INDICATORS OF INDIVIDUAL AND POPULATION HEALTH IN THE VANCOUVER ISLAND MARMOT (*MARMOTA VANCOUVERENSIS*)

BY

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D.V.M., Western College of Veterinary Medicine, 1987

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SCIENCE IN ENVIRONMENTAL SCIENCES

Thompson Rivers University

Kamloops, British Columbia, Canada

November 2018

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ABSTRACT

The Vancouver Island Marmot (*Marmota vancouverensis*) is an endangered rodent endemic to the mountains of Vancouver Island, British Columbia, Canada. Following population declines in the 1980s and 1990s, an intensive captive breeding and reintroduction program was initiated involving three Canadian zoos and a purpose-built, subalpine facility on Vancouver Island.

From 1997 to 2017, 660 marmots were associated with the captive program, including 63 wild-born individuals captured for breeding and 597 marmots born and weaned in captivity. Reintroductions began in 2003 and by 2017 a total of 501 marmots had been released. Although this significantly increased the wild population from its low point in 2003, conservation of the Vancouver Island Marmot (VIM) continues to involve intensive *ex situ* management, reintroductions, and translocations.

Health and disease surveillence is fundamental to the success of conservation programs like the marmot recovery project. This thesis builds upon our understanding of VIM health by describing and evaluating select health parameters, to determine baseline characteristics for VIM under its different treatments (wild, captive and captive-release) and to identify potential risk factors that may influence the health of the marmot's population and its capacity to achieve recovery objectives. The analysis involved data that was collected between 1992 and 2016, and included 1,106 VIM blood profiles, 3,174 physical examinations, 140 post mortem examinations and 533 field mortality records.

VIM hematology and serum biochemistry reference ranges were calculated as a baseline metric and were qualitatively comparable to published values for other

rodent species. Leukogram and protein values were found to have potential utility as a quantitative measure for comparing VIM management groups.

There were significant differences in the clinical and pathological data collected from captive and free-ranging (captive-release and wild) marmots. Captives could be monitored with greater intensity and to an older age, due to increased longevity. A host of clinical and pathological disorders were described in captive marmots, including age-related, management-related, and congenital problems. There was a paucity of health conditions identified in free-ranging VIM and this could be due to a fundamental lack of disease, or limited opportunities to conduct post mortem examinations or evaluate compromised individuals in the field.

The analysis did not identify any specific infectious agents that represented a generalized population threat to VIM. Cardiomyopathy and neoplasia, which occurred in older individuals, were the most consequential health complications for captive marmots. Implantation of abdominal radio-transmitters was not found to impact marmot health and was important for identifying mortalities in the field. The first wild hibernation represented a time of significant mortality for VIM released from captivity. However, reintroduced marmots that survived their first year in the wild were comparable to their wild-born counterparts with respect to hibernation success and clinical presentation. Predators continued to represent a major cause of mortality for free-ranging marmots. In the absence of other identified health threats, predation and reduced hibernation success of captive-release marmots appear to be significant factors limiting the health and potential recovery of the *in situ* population.

Keywords: marmot, endangered, recovery, health, disease, surveillance

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ACKNOWLEDGEMENTS

Dave Fraser, the Endangered Species Specialist for British Columbia's Ministry of Environment (and a former member of the Vancouver Island Marmot Recovery Team), once told me that the most successful conservation efforts were the ones driven by individuals who are passionate about the cause. Over many years, marmot recovery, and my own humble thesis, have benefited greatly from the hard work, expertise, and passion of many people.

This includes the animal care, curatorial, and veterinary staffs at the Toronto Zoo and the Calgary Zoo, and Gordon Blankstein and the workforce at the Mountain View Conservation and Breeding Society. Collette Howell, Rick Wenman and Debbie Rempel were instrumental in developing the initial protocols for marmot husbandry and breeding. Dr. Doug Whiteside, Dr. Sandie Black, Dr. Graham Crawshaw, Dr. Chris Dutton and many other veterinarians, have made important contributions to marmot health. The staff at the Tony Barrett Mount Washington Marmot Recovery Centre, particularly Louise Dykslag and Alana Buchanan, contributed many years of diligent animal care.

A number of pathologists at the University of Guelph, the Calgary Zoo and the Animal Health Centre (AHC) in Abbotsford conducted marmot post mortems. Dr. Stephen Raverty (AHC) deserves special recognition for his thoroughness, expertise and the number of marmot cases he examined. He went out of his way on many occasions to provide us with his insights and support.

The Vancouver Island Marmot Recovery Team and the Captive Management Group grappled with difficult decisions and dealt with contentious issues in a consistently thoughtful and respectful manner. Tremendous leadership was shown by the Recovery Team Chairs - Doug Janz, Don Doyle and Sean Pendergast (current)

- and by the Executive Directors of the Marmot Recovery Foundation (MRF), the late Tony Barrett, the late Robert Huber, Viki Jackson and Adam Taylor (current). The late Jim Walker, former Chair of the MRF Board, and many MRF board members, were vital advocates for the marmots. An army of field personnel endured extreme temperatures, biting insects, lumpy ground, challenging terrain, predators and many long days and nights. This included Cheyney Jackson (current MRF Field Co-ordinator), Mike Lester, Jerry MacDermott, Sean Pendergast, Crystal Reid, Chris White and many, many others.

Dr. Andrew Bryant was important to VIM and the project in many ways, and along with Tony Barrett and Stan Coleman, was instrumental in getting the MRF off the ground. Dr. Ken Langelier developed many of the original techniques used for surgically implanting radio-transmitters. Robin Campbell and the North Island Wildlife Recovery Association provided support in a multitude of ways. John Carnio was instrumental in starting the captive breeding program at the Toronto Zoo and has acted as the VIM Studbook Keeper since its inception. His sage wisdom has been valued for many years. His successor at the Toronto Zoo, Maria Franke, has been a strong voice for the marmots. Dr. Helen Schwantje has been an instrumental and multi-faceted contributor and promoter of marmot health.

Dr. David Hill (a member of my thesis committee) spent valuable time helping me learn the basics of statistics, and Dr. Todd Shury was a welcome addition as my external examiner. I would like to thank my supervisors, Dr. Craig Stephen and Dr. Karl Larsen, for their friendship, guidance and patience. It was very much appreciated.

And a thanks to the VIM themselves, who live their lives as marmots, and do not stress about being critically endangered. A lesson to us all.

DEDICATION

This thesis is dedicated to Marnie, Steven, Greg, Laura, Vern and Margaret. You didn't always understand my passion for wildlife, but you accepted and supported it nevertheless.

Haida (right) was born in 2002 at the Mountain View Conservation and Breeding Society in Langley, British Columbia and became the first captive-release female to breed in the wild. Her descendants continue to survive at the Haley Lake Ecological Reserve on Vancouver Island. Photo: Oli Gardner

People often ask me 'What are marmots good for?', I ask them "What are you good for?"

Dr. Kenneth B. Armitage

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CHAPTER 1

THESIS INTRODUCTION

To prevent extinction and facilitate recovery, many conservation programs have undertaken intensive management of endangered species. In the case of the critically endangered Vancouver Island Marmot (*Marmota vancouverensis*) this has involved captive breeding and conservation translocations, the latter defined as the deliberate movement of individuals from one location, either captive or wild, to free release in another location, to restore extirpated populations or to support existing small populations (Convention on Biological Diversity, 2013; Ewan *et al.* 2012; Williams & Hoffman, 2009; Teixeira, *et al*. 2007; Rout *et al*., 2007; IUCN, 2002; Fischer & Lindenmayer, 2000; Ebenhard, 1995). Dwindling numbers and artificial manipulation, including animal movements and *ex situ* management, have the potential to negatively impact the health of a threatened species (Daszak, *et al.* 2001; Snyder, *et al.* 1996). Although comprehensive tracking of health and health effects in imperiled wildlife populations is essential for their successful long-term maintenance and recovery, and for the timely recognition and mitigation of potential threats, effective health monitoring and its implications represents a significant challenge for many conservation programs (Delahay *et al.* 2009).

The objective of this thesis is to build upon our current understanding of Vancouver Island Marmot (VIM) health by describing and evaluating select health parameters, to determine what is expected for this species under different treatments (wild, captive and captive-release) and to identify possible risk factors associated with small population size and conservation management that may be

influencing the health of the marmot's population and its relative capacity to achieve defined recovery objectives.

HEALTH RISKS IN THREATENED SPECIES

Declines in population size can result in a loss of genetic diversity that may compromise an endangered species' capacity to cope with current conditions or adapt to future change (Jamieson, 2010). Decreased heterozygosity can have multiple consequences including a reduction in rigour, growth, fecundity, survival, and immunocompetence, and it may also lead to the accumulation and expression of deleterious alleles (Ewan *et al.* 2012; Williams and Hoffman, 2009). Captivity can significantly alter animals (Darwin, 1859) and artificial conditions may promote the occurrence of heritable or acquired traits that reduce the fitness of individuals being returned to the wild (Kreger *et al.* 2005). Many translocation programs using captive-bred animals have been less successful than those ultilizing wild-born animals (Robert, 2009; Teixeira *et al.* 2007, Jule, Leavor, & Lea, 2008; Fischer & Lindenmayer, 2000). The post-release success of captive animals may be limited by physical, physiological and behavorial deficiencies, including inadequate physical conditioning, underdeveloped locomotor abilities, morphological changes, alteration of reproductive and metabollic cycles, increased stress, inadequate or inappropriate anti-predator or threat responses, overt boldness or aggression, increased docility, alterred sociocognitive abilities, and an inability to recognize or procure appropriate foods (Bonato *et al*. 2013; Teixeira *et al*. 2007; Hare, *et al*., 2005; McPhee, 2003; Concannon *et al*. 1997). Rapid genetic changes associated with domestication may occur within a single generation in certain taxa (De Mestral & Herbinger, 2013) and

some abberant characteristics may become more pronounced or more variable following multiple generations in captivity (McPhee, 2003).

Captive confinement also can result in the artificial intensification of endemic parasites, or alternatively it may result in a reduction of acquired resistence due to the disruption of natural relationships that are normally maintained between a host and its commensal pathogens and parasites (Mathews *et al*. 2005). Most zoological institutions maintain geographically-varied collections of animals from a diversity of sources, each with their own spectrum of infectious agents (Snyder, *et al*. 1996). In addition, captive facilities may inadvertently harbour pest species, which provide an additional source for the introduction of exotic pathogens. Even with the safeguard of good biosecurity, there is always the potential risk that an *ex situ* recovery population maintained within a zoo will be exposed to a novel pathogen. This potential risk cannot be fully quantified because it is not realistically possible to determine the full spectrum of suseptibilty that an endangered species has to novel infectious agents.

Whenever animals are translocated from one environment to another (be it wild to captive, captive to captive, captive to wild or wild to wild) there is a risk to the health of the individuals being moved and a risk to the health of extant individuals or populations (Leighton, 2002; Woodford, 1993). Translocated animals may serve as vectors for the introduction of novel pathogens to naïve recipient populations, or alternatively, they may be naïve to the transmissible agents that exist within the extant conspecific or sympatric populations at their release site.

INDIVIDUAL HEALTH

To survive and propagate, organisms must co-ordinate complex assemblages of functional and metabolic characteristics that allow for the maintenance of intrinsic equilibrium, or homeostasis. To be effective, this physiological balance must be maintained within the typical range of ambient conditions to which a species has become adapted, and to some extent, it must also be preserved in response to atypical perturbations (Ryser-Degiorgis, 2013). Within a species, there is individual variability in the capacity to cope or self-manage. Those individuals that are less competent at regulating biological integrity or those that are less able to maintain function during times of challenge or stress, have diminished resilience and are potentially inferior with respect to growth, reproduction, and survival (Darwin, 1859). An individual's capacity to maintain homeostasis, well-being, and normal life functions, can be equated to its comparative state of health. A reduced ability to support homeostasis can result in biological impairment and this can be associated with imbalance or disease (Ryser-Degiorgis, 2013). If health is used in the context of a continuum or relative state (i.e. an individual's ability to sustain biological processes and its productivity at specific points in life, or cumulatively over a lifetime, compared to conspecifics) it does not merely represent the absence of observable dysfunction or disease (Gunnarsson, 2006).

POPULATION HEALTH

Although health in individuals can be quantified by measurable characteristics or outcomes relative to those of conspecifics or even individuals of other species, it is much more difficult to define and evaluate the health of populations, species, and the intricate ecosystems of which they are a part (Deem *et*

al. 2008). Many wildlife studies evaluating health have emphasized the description of dysfunction, pathogens, and mortality in populations (Ryser-Degiorgis, 2013). Although the occurrence of infectious and non-infectious disease and the factors that predispose populations to disease are important to comprehensive monitoring and conservation, this type of data represents only one component in the multifaceted context of population health. Health surveillance also can involve the evaluation of many other parameters and condition indices, including growth, physical characteristics and body condition, fecundity, longevity, survival rates, recruitment rates, commensal microflora, behavior, demographics, physiological and immunological measures, and genetic diversity (Robert & Schwanz, 2013; Sackett *et al*. 2013; Brashares *et al*. 2010; Mellish *et al.* 2010; Villers *et al.* 2008; Mathews *et al.* 2006; Stevenson & Woods, Jr., 2006; Ostermann *et a.*, 2001). A workable health construct for a species can be based on two basic components - a normative component and a descriptive component (Hanisch, *et al*. 2012).

The normative component of population health seeks to address what should be, by identifying an ideal standard or holistic condition of population well-being, and by determining what is required to restore or maintain that condition (Hanisch, *et al*. 2012). The ideal, healthy wildlife population could be one that is self–sustaining, and able to survive within its natural habitat, while maintaining its intricate ecological relationships without the need for artificial support or intervention. Such a population should have the capacity to persist for a prolonged, but indeterminate period and avoid premature extinction. It must have the intrinsic ability to cope with existing and future stressors, and demonstrate resiliency and sustainability. Threatened populations, by their very definition, lack the capacity to autonomously achieve these normative ideals of integrity and health. From a

conservation and management perspective, the comparative state of fitness or health of a threatened or recovering wildlife population could be its relative ability to achieve and maintain discrete, biologically feasible, recovery objectives. Recovery involves the identification and mitigation of threats to increase the likelihood that a threatened species will continue to persist in the wild (Vancouver Island Marmot Recovery Team, 2008). To achieve recovery there is a need for appropriate intervention and this needs to be supported by social and political will, adequate resources, and technical wherewithal. Therefore, an endangered species' current and future health also is dependent upon the presence or absence of tangible remedial actions and upon the logistical feasibility of implementing them.

