ was not supported.

Mean respiratory rate was significantly higher in bears captured by helicopter in Sweden than in bears captured by culvert trap in Alberta (Table 4). Respiratory rates were also affected by an interaction between rectal temperature and age in bears from Sweden (i.e., higher respiratory rates with increasing rectal temperatures in two-year-old bears), but this effect was not evident in the model derived from the combined datasets. Respiratory rates in bears from Sweden were significantly lower at 45 min than the first recording at 15 min following drug administration, and significantly higher at all time points from 90 to 135 min after drug administration. Respiratory rate was not differentially affected by anesthetic protocol (Fig 3).

Table 4. Regression coefficients (β) and significance (p) of the predictor variables in the best model explaining variation in the response variables for hypothesis (H) 5 in brown bears anesthetized with either medetomidine-tiletamine-zolazepam (MTZ) or dexmedetomidine-tiletamine-zolazepam (DTZ) in Sweden (n = 34) and Alberta, Canada (n = 6) in 2014–2015.

Predictors[a]	H5: Heart rate				H5: Respiratory rate				H5: Rectal temperature			
	Sweden		Sweden + Alberta		Sweden		Sweden + Alberta		Sweden		Sweden + Alberta	
	β	p	β	p	β	p	β	p	β	p	β	p
Area (Sweden)							0.644	<0.001				
Age (Yearlings)			37.415	0.092							0.529	0.161
Age (Two year olds)	-23.334	0.013	8.200	0.696	0.004	0.976					1.161	0.002
Sex (Male)	6.232	0.215	4.837	0.247								
Drug (MTZ)	-0.694	0.869										
Length	-5.948	0.142	0.620	0.946								
Length*Age (Yearlings)			-9.142	0.508								
Length*Age (Two year olds)			4.452	0.812								
CD time	4.043	0.096										
Ordinal day of capture	9.313	0.002	7.909	0.001								
Induction time	-4.40	0.242										
Induction time*Sex (Male)	6.903	0.153										
Surgery (Yes)	-1.824	0.718										
Ketamine dose level	-3.324	0.175	-3.280	0.121								
RT	5.134	<0.001	5.637	<0.001	-0.003	0.946						
RT*Age (Two year olds)					0.381	<0.001						
HR									0.370	<0.001	0.479	<0.001
RR	1.496	0.018	1.378	0.090								
Time (30 minutes)	-5.689	0.009	-2.985	0.154	-0.154	0.093			0.112	0.390	0.074	0.553
Time (45 minutes)	-8.032	<0.001	-5.374	0.009	-0.182	0.044			-0.005	0.969	-0.003	0.982
Time (60 minutes)	-7.205	0.002	-4.858	0.034	-0.148	0.119			-0.251	0.065	-0.234	0.083
Time (75 minutes)	-6.866	0.003	-5.780	0.029	0.047	0.616			-0.523	<0.001	-0.428	<0.001
Time (90 minutes)	-6.969	0.005			0.230	0.025			-0.695	<0.001		
Time (105 minutes)	-5.252	0.05			0.299	0.006			-0.966	<0.001		
Time (120 minutes)	-7-726	0.009			0.391	0.001			-1.024	<0.001		
Time (135 min)	-8.603	0.008			0.438	<0.001			-1.216	<0.001		

[a] Predictor variables–(i) Area: Sweden, Alberta; (ii) Age: yearlings, two year olds, adults (≥5 yr); (iii) Sex: male, female; (vi) Drug: MTZ or DTZ in mg/kg body weight; (v) Length: contour body length in cm; (vi) CD time: time interval in minutes from when active pursuit began to when the bear was darted; (vii) Induction time: time interval in minutes from when a bear was darted to recumbency (viii) Surgery: yes or no; (ix) Ketamine dose level: in mg/kg body weight; (x) RT: rectal temperature; (xi) HR: heart rate; (xii) RR: respiratory rate; (xii) Time: minutes after darting when measurements were recorded. Regression coefficients for factors are relative coefficients such that: (i) β for Area (Sweden) was determined with β for Area (Alberta) set to 0; β for Age (Two year olds) was determined with β for Age (Yearlings) set to 0 for the Sweden dataset; (iii) β for Age (Yearlings) and for Age (Two year olds) were determined with β for Age (Adults) set to 0 for the Sweden + Alberta dataset; (iv) β for Sex (Male) was determined with β for Sex (Female) set to 0; (v) β for Drug (MTZ) was determined with β for Drug (DZT) set to 0; (vi) β for Surgery (Yes) was determined with β for Surgery (No) set to 0; and (vii) β for Times (30–135 minutes) were determined with β for Time (15 minutes) set to 0.

Therefore, our prediction that DTZ would produce less depression of the respiratory function (respiratory rate) than MTZ was not supported.

Rectal temperature was influenced positively by heart rate and negatively by time following drug administration. For the combined datasets, two-year-old bears had significantly higher rectal temperatures than adult bears (Table 4). Rectal temperature was not differentially affected by anesthetic protocol. Therefore, our prediction that MTZ would increase rectal
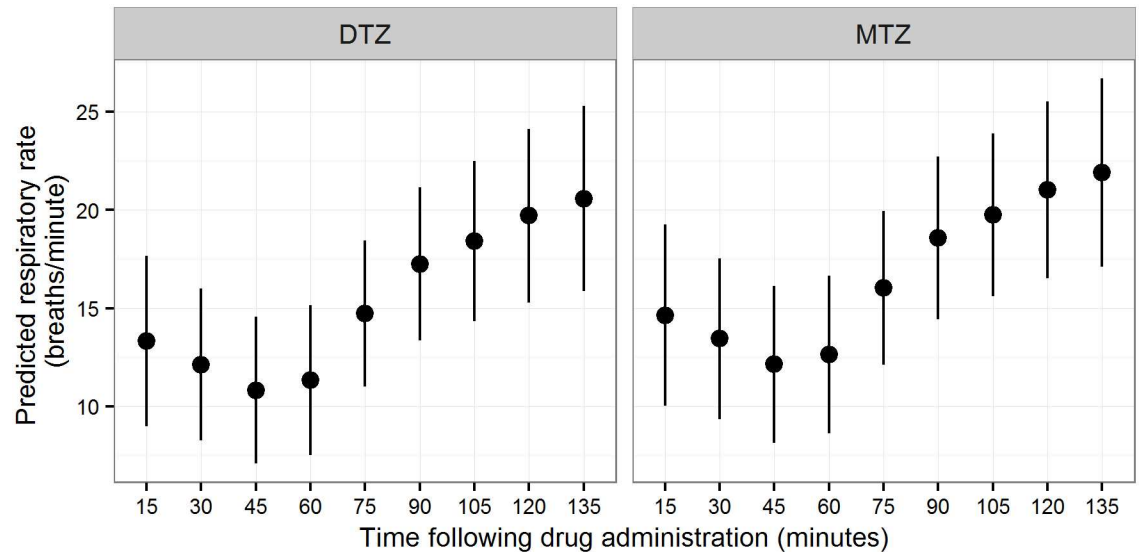
Fig 3. Respiratory rate (breaths/minute) over time following administration by drug combination (MTZ: medetomidine-tiletamine-zolazepam; DTZ: dexmedetomidine-tiletamine-zolazepam) in 34 anesthetic events of free-ranging brown bears captured in Sweden in 2014–2015.

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temperature more than DTZ was not supported and, more generally, all three predictions under hypothesis 5 were not supported.

Atipamezole was used to end anesthesia in the two study areas (Sweden: 0.48 ± 0.21 mg/kg body weight, Alberta: 0.27 ± 0.1 mg/kg body weight). The duration of anesthesia (time interval from when a bear was darted to atipamezole administration) was longer in the bears captured in Sweden (132 ± 43 min) compared to Alberta (83 ± 25 min). The time interval from atipamezole administration until the bear showed the first signs of recovery was only documented in the bears captured with culvert trap in Alberta. Time of recovery was shorter in the DTZ group (median of 13 (8–26) min vs. 28 (26–54) min in the MTZ group) but, due to the small sample size, we did not perform a statistical analysis. No capture-related mortalities occurred in the study bears during or within one month following anesthesia as determined from movement data collected by GPS radio collars on study animals.

Discussion

Both MTZ and DTZ proved to be safe and reliable drug combinations for anesthetizing free-ranging brown bears captured by helicopter and by culvert trap. However, we found no evidence to support use of DTZ as the better anesthetic combination. Both protocols produced a rapid onset of anesthesia, smooth induction, good analgesia and muscle relaxation, and smooth predictable recovery. Furthermore, the bears achieved an adequate plane of anesthesia for abdominal and subcutaneous surgeries, and muscle biopsies. We did not detect any bears' reaction (i.e., increase in heart rate) to surgery.

Induction was smooth and adverse effects that could not be effectively treated were not encountered with either combination. The induction time in the study bears increased with an increasing dose level of TZ. This result could be explained since the bears receiving more than one dart (i.e., a higher dose level of the anesthetic combination) were the bears that took longer to achieve recumbency. When only bears anesthetized with a single dart were considered, the induction time decreased with an increasing dose level of TZ in yearlings and adults. This is in agreement with the results reported by Painer et al. (2012), where the length of the induction

time in yearling brown bears anesthetized with one dart decreased with an increasing dose of M. In our study, we did not prove that induction occurs faster in bears receiving DTZ than MTZ. Therefore, we rejected our first hypothesis. Selmi et al. (2004) reported shorter times to initial sedative effects in golden-lion tamarins anesthetized with D-ketamine compared to M-ketamine. However, the same study found no difference in the time to lateral recumbency. In addition, the time from darting to first signs of sedation and recumbency were similar in Bennett's wallabies (*Macropus rufogriseus*) and Chinese water deer (*Hydropotes inermis*) comparing two groups of animals receiving M-ketamine or D-ketamine [17, 18]. Although there are no previous comparisons of the effects of M and D in ursids, Teisberg et al. (2014) described induction times in bears captured with helicopter and anesthetized with DTZ similar to times found in studies using other drug combinations (xylazine-tiletamine-zolazepam, MTZ) [29, 41].

In accordance with previous studies in brown bears [42], the need for supplemental drugs to sustain anesthesia increased as the handling time increased. Using the same doses of MTZ for subadults and slightly lower doses for yearlings, Fahlman et al. (2011) reported that bears were sufficiently anesthetized to allow one hour of handling time. In our study in Sweden, the mean handling time was 128 ± 42 min, and supplemental drugs were necessary to sustain anesthesia in 62% of the bears. However, the need for supplemental drug administration was similar between anesthetic protocols. Thus, we rejected our second hypothesis. In wildlife species, a longer lasting anesthetic effect of D-ketamine over M-ketamine was discovered in golden-lion tamarins [16]. On the contrary, no difference in the duration of anesthesia was observed in wallabies and Chinese water deer at the time atipamezole was administered as reversal [17, 18]. Comparative studies between M and D have shown a longer lasting sedative effect of D in dogs and cats [13, 43]. Although, more recent studies have failed to prove any difference, and have concluded that M and D possess comparable sedative effects [44, 45].

