DEVELOPMENT AND APPLICATION OF A HEALTH FUNCTION SCORE SYSTEM FOR GRIZZLY BEARS (*URSUS ARCTOS*) IN WESTERN ALBERTA

A Thesis Submitted to the College of
Graduate Studies and Research
In Partial Fulfillment of the Requirements
For the Degree of Master of Science
In the Department of Veterinary Pathology
University of Saskatchewan
Saskatoon

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ABSTRACT

The persistence of grizzly bears (*Ursus arctos*) in western Alberta is threatened by increasing human activities on the landscape. The Foothills Research Institute Grizzly Bear Program (FRIGBP) hypothesizes human-caused landscape change in Alberta causes long-term stress in individual bears, resulting in impaired biological functions and, when many bears are affected, decreased population performance. To facilitate the evaluation of individual grizzly bear health within the FRIGBP, the objective of my research was to develop and assess the usefulness of a health function score system for grizzly bears. From a large set of complex biological data collected from grizzly bears from 1999 to 2007, I merged 14 " constituent" variables into four health functions; growth, immunity, movement, and stress. For each health function, I calculated individual scores by adding ranked and weighted variable percentiles. I found that health function scores corresponded well with health status of individual bears based on values for multiple constituent variables. The score system facilitated quick screening of health in individual bears, identification of bears with reduced health, and comparison of health profiles between bears. I examined the usefulness of the score system by evaluating relationships presumed to exist under the working hypothesis of the FRIGBP. Results generated from health function scores were compared with those from constituent variable values using statistical and graphical techniques. I concluded that scores likely provided clearer depiction of wildlife health relationships than did constituent variables because they were not influenced by capture method, sex, or outlying observations. By using the score system, I found support for the proposed positive relationship between human-affected landscape condition and stress, but not for inverse relationships between stress and other health functions. The usefulness of the score system could be increased by minimizing use of redundant constituent variables, e.g., in growth and immunity, and removing the influence of potential confounding factors, e.g., capture.

ACKNOWLEDGEMENTS

I would like to thank my supervisor Marc Cattet for introducing me to the exciting area of wildlife health, and for his guidance, support, and patience throughout this study. I thank my advisory committee, David Janz, Gord Stenhouse, and Gary Wobeser, and the graduate chairs Andy Allen and Marion Jackson from the Department of Veterinary Pathology, for their support and advice during my project. I am grateful to the Foothills Research Institute Grizzly Bear Program (FRIGBP) for giving me the opportunity to pursue this study and experience the remaining wilderness and changing landscape of Alberta. I thank Rick Booker, Marc Cattet, Erin Geymonat, Bernie Goski, Dave Hobson, Jay Honeyman, Terry Larsen, Saundi Norris, John Saunders, Codi Schmidt, and Gord Stenhouse for their assistance, guidance, and companionship during field work. I thank Jason Hamilton, Edna Leung, Zsuzsanna Papp, Jennifer Town, Brenda Trask, and Mathilakath Vijayan for analyzing samples, and Jerome Cranston, Julie Duval, and Karen Graham for help with movement data. I am grateful to Jennifer Cowell and Ian Shirley for help with presentation of research results, and Jan Diedrichs, Nadine Kozakevich, Sandy Mayes, and Betty Werbicki for administrative support. My appreciation is also extended to faculty, Prairie Diagnostics Service staff, and fellow graduate students who always were available for consultation and advice.

I also thank my life partner Aleksija, my brother Markus and sister-in-law Sara, and my parents for their unwavering support and love throughout my program. Friends in Sweden, Saskatoon, and Grand Manan – thank you for your encouragement and friendship.

Funding for this project was provided by Alberta Innovation and Science, Natural Sciences and Engineering Research Council, collaborative partners of FRIGBP, and Western College of Veterinary Medicine Interprovincial Graduate Fellowships. Financial support for the study in appendix B was also provided by Animal Welfare Foundation of Canada.

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LIST OF ABBREVIATIONS

ANCOVA	analysis of covariance
BCI	body condition index
CBG	cortisol-binding globulin
CMF	free range capture
CMT	trap capture
COSEWIC	Committee on the Status of Endangered Wildlife in Canada
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
ESCC	Endangered Species Conservation Committee
FRIGBP	Foothills Research Institute Grizzly Bear Program
GIS	Geographic Information System
GPS	Global Positioning System
HFS	health function score
hsp	heat shock protein
IUCN	International Union for Conservation of Nature and Natural Resources
PPHR	percent protected home range
SLBL	straight-line body length
TBM	total body mass

CHAPTER 1

INTRODUCTION

1.1 Wildlife Health

1.1.1 The Concept of Health

The concept of health can be complex and confusing. In health-related research, a clear explanation of what constitutes health, what level of health is being addressed (individual or population level), and how health is being assessed often is lacking. The health of an animal can be defined as "the state of an organism's existence that is characterized by unimpaired biological functioning, complete physical and physiological adjustment to its surroundings, and uncompromised well-being" (Hurnik et al. 1995). At the individual level, health is assessed by detecting disease and injury through clinical and pathological examination and tissue sample analyses (Pasquini and Pasquini 1999, McGavin et al. 2001), observing behaviour (Jensen 2002), and measuring functioning and productivity in a solitary animal (Blood and Radostits 1989).

Other definitions of animal health also include the state, i.e., the overall health, of "groups of animals" or "populations" (Department for Environment, Food and Rural Affairs 2004, National Animal Health Strategy 2007). There are different approaches to population health assessment. The current, and future, population health status can be derived by collating health information from individual animals, for example, from clinical and pathological examination and tissue sample analyses (Munson and Karesh 2002). Detection of mastitis in a cow (*Bos taurus*), pleuropneumonia in a pig (*Sus scrofa*) (Blood and Radostits 1989), tuberculosis in a captive wood bison (*Bison bison athabascae*) (Lutz-Wallace et al. 2006), lead poisoning of

individual waterfowl (Locke and Thomas 1996), and capture myopathy in a grizzly bear (*Ursus arctos*) (Cattet et al. 2008b) are examples of how assessment of individual animals can reveal potential health problems at the population level. Based on the knowledge of individual animal health, epidemiological studies can be conducted to evaluate the health status and the cause and distribution of disease in a population (Thrusfield 2005, Smith 2006). Another approach is based on association between a population's health and its performance. For example, domestic animal production results, such as milk, egg, and meat yield (Radostits et al. 1994, Thrusfield 2005), and wildlife reproductive and survival rates, abundance, and population composition (Wobeser 2006), provide indirect measurements of health at the population level.

1.1.2 What is Wildlife Health?

The term "wildlife health" often is used or referred to in many research areas that relate to animal and human health, conservation, and ecology (Aguirre et al. 2002, Friend 2006). The term, however, often is not clearly defined in the scientific literature or by educational organizations, governmental and non-governmental agencies, professional associations, or research institutions. Most commonly, "wildlife health" refers to disease status in wild animals and populations. According to Munson and Karesh (2002), disease is "a disorder of body functions, systems, or organs", whereas Wobeser (1997) defined disease as "any impairment that interferes with or modifies the performance of normal functions, including responses to environmental factors such as nutrition, toxicants, and climate; infectious agents; inherent or congenital defects; or combinations of these factors".

By using disease status to represent wildlife health, Deem et al. (2001) and Munson and Karesh (2002) considered the need to incorporate wildlife health into successful conservation, Kirkwood (1993) discussed wildlife health in the context of wildlife rehabilitation, and Leighton

(2007) emphasized that wildlife health is an essential part of wildlife management, conservation, and, more recently, of the world-wide emerging diseases and associated public health issues. Balch and Sang (2005) connected wildlife health with health of the arctic ecosystem and public health, whereas Kock (1996) gave several examples of how wildlife health is integrated into areas such as animal welfare, conservation and sustainable wildlife use, game farming, and investigating the interface between domestic and wild animals in South Africa.

Several researchers equate wildlife health assessment with disease monitoring. Karesh et al. (1999) evaluated health in rockhopper penguins (*Eudyptes chrysocomes*) by collecting base-line data on hematology, serum chemistry, metal, mineral, and toxic chemical levels, and serologic evidence of exposure to infectious agents. Fiorello et al. (2007) tested wild Bolivian carnivores for antibodies to common pathogens of domestic carnivores, and Merianos (2007) advocated intensified monitoring of zoonotic diseases in wildlife to increase the understanding of the role of wildlife health in emerging diseases. Sainsbury et al. (2001) promoted increased monitoring of wildlife health in a coordinated national scheme, through clinical and pathological examination and tissue analyses for infectious disease agents and toxic contaminants, to help understand the population dynamics of endangered species and to detect human-induced animal welfare problems.

Health and disease are relative terms representing opposite ends of a continuum – as health increases, signs of disease diminish, and vice versa. A useful way to conceptualize an animal's health is in terms of energy (Wobeser 2006). An animal's acquired and stored energy is allocated to its biological functions, e.g., maintenance, activity, thermoregulation, growth, reproduction, and defense. Any change in the acquired energy amount alters the quantity of energy available for maintaining these functions. Moreover, increased energy use for one biological function leads to less energy available for another (McNamara and Buchanan 2005,

Wobeser 2006). The allocation of available energy among different biological functions is regulated in an "energy trade-off model" (Wobeser 2006). By analyzing the flow of energy, it is possible to determine where an animal sits on the health-disease continuum (Stevenson 2006). For example, Soler et al. (2002) showed that growth is negatively affected in magpies (*Pica pica*) when an immune response is built up, i.e., energy from growth is re-allocated to immunity.

Every disease-causing agent or factor comprises an energy cost to the animal. Wobeser (2006) described sarcoptic mange in coyotes (*Canis latrans*), where decreased food intake because of intensive itching, coupled with increased energy expenditure from scratching, production of an inflammatory response to the mite (*Sarcoptes scabiei*), and thermoregulation because of hair loss, result in less energy available for growth and reproduction.

The energy trade-off model is not only affected by disease-causing agents and factors. It is also influenced by environmental factors, such as habitat conditions, anthropogenic environmental change, weather, population densities, and presence of predators (McEwen and Wingfield 2003, Stevenson 2006). Wobeser (2006) provided an example of how environmental factors, a disease-causing agent, and wild animals are connected with regards to energy availability and trade-off. He described the interaction between weather, food abundance, population density, parasite numbers, and disease in grazing animals, and the consequences for growth, reproduction, and immunity. Creel et al. (2007) suggested that the effect of increased anti-predator behaviour on habitat selection, foraging pattern, and sensitivity to environmental conditions in female elk (*Cervus canadensis*) in response to increased wolf (*Canis lupus*) population size, is costing them energy that could have been devoted to reproduction. Furthermore, Derocher and Stirling (1998) proposed that polar bears (*Ursus maritimus*) in colder climate, and with larger home ranges, use a greater proportion of their energy for thermoregulation and movement, leaving less energy for growth.

The availability of energy enables a prioritization of the most essential biological functions under certain conditions. The prioritization may come, however, at the expense of one or several other functions. Hence, responding to disease-causing agents or environmental components, or both, to avoid disease (e.g., injury, starvation, and hypothermia) and predation may result in other manifestations of decreased biological functions (e.g., decreased growth, reproduction, and activity), thereby demoting an animal's position on the health-disease continuum (McEwen and Wingfield 2003, Wobeser 2006).

The energy availability and trade-off model play an important role in wildlife health within the context of conservation. McEwen and Wingfield (2003) and Wingfield (2005) suggested that human activities in the environment can lead either to increased energy requirements that are beyond the capacity of a wild animal to replace from environmental resources or to an imbalance due to decreased energy intake. For example, animals may avoid habitat with good food resources, provide less food and care for the offspring, change their interspecific behaviour and migration pattern, and become more exposed to predation and disease-causing agents and factors (McEwen and Wingfield 2003, Walker et al. 2005). Energy deficits and imbalances may lead to decreased biological functions (Wobeser 2006). When biological functions are negatively affected in several animals, it can adversely affect the population's viability and persistence (Stevenson and Woods 2006, Wikelski and Cooke 2006).

There is concern that wildlife populations, at both a local and global level, are failing to maintain their viability or fulfill their ecological role in the face of current levels of anthropogenic environmental change, e.g., resource extraction, urban development, climate change, pollution, and recreation (Stevenson et al. 2005, Walker et al. 2005). For the purposes of my thesis, I have chosen to define wildlife health at the individual level as "the capacity of a wild animal to maintain biological functioning when challenged by environmental change" and at the

population level as “the capacity of a wildlife population to adapt to or respond to environmental challenges and changes” (Cattet, personal communication 2006).

1.1.3 The Role of Stress in Wildlife Health

What underlying mechanism explains how the perception of environmental changes by an animal affects its biological functions, and subsequently, the animal population? Along with regular, predictable life routines, such as obtaining food and water, breeding, interacting socially, migrating, and hibernating, wild animals are exposed to unpredictable, perturbating challenges, such as adverse weather events, natural disasters, predation, disease-causing agents and factors, and anthropogenic environmental change (McEwen and Wingfield 2003, Reeder and Kramer 2005). Animals maintain stability of essential physiological systems (homeostasis) through predictable and unpredictable change by acquiring and re-allocating energy through adjustment of physiology and behaviour. This is termed allostasis (McEwen and Wingfield 2003). Adjustments caused by unpredictable changes, however, may require extra energy (section 1.1.2). If the energy demand is higher than the animal’s energy stores and energy uptake from the environment, allostatic overload, i.e. a state in which an animal can no longer cope with external demands, occurs. The animal responds with increased glucocorticoid secretion, which, along with other hormones of the hypothalamic-pituitary-adrenal stress response, facilitates behavioural and physiological changes to allow the animal to escape or tolerate the disturbance, or stressor. Initially, the hormonal activity results in increased energy metabolism (gluconeogenesis), promotion of escape, enhanced immune functions, and, in some cases, increased foraging. Other non-essential activities, such as reproductive behaviour and territorial defense, are suppressed temporarily. These alternate physiological and behavioural paths divert available energy and reduce the allostatic overload. Following this, levels of circulating corticosteroids decrease

(Wingfield 2005). If the animal perceives long-term, continuous stress or repeated exposure to one or several stressors, the activation of the adrenocorticoid stress response may be prolonged, and circulating glucocorticoid levels remain high (Moberg 2000, McEwen and Wingfield 2003).

A long-term stress response can have deleterious effects on an animal. For example, the animal may run into energy debt if continued mobilization of energy stores is paralleled with inhibited feeding capacity or decreased access to food sources. If the animal is below its peak physical fitness, e.g., during pregnancy, lactation, with aging, or with high parasite load, marked negative energy balance can be fatal (Wobeser 2006, Hamilton 2007). Further, several biological functions may be negatively affected by long-term increases in glucocorticoid levels, including suppression of the immune (Goodman 1998, Maule and VanderKooi 1999) and reproductive systems (Pottinger 1999), muscle wasting (Wingfield 2005), impaired growth (Sjaastad et al. 2003), and malfunction of neuronal cells (McEwen and Wingfield 2003). These effects also can be due to diversion of energy rather than direct pathological influence of chronically elevated glucocorticoid levels (Moberg 2000, Wobeser 2006).

Anthropogenic changes of the environment contribute significantly to the allostatic overload in a wild animal (Wingfield 2005). If extensive or prolonged, change can cause a long-term stress response with subsequent impairment of biological functions. When one or several biological functions are affected in many individuals, negative consequences may manifest at the population level, e.g., reduced reproductive and survival rates and decline in abundance (Walker et al. 2005, Wikelski and Cooke 2006). Long-term physiological stress is believed to be an underlying mechanism linking human-caused environmental change (e.g., resource extraction, urban development, recreation, pollution, and climate change) with wildlife health (Cattet et al. 2006) (Figure 1.1).

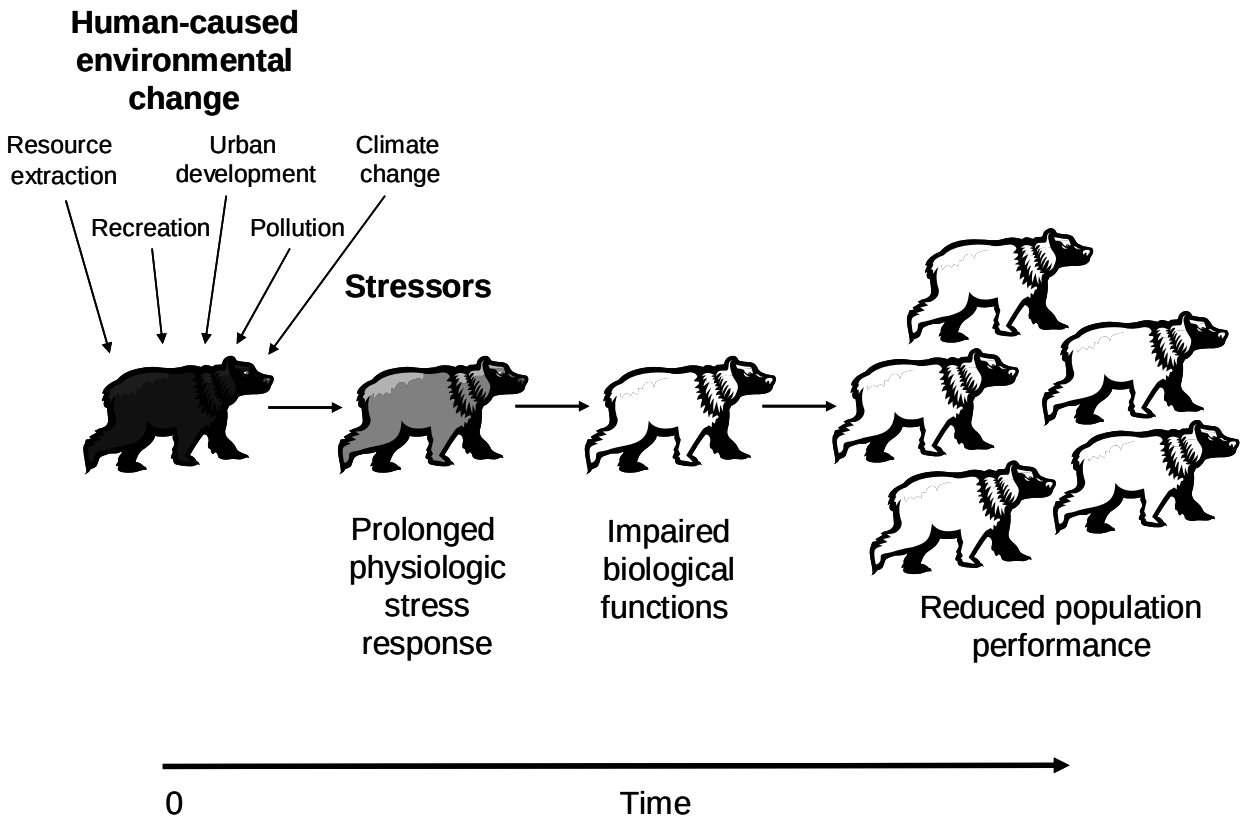

Figure 1.1. The proposed relationship between human-caused environmental change, long-term physiological stress, and wildlife health. Environmental change caused by human activities can cause long-term physiologic stress in wild animals, which eventually results in impaired biological functions, i.e., decreased health, at the individual level, and poor performance at the population level.

1.1.4 Evaluation of Wildlife Health

Historically, demographic parameters, such as reproductive and survival rates and population composition, density, and size, have been used to measure wildlife health indirectly (Garshelis et al. 2005, Stevenson and Woods 2006). Although assessment of demographic variables provides quantitative information, it provides limited knowledge of underlying mechanisms driving wildlife population health (Stevenson 2006, Wikelski and Cooke 2006). In addition, populations are impacted by many factors that can change population demography.