The descriptive component of health incorporates the empirical, bio-statistical elements of health and health surveillance (Hanisch *et al*. 2012). This includes the establishment of baseline parameters to delineate what is normal or expected for a species, how these measures compare between populations exposed to different conditions, and how they reflect on the wellness and population viability of the species in question (Vitali *et al*, 2011). These parameters should be monitored over time to identify how they change in response to existing conditions (which may be indicative of health deterioration and reduced coping capacity) or to new stresses or challenges.

THE VANCOUVER ISLAND MARMOT

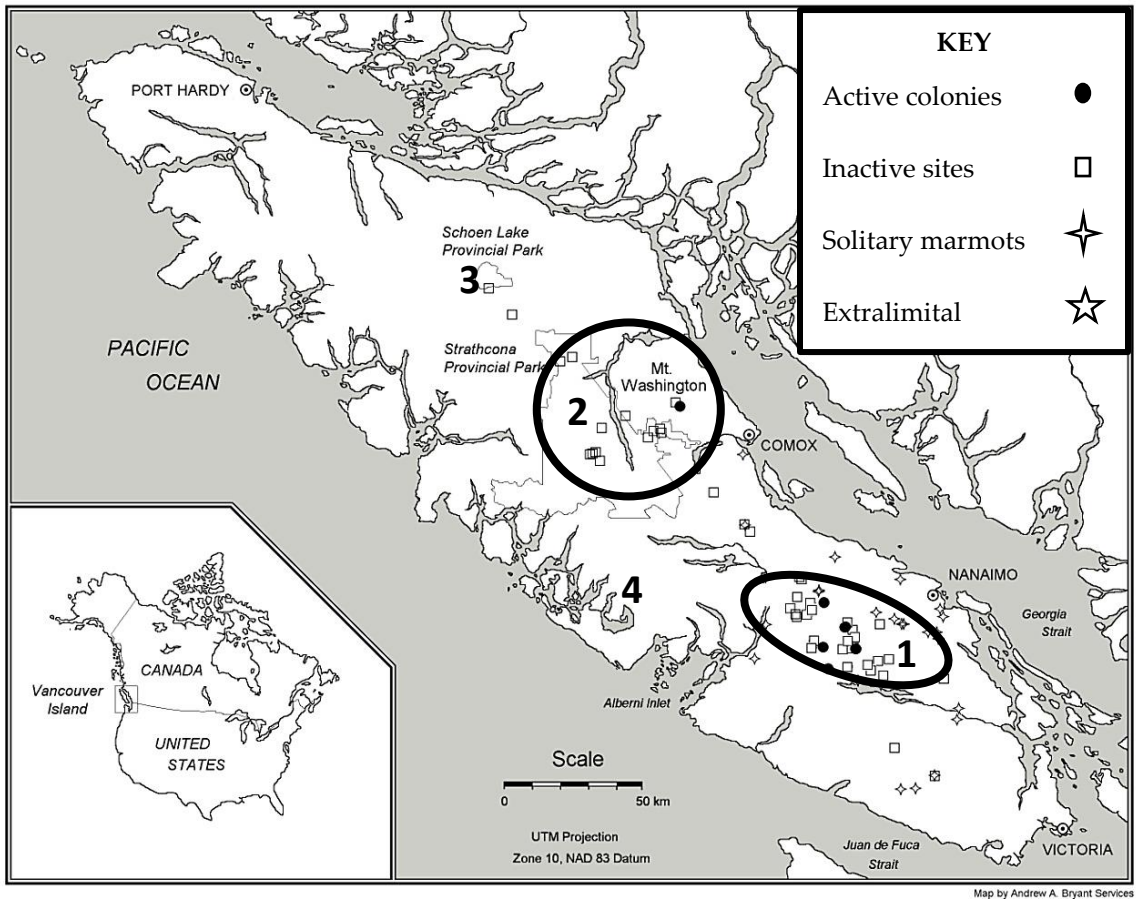
As both its common and scientific names suggest, the Vancouver Island Marmot (VIM) is a species that is endemic to the insular mountains of Vancouver Island (Swarth, 1912; Swarth, 1911). This large, fossorial sciurid naturally inhabits small, moderately to steeply sloped, south to west facing subalpine meadows

between 1000 and 1450 metres elevation (Bryant and Janz 1996). Within recent historical times (i.e. within the last century) its range has extended from several mountains to the immediate north of Lake Cowichan (Latitude 48.94 N, Longitude 124.16 W) on south-central Vancouver Island to Mount Schoen (Latitude 50.16 N, Longitude 126.23 W) which lies approximately 200 kilometers to the northwest (Janz *et al*. 2000) (Figure 1.1).

Due to the low numbers of VIM, limited available natural habitat, restricted geographical range, and endemic status, the species was first listed as endangered in 1978 by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 1979). Currently, the marmot is listed as critically endangered by the International Union for the Conservation of Nature's (IUCN) Red List. It also receives legal protection under the federal Species at Risk Act (SARA), the British Columbia Wildlife Act, and the United States Endangered Species Act (Vancouver Island Marmot Recovery Team, 2008).

Field inventory conducted in the early to mid-1980s, concentrating primarily in the Nanaimo Lakes region (identified as the "core" area for marmots - which encompasses mountains to the north and north-west of Lake Cowichan and to the east and north-east of the Alberni Inlet) determined that there were approximately 300 to 350 marmots in existence and that the population was stable or increasing. However, beginning in the late 1980s and throughout the 1990s, VIM numbers demonstrated precipitous and progressive declines (Bryant & Page, 2005). By 1998 the wild population had been reduced to less than 100 individuals and by 2003 it was less than 30 (Jackson *et al.* 2015; Janz *et al.* 2000).

Figure 1.1. Historical distribution of the Vancouver Island Marmot (*Marmota vancouverensis*), from the time it was first scientifically collected by Harry Swarth in 1910 up to the early 2000s, prior to intensive reintroduction efforts which began in 2003. The elipses delineate the two geographically distinct metapopulations that have been delineated for species recovery; (1) Nanaimo Lakes and (2) Strathcona Park / Mount Washington. Two other sites, (3) Mount Cain and (4) Steamboat Mountain on the Clayoquot Plateau are locations of extralimital, assisted colonization. Map prepared by A.A. Bryant and adapted from the 2000 Update of the National Recovery Plan for the V.I. Marmot



In the early 1980s it was first determined that a significant portion of the marmot population had recently become established in higher tracts of logged habitat (at elevations above 700 meters) that in the early stages of seral succession, mimicked the characteristics of the marmots' natural subalpine meadows. However, rapid regeneration of conifers and alders at these sites altered the formerly meadow-like habitat, making them unsuitable for the colonizing marmots. Increased tree cover may also have served to increase the stalking advantages for Cougars (*Puma concolor*). The increased open habitat created by logging may have facilitated an increase in Golden Eagle (*Aquila chrysaetos*) numbers and extensive logging roads may have acted as travel corridors for Grey Wolves (*Canis lupus*), thereby increasing their access and contact with marmots who commonly used these same roads as burrowing substrate. Increased vulnerability to predation from these naturally-occurring predators may have resulted in marmot declines at these anthropogenic, ephemeral sites (Aaltonen *et al*. 2009). This resulted in a typical pattern of cut-block colonization and population growth, followed by colony attrition, collapse and eventual extirpation (Bryant, 1996).

Most colonies in logged habitat became established in close proximity to natural colonies, which started to exhibit parallel declines, and in many cases, extirpation. It is possible that the logged areas disrupted colony connectivity, genetic exchange and "rescue" of natural colonies by intercepting dispersing marmots, thus interfering with the natural mechanisms that had traditionally perpetuated the marmots' meta-population (Bryant, 1998). Altered predator-prey relationships on Vancouver Island, including declines in Black-tailed Deer (*Odocoileus hemionus*), may have also led to increased predation pressure that affected marmots in both natural and logged habitat (Bryant and Page, 2005).

Limited historical data exists for the populations in Strathcona Park and Forbidden Plateau, which lie to the northwest of the Alberni Inlet. It is believed that these populations declined over the last few decades, leaving only a single extant colony inhabiting the artificially managed ski-runs at Mount Washington (Bryant, 1998). These northern sites were not under the same development pressures as the southern "core" and the proximate causes of marmot declines in these areas and in the Beaufort Range, which connected the north-west and south-east populations, have not been well established.

There is compelling paleontological and archaeological evidence to suggest that this species was once more widespread on Vancouver Island and that its subsequent range contraction was not entirely related to modern anthropogenic influences (Nagorsen *et al.* 1996).

MARMOT RECOVERY AND CURRENT STATUS

Based upon extremely low numbers and ongoing population declines, the 2000 National Recovery Plan concluded that although sufficient natural habitat remained, recovery efforts involving only *in situ* management were unlikely to save the species from extinction. This document stated that "*few animals exist for reintroductions or other management activities*" and that "*it is unlikely that wild populations will suddenly rebound of their own accord* (and therefore) *Captive breeding and reintroduction present the only chance of increasing populations within a reasonable period of time and minimizing the risk of extinction*".

As a result of these determinations, an intensive captive breeding program was initiated for this species in 1997, with the intention of (i) establishing a safeguard against potential catastrophic or stochastic events in the wild, (ii) acting as a long-term genetic reservoir, (iii) determining appropriate management and

husbandry techniques for the successful captive maintenance and propagation of Vancouver Island Marmots, (iv) conducting directed research, and (v) providing sufficient numbers of individuals for release and eventual restoration of the wild population (Janz *et al*. 2000). Since its inception, this *ex situ* program has involved the participation of three zoological institutions, the Toronto Zoo (TZ), the Calgary Zoo (CZ), and the Mountain View Conservation and Breeding Society in Langley, British Columbia (MVF) along with the construction of a dedicated $1.2 million marmot facility at Mount Washington on Vancouver Island, the Tony Barrett Mount Washington Marmot Recovery Centre (MRC).

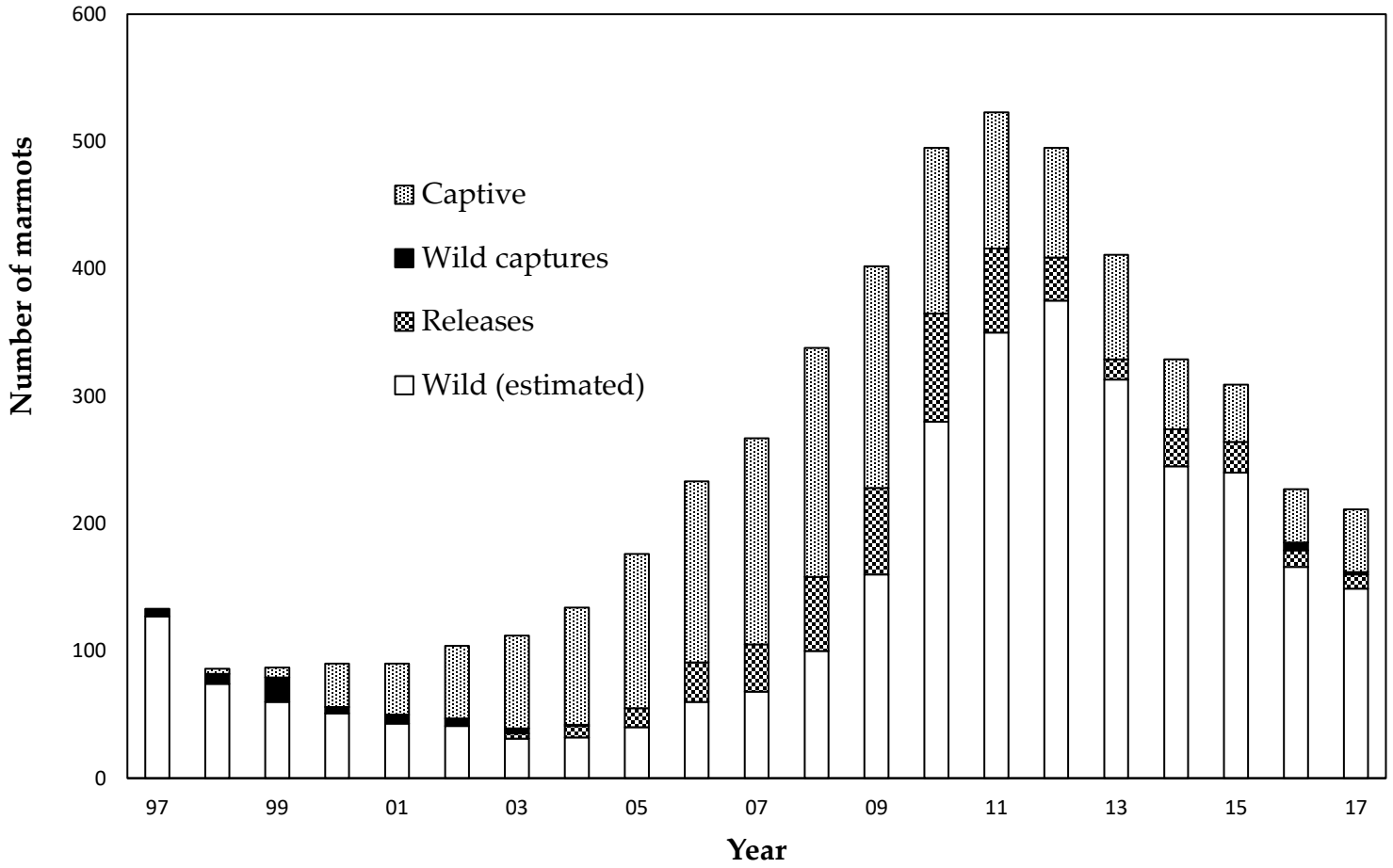
The VIM captive breeding program reached its numerical peak in 2008 with 177 marmots and 46 breeding pairs. From 2009 to 2015 the program was intentionally down-sized due to diminishing resources and some early reintroduction success (Jackson *et al.* 2015). Subsequent declines in the wild population following 2012 have prompted a recent restoration of the captive program. As of December 2017, the captive population consists of 49 surviving marmots, including 12 breeding pairs. Overall, there has been a total of 660 individual marmots maintained in captivity, including 63 marmots originally captured from the wild and 597 captive-born marmots. One hundred and twelve mortalities have occurred in captivity and a total of 501 marmots (8 wild-born, 493 captive-born) have been released back to the wild (Vancouver Island Marmot Captive Management Group, 2017). Releases include 167 captive individuals into the Nanaimo Lakes area, 187 into Strathcona Park, 109 to Mount Washington, 16 into the Clayoquot Plateau Provincial Park, and 22 into the Mount Cain / Mount Schoen area.