Blood concentrations of cortisol, and glucose to a lesser extent, are widely-used parameters to assess the stress response to capture and handling in free-ranging wild animals [23, 24]. During the stress response to capture, glucocorticoid steroid hormones (including cortisol) are released into the blood circulation, and among their many effects is a sudden rise in blood glucose levels (i.e., hyperglycemia) [46]. Alpha-2 adrenoceptor agonists can reduce the stress of physical capture and handling due to their sedative effects (reduction of struggling and improvement of muscle relaxation) [47]. On the other hand, it is well documented that the use of alpha-2 adrenoceptor agonists increases plasma glucose concentrations through insulin release inhibition [26, 48]. The role of alpha-2 adrenoceptor agonists on cortisol concentrations is controversial, and varies among species [26, 27, 48–53]. Additionally, these studies suggest that the drug effect might be age and dose-dependent. Based on our results, we would suggest that bears with longer inductions, males, bears that weighed less, and bears captured by culvert trap vs. helicopter were more stressed by the capture event. However, blood cortisol concentrations did not support a lower stress response when using DTZ than when using MTZ, thus rejecting our third hypothesis. However, due to a paucity of information on the effect of alpha-2 adrenoceptor agonists, as well as TZ, in ursid species, caution should be taken. When drawing conclusions about capture-related stress by using cortisol concentrations in anesthetized animals, the potential for drug-induced effects should be considered.

We discovered acidemia (S3 Table) at similar levels to previous studies on brown bears captured by helicopter and anesthetized with MTZ in Scandinavia [29]. The reduction in pH values in our study can be attributed to a combination of respiratory and metabolic causes. The physical exertion during capture was probably responsible for acid lactic production and decrease of base excess values. This lead to a reduction in pH values due to metabolic acidosis in the early stages of the capture. A reduction in the respiratory rate due to the alpha-2

adrenoceptor agonists increased $PaCO_2$ values causing respiratory acidosis. In our study, we rejected our fourth hypothesis as higher pH did not occur in bears anesthetized with DZT than MTZ.

We also documented hypoxemia (inadequate oxygen levels in the blood) which is a common physiological alteration found during the anesthesia of ursid species [7, 28, 29, 54]. The use of alpha-2 adrenoceptor agonists can cause respiratory depression and produce intrapulmonary changes that may result in hypoxemia [29, 55–57]. Hypoxemia can lead to hypoxia (inadequate oxygen levels in the body) that can have life-threatening consequences, such as myocardial ischemia, brain cell death and multi-organ damage [56, 58]. In the bears of the study, oxygen supplementation improved oxygenation and effectively treated hypoxemia as previously reported in brown bears [54, 59]. We found a decrease in PaO_2 values with increasing rectal temperatures, as hyperthermia increases oxygen consumption [58]. Additionally, PaO_2 values decreased with an increasing body length (significant correlated to dose level of alpha-2 adrenoceptor agonist). It is widely documented that effects of alpha-2 adrenoceptor agonists (i.e., sedation, analgesia, cardiovascular function) are dose-dependent [42, 55, 60, 61]. The alteration of the central and peripheral response to CO_2 and oxygen is also dose-dependent [62]. A previous study in brown bears suggested that the hypoxemia caused by M could be dose-dependent [29]. Moreover, significantly lower PaO_2 values were found when high doses of M and D were administered to dogs compared to lower doses [15]. Recently, studies using D in the anesthesia of bears found normal respiratory rates and high oxygen saturations [21, 22]. These authors suggested a potential benefit of D over M in bears due to less respiratory depression (i.e., hypoventilation, hypoxemia). However, these studies did not include a comparison of performance or efficacy with equivalent doses of M. In our study bears, contrary to Teisberg et al. (2014), both MTZ and DTZ caused hypoxemia ($PaO_2 < 80$ mm Hg). We rejected our fourth hypothesis, as bears anesthetized with DTZ did not show higher PaO_2 than bears anesthetized with MTZ. We argue that the different findings between Teisberg et al. (2014) and our study is due to the dose-dependent effect of alpha-2 adrenoceptor agonists on PaO_2. The mean D dose level used in our study (21.97 ± 10.12 μg/kg in Alberta, 57.51 ± 38.37 μg/Kg in Sweden) was two to five times higher than in Teisberg et al. (2014) (10.11 ± 1.04 μg/Kg).

The hemoglobin oxygen saturation measured with pulse oximeter (SpO_2) in the bears captured by culvert trap proved to be an unreliable indicator for hypoxemia in the study bears, as shown in other studies involving wildlife species [59, 63, 64]. For example, in one bear we measured 95% SpO_2 that corresponded with PaO_2 value of 59 mmHg recorded at the same point in time.

Values of $PaCO_2$ represent the balance between cellular production of carbon dioxide (CO_2) and ventilatory removal of CO_2. CO_2 elimination depends on the respiratory rate and the volume of inspired or expired air in one breath (tidal volume) [62]. Thus, we reported a reduction in $PaCO_2$ caused by increasing respiratory rates. Nevertheless, hypercapnia was a more common physiological alteration documented in the study. $PaCO_2$ values in our study were similar to previously reported values in brown bears anesthetized with MTZ in Scandinavia [29]. Mild to moderate hypercapnia may be beneficial in that it enhances the release of oxygen from hemoglobin into the tissues. However, severe hypercapnia, can lead to impaired myocardial contractility, narcosis, and coma [58]. $PaCO_2$ values increased with increasing PaO_2 values (correlated to time from darting to sampling time). Although provision of supplemental oxygen causes PaO_2 values to increase, it has little effect on hypercapnia. The elevation of $PaCO_2$ values usually indicates low respiratory rates (hypoventilation) that, in the study bears, was probably caused by the alpha-2 adrenoceptor agonists [29, 55]. In relation to $PaCO_2$ values, we observed a differential effect of the anesthetic protocol. In the DTZ group, $PaCO_2$

values decreased with increasing respiratory rates due to increased elimination of CO_2. In contrast, $PaCO_2$ values remained constant with increasing respiratory rates in the MZT group. Additionally, we found, although not significant, higher $PaCO_2$ values with increasing rectal temperatures in the MTZ when data from Sweden and Alberta were combined. We believe that the greater variation in rectal temperature in the combined datasets was due to the different capture methods used, and therefore, made this interaction relevant. Furthermore, we believe that increasing rectal temperatures reflect increasing respiratory rates, as demonstrated in other studies with bears, where concurrent high respiratory rates and hyperthermia were documented [9, 29]. Surprisingly, these findings were not supported by significantly different respiratory rates between anesthetic protocols (i.e., higher respiratory rate in the DTZ group). Thus, we suggest that the results regarding $PaCO_2$ values may be caused by a differential drug effect on the tidal volume (i.e., alveolar volume) and ventilation. The use of DTZ in the anesthesia of giant pandas (*Ailuropoda melanoleuca*) revealed changes in SpO_2 with constant respiratory rates [19], supporting the fact that changes in ventilation might occur independently of respiratory rates. Anesthetic drugs can influence tidal volume by causing ventilation-perfusion problems [62]. Ventilation-perfusion problems lead to a decrease in PaO_2 levels before any changes in $PaCO_2$ levels. The administration of supplemental oxygen during anesthesia prevented us from detecting this effect. These results provide partial support to our fourth hypothesis that bears anesthetized with DTZ would have lower $PaCO_2$ values than bears anesthetized with MTZ. We believe that D resulted in better ventilation than M, but only when respiratory rates increased. If this is true, D could prove more beneficial than M in situations when respiratory rates are anticipated to increase as in captures involving pursuit with a helicopter, captures with high ambient temperatures, or in later stages of anesthesia and during recovery. Nevertheless, we acknowledge that other comparative studies have not revealed differences between the use of M and the use of D on arterial blood gases and acid-base status [15, 17, 18].

In this study, mean heart rates remained within normal ranges (50–120 beats per min, S4 and S5 Tables) during the anesthetic period although we did observe bradycardia and tachycardia in some individual bears. Bradycardia secondary to vasoconstriction and hypertension is a common effect of the administration of alpha-2 adrenoceptor agonists [55, 65, 66]. Heart rates decreased over time as reported in previous studies [16, 20]. We also found lower heart rates in two-year-old bears than in yearlings in Sweden. Similarly, age differences have been previously reported in brown bears [29]. Brown bears in Scandinavia hibernate over a six-month period [67]. During this period, the bears do not eat, drink, defecate or urinate, and their metabolism is reduced. When bears emerge from the den after the hibernation period, their metabolic rate is approximately 50% of its normal rate which occurs sometime in the weeks following den emergence. For example, metabolic rate increased and stabilized 3 weeks following den emergence in black bears [68]. During this period of increased metabolism, heart rate, respiratory rate, body temperature, and movement rates increase [68, 69]. The bears of the study were captured from April, shortly after den emergence, to July. Thus, an increase in ordinal day of capture, accompanied by increasing rectal temperature and respiratory rate, would explain the increase in heart rate (used as an indicator of metabolic rate) [70]. We did not find fewer occurrences of bradycardia in bears receiving DTZ than in bears receiving MTZ. Therefore, we rejected our fifth hypothesis. Similarly, studies on other wildlife species have not found differences in the effect of M or D on heart rates [17, 18]. Selmi et al. (2004) showed that the heart rate in tamarins receiving D-ketamine was significantly lower than in the M-ketamine group. However, the authors attributed this result to different degrees of sedation and analgesia. In cats and dogs, numerous studies have reported contradictory results in comparing the effect of different doses of M and D on heart rate. For example, one study with domestic cats concluded that D and M have equivalent therapeutic effects [13], while another

reported greater mean heart rates for M compared with D five min after drug administration, but mean heart rates were greater for D than for M at 180 min [44]. In dogs, Kuusela et al. (2001) reported a lower overall heart rate (area under the heart rate versus time) for D versus M in one of the dose levels (mg/kg) assessed but not in the others. These results suggest that the effects of alpha-2 adrenoceptor agonists on heart rates depend upon species, dose level and the time of measurement.

In this study, despite mean respiratory rates remaining within normal range (5–30 breaths/min) during anesthesia, hypoventilation likely occurred based on the magnitude of increases of $PaCO_2$ values, and based on the respiratory rates reported in previous studies [29]. Similar to what has been reported in other studies, respiratory rate decreased over the first hour of anesthesia [16, 20, 29]. Respiratory rates increased after 90 min of anesthesia, probably due to a compensatory mechanism for hypercapnia and/or a light plane of anesthesia. We discovered higher respiratory rates in the Swedish bears than in the Alberta bears. This likely reflects the use of different captured methods, helicopter in Sweden vs. culvert trap in Alberta. Captures from helicopter often involve greater physical exertion with consequential increases in rectal temperature and respiratory rate prior to drug administration [71]. Bears receiving DTZ did not present lower respiratory rates than bears receiving MTZ. Hence, we rejected our fifth hypothesis. As previously mentioned, studies using D found normal respiratory rates during the anesthesia of bears [21, 22]. Bouts et al. (2011) also suggested that D would cause less respiratory depression compared to M. Nevertheless, studies in other wildlife species, as well as in domestic dogs and cats, have reported no differences in respiratory rates when comparing the two alpha-2 adrenoceptor agonists [13, 16, 17, 44, 45]. However, a study of laboratory mice reported higher respiratory rates in mice anesthetized with M-ketamine vs. D-ketamine [72].