Seasonal cycles (e.g., breeding, food availability, and climate), migration, predation, presence of disease-causing agents and factors, and density-dependent compensation are examples of factors that have to be considered when demographic variables are evaluated (Stevenson and Woods 2006, Wobeser 2006). Moreover, demographic analyses typically provide point in time estimates, but no measures of trend, which prevents adequate assessment of population dynamics (Garshelis et al. 2005). Long-term collection of data is often required for accurate estimation of demographic variables, especially in species with a long generation time and large range (Ross 2002, McLoughlin et al. 2003). Overall, a population demography approach may be too slow and insensitive to provide early warning of potential impact of environmental changes on wildlife populations (Reaser et al. 2002, Nielsen et al. 2006).

In contrast, evaluation of biological functions (e.g., growth, immunity, and stress), through measures of physiological and physical variables of individual animals, enable a mechanistic understanding of how wild animals are affected by human environmental change. This provides not only a quantitative, but also a qualitative, assessment of wildlife health (Wikelski and Cooke 2006). Physiological and physical qualities measured to assess wildlife health include hematology and serum biochemistry values, levels of vitamins and minerals (Deem et al. 2001), sex, growth, and glucocorticoid hormones (Wikelski and Cooke 2006), and bodymass, length, and body condition (Cattet et al. 2002). Occurrence of infectious agents (e.g., bacteria, viruses, and parasites) and non-infectious factors (e.g., toxins, chemicals, physical agents, degenerative changes, and nutritional deficiencies) can also be determined (Munson and Karesh 2002, Wobeser 2006). This information is crucial for establishment of comparative health baseline data (Deem et al. 2001, Wikelski and Cooke 2006), and is obtained through evaluation of blood, fecal, and tissue samples (Dunbar et al. 1999) and weight and morphometric measures (Windberg et al. 1991) collected from live-captured or dead animals. Given that the information

consists of physiological and physical data obtained at a specific point in time, evaluation of traditional baseline information exclusively may not fully address a wild animal's ability to cope with long-term environmental challenges and changes. Moreover, effects of capture and handling, such as acute stress, dehydration (Cattet et al. 2003b), physical injuries, changed movement rates, and behaviour (Wikelski and Cooke 2006, Cattet et al. 2008a), may confound interpretation of collected health data.

More recent methods to assess wildlife health reflect long-term physiological conditions with greater accuracy, either because of long-term characteristics of the measured physiological variable, or use of less invasive sampling techniques. These methods include measures of cortisol-binding globulin (Reeder and Kramer 2005, Hamilton 2007) and heat shock proteins in serum (Bierkens 2000, Hamilton 2007), sex hormone and corticosteroid levels in feces (Wasser et al. 1996, Wasser et al. 2000), corticosteroid levels in saliva, urine (Hernández-Jáuregui et al. 2005), hair (Davenport et al. 2006), and feathers (Bortolotti et al. 2008), and stress proteins in skin and muscle (Haab et al. 2001, Cattet et al. 2006).

To improve the ability to measure and understand wildlife health, the two described approaches to wildlife health assessment, i.e., individual measures of health (physiological and physical data) and population performance (demographic data) can be combined (Wikelski and Cooke 2006). Individual health properties may not only explain population performance, but also be used to predict it in models. For example, through testing of competing hypotheses, Peery et al. (2004) demonstrated an association between low food availability because of climate change and overfishing, low levels of plasma vitellogenin and calcium, impaired reproduction in marble murrelets (*Brachyramphus marmoratus*), and subsequent decrease in population numbers. Other researchers have investigated relationships between human activities, stress response in individual animals, and population performance. For example, Wikelski et al. (2001) found that

increased serum corticosterone predicted mass mortality in a marine iguana (*Amblyrhyncus cristatus*) population affected by an oil spill, and Müllner et al. (2004) showed that serum corticosterone levels was negatively correlated with survival in juvenile hoatzins (*Opisthocomus hoazin*) when the population was exposed to ecotourism.

Knowledge of the relationship between animals and their habitat is essential for understanding the consequences of anthropogenic environmental change (Wikelski and Cooke 2006). Hence, to further enhance the assessment of wildlife health, it can be placed in a spatial and temporal context (Clark et al. 2001, Stenhouse and Graham 2005). Geospatial analyses and radiotelemetry observations provide information about landscape structure and change, and animal activity and range, respectively. Linking long-term stress response and other biological functions with landscape and activity characteristics enables early recognition of reactions in wildlife to environmental changes and challenges. This is important, as it permits development of models to forecast the effects of environmental change on wildlife populations before its occurrence (Walker et al. 2005, Wikelski and Cooke 2006). With this knowledge, wildlife managers, conservationists, governments, and industry can implement immediate and longer term measures to prevent or mitigate negative consequences on wildlife populations (Clark et al. 2001, Wikelski and Cooke 2006).

1.2 Foothills Research Institute Grizzly Bear Program (FRIGBP)

1.2.1 Background – Grizzly Bear Status and Conservation

Historically, the grizzly bear was found from the Pacific Ocean to the Mississippi River and from central Mexico to the Arctic Ocean. Extensive agricultural land conversion, high-density human settlement, and unrestricted hunting, however, resulted in extirpation or considerable decline of grizzly bear populations throughout their historical distribution. In the

United States, the grizzly bear has disappeared from 99 % of its former range south of the Canadian border. Today, the species can be found only in Alaska and unsettled areas in northwestern USA (Kansas 2002). Within Canada, grizzly bears currently exist in the Yukon, the Northwest Territories, Nunavut, British Columbia, and in mountainous areas, slopes, and low-land boreal forests in western Alberta. The species is extirpated from the prairies of Saskatchewan, Manitoba, and Alberta. It also has disappeared from the boreal regions of Saskatchewan, Manitoba, and northern/eastern Alberta (Ross 2002) (Figure 1.2). It is uncertain how many grizzly bears exist throughout Canada in the 21st century. The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) reported that there are between 26,900 and 29,150 grizzly bears in Canada, based on figures compiled in 2001/2002 from provincial and territorial jurisdictions (Ross 2002). British Columbia has the largest provincial population, approximately 17,000 bears, according to Hamilton et al. (2004). Information from 2001/2002 suggests that there are 6000 to 7000 bears in the Yukon and probably between 800 and 2000 bears in Nunavut (Ross 2002). The population estimate for the Northwest Territories is 3500-4000 grizzly bears (Northwest Territories Environment and Natural Resources Wildlife Division 2008), whereas Alberta Sustainable Resource Development Fish and Wildlife Division (2007) estimates 500 to 1000 grizzly bears exist in Alberta.

Grizzly bear populations are considered stable throughout various parts of their Canadian range, i.e., in parts of British Columbia and the northern territories (Ross 2002, McLoughlin et al. 2003). The species, however, is highly susceptible to anthropogenic activities on the landscape (Weaver et al. 1996, Johnson et al. 2005), and there is increased concern that some life history traits of grizzly bears, such as low reproductive potential and low dispersion, restrict the resilience of populations threatened by human disturbance (Kansas 2002, McLoughlin et al. 2003). Low reproductive rates (i.e., late maturity age, small litter sizes, and long interbirth

intervals) result in low rates of increase or recovery for the species (McLoughlin et al. 2003, Munro et al. 2006). Grizzly bears, especially subadult females, have low dispersal capabilities, which may reduce the ability to re-colonize areas where breeding populations have diminished (Weaver et al. 1996, Kansas 2002). Fragmenting of undisturbed habitats and increased human access to remote areas may decrease the viability of affected populations (Kansas 2002, Johnson et al. 2005). For these reasons, all grizzly bear populations in Canada have been listed under "special concern" by COSEWIC since 1991 (McLoughlin et al. 2003, Committee on the Status of Endangered Wildlife in Canada 2007).

Recently, western Alberta has undergone an unparalleled increase in resource extraction activities and population growth (Schneider et al. 2003). Expanding human activities on the landscape, such as forestry, oil and gas activities, mining, associated road development, residential spread, recreation, and fire suppression, constitute a threat to grizzly bears (Kansas 2002, Nielsen et al. 2004a). Nielsen et al. (2004a) suggested that clearcuts may favor grizzly bears in certain situations by providing important food sources. Human-caused environmental perturbation, however, can also lead to habitat avoidance, act as barriers to food resources and migration, disturb breeding and rearing activities, change rates of interspecific interaction (e.g., predation), and fragment and isolate populations genetically (Kansas 2002, Ross 2002). Cumulative effects of limiting factors, such as human activities on the landscape, may affect negatively the grizzly bear carrying capacity, i.e., the number of individuals that can be supported in a given area within natural resource limits and without degrading the natural environment (Alberta Grizzly Bear Recovery Plan 2008-2013 2008). In addition, the subsequent increase of human-caused mortality because of poaching, vehicle accidents, self-defense kills, and removal of bears because of human-bear conflict, contributes to the uncertainty of the grizzly bear persistence in the province (McLellan et al. 1999, Nielsen et al. 2004b).

Although considered broadly across Canada as a species under "special concern", grizzly bears in Alberta are considered "may be at risk" by Alberta's Endangered Species Conservation Committee (ESCC) (Alberta Sustainable Resource Development Fish and Wildlife Division 2006). In 2002, the ESCC recommended that the grizzly bear be listed as "threatened" under the province's Wildlife Act. The recommendation was based on the International Union for Conservation of Nature and Natural Resources (IUCN) criterion that wildlife populations containing fewer than 1000 mature breeding animals be listed as threatened (International Union for Conservation of Nature and Natural Resources 2001), coupled with consideration of the slow reproductive rate of grizzly bears, limited immigration from populations outside Alberta, and the species high susceptibility to human activities (Alberta Sustainable Resource Development Fish and Wildlife Division 2005).

After reviewing the ESCC recommendation, the provincial government appointed the Alberta Grizzly Bear Recovery Team in 2002 to research and develop a management plan to support conservation of grizzly bears in Alberta (Alberta Sustainable Resource Development Fish and Wildlife Division 2005). The recovery team, represented by stakeholders and government staff, found that licensed hunting, poaching, and self-defense kills were the main sources of grizzly bear mortality. The team also recognized that increasing human activity in grizzly bear range plays a significant role in increasing human-bear conflicts, which can result in removal of problem bears. Finally, the team concluded that for the implementation of adequate bear management and species recovery, it is crucial to obtain reliable population numbers and to understand how grizzly bears are affected by human activity, concurrently with reducing human-caused grizzly bear mortality (Alberta Grizzly Bear Recovery Plan 2008-2013 2008).

A Grizzly Bear Recovery Plan, with recommendations to ensure the conservation of the species, was presented to the provincial government in draft form in February 2005 (Alberta

Grizzly Bear Recovery Plan 2008-2013 2008). After final revision, the Alberta Grizzly Bear Recovery Plan 2008-2013 was released in March 2008, although several of the Plan's recommendations were implemented prior to this date. Since 2004, DNA inventories have been conducted annually in different grizzly bear populations to estimate population sizes and bear density across grizzly bear range in Alberta. A mortality data base has been established, grizzly bear health and landscape research have been initiated by the Foothills Research Institute Grizzly Bear Program (FRIGBP), important grizzly bear habitat is being identified for protection from unregulated public access and other human activities and for habitat enhancement, and a public education program about human-bear interface is now in place. The spring grizzly bear hunt has been temporarily suspended for three years (2006-2008) with any decision to resume the hunt pending results of ongoing research (Alberta Grizzly Bear Recovery Plan 2008-2013 2008, Alberta Sustainable Resource Development Fish and Wildlife Division 2008). Eventually, Alberta's ESCC will review the recovery plan and associated research results and decide upon a status recommendation for the grizzly bear in Alberta (Alberta Sustainable Resource Development Fish and Wildlife Division 2005).

The ongoing DNA-based population survey has, so far, covered much of the core grizzly bear habitat in Alberta. It is estimated 228 grizzly bears reside full-time in the surveyed areas (Boulanger et al. 2005a, Boulanger et al. 2005b, Alberta Grizzly Bear Inventory Team, 2007 2007, Grizzly Bear Inventory Team, 2007 2008). DNA data collection for north of Highway 16 occurred in 2008 with results expected early in 2009 (Alberta Grizzly Bear Recovery Plan 2008-2013 2008). The results from the completed provincial population inventories will play an essential role in how the grizzly bear will be listed next in Alberta, with potential implications for management and conservation (Alberta Sustainable Resource Development Fish and Wildlife Division 2008).

Figure 1.2. Current distribution of grizzly bears in Canada (dark). Adapted from COSEWIC assessment and update status report on the grizzly bear *Ursus arctos* in Canada (Ross 2002).

1.2.2 Introduction to Foothills Research Institute Grizzly Bear Program

The FRIGBP (http://foothillsresearchinstitute.ca/pages/Programs/Grizzly_Bear.aspx) was started in 1998 by the Foothills Model Forest, Hinton, Alberta. The cause of its initiation was the concern over the cumulative effects of the Cheviot Coal Mine and other resource extraction activity on grizzly bears in west-central Alberta. The FRIGBP is a multi-disciplinary research program, which is supported by industrial partners and provincial and federal governments. Its primary goal is to provide science-based information to enable resource managers to plan landscape activities without threatening the persistence of grizzly bear populations in Alberta. Field work began in 1999, and during the following five years the research program focused on identifying habitat conditions and probability of grizzly bear occurrence on the landscape, grizzly

bear response to human activities, and assessing grizzly bear population performance (e.g., reproductive and survival rates) in light of different landscape metrics (Stenhouse and Graham 2005). In 2005, the FRIGBP expanded research efforts to evaluate and predict relationships between landscape change and grizzly bear population health (Stenhouse and Graham 2005, Cattet et al. 2006).

1.2.3 Working Hypothesis

Since 2005, the FRIGBP has been assessing grizzly bear health and landscape conditions in grizzly bear habitat to establish linkages between human-caused landscape change and the status of grizzly bear populations throughout western Alberta (Stenhouse and Graham 2005, Cattet et al. 2006) (Figure 1.3). Resource extraction and associated road development are occurring at an unprecedented rate in western parts of the province (Gibeau et al. 2002, Schneider et al. 2003), much of which is prime grizzly bear habitat. By understanding mechanisms underlying poor population performance, the FRIGBP will be able to provide industry and government with science-based information that can be used to help conserve grizzly bears. The working hypothesis of the FRIGBP is that human-caused landscape changes are perceived as long-term stressors by individual grizzly bears. As the long-term stress response persists, other biological functions are negatively affected, i.e., reproduction, immunity, growth, movement, and possibly longevity. As health deteriorates in increasing numbers of animals, negative effects, such as decreased reproduction and survival rates, may become apparent at the population level, eventually leading to a decline in abundance (Cattet et al. 2006) (Figure 1.1).

Figure 1.3. The FRIGBP study area in western Alberta 1999-2007 (dark) (Foothills Research Institute Grizzly Bear Program, June, 2008).

1.2.4 Approach

To enable determination and forecasting of potential effects of landscape change on grizzly bear health, the FRIGBP is combining several research components, i.e., geospatial mapping, Global Positioning System (GPS) radiotelemetry, and wildlife health (Stenhouse and Graham 2005). High resolution spatial data are obtained from satellite remote sensing imagery and used to classify landscape structure and change, e.g., food availability, tree cover, cut blocks, road density, seismic cut lines, and habitat fragmentation (Franklin 2005, Linke et al. 2005). GPS radiotelemetry collars are fitted to captured bears to provide location data, such as bear occurrence on the landscape and home range, as well as individual bear activity (Stenhouse et al. 2005, Stenhouse and Graham 2005).

Evaluation of wildlife health, including long-term stress, requires capturing grizzly bears, recording physical and physiological measurements, and sampling of different tissues. The research team of the FRIGBP captures bears annually, primarily from April to June. Depending on terrain canopy coverage and accessibility by road, bears are captured either by remote drug delivery from helicopter, by leg-hold snare, or by culvert trap, with some bears captured more than once (Hobson 2005).

Long-term physiological stress in grizzly bears is measured by analyzing biological samples using several laboratory techniques, including measurement of serum-based stress biomarkers (total cortisol, bear specific cortisol-binding globulin [CBG], and heat shock proteins [hsps] 60 and 70) (Hamilton 2007), an antibody-based protein microarray for measuring multiple stress proteins in different bear tissues (Cattet et al. 2006), and measurement of cortisol entrapped in growing hair (Davenport et al. 2006). Some of these biomarkers are robust to potential confounding effects from capture and handling (acute stress response), while others can also be measured in samples that can be collected without capturing bears. CBG, a protein that binds and transports cortisol in blood, is less labile and more long-lasting in response to stressors than serum cortisol (Reeder and Kramer 2005, Hamilton 2007). Syntheses of hsp60 and 70 are increased in cells that are exposed to different kinds of long-term stressors as a part of the affected cell's repair system (Feder and Hoffman 1999, Kültz 2005). The protein array yields expression profiles for multiple stress-activated proteins and thereby provides insight into the characteristics of the long-term stressors and their associated health effects. Small portions of tissue (skin and muscle) for these analyses can be obtained entirely by remote biopsy techniques (Cattet et al. 2006). Cortisol deposited in hair shafts reflects the corticosteroid response to stressors over the duration of hair growth (Davenport et al. 2006), and its measurement is another technique that does not require capture and handling of bears.

The research team obtains detailed individual information on biological functions, i.e., immunity, reproduction, growth, and movement, by comprehensive measures of physiological, physical, and movement data of captured bears. Laboratory evaluation of hematological and biochemical variables and sex hormones provides information about innate and acquired immunity and reproduction, respectively. Physical characteristics, such as weight, length, axillary girth, and body condition are quantified to assess growth. Sequential locations recorded by GPS collars are used to estimate movement rates as an index of activity in individual bears. Sex is decided by examination of external genitalia, and age is determined by counting cementum annuli in an extracted premolar tooth (Cattet et al. 2006). To increase the comprehensiveness of the health status at the individual level, long-term stress data are integrated with biological function data (Cattet et al. 2006). Performance at the population level is measured by demographic methods, such as evaluation of adult and cub survival, reproductive rate (Boulanger 2005), and estimation of population size by DNA identification from collected hair (Alberta Grizzly Bear Inventory Team, 2007 2007).

The relationship between landscape and health data is established by connecting individual health information with landscape structure and change in a spatial and temporal context. The spatial connection is established by quantifying landscape attributes and human activities in individual bears' home ranges as determined by GPS radiotelemetry, whereas the temporal connection is often the home range conditions, or rate of change in conditions, in the year(s) preceding capture (Cattet et al. 2006). Geographic Information System (GIS), geospatial, location, and health data are combined to form predictive models and eventually to generate maps similar in format to resource selection function maps (Nielsen et al. 2002). These maps will show the relative probability of healthy vs. unhealthy (stressed) grizzly bear occurrence on the landscape. By incorporating demographic measures, it should be possible to determine where in

Alberta are grizzly bear populations most likely to persist, decline, or disappear. Consistent with the primary goal of the FRIGBP, these data and products will allow resource managers to make scientifically sound decisions to ensure long-term conservation of grizzly bears when planning development of the landscape (Stenhouse and Graham 2005, Cattet et al. 2006). Results from this research will also be used to refine the Alberta Grizzly Bear Recovery Plan (Alberta Grizzly Bear Recovery Plan 2008-2013 2008).

1.3 M.Sc. Project

1.3.1 Objectives

My M.Sc. project was part of the FRIGBP. Based on the research program's working hypothesis, I focused on evaluating the health of individual bears. To facilitate the evaluation and application of a large set of complex biological data, I sought to develop a method to compress information from functional groupings of biological variables into single scores to quantify different health functions, e.g., stress, growth, immunity, and movement. My two specific objectives were:

1. To develop a health function score system based on grizzly bear biological data collected from 1999 to 2007 (Chapter 2); and
2. To test the usefulness of the health function score system by comparing results provided by the score system with results provided by more conventional health variables from the evaluation of associations presumed to exist under the working hypothesis of the FRIGBP (Chapter 3).