Based upon historical occurrence records, the estimated carrying capacity of suitable habitat patches, and population simulation models, recovery for the

Vancouver Island marmot was initially defined as a self-sustaining wild population of 400 to 600 marmots, distributed in three geographically separate meta-populations on Vancouver Island (Vancouver Island Marmot Recovery Team, 2008; Janz, *et al*. 2000). The potential existence of three independent meta-populations, identified as (i) the Nanaimo Lakes region, (ii) western Strathcona Park and (iii) Mount Washington / Forbidden Plateau, would likely serve to reduce the population's vulnerability to stochastic or localized threats, and were delineated by the presence of large water bodies, primarily the Alberni Inlet and Buttle Lake, that would act as barriers to dispersing marmots. Due to the potential for habitat connectivity recently identified along the southern end of Buttle Lake, the western Strathcona Park and Mount Washington / Forbidden Plateau were deemed to be a single recovery population in the 2017 Recovery Strategy. Based upon the limitations imposed by a discrete numerical recovery goal (i.e. one without a temporal component), this strategy also re-defined marmot recovery as both geographically distinct meta-populations having a greater than 90% probability of persisting for over 100 years, without augmentation from the captive program (Vancouver Island Marmot Recovery Team, 2017; Jackson *et al.* 2015).

Although there has been some population restoration from its low point in 2003, the VIM continues to be managed by an intensive program involving captive breeding, reintroductions, and translocations. At the end of the 2017 field season population estimates were 70 to 80 individuals in the Nanaimo Lakes meta-population, 70 to 80 in the Strathcona Park meta-population (including Mount Washington), and an unknown number surviving within Clayoquot Plateau Provincial Park, and at Mount Seth in Schoen Lake Provincial Park. The total wild population is currently estimated to be approximately 150 individuals (Vancouver Island Marmot Recovery Team, November 2017).

Figure 1.2. Annual total of individuals in the Vancouver Island Marmot (*Marmota vancouverenesis*) population from 1997 to 2017.



THESIS STRUCTURE

For the foreseeable future, the critically endangered VIM will be managed by an intensive program of captive breeding and conservation translocations. Low numbers and artificial manipulation pose potential risks that could negatively impact individual and population health and could prevent this species from achieving recovery objectives. Effective health monitoring and a better understanding of normality or baseline characteristics, and identification of possible threats, is essential for the successful long-term conservation of this species. This recovery program has gathered a great range of health information from the marmots' wild, captive, and captive-release populations. Without further collation of these disparate data, their usefulness for future research, learning, comparison, evidence-based decisions, and management will not be fully realized. In this thesis I compile and analyse these data by describing and evaluating select health parameters. Individual condition indices (hematology and serology, chapter two), clinical assessments (physical examinations, chapter three), and health outcomes (morbidity and mortality, chapter three) are used to establish and compare baseline health characteristics for the VIM under different treatments (wild, captive and captive-release) and to identify possible health threats. This information can be used in the future to recognize changes in VIM health and to guide management decisions.

LITERATURE CITED

Aaltonen, K., Bryant, A. A., Hostetler, J. A., & Oli, M. K. (2009). Reintroducing endangered Vancouver Island marmots: survival and cause-specific mortality rates of captive-born versus wild-born individuals. *Biological Conservation 142*, 2181-2190.

Bonato, M., Malecki, I. A., Wang, M. D., & Cloete, S. W. (2013). Extensive human presence at an early age of ostriches improves the docility of birds at a later stage of life. *Applied Animal Behaviour Science 148*, 232-239.

Brashares, J. S., Werner, J. R., & Sinclair, A. (2010). Social 'meltdown' in the demise of an island endemic: Allee effects and the Vancouver Island marmot. *Journal of Animal Ecology 79*, 965-973.

Bryant, A. A. (1996). Reproduction and persistence of Vancouver Island marmots (*Marmota vancouverensis*) in natural and logged habitats. *Canadian Journal of Zoology*, 678-687.

Bryant, A. A. (1998). *Metapopulation ecology of Vancouver Island marmots (Marmota vancouverensis), PhD thesis.* Victoria, B.C.: University of Victoria.

Bryant, A. A., & Janz, D. W. (1996). Distribution and abundance of Vancouver Island marmots (*Marmota vancouverenisis*). *Canadian Journal of Zoology (74)*, 667-677.

Bryant, A. A., & Page, R. E. (2005). Timing and causes of mortality in the endangered Vancouver Island marmot (*Marmota vancouverensis*). *Canadian Journal of Zoology (83)*, 674-682.

Concannon, P., Roberts, P., Baldwin, B., & Tennant, B. (1997). Long-term entrainment of circannual reproductive and metabolic cycles by northern and southern hemisphere photoperiods in woodchucks (*Marmota monax*). *Biology of Reproduction 57*, 1008-1015.

Convention on Biological Diversity. (2013, January 25). Retrieved from Convention on Biological Diversity: http://www.cbd.int/convention/text/default.shtml

COSEWIC. (1979). *Status of the Vancouver Island Marmot in Canada.* Ottawa, Ontario: Committee on the Status of Endangered Wildlife in Canada.

Darwin, C. (1859). *The Origin of Species (First Edition).* London: William Pickering.

Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2001). Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Tropica 78*, 103-116.

De Mestral, L. G., & Herbinger, C. M. (2013). Reduction in antipredator response detected btween first and second generations of endangered juvenile Atlantic salmon Salmo salar in a captive breeding and rearing programme. *Journal of Fish Biology 83 (5)*, 1268-1286.

Deem, S. L., Parker, P. G., & Miller, R. E. (2008). Building bridges: connecting the health and conservation professions. *The Journal of Tropical Biology and Conservation*, 662-665.

Delahay, R. J. et al. (eds). (2009). *Management of Disease in Wild Mammals.* New York: Springer.

Ebenhard, T. (1995). Conservation breeding as a tool for saving species from extinction. *Tree 10 (11)*, 438-443.

Ewan, J. C., Armstrong, D. P., Parker, P. A., & Seddon, P. J. (2012). In *Reintroduction Biology: Integrating Science and Management.* Wiley- Blackwell.

Fischer, J., & Lindenmayer, D. B. (2000). As assessment of the published results of animal relocations. *Biological Conservation 96*, 1-11.

Gunnarsson, S. (2006). The conceptualisation of health and disease in veterinary medicine. *Acta Veterinaria Scandinavica 48 (20)*.

Hanisch, S. L., Riley, S. J., & Nelson, M. P. (2012). Promoting wildlife health or fighting disease: insights from history, philosophy, and science. *Wildlife Society Bulletin 36 (3)*, 477-482.

Hare, B., Plyusnina, I., Ignacido, N., Schepina, A., Wrangham, R., & Trut, L. (2005). Social cognitive evolution in captive foxes is a correlated by-product of experimental domestication. *Current Biology (15)*, 226-230.

IUCN Technical Guidelines on the Management of Ex-situ Populations for Conservation. (2002). Retrieved from ICUN: http://www.iucn.org/about/work/programmes/species/publications/iucn_guidelines_and__policy__statements/

Jackson, C.; Baker, A.; Doyle, D.; Franke, M.; Jackson, V.; Lloyd, N.; McAdie, M.; Stephens, T.; Traylor-Holzer, K.;. (2015). *Vancouver Island Marmot Population and Habitat Viability Assessment Workshop Final Report.* Apple Valley, MN: IUCN SSC Conservation Breeding Specialist Group.

Jamieson, I. G. (2010). Founder effects, inbreeding, and loss of genetic diversity in four avian reintroduction programs. *Conservation Biology 25 (1)*, 115-123.

Janz, D. W., Bryant, A. A., Dawe, N. K., Schwantje, H., Harper, B., Nagorsen, D., . . . Simmons, R. (2000). *National Recovery Plan for the Vancouver Island Marmot (Marmota vancouverensis) 2000 Update: RENEW Report No.19.* Ottawa, Ontario: Environment Canada.

Jule, K. R., Leavor, L. A., & Lea, S. E. (2008). The effects of captive experience on reintroduction survival in carnivores: A review and analysis. *Biological Conservation 141*, 355-363.

Kreger, M. D., Hatfield, J. S., Estevez , I., Gee, G. F., & Clugston , D. A. (2005). The effects of captive rearing on the behavior of newly-released whooping cranes (*Grus americana*). *Applied Animal Behavior Science 93*, 165-178.

Leighton, F. A. (2002). Health risk assessment of the translocation of wild animals. *Rev. Sci. Tech. Off. Int. Epiz. 21(1)* , 187-195.

Mathews, F., Moro, D., Strachan, R., Gelling, M., & Buller, N. (2006). Health surveillance in wildlife reintroductions. *Biological Conservation 131*, 338-347.

Matthews, F., Orros, M., McLaren, G., Gelling, M., & Foster, R. (2005). Keeping fit on the ark: assessing the suitability of captive-bred animals for release. *Biological Conservation 121*, 569-577.

McPhee, M. E. (2003). Generations in captivity increases behavioral variance: considerations for captive breeding and reintroduction programs. *Biological Conservation 115* , 71-77.

Mellish, J. E., Hindle, A. G., & Horning, M. (2010). A preliminary assessment of the impact of disturbance and handling on Weddell seals of McMurdo Sound, Antarctica. *Antarctic Science 22 (1)*, 25-29.

Nagorsen, D. W., Keddie, G., & Luszcz, T. (1996). *Vancouver Island Marmot Bones from Subalpine Caves: Archaeological and Biological Significance, Occasional Paper No. 4.* Victoria, British Columbia: Ministry of Environment, Lands and Parks.

Ostermann, S. D., Deforge, J. R., & Edge, W. D. (2001). Captive breeding and reintroduction evaluation criteria: a case study of peninsular bighorn sheep. *Conservation Biology 15(3)*, 749-760.

Robert, A. (2009). Captive breeding genetics and reintroduction success. *Biological Conservation 142*, 2915-2922.

Robert, K. A., & Schwanz, L. E. (2013). Monitoring the health status of free-ranging Tammar wallabies using hematology, serum biochemestry, and parasite loads. *The Journal of Wildlife management 77 (6)*, 1232-1243.

Rout, T. M., Hauser, C. E., & Possingham, H. P. (2007). Minimize long-term loss or maximize short-term gain? Optimal translocation strtaegies for threatened species. *Ecological Modelling 201* , 67-74.

Ryser-Degiorgis, M.-P. (2013). Wildlife health investigations: needs, challenges and recommendations. *BMC Veterinary Research 9 9223)*.

Sackett, L. C., Collinge, S. K., & Martin, A. P. (2013). Do pathogens reduce genetic diversity of their hosts? Variable effects of sylvatic plague in black-tailed prairie dogs. *Molecular Ecology 22*, 2441-2455.

Snyder, N. F., Derrickson, S. R., Bessinger, S. R., Wiley, J. W., Smith, T. B., Toone, W. D., & Miller, B. (1996). Limitations of captive breeding in endangered species recovery. *Conservation Biology 10 (2)*, 338-348.

Stevenson, R. D., & Woods, Jr., W. A. (2006). Condition indices for conservation: new uses for evolving tools. *Integrative and Comparative Biology 46 (6)*, 1169-1190.

Swarth, H. S. (1911). Two new species of marmots from North America. *University of California Publications in Zoology (7)*, 201-204.

Swarth, H. S. (1912). Report on a collection of birds and mammals from Vancouver Island. *University of California Publications in Zoology (10)*, 1-124.

Teixeira, C. P., De Azevedo, C. S., Mendl, M., Cipreste, C. F., & Young, R. J. (2007). Revisiting translocation and reintroduction programmes: the importance of considering stress. *Animal Behaviour 73*, 1-13.

Vancouver Island Marmot Captive Management Group (2017). *Vancouver Island Marmot Captive Management Group Meeting Minutes and Annual Summary*. Internal Report, Vancouver Island Marmot Recovery Team. Unpublished.

Vancouver Island Marmot Recovery Team (2017). *Recovery Plan for the Vancouver Island Marmot in British Columbia.* Victoria, B.C.: B.C. Ministry of Environment.

Vancouver Island Marmot Recovery Team (2008). *Recovery Strategy for the Vancouver Island Marmot (Marmota vancouverensis).* Victoria, B.C.: B.C. Ministry of Environment.

Villers, L. M., Jang, S. S., Lent, C. L., Sock-Cheng, L.-K., & Norosoarinaivo, J. A. (2008). Survey and comparison of major intestinal flora in captive and wild ring-tailed lemur (*Lemur catta*) populations. *American Journal of Primatology 70*, 175-184.

Vitali, S., Reiss, A., & Eden, P. (2011). Conservation medicine in and through zoos. *International Zoo Yearbook 45*, 160-167.

Williams, S. E., & Hoffman, E. A. (2009). Minimizing genetic adaptation in captive breeding programs: A review. *Biological Conservation 142*, 2388-2400.

Woodford, M. H. (1993). International disease implications for wildlife translocations. *Journal of Zoo and Wildlife Medicine 24(3)*, 265-270.

CHAPTER 2

HEMATOLOGICAL AND SERUM BIOCHEMICAL PARAMETERS IN THE VANCOUVER ISLAND MARMOT (*MARMOTA VANCOUVERENSIS*): REFERENCE RANGES AND A COMPARISON OF VALUES BETWEEN MANAGEMENT GROUPS

INTRODUCTION

The Vancouver Island marmot (*Marmota vancouverensis)* is a critically endangered member of the family Sciuridae, endemic to the subalpine meadows of central Vancouver Island, located off the southwest coast of British Columbia, Canada (Swarth, 1912; Swarth, 1911). In the late 1980s this rare species started to exhibit serious population declines (Bryant & Page, 2005). By 1998 marmot numbers had dropped below 100, and by 2003 there were fewer than 30 wild individuals (Jackson *et al.* 2015; Janz *et al.* 2000). In response to this dramatic attrition, a concerted captive breeding and reintroduction program was initiated in 1997. Since its inception, this program has included the participation of the Toronto Zoo (TZ, 1997 to present), the Calgary Zoo (CZ, 1998 to present), and the Mountain View Conservation and Breeding Society in Langley, British Columbia (MV, 2000 to 2014). In 2001 a purpose-built, quarantine and breeding facility, the Tony Barrett Mount Washington Marmot Recovery Centre (MRC), became operational at Mount Washington, Vancouver Island.