We recorded body temperatures $\geq 40°C$ in the bears of the study. The highest body temperature recorded was 41.3°C in the MTZ group in Sweden. Hyperthermia has been previously reported in brown bears captured with helicopter [9, 29]. We found a significantly positive effect of age on rectal temperatures, two-year-old bears presented higher temperatures than yearlings and adult bears. This probably reflects the combined effect of a different capture method (helicopter in Sweden vs. culvert trap in Alberta) and the age difference among the bears of the two study areas (young bears in Sweden vs. adult bears in Alberta). Rectal temperatures in the Swedish bears were higher than in the Alberta bears due to physical exertion during helicopter pursuit [71]. Fahlman et al. (2011) reported lower rectal temperatures in yearling brown bears in comparison to subadults and adults. In our study, helicopter pursuit caused an increase in rectal temperature that masked the age effects on body temperature between yearlings in Sweden and adult bears captured in Alberta with culvert traps. Additionally, ambient temperature could also be an influencing factor as all yearlings were captured in April-May shortly after den emergence, while some two year olds were captured in July. Rectal temperatures significantly decreased over time in accordance with previous reports [18, 20, 29]. However, hypothermia was not observed at any time. The lowest body temperature recorded was 36.5°C in the DTZ group in Sweden. The alteration of thermoregulatory mechanisms by the alpha-2 adrenoceptor agonists [73], the cessation of physical activity, the onset of drug-induced muscle relaxation, and the application of corrective measures to reduce body temperature probably contributed to the decrease in body temperature [74]. Rectal temperature was not differentially affected by the drug combination used, hence, rejecting our fifth hypothesis that bears anesthetized with DTZ would show a quicker recovery of normal body temperature than MTZ. None of the studies comparing the effects of alpha-2 adrenoceptor agonists on thermoregulation in wildlife species have demonstrated any difference [16–18, 20]. However, these studies were performed in captive settings, where the animals were not subjected to high levels of physical exertion, and body temperatures were normal or close to

normal at induction [17]. In free-ranging animals, especially those pursued during capture, we expect hyperthermia at early stages, irrespective of the anesthetic protocol used, due to stress and physical exertion. We also expect temperature to decrease and return to normal values over time. This decrease, however, might be affected by the anesthetic protocol used through the alteration of thermoregulatory mechanisms or changes in the respiratory rates [41, 75]. Drugs producing less depression of the respiratory function, would allow animals to better dissipate heat, and return to normal temperature values quicker [76]. In our study, we observed initial hyperthermia, and a decrease of rectal temperature over time as expected. Contrary to our hypothesis, both MZT and DZT produced the same level of respiratory depression on the bears, and therefore, no differences in rectal temperature between groups were detected at any time.

In Alberta, the time of recovery was shorter in the DTZ group. However, the dose level of atipamezole administered to the bears to reverse anesthesia was higher in the DZT (9.23 ± 1.08) than the MTZ group (7.85 ± 4.70). Furthermore, the sample size was small (n = 6). Thus, no definitive conclusions can be drawn. Results of previous studies in regards to recovery time are not in agreement. Some studies showed no difference in the recovery times [13, 72]. Other studies found a faster recovery with M than D when using a half-dose of atipamezole to reverse the effects of D [18]. Thus, the use of a full dose of atipamezole for D is recommended [18, 21]. When no reversal agent was used, Selmi et al. (2004) reported no differences in the time interval between the end of anesthesia and the animal standing, but longer times from standing until the animal could walk when using D in the anesthetic combination.

In summary, DZT and MZT produced reliable anesthesia without detectable differences in induction time, the need for supplemental drugs to sustain anesthesia, capture-related stress, acid-base status, PaO_2, and physiological responses in free-ranging brown bears captured by helicopter or by culvert trap. DZT provided an apparent benefit by decreasing $PaCO_2$ levels with increasing respiratory rates. However, this advantage was not supported by differential respiratory rates between anesthetic protocols. We recommend the use of supplemental oxygen to treat hypoxemia at the dose levels of alpha-2 adrenoceptor agonists used in the study. We conclude that dexmedetomidine offers no advantage over the use of medetomidine in the anesthesia of free-ranging brown bears when combined with tiletamine-zolazepam.

Supporting Information

S1 Text. Detailed results of pH, partial pressure of arterial oxygen (PaO₂), and partial pressure of arterial carbon dioxide (PaCO₂) in free-ranging brown bears (*Ursus arctos*) undergoing anesthesia with medeteomidine-tiletamine-zolazepam (MTZ) or dexmedeteomidine-tiletamine-zolazepam (DTZ) in Sweden (N = 34) and Alberta, Canada (N = 6) in 2014–2015.
(DOCX)

S2 Text. Detailed results of physiological responses in free-ranging brown bears (*Ursus arctos*) undergoing anesthesia with medeteomidine-tiletamine-zolazepam (MTZ) or dexmedeteomidine-tiletamine-zolazepam (DTZ) in Sweden (N = 34) and Alberta, Canada (N = 6) in 2014–2015.
(DOCX)

S1 Table. Capture date, age (years), sex (M: male; F: female), body weight (kg), body length (cm), drug combination used for anesthesia (DTZ: dexmedeteomidine-tiletamine-zolazepam; MTZ: medeteomidine-tiletamine-zolazepam), alpha-2 adrenoceptor agonist dose

level (µg/kg), tiletamine-zolazepam dose level (TZ dose level, mg/kg), induction time (minutes), use and dose level of supplemental drugs (Suppl.drugs, Y: yes; N: no; Suppl. dose level, mg/kg) in 34 anesthetic events of free-ranging brown bears (*Ursus arctos*) captured in Sweden in 2014–2015.
(DOCX)

S2 Table. Capture date, age (years), sex (M: male; F: female), body weight (kg), body length (cm), drug combination used for anesthesia (DTZ: dexmedeteomidine-tiletamine-zolazepam; MTZ: medeteomidine-tiletamine-zolazepam), alpha-2 adrenoceptor agonist dose level (µg/kg), tiletamine-zolazepam dose level (TZ dose level, mg/kg), induction time (minutes), use and dose level of supplemental drugs (Suppl.drugs, Y: yes; N: no; Suppl. dose level, mg/kg) in six free-ranging brown bears (*Ursus arctos*) captured in Alberta, Canada in 2014–2015.
(DOCX)

S3 Table. Arterial blood gases, acid-base status, and oxygen saturation (mean ± standard deviation) in free-ranging brown bears (*Ursus arctos*) undergoing anesthesia with medeteo-midine-tiletamine-zolazepam or dexmedeteomidine-tiletamine-zolazepam in Sweden (N = 34) and Alberta, Canada (N = 6) in 2014–2015. For the bears captured in Alberta, the median value and range are shown in parenthesis. Arterial blood gases and acid-base status were not measured in all bears at both sampling times.
(DOCX)

S4 Table. Physiological responses (mean ± standard deviation) in 34 anesthetic events of free-ranging brown bears (*Ursus arctos*) using medeteomidine-tiletamine-zolazepam or dexmedeteomidine-tiletamine-zolazepam in Sweden in 2014–2015. Measurements were not recorded from all bears at all time points.
(DOCX)

S5 Table. Physiological responses (mean ± standard deviation) in six free-ranging brown bears (*Ursus arctos*) undergoing anesthesia with medeteomidine-tiletamine-zolazepam or dexmedeteomidine-tiletamine-zolazepam in Alberta, Canada in 2014–2015. The median value and range are shown in parenthesis. Measurements were not recorded from all bears at all time points.
(DOCX)

S6 Table. Hematological and biochemical parameters (mean ± standard deviation) in arterial blood from free-ranging brown bears (*Ursus arctos*) undergoing anesthesia with mede-teomidine-tiletamine-zolazepam or dexmedeteomidine-tiletamine-zolazepam in Sweden (N = 34) and Alberta, Canada (N = 6) in 2014–2015. For the bears captured in Alberta, the median value and range are shown in parentheses. Blood parameters were not measured in all bears.
(DOCX)

Acknowledgments

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Supporting information

S1 Text. Detailed results of pH, partial pressure of arterial oxygen (PaO_2), and partial pressure of arterial carbon dioxide ($PaCO_2$) in free-ranging brown bears (*Ursus arctos*) undergoing anesthesia with medeteomidine-tiletamine-zolazepam (MTZ) or dexmedeteomidine-tiletamine-zolazepam (DTZ) in Sweden (N=34) and Alberta, Canada (N=6) in 2014-2015.

pH

Arterial blood gases and acid-base status were not measured in all bears at both sampling times. We obtained the first and second arterial blood samples at 32 ± 5 and 63 ± 5 min after drug administration from bears in Sweden. Acidemia (pH < 7.35) occurred in 28 bears at 30 min after darting (13 of 14 bears in the MTZ group, 15 of 16 bears in the DTZ group). These included two bears in the DTZ group with severe acidemia (pH < 7.25). After one hour of anesthesia, 27 bears had acidemia (13 of 16 bears in the MTZ group, 14 of 18 bears in the DTZ group).

We obtained the first and second arterial blood samples at 34 ± 6 and 60 ± 2 min after drug administration from six bears in Alberta. Acidemia occurred in two bears (one of three bears in each group) 30 min after darting, but was not detected in any of the bears at 60 min.

PaO_2

We recorded hypoxemia (PaO_2 < 80 mmHg) in 27 bears captured in Sweden (13 of 14 bears in the MTZ group, 14 of 16 bears in the DTZ) at 30 min following drug administration. Of these, 20 bears (11 of 14 bears in the MTZ group, nine of 16 bears in the DTZ) had mild hypoxemia (PaO_2 from 60-80 mmHg), and seven bears (two bears in the MTZ group, five bears in the DTZ) had marked hypoxemia (PaO_2 from 40-60 mmHg). All 27 bears were supplemented with oxygen. At 60 min, four of seven bears not receiving oxygen (two bears in each anesthetic protocol) were hypoxemic.

Hypoxemia occurred in all Alberta bears at both sampling times. We recorded mild hypoxemia in three bears (one bear in the MTZ group, two bears in the DTZ group) and marked hypoxemia in the other three bears (two bears in the MTZ group, one bear in the DTZ group) at 30 min following drug administration. The PaO_2 values increased slightly over time in all bears except one without provision of

oxygen. One hour following drug administration, hypoxemia was mild in four bears (two bears in each group), and marked in two bears (one bear in each group). Values of hemoglobin oxygen saturation readings recorded by pulse oximeter (SpO_2) were below 90% with both drug combinations.

$PaCO_2$

We documented hypocapnia ($PaCO_2 < 35$ mmHg) in one of 14 bears that received MTZ at 30 min following drug administration in Sweden. We found mild hypercapnia ($PaCO_2$ from 45-60 mmHg) in three bears at 30 min after darting (one of 14 bears in the MZT, two of 16 bears in the DTZ group), and in 10 bears (six of 16 bears in the MTZ group, four of 18 bears in the DTZ group) at one hour following drug administration. Hypercapnia was severe ($PaCO_2 > 60$ mmHg) in one of the bears in the MTZ group.