CHAPTER 2

HEALTH FUNCTION SCORE SYSTEM – DEVELOPMENT

2.1 Abstract

A primary objective of the Foothills Research Institute Grizzly Bear Program is to evaluate relationships between measures of health in grizzly bears (*Ursus arctos*) with human-caused change of their habitat. As one component of the research program, I developed a health function score system based on biological information from grizzly bears in western Alberta, collected from 1999 to 2007. From the extensive health data set, I selected 14 "constituent" variables and merged them into four health functions that reflected growth, immunity, movement, and stress. By adding weighted variable percentiles, I calculated individual, standardized scores for each health function, ranging from 0.00 to 1.00. My calculation method ensured scores were independent of sex and capture method. I demonstrated overall good agreement between health function scores and health status of individual bears based on values for multiple constituent variables. I could, therefore, use health function scores to quickly evaluate individual bear health, identify bears with reduced health, and compare health profiles between bears. Limitations of the health function score system included potential influence of capture, correlation among constituent variables, subjective variable selection and weighting, and missing values for constituent variables. Nevertheless, the system appears to be a practical tool to quickly screen and compare health of individual grizzly bears based on elements of their biological functions. The system also has potential for application in other wild species.

2.2 Introduction

Grizzly bears (*Ursus arctos*) in western Alberta are negatively affected by human-caused landscape disturbance that results in fragmentation and loss of habitat, as well as decrease in quality of available habitat (Kansas 2002, Nielsen et al. 2004a). In 2005, the research team of the Foothills Research Institute Grizzly Bear Program (FRIGBP) hypothesized that negative consequences of landscape change on grizzly bear population performance in Alberta are emerging primarily as a result of long-term physiological stress in individual bears (Cattet et al. 2006). In many animals, long-term stress is known to have adverse impact on biological functions, including growth, immunity, and reproduction (Balm 1999, Wingfield 2005). When many individuals in a population are affected by long-term stress, negative effects may appear at the population level as reduced reproduction and survival rates and eventually loss of abundance (Stevenson 2006, Wikelski and Cooke 2006). Since 2005, the FRIGBP has concentrated significant effort toward detection of stress and assessment of health in grizzly bears.

For my M.Sc. project, I focused on developing a practical technique to evaluate health of grizzly bears. I defined grizzly bear health, or more generally wildlife health, as the capacity of an individual animal to maintain biological functioning when challenged by environmental change (section 1.1.2). I had available an extensive database of health information collected by the FRIGBP from 1999 to 2007 that comprised several hundred cases (n = 280) with as many as 129 variables per case. With so much health information, I was challenged to evaluate health of individual bears in accordance with my definition based on biological functions. This difficulty was further compounded by the database containing incomplete records and records from repeated captures of individual bears, and occurrence of health variables influenced by sex and age of bear, method of capture, and date of capture. To circumvent these difficulties, I developed a health function score system to enable quick screening of health profiles for individual bears, to

identify bears with reduced health, and to explore associations between health, stress, and landscape condition. In this chapter, I focus on the development and verification of a health function score system for grizzly bears. In chapter 3, I assess the usefulness of the health function score system by applying it to explore associations presumed to exist under the working hypothesis of the FRIGBP.

2.3 Methods

2.3.1 Capture, Data Collection, and Laboratory Analyses

We captured 165 grizzly bears, 75 females (one to 21 years old at first capture) and 90 males (one to 21 years old at first capture), 280 times within the FRIGBP study area in western Alberta (49°00'–58°00'N and 113°50'–120°00'W) from 1999 to 2007 (Figure 2.1). Captures occurred annually from late March (den emergence) to November (den entry) with most occurring in May and June. We used Aldrich leg-hold snares (Aldrich Snare Co., Clallam Bay, Washington) for 154 captures, remote drug delivery from helicopter for 96 captures or from ground for 4 captures, and culvert traps for 26 captures with selection of capture method based on terrain openness and accessibility (Hobson 2005, Hobson et al. 2007).

We immobilized grizzly bears by remote drug delivery (Pneudart® Inc., Williamsport, Pennsylvania, USA, Paxarms® N.Z. Ltd., Timaru, New Zealand or Daninject®, Børkop, Denmark) with either (i) a combination of xylazine hydrochloride (Cervizine 300®, Wildlife Pharmaceuticals Inc., Ft. Collins, Colorado, USA) at 2-3 mg/kg estimated body weight and zolazepam hydrochloride + tiletamine hydrochloride in a 1:1 ratio (Telazol®, Fort Dodge Animal Health, Fort Dodge, Iowa, USA) at 3.0-4.5 mg/kg (Cattet et al. 2003a) or (ii) zolazepam hydrochloride + tiletamine hydrochloride at 8 - 10 mg/kg (Taylor et al. 1989). We reversed the immobilization with (i) atipamezole hydrochloride (Antisedan®, Novartis Animal Health Canada

Inc., Mississauga, Ontario, Canada) intramuscularly (IM) or half volume intravenously (IV) and half volume IM, at 0.2-0.3 mg/kg or (ii) yohimbine hydrochloride (Antagonil®, Wildlife Pharmaceuticals, Inc., Fort Collins, Colorado, USA) IM or half volume IV and half volume IM, at 0.15-0.20 mg/kg (Cattet et al. 2003a, Cattet et al. 2008a).

We recorded pulse and respiratory rates, rectal temperature, and hemoglobulin oxygen saturation (Nellcor NPB-40 pulse oximeter, Nellcor, Pleasanton, California, USA) of anesthetized bears every 10-15 minutes during the 45-75 minutes required for sample collection and measurements. We determined sex by examination of external genitalia. Following application of a mental nerve block using bupivicaine (Marcaine®, Sanofi, Markham, Ontario, Canada) at a dose of 10-15 mg, we extracted a premolar tooth to estimate age by counting of cementum annuli (Stoneberg and Jonkel 1966). We weighed bears in a sling beneath a load scale (MSI-7200 Dynalink, Precision Giant Systems Inc., Edmonton, Alberta, Canada). With bears positioned in sternal recumbency, we measured body length as straight-line distance from tip of nose to end of last vertebra and axillary girth as circumference of chest at level of axilla. We collected blood from the femoral or jugular vein into an EDTA tube for hematological measurements and into sterile serum tubes for biochemical and hormonal analyses. We chilled blood in EDTA tubes for determination of complete blood counts with an Abbott Cell-Dynn® 3200 hematology analyzer (Abbott Laboratories Diagnostic Division, Abbott Park, Illinois, USA) within 24 hours of collection. We centrifuged blood samples in serum tubes within eight hours of collection and froze the extracted serum (-20° C) for biochemical analysis with an Abbott Spectrum® Series II biochemistry analyzer (Abbott Laboratories Diagnostic Division, Abbot Park, Illinois, USA). We measured levels of serum cortisol using a 125 I cortisol radioimmunoassay (RIA) kit (#07-221102 MP Biomedicals, Irvine, California, USA) and determined serum heat shock protein 60 and 70 levels using enzyme-linked immunosorbent assay

(ELISA) kits (#EKS-600, #EKS-700 StressGen Biotechnologies, Victoria, British Colombia, Canada) validated for grizzly bears (Hamilton 2007). We fitted grizzly bears with one of the following Global Positioning System (GPS) radiocollars: Televilt Simplex, Televilt Tellus (Televilt®, TVP Positioning AB, Lindesberg, Sweden), or Advanced Telemetry Systems (Advanced Telemetry Systems, Inc., Isanti, Minnesota, USA) to acquire sequential locations at one to four-hour intervals (Hobson et al. 2007, Cattet et al. 2008a).

The capture and handling protocol was approved by the Animal Care Committee at the University of Saskatchewan and was in accordance with guidelines provided by the American Society of Mammalogists Animal Care and Use Committee (1998) and the Canadian Council on Animal Care (2003).

Figure 2.1. The FRIGBP study area in western Alberta 1999-2007 (dark) (Foothills Research Institute Grizzly Bear Program, June, 2008).

2.3.2 Identification of Health Functions and Constituent Variables

I identified growth, immunity, movement, and stress as health functions for which data were sufficient to evaluate. For each health function, I selected two to five constituent variables that were representative of the health function, but minimally correlated with other constituent variables (Table 2.1).

I selected total body mass, straight-line body length, axillary girth, and body condition index (BCI) as constituent variables that represent growth. Total body mass and axillary girth reflect body (i.e., nutritional) condition, but are also influenced by body size (Nagy et al. 1984, Cattet et al. 1997), straight-line body length reflects body size, and BCI indicates body condition

independent of body size (Cattet et al. 2002).

I chose neutrophil, lymphocyte, monocyte, and eosinophil counts and total serum globulin concentration as constituent variables that represent immunity. Neutrophil, monocyte, and eosinophil counts are measures of innate immunity, whereas number of lymphocytes is an indicator of acquired immunity. Serum concentration of globulin is a measure of both acquired and innate immunity (Tizard 1996, Stockham and Scott 2002).

For movement, I estimated average daily movement rates for individual bears during breeding and non-breeding seasons based on consecutive GPS locations recorded every 1-4 hours within a 24 hour period (midnight to midnight) (Cattet et al. 2008a). Movement rate is an indicator of general grizzly bear activity patterns (Heard et al. 2008) and may vary due to seasonal breeding status (McLoughlin et al. 1999, Ross 2002). I defined breeding season as the period from May 16 to July 31 and non-breeding season as all other dates between den emergence and entry (late March to early November) (Schwartz et al. 2003, Stenhouse et al. 2005).

For stress, I selected serum concentrations of total cortisol, heat shock protein 60 (hsp60), and heat shock protein 70 (hsp70) as constituent variables. Total cortisol concentration is a measure of systemic stress response (McEwen and Wingfield 2003), whereas hsps 60 and 70 concentrations are indicators of cellular stress response (Kültz 2005, Calderwood et al. 2007).

Table 2.1. Constituent variables used to represent health functions for grizzly bears captured by FRIGBP 1999-2007.

Health function	Constituent variables				
Growth	Total body mass (kg)	Straight-line body length (cm)	Axillary girth (cm)	Body condition index[a]	
Immunity	Neutrophil count (x 10^9/L)	Lymphocyte count (x 10^9/L)	Monocyte count (x 10^9/L)	Eosinophil count (x 10^9/L)	Globulin (g/L)
Movement	Average daily movement rate, breeding season (m/h)	Average daily movement rate, non-breeding season (m/h)			
Stress	Total cortisol (ng/ml)	Heat shock protein 60 (ng/ml)	Heat shock protein 70 (ng/ml)		

[a] Body condition index is based on standardized residuals from the regression of body mass against a linear measure of size, ranging from -3.00 to +3.00 (Cattet et al. 2002).

2.3.3 Determination of Health Function Scores

2.3.3.1 Effects of Sex, Age, and Capture on Constituent Variables

Constituent variables typically are affected by an animal's sex and age (Schwartz et al. 2003, Reeder and Kramer 2005), as well as method and timing of capture (Cattet et al. 2003b, Cattet et al. 2008a). I used two-way analysis of covariance (ANCOVA) to determine if variables differed by sex and capture method or correlated with age of bear and Julian day of capture. Statistical analysis was performed using SPSS 16.0 for Windows® (SPSS, Inc., Chicago, Illinois). To maintain independence among data points, I used only data from the first capture of a bear within the project for analyses involving growth variables. For analyses of immunity and stress

variables, I used data from the first capture within a given year. Because the effect of occasional acute stressful stimuli, such as capture, on stress and immunity variables is transient (Stockham and Scott 2002, Wingfield 2005), I assumed independence between results from different years. So, it was possible to use data collected from the same individual over multiple captures provided the captures occurred in different years. For analyses of movement variables, I used data collected > 17 days following capture to reduce the influence of capture on bear movements (Cattet et al. 2008a). Because of the small numbers of bears captured by culvert trap and ground capture, data were merged into two capture methods based on the nature of the capture method: (i) free range, i.e., helicopter and ground capture, vs. (ii) trap, i.e., leg-hold snare and culvert trap. Findings by Cattet et al. (2003b) and Cattet et al. (2008a) also provided support for this division. Sample sizes varied between analyses depending on completeness of records. Where statistical assumptions of parametric statistics were violated, I used transformed data, as necessary (Norman and Streiner 2008). Statistical significance was assigned when the probability (p) of a Type I error was ≤ 0.05. I report all results as mean, or mean adjusted for covariates, and 95 % confidence interval.

2.3.3.2 Calculation of Health Function Scores

I calculated health function scores (HFSs) using all available data. To standardize the scores, I first ranked all values in ascending order for each constituent variable if the variable was not affected by sex or method of capture. If the variable was affected, I ranked values in ascending order within sub-groupings based on sex, method of capture, or both factors. Contrary to this procedure, values for lymphocyte and eosinophil counts were ranked in descending order instead. I did this because values for these variables often decrease while values for other constituent immunity variables (neutrophil and monocyte counts and globulin concentration)

increase in situations of stress or disease (Stockham and Scott 2002). By inversing ranks, I was able to ensure all immunity variables changed in the same direction (increased or decreased) under similar circumstances. I then converted ranks to percentiles using the formula: (Rank – 1) ÷ N (Sullivan III 2007). Based on a scaling method developed by Saaty (1977), percentiles were weighted according to the relative qualitative importance of the particular variable for the HFS (Appendix A). Finally, I added the weighted percentiles (w_iPc_i) of constituent variables to calculate scores, ranging from 0.00 to 1.00, for each health function: HFS = ($w_1Pc_1 + w_2Pc_2$+… + w_iPc_i).

2.3.4 Effects of Sex and Capture Method on Health Function Scores

I investigated if the effect of sex and capture method was removed when HFSs were calculated. I used two-way ANCOVA with sex and capture method as factors and age of bear and Julian day of capture as covariates. Statistical analysis was performed using SPSS 16.0 for Windows® (SPSS, Inc., Chicago, Illinois). To maintain independence among data points in the statistical analyses, I used only data from the first capture of a bear within the project for analyses involving growth score, data from the first capture of a bear within a given year for analyses involving immunity and stress scores, and all data for analyses involving movement score. Sample sizes varied between analyses depending on completeness of records. Where statistical assumptions of parametric statistics were violated, I used transformed data, as necessary (Norman and Streiner 2008). Statistical significance was assigned when the probability (p) of a Type I error was ≤ 0.05. I report all results as mean, or mean adjusted for covariates, and 95 % confidence interval.

2.3.5 Comparisons Between Health Function Scores and Constituent Variable Values

To determine if differences in HFSs among individual bears were mirrored by similar differences in constituent variable values, I examined the correspondence between HFSs and constituent variable values within each health function for four grizzly bears that were of same sex, age class, and reproductive status and captured by the same method during the same month/season.

2.4 Results

2.4.1 Effects of Sex, Age, and Capture on Constituent Variables

I found all growth variables were affected by sex, capture method, and age (Table 2.2). Age-adjusted mean values of males were greater than those of females for total body mass (128.2 kg [95 % confidence interval: 116.2-140.2] vs. 77.6 kg [66.6-88.5]; $F = 46.61$, $p \leq 0.001$), straight-line body length (161 cm [158-164] vs. 149 cm [144-154]; $F = 37.17$, $p \leq 0.001$), axillary girth (110 cm [106-113] vs. 93 cm [89-96]; $F = 50.73$, $p \leq 0.001$), and BCI (0.78 [0.49-1.08]: vs. -0.02 [-0.28-0.24]; $F = 20.36$, $p \leq 0.001$). Age-adjusted mean values for all growth variables were also greater for bears captured by trap than for bears captured while free-ranging ($F \geq 4.06$, $p \leq 0.046$). The effect of capture method, however, was caused by a bias in sampling design (see Discussion for explanation). So, I calculated percentile values for female and male groupings, but not for capture method groupings.

I found immunity variables were affected by sex, age, and capture, but the significance of these effects differed between variables (Table 2.3). The neutrophil count was greater, and lymphocyte and eosinophil counts were lower, for trap-captured bears than for free range-captured bears ($F \geq 4.40$, $p \leq 0.037$). These variables, however, did not differ between sexes. In contrast, monocyte count and serum globulin concentration differed between sexes, as well as

capture methods. Monocyte counts were greater in trap-captured bears than in free range-captured bears ($F = 10.71$, $p = 0.001$) and in males than in females ($F = 11.48$, $p = 0.001$). I found the same pattern with globulin concentration, but the effects were weaker ($F_{CM} = 4.62$, $p = 0.033$, and $F_{sex} = 4.54$, $p = 0.034$). Given these differences among immunity variables, I calculated percentile values for neutrophils, lymphocytes, and eosinophils based only on capture method groupings, whereas I calculated percentile values for monocytes and globulin by groupings based on both sex and capture method.

I found movement rates were greater for males than females during breeding ($F = 16.21$, $p \leq 0.001$) and non-breeding seasons ($F = 8.45$, $p = 0.005$) (Table 2.4). Movement rates were not affected by capture method ($F \leq 2.27$, $p \geq 0.135$). I, therefore, calculated percentile values for female and male groupings.

I found stress variables were affected by capture method only (Table 2.5). This effect was significant for total cortisol ($F = 13.67$, $p \leq 0.001$) and hsp70 ($F = 8.30$, $p = 0.004$), but not for hsp60 ($F = 0.04$, $p = 0.842$). Thus, I calculated percentile values for total cortisol and hsp70 by capture method groupings.

Table 2.2. Effects[a] of sex, age, and capture on growth variables for grizzly bears captured by FRIGBP 1999-2007.

Variable	F&CMF[b]	F&CMT	M&CMF	M&CMT	Effect of Sex	Effect of CM	Effect of Age	Effect of J.day
Total body mass (kg)	69.0 (49.9-88.2) [16]	92.3 (80.6-104.0) [42]	90.9 (64.1-117.6) [8]	149.4 (138.6-160.3) [49]	S^{***}	S^{***}	S^{***}	NS
Straight-line body length (cm)	144 (138-150) [18]	153 (149-157) [46]	154 (146-162) [12]	169 (165-172) [57]	S^{***}	S^{***}	S^{***}	NS
Axillary girth (cm)	89 (83-95) [18]	97 (93-101) [46]	103 (95-111) [11]	115 (111-118) [58]	S^{***}	S^{***}	S^{***}	NS
Body condition index	-0.06 (-0.51-0.38) [16]	0.13 (-0.15-0.41) [40]	0.18 (-0.48-0.85) [7]	1.06 (0.79-1.33) [43]	S^{***}	S^{*}	S^{***}	NS

[a] Values reported as mean, 95 % confidence interval in round brackets, and sample size in square brackets. Statistical comparison made by two-way ANCOVA with sex and capture method (CM) as factors, and age of bear in years (Age) and Julian day of capture (J.day) as covariates. Significance is presented as S^{*} for $p \leq 0.05$, S^{**} for $p \leq 0.01$, S^{***} for $p \leq 0.001$, and NS for non-significance ($p > 0.05$).

[b] F&CMF = female grizzly bears captured free range, F&CMT = female grizzly bears captured with traps, M&CMF = male grizzly bears captured free range, and M&CMT = male grizzly bears captured with traps.

Table 2.3. Effects[a] of sex, age, and capture on immunity variables for grizzly bears captured by FRIGBP 1999-2007.