As of December 2017, 660 marmots have been associated with the captive program, including 63 wild-born individuals originally captured for breeding purposes and 597 marmots born and weaned in captivity. Reintroductions of captive animals began in 2003 and by 2017 a total of 501 marmots (8 of the original wild-born and 493 of the captive-born) or 75.9% of the overall captive total, have been

released to the wild. Although these recovery efforts have helped to increase the wild population from its low point in 2003, conservation of the Vancouver Island Marmot (VIM) continues to involve an intensive program of *ex situ* management, reintroductions, and translocations (Vancouver Island Marmot Recovery Team, 2017).

The health of an endangered species like the VIM can be negatively affected by many biotic and abiotic factors, including environmental perturbations, low population numbers, and artificial manipulation (Jackson *et al.* 2015; Moorhouse *et al.*, 2006). Although monitoring health and changes in health is important for the vigilent recognition, ellucidation, and mitigation of potential threats that may jeopardise species viability and recovery, effective health surveillence represents a significant challenge for conservation programs (Jackson *et al.* 2015; Robert & Schwanz, 2013; Delahay *et al.,* 2009; Miller, 2007; Daszak, *et al.* 2001; Snyder, *et al.* 1996).

Many studies characterising wildlife health have emphasized dysfunction, pathogens, and mortality in populations (Ryser-Degiorgis, 2013). However, the ability to recognize and contextualize abnormality or disease requires an appreciation of 'normal' (Dimauro, *et al.*, 2008). Baseline measurements of health parameters, often lacking in many wildlife species (Maceda-Veiga, *et al*., 2015), can be used to delineate variability and to compare what is expected for a species under different management regimes. This in turn may identify determinants and possible risk factors that are influencing health in individuals and populations (Hanisch, *et al*. 2012; Vitali *et al.,* 2011; Mathews *et al.,* 2006; Deem *et al*. 2001). A wide variety of morphological, physiological, biochemical, and condition indices have been used to quantify health and fitness at both the individual and population level (Peig & Green, 2009; Stevenson & Woods, Jr., 2006; Wisely, et al., 2005). This includes

hematology and serum biochemistry, which have been used to describe and compare physiological state, adaptation to different habitat conditions, nutritional status, body condition, organ function, relative stress, immune status, biological integrity, the presence of infectious or non-infectious disease, inflammation, and parasite burdens (Ruykys *et al*., 2012; Mellish *et al*., 2010; Moorhouse *et al*., 2007; Masello & Quillfeldt, 2004).

In this project, VIM hematology and serum biochemistry metrics were analysed to delineate expected values and their range of variability in clinically normal animals. Hematology and serology parameters also were compared between different management conditions (wild, captive and captive-release, un-implanted and implanted). The goals of this project were to compile the first set of hematology and serum reference values for the VIM and assess the potential role of these parameters for comparing and monitoring health in this species.

MATERIALS AND METHODS

Classification of marmot management groups

1. *Captive marmots* were defined as any captive-born marmot originating from one of the four captive facilities or any wild-born marmot that had been captured for the captive breeding program and had therefore spent at least one hibernation in any of these captive facilities.
2. *Captive-release marmots* were animals released to the wild from captivity, including those originally born in captivity and any wild-born marmot that had been maintained in captivity for more than a single active season prior to release.
3. *Wild marmots* were wild-born individuals that had never been maintained in a captive facility or any wild-born marmot that had been temporarily maintained at the MRC for less than a single, active season and then returned to the wild.

Captive-release and wild marmots were collectively defined as free-ranging and with a few exceptions were surgically implanted with a very high frequency (VHF) radio-transmitter (Appendix E).

Classification of marmot ages

Marmot ages were categorized as young-of-the-year (pups or juveniles in their first summer), yearling (individuals in their second summer), two-year old (third summer), and adult (fourth or subsequent summers). The age of all captive-born individuals was known with certainty. The age categories of wild-born marmots were known or extrapolated from previously described pelage characteristics (Bryant 1998) and relative size and appearance. In the field, age categories could be determined with relative certainty, except in the case of some individuals identified as two year-olds that exhibited characteristics which overlapped with those of yearlings and adults. Wild adults that were handled and identified for the first time could only be categorized as being three years of age or older, due to the non-specificity of their size and pelage characteristics.

Sample collection and analysis

Comprehensive physical examinations were conducted on all marmots at the time of blood collection. These examinations were conducted by a veterinarian and routinely involved an evaluation of the cardiovascular, respiratory, musculoskeletal, nervous, integumentary, and urogenital systems and an assessment of the marmot's body condition. Marmots that did not display identifiable abnormalities at the time of examination were classified as clinically normal. For the purposes of this analysis, blood samples collected from captive marmots with pre-existing or newly identified health concerns at the time of sampling and those from quarantined, wild-caught marmots undergoing the chronic stress associated with transitioning to captive conditions (Cabezas *et al*., 2007), were categorized as clinically abnormal. These

clinically abnormal samples were excluded from calculation of the reference values and in analyses comparing age, sex, and management groups. They only were used to explore the possibility that there were group differences between clinically normal and abnormal animals. Samples were collected in captivity or at field sites in association with the following circumstances (Figure 2.1):

A. Captive Marmots:

i. Annual or biennial health evaluation of captive marmots maintained at the TZ, CZ, MV and MRC (258 individuals, 606 samples). These marmots did not have implanted abdominal radio-transmitters at the time of sampling (*un-implanted*).

ii. Pre-operative evaluation of captive marmots prior to surgical implantation with abdominal radio-transmitters (188 individuals, 188 samples). These marmots did not have implants at the time of sampling. Categories i. and ii. (healthy, un-implanted, captive marmots) represented the largest number of samples, accounting for 76.4% of the overall total.

iii. Evaluation of implanted, captive-release marmots on the day of release or on the day that preceded it (133 individuals, 133 samples). Following surgery, captive, implanted marmots were afforded a period of convalescence in captivity before being released to the wild. The interval between captive surgery and release ranged from 11 to 93 days (average = 28 days).

iv. Examinations that identified or addressed specific health concerns in captive marmots (30 individuals, 44 samples). Categories of health concerns included congenital or early onset problems, infections / inflammation, heart disorders, neoplasia, and trauma.

v. Health evaluation of marmots during quarantine. These wild-caught marmots were being transitioned into the captive program (23 individuals, 23 samples).

Samples from (iv.) and (v.) were categorized as clinically abnormal.

B. Free-ranging Marmots:

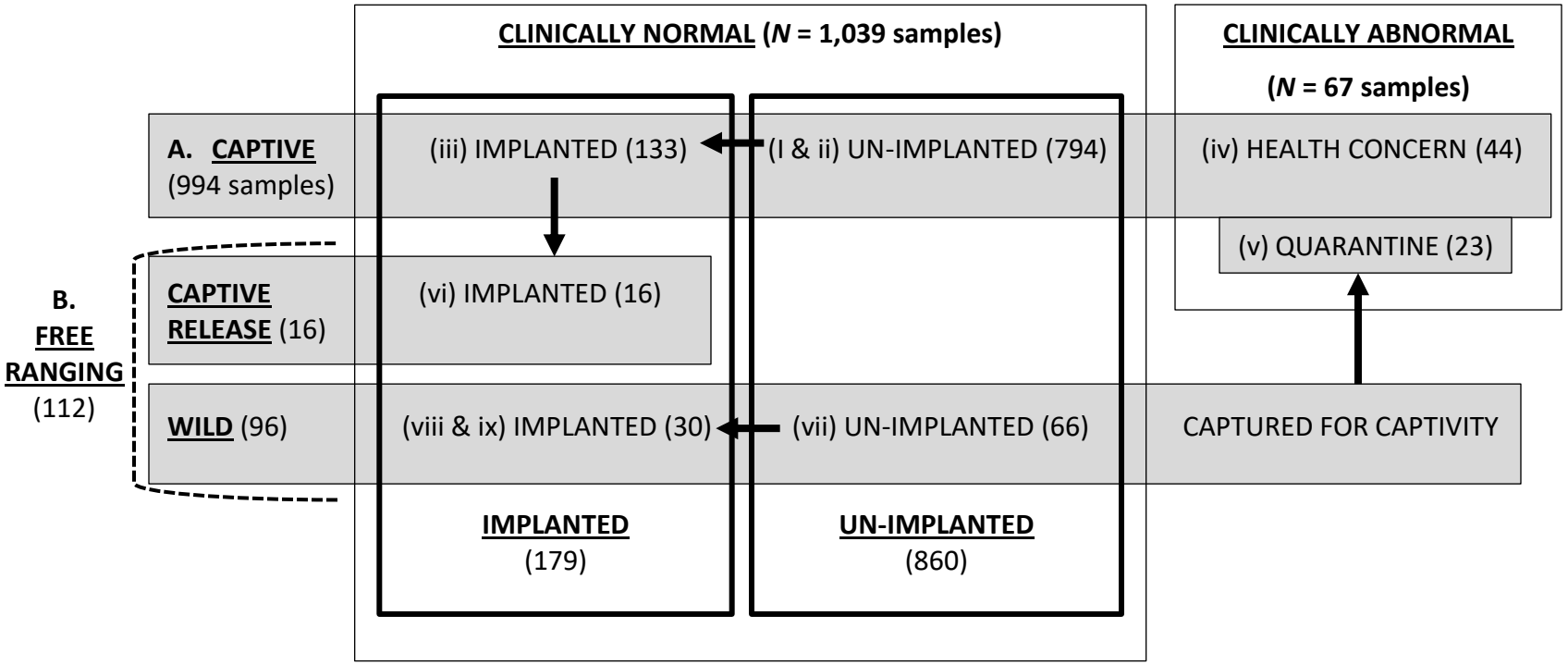
vi. Evaluation of implanted, post-release marmots prior to surgical replacement of radio-transmitters (16 individuals, 16 samples).

vii. Pre-operative evaluation of wild marmots prior to surgical implantation with abdominal radio-transmitters (66 individuals, 66 samples). Marmots did not have an implanted radio-transmitter at the time of sampling.

viii. Evaluation of implanted, wild marmots prior to replacement of radio-transmitters (14 individuals, 19 samples).

ix. Health evaluation of implanted, wild marmots which were opportunistically recaptured for evaluation or translocation (11 individuals, 11 samples).

Figure 2.1. Schematic diagram illustrating the relationships and sample size of the Vancouver Island Marmot (*Marmota vancouverensis*) blood sampling categories used in the analyses. "Implanted" refers to individuals with previously implanted radio-transmitters. The arrows indicate groups with the potential for overlap (i.e. an individual may have been sampled as part of a pre-surgical examination and then again prior to release, or at the time of recapture or radio-transmitter replacement).



Whole blood samples (up to 6 millilitres in volume) were obtained following immobilization with an intramuscular injection of ketamine hydrochloride combined with midazolam hydrochloride (various manufacturers) at approximately 10 mg/kg and 0.25 mg/kg, respectively and then maintained on inhaled isoflurane. In some instances, marmots were mask induced with isoflurane without receiving any injectable immobilization agents (Graham Crawshaw, Toronto Zoo, personal communication).

Blood samples were collected from the cephalic, saphenous, femoral, or tarsal veins. Following venipuncture, a portion of the whole blood was promptly transferred into a vacuum tube containing the anticoagulant ethylenediamine-tetra-acetic acid (EDTA) and into a serum-separating vacuum tube (SST) containing gel. The SST samples were allowed to clot, and then centrifuged. A small portion of whole blood was used to make two blood smears, which were subsequently air-dried at room temperature or in the field, at ambient temperature. Captive and field samples were kept cool and protected from heat, direct sunlight, and freezing temperatures. The data used in this analysis were derived from samples collected over a 23-year period from marmots at geographically disparate locations. Because of lab changeover and logistical practicalities, samples were submitted to one of four diagnostic laboratories; the in-house laboratory at the Toronto Zoo (n=122), Central Laboratory for Veterinarians in Calgary, Alberta or Langley, British Columbia (combined, n=648) and Idexx Reference Laboratories in Delta, British Columbia (n=268). Ninety-eight per cent or 1,084 of the submitted samples were analyzed within three days of collection.

The 16 hematological parameters included total white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin

concentration (MCHC), red cell distribution width (RDW), platelet count, mean platelet volume, segmented neutrophils, band neutrophils, lymphocytes, monocytes, eosinophils and basophils. The 30 serological parameters included glucose, blood urea nitrogen (BUN), creatinine, BUN / creatinine ratio, sodium (Na), potassium (K), sodium / potassium ratio, chloride (Cl), bicarbonate, carbon dioxide, anion gap, calcium, phosphorous (P), calcium / phosphorous ratio, total protein, albumin, globulin, albumin / globulin ratio (A/G ratio), total bilirubin, alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST), gamma glutamyl transferase (GGT), creatinine phosphokinase (CK), calculated osmolality, lactose dehydrogenase (LDH), amylase, lipase, cholesterol and tetra iodothyronine.

In keeping with management activities, all blood samples were collected during the marmots' active season. Although the blood parameters of other true hibernators have been found to exhibit changes during hibernation, including decreases in leukocytes, total bilirubin, glucose, and creatinine, and increases in total protein and albumin (Feoktistova *et al*., 2016), these trends could not be investigated with the availible data. Free-ranging marmots were not accessible for sampling during the hibernation period. Although there was the potential to sample captive marmots, which were allowed to hibernate as a standard management protocol, this did not occur due to concerns about prolonged clotting times associated with physiological changes during torpor, such as thrombocytopenia (Cooper *et al*., 2012).

Baseline reference values were calculated in accordance with guidelines established by Species 360 (formerly the International Species Information System), which incorporate several steps to minimize the potential influence of multiple laboratories and repeated sampling of the same individuals (Teare, 2002). In accordance with these guidelines, the mean and standard deviation are calculated for each of the hematological and serological variables generated from clinically

normal or healthy animals. The standard deviation for these variables was multiplied by three, and the resulting value (representing 3 standard deviations or 99.7% of the values) was subtracted and added to the calculated mean. Any values lying outside this calculated lower and upper range were deemed to be outliers and were removed. The mean and standard deviation were then re-calculated without the outliers (Teare, 2002; de With *et al*., 1999). For the VIM baseline reference values, all clinically normal marmots from all groups, including captive, captive-release, and wild (inclusive of implanted and non-implanted animals) were included in the analysis.