With the Alberta bears, one of three bears in the MTZ group was hypocapnic at one hour following drug administration, while another bear in the MTZ group was mildly hypercapnic at both sampling times.

S2 Text. Detailed results of physiological responses in free-ranging brown bears (*Ursus arctos*) undergoing anesthesia with medeteomidine-tiletamine-zolazepam (MTZ) or dexmedeteomidine-tiletamine-zolazepam (DTZ) in Sweden (N=34) and Alberta, Canada (N=6) in 2014-2015.

Heart rate

We detected bradycardia (< 50 beats per min) in three bears (one of 16 bears in the MTZ group, two of 18 bears in the DTZ group) at 75 min following drug administration in Sweden. Heart rates lower than 50 beats per min were sustained until the end of the anesthesia in the bear belonging to the MTZ group, but increased above this rate in the other two bears. We detected tachycardia (> 120 beats per min) in three bears (two in the MTZ, one in the DTZ group). The elevated heart rate persisted longest in the bear belonging to the DTZ group.

We did not detect tachycardia at any time in the bears captured using culvert traps in Alberta. However, bradycardia was detected in four bears (one of three bears in the MTZ group, all three bears in the DTZ group) as early as 15 min after drug administration, and sustained until the end of the anesthesia.

Respiratory rate

We detected bradypnea (< 5 breaths per min) in two of 16 bears in the MTZ group at various times following drug administration in Sweden. Tachypnea (> 30 breaths per min) occurred in eight bears (five of 16 in the MTZ group, three of 18 in DTZ group) during anesthesia.

Respiratory rates were within the normal range (5-30 breaths per min) throughout anesthesia in the bears captured by culvert trap in Alberta.

Body temperature

Hypothermia (T < 35°C) was not recorded at any time during anesthesia in the Swedish bears. However, hyperthermia (T ≥ 40°C) was recorded in bears receiving both drug combinations. Five bears within each drug group were hyperthermic at 30 min after darting, and two bears within each drug group were still hyperthermic at 60 min.

Rectal temperature was within the considered normal range (35-40°C) throughout anesthesia in the bears captured by culvert trap in Alberta.

S1 Table. Capture date, age (years), sex (M: male; F: female), body weight (kg), body length (cm), drug combination used for anesthesia (DTZ: dexmedeteomidine-tiletamine-zolazepam; MTZ: medeteomidine-tiletamine-zolazepam), alpha-2 adrenoceptor agonist dose level (µg/kg), tiletamine-zolazepam dose level (TZ dose level, mg/kg), induction time (minutes), use and dose level of supplemental drugs (Suppl.drugs, Y: yes; N: no; Suppl. dose level, mg/kg) in 34 anesthetic events of free-ranging brown bears (*Ursus arctos*) captured in Sweden in 2014-2015.

Bear ID	Capture date	Age	Sex	Weight	Length	Drug combination	Alpha-2 dose level	TZ dose level	Induction	Suppl. drugs	Suppl. dose level
1	4/23/2014	2	M	31.5	116	MTZ	105.4	5.29	13	N	0
2	4/23/2014	2	M	39	117	DTZ	21.3	2.14	4	N	0
3	4/24/2014	2	M	56	131	DTZ	22.3	2.23	6	Y	1.61
4	4/24/2014	2	M	51.5	132	MTZ	48.5	2.43	1	Y	1.75
5	4/26/2014	1	F	14.5	90	DTZ	57.2	5.74	2	Y	2.07
6	4/26/2014	1	M	17	93	MTZ	97.6	4.9	2	Y	1.76
7	4/26/2014	1	M	18	92	MTZ	92.2	4.63	3	N	0
8	4/26/2014	1	M	16	82	DTZ	51.9	5.21	1	N	0
9	4/27/2014	1	F	15	90	MTZ	110.7	5.55	1	N	0
10	4/27/2014	1	M	15.5	91	DTZ	53.5	5.37	2	N	0
11	4/27/2014	1	F	22	100	DTZ	37.7	3.79	2	Y	2
12	4/27/2014	1	F	25	109	DTZ	33.2	3.33	2	N	0
13	4/27/2014	1	M	27	110	MTZ	61.5	3.09	3	N	0
14	4/28/2014	1	M	22	108	DTZ	37.7	3.79	2	N	0
15	4/28/2014	1	M	16	87	MTZ	103.8	5.21	7	Y	1
16	4/28/2014	1	M	19	92	DTZ	43.7	4.38	2	N	0
17	7/1/2014	2	F	49	121	MTZ	33.9	1.7	3	Y	2.04
18	7/1/2014	2	M	42	115	MTZ	59.5	2.98	4	Y	1.67
19	7/1/2014	2	M	40	115	DTZ	31.3	3.13	4	Y	1.25
20	7/2/2014	2	F	43	116	MTZ	58.1	2.91	2	Y	2.33
21	7/2/2014	2	F	33	115	DTZ	37.9	3.79	4	Y	3.03
5	4/22/2015	2	F	23	103	DTZ	54.3	5.43	4	N	0
8	4/23/2015	2	M	37	118	MTZ	67.6	3.38	5	Y	1.08
7	4/23/2015	2	M	41	119	DTZ	30.5	3.05	4	Y	0.85
22	4/23/2015	1	F	18	98	MTZ	92.2	4.63	2	N	0
23	4/23/2015	1	F	14	86	DTZ	118.6	11.9	10	N	0
24	4/24/2015	1	M	21	97	DTZ	79	7.93	6	Y	1.9
25	4/24/2015	1	M	20.5	102	MTZ	162	8.13	10	Y	1.95
26	5/15/2015	1	F	18.4	93.5	DTZ	60	3.02	3	Y	2.17
27	5/15/2015	1	F	13.2	87	MTZ	126	6.31	2	Y	1.89
28	5/16/2015	1	F	11	81	MTZ	151	7.57	2	Y	2.27
29	5/16/2015	1	F	9.5	83	DTZ	175	8.77	1	Y	2.63
30	5/16/2015	1	F	13	89	MTZ	128	6.41	2	Y	1.92
31	5/16/2015	1	F	18	95	DTZ	90	4.63	6	Y	1.38

S2 Table. Capture date, age (years), sex (M: male; F: female), body weight (kg), body length (cm), drug combination used for anesthesia (DTZ: dexmedeteomidine-tiletamine-zolazepam; MTZ: medeteomidine-tiletamine-zolazepam), alpha-2 adrenoceptor agonist dose level (µg/kg), tiletamine-zolazepam dose level (TZ dose level, mg/kg), induction time (minutes), use and dose level of supplemental drugs (Suppl.drugs, Y: yes; N: no; Suppl. dose level, mg/kg) in six free-ranging brown bears (*Ursus arctos*) captured in Alberta, Canada in 2014-2015.

Bear ID	Capture date	Age	Sex	Weight	Length	Drug combination	Alpha-2 dose level	TZ dose level	Induction	Suppl. drugs	Suppl. dose level
1	5/14/2014	6	M	169.6	198	MTZ	31	1.5	9	N	0
2	5/19/2014	9	M	222.2	209	DTZ	10	1	5	Y	2.7
3	5/14/2015	9	M	118.8	176	DTZ	26	2.5	5	N	0
4	5/14/2015	15	M	298.6	221	MTZ	60	2.9	6	N	0
5	5/18/2015	8	M	115.2	175	DTZ	29	1.4	NR	Y	1.74
6	5/18/2015	8	M	167.8	196	MTZ	66	3.1	NR	N	0

S3 Table. Arterial blood gases, acid-base status, and oxygen saturation (mean ± standard deviation) in free-ranging brown bears (*Ursus arctos*) undergoing anesthesia with medeteomidine-tiletamine-zolazepam or dexmedeteomidine-tiletamine-zolazepam in Sweden (N=34) and Alberta, Canada (N=6) in 2014-2015. For the bears captured in Alberta, the median value and range are shown in parenthesis. Arterial blood gases and acid-base status were not measured in all bears at both sampling times.

				Minutes following drug administration				
		30 min					**60 min**	
	N	Sweden	N	Alberta	N	Sweden	N	Alberta
pH	30	7.30 ± 0.04	6	7.35 ± 0.03 (7.36 (7.30-7.38))	34	7.33 ± 0.042	6	7.36 ± 0.02 (7.36 (7.34-7.40))
PaO$_2$	30	70 ± 10	6	60 ± 7 (59 (52-70))	34	121 ± 33	6	66 ± 10 (67 (50-79))
PaCO$_2$	30	41 ± 4	6	42 ± 4 (41 (36-49))	34	44 ± 4	6	41 ± 5 (41 (33-49))
HCO$_3$	30	20 ± 3	6	23 ± 3 (22 (20-28))	34	23 ± 4	6	23 ± 3 (23 (20-28))
BE	30	-5 ±4	6	-2 ± 3 (-3 (-6-3))	34	-2 ± 4	6	-2 ± 3 (-2 (-5-3))
TCO$_2$	30	21 ± 3	6	24 ± 3 (23 (21-29))	34	24 ± 4	6	24 ± 3 (24 (21-29))
SaO$_2$	30	87 ± 6	6	87 ± 5 (88 (79-93))	34	95 ± 10	6	90 ± 6 (91 (77-95))
Lac	30	3.65 ± 2.13	6	1.2 ± 0.58 (1.1 (0.7-2.3))	33	1.72 ± 0.80	6	0.93 ± 0.49 (0.76 (0.6-1.9))
SpO$_2$	NR	NR	5	90 ± 4 (90 (85-97))	NR	NR	6	92 ± 7 (94 (78-96))

N: Sample size; PaCO2: partial pressure of arterial carbon dioxide, in mm Hg; PaO2: partial pressure of arterial oxygen, in mm Hg; BE: base excess, in mmol/L;

HCO3: bicarbonate, in mmol/L; TCO2: total carbon dioxide, in mmol/L; SaO2: arterial oxygen saturation, in %; Lac: lactate concentration, in mmol/L; SpO2: Oxygen

saturation readings obtained with a pulse oximeter, in % (only in Alberta); NR: not recorded

S4 Table. Physiological responses (mean ± standard deviation) in 34 anesthetic events of free-ranging brown bears (*Ursus arctos*) using medeteomidine-tiletamine-zolazepam or dexmedeteomidine-tiletamine-zolazepam in Sweden in 2014-2015. Measurements were not recorded from all bears at all time points.