Variable	F&CMF[b]	F&CMT	M&CMF	M&CMT	Effect of Sex	Effect of CM	Effect of Age	Effect of J.day
Neutrophil count (x 10^9/L)	5.57 (4.35-6.79) [43]	11.94 (10.81-13.09) [49]	5.67 (3.99-7.36) [23]	12.98 (12.01-13.95) [68]	NS	S^{***}	S^{*}	NS
Lymphocyte count (x 10^9/L)	1.09 (0.93-1.25) [44]	0.71 (0.58-0.84) [54]	1.04 (0.71-1.36) [25]	0.95 (0.77-1.14) [72]	NS	S^{*}	NS	NS
Monocyte count (x 10^9/L)	0.34 (0.26-0.43) [44]	0.46 (0.37-0.55) [54]	0.41 (0.28-0.55) [25]	0.73 (0.61-0.86) [72]	S^{***}	S^{***}	NS	NS
Eosinophil count (x 10^9/L)	0.24 (0.15-0.33) [44]	0.17 (0.09-0.26) [54]	0.32 (0.19-0.45) [25]	0.17 (0.09-0.24) [72]	NS	S^{*}	NS	S^{*}
Globulin (g/L)	28 (26-29) [48]	29 (28-30) [59]	29 (27-31) [24]	31 (30-32) [72]	S^{*}	S^{*}	S^{*}	S^{*}

[a] Values reported as mean, 95 % confidence interval in round brackets, and sample size in square brackets. Statistical comparison made by two-way ANCOVA with sex and capture method (CM) as factors, and age of bear in years (Age) and Julian day of capture (J.day) as covariates. Significance is presented as S^{*} for $p \leq 0.05$, S^{**} for $p \leq 0.01$, S^{***} for $p \leq 0.001$, and NS for non-significance ($p > 0.05$).

[b] F&CMF = female grizzly bears captured free range, F&CMT = female grizzly bears captured with traps, M&CMF = male grizzly bears captured free range, and M&CMT = male grizzly bears captured with traps.

Table 2.4. Effects[a] of sex, age, and capture on movement variables for grizzly bears captured by FRIGBP 1999-2007.

Variable	F&CMF[b]	F&CMT	M&CMF	M&CMT	Effect of Sex	CM	Age	J.day
Average daily movement rate, breeding season (m/h)	257.0 (218.5-295.5) [31]	275.1 (236.8-313.4) [27]	325.7 (259.2-392.2) [16]	398.6 (329.8-467.3) [32]	S^{***}	NS	NS	NS
Average daily movement rate, non-breeding season (m/h)	241.3 (193.6-289.0) [35]	261.0 (204.7-317.3) [24]	304.6 (232.5-376.8) [15]	355.1 (297.4-412.8) [23]	S^{**}	NS	NS	S^{*}

[a] Values reported as mean, 95 % confidence interval in round brackets, and sample size in square brackets. Statistical comparison made by two-way ANCOVA with sex and capture method (CM) as factors, and age of bear in years (Age) and Julian day of capture (J.day) as covariates. Significance is presented as S^{*} for $p \leq 0.05$, S^{**} for $p \leq 0.01$, S^{***} for $p \leq 0.001$, and NS for non-significance ($p > 0.05$).

[b] F&CMF = female grizzly bears captured free range, F&CMT = female grizzly bears captured with traps, M&CMF = male grizzly bears captured free range, and M&CMT = male grizzly bears captured with traps.

Table 2.5. Effects[a] of sex, age, and capture on stress variables for grizzly bears captured by FRIGBP 1999-2007.

Variable	F&CMF[b]	F&CMT	M&CMF	M&CMT	Effect of Sex	Effect of CM	Effect of Age	Effect of J.day
Total cortisol (ng/ml)	49.5 (38.4-60.7) [46]	86.6 (67.3-105.9) [61]	47.6 (30.0-65.2) [26]	86.3 (66.8-105.8) [77]	NS	S^{***}	NS	NS
Heat shock protein 60 (ng/ml)	2.95 (1.40-4.50) [46]	3.31 (1.81-4.81) [56]	3.98 (2.19-5.78) [25]	3.11 (2.01-4.21) [67]	NS	NS	NS	NS
Heat shock protein 70 (ng/ml)	2.24 (1.51-2.97) [46]	3.03 (2.26-3.80) [56]	2.13 (1.11-3.14) [25]	3.79 (2.99-4.59) [67]	NS	S^{**}	NS	NS

[a] Values reported as mean, 95 % confidence interval in round brackets, and sample size in square brackets. Statistical comparison made by two-way ANCOVA with sex and capture method (CM) as factors, and age of bear in years (Age) and Julian day of capture (J.day) as covariates. Significance is presented as S^{*} for $p \leq 0.05$, S^{**} for $p \leq 0.01$, S^{***} for $p \leq 0.001$, and NS for non-significance ($p > 0.05$).

[b] F&CMF = female grizzly bears captured free range, F&CMT = female grizzly bears captured with traps, M&CMF = male grizzly bears captured free range, and M&CMT = male grizzly bears captured with traps.

2.4.2 Calculation of Health Function Scores

I found that mean scores were near 0.50, the 95 % confidence intervals were narrow, and minimum and maximum scores were similar among the four health function groups (Figure 2.2).

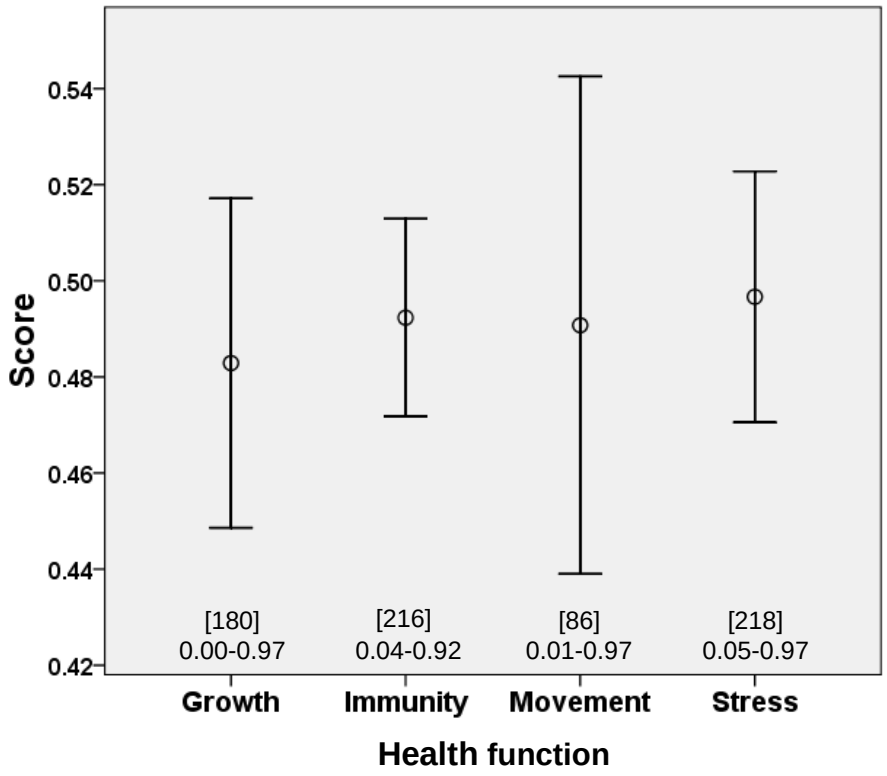

Figure 2.2. Descriptive statistics for health function scores calculated for grizzly bears captured by FRIGBP 1999-2007. Mean scores and 95 % confidence intervals are represented by open circles and capped vertical lines, respectively. Sample sizes are presented in square brackets with minimum and maximum values presented below.

2.4.3 Effects of Sex and Capture Method on Health Function Scores

I found that HFSs were not affected by sex ($F \leq 1.38$, $p \geq 0.242$) or capture method ($F \leq 1.02$, $p \geq 0.314$) in most cases (Table 2.6). Two exceptions were that trap-captured bears had higher growth and movement scores than free range-captured bears (growth: $F = 14.27$, $p \leq 0.001$, movement: $F = 4.33$, $p = 0.041$). These effects, however, were likely caused by biases in sampling design (see Discussion for explanation).

Table 2.6. Effects[a] of sex, age, and capture on health function scores for grizzly bears captured by FRIGBP 1999-2007.

Health function	F&CMF[b]	F&CMT	M&CMF	M&CMT	Effect of Sex	CM	Age	J. day
Growth	0.31 (0.23-0.40) [16]	0.42 (0.37-0.48) [40]	0.27 (0.13-0.41) [6]	0.50 (0.45-0.56) [43]	NS	S^{***}	S^{***}	NS
Immunity	0.44 (0.40-0.49) [43]	0.50 (0.46-0.54) [49]	0.53 (0.46-0.59) [23]	0.49 (0.46-0.53) [68]	NS	NS	S^{*}	S^{*}
Movement	0.45 (0.35-0.54) (*n*=27)	0.56 (0.44-0.67) (*n*=23)	0.41 (0.29-0.52) (*n*=13)	0.52 (0.42-0.62) (*n*=23)	NS	S^{*}	NS	NS
Stress	0.50 (0.44-0.56) [46]	0.47 (0.43-0.52) [56]	0.47 (0.38-0.56) [25]	0.52 (0.48-0.57) [67]	NS	NS	NS	NS

[a] Health function scores reported as mean, 95 % confidence interval in round brackets, and sample size in square brackets. Statistical comparison made by two-way ANCOVA with sex and capture method (CM) as factors, and age of bear in years (Age) and Julian day of capture (J.day) as covariates. Significance is presented as S^{*} for $p \leq 0.05$, S^{**} for $p \leq 0.01$, S^{***} for $p \leq 0.001$, and NS for non-significance ($p > 0.05$).
[b] F&CMF = female grizzly bears captured free range, F&CMT = female grizzly bears captured with traps, M&CMF = male grizzly bears captured free range, and M&CMT = male grizzly bears captured with traps.

2.4.4 Comparisons Between Health Function Scores and Constituent Variable Values

Generally, I found poor correspondence between HFSs and individual variable values (Tables 2.7-2.10). Correspondence was more often poor for values of variables with lower weights, e.g., monocyte and eosinophil counts and hsp60 ($w < 0.1$, Appendix A) (Tables 2.8 and 2.10), than for values of variables with higher weights. Still, by considering each score in regard to multiple constituent variable values for an individual bear, I found good correspondence between HFSs and individual bear health status. Overall, bears with higher scores had multiple variable values that were higher (or lower for lymphocyte and eosinophil counts) and vice versa.

Table 2.7. Correspondence between growth scores and values of constituent variables[a] for four 6-year old male grizzly bears captured in traps during May by FRIGBP 1999-2007.

Bear ID	Growth score	TBM[b] (kg)	SLBL (cm)	Axillary girth (cm)	BCI
G242	0.75	255.0	196	133	1.19
G045	0.73	212.2	181	125	1.54
G209	0.60	175.1	174	122	1.21
G024	0.38	155.1	181	118	-0.04

[a] First capture by FRIGBP.
[b] TBM = total body mass, SLBL = straight-line body length, and BCI = body condition index.

Table 2.8. Correspondence between immunity scores and values of constituent variables[a] for four 5-year old female grizzly bears without accompanying cubs, captured free range in April-May by FRIGBP 1999-2007.

Bear ID	Immunity score	Neutrophil count (x 10^9/L)	Lymphocyte count (x 10^9/L)	Monocyte count (x 10^9/L)	Eosinophil count (x 10^9/L)	Globulin (g/L)
G100	0.73	8.01	0.63	0.18	0.18	31
G037	0.60	8.37	0.94	0.09	0.00	27
G004	0.45	3.79	0.53	0.29	0.19	26
G020	0.30	4.83	1.16	0.27	0.54	25

[a] First capture within a given year.

Table 2.9. Correspondence between movement scores and values of constituent variables[a] for four 9-11 year old male grizzly bears captured in traps by FRIGBP 1999-2007.

Bear ID	Movement score	Average daily movement rate, breeding season (m/h)	Average daily movement rate, non-breeding season (m/h)
G017	0.77	648	348
G098	0.45	310	326
G014	0.44	376	274
G217	0.15	258	181

[a] All captures included.

Table 2.10. Correspondence between stress scores and values of constituent variables[a] for four 7-8 year old female grizzly bears without accompanying cubs, captured free range in April-July by FRIGBP 1999-2007.

Bear ID	Stress score	Total cortisol (ng/ml)	Hsp60[b] (ng/ml)	Hsp70 (ng/ml)
G093	0.82	104.4	5.96	2.66
G89K	0.49	31.1	0.00	2.70
G003	0.27	12.2	0.35	1.41
G028	0.20	8.3	0.74	1.09

[a] First capture within a given year.
[b] Hsp60 = heat shock protein 60 and Hsp70 = heat shock protein 70.

2.5 Discussion

2.5.1 Effects of Sex and Capture Method on Constituent Variables and Health Function Scores

I found many constituent variables used to calculate health scores were influenced by sex or method of capture or, in some cases, by both factors. In general, growth and movement variables were influenced by sex, whereas immunity and stress variables were influenced by method of capture. The influence of biological and anthropogenic factors is also taken into account in other studies of wildlife health. For example, Wells et al. (2004) considered the effect of sex, reproductive class, age, sample type, and use of analytical laboratory when they developed a health monitoring system for bottlenose dolphins (*Tursiops truncatus*) based on physiological variables. Gagné et al. (2008) included sex as a factor when they measured the impact of anthropogenic activity on populations of the soft-shell clam (*Mya arenaria)* with a multi-biomarker approach.

I found male grizzly bears had higher growth variable values than female bears. These findings were supported by Hilderbrand et al. (1999) and Schwartz et al. (2003), who stated that sexual dimorphism is apparent in grizzly bears with males being up to two times bigger than females. Sex differences in body mass and size are influenced by food abundance and quality, age at sexual maturity, reproductive status, season of sampling (Schwartz et al. 2003), and competition between sexes whereby males may displace females from productive habitat (Herrero 2005). Growth variables also differed significantly between capture methods. I attributed these findings, however, to the fact that capture by trap occurred more in forested, closed terrain at lower elevation where grizzly bears were larger, whereas capture by helicopter was used more in open terrain at higher elevation where grizzly bears were smaller (Boulanger et al., unpublished data).

I found neutrophil and monocyte counts were higher, and lymphocyte and eosinophil counts were lower, in grizzly bears captured by trap compared to bears captured free range. These findings were consistent with a stress leukogram found in several species following stress-induced corticosteroid release (Stockham and Scott 2002, Jackson 2007). Trap-captured bears could have spent up to 24 hours in the trap prior to chemical immobilization (Cattet et al. 2003b), whereas free range-captured bears were chased less than one minute prior to initiation of chemical immobilization and subsequent collection of samples (Hobson 2005). Because it takes four to eight hours before a stress leukogram is apparent in many domestic species after a single administration of corticosteroids (Latimer et al. 2003), differences in leukocyte numbers were most likely the result of longer duration of stress in trap-captured bears compared to free range-captured bears (Cattet et al. 2003b, Kusak et al. 2005). Still, I cannot rule out the possibility that differences occurred because the time interval between onset of stress and blood collection was much longer for bears captured by trap than bears captured from helicopter. In other words, it is

plausible that leukocyte numbers could have been more similar if bears captured free range were sampled 12-24 hours following capture.

Monocyte count was higher for males than females. Other references, however, did not report sex differences in monocyte numbers in American (grizzly) and European brown bears (Pearson and Halloran 1972, Kusak et al. 2005).

I found greater globulin concentration in trap-captured bears than in free range-captured bears. This may be explained by mild dehydration in trap-captured bears from deprivation of water and insensible water loss while restrained (Cattet et al. 2003b). Globulin concentration also differed between females and males. Neither Brannon (1985) nor Huber et al. (1997), however, found globulin concentration differed between sexes in brown bears.

My findings of higher movement rates for males compared to females in both breeding and non-breeding season were consistent with other observations (Grogan 2001, Ross 2002). McLoughlin et al. (1999) suggested that male grizzly bears in the central Canadian Arctic tend to wander more in search for breeding mates, and, because of having a larger energy demand, for food sources.

Capture method affected some of the constituent stress variables. My findings of higher serum cortisol concentrations in grizzly bears captured with trap than in bears captured free range were in agreement with earlier observations of capture effects on bears and other wild mammals (Cattet et al. 2003b, Iossa et al. 2007). The dissimilarities between capture methods may have been a consequence of greater physical and physiological stress in trap-captured grizzly bears (Cattet et al. 2003b, Iossa et al. 2007). Similarly, serum concentration of hsp70 was also significantly higher in trap-captured bears compared to free range-captured bears. Although the underlying mechanism is unclear, Fleshner et al. (2004) demonstrated that serum hsp70 concentration increases in rodents exposed to predatory fear over 90 minutes, whereas Walsh et

al. (2001) and Febbraio et al. (2002) observed similar changes in serum hsp70 concentrations in humans after 30 minutes of physical exercise. These findings may be compatible with higher hsp70 levels in trap-captured grizzly bears, which were exposed to prolonged stress and intense physical activity (Cattet et al. 2003b). Increased serum hsp60 concentration is found with chronic cellular stress associated with inflammatory disease (Pockley 2002) rather than acute stress (Hamilton 2007), which may explain why this variable did not differ between capture groups. Neither total cortisol, nor hsp concentrations, differed between sexes. Total cortisol concentration, however, differs between males and females in other studies of captured wild animals (Creel 2005, Reeder and Kramer 2005).

I was able to remove potential influences of sex and capture method on HFSs by adjusting for these factors as a step in the score calculation procedure. Removing the effect of these factors should allow for clearer interpretation of HFSs (Petrie and Watson 2006, Cattet et al. 2008a). Further, because HFSs can be used to compare bears independent of sex and capture method, all animals with health function scores can be included in analyses without sub-dividing them into smaller groups, improving statistical power. Maintaining as many individuals as possible in analyses also is more representative of the general population (Petrie and Watson 2006).

As mentioned previously, growth variables, and thus growth score, were likely affected by capture method as a result of sampling bias. I also found a similar effect of capture method on movement score. Bears living in foothill areas where traps were mostly used were able to move across the landscape easier and would be predicted to have greater movement rates than bears living in mountainous areas where capture by helicopter was used more frequently and where bear movements would be more constrained by topography (Boulanger et al., unpublished data). Different sample sizes among movement score (n = 86) and constituent movement variables (movement rate, breeding season: n = 106, movement rate, non-breeding season: n = 97), and

influence of outlying observations in the comparisons of movement rates, may explain why effect of capture method was statistically non-significant for movement rates, but significant for movement score.

2.5.2 Assessment of Grizzly Bear Health Status with Health Function Scores

Although I found correspondence between HFSs and individual variable values was generally poor, there was good correspondence between HFSs and health status of individual bears based on values for multiple constituent variables. As a result, I could use HFSs to evaluate overall health status of individuals and identify bears with reduced health. Further, I could compare health profiles between bears. This is a quicker method than evaluating health using one constituent variable at a time. For example, the five-year old grizzly bear G070, captured in June, had the following scores – stress: 0.65, growth: 0.32, immunity: 0.92, and movement: 0.42. These results suggested increased stress, reduced growth, and that immune function was affected by stress, potentially with a concurrent infectious disease, and movement was decreased. As a comparison, the following scores in another 5-year old bear captured in June, G075F – stress: 0.32, growth: 0.71, immunity: 0.41, and movement: 0.31, suggested less stress, good growth status, no effects of stress/infectious disease on immunity, and decreased movement. My comparison of HFSs implied that G075F was healthier than G070 with regard to several specific biological functions and overall health.

2.5.3 Applications and Limitations of the Health Function Score System

I found the health function score system a practical tool to evaluate health of individual grizzly bears, but recognized some limitations on its use.

I recognized the following advantages with the system:

1. I could use the health function score system as a screening tool to quickly evaluate individual grizzly bear health, identify bears in poor health, and compare differences of overall health or specific biological functions between grizzly bears.
2. My calculation method ensured HFSs were independent from the effects of sex and capture method. Wells et al. (2004) reported other potentially confounding factors in their health monitoring system for bottle-nose dolphins. The authors were concerned that inter-laboratory variability hampers valid comparisons over time and between different populations. Because we used the same laboratories consistently for each analysis, this concern was not an issue in my M.Sc. project. Further, Wells et al. (2004), who added separately scored variable values into a health grade per animal, also proposed that missing values potentially bias grades downwards. Merged HFSs for grizzly bears, however, contained all constituent variables.
3. Researchers working with other bear populations can easily replicate the health function score system when similar data are available.
4. Because constituent variables reflect similar measures of biological functions in most wild mammals (Cunningham 1992, Feldhamer et al. 2003), the health function score system can be adapted to other wild species.