Subsequent analyses focused on comparing the major management groups, specifically wild, captive, and captive-release marmots. Values for clinically normal marmots without abdominal radio-transmitters were compared to clinically normal, implanted marmots and to values for marmots in the clinically-abnormal group. Data were visually examined using dot plots and box plots to identify potential trends or differences with respect to marmot age, sex, and season.

Statistical analyses were performed using R (version 3.3.1, R Development Project, https://www.r-project.org). Normality of data distribution was assessed visually using histograms. Statistical comparison of parameters was performed using Student's unpaired *t*-test. All tests were two-tailed and statistical significance was assigned at $\alpha = 0.01$.

RESULTS

From August 1992 to June 2015, 1,106 blood samples were opportunistically collected from 439 (243 males, 196 females) captive, captive-release, and wild VIM. Individual marmots were sampled between 1 and 14 times. The overall average was 2.52 samples per individual marmot. The sampling mean for captive marmots was

2.85 (range 1 to 14) and for wild individuals it was 1.06 (range 1 to 4). Sixty-seven of the samples were categorized as clinically abnormal. The remaining 1,039 blood samples were obtained from a total of 429 individual marmots determined to be clinically normal or healthy at the time of sampling. Blood sample distribution with respect to year of collection is presented in Figure 2.2. The blood samples were collected over a 23 year-period, indicating the potential for differences with respect to technicians, laboratories and analytical techniques over time. Monthly distribution of sample collection is presented in Figure 2.3. All blood samples were collected during the marmots' active season with over 90% being obtained between June and September. Most of the routinely scheduled annual or biennial health evaluations of captive marmots, which represented the largest category of blood samples, occurred in September, and this is reflected in the graph.

The sex-age distribution of sampled marmots is presented in Figure 2.4. Although there is relatively comparable representation of males and females, the sample set was biased towards younger individuals. The age distributions of free-ranging and captive samples are compared in Figure 2.5. The greater longevity and accessibility of captive marmots allowed more opportunities for collection of blood samples from older animals, in contrast to their free-ranging counterparts

Hematology and serum biochemistry reference values derived from all clinically normal VIM and following Species 360 guidelines are presented in Tables 2.1 and 2.2. The means of VIM reference values are compared to values that have been generated for other small to medium sized rodents in Table 2.3. The general characteristics of the VIM reference ranges appear to be qualitatively comparable to values that have been generated for these other species of rodents.

Figure 2.2. Annual distribution of 1,106 blood samples collected from Vancouver Island Marmots (*Marmota vancouverensis*) 1992 to 2015.

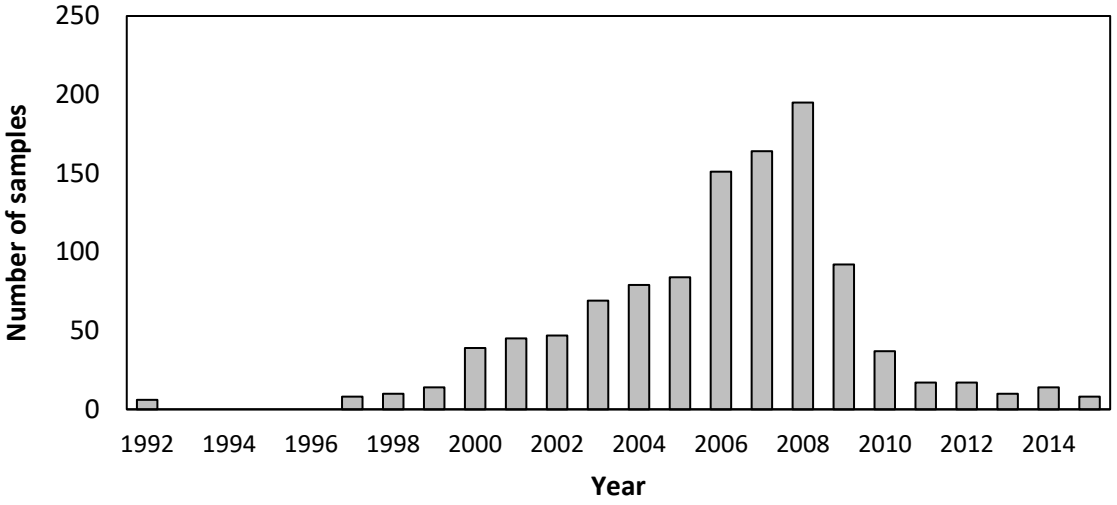


Figure 2.3. Monthly distribution of 1,106 blood samples collected from captive and free-ranging Vancouver Island Marmots (*Marmota vancouverensis*).

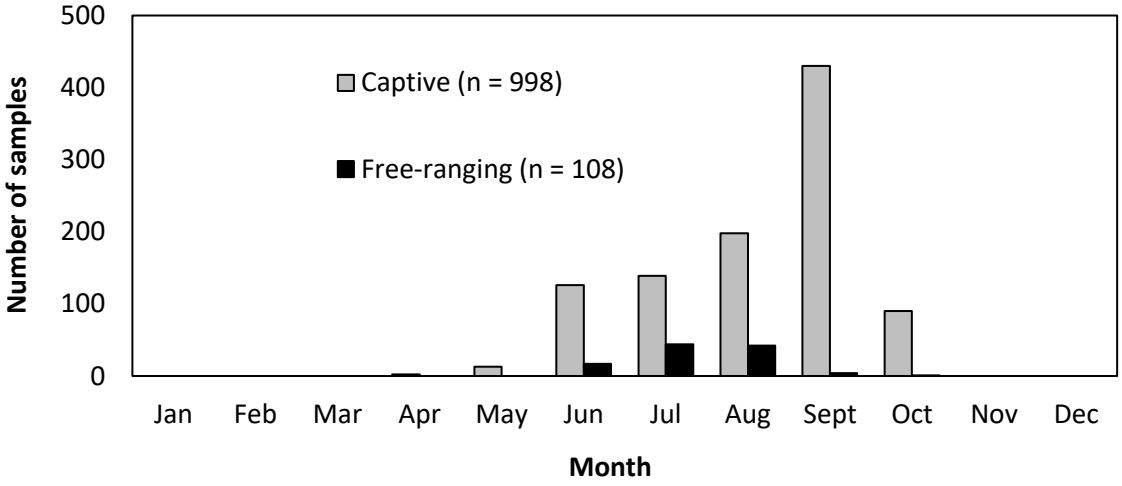


Figure 2.4. Sex and age distribution of 1,106 blood samples collected from captive and free-ranging Vancouver Island Marmots (*Marmota vancouverensis*).

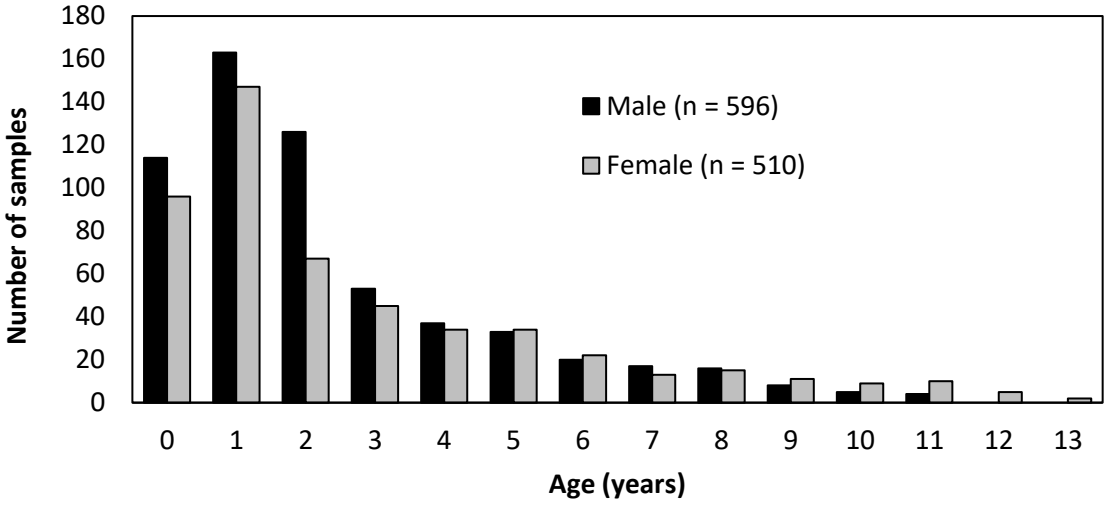


Figure 2.5. Comparison of age distribution of 1,106 blood samples collected from captive and free-ranging Vancouver Island Marmots (*Marmota vancouverensis*).

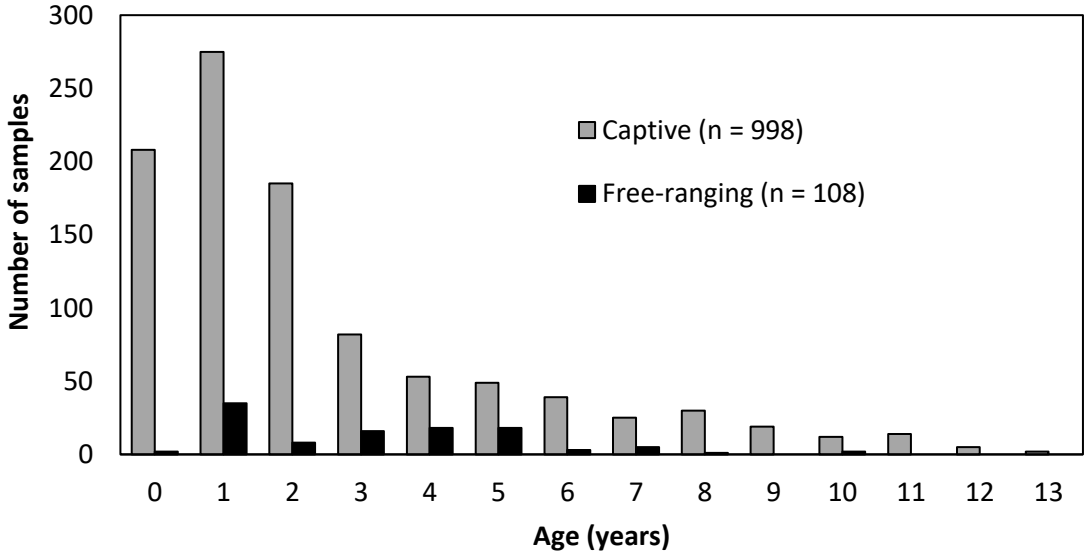


Table 2.1. Reference intervals for 16 hematological parameters from clinically healthy Vancouver Island Marmots (*Marmota vancouverenis*).

Parameter	Units	Mean	Min	Max	*N*	S.D.
WHITE BLOOD CELLS						
White blood cells	x 10^9/L	4.96	0.60	12.20	1018	1.89
Segmented neutrophils	x 10^9/L	2.64	0.25	7.56	1017	1.20
Band neutrophils	x 10^9/L	0.00	0.00	0.00	1012	0.00
Lymphocytes	x 10^9/L	1.93	0.01	5.55	1018	1.13
Monocytes	x 10^9/L	0.31	0.00	1.40	1012	0.25
Eosinophils	x 10^9/L	0.03	0.00	0.25	1011	0.05
Basophils	x 10^9/L	0.01	0.00	0.12	1003	0.02
ERYTHROCYTES						
Red blood cells	x 10^{12}/L	6.44	4.2	8.49	1010	0.63
Hemoglobin	g/L	145.10	98	187	1004	14.32
Hematocrit	L/L	0.43	0.29	0.557	1021	0.04
Mean corpuscular volume	fl	65.90	50	82.3	977	3.51
Mean corpuscular hemoglobin	pg	22.44	16.3	33.1	986	1.60
Mean corpuscular hemoglobin concentration	g/L	339.29	294	384	983	12.26
Red cell distribution width	%CV	14.35	10.1	20.3	877	1.52
HEMOSTASIS						
Platelet count	x 10^9/L	318.70	37	625	761	91.68
Mean platelet volume	fl	8.91	6.7	14	694	1.26

Table 2.2. Reference intervals for 30 serum biochemistry parameters from clinically healthy Vancouver Island Marmots (*Marmota vancouverensis*).

Parameter	Units	Mean	Min	Max	*N*	S.D.
ELECTROLYTES AND ACID-BASE						
Sodium	mmol/L	143.84	130	155.8	525	4.05
Potassium	mmol/L	5.37	3.3	13.3	522	1.42
Sodium / Potassium ratio	ratio	28.14	8.6	43.6	525	6.08
Chloride	mmol/L	102.11	88	112	528	4.11
Bicarbonate	mmol/L	26.37	9	39	147	5.88
Carbon Dioxide	mmol/L	26.37	5	38	357	5.60
Anion Gap		20.71	6	41	512	6.56
Calcium	mmol/L	2.39	1.44	3.33	598	0.21
Phosphorous	mmol/L	1.83	0.66	3.48	594	0.47
Calcium / Phosphorous ratio	ratio	1.39	0.44	4.25	573	0.41
Calculated Osmolality	mmol/kg	297.25	276	319	508	6.73
PROTEINS						
Total Protein	g/L	61.97	42	90	643	8.97
Albumin	g/L	27.30	17	43	555	3.56
Globulin	g/L	32.85	18.6	53	553	6.03
Albumin / Globulin ratio	ratio	0.86	0.3	1.9	561	0.20
LIVER AND MUSCLE						
Total Bilirubin	µmol/L	2.63	0	7	428	1.37
Alkaline Phosphatase	IU/L	71.58	5	266	556	41.35
Alanine Aminotransferase	IU/L	18.64	1	117	512	16.97
Aspartate Aminotransferase	IU/L	37.08	1	222	449	34.51
Gamma Glutamyltransferase	IU/L	3.96	0	18	516	3.01

Table 2.2. (cont.) Reference intervals for 30 serum biochemistry parameters from clinically healthy Vancouver Island Marmots (*Marmota vancouverensis*).