Time after darting	N	Heart rate (beats/minute)	N	Respiratory rate (breaths/minute)	N	Body temperature (°C)
15 min	16	94 ± 17	16	17 ± 5	16	39.4 ± 0.8
30 min	31	88 ± 17	31	16 ± 13	31	39.5 ± 0.9
45 min	34	84 ± 19	34	14 ± 8	34	39.4 ± 0.8
60 min	30	84 ± 20	30	13 ± 6	29	39.1 ± 0.8
75 min	34	82 ± 22	34	16 ± 7	34	38.8 ± 1.0
90 min	25	83 ± 20	25	19 ± 7	25	38.8 ± 0.8
105 min	25	86 ± 21	25	20 ± 7	24	38.5 ± 0.8
120 min	17	82 ± 24	17	20 ± 6	17	38.5 ± 0.6
135 min	14	77 ± 23	14	20 ± 6	14	38.2 ± 0.7

N: Sample size

S5 Table. Physiological responses (mean ± standard deviation) in six free-ranging brown bears (*Ursus arctos*) undergoing anesthesia with medeteomidine-tiletamine-zolazepam or dexmedeteomidine-tiletamine-zolazepam in Alberta, Canada in 2014-2015. The median value and range are shown in parenthesis. Measurements were not recorded from all bears at all time points.

Time after darting	N	Heart rate (beats/min)	N	Respiratory rate (breaths/min)	N	Body temperature (°C)
15 min	4	52 ± 5 (51 (47-58))	4	7 ± 1 (6 (6-8))	4	38.2 ± 0.7 (38.1 (37.5-39.2))
30 min	5	54 ± 8 (51 (45-64))	6	7 ± 1 (7 (5-9))	6	37.8 ± 0.9 (37.7 (36.7-39.4))
45 min	6	54 ± 7 (53 (43-62))	6	8 ± 1 (8 (6-10))	6	37.8 ± 0.9 (37.7 (36.6-39.4))
60 min	6	51 ± 9 (52 (40-65))	6	7 ± 2 (6 (5-10))	6	37.8 ± 1.3 (37.9 (35.7-39.7))
75 min	6	51 ± 9 (45 (36-55))	6	7 ± 2 (8 (6-10))	6	37.8 ± 1.3 (36.8 (36.6-37.1))

N: Sample size

S6 Table. Hematological and biochemical parameters (mean ± standard deviation) in arterial blood from free-ranging brown bears (*Ursus arctos*) undergoing anesthesia with medeteomidine-tiletamine-zolazepam or dexmedeteomidine-tiletamine-zolazepam in Sweden (N=34) and Alberta, Canada (N=6) in 2014-2015. For the bears captured in Alberta, the median value and range are shown in parentheses. Blood parameters were not measured in all bears.

	N	Sweden	N	Alberta
Sodium (mmol/L)	23	129 ± 3	6	138 ± 1 (138 (136-140))
Potassium (mmol/L)	22	3.9 ± 0.4	6	3.9 ± 0.8 (4.0 (2.6-4.7))
Chloride (mmol/L)	18	103 ± 3	4	109 ± 4 (110 (104-113)
Blood Urea Nitrogen (mg/dL)	18	10 ± 10	4	30 ± 15 (27 (16-49))
Glucose (mg/dL)	20	133 ± 35	6	193 ± 26 (192 (154-224))
Hematocrit (%PCV)	23	39 ± 4	6	45 ± 1 (44 (43-47))
Hemoglobin (g/dl)	23	13.2 ± 1.3	6	15.2 ± 0.5 (15.1 (14.6-16.0))
Cortisol (nmol/L)	34	293 ± 158	5	249 ± 161 (337 (33-406))

N: Sample size

Paper III

Fandos Esteruelas, N., Cattet, M., Zedrosser, A., Stenhouse, G. B., Swenson, J. E., Evans, A. L., Arnemo, J. M.. (2017). Evaluation of the long-term effects of capture, handling, and surgery on body condition in male brown bears (*Ursus arctos*). *Manuscript*.

Photo: Amy Stenhouse

Evaluation of the long-term effects of capture, handling, and surgery on body condition in male brown bears (*Ursus arctos*)

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Abstract

Body condition is an important determinant of an individual animal's health and fitness. It is a measure of the stored energy that is available to fuel essential behaviors and physiological processes in accordance with an animal's life history, while counteracting the energetic costs of natural and anthropogenic environmental factors. In this study, we evaluated the impact of capture, handling, and surgery on body condition index (BCI) values of independent male brown bears (*Ursus arctos*) from two long-term research projects, one in Scandinavia, and the other in Alberta, Canada. We used data collected from 551 captures of 302 unique individuals from 1988 to 2015. We accounted for the potential impact of research activities using generalized linear mixed models, and compared capture-related variables against other potential determinants of body condition including age, ordinal day of capture, and study area. We found that age, ordinal day of capture and study area were significant determinants of BCI values in the study bears ($R^2 = 0.46$). Age had a curvilinear association with BCI, whereas ordinal day of capture was positively correlated with BCI. Study area also explained some of the variation in BCI values among bears in that values tended to be higher for bears in Scandinavian than bears in Alberta. Capture-related variables did not have a significant impact on BCI values. Although we were unable to detect any effect of capture, handling and surgery on the BCI of independent male bears, we would like to stress the importance of the evaluation of the potential impacts of capture and handling as part of the health assessment in studies involving wildlife, and for the interpretation of research results.

Introduction

Body condition is an important determinant of an individual animal's health and fitness (Cattet et al. 2002; Peig and Green, 2010). In theory, it is a measure of the stored energy that is available to fuel essential behaviors and physiological processes in accordance with an animal's life history, while counteracting the energetic costs of natural and anthropogenic environmental factors. According to life history theory, individuals will allocate resources optimally among life-history traits over their lifetime (Stearns, 1992). Physiological and behavioral responses to capture and handling impose energetic costs (Morellet et al., 2009) and could, therefore, impact other vital processes (e.g., growth, reproduction, immune function). If the energetic costs of capture and handling are long lasting, a loss of body condition could lead to reduced survival and reproductive rates, as has been reported in ursids (Noyce and Garshelis, 1994; Atkinson and Ramsay, 1995). Thus, changes in body condition may have an effect at the individual level, but could also influence population dynamics through changes in birth (i.e., reproduction) (Stirling et al., 1999) and death rates (i.e. survival) (Robbins et al., 2012).

In practice, body condition is estimated using indirect methods such as morphological, biochemical or physiological metrics. Estimates of body condition are widely used by ecologists as one of many measures to describe ecological interactions (e.g., diet, density, parasite load), environmental degradation (e.g., habitat loss, pollution, climate change), as well as life-history patterns (e.g., reproduction, survival) (Stevenson and Woods, 2006). Despite their widespread use, some methods to estimate body condition are inaccurate and time consuming, or are used without empirical validation (Green, 2001). In addition, there is no consensus about the most appropriate method, and a diversity of estimates have been reported (Peig and Green, 2010; Labocha et al., 2014). In bears, body condition has been estimated using morphometric measurements (e.g., body mass, body length) (Cattet, 1990), blood analyses (e.g., albumin, total protein) (Noyce and Garshelis, 1994), chemical analyses of the carcass (Watts and Hansen, 1987), measurement of fat in bone marrow and muscles (Cattet, 1990), and bioelectrical impedance and isotope dilution (Farley and Robbins, 1994).

Extensive literature exists reporting the influence of numerous factors (e.g., age, environmental conditions, etc.) on body condition in mammals. The growth pattern and body condition of large mammals is largely determined by biological factors such as age and sex (Garlich-Miller and Stewart, 1998; Solberg et al., 2004; Nielsen et al., 2013). In species with sexual size dimorphism, such as the brown bear (*Ursus arctos*), the different growth rate of males and females is the result of differences in energy utilization. Males maximize growth rate, whereas females balance energy use between growth and reproduction (Isaac, 2005). Also, spatial and temporal heterogeneity of the environment is recognized as a major force influencing life-history traits of individuals (Stearns, 1992), and ultimately, population dynamics (Grenfell et al., 1998; Dobson and Oli, 2001). Density-independent (e.g., temperature, precipitation) and density dependent factors (i.e., population density) may have an impact on body condition by affecting food quality and availability (Stirling et al., 1999; MacDonald et al., 2002).

Only a few studies have focused on the long-term effects of capture and handling on wildlife, with inconsistent results. Some have reported a negative effect of capture and handling on the animal's reproduction, physiology, behavior and/or activity (Alibhai et al., 2001; Cattet et al., 2008; Morellet et al., 2009), whereas others have not detected significant long-term effects (McMahon et al., 2008; Omsjoe et al., 2009; Harcourt et al., 2010; Thiemann et al., 2013). In some studies, where effects have been observed, the presence or absence of effects has been dependent upon the sex, age, and reproductive status of the target animals (Lunn et al., 2004). For example, Ramsay and Stirling (1986) found that recapture had a negative influence on the weight of female polar bears (*Ursus maritimus*) with cubs, but no effect was detected in male bears.

The overall impact of capture and handling cannot be determined without fully evaluating physical, behavioral, and physiological effects on captured and handled animals in the weeks and months following capture. The failure to recognize potential long-term effects of capture and handling on study animals has implications both for animal welfare and the interpretation of research results (Powell and Proulx, 2003; Cattet et al., 2008). Recently, the evaluation of long-term effects of capture and handling on body condition has been conducted in a few wildlife species, including bears (Tuyttens et al., 2002; Moorhouse and

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MacDonald, 2005; Cattet et al., 2008). However, the results of these studies are not in agreement. Some studies report a negative effect of capture and handling (i.e., number of captures, carrying a radio-collar) on the body condition of target animals (Tuyttens et al., 2002; Moorhouse and MacDonald, 2005; Cattet et al., 2008; Nielsen et al., 2013), whereas others report no effects (Rode et al., 2014).

In this study, we evaluated the potential impact of capture, handling, and surgery on body condition index values of independent male brown bears from two long-term research projects, one in Scandinavia, and the other in Alberta, Canada. We focused on a single subset of bears (i.e., independent males), because demands on body condition, and the influence of different factors on body condition, might vary among sex, age, and reproductive classes, due to differences in their respective life histories (Coulson et al., 2001; Bonenfant et al., 2009; Nielsen et al., 2013). For example, meeting the nutritional demands of having a large body size might predispose male bears to being more sensitive to scarcity in food resources (Atkinson et al., 1996; Isaac, 2005).

Our primary objective was to evaluate and compare associations between body condition, as estimated by a body condition index (Cattet et al., 2002), and age of the bear, ordinal day of capture, study area, and several measures of capture and handling. Previous studies of body condition in brown bears have identified associations between body condition and age (Cattet et al., 2008; Nielsen et al., 2013), body condition and season (Hilderbrand et al., 2000), and body condition and environmental factors (i.e., habitat quality, anthropogenic factors) (Boulanger et al., 2013; Nielsen et al., 2013). Our measures of capture and handling included method of capture, the number of captures, the time interval between captures, and the performance of abdominal surgeries for the implantation or removal of bio-logging devices.

Material and methods

Study areas

We used data from two long-term research projects, the Scandinavian Brown Bear Research Project, and the fRI Research Grizzly Bear Program in Alberta, Canada. The Scandinavian Brown Bear Research Project started in Sweden in 1984, and expanded to include Norway in 1987. The main goals of

the project are to understand the ecology of the Scandinavian brown bear, to provide the scientific basis for the management of the species in Sweden and Norway, and to provide information to the general public. The study area in Scandinavia consists of an area of 13,000 km^2 of intensively managed boreal forest in the south (61°N, 14°E), and 6,000 km^2 with deep valleys in the north (67°N, 18°E). Details on the study area, trends and status of the brown bear population are presented in Zedrosser et al. (2006) and Swenson et al. (2017).