I identified the following limitations with the health function system:

1. Some variable values changed frequently in response to different stimuli. Because the characteristics of constituent variables determine the accuracy of a HFS, such influences may prevent correct reflection of a biological function. For example, the acute stress response affected total cortisol concentration, irrespective of capture method (Boonstra 2005). In contrast, use of variables robust to capture effect, e.g., measure of serum concentrations of cortisol-binding globulin (Hamilton 2007), hair cortisol (Davenport et al. 2006), and fecal glucocorticoids (Hunt

and Wasser 2003), can provide a score that more accurately reflects long-term measures of stress. Further, capture stress, especially from trap captures, dehydration, and organ dysfunction can influence immunity variables (Cattet et al. 2003b, Latimer et al. 2003). Rather than relying on white blood cell counts, Smits (2007) suggested challenging the immune system to measure an animal's immune competence. Serological analyses after antigen challenge (Lie et al. 2004), lymphocyte proliferation test (Lie et al. 2005), and whole blood chemiluminescence (Papp and Smits 2007) have been used for bears. If practical, challenge protocols could provide useful variables for immunity scores for grizzly bears.

2. There was lack of independence between some constituent variables (e.g., growth variables and white blood cells), which could have biased HFSs. Wells et al. (2004) expressed similar concern in their study of bottle-nose dolphin health. By using correlation and factor analyses, it is possible to reduce variable redundancy (Norman and Streiner 2008). This type of approach could be pursued in future with this health function score system.

3. Even though I selected and weighted constituent variables, and included independent factors and covariates, based on published findings, my choices were still subjective to a certain degree. Another person might make different choices, which may lead to other outcomes of the score and affect the system's replicability.

4. Missing values for constituent variables limited the calculation of HFSs.

2.5.4 Conclusion

In this chapter, I developed a health function score system for grizzly bears using several steps: I identified growth, immunity, movement, and stress as health functions, and then selected two to five constituent variables representative for each health function. I determined if sex or capture method had an effect on constituent variables. I ranked constituent variable values, for

corresponding sub-groups if warranted, and calculated variable percentiles. Percentiles were weighted according to relative qualitative importance of the variable for the health function. By adding weighted percentiles, I calculated scores, ranging from 0.00 to 1.00, for the four health functions. Finally, a score for each health function was assigned to every bear in a given capture. In contrast to using constituent variables, the health function score system enabled quick and easy screening of individual grizzly bear health, identification of bears with reduced health, and comparison of certain biological functions or overall health between grizzly bears. The effect of capture and other stimuli on variable values, lack of independence between some variables, subjective selections of weighted variables, factors, and covariates, and missing variable values, however, constituted limitations of the health function score system.

To assess the usefulness of the health function score system, in chapter 3 I will use the working hypothesis of the FRIGBP as a framework to compare results provided by HFSs with results provided by constituent variable values. I will perform comparative statistical analyses to seek proposed relationships between human-affected landscape condition and stress and between stress and other measures of health.

CHAPTER 3

DETERMINATION OF THE USEFULNESS OF THE HEALTH FUNCTION SCORE SYSTEM

3.1 Abstract

I evaluated the usefulness of the health function score system for grizzly bears (*Ursus arctos*) by seeking proposed relationships between human-affected landscape condition (percent protected home range) and stress and between stress and other measures of health (growth, immunity, and movement). I used statistical and graphical techniques to compare results provided by health function scores with results provided by constituent variable values, using four criteria: (i) strength and direction of association, (ii) influence of sex, capture method, age of bear, and Julian day of capture, (iii) occurrence of outlying observations, and (iv) sample size. Health function scores provided among the strongest associations between percent protected home range and stress and between stress score and growth. Health function scores were unaffected by capture method, sex, and outlying observations. The score system, therefore, likely provided clearer evaluation of relationships in wildlife health than did analyses using constituent variables. Small sample sizes in analyses with health function scores, however, potentially resulted in less statistical power. I found some support for the proposed positive relationship between human-affected landscape condition and stress, but not for inverse relationships between stress and other health functions, by using the score system. Overall, I found the score system to be a useful tool for evaluating relationships in wildlife health.

3.2 Introduction

The persistence of grizzly bears (*Ursus arctos*) in western Alberta is threatened by human activity, including resource extraction, agriculture, urbanization, and recreation (Gibeau et al. 2002, Nielsen et al. 2004b). In 2005, the research team of the Foothills Research Institute Grizzly Bear Program (FRIGBP) hypothesized that long-term physiological stress is the predominant mechanism linking environmental change with impaired health in individual animals and subsequent declines in wildlife population performance (Cattet et al. 2006). Other studies have suggested similar relationships between human activity on the landscape, stress, and health. For example, Wasser et al. (1997) found that logging traffic and timber harvesting increase fecal corticosterone levels of male northern spotted owl (*Strix occidentalis caurina*), Creel et al. (2002) demonstrated that snowmobile activity elevates serum glucocorticoid levels in elk (*Cervus canadensis*), and Walker et al. (2005) proposed that increased adrenocortical activity in Magellanic penguin chicks (*Spheniscus magellanicus)* exposed to ecotourism could impair growth and reproduction status later in life.

To assess the usefulness of the health function score system (chapter 2), I used the working hypothesis of the FRIGBP as a framework to compare and contrast results provided by health function scores (HFSs) with results provided by constituent variable values. Specifically, I conducted comparative statistical analyses directed toward seeking proposed relationships between human-affected landscape condition and stress, and between stress and other measures of health.

3.3 Methods

I used percent protected home range (PPHR) as a measure of landscape condition influenced by human activity. PPHR is a measure of the proportion of an individual grizzly

bear's 95 % Kernel home range that is protected in National Parks or provincially protected areas (Cattet et al. 2006, Hamilton 2007). Three National Parks, five Provincial Parks, and three Wilderness Areas protect 19,928 km^2 (Ross 2002), or approximately 9 % of all grizzly bear habitat in western Alberta (Stenhouse and Graham 2005). PPHR has been considered an indicator of human disturbance levels in grizzly bear home ranges (Boulanger 2005). Protected areas generally are less affected by resource extraction (e.g., oil and gas extraction, forestry, mining, and road development) and associated human-caused grizzly bear mortalities than non-protected areas (Gibeau et al. 2001, Nielsen et al. 2006). I was provided PPHR-values for 101 bears by the FRIGBP.

I used several criteria to compare and contrast the association between PPHR and stress score with associations between PPHR and constituent stress variables (Table 3.1). They were: (i) strength and direction of association, (ii) effect of sex, capture method, age of bear, and Julian day of capture, (iii) occurrence of outlying observations, and (iv) sample size. I evaluated these criteria using statistical and graphical techniques. Multiple linear regression and partial correlation analyses were used to determine strength and direction of associations between PPHR (independent variable) and stress score or constituent stress variable (dependent variable). I determined the effects of age of bear and Julian day by including these as independent variables in the regression model. Scatter plots, as well as results from analyses in Chapter 2, were used to evaluate effects of sex and capture method and occurrence of outlying observations in the analyses. I defined outlying observations as values greater than three standard deviations from the mean, according to Petrie and Watson (2006). If the dependent variable (stress score or constituent stress variable) was affected by age or Julian day of capture, I calculated adjusted values for presentation in scatter plots.

I used the same criteria and approach described above to compare and contrast associations between stress score and other HFSs (growth, immunity, and movement) with associations between stress score and constituent variables of other HFSs (Table 3.1).

Sample size varied between analyses depending on completeness of records. To maintain independence among data points in the analyses, I used only results from the first capture of a bear by the FRIGBP in analyses involving growth score/variable as dependent variable. For analyses with stress score and immunity score/variable as dependent variable, results from multiple captures were used. If multiple captures occurred within the same year, however, I used only results from the first capture. Analyses with movement score/variable as dependent variable used all results. Statistical analysis was performed using SPSS 16.0 for Windows® (SPSS, Inc., Chicago, Illinois). I expected associations would be weak, but biologically significant, because they were affected by several factors not accounted for (related to life-history, environment, and capture). Statistical significance was, therefore, assigned when probability (p) of a Type I error (α) was ≤ 0.10 (Petrie and Watson 2006).

Table 3.1. Constituent variables used to represent health functions for grizzly bears captured by FRIGBP 1999-2007.

Health function	**Constituent variables**				
Growth	Total body mass (kg)	Straight-line body length (cm)	Axillary girth (cm)	Body condition index	
Immunity	Neutrophil count (x 10^9/L)	Lymphocyte count (x 10^9/L)	Monocyte count (x 10^9/L)	Eosinophil count (x 10^9/L)	Globulin (g/L)
Movement	Average daily movement rate, breeding season (m/h)	Average daily movement rate, non-breeding season (m/h)			
Stress	Total cortisol (ng/ml)	Heat shock protein 60 (ng/ml)	Heat shock protein 70 (ng/ml)		

3.4 Results

3.4.1 Comparative Analysis of the Proposed Relationship between Landscape Condition and Stress

I found PPHR was inversely associated with stress score, as well as with total cortisol and heat shock protein 70 (hsp70) (Table 3.2, Figure 3.1 [a], [b], and [d]). The strength and direction of association between PPHR and these three independent variables were similar. PPHR was not associated, however, with heat shock protein 60 (hsp60) (Table 3.2, Figure 3.1 [c]). In general, trap-captured (CMT) bears had less PPHR than free range-captured (CMF) bears, both in the analysis with stress score and analyses with constituent variables ($PPHR_{CMT}$: mean = 7 % [95 % confidence interval = 2-13], n = 51 and 52, $PPHR_{CMF}$: 51 % [41-61], n = 54 and 56, $t \geq 7.41$, $p \leq 0.001$) (Figure 3.1 [a]-[d]). I found, with a few exceptions for hsp70, both total cortisol and hsp70

included much higher values for trap-captured bears than for free range-captured bears (Figure 3.1 [b] and [d]). Results from analyses in chapter 2 (total cortisol – CMT: mean = 86.4 ng/ml [95 % confidence interval = 72.7-100.1], n = 138, CMF: 48.8 ng/ml [39.5-58.1], n = 72, F = 13.67, $p \leq 0.001$, hsp70 – CMT: 3.44 ng/ml [2.89-4.00], n = 123, CMF: 2.20 ng/ml [1.62-2.78], n = 71, F = 8.30, p = 0.004) (section 2.4.1) supported my impression from the scatter plots that capture method had a significant effect on total cortisol and hsp70 concentrations. My impression from Figure 3.1 [a] was that stress scores did not differ between capture methods because they covered a more similar range of values across PPHR for trap- and free range-captured bears than did values of the two constituent variables. This finding was supported by results in chapter 2 (Stress score – CMT: mean = 0.50 [95 % confidence interval = 0.47-0.53], n = 123, CMF: 0.49 [0.44-0.54], n = 71, F = 0.13, p = 0.715) (section 2.4.3). Age influenced stress score, but none of the constituent variables. In contrast to the analysis with stress score, I identified outlying observations in all analyses with constituent variables (Figure 3.1 [b]-[d]). Sample sizes were similar for all analyses (Table 3.2).

Table 3.2. Associations[a] between percent protected home range and stress variables for grizzly bears captured by FRIGBP 1999-2007.

Dependent variable	Final regression model	$r_{partial}$[b]	t (p) [n][c]
Stress score	0.483 - 0.001*%protected + 0.007*Age	-0.24	-2.50 (0.014) [105]
Total cortisol (ng/ml)	87.546 - 0.473*%protected	-0.25	-2.60 (0.010) [108]
Heat shock protein 60 (ng/ml)	No significant model	-0.03	-0.30 (0.762) [108]
Heat shock protein 70 (ng/ml)	3.585 - 0.020*%protected	-0.26	-2.72 (0.008) [108]

[a] Associations between stress score, total cortisol, heat shock protein 60, or heat shock protein 70 (dependent variables) and percent protected home range area (%protected), age of bear (Age), and Julian day of capture (Jday) (independent variables) determined by multiple regression analysis (backward step-down selection model). Statistical significance was assigned when $p \leq 0.10$.

[b] Partial correlation coefficient ($r_{partial}$) for association between percent protected home range and stress variable.

[c] Test-statistic (t), significance level (p), and sample size [n] in partial correlation analysis of percent protected home range and stress variable.

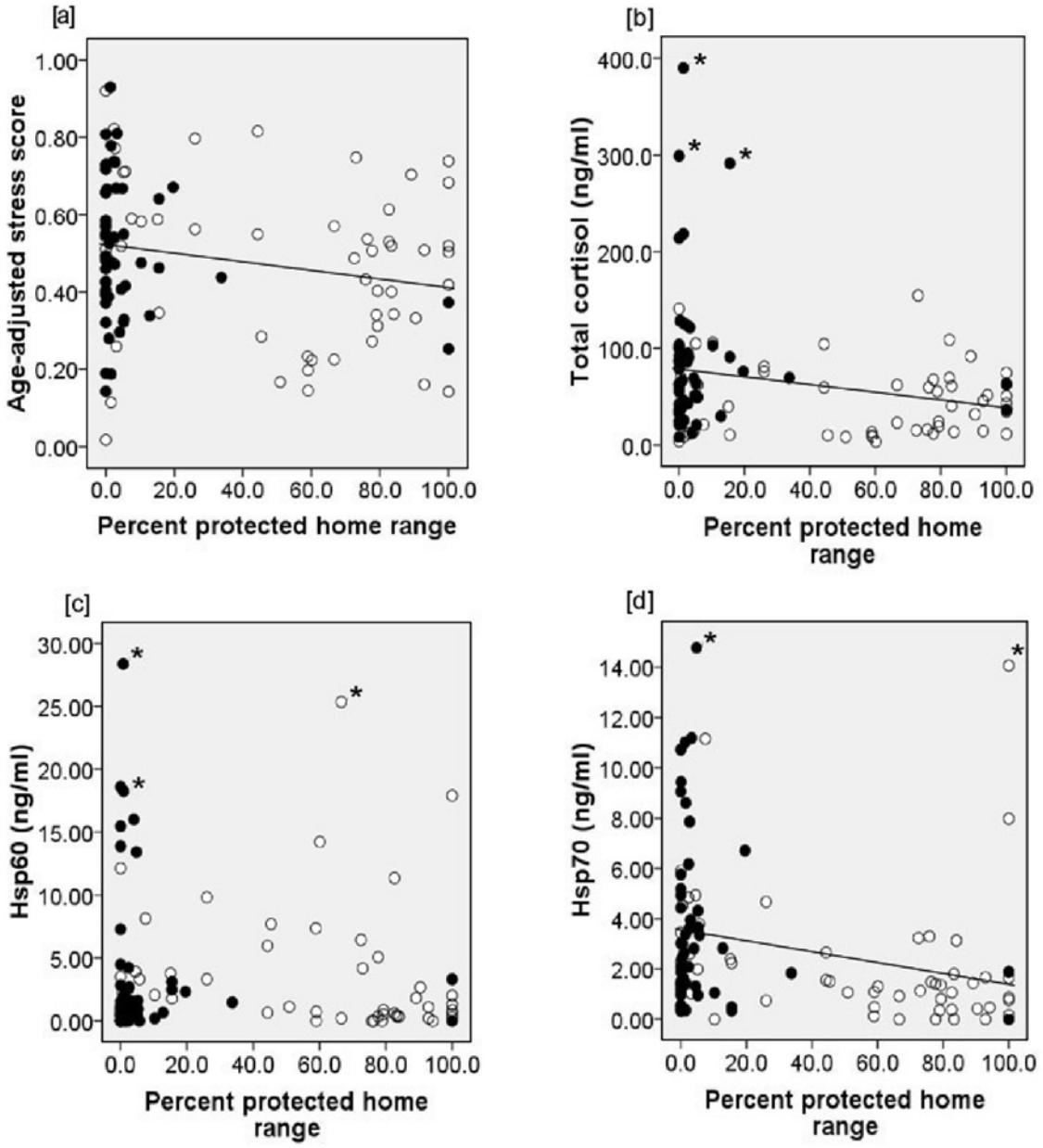

Figure 3.1. Associations between percent protected home range and stress variables in grizzly bears captured free range ○ or captured in traps ● by FRIGBP 1999-2007. Stress variables are [a] age-adjusted stress score and serum concentrations of [b] total cortisol, [c] heat shock protein 60 (hsp60), and [d] heat shock protein 70 (hsp70). Outlying observations (> 3 standard deviations from the mean) are indicated with*****. Linear regression lines are included in plots representing significant associations.

3.4.2 Comparative Analysis of the Proposed Relationship between Stress and Health

I found stress scores were positively associated with growth scores and values for the four constituent growth variables (Table 3.3, Figure 3.2 [a]-[e]). Stress score had stronger associations with growth score and total body mass (TBM) than with other constituent variables. I identified associations of stress score with growth score, TBM, and axillary girth (Ax. girth) for male (M) bears, but not for females (F) (Growth score$_M$: $r_{partial} = 0.38$, $t = 2.58$, $p = 0.014$, $n = 38$, Growth score$_F$: $r_{partial} = 0.19$, $t = 1.31$, $p = 0.198$, $n = 45$; TBM$_M$: $r_{partial} = 0.39$, $t = 2.94$, $p = 0.005$, $n = 46$, TBM$_F$: $r_{partial} = 0.11$, $t = 0.73$, $p = 0.468$, $n = 47$; Ax. girth$_M$: $r_{partial} = 0.30$, $t = 2.36$, $p = 0.022$, $n = 55$, Ax. girth$_F$: $r_{partial} = -0.06$, $t = -0.45$, $p = 0.653$, $n = 51$) (Figure 3.2 [a], [b] and [d]). In contrast, sex did not affect the associations between stress score and straight-line body length (SLBL) or body condition (BCI) (SLBL$_M$: $r_{partial} = 0.12$, $t = 0.91$, $p = 0.364$, $n = 55$, SLBL$_F$: $r_{partial} = 0.10$, $t = 0.72$, $p = 0.476$, $n = 51$, BCI$_M$: $r_{partial} = 0.20$, $t = 1.31$, $p = 0.198$, $n = 39$, BCI$_F$: $r_{partial} = 0.14$, $t = 0.97$, $p = 0.335$, $n = 45$) (Figure 3.2 [c] and [e]). Age affected growth score and all constituent variables. I identified an outlying observation in the analysis with axillary girth (Figure 3.2 [d]), but none in the other analyses. Analyses involving SLBL and axillary girth had the largest sample sizes and the analysis involving growth score the smallest (Table 3.3).

I found stress score was positively associated with neutrophil count, but not with any other immunity variables (Table 3.4, Figure 3.3 [a]-[f]). This association, however, was only evident for trap-captured (CMT) bears, not free range-captured (CMF) bears (Neutrophil count$_{CMT}$: $r_{partial} = 0.28$, $t = 2.97$, $p = 0.004$, $n = 109$, Neutrophil count$_{CMF}$: $r_{partial} = -0.12$, $t = -0.92$, $p = 0.362$, $n = 65$) (Figure 3.3 [b]). Age and Julian day of capture affected immunity score and several constituent variables, but the significance of these effects on dependent variables differed between analyses. I found outlying observations in all analyses involving constituent variables (Figure 3.3 [b]-[f]), but none in the analysis involving immunity score (Figure 3.3 [a]). The

analysis involving globulin concentration included the largest number of samples and the analyses involving immunity score and lymphocyte count the smallest (Table 3.4).