Parameter	Units	Mean	Min	Max	*N*	S.D.
LIVER AND MUSCLE						
Creatinine Phosphokinase	IU/L	526.24	56	2970	583	366.23
Cholesterol	mmol/L	7.24	3.71	13.05	72	1.98
Glucose	mmol/L	8.78	2.2	15.8	630	2.29
RENAL FUNCTION						
Blood Urea Nitrogen	mmol/L	10.92	3.1	22.2	632	2.60
Creatinine	µmol/L	81.91	18	150	617	21.80
Bun / Creatinine ratio	ratio	0.14	0.025	0.376	613	0.06
OTHER						
Lactose Dehydrogenase	IU/L	1784.97	865	4068	30	822.29
Amylase	IU/L	839.79	147	2086	77	423.02
Lipase	IU/L	191.79	60	856	73	137.25
Tetra Iodothyronine	nmol/L	57.17	20	91	151	16.82

Table 2.3. Vancouver Island Marmot (*Marmota vancouverenis*) hematological and serological mean values compared to published means for the Woodchuck (Bellezza, et al., 2015) and normal values published for Mice (*Mus musculus*), Rats (*Rattus norvegicus*), Gerbils (*Meriones unguiculatus*), and Guinea Pigs (*Cavia porcellus*) (Harkness & Wagner, 1995).

Parameter	Units	VIM mean	Woodchuck low / high mean (a)	Mouse range	Rat range	Gerbil range	Guinea Pig range
WHITE BLOOD CELLS							
White blood cells	x 10^9/L	5	8.7 / 10.4	6 - 15	6 - 17	7 - 15	7 - 18
Segmented neutrophils	%	53	63 (b)	10 - 40	9 - 34	5 - 34	28 - 44
Lymphocytes	%	39	26 (b)	55 - 95	65 - 85	60 - 95	39 - 72
Monocytes	%	6	6 (b)	0.1 - 3.5	0 - 5	0 -3	3 - 12
Eosinophils	%	0.6	4 (b)	0 - 4	0 - 6	0 - 4	1 - 5
Basophils	%	0.2		0 - 0.3	0 - 1.5	0 - 1	0 - 3
ERYTHROCYTES							
Red blood cells	x 10^{12}/L	6.4	4.7 / 5.3	7.0 - 12.5	7 - 10	8 - 9	4.5 - 7
Hemoglobin	g/L	145	122 / 132	102 - 166	110 - 180	126 - 162	110 - 150
Hematocrit	L/L	0.43	0.36 / 0.41	0.39-0.49	0.36-0.48	0.43-0.49	0.37-0.48
Mean corpuscular volume	fl	66	73 / 77				
Mean corpuscular hemoglobin	pg	22	25 / 26				
Mean corpuscular hemoglobin concentration	g/L	340	340 / 360				
Red cell distribution width	%CV	14	14 / 17.5				

(a) High and low means arising from multiple studies as reported in Bellezza, *et al*., 2015

(b) Published values in which the collective mean of the differential count equals the mean of the total white blood cells

Table 2.3. (cont.) Vancouver Island Marmot (*Marmota vancouverenis*) hematological and serological mean values compared to published means for the Woodchuck (Bellezza, et al., 2015) and normal values published for Mice (*Mus musculus*), Rats (*Rattus norvegicus*), Gerbils (*Meriones unguiculatus*), and Guinea Pigs (*Cavia porcellus*) (Harkness & Wagner, 1995).

Parameter	Units	VIM mean	Woodchuck low / high mean (a)	Mouse range	Rat range	Gerbil range	Guinea Pig range
HEMOSTASIS							
Platelet count	x 10^9/L	319	451 / 525	800-1100	500-1300	400 - 600	250 - 850
Mean platelet volume	fl	8.9	6.8 / 7.1				
ELECTROLYTES AND ACID-BASE							
Sodium	mmol/L	144	143 / 151	112 - 193	135 - 155	144 - 158	132 - 156
Potassium	mmol/L	5.4	3.7 / 4.7	5.1 - 10.4	4 - 8	3.8 - 5.2	4.5 - 8.9
Sodium / Potassium ratio	ratio	28	34 / 40				
Chloride	mmol/L	102	97 / 102	82 - 114	94 - 116	93 - 118	98 - 115
Bicarbonate	mmol/L	26	30 / 34				
Carbon Dioxide	mmol/L	26	31 / 34				
Anion Gap		20	15 / 20				
Calcium	mmol/L	2.4	2.3 / 2.6	0.8 - 2.1	1.3 - 3.2	0.9 - 1.6	1.3 - 3.0
Phosphorous	mmol/L	1.8	1.2 / 1.7	0.7 - 3.0	1.7 - 2.7	1.2 - 2.3	1.0 - 3.9
PROTEINS							
Total Protein	g/L	62	55 / 69	35 - 72	56 - 76	43 - 125	46 - 62
Albumin	g/L	27	23 / 37	25 - 48	38 - 48	18 - 55	21 - 39
Globulin	g/L	33	30 / 36	6	18 - 30	12 - 60	17 - 26
Albumin / Globulin ratio	ratio	0.9	0.7 / 1.1				

(a) High and low means arising from multiple studies as reported in Bellezza, *et al*., 2015

Table 2.3. (cont.) Vancouver Island Marmot (*Marmota vancouverenis*) hematological and serological mean values compared to published means for the Woodchuck (Bellezza, et al., 2015) and normal values published for Mice (*Mus musculus*), Rats (*Rattus norvegicus*), Gerbils (*Meriones unguiculatus*), and Guinea Pigs (*Cavia porcellus*) (Harkness & Wagner, 1995).

Parameter	Units	VIM mean	Woodchuck low / high mean (a)	Mouse range	Rat range	Gerbil range	Guinea Pig range
LIVER AND MUSCLE							
Total Bilirubin	µmol/L	2.6	1.9 / 5.5	1.71-15.4	3.4 - 9.4	3.4 - 10.3	5.1 - 15.4
Alkaline Phosphatase	IU/L	72	7.2 / 19.3	45 - 222	16 - 125	12 - 37	18 - 28
Alanine Aminotransferase	IU/L	19	1.0 / 3.5	26 - 77	16 - 89		10 - 25
Aspartate Aminotransferase	IU/L	37	21 / 34	54 - 269	192 - 262		45.5-48.2
Gamma Glutamyltransferase	IU/L	4	1.7 (b)				
Creatinine Phosphokinase	IU/L	526	478 / 690				
Cholesterol	mmol/L	7.2	3.6 / 5.4	0.7 - 2.1	1 - 3.4	2.3 - 3.9	0.5 - 1.1
Glucose	mmol/L	8.8	10.2 / 12.1	3.4 - 9.7	2.8 - 7.5	2.8 - 7.5	3.3 - 7
RENAL FUNCTION							
Blood Urea Nitrogen	mmol/L	11	4.6 / 9	4.3 - 10	5.4 - 7.5	6 - 9.6	3.2 - 11.3
Creatinine	µmol/L	82	79.5 / 133	26 - 88	18 - 71	53 - 124	53 - 194
OTHER							
Amylase	IU/L	840	2210 / 2645				
Lipase	IU/L	192	201 / 361				

(a) High and low means arising from multiple studies as reported in Bellezza, *et al*., 2015

(b) One mean reported in Bellezza, *et al*., 2015

Seasonal Trends. Total white blood cells, neutrophils, lymphocytes, monocytes, total protein and albumin increased during the course of the active season (Figure 2.6), while platelet count, total bilirubin, glucose and creatinine did not exhibit change. In laboratory Woodchucks (*Marmota monax*), the hematocrit has been reported to be higher in the spring than in the autumn (Bellezza, *et al*., 2015), but this was not apparent from these data. Woodchucks and Yellow-bellied Marmots (*Marmota flaviventris*), also are reported to have an increase in red blood cells, hematocrit, and hemoglobin from early to late summer in preparation for hibernation (Armitage, 2014). VIM exhibited a similar trend in all three parameters from June to October, but the magnitude of the difference was relatively small (Figure 2.7).

Age trends. Red blood cells, hematocrit, and hemoglobin appeared to decline with marmot age (Figure 2.8) whereas the leukogram (including total white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, and basophils) showed no age pattern. Glucose decreased with age whereas amylase increased with age (Figure 2.9).

Elevation trends. Acclimatization to lower elevations has resulted in decreased hematocrit levels in Yellow-bellied Marmots (Armitage, 2014). The mean of hematocrit values from captive marmots maintained at the MV (elevation 25 meters) were 5.5, 5.6, and 7.6% lower than respective values from the CZ (elevation 1027 meters), TZ (elevation 145 meters), and the MRC (elevation 1244 meters), and these differences were statistically significant ($P < 0.0001$). However, there was no significant difference or discernable elevation trend between the other three institutions and this could indicate that the MV values are the result of some facility effect rather than elevation.

Sex trends. In several species, female hematocrit values are lower than those of males (Probst, et al., 2006). A sex difference in hematocrit was seen in these data and

was statistically significant (male mean = 0.434, female mean = 0.416, P < 0.0001). Amylase was lower in males than females (male mean = 711.11, female mean = 933.10, P = 0.0408) (Figure 2.10). There was no apparent or statistically-significant sex difference with respect to white blood cells (P = 0.4014) or glucose (P = 0.1779).

Group comparisons. Parameters for clinically normal captive, wild, and captive-release marmots are compared in Tables 2.4 and 2.5. Values for non-implanted, implanted, and clinically abnormal marmots are compared in Table 2.6 and 2.7. The statistical and clinical significance of differences between hematology and serum biochemistry parameters with respect to specific groups are summarised in Table 2.8 and 2.9.

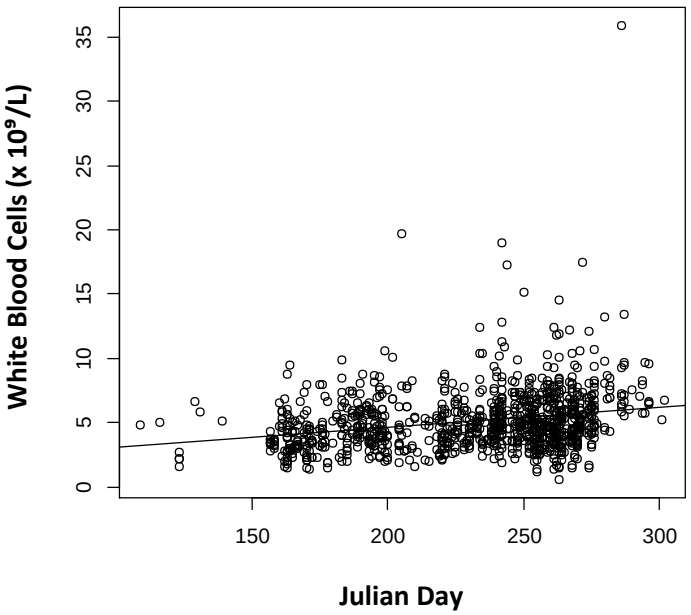
Eleven of the 46 parameters showed no clinical or statistical differences between any of the groups that were compared, including values which were derived from clinically abnormal animals. This included 2 hematology parameters; band neutrophils and eosinophils, and 9 serum parameters; anion gap, calcium, calculated osmolality, aspartate aminotransferase, gamma glutamyltransferase, creatinine phosphokinase, BUN / creatinine ratio, lactose dehydrogenase (data limited for some groups), and lipase (data limited for some groups).

Twenty-eight of the 46 parameters showed evidence of statistically significant differences between certain groups, but the small magnitude and nature of these differences did not suggest physiological or clinical significance.

Eight of the 46 parameters had biologically plausible and statistically significant differences between some of the compared groups (Table 2.8). This included 5 leukogram parameters; white blood cells, segmented neutrophils, lymphocytes, monocytes, basophils, and 3 serum biochemistry parameters; total protein, albumin, and globulin. Potentially significant differences in leukogram and protein parameters occurred between the captive and free-ranging groups (both

wild and captive-release). Also, leukogram parameters of clinically normal, un-implanted marmots differed from the clinically abnormal group.

Figure 2.6. Changes in total white blood cells and total protein from April to October for clinically normal Vancouver Island Marmots (*Marmota vancouverensis*).



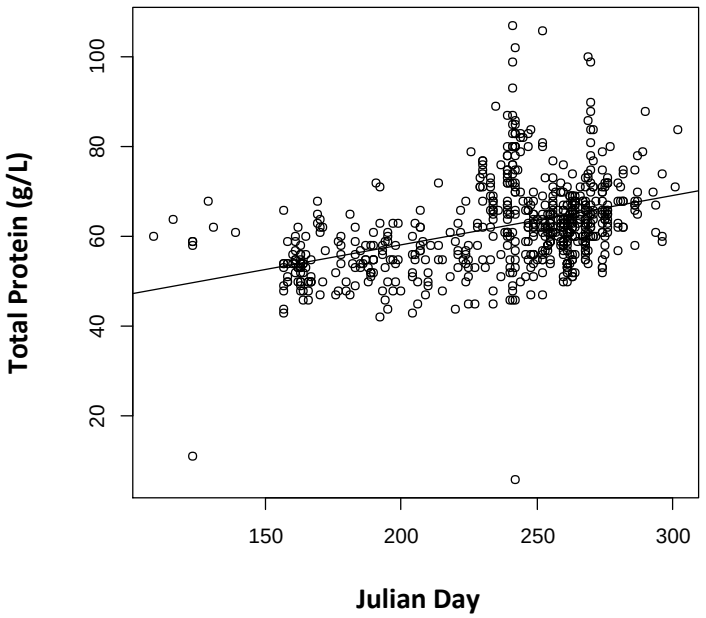
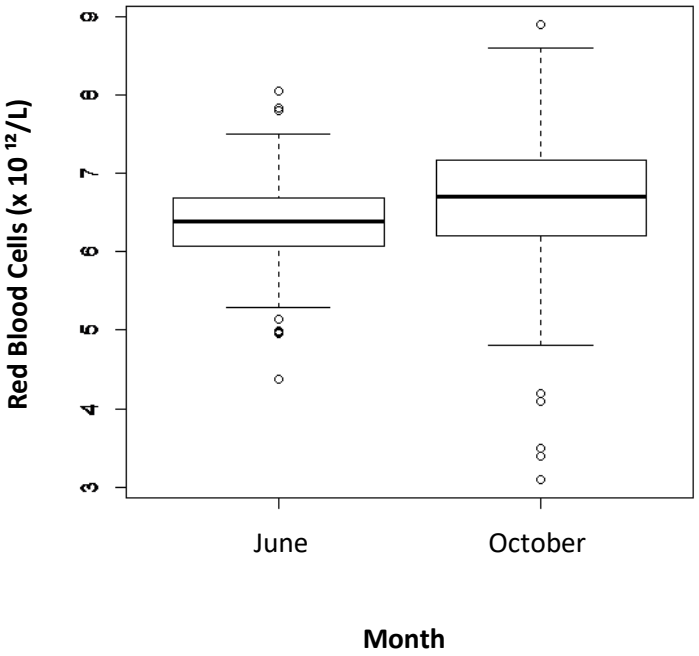


Figure 2.7. Boxplot comparisons of red blood cells and hemoglobin between June and October for clinically normal Vancouver Island Marmots (*Marmota vancouverensis*).