The fRI Research Grizzly Bear Program began in 1999 to provide knowledge and planning tools to land and resource managers to ensure the long-term conservation of brown bears in Alberta. The main focus of the program is applied scientific field research with a large-scale approach towards brown bear conservation and recovery. The study area in Alberta consists of ~300,000 km^2 along the eastern slopes of the Canadian Rocky Mountains (49-58°N, 113-120°W) encompassing mountains and foothills. Details on the study area and the status of the brown bear population are available in Nielsen et al. (2006), Natural Regions Committee (2006), and ASRD and ACA (2010). The fRI Research Grizzly Bear Program also provided us with a subset of archived data collected by the Eastern Slopes Grizzly Bear Project from 1993 to 2002 (Herrero, 2005).

Capture and handling of study animals

Study animals consisted of independent male brown bears defined as males that were independent of their mothers at the time of the capture.

Scandinavian Brown Bear Research Project

We evaluated data from the captures of 157 individual male bears in Scandinavia, from March to October between 1988 and 2014, with 219 captures involving adult bears (≥ 5 years) and 152 captures involving juvenile bears (< 5 years). All bears were anesthetized by remote drug delivery from a helicopter. Body weight was determined by suspending bears from a spring scale. Body length was measured along the curvature of the dorsum as the distance from tip of nose to end of last tail vertebra with the bear in sternal or lateral recumbency. Additional details on capture and handling procedures are available in Arnemo and Evans (2017).

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In 82 capture events, brown bears had undergone one or more previous abdominal surgeries. Surgeries consisted in the implantation or removal of intraperitoneal devices, such as radio transmitters (Telonics®, Telonics Inc., Mesa, Arizona, USA), physiological sensors (Vectronic Aerospace®, Berlin, Germany), and temperature loggers (Star-Oddi®, Gardabaer, Iceland). We administered carprofen (Rimadyl® vet. 50 mg/ml, Orion Pharma Animal Health, FI-02200 Espoo, Finland) or meloxicam (Metacam® 5mg/ml, Boehringer Ingelheim, Reihn, Germany) to provide analgesia.

The capture and handling protocols in Scandinavia were approved by wildlife authorities (the Swedish Environmental Protection Agency (Stockholm, Sweden), and the Norwegian Environment Agency (Trondheim, Norway)), and ethical committees (the Swedish Ethical Committee on Animal Research (Uppsala, Sweden), and the National Animal Research Authority (Brumunddal, Norway)).

fRI Research Grizzly Bear Program

Data were collated from the captures of 145 individual male bears in Alberta, from April to October between 1993 and 2015, with 109 captures involving adult bears and 71 captures involving juvenile bears. These included data from 81 captures using Aldrich leg hold snares (Aldrich Snare Co., Clallam Bay, Washington), 77 captures using culvert traps, and 22 captures using remote drug delivery from a helicopter. Body weight was determined by suspending bears from an electronic load scale. Body length was measured as described previously. Additional details on capture and handling procedures are presented in Cattet et al. (2003 and 2008).

Captures were authorized by the Alberta Department of Environment and Sustainable Resource Development (provincial jurisdiction lands), Alberta Tourism and Parks (provincial parks and protected areas jurisdiction lands), and Parks Canada (federal jurisdiction lands). Capture protocols were approved by the University of Saskatchewan's Committee on Animal Care and Supply (Animal Use Protocol # 20010016).

All bear captures in both study areas were in accordance with guidelines provided by the American Society of Mammalogists' Animal Care and Use Committee (Sikes and Gannon, 2011) and the Canadian Council on Animal Care (2003) for the safe handling of wildlife.

Statistical analysis

Response variable

The response variable was a body condition index (BCI) that has been validated for ursids. It is essentially the standardized residual derived from the regression of body mass against body length (Cattet et al. 2002). We evaluated various types of relationships between body mass (M) and length (L) using the Curve Estimation procedure in SPSS (IBM SPSS Statistics Version 20, IBM, Armonk, New York, USA), and determined that a power (polynomial) model of the form, $M = \beta_0 * L^{\beta_1}$, best described the data, based on highest F- and adjusted R^2-values. Applying this model to the data, we saved the standardized residuals as BCI values for the study bears.

Predictor variables

We evaluated seven variables, both individually and combined as two-way interactions, as predictors of the BCI (Table 1). Age of bear (age) was calculated as age in years + (ordinal day of capture/365) and was evaluated as both linear (age) and polynomial (age^2, age^3) terms, as in Nielsen et al. (2013). Age in years was estimated from the mother's reproductive history or by extracting a premolar tooth and counting cementum annuli (Stoneberg and Jonkel, 1966). Ordinal day of capture (day) was also evaluated as both linear and polynomial terms (day, day^2, day^3). Time measures, including day, month and season of capture have been evaluated previously as predictors of body condition in brown bears (Hilderbrand et al., 2000; Cattet et al., 2008). Study area (Scandinavia; Alberta) was used as a coarse-level variable to account for potential differences between study groups with respect to natural environmental conditions and human activities other than capture and handling (Boulanger et al., 2013; Nielsen et al., 2013). For measures of capture and handling, we included the method of capture used in the previous capture, the number of times a bear had been previously captured, the time interval between the previous

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and the current capture event, and whether or not a bear had undergone abdominal surgery in a previous capture event as predictors in the analysis of BCI in the bears.

We also considered the potential effect of several two-way interactions on BCI, including age x day, age x study area, age x capture number, age x capture interval, day x study area, day x capture number, study area x capture number, and study area x capture interval. Interactions between biological and environmental factors are selective forces affecting life-history traits in mammals (Coulson et al., 2001; Martínez-Jauregui et al., 2009). Also, there is evidence that populations of the same species in different areas can be driven by different environmental drivers or be influenced by the same environmental driver in contrasting ways (Ginett and Young, 2000; Martinez-Jauregui et al., 2009). Furthermore, the interactions terms including capture-related variables allowed changes in BCI to differ as a function of the number of captures, as previously reported in brown bears (Cattet et al., 2008).

Statistical approach

We approached the statistical analyses in three sequential phases, data exploration, model development, and model validation using the software R 3.3.2 (R Core Team, 2016). For data exploration, we evaluated the raw data for (i) missing values, (ii) presence of outliers, (iii) collinearity among potential predictor (independent) variables, and (iv) relationships or associations between response (dependent) and predictor variables (Zuur and Ieno, 2016). Collinearity among predictor variables was evaluated using both variance inflation factors (VIF $\geq$ 3.0) and pairwise correlations (r $\geq$ 0.7). We standardized continuous predictor variables (covariates), by subtracting the mean from the individual observed values and then dividing by the standard deviation, prior to model development to facilitate comparisons among different models (Zuur et al., 2009).

For model development, we used generalized linear mixed models (GLMM) (Zuur et al., 2009) to evaluate the variation in BCI values in association with the various predictor variables. The unique identification for individual bears and the years in which they were captured were assigned as random effects, and included in all models. We used an Information Theoretic approach to compare among different model structures (Gaussian or Gamma distribution; identity, inverse, or log links), and to select an

appropriate model structure. A GLMM with a Gamma distribution and identity-link function was selected based on the Akaike's Information Criterion corrected for small sample sizes (AICc) (Burnham and Anderson, 2002). After the model structure was determined, we built multiple models for each predictor variable or group of variables (i.e., age, day, study area, and capture-related variables) in order to determine whether to use a linear or polynomial associations between BCI and age, and BCI and day, and to identify potentially significant interactions. We compared these models, and selected the most parsimonious (based on AICc) for further comparisons. These models were used as candidate models themselves, but they were also used to build candidate models by combining with other models, e.g., age model + day model = age + day model. Finally, we obtained 16 candidate models (including null and global models) (Table 2). The candidate models were compared using AICc and AIC weights and those with ΔAICc ≤ 2.00 were considered (Burnham and Anderson, 2002). Within the best model, we considered a term or interaction informative when its 95% confidence interval did not include the value 0.

For model validation, we plotted the standardized residuals of the best model against the fitted values, and all predictor variables to assess normality and identify violations of homogeneity. We present the mean ± standard deviation for all variables, unless otherwise stated.

Results

The mean, standard deviation, and range in body mass and body length for the study bears were 138.9 ± 61.89 kg (22-311) and 177.9 ± 22.96 cm (96-229), respectively. Mean BCI was 0.0 ± 1.00, and ranged from -3.08 (poor) to + 3.83 (excellent). Values by study area and age category are presented in Table 3.

The highest-ranked candidate model (M12) indicated that age, day, and study area were the main factors associated with BCI values for the study bears (Table 4). The fixed effects in our best model explained 46% in BCI variation among bears. Age had a positive curvilinear association with BCI (Table 5; Fig. 1). The mean BCI of bears increased with age until they reached 15.7 years old. From 15.7 to approximately 23 years old, the mean BCI was positive (≥ 0.00) and stable. After 23 years, the mean BCI

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trajectory declined precipitously, but our data set included only three bears that were >23 years. The association between ordinal day of capture and mean BCI was also positive and curvilinear, although the shape of the curve was different than that describing the association between age and BCI (Table 5; Fig. 2). The mean BCI increased as ordinal day of capture increased (i.e., bears captured later in the year). The increase was slow from den emergence in spring until the beginning of summer (approximate breaking point July 6th), and the mean BCI was ≤ 0.00 throughout this time. However, the mean BCI increased to positive values over summer, and increased markedly during fall (after September 21st) before the bears began hibernation. Our highest-ranked model (M12) also indicated that bears in Scandinavia were likely to be in better body condition than bears in Alberta (Table 5, Fig.3). The differences in body condition between study areas did not appear to be attributable to differences between projects in the annual timing of captures, the year of capture, or the age composition of captured animals, because when we controlled for these potential sources of variation, the differences in body condition between study areas persisted. Models that included capture-related variables (i.e., number of previous captures, capture interval, age x number of previous captures) were not supported ($\Delta AIC_C > 2$; Table 4).

Discussion

The results of our study showed that variation in the BCI values of independent male brown bears was primarily associated with the age of the bears, the day they were captured, and the area of study. However, we did not find any associations between capture-related variables and the bears' BCI values.

We assessed the independence of our body index from body size (Cattet et al., 2002), by plotting the bears' BCI against body length. We did not observe heterogeneity, which supported the BCI as a valid estimate of body condition. Therefore, we confirmed that the associations found between BCI and the predictor variables used were not artefactual.