I found stress score was not associated with movement score or with constituent movement variables (Table 3.5, Figure 3.4 [a]-[c]). The scatter plots suggested lack of association was similar for female and male bears (Figure 3.4 [a]-[c]). Julian day of capture influenced movement score and average daily movement rate in non-breeding, but not in breeding, season. Outlying observations were present in the analyses with constituent variables, but absent in the analysis with movement score (Figure 3.4 [a]-[c]). The analysis involving average daily movement rate in breeding season had the largest number of samples, whereas movement score had the smallest (Table 3.5).

Table 3.3. Associations[a] between stress score and growth variables for grizzly bears captured by FRIGBP 1999-2007.

Dependent variable	Final regression model	$r_{partial}$[b]	t (p) [n][c]
Growth score	0.141 + 0.269*Stress + 0.024*Age	0.27	2.61 (0.011) [83]
Total body mass (kg)	46.199 + 65.618*Stress + 5.093*Age	0.26	2.61 (0.011) [93]
Straight-line body length (cm)	139.449 + 14.594*Stress + 1.806*Age	0.17	1.77 (0.080) [106]
Axillary girth (cm)	82.985 + 17.035*Stress + 1.987*Age	0.19	2.05 (0.043) [106]
Body condition index	-0.338 + 1.133*Stress + 0.041*Age	0.21	2.00 (0.048) [84]

[a] Associations between growth score, total body mass, straight-line body length, axillary girth, and body condition index (dependent variables) and stress score (Stress), age of bear (Age), and Julian day of capture (Jday) (independent variables) determined by multiple regression analysis (backward step-down selection model). Statistical significance was assigned when $p \leq 0.10$.
[b] Partial correlation coefficient ($r_{partial}$) for association between stress score and growth variable.
[c] Test-statistic (t), significance level (p), and sample size [n] in partial correlation analysis of stress score and growth variable.

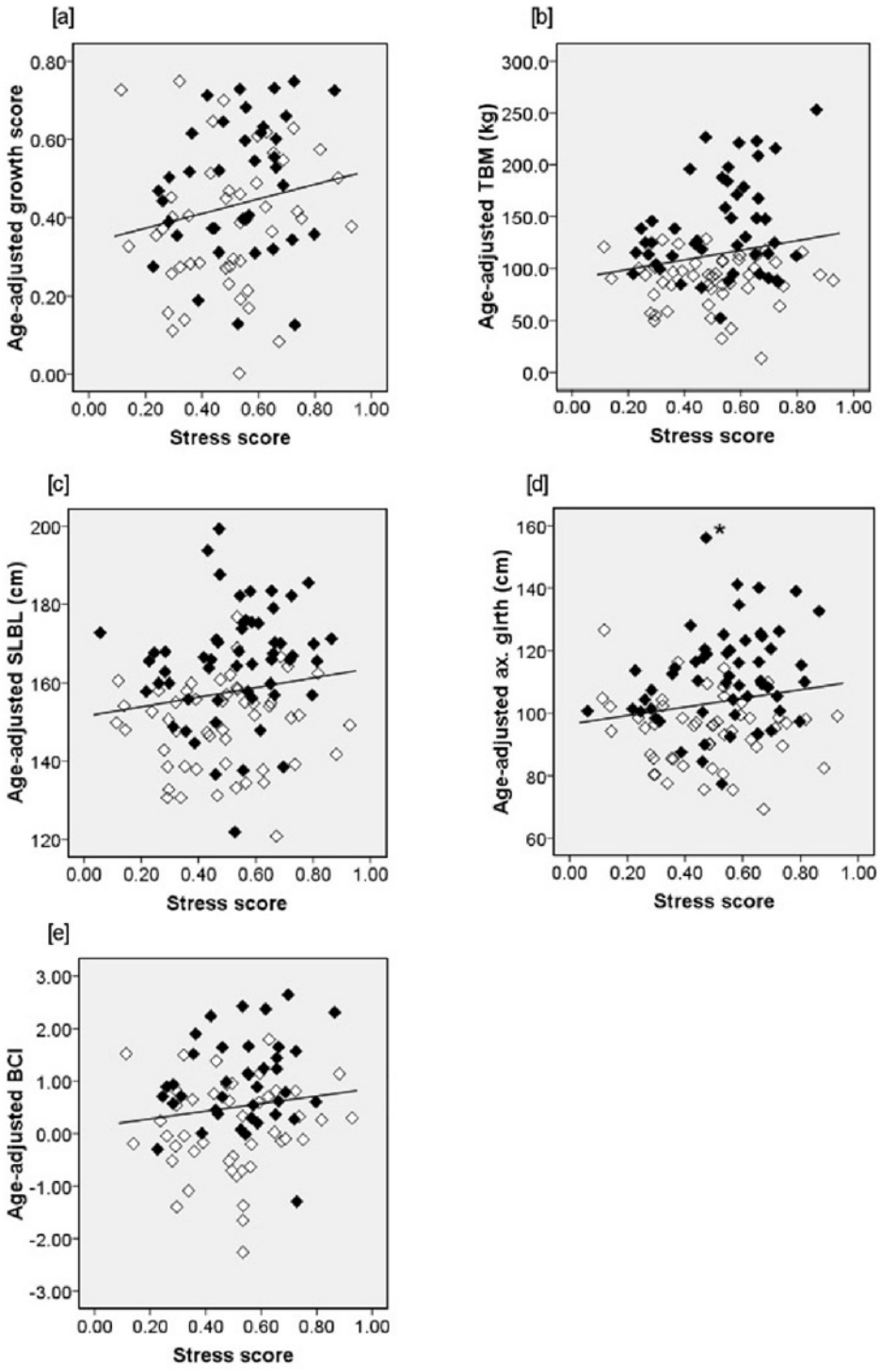

Figure 3.2. Associations between stress score and growth variables in female ◇ and male ◆ grizzly bears captured by FRIGBP 1999-2007. Growth variables are [a] age-adjusted growth score, [b] age-adjusted total body mass (TBM), [c] age-adjusted straight-line body length (SLBL), [d] age-adjusted axillary girth (ax. girth), and [e] age-adjusted body condition index (BCI). Outlying observations (> 3 standard deviations from the mean) are indicated with*****. Linear regression lines are included in plots representing significant associations.

Table 3.4. Associations[a] between stress score and immunity variables for grizzly bears captured by FRIGBP 1999-2007.

Dependent variable	Final regression model	$r_{partial}$[b]	t (p) [n][c]
Immunity score	0.358 + 0.004*Age + 0.001*Jday	0.01	0.12 (0.905) [164]
Neutrophil count (x 10^9/L)	5.812 + 3.733*Stress + 0.016*Jday	0.14	1.87 (0.063) [174]
Lymphocyte count (x 10^9/L)	0.786 + 0.019*Age	0.02	0.25 (0.804) [164]
Monocyte count (x 10^9/L)	No significant model	0.08	1.08 (0.283) [174]
Eosinophil count (x 10^9/L)	0.030 + 0.001*Jday	0.01	0.18 (0.860) [174]
Globulin (g/L)	25.936 + 0.230*Jday	-0.05	-0.66 (0.514) [192]

[a] Associations between immunity score, neutrophil, lymphocyte, monocyte, and eosinophil counts, and serum globulin concentration (dependent variables) and stress score (Stress), age of bear (Age), and Julian day of capture (Jday) (independent variables) determined by multiple regression analysis (backward step-down selection model). Statistical significance was assigned when $p \leq 0.10$.
[b] Partial correlation coefficient ($r_{partial}$) for association between stress score and immunity variable.
[c] Test-statistic (t), significance level (p), and sample size [n] in partial correlation analysis of stress score and immunity variable.

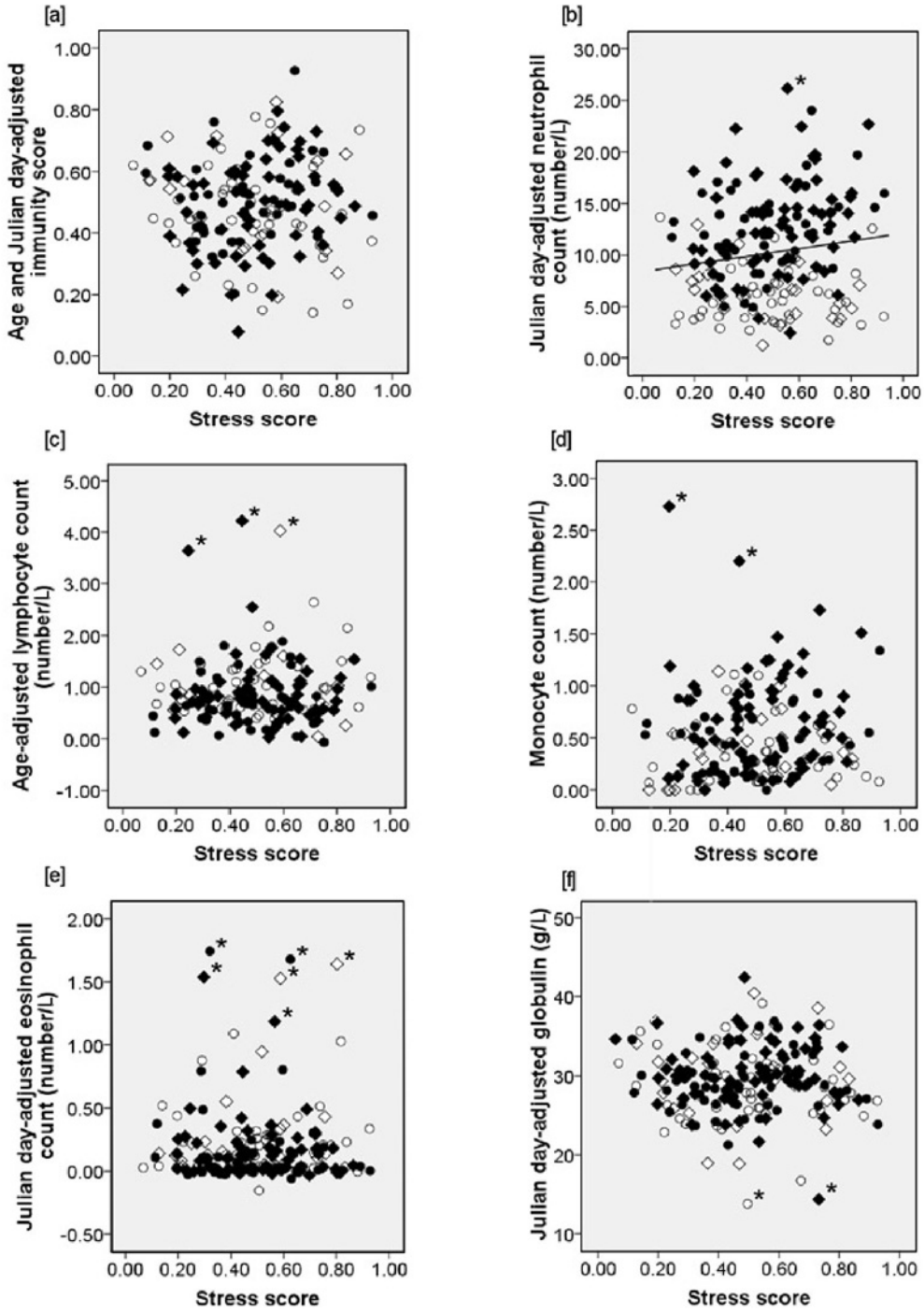

Figure 3.3. Associations between stress score and immunity variables in female grizzly bears captured free range ○, female grizzly bears captured in traps ●, male grizzly bears captured free range ◇, and male grizzly bears captured in traps ◆ by FRIGBP 1999-2007. Immunity variables are [a] age and Julian day-adjusted immunity score, [b] Julian day-adjusted neutrophil, [c] age-adjusted lymphocyte, [d] monocyte, and [e] Julian day-adjusted eosinophil counts (number x 10^9/L), and [f] Julian day-adjusted serum globulin concentration. Outlying observations (> 3 standard deviations from the mean) are indicated with*****. Linear regression line is included in the plot representing a significant association [b].

Table 3.5. Associations[a] between stress score and movement variables for grizzly bears captured by FRIGBP 1999-2007.

Dependent variable	Final regression model	$r_{partial}$[b]	t (p) [n][c]
Movement score	0.663 - 0.001*Jday	-0.15	-1.28 (0.205) [79]
Average daily movement rate, breeding season (m/h)	No significant model	-0.14	-1.33 (0.187) [97]
Average daily movement rate, non-breeding season (m/h)	413.5 - 0.801*Jday	-0.07	-0.67 (0.504) [85]

[a] Associations between movement score, average daily movement rate in breeding and non-breeding season, respectively (dependent variables) and stress score (Stress), age of bear (Age), and Julian day of capture (Jday) (independent variables) determined by multiple regression analysis (backward step-down selection model). Statistical significance was assigned when $p \leq 0.10$.
[b] Partial correlation coefficient ($r_{partial}$) for association between stress score and movement variable.
[c] Test-statistic (t), significance level (p), and sample size [n] in partial correlation analysis of stress score and movement variable.

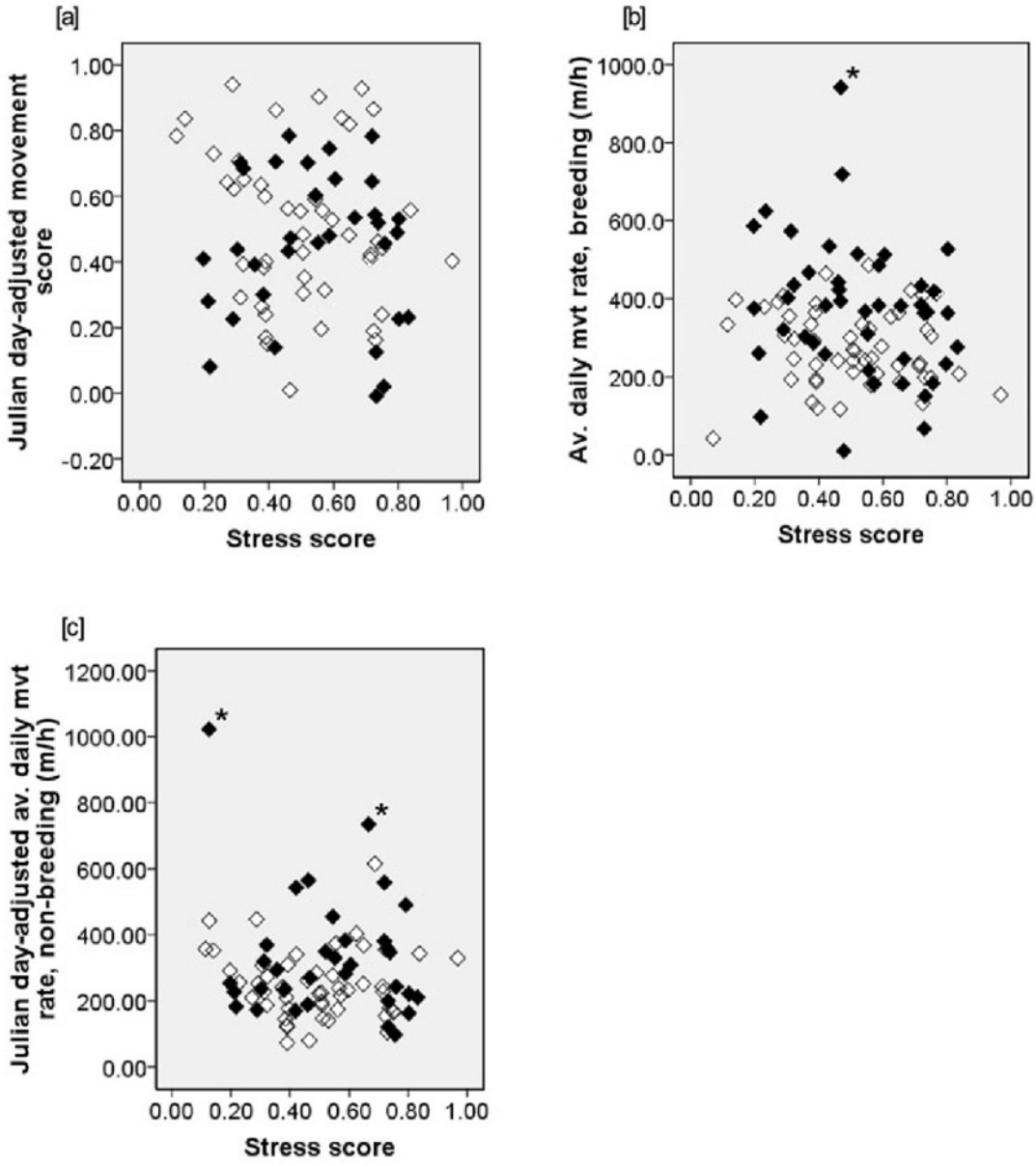

Figure 3.4. Associations between stress score and movement variables in female ◇ and male ◆ grizzly bears captured by FRIGBP 1999-2007. Movement variables are [a] Julian day-adjusted movement score, [b] average daily movement rate, breeding season (av. daily mvt rate, breeding), and [c] Julian day-adjusted average daily movement rate, non-breeding season (av. daily mvt rate, non-breeding). Outlying observations (> 3 standard deviations from the mean) are indicated with*****.

3.5 Discussion

3.5.1 Usefulness of the Health Function Score System

I found the HFSs to be useful for evaluating relationships proposed in the working hypothesis of the FRIGBP because they were not influenced by capture method, sex, or outlying observations. I base this conclusion on my findings under four criteria as follows:

(i) Strength and direction of association: I found HFSs provided among the strongest associations between PPHR and stress and between stress score and growth. This supported use of HFSs for identifying relationships in wildlife health. Neutrophil count, however, provided the only association between stress score and immunity.

My finding that the direction of association was similar between HFSs and constituent variables suggested HFSs accurately reflected biological functions when evaluating associations in wildlife health.

(ii) Effects of factors: I found capture method likely influenced analyses of PPHR and two constituent stress variables. Stress perception from anthropogenic environmental change (Chruszcz et al. 2003, Hamilton 2007) may have increased stress score and variable values in bears with less protected home ranges. The majority of bears with no or little percent protected home range, however, were captured with traps, which may have elevated their total cortisol and hsp70 concentrations further (Hernández-Jáuregui et al. 2005, Hamilton 2007). Moreover, I found the association between stress score and neutrophil count was based on observations for trap-captured, but not free range-captured, bears. Greater stress response (Latimer et al. 2003, Iossa et al. 2007) or prolonged time interval between onset of stress and collection of blood (Cattet et al. 2003b, Hobson 2005) in trap-captured bears probably explained the effect of capture method on neutrophil count and its association with stress score. In contrast, the effect of capture method was removed in analyses with stress score and immunity score as dependent variables.

HFSs, therefore, likely provided clearer evaluations of relationships between PPHR and stress and between stress and immunity.

Scatter plots and results in chapter 2 (sections 2.4.1 and 2.4.3) suggested the effect of sex on constituent growth variables, i.e., higher values for males than females, was removed in growth score. I found sex influenced the associations between stress score and growth score, body mass, and axillary girth, respectively. My finding that the association between stress score and growth score was based on male bears demonstrated that HFSs, unbiased with respect to sex, can be used to detect different association patterns between females and males. In other words, we may be able to identify other factors than sexual dimorphism that would explain association patterns in growth for females and males (Schwartz et al. 2003). For example, Derocher and Wiig (2002) compared sexes when they discussed a potential association between growth and pollution load in different polar bear (*Ursus maritimus*) populations.