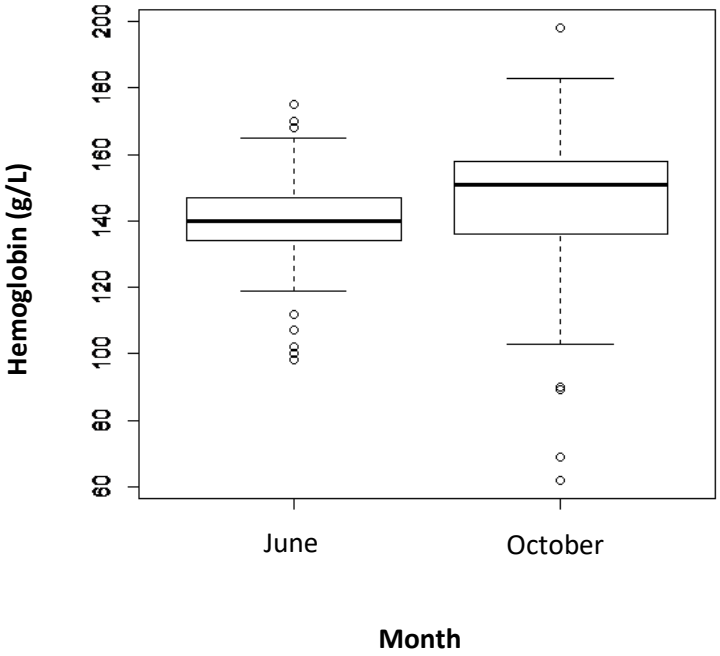
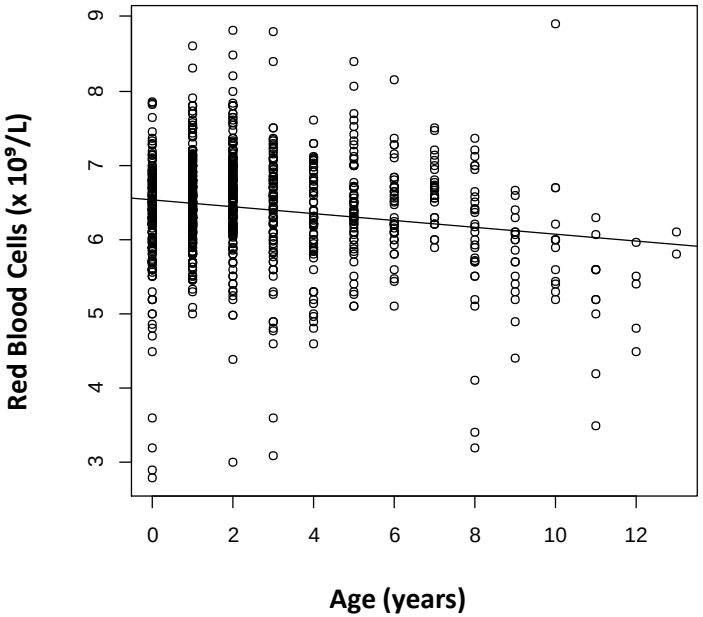


Figure 2.8. Change in red blood cells and hemoglobin for clinically normal Vancouver Island Marmots (*Marmota vancouverensis*) according to age.



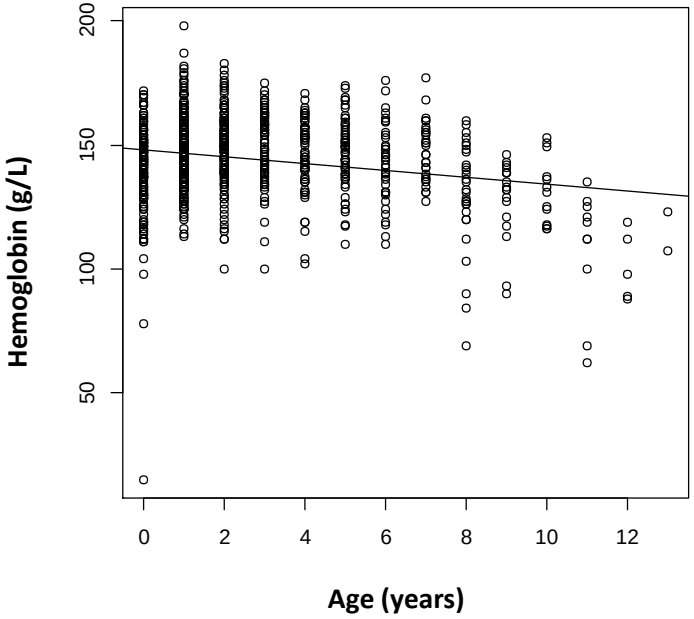
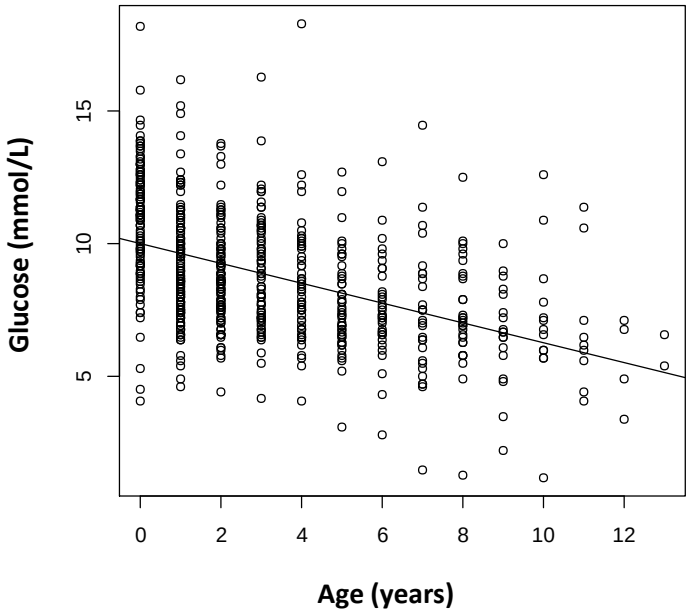


Figure 2.9. Change in glucose and amylase for clinically normal Vancouver Island Marmots (*Marmota vancouverensis*) according to age.



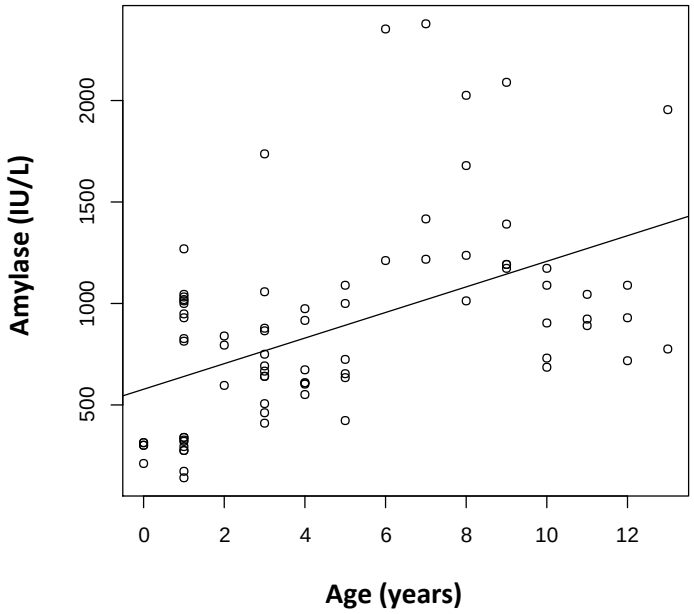
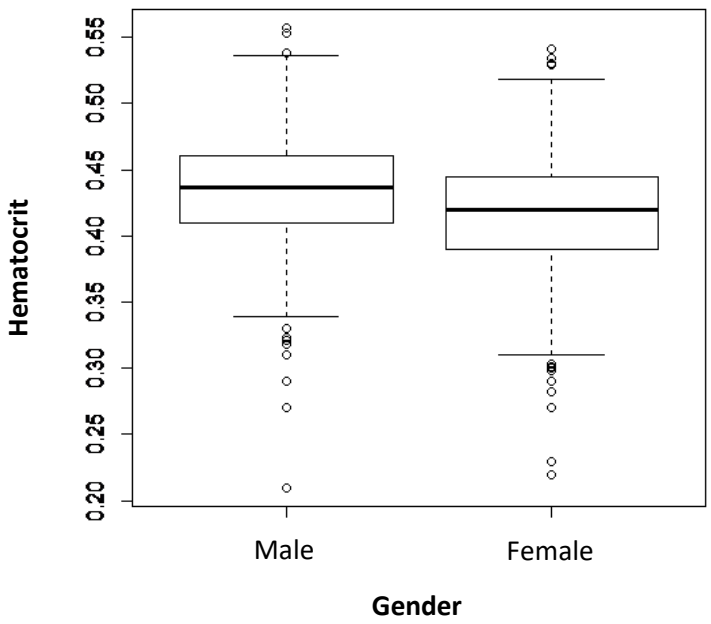


Figure 2.10. Boxplots comparing male and female values for hematocrit and amylase in Vancouver Island Marmots (*Marmota vancouverensis*).



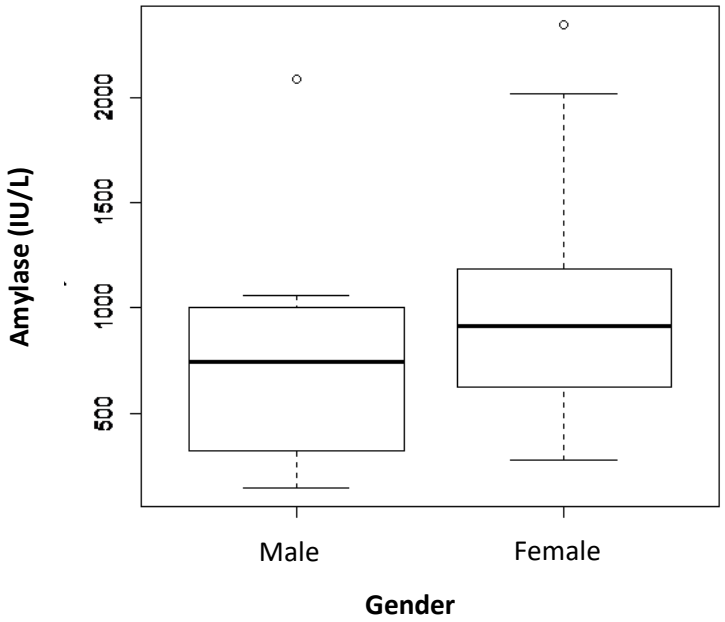


Table 2.4. Hematology values comparing healthy captive (un-implanted), wild and captive-release Vancouver Island Marmots (*Marmota vancouverensis*). Bold indicates comparisons with statistical and potential clinical significance (a).

Parameter	Units	Captive Mean	S.D.	*N*	Wild mean	S.D.	*N*	Captive-release mean	S.D.	*N*
WHITE BLOOD CELLS										
White blood cells	x 10^9/L	**5.506**	**2.619**	**601**	**4.401**	**2.376**	**94**	**3.825**	**1.000**	**16**
Segmented neutrophils	x 10^9/L	2.949	1.803	598	2.973	1.805	94	2.841	0.808	16
Band neutrophils	x 10^9/L	0.004	0.032	598	0.000	0.000	94	0.000	0.000	16
Lymphocytes	x 10^9/L	**2.102**	**1.268**	**598**	**1.161**	**0.992**	**94**	**0.618**	**0.204**	**16**
Monocytes	x 10^9/L	**0.404**	**0.415**	**598**	**0.194**	**0.164**	**94**	0.304	0.454	16
Eosinophils	x 10^9/L	0.034	0.074	598	0.049	0.090	94	0.031	0.045	16
Basophils	x 10^9/L	0.016	0.038	598	0.010	0.021	94	0.028	0.037	16
ERYTHROCYTES										
Red blood cells	x 10^{12}/L	6.38	0.789	597	6.32	0.613	94	6.28	0.530	16
Hemoglobin	g/L	145.206	17.999	587	146.36	14.308	94	145.00	12.329	16
Hematocrit	L/L	0.43	0.048	601	0.43	0.043	94	0.43	0.039	16
Mean corpuscular volume	fl	66.356	4.782	564	67.93	2.752	94	68.63	2.752	16
Mean corpuscular hemoglobin	pg	22.614	1.846	557	23.22	1.192	94	23.08	0.728	16
Mean corpuscular hemoglobin concentration	g/L	340.397	18.244	567	341.00	10.327	94	336.81	11.397	16
Red cell distribution width	%CV	14.471	1.623	475	12.99	3.576	94	14.76	2.059	16
HEMOSTASIS										
Platelet count	x 10^9/L	322.683	121.355	341	312.93	108.046	92	302.06	51.177	16
Mean platelet volume	fl	8.980	1.653	332	8.13	0.933	86	8.99	1.829	16

(a) Potential clinical significance was considered if the magnitude of the difference in the parameter comparison had practical relevance with respect to physiological or clinical state.

Table 2.5. Serum biochemistry values comparing healthy, captive (un-implanted), wild and captive-release Vancouver Island marmots (*Marmota vancouverensis*). Bold indicates comparisons with statistical and potential clinical significance (a).