In brown bears, age-specific growth curves have been described for males of different populations (Swenson et al., 2007; Zedrosser et al., 2007; Bartareau et al., 2011). These curves consistently show an increase in body weight and body length with age. Previous research on brown bears (Cattet et al., 2008),

and polar bears (Macbeth et al., 2012) supports our findings of a curvilinear relationship of age with body condition. Differences in physiological condition among animals within a population might be due to differences in age classes, i.e., certain classes outcompete others for limited resources. In American black bears, Schroeder (1987) concluded that differences in hematological patterns and the ratio of body weight/body length reflected the competitive ability of bears to successfully forage on limited food resources, and produced a ranking of condition within a sex and age class (i.e., highest to lowest: adult males, adult females, subadult males, subadult females). A biological explanation for our result is that juvenile animals that are poor at acquiring food do not survive. Consequently, animals that become older are animals that were successful at acquiring food and are, therefore, in better body condition. The drop off in BCI in bears > 23 years could reflect senescence, where animals of advanced age have reduced the ability to acquire food. Senescence could be defined as a biological deterioration in physiological functions which predicts that older individuals will show an age-specific increase in mortality and a decline in somatic and reproductive investment (Broussard et al., 2003). Thus, body condition would initially increase with age, reach a maximum at intermediate age, and declined at the oldest ages. In Weddell seals (*Leptonychotes wedellii*), Proffitt et al. (2007) attributed declines in body mass at the oldest age to senescence. In brown bears, evidence of senescence has been found in females, which show a relatively high reproductive performance until 25 years (Schwartz et al., 2003). However, we focused our study on male brown bears, for which studies have found no evidence of reproductive senescence (Zedrosser et al., 2007). Further, as we have already mentioned, our dataset included only three individuals > 23 years.

The brown bear is an omnivorous mammal that inhabits highly variable environments (Ferguson and McLoughlin, 2000; Munro et al., 2006), and has developed a life strategy to cope with seasonal food scarcity. Brown bears are active from spring to autumn and during this period they consume large amounts of high-energy food to accumulate fat for hibernation (Swenson et al., 2000). From spring to late summer, bears feed on roots, green vegetation, insects, and ungulate neonates. In late summer and autumn, bears enter a phase of high food consumption (hyperphagia) based on berries, fruits, and hard masts when available (Munro et al., 2006; Stenset et al., 2016). This period is essential to accumulate adipose tissue for

hibernation and reproduction (Hilderbrand et al., 1999; López-Alfaro et al., 2013). Bears enter the den in late autumn and exit after 3-7 months (Swenson et al., 2000). During hibernation they rely on the energy provided by the fat and lean reserves acquired during autumn (Farley and Robins, 1995; Robbins et al., 2012). Not surprisingly, we found an increase in the bears' BCI with the ordinal day of capture. Moreover, the rate of increase coincided with a temporal division that has been previously used in studies on brown bears, spring (from April to mid-July) and summer/fall (from mid-July to mid-October) (Moe et al., 2007; Heard et al., 2008). We documented a slow increase in the bears' BCI in spring until the beginning of summer coinciding with bears digging roots and preying on ungulates. Over summer and fall, when hyperphagia occurs, bears transition from feeding on graminoids, forbs, and protein sources (ants and ungulates) to eating berries and fruits, and their BCI increased markedly.

In this study, we used data collected from two independent brown bear populations that inhabit boreal forest ecosystems in Europe and North America. Both areas are similar in that they are characterized by a continental climate with cold winters and short, warm summers, and have similar values of average precipitation, snow cover and growing season (Natural Regions Committee, 2006; Zedrosser et al., 2006). In addition, both bear populations are interior and have similar diets with no access to spawning salmon (*Oncorhynchus* spp.) (Munro et al., 2006; Stenset et al., 2016). Some authors state that there is no reason to believe that the same species living in different areas will respond in the same way to climate, as the forms of regulation may differ among populations or populations may experience limiting factors at different times of the year (Martínez-Jauregui et al., 2009). In fact, our findings suggest a difference in body condition in brown bears due to study area, i.e., brown bears in Scandinavia were likely to be in better body condition than bears in Alberta. While their respective habitats and weather exposure may be similar, brown bear populations in Scandinavia and Alberta differ in a wide range of factors such as genetics (Taberlet and Bouvet 1994, Waits et al., 1998), temporal trends in population numbers and current population status (ASRD and ACA, 2010; Swenson et al., 2017), and human-pressure activities (Nielsen et al., 2006; Zedrosser et al., 2006). These factors likely also contribute to our findings. However, without the findings

from comparative studies, we cannot be certain of the specific factor or combination of factors that explain the study area difference in mean BCI values.

Our results indicated that capture, handling, and surgery of independent male brown bears did not influence the variation in their body condition estimated as a BCI. Our results agree with Rode et al. (2014), who concluded that repeated captures were not related to long-term negative effects on body condition in polar bears. Conversely, there are a few studies demonstrating a negative effect of capture and handling on body condition in mammals. Tuyttens et al. (2002) found that European badgers (*Meles meles*) that had been carrying a radio-collar for up to 100 days were more likely to have a low body condition score compared to control badgers that had never been fitted with a collar. In water voles (*Arvicola amphibius*), the attachment of radio-collars to females caused a male-skewed sex ratio of the offspring (Moorhouse and MacDonald, 2005). The authors attributed this finding to a deterioration in maternal condition in response to radio-collaring.

In brown bears, Cattet et al. (2008) reported long-term effects of capture and handling on behavior (i.e., reduction in movement rates) and body condition. They found that the age-specific body condition of bears captured twice or more tended to be poorer than that of bears captured only once. Moreover, they found that the negative effect of capture and handling was proportional to the number of times a bear had been captured, and this effect became more apparent with age. The fact that Cattet et al., (2008) identified significant capture effects, not only in the same species, the brown bear, but also within the same Alberta population of bears used in our study brings to question the apparent disparity in findings between this study and our study. This might be due to 1) calculating BCI based on different measurements of body length, and/or 2) studying different sex-reproductive classes. First, Cattet et al. (2008) calculated the bears' BCI values based on straight-line body length, which is measured as the straight-line distance, from the tip to the nose to the end of the last tail vertebra, using a measuring tape extended above the bear in sternal recumbency. This follows from the procedure recommended by Cattet et al. (2002) in their validation study of the BCI. In our study, body length was measured along the curvature of the dorsum with the bear in either sternal or lateral recumbency. In order to compare the two methods to measure body length, we used

294 records from the fRI Research Grizzly Bear Program in Alberta, Canada, where both straight-line and curvature body length were measured in the same bear (fRI, unpublished data). The regression of body weight against straight-line body length showed a lower coefficient of variation than the regression against curvature body length. Thus, precision would be lower when measuring the curvature of the dorsum, likely because of the fur's interference. Although, straight-line body length seems to be a slightly more precise method to measure body length, poor repeatability is found with both methods. The BCI method has been validated for ursids, and has been demonstrated to reflect true body condition (Cattet et al., 2002). However, we should take into consideration that body length measurements have poor repeatability (i.e., inter- and intra-individual errors in the measurement of body length), and/or that the presence/absence of food in the digestive tract may lead to wrong estimates of body mass, and thus, body condition (Cattet et al., 1997). Moreover, some authors state that the best body condition may vary across species, populations, and even across sexes (Labocha et al., 2014). Second, we focused on a single group of animals in the population, the independent males, whereas Cattet et al., (2008) and Nielsen et al. (2013) included both sexes, and several reproductive classes (i.e., male, female, female with dependent offspring). Both studies concluded that BCI values varied as a function of sex and reproductive class. Nielsen et al. (2013) found that adult females were more likely to have a lower BCI than subadult or adult male bears, and this association was more pronounced with the presence of dependent young. Also, in polar bears, Macbeth et al., (2012) recorded the lowest BCI values in females with dependent cubs in comparison with other sex-reproductive classes. However, in the studies of brown bears, potential interactions between sex-reproductive class and capture variables were not evaluated. Nevertheless, given the different energetic demands among sex-reproductive classes, it is possible that the capture effects identified in these studies were not the same for all groups. In polar bears, Ramsay and Stirling (1986) only found a detectable negative effect of capture and handling on the weight of females with cubs, and suggested that the additional energetics costs of capture to a pregnant female might reduce their weight, and could potentially reduce the weight and size of her offspring. Thus, the cumulative cost of reproduction and provisioning offspring (i.e., lactation and maternal care) in female bears might result in the energetic response to capture and handling having a measurable effect on their

body condition. In contrast, the energetic response to capture and handling may have a negligible effect on the body condition of males, as was found in our study, because they are not additionally burdened by the energetic demands of pregnancy and lactation.

In summary, we found that the body condition of independent male brown bears, as estimated by the BCI, did not appear to be influenced by capture, handling, and surgery. However, we did find the BCI to be positively associated with age of bear and ordinal day of capture, as has been reported in previous studies of brown bears. We also found a weak difference in mean BCI values between study areas, with bears captured in Scandinavia tending to be in better condition than bears captured in Alberta. More studies like this are needed to evaluate if capture and handling procedures are inadvertently biasing research results. Although we did not identify any capture-induced biases in this study of body condition in independent male brown bears, future studies should be conducted to determine if the same holds true for other sex, age, and reproductive classes.

Acknowledgements

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Table 1. Explanatory variables used to predict body condition in free-ranging independent male brown

bears captured either in Scandinavia (N=371) or Alberta, Canada (N=180) between 1988 and 2015.

Variable	Variable type	Variable description range
1) Fixed effects		
Age	continuous	age in years + ordinal date of capture/365 – 1.7-29.3
Day	continuous	ordinal day of capture with January 1 set as 1 – day 81 to day 292
Study area	categorical	Scandinavia or Alberta, Canada
Capture method	categorical	method used in the previous capture = not applicable (i.e., first-time-captured bears; $n = 203$), bears captured by culvert trap ($n = 15$), snare ($n = 22$) or from helicopter ($n = 311$)
Capture number	continuous	number of times a bear had been previously captured (0-12 times)
Capture interval	continuous	days between the previous and current capture events, 0 for bears captured once – 0-4033
Previous surgery	categorical	whether a bear had experienced or not a previous abdominal surgery – no ($n = 469$) or yes ($n = 82$)
2) Random effects		
Bear ID	categorical	302 individual bears
Year of capture	categorical	bears captured from 1988 to 2015

Table 2. Selected candidate models (based on Akaike's Information Criterion corrected for small sample sizes) and explanatory variables[a] used to predict drivers of body condition in free-ranging independent male brown bears captured either in Scandinavia (N=371) or Alberta, Canada (N=180) between 1988 and 2015.

Model	Age	Day	Study area	Capture and handling	Interactions
1) Intercept (Null)					
2) Age	$Age + Age^2 + Age^3$				
3) Day		Day^3			
4) Study area			Area		
5) Capture				Capture number + Capture interval	
6) Age + Day	$Age + Age^2 + Age^3$	Day^3			
7) Age + Study area	$Age + Age^2 + Age^3$		Area		
8) Age + Capture	$Age + Age^2 + Age3$			Capture number + Capture interval	Age x Capture number
9) Day + Study area		Day^3	Area		Day^3 x Area
10) Day + Capture		Day^3		Capture number + Capture interval	
11) Study area + Capture			Area	Capture number + Capture interval	
12) Age + Day + Study area	$Age + Age^2 + Age^3$	Day^3	Area		
13) Age + Day + Capture	$Age + Age^2 + Age^3$	Day^3		Capture number + Capture interval	Age x Capture number
14) Age + Study area + Capture	$Age + Age^2 + Age^3$		Area	Capture number + Capture interval	Age x Capture number
15) Day + Study area + Capture		Day^3	Area	Capture number + Capture interval	
16) Age + Day + Study area + Capture (Global)	$Age + Age^2 + Age^3$	Day^3	Area	Capture number + Capture interval	Age x Capture number

[a] Variables include: Age (age adjusted calculated as age in years + ordinal date of capture/365), Day (ordinal day of capture), Area (study area: Scandinavia, Alberta), Capture number (number of times a bear was previously captured), and Capture interval (days between the previous and current capture events).