Factors that were not accounted for in this study, including genetic make-up, inherent habitat and food quality, reproductive status, social structure, population density, migration, local climate, and topography (Schwartz et al. 2003, Wobeser 2006), may have influenced HFSs. Such factors should be considered to avoid erroneous interpretation of wildlife health (Boulanger 2005, Petri and Watson 2006). For example, my findings of positive associations between stress score and growth contradicted findings in the literature. Long-term, systemic (Wingfield and Raminofsky 1999, Sjaastad et al. 2003) and cellular (Feder and Hoffman 1999) stress is known to have negative effects on growth. Confounding factors may have masked a more clear evaluation of the relationship between stress and growth scores. For example, I cannot rule out an association between (i) better growth because of higher productivity and nutritional quality of foods (Schwartz et al. 2003, Munro et al. 2006) and (ii) greater perception of stress due to higher

level of anthropogenic disturbance (Chruszcz et al. 2003, Garshelis et al. 2005) in bears captured at lower elevation.

Further, capture-related stress may have influenced stress score by its effect on constituent stress variables. For example, cortisol concentration starts to increase within three minutes after the initiation of capture or pursuit, irrespective of capture method (Boonstra 2005). In addition, I found serum hsp70 concentration was increased in trap-captured bears possibly due to prolonged stress and physical exertion (Fleshner and Johnson 2005, Hamilton 2007). Hence, inclusion of stress variables robust to capture-related stress would ensure that stress score only reflected long-term stress (section 2.5.3). To improve the evaluation of the association between stress and immunity, immunity score would preferably consist of variables unaffected by capture-related stress. Nevertheless, alternative immunity measures, e.g., serological analyses (Silberman et al. 2003), lymphocyte proliferation test (Hangalapura et al. 2004), whole blood chemiluminescence assay (McLaren et al. 2003), and cutaneous delayed type hypersensitivity test (Dhabhar 1998) may also be influenced by acute stress. From the standpoint of further reducing the effect of capture-related stress on immunity score, replacing current immunity variables with these challenge-based measures may not be warranted.

(iii) Effect of outlying observations: I identified outlying observations in analyses with constituent variable values. These may distort the outcome of the analyses (Petrie and Watson 2006). For example, I found two outlying observations prevented a significant positive association between stress score and monocyte count for trap-captured bears (monocyte count without outlying observations: $r_{\text{partial}} = 0.21, t = 2.23, p = 0.028, n = 107$). Further, the association pattern changed direction from negative to positive in the analysis with stress score and average daily movement rate, non-breeding season after I removed the most extreme of the two outlying

observations (data not shown). In contrast, outlying observations were absent in analyses with HFS as dependent variable because I had standardized the HFSs.

(iv) Effect of sample size: I found analyses involving HFSs consistently had the smallest sample sizes. Sample size is closely associated with the statistical power of an analysis, i.e., the chance to detect a statistically significant difference between groups if it exists. Generally, greater sample size results in greater power (Petrie and Watson 2006). Sample sizes were similar in analyses of PPHR and stress score and constituent variable values. Nevertheless, in other analyses with HFS as dependent variable, smaller sample sizes potentially may have contributed to less statistical power than in analyses with constituent variable values. Other factors than sample size, e.g., variability among observations and magnitude of treatment effect (Olsen 2003), however, also affect statistical power. I, therefore, considered this criterion to be of minor importance for the comparisons of association results.

3.5.2 Conclusion

In this chapter, I used statistical and graphical techniques to determine the usefulness of the health function score system for grizzly bears. I compared and contrasted results provided by HFSs with results provided by constituent variable values in analyses aimed at seeking proposed relationships between human-affected landscape condition (PPHR) and stress, and between stress and other health functions (growth, immunity, and movement). I used the following criteria: (i) strength and direction of association, (ii) influence of sex, capture method, age of bear and Julian day of capture, (iii) occurrence of outlying observations, and (iv) sample size. HFSs provided among the strongest associations between PPHR and stress and between stress score and growth, whereas neutrophil count provided the only association between stress score and immunity. Capture method, sex, and outlying observations did not affect HFSs. Hence, the health function

score system likely provided clearer evaluations of relationships in wildlife health than values of constituent variables did. Analyses with HFS had the smallest sample sizes, which potentially could have resulted in less statistical power compared to analyses with constituent variables. Nevertheless, HFSs appeared more useful than constituent variables to evaluate relationships between landscape condition, stress, and other health functions.

Stress score provided the clearest evidence of an association between PPHR and stress. This finding provided some support for the proposed positive relationship between human-affected landscape condition and stress. In contrast, I found no support for the proposed inverse relationships between stress and growth, immunity, and movement. Factors other than the ones I removed in the analyses may have masked such relationships, e.g., elevation (between stress and growth) or capture-related stress. Independence of capture effect would improve the possibilities to confirm relationships between stress and other health functions, if such relationships exist.

CHAPTER 4

GENERAL DISCUSSION

4.1 Introduction

Human activities, including resource extraction, agriculture, urbanization, and recreation, are threatening grizzly bears (*Ursus arctos*) in western Alberta (Gibeau et al. 2002, Nielsen et al. 2004b). The Foothills Research Institute Grizzly Bear Program (FRIGBP) therefore is evaluating effects of landscape change on grizzly bear health. Assessment of individual grizzly bear health based on stress and other biological functions may enable detection of negative changes in individuals before population performance is impaired (Walker et al. 2005, Wikelski and Cooke 2006). With this knowledge, resource managers can prevent adverse effects on grizzly bears when planning development on the landscape (Stenhouse and Graham 2005, Cattet et al. 2006).

The aim of the work presented in this thesis was to measure individual health in grizzly bears within the FRIGBP study area in western Alberta. The data set available for this study, however, was large and included incomplete records, records from repeated captures, and health variables that were influenced by sex, age of bear, and capture. To facilitate assessment of individual grizzly bear health, I developed a health function score system based on merged biological information from bears captured by FRIGBP 1999-2007 (chapter 2). I calculated individual scores for each of four health functions (i.e., growth, immunity, movement, and stress) by adding ranked and weighted percentiles of two to five constituent variables. I found the health function score system to be a practical screening tool to assess individual bear health, identify bears with reduced health, and compare health profiles between bears. I determined the

usefulness of the score system by evaluating relationships presumed to exist under the working hypothesis of the FRIGBP (chapter 3). I found the score system to be more useful than constituent variables for evaluating relationships between landscape condition, stress, and other health functions because health function scores (HFSs) were unaffected by capture method, sex, and outlying observations.

4.2 Improvement of the Health Function Score System

Although I found the health function score system a quick and useful tool to evaluate health in individual grizzly bears and relationships in wildlife health, the following improvements would increase its usefulness:

1. A reproduction score would provide further insight into overall health because this health function is directly linked to population performance (Hilderbrand et al. 1999, Garshelis et al. 2005). Measures of serum (Feldman and Nelson 1996), fecal (Wasser et al. 1996), and urine (Lasley and Shideler 1993) sex hormone concentrations, evaluation of female and male reproductive organs with ultrasonography, and investigation of sperm quality (Feldman and Nelson 1996) could provide potential constituent variables for a reproduction score.
2. The removal of effects of additional confounding factors on HFSs would enhance the score system. I removed the effects of sex and capture method when I calculated the scores. Other factors, however, could influence HFSs and their use in analyses of wildlife health. For example, the physiological circadian pattern of total cortisol concentration (Reeder and Kramer 2005), other environmental stressors besides human-affected landscape condition (including severe weather events, predation, and disease), and unpredictable changes in the social environment (intruders in the home range and changes in dominance status) (McEwen and Wingfield 2003, Reeder and Kramer 2005) may influence stress. Organ failure and dehydration can affect

immunity (Tizard 1996, Stockham and Scott 2002), genotype and food productivity may affect growth (Schwartz et al. 2003), and habitat quality, dispersal, and reproductive status can influence movement (Schwartz et al. 2003, Munro et al. 2006). More extended or advanced statistical models than I used in this study, such as multiple regression models and factor analyses (Norman and Streiner 2008), could identify and largely account for effects of many confounding factors on HFSs.

I accounted for differences in capture method, but the effect of capture in general on stress (Boonstra 2005) and immunity (Latimer et al. 2003) scores may obscure influences of other stressors. Replacing constituent variables affected by capture-related stress with variables that are not could remove this effect. In fact, the research team of the FRIGBP currently is developing alternative techniques to measure stress unaffected by capture and handling, which can be used in an improved health function score system. These techniques include assessment of cortisol-binding globulin concentration in serum (Hamilton 2007), stress proteins in skin and muscle, using an antibody-based microarray (Cattet et al. 2006), and corticosteroid concentrations in hair (Davenport et al. 2006).

3. The removal of dependent and redundant variables in HFSs would further improve the score system. I found associations between constituent variables within growth, immunity, and movement. Lack of independence between variables may hamper the application of a score system (Wells et al. 2004). With correlation and factor analyses (Norman and Streiner 2008) it would be possible to identify independent, or less dependent, variables and thereby reduce dependency and redundancy among constituent variables.

4.3 Potential Applications and Limitations of the Health Function Score System

I believe the health function score system has many applications in wildlife health, but also recognize certain limitations.

I found the following applications for the score system:

1. The health function score system provides a quick screening of health in individual bears and identification of bears with reduced health. The score system could also be used to identify differences in individual health between years and reproductive class and to identify potential effects of repeated captures. It was possible to compare HFSs within a bear and between bears because I had standardized the scores. This would also enable comparison of HFSs in bears from different populations, even if the populations were handled in different ways.
2. Results provided by the score system could serve as the health variables when relationships between health and landscape condition and change are evaluated. For example, the FRIGBP research team is integrating Geographic Information System (GIS), geospatial, location, and health data to develop predictive models and, eventually, to generate maps similar in format to resource selection function maps (Nielsen et al. 2002). These maps will show the relative probability of healthy vs. unhealthy grizzly bear occurrence on the landscape. Over time, the research team will assess changes in health functions of bears inhabiting landscapes undergoing different rates of human-caused alteration. Further, the research team should be able to forecast where in Alberta are grizzly bear populations most likely to persist or disappear by incorporating demographic measures in the analyses (Boulanger 2005, Cattet et al. 2006).
3. The health function score system can be adapted and applied to other wild species. For example, in Alberta and elsewhere, resource extraction threatens the conservation of woodland caribou (*Rangifer tarandus caribou)* (Dzus 2001), wolverine (*Gulo gulo*) (Carroll et al. 2001), and swift fox (*Vulpes velox*) (Alberta Swift Fox Recovery Team 2007).

I identified the following limitations of the score system:

1. It is difficult to determine more precisely where an animal sits on the health-disease continuum because there are no comparative baseline data for the HFSs. Instead, the ranked HFSs are relative measures of health.
2. It is not possible to use the score system to identify outlying observations because I had ranked variable values to standardize the HFSs. Wells et al. (2004) proposed that changes of the proportion of outlying individual health grades over time may provide sensitive assessment of population trends. They suggested this because the outliers themselves may represent potential sentinels of environmental problems. Their health grades, however, were based on added point scores for each physiological parameter, which allowed for identification of outlying observations.
3. The replicability of the score system, and the comparison between HFSs provided by different studies, may be hampered by the weighting process. I introduced subjectivity in the calculation process, even though I weighted constituent variables largely based on literature. In other studies, interpretation and experience of the relative qualitative importance of constituent variables may differ or be lacking. This could lead to different scores for similar variable percentiles.
4. The score system would not provide a complete picture of the overall health in studies with a limited number of variables. The overall health is based on information from all health functions. Feasibility, logistics, and cost of sampling and analyzing data for constituent variables, however, may limit the number of health functions examined.

4.4 Future Directions

We can improve our understanding of grizzly bear health, and more generally wildlife health, by using the health function score system. I recommend the following actions to increase

the accuracy and usefulness of the system:

1. Identification of feasible reproduction variables for grizzly bears to be applied in a reproduction score.
2. Development of practical measures of immunity robust to capture-related stress to be included in the immunity score.
3. Use of statistical procedures to remove effects of additional confounding factors on HFSs and remove redundant variables in HFSs.
4. Utilization of HFSs in predictive models and maps showing the relative probability of healthy vs. unhealthy grizzly bear occurrence on the landscape.
5. Application of the health function score system to other species.

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APPENDIX A

WEIGHTING OF CONSTITUENT VARIABLES

Constituent variables may be of different importance to a score reflecting a certain health function (Wells et al. 2004). Hence, when the health function scores (HFSs) for grizzly bears were calculated, I weighted percentiles according to the relative qualitative importance of the particular variable to the HFS (section 2.3.3.2). This method was based on a ranking process developed by Saaty (1977). A nine point continuous scale was used in a subjective pairwise comparison of variables (Table A.1). I determined the relative importance of paired variables (section 2.3.2) based on published data and experience. I first recorded the weight, k, on the variable that was more important (a) of the two to the HFS (Tables A.2-5). The least important variable (b) was then given the inverse weight, $1/k$, representing that this latter variable was only $1/k$ as important as the former to the score. If no differences between the variables could be established, both variables were assigned a weight of 1. For each health function, I summed weights ($\sum w$) across rows and calculated proportional weights (w) for each constituent variable.

Table A.1. The weighting scale for constituent variables with definitions and explanations. Adapted from Saaty (1977).

Definition	Explanation	w[a]
Equal or unknown importance	Two variables contribute equally to the objective, or the relationship between the two variables is unknown	1
Weak importance of one over another	Experience and judgement slightly favour one variable over another	3
Essential or strong importance	Experience and judgement strongly favour one variable over another	5
Demonstrated importance	A variable is strongly favoured and its dominance is demonstrated in practice	7
Absolute importance	The evidence favouring one variable over another is of the highest possible order of affirmation	9
Intermediate values between the two adjacent judgements	Intermediate values between two adjacent judgements	2, 4, 6, 8
Reciprocal values	If variable *a* is assigned a value of $k = 5$ when compared with variable *b*, then *b* is assigned 1/5 when compared with *a*	

[a] w = weight.

Table A.2. Weights (w) for constituent variables in growth.

	Variable comparison				$\sum w^b$	w^c
Variables[a]	TBM	SLBL	Ax. girth	BCI		
TBM	1	2	5	1/5	8.20	0.24
SLBL	1/2	1	5	1/5	6.70	0.20
Ax. Girth	1/5	1/5	1	1/6	1.57	0.05
BCI	5	5	6	1	17.00	0.51
Total					33.47	1.00

[a] Variables are total body mass (TBM), straight-line body length (SLBL), axillary girth (Ax. girth), and body condition index (BCI).
[b] Summed weights.
[c] Proportional weights.

Table A.3. Weights (w) for constituent variables in immunity.

	Variable comparison					$\sum w^b$	w^c
Variables[a]	Neutro.ct	Lympho.ct	Mono.ct	Eos.ct	Globulin		
Neutro.ct	1	2	5	5	3	16.00	0.35
Lympho.ct	1/2	1	5	4	3	13.50	0.29
Mono.ct	1/5	1/5	1	1/2	1/5	2.10	0.05
Eos.ct	1/5	1/4	2	1	1/4	3.70	0.08
Globulin	1/3	1/3	5	4	1	10.67	0.23
Total						45.97	1.00

[a] Variables are neutrophil count (Neutro.ct), lymphocyte count (Lympho.ct), monocyte count (Mono.ct), eosinophil count (Eos.ct), and serum globulin concentration (Globulin).
[b] Summed weights.
[c] Proportional weights.

Table A.4. Weights (w) for constituent variables in movement.

	Variable comparison		$\sum w^a$	w^b
Variables	Average daily movement rate, breeding season	Average daily movement rate, non-breeding season		
Average daily movement rate, breeding season	1	1	2	0.50
Average daily movement rate, non-breeding season	1	1	2	0.50
Total			4.00	1.00

[a] Summed weights.
[b] Proportional weights.

Table A.5. Weights (w) for constituent variables in stress.

	Variable comparison			$\sum w^b$	w^c
Variables[a]	Total cortisol	Hsp60	Hsp70		
Total cortisol	1	5	3	9.00	0.57
Hsp60	1/5	1	1/4	1.45	0.09
Hsp70	1/3	4	1	5.33	0.34
Total				15.78	1.00

[a] Variables are serum concentrations of total cortisol, heat shock protein 60 (Hsp60), and heat shock protein 70 (Hsp70).
[b] Summed weights.
[c] Proportional weights.

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APPENDIX B

EVALUATION OF CAPTURE WITH CULVERT TRAP BY COMPARISON WITH LEG-HOLD SNARE IN THE FOOTHILLS RESEARCH INSTITUTE GRIZZLY BEAR PROGRAM: EFFECTS ON GRIZZLY BEAR HEALTH AND WELFARE

B.1 Abstract

As a research project within the Foothills Research Institute Grizzly Bear Program, I evaluated health and welfare effects of capture with culvert trap by comparison of physiological data and physical injuries between grizzly bears (*Ursus arctos*) captured with culvert trap and grizzly bears captured with leg-hold snare. I found that both capture methods can have negative short- and long-term effects on health and welfare. Bears captured with culvert trap were less likely to develop muscle, joint, and bone injuries, and capture myopathy than bears captured with leg-hold snare, but were more likely to develop injuries to teeth and gums. Irrespective of capture method, bears were affected by capture-related stress, acid-base imbalance, and mild dehydration. To prevent mouth injury, culvert traps can be designed with smooth interiors with nothing for contained animals to bite. Quick attendance to captured bears may decrease adverse effects of stress and exertion and risk for injury.

B.2 Introduction

Since 1999, the Foothills Research Institute Grizzly Bear Program (FRIGBP) has been conducting research on grizzly bear (*Ursus arctos*) populations, health, and habitat conditions in

western Alberta to provide resource managers with tools to integrate grizzly bear "needs" into land management decisions. To acquire health, movement, and home range data, the FRIGBP research team captures 15 to 40 grizzly bears annually. Because of remote research locations, often with extensive tree cover, many of these bears are captured with baited spring-activated leg-hold snares. Upon activation of the spring, the snare is tightened around a lower limb, restraining the bear. Although considered a humane capture method for carnivores by many wildlife specialists (Powell 2005, Iossa et al. 2007), FRIGBP researchers have identified several welfare concerns with use of leg-hold snare. These include visible physical injuries (Lemieux and Czetwertynski 2006, Cattet et al. 2008b), muscle injury and adverse physiological responses indicated by serum biochemistry and hematology (Cattet et al. 2003b, Powell 2005), and sub-normal movement rates for 2-3 weeks following capture (Cattet et al. 2008a). With this knowledge, the FRIGBP research team is striving to minimize negative effects of capture on grizzly bear health and welfare.

As one alternative to using leg-hold snare, the research team is also capturing grizzly bears using culvert traps. A culvert trap consists of a metal cylinder, into which the bear enters through an opening, attracted by bait. By moving the bait, the bear triggers closure of the trap door. Animals captured by culvert trap are contained, but not restrained, and appear to undergo less trauma than animals captured by snares and other leg-restraining traps (Powell and Proulx 2003, Iossa et al. 2007). Iossa et al. (2007) concluded the generally low number of physical injuries and lower level of stress caused by culvert trap capture favor this capture method over other trap capture techniques. Additionally, in contrast to capture by leg-hold snare, bears captured in culvert trap are protected from attack by other animals, and non-target species can be released without chemical immobilization and handling (Iossa et al. 2007).

In this study, I evaluated health and welfare effects of capture with culvert trap by

comparison of physiological data and physical injuries between grizzly bears captured with culvert trap and grizzly bears captured with leg-hold snare.

B.3 Methods

To meet objectives of the FRIGBP, physiological, sex, and age data were collected, and capture-related physical injuries were documented in grizzly bears captured with culvert trap (CT) and leg-hold snare (LS) (Aldrich Snare Co., Clallam Bay, Washington) in western Alberta between May 1999 and September 2007. With both methods, bears were captured up to 24 hours prior to chemical immobilization. Complete blood cell counts, serum biochemistry panels, and serum levels of stress biomarkers were analyzed subsequently (For complete details of capture, data collection, and laboratory analyses; see section 2.3.1).