Table 3. Mean value, standard deviation and range in body mass (BM, in kg), body length (BL, in cm), and body condition index (BCI) by study area and age category in free-ranging independent male brown bears captured either in Scandinavia (N=371) or Alberta, Canada (N=180) between 1988 and 2015. Bears < 5 years old were considered juveniles, whereas bears ≥ 5 years old were adults. N denotes sample size.

	All bears		Juveniles		Adults	
	Scandinavia, N=371	Alberta, N=180	Scandinavia, N=152	Alberta, N=71	Scandinavia, N=219	Alberta, N=109
BM	132.9 ± 59.66 (22-290)	151.3 ± 64.65 (38-311)	76.3 ± 29.66 (22-147)	99.9 ± 35.59 (38-209)	172.2 ± 40.61 (78-290)	184.8 ± 57.71 (70-311)
BL	175.4 ± 23.29 (96-225)	183.0 ± 21.46 (122-229)	156.2 ± 20.60 (96-207)	164.7 ± 16.34 (122-222)	188.8 ± 13.76 (148-225)	194.9 ± 15.04 (144-229)
BCI	-0.005 ± 0.99 (-3.08-3.5)	0.012 ± 1.03 (-2.54-3.83)	-0.477 ± 0.67 (-2.53-1.46)	-0.171 ± 0.72 (-2.06-2.07)	0.323 ± 1.04 (-3.08-3.5)	0.131 ± 1.74 (-2.54-3.83)

Table 4. Comparison of the ten most supported candidate models (based on Akaike's Information Criterion corrected for small sample sizes[a]) to predict drivers of BCI in free-ranging independent male brown bears captured either in Scandinavia (N=371) or Alberta, Canada (N=180) between 1988 and 2015. The null model (intercept-only) is also shown for comparison. All models include an intercept and random effect[b]. Bold typeface denotes models with $\Delta AIC_C \leq 2.00$.

Model	k	AIC_c	ΔAIC_c	w_i	R^2_{LR}
12) Age + Day + Study area	**9**	**1243.72**	**0**	**0.67**	**0.463**
6) Age + Day	8	1246.66	2.94	0.15	0.458
16) Day + Study area + Capture interval + (Age*Capture number) (Global)	12	1247.39	3.67	0.11	0.466
13) Day + Capture interval + (Age*Capture number)	11	1248.43	4.71	0.06	0.463
7) Age + Study area	8	1256.37	12.65	0	0.449
2) Age	7	1256.79	13.07	0	0.446
8) Capture interval + (Age*Capture number)	10	1258.99	15.27	0	0.45
14) Study area + Capture interval (Age*Capture number)	11	1259.83	16.11	0	0.451
10) Day + Capture number + Capture interval	7	1282.18	38.46	0	0.42
15) Day + Study area + Capture number + Capture interval	8	1282.93	39.22	0	0.421
1) Intercept (Null)	4	1319.15	75.43	0	0.373

[a] Statistics include number of estimable parameters in model (K), sample-size–adjusted Akaike information criterion (AIC_C), difference in AIC_C between top model and model i (ΔAIC_C), Akaike weight for model i (w_i), and a coefficient of determination based on the likelihood-ratio test (R^2_{LR}).

[b] Bear ID and year of capture were included as random effects (intercept) in all models.

Table 5. Regression coefficients (β), standard deviation (SE), and confidence intervals (LCI = lower limit of the 95% confidence interval, UCI = upper limit of the 95% confidence interval) of the predictor variables[a] in the most supported model (Age + Day + Study area) explaining variation in body condition index in free-ranging male brown bears captured either in Scandinavia (N=371) or Alberta, Canada (N=180) between 1988 and 2015.

Predictor variables	β	SE	LCI	UCI
Age	0.427	0.063	0.304	0.549
Age^2	0.144	0.056	0.035	0.253
Age^3	-0.057	0.013	-0.083	-0.032
Day^3	0.017	0.005	0.008	0.026
AreaScandinavia	0.316	0.141	0.040	0.592

[a] Variables include: Age (age adjusted calculated as age in years + ordinal date of capture/365), Day (ordinal day of capture), and Area (study area: Scandinavia, Alberta).

Figure 1. Body condition index by adjusted age in free-ranging independent male brown bears captured either in Scandinavia (N=371) or Alberta, Canada (N=180) between 1988 and 2015.
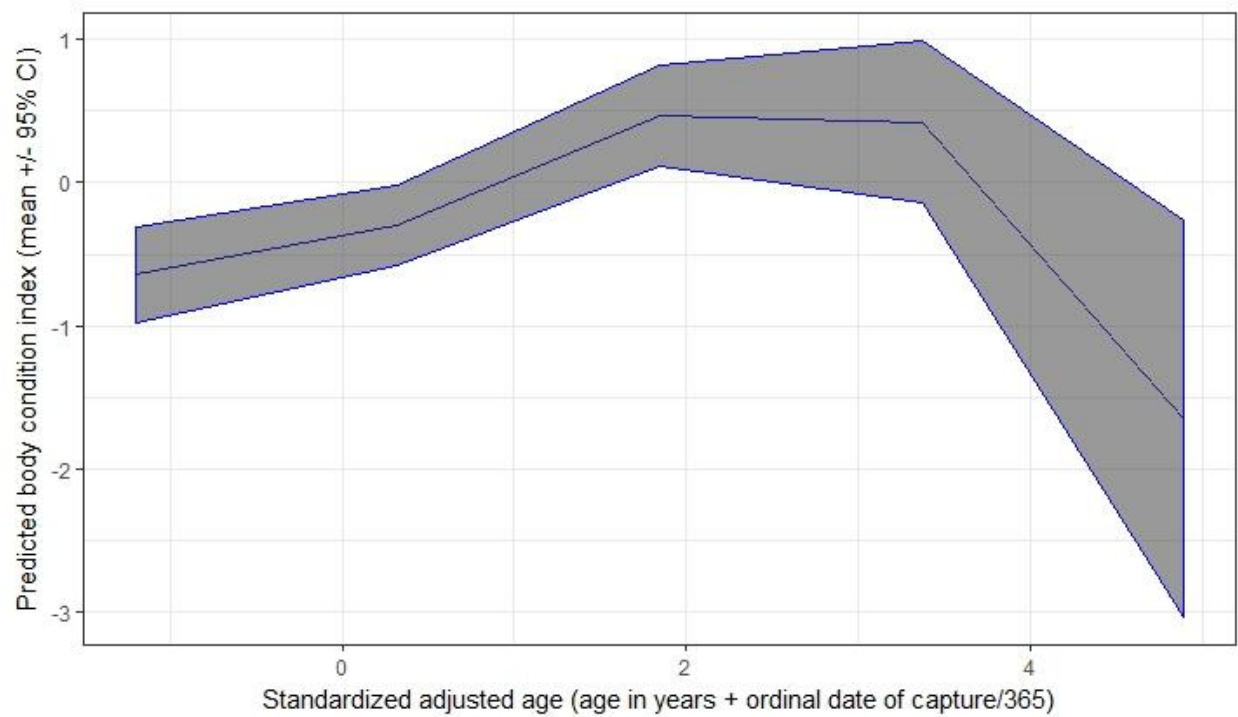
Figure 2. Body condition index by ordinal day of capture in free-ranging independent male brown bears captured either in Scandinavia (N=371) or Alberta, Canada (N=180) between 1988 and 2015.
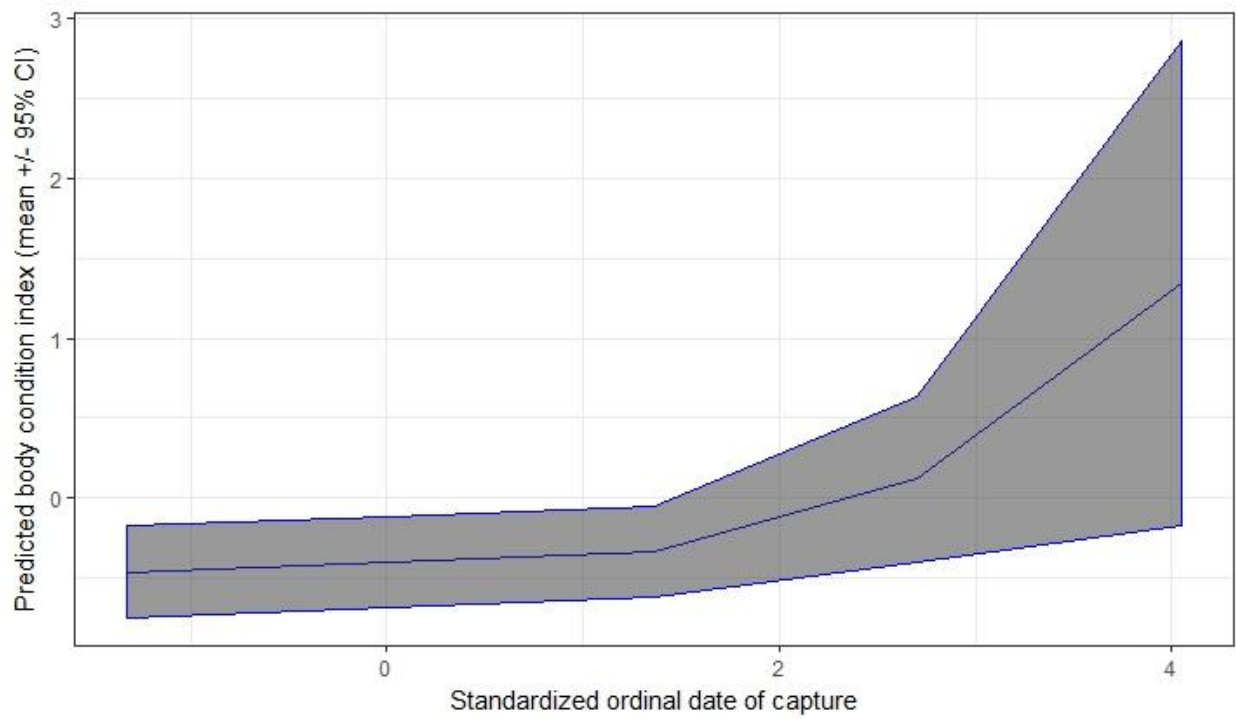
Figure 3. Body condition index by study area in free-ranging independent male brown bears captured either in Scandinavia (N=371) or Alberta, Canada (N=180) between 1988 and 2015.