I compared health data from 21 grizzly bears captured by CT with a random subset of health data from 20 grizzly bears captured by LS. Both capture groups were similar with respect to capture year, but there were differences in sex (female = F, male = M) and age (juvenile = J [< 5 years old], adult = A [≥ 5 years old]) composition between groups (CT – F_J: 5 bears, F_A: 5, M_J: 9, M_A: 2, LS – F_J: 2, F_A: 4, M_J: 7, M_A: 7). Not all data were available for all bears. To maintain independence among data points, I used only data from the first capture of a bear within a given year. Mann-Whitney U-test was used to compare physiological data between the two methods of capture (Petrie and Watson 2006). Because Cattet et al. (2003a) reported serum glucose concentration in grizzly bears differed due to immobilization drug combination, I compared serum glucose concentrations only from bears immobilized with xylazine hydrochloride and zolazepam hydrochloride + tiletamine hydrochloride, whereas I included results from all bears irrespective of drug combination (section 2.3.1) for the other analyses. I investigated if changes in the leukogram were correlated with capture-related stress or physical exertion and muscle

damage by calculating the Spearman correlation coefficient (Petrie and Watson 2006). SPSS 16.0 for Windows® (SPSS, Inc., Chicago, Illinois) was used for all statistical analyses, and statistical significance was assigned when the probability (p) of a type I error was ≤ 0.05. I report results from the Mann-Whitney U-test as median and minimum and maximum values and from the correlation analyses as Spearman correlation coefficient (r_s). For select physiological variables, I compared values with reference intervals for captive brown bears (Teare 2002). These reference intervals are appropriate for wild bears, as demonstrated in Cattet et al. (2008a). Lymphocyte and eosinophil counts were compared with minimum and maximum values for captive brown bears (Teare 2002) instead of reference intervals. I categorized acute capture-related physical injuries as follows: [i] no visible injury, [ii] swollen body part or superficial cut/scrape, [iii] claw injury, exposed pulp, [iv] deep laceration, exposed muscle/bone, [v] tooth injury, and [vi] bone fracture or loss of foot/toe.

B.4 Results

B.4.1 Physiological Measures

I found total white blood cell (WBC) and neutrophil counts (NC) were higher in bears captured by LS than bears captured with CT (Table B.1). WBC and NC exceeded the upper limit of reference intervals for captive brown bears (WBC: 13.25 x 10^9/L, NC: 9.50 x 10^9/L) in 11 out of 16 (11/16) and 12/15 LS bears and in 3/10 and 4/9 CT bears, respectively. Other white blood cell counts did not differ significantly between the two capture groups. Both lymphocyte and eosinophil counts, however, were low in several LS and CT bears, with lymphocyte count below the minimum value (0.36 x 10^9/L) in 3/15 LS bears and 0/9 CT bears and eosinophil count below the minimum value (0.04 x 10^9/L) in 11/15 LS bears and 4/9 CT bears. I found serum activities of creatine kinase (CK) and aspartate aminotransferase (AST) were higher in LS than in CT bears

(CK: 1109 U/L vs. 121 U/L, $U = 30.00$, $Z = -3.41$, $p = 0.001$, AST: 207 U/L vs. 102 U/L, $U = 41.50$, $Z = -2.74$, $p = 0.006$) (Figure B.1). CK concentrations in 13/16 LS bears and 2/14 CT bears, and AST concentrations in 10/16 LS bears and 2/13 CT bears, exceeded the upper limit of the reference intervals (CK: 387 U/L, AST: 142 U/L). In contrast, alanine aminotransferase and α-glutamyltransferase concentrations did not differ between capture methods (Table B.2). Bicarbonate concentration tended to be lower in LS bears than in CT bears, but anion gap did not differ between capture groups. Bicarbonate concentration was at the low end of the reference interval in several bears in both capture groups and below the lower limit (15.0 mmol/L) in 6/16 LS and 2/14 CT bears. Compared to findings in Cattet et al. (2003b), anion gap was high (≥ 23) in a majority of bears, irrespective of capture method (LS: 12/16, CT: 9/14). Serum electrolyte, urea, creatinine, total protein, and glucose concentrations did not differ by capture method. Nevertheless, sodium (S), chloride (C), and urea (U) concentrations exceeded the upper limit of the reference intervals (S: 146 mmol/L, C: 111 mmol/L, and U: 11.4 mmol/L) in a small proportion of bears (CT: 4/14, 4/14, and 5/14, LS: 4/16, 2/16, and 3/16). I found no significant differences in total cortisol and heat shock protein 70 (hsp70) concentrations between capture groups. Serum values of total cortisol and hsp70 from captive bears were not available for comparison.

Among all bears, WBC and NC were correlated with CK (WBC: $r_s = 0.43$, $p = 0.028$, $n = 26$, NC: $r_s = 0.51$, $p = 0.011$, $n = 24$) and AST (WBC: $r_s = 0.49$, $p = 0.013$, $n = 25$, NC: $r_s = 0.60$, $p = 0.002$, $n = 24$). In contrast, WBC and NC did not correlate with total cortisol (WBC: $r_s = -0.04$, $p = 0.833$, $n = 26$, NC: $r_s = -0.09$, $p = 0.688$, $n = 24$). Hsp70 tended to correlate with WBC, NC, and CK (WBC: $r_s = 0.36$, $p = 0.103$, $n = 22$, NC: $r_s = 0.38$, $p = 0.095$, $n = 20$, CK: $r_s = 0.36$, $p = 0.082$, $n = 24$), but not with AST ($r_s = 0.27$, $p = 0.216$, $n = 23$).

Table B.1. Comparison of white blood cell counts[a] between grizzly bears captured by culvert trap and grizzly bears captured by leg-hold snare for the FRIGBP 1999-2007.

Variable	Culvert trap	Leg-hold snare	*U*, *Z*, *p*[b]
White blood cell count (x 10^9/L)	11.21 (8.30-21.80) [10]	14.70 (6.60-25.50) [16]	40.00, -2.11, 0.035
Neutrophil count (x 10^9/L)	9.13 (6.04-19.62) [9]	13.71 (5.61-23.29) [15]	33.00, -2.06, 0.040
Lymphocyte count (x 10^9/L)	1.02 (0.54-1.45) [9]	0.73 (0.24-3.83) [15]	49.00, -1.10, 0.270
Eosinophil count (x 10^9/L)	0.08 (0.00-1.53) [9]	0.00 (0.00-0.59) [15]	47.00, -1.41, 0.160
Monocyte count (x 10^9/L)	0.71 (0.08-1.14) [9]	0.55 (0.15-1.53) [15]	54.50, -0.77, 0.438

[a] Values reported as median, minimum and maximum values in round brackets, and sample size in square brackets. Statistical comparison made by Mann-Whitney U-test with culvert trap and leg-hold snare as groups. Statistical significance was assigned when $p \leq 0.05$.
[b] Test statistic reported as *U*, *Z*-value as *Z*, and significance level as *p*.

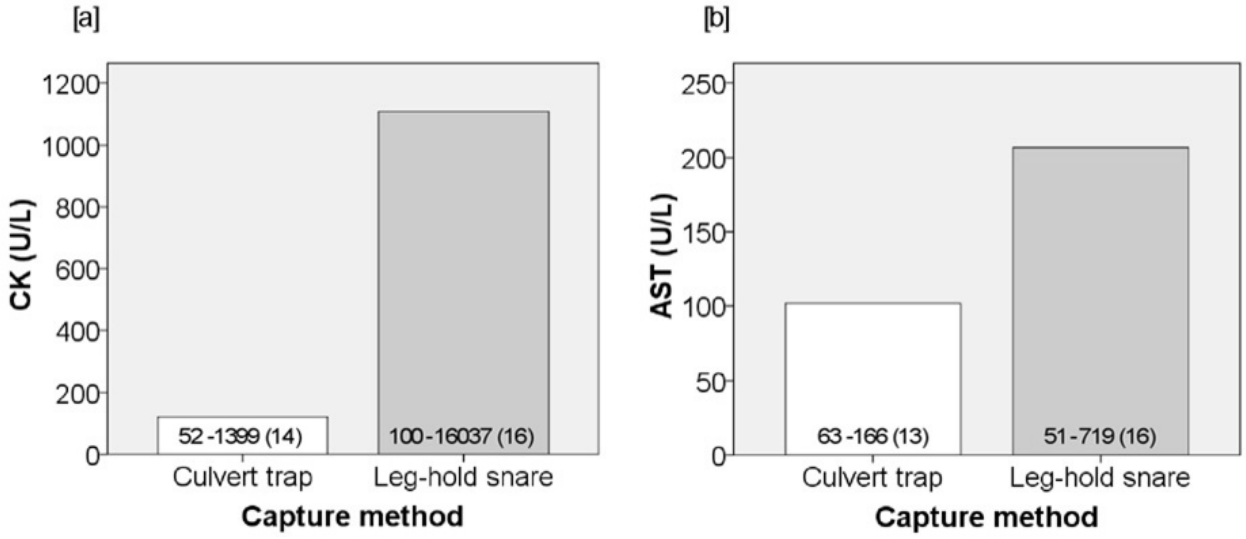

Figure B.1. Comparison of median muscle enzyme activity between grizzly bears captured by culvert trap and grizzly bears captured by leg-hold snare for the FRIGBP 1999-2007. Muscle enzymes are [a] creatine kinase (CK) and [b] aspartate aminotransferase (AST). Minimum and maximum values and sample size (round brackets) are within bars.

Table B.2. Comparison of serum biochemistry concentrations[a] between grizzly bears captured by culvert trap and grizzly bears captured by leg-hold snare for the FRIGBP 1999-2007.

Variable	Culvert trap	Leg-hold snare	*U*, *Z*, *p*[b]
Sodium (mmol/L)	145 (117-151) [14]	140 (123-150) [16]	86.50, -1.06, 0.288
Chloride (mmol/L)	105 (84-115) [14]	102 (91-117) [16]	98.00, -0.58, 0.560
Potassium (mmol/L)	4.3 (3.3-5.7) [14]	4.0 (2.9-5.0) [16]	75.00, -1.54, 0.123
Calcium (mmol/L)	2.27 (1.93-2.61) [14]	2.23 (2.02-2.56) [16]	83.00, -1.21, 0.227
Bicarbonate (mmol/L)	17.5 (12.0-22.0) [14]	15.0 (13.0-22.0) [16]	66.00, -1.93, 0.054
Anion gap	24.5 (13.0-41.0) [14]	24.0 (14.0-36.0) [16]	110.50, -0.06, 0.950
Urea (mmol/L)	8.8 (2.1-18.9) [14]	7.5 (3.0-18.9) [16]	87.50, -1.02, 0.308
Creatinine (μmol/L)	68 (57-107) [14]	75 (47-113) [16]	93.00, -0.79, 0.429
ALT (U/L)[c]	51 (14-66) [14]	51 (10-102) [16]	89.50, -0.94, 0.349
GGT (U/L)	14 (4-28) [14]	16 (3-70) [16]	88.50, -0.98, 0.327
Total protein (g/L)	67 (50-86) [14]	65 (57-77) [16]	93.50, -0.77, 0.441
Glucose (mmol/L)	8.3 (2.9-13.2) [11]	7.9 (5.7-12.1) [14]	73.50, -0.19, 0.848
Total cortisol (ng/ml)	49.6 (6.8-188.4) [14]	58.9 (13.6-265.9) [16]	94.00, -0.75, 0.454
Hsp70 (ng/ml)	2.62 (0.81-12.73) [12]	2.24 (0.46-5.95) [14]	74.00, -0.19, 0.852

[a] Values reported as median, minimum and maximum values in round brackets, and sample size in square brackets. Statistical comparison made by Mann-Whitney U-test with culvert trap and leg-hold snare as groups. Statistical significance was assigned when $p \leq 0.05$.
[b] Test statistic reported as *U*, *Z*-value as *Z*, and significance level as *p*.
[c] ALT = alanine aminotransferase, GGT = α-glutamyltransferase, and Hsp70 = heat shock protein 70.

B.4.2 Physical Injuries

I found that, compared to bears captured with LS, a larger proportion of bears captured with CT had no visible injuries reported, and claw injuries, deep lacerations, bone fractures, and

amputations were not documented (Table B.3). Capture by CT, however, resulted in damage to teeth and gums in 5 out of 21 (5/21) and superficial cuts and scrapes in 3/21 bears. Tooth damage was reported in two LS bears, but more often LS bears developed minor (cuts, scrapes, and paw swelling) to major (deep lacerations, bone fracture, and toe amputation) injuries to the snared limb.

Table B.3. Physical injuries observed in grizzly bears captured by culvert trap or by leg-hold snare for the FRIGBP 1999-2007.

Physical injury	Culvert trap	Leg-hold snare
No visible injury	15/21[a]	8/17
Swollen body part or superficial cut/scrape	3/21	8/17
Claw injury, exposed pulp	0/21	0/17
Deep laceration, exposed muscle/bone	0/21	1/17
Tooth injury[b]	5/21	2/17
(Tooth injury with exposed pulp)	(2/21)	0/17
Bone fracture or loss of foot/toe	0/21	2/17

[a] Proportion of bears affected.
[b] Tooth injury = all tooth lesions.

B.5 Discussion

By evaluating health data in grizzly bears captured by FRIGBP, I found differences between effects of CT and LS capture on health and welfare. In general, bears captured by CT were less likely to injure limbs (muscles, joints, and bones) and develop capture myopathy than bears captured by LS. Bears captured by CT, however, were more likely to damage their teeth

and gums. Bears in both capture groups were affected by capture-related stress, altered acid-base balance suggestive of metabolic acidosis, and mild dehydration. Hence, both capture methods can have negative short- and long-term effects on health and welfare in grizzly bears.

I found several LS bears and a small number of CT bears had increased WBCs and NCs. Lymphocyte and eosinophil counts were low in bears captured with both capture methods. These findings were consistent with a stress leukogram, most likely caused by adrenal stimulation and subsequent increase of cortisol concentration (Latimer et al. 2003). Generally, greater corticosteroid concentrations result in greater alteration of the leukogram (Stockham and Scott 2002). Despite differences in leukogram profiles between capture methods in this study, cortisol concentrations were similarly elevated (Cattet et al. 2003b, Hamilton 2007). This suggested stress levels were similar between capture methods, and the greater WBC and NC response in LS bears was exacerbated by inflammation as a consequence of tissue (muscle) injury (Latimer et al. 2003). This was supported by correlations of WBC and NC with serum concentrations of the muscle enzymes CK and AST, but no correlations with total cortisol. Similarly, Schroeder (1987) found greater WBC and muscle enzyme activity in LS-captured than in CT-captured American black bears (*Ursus americanus*).

My finding that hsp70 tended to correlate with WBC, NC, and CK, suggested hsp70 concentration in bears in this study increased as a result of excessive physical activity instead of capture-related stress. This observation was supported by Walsh et al. (2001) and Febbraio et al. (2002), who reported increased serum hsp70 concentrations in humans after 30 minutes of physical exercise. I found no difference in hsp70 concentration between CT and LS bears, neither did Hamilton (2007). He demonstrated, however, that hsp70 concentration was significantly elevated in LS bears by comparing with lower values in helicopter-captured bears.

I found CK and AST concentrations were generally higher in LS than in CT bears, a finding consistent with muscle injury (i.e., necrosis and ischemia) caused by tightening of the snare around the forelimb and straining of muscles and joints while attempting to escape (Cattet et al. 2003b). CK activity increases rapidly (peaking within hours), whereas AST activity increases more slowly (peaking within 24 to 36 hours) as a consequence of muscle injury and return to baseline within two to three days or over several days to weeks, respectively (Jackson 2007). Still, minor muscle injuries can take four to eight weeks to repair, and more severe injuries can affect strength and range of motion for longer duration (Cattet et al. 2008a). Increased serum muscle enzyme concentrations have been associated with exertional (or capture) myopathy in bears and other species (Kreeger et al. 1990, Cattet et al. 2008a). This is a non-infectious, sometimes fatal, disease of wild and domestic animals characterized by damage to skeletal and cardiac muscles and associated with physiological imbalances (i.e., shock, ischemia, metabolic acidosis, and azotemia) following strenuous exertion, struggle, and stress (Williams and Thorne 1996, Hartup et al. 1999). Cattet et al. (2008a) suggested extreme AST concentrations (> 710 U/L) in grizzly bears due to muscle exertion and injury was consistent with occurrence of capture myopathy. Based on this suggestion, 1 out of 16 LS bears, but none of the CT bears, in this study may have developed capture myopathy. Although potentially fatal in some species, Cattet et al. (2008a, b) suggested the disease more likely affects survival in grizzly bears through changed behaviour, leading to increased exposure to hunting and poaching and less acquirement of food and shelter.

Excessive muscle activity most likely also explained why anion gap was high and bicarbonate concentration was low in several LS and CT bears. Anaerobic glycolysis due to extreme muscle exertion provides energy, but also results in increased levels of lactic acid and decreased bicarbonate concentration in serum, consistent with metabolic acidosis (Jackson 2007).

These findings were supported by Fahlman (2008), who identified metabolic (lactic) acidosis in European brown bears and other carnivores as a consequence of capture-induced physical exertion. This acid-base imbalance can, if severe and not compensated, result in circulatory collapse and cardiac arrest (Schaer 1986). My findings of increased sodium, chloride, and urea concentrations suggested some bears in both capture groups were mildly dehydrated (Stockham and Scott 2002), likely as a result of water deprivation when trapped (up to 24 hours), coupled with increased water loss due to exertion (Cattet et al. 2003b, Powell 2005). Although CT and LS bears may behave differently in their attempts to escape (contained vs. restrained), my observations of metabolic acidosis and dehydration suggested physical activity levels were probably similar between CT and LS bears. In contrast, Powell and Proulx (2003) concluded bears captured by LS often struggle much more than bears in CT. Further, White et al. (1991) and Warburton et al. (1999) observed that physical activity was greater in red foxes (*Vulpes vulpes*) and Australian brushtail possums (*Trichosurus vulpecula*) captured with leg-hold traps than box traps.

I found reports of physical injury were less frequent in CT bears than in LS bears. Claw injuries, deep lacerations, bone fractures, or amputations were not reported for CT bears. These findings were in agreement with studies of culvert-trapped American black bears (Reagan et al. 2002) and other carnivores (Iossa et al. 2007). Capture by CT, however, resulted in damaged teeth and gums in 5 of 21 bears. Tooth injuries resulted from bears biting on protruding objects or edges inside the trap or from teeth getting stuck in ventilation/observation holes. A fractured tooth causes intense pain and can result in local infections, even when the pulp is not exposed (Gorrel 2004). Bears with mouth injuries may decrease or stop feeding until injuries heal. Similar to my findings, Powell and Proulx (2003) reported tooth damage occurred in several species captured with CT.

I found tooth damage was reported in a smaller proportion of LS bears. Instead, minor cuts, scrapes, and paw swelling, as well as deep lacerations, bone fractures, and amputations, were more commonly reported for LS bears. These types of injuries have been found in previous studies of LS-captured black bears (Powell 2005, Lemieux and Czetwertynski 2006) and other carnivores (Logan et al. 1999, Shivik et al. 2000) and can potentially result in short- or long-term disablement.

In conclusion, although CT bears were less likely to injure muscles, joints, and bones, they were more likely to damage teeth and gums. To prevent this type of injury, culvert traps can be designed with smooth interiors with nothing for contained animals to bite (Iossa et al. 2007). Longer periods of time spent in a trap are often associated with greater stress, exertion, and more serious injuries (Powell and Proulx 2003). Regardless of capture method, captured bears should be attended to as quickly as possible to reduce the impact of capture on health and welfare. In addition to frequent capture-site visits, remote trap-monitoring devices that signal when a bear is captured allow quick attendance (Kaczensky et al. 2002, Goski et al. 2007). A new method for capture in forested areas, so called remote-controlled teleinjection (Ryser et al. 2005), is selective and appears to be less stressful and cause less injury than CT and LS captures. Application of this technique for capture of grizzly bears warrants investigation.

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