Parameter	Units	Captive Mean	S.D.	*N*	Wild mean	S.D.	*N*	Captive-release mean	S.D.	*N*
ELECTROLYTES AND ACID-BASE										
Sodium	mmol/L	144.16	4.811	335	142.05	4.148	79	139.45	6.729	11
Potassium	mmol/L	5.241	2.771	334	7.26	3.043	79	9.98	5.900	11
Sodium / Potassium ratio	ratio	29.43	5.842	334	22.13	6.779	79	16.91	6.039	11
Chloride	mmol/L	101.32	6.094	332	103.10	4.205	79	99.27	4.650	11
Bicarbonate	mmol/L	26.29	6.624	96	25.93	4.131	15	27.44	2.297	9
Carbon Dioxide	mmol/L	26.66	5.979	211	24.33	5.673	64	28.50	3.536	2
Anion Gap		21.39	7.333	318	21.65	7.968	79	22.55	4.712	11
Calcium	mmol/L	2.40	0.222	404	2.42	0.979	79	2.28	0.109	11
Phosphorous	mmol/L	1.70	0.427	405	2.14	0.740	79	1.82	0.526	11
Calcium / Phosphorous ratio	ratio	1.50	0.385	379	1.26	0.709	79	1.34	0.364	11
Calculated Osmolality	mmol/kg	298.73	7.913	317	297.20	6.464	79	294.36	5.801	11
PROTEINS										
Total Protein	g/L	**65.55**	**9.919**	**455**	**54.00**	**6.278**	**79**	**55.18**	**6.080**	**11**
Albumin	g/L	**29.09**	**4.833**	**369**	**25.68**	**7.185**	**79**	**23.45**	**3.078**	**11**
Globulin	g/L	**35.29**	**7.131**	**365**	29.01	4.648	**78**	**31.73**	**3.636**	**11**
Albumin / Globulin ratio	ratio	0.90	0.619	366	0.88	0.139	79	0.75	0.082	11
LIVER AND MUSCLE										
Total Bilirubin	µmol/L	2.81	1.848	263	3.16	1.718	64	4.56	1.236	9
Alkaline Phosphatase	IU/L	75.70	60.877	367	94.48	123.009	79	75.00	26.680	11
Alanine Aminotransferase	IU/L	28.35	40.501	333	17.46	12.506	79	13.09	7.739	11
Aspartate Aminotransferase	IU/L	49.89	72.258	311	36.60	24.691	52	54.09	38.454	11
Gamma Glutamyltransferase	IU/L	4.77	6.046	333	4.66	3.293	76	3.36	2.420	11
Creatinine Phosphokinase	IU/L	649.92	987.135	398	813.01	1193.698	79	522.00	111.966	11

(a) Potential clinical significance was considered if the magnitude of the difference in the parameter comparison had practical relevance with respect to physiological or clinical state.

Table 2.5. (cont.) Serum biochemistry values comparing healthy, captive (un-implanted), wild and captive-release Vancouver Island marmots (*Marmota vancouverensis*). Bold indicates comparisons with statistical and potential clinical significance (a).

Parameter	Units	Captive Mean	S.D.	*N*	Wild mean	S.D.	*N*	Captive-release mean	S.D.	*N*
Cholesterol	mmol/L	7.94	1.994	37	6.32	1.388	33	No data	No data	No data
Glucose	mmol/L	8.97	2.504	440	8.75	2.669	79	6.81	2.071	11
RENAL FUNCTION										
Blood Urea Nitrogen	mmol/L	11.85	3.967	441	10.48	2.712	79	9.63	2.633	11
Creatinine	µmol/L	83.31	24.741	427	73.59	22.758	79	65.55	10.211	11
Bun / Creatinine ratio	ratio	0.16	0.086	427	0.16	0.067	79	0.151	0.052	11
OTHER										
Lactose Dehydrogenase	IU/L	1784.97	822.289	30	No data	No data	No data	No data	No data	No data
Amylase	IU/L	1105.81	496.744	43	587.21	279.358	34	No data	No data	No data
Lipase	IU/L	181.11	134.140	38	204.91	145.485	33	No data	No data	No data
Tetra Iodothyronine	nmol/L	61.88	14.315	77	42.50	14.470	46	No data	No data	No data

(a) Potential clinical significance was considered if the magnitude of the difference in the parameter comparison had practical relevance with respect to physiological or clinical state.

Table 2.6. Hematology values comparing (i) clinically normal, un-implanted, (ii) clinically normal, implanted, and (iii) clinically abnormal Vancouver Island Marmots (*Marmota vancouverensis*). Bold indicates comparisons with statistical and potential clinical significance (a).

Parameter	Units	Un-implanted mean	S.D.	*N*	Implanted mean	S.D.	*N*	Non-representative mean	S.D.	*N*
WHITE BLOOD CELLS										
White blood cells	x 10^9/L	**5.081**	**2.447**	**857**	5.213	1.970	174	**6.468**	**3.718**	**65**
Segmented neutrophils	x 10^9/L	**2.703**	**1.642**	**854**	2.909	1.428	174	**3.726**	**2.583**	**65**
Band neutrophils	x 10^9/L	0.003	0.027	854	0.000	0.000	174	0.000	0.000	65
Lymphocytes	x 10^9/L	1.978	1.237	854	1.936	1.169	174	2.111	1.406	65
Monocytes	x 10^9/L	**0.346**	**0.373**	**854**	0.311	0.283	174	**0.505**	**0.673**	**65**
Eosinophils	x 10^9/L	0.035	0.075	854	0.035	0.062	174	0.037	0.069	65
Basophils	x 10^9/L	**0.015**	**0.035**	**854**	0.019	0.036	174	**0.041**	**0.152**	**65**
ERYTHROCYTES										
Red blood cells	x 10^{12}/L	6.35	0.74	853	6.73	0.55	173	6.31	0.87	60
Hemoglobin	g/L	143.47	16.94	843	148.87	11.07	174	138.35	20.54	60
Hematocrit	L/L	0.42	0.05	857	0.44	0.03	174	0.41	0.06	64
Mean corpuscular volume	fl	66.13	4.39	819	65.73	2.76	174	64.62	4.30	60
Mean corpuscular hemoglobin	pg	22.49	1.68	813	22.17	1.11	174	21.95	1.45	60
Mean corpuscular hemoglobin concentration	g/L	340.06	16.18	823	337.01	9.87	174	340.05	14.49	60
Red cell distribution width	%CV	14.24	2.14	731	14.62	2.01	174	14.68	3.17	57
HEMOSTASIS										
Platelet count	x 10^9/L	314.15	106.686	595	354.08	92.153	174	394.21	172.393	43
Mean platelet volume	fl	8.92	1.500	570	8.94	1.204	174	8.61	1.500	40

(a) Potential clinical significance was considered if the magnitude of the difference in the parameter comparison had practical relevance with respect to physiological or clinical state.

Table 2.7. Serum biochemistry values comparing clinically normal un-implanted and implanted and clinically abnormal Vancouver Island Marmots (*Marmota vancouverensis*). Bold indicates comparisons with statistical and potential clinical significance (a).

Parameter	Units	Un-implanted mean	S.D.	*N*	Implanted mean	S.D.	*N*	Clinically abnormal mean	S.D.	*N*
ELECTROLYTES AND ACID-BASE										
Sodium	mmol/L	143.79	4.69	461	143.35	4.31	72	144.19	3.82	54
Potassium	mmol/L	5.46	2.67	460	6.74	3.53	72	5.38	1.15	54
Sodium / Potassium ratio	ratio	28.38	6.08	460	24.77	7.86	72	27.78	5.86	52
Chloride	mmol/L	101.86	5.62	458	102.58	4.63	72	101.23	3.58	52
Bicarbonate	mmol/L	26.48	6.33	117	25.97	3.67	30	25.87	4.67	15
Carbon Dioxide	mmol/L	26.46	5.67	316	25.64	4.97	42	25.88	8.29	33
Anion Gap		20.78	7.06	444	21.73	7.50	72	22.71	8.72	48
Calcium	mmol/L	2.44	0.92	530	2.48	0.72	72	2.48	0.18	56
Phosphorous	mmol/L	1.82	0.51	531	2.15	0.72	72	2.00	0.46	56
Calcium / Phosphorous ratio	ratio	1.49	1.38	505	1.26	0.60	72	1.29	0.42	54
Calculated Osmolality	mmol/kg	297.58	7.91	443	297.17	6.10	72	298.24	7.48	49
PROTEINS										
Total Protein	g/L	62.89	10.47	581	56.72	5.53	72	60.59	7.92	58
Albumin	g/L	28.04	4.72	495	26.06	7.47	72	26.89	4.36	55
Globulin	g/L	33.50	7.22	491	31.47	4.22	71	33.42	6.96	55
Albumin / Globulin ratio	ratio	0.90	0.54	492	0.81	0.13	72	0.85	0.22	53
LIVER AND MUSCLE										
Total Bilirubin	µmol/L	2.70	1.73	370	3.08	1.51	66	3.44	3.18	48
Alkaline Phosphatase	IU/L	75.14	55.83	493	90.07	125.44	72	62.24	38.63	54
Alanine Aminotransferase	IU/L	24.46	35.46	455	14.68	11.50	72	18.94	53.38	51
Aspartate Aminotransferase	IU/L	45.51	64.45	405	31.83	23.88	54	91.32	361.58	28

(a) Potential clinical significance was considered if the magnitude of the difference in the parameter comparison had practical relevance with respect to physiological or clinical state.

Table 2.7. (cont.) Serum biochemistry values comparing clinically normal un-implanted and implanted and clinically abnormal Vancouver Island Marmots (*Marmota vancouverensis*). Bold indicates comparisons with statistical and potential clinical significance (a).

Parameter	Units	Un-implanted mean	S.D.	*N*	Implanted mean	S.D.	*N*	Clinically abnormal mean	S.D.	*N*
ELECTROLYTES AND ACID-BASE										
Gamma Glutamyltransferase	IU/L	4.61	5.35	455	3.86	3.03	72	3.26	3.28	53
Creatinine Phosphokinase	IU/L	650.75	897.44	524	568.46	1103.35	72	610.11	525.89	54
Cholesterol	mmol/L	7.23	2.02	68	7.38	1.24	4	7.91	2.88	11
Glucose	mmol/L	8.88	2.43	56	8.13	2.32	72	8.69	2.71	57
RENAL FUNCTION										
Blood Urea Nitrogen	mmol/L	11.30	3.89	567	9.84	2.85	72	11.14	4.16	57
Creatinine	µmol/L	83.20	24.00	553	77.32	19.25	71	88.26	29.88	57
Bun / Creatinine ratio	ratio	0.15	0.08	553	0.13	0.05	71	0.14	0.06	56
OTHER										
Lactose Dehydrogenase	IU/L	1784.97	822.29	30	No data	No data	No data	2590.50	587.61	2
Amylase	IU/L	893.31	490.28	74	655.60	269.49	5	753.60	388.75	15
Lipase	IU/L	181.60	115.37	68	330.40	300.58	5	145.92	43.34	12
Tetra Iodothyronine	nmol/L	57.70	16.40	128	56.48	21.64	25	65.22	27.92	13

(a) Potential clinical significance was considered if the magnitude of the difference in the parameter comparison had practical relevance with respect to physiological or clinical state.

Table 2.8. Summary of significance levels in hematology parameters between specific treatments or groups of Vancouver Island Marmots (*Marmota vancouverensis*). Grey indicates statistical significance at $\alpha < 0.01$ and black indicates that there is both statistical significance at $\alpha < 0.01$ and potential clinical significance (a).

	White blood cells	Segmented neutrophils	Band neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Red blood cells	Hemoglobin	Hematocrit	Mean corpuscular volume	Mean corpuscular hemoglobin	Mean corpuscular hemoglobin	Red cell distribution width	Platelet count	Mean platelet volume
Captive versus Wild	**<0.01**	0.90	0.23	**<0.01**	**<0.01**	0.08	0.14	0.48	0.55	1.00	<0.01	<0.01	0.76	<0.01	0.48	<0.01
Captive versus Captive-Release	**<0.01**	0.81	0.62	**<0.01**	0.34	0.87	0.45	0.61	0.96	1.00	<0.01	0.31	0.43	0.49	0.50	0.98
Wild versus Captive-Release	0.34	0.77	1.00	0.03	0.08	0.44	0.21	0.81	0.72	1.00	0.35	0.69	0.14	0.06	0.69	0.01
Un-Implanted versus Implanted	0.50	0.12	0.14	0.68	0.24	1.00	0.17	<0.01	<0.01	<0.01	0.25	0.02	0.02	0.03	<0.01	0.87
Un-implanted versus Non-representative	**<0.01**	**<0.01**	0.37	0.41	**<0.01**	0.84	**<0.01**	0.69	0.03	0.15	0.01	0.02	1.00	0.15	<0.01	0.21

(a) Potential clinical significance was considered if the magnitude of the difference in the parameter comparison had practical relevance with respect to physiological or clinical state.

Table 2.9. Summary of significance levels in serum biochemistry parameters between specific treatments or groups of Vancouver Island Marmots (*Marmota vancouverensis*). Grey indicates statistical significance at $\alpha < 0.01$ and black indicates that there is both statistical significance at $\alpha < 0.01$ and potential clinical significance (a).

	Sodium	Potassium	Sodium / Potassium ratio	Chloride	Carbon Dioxide	Anion Gap	Calcium	Phosphorous	Calcium / Phosphorous ratio	Calculated Osmolality	Total Protein	Albumin	Globulin	Albumin / Globulin ratio	Total Bilirubin	Alkaline Phosphatase
Captive versus Wild	<0.01	<0.01	<0.01	0.01	<0.01	0.78	0.71	<0.01	<0.01	0.11	**<0.01**	**<0.01**	**<0.01**	0.78	0.17	0.05
Captive versus Captive-Release	<0.01	<0.01	<0.01	0.27	0.67	0.60	0.07	0.36	0.17	0.07	**<0.01**	**<0.01**	0.10	0.42	0.01	0.97
Wild versus Captive-Release	0.08	0.02	0.02	<0.01	0.31	0.71	0.64	0.17	0.72	0.17	0.56	0.31	0.06	<0.00	0.02	0.60
Un-implanted versus Implanted	0.45	<0.01	0.82	0.96	0.96	0.96	0.99	0.82	0.95	0.98	0.84	0.79	0.92	0.95	0.93	0.92
Un-implanted versus Non-representative	0.55	0.83	0.97	0.97	0.97	0.93	0.99	0.85	0.96	0.98	0.94	0.94	1.00	0.98	0.88	0.94

(a) Potential clinical significance was considered if the magnitude of the difference in the parameter comparison had practical relevance with respect to physiological or clinical state.

Table 2.9. (continued) Summary of significance levels in serum biochemistry parameters between specific treatments or groups of Vancouver Island Marmots (*Marmota vancouverensis*). Grey indicates statistical significance at $\alpha < 0.01$ and black indicates that there is both statistical significance at $\alpha < 0.01$ and potential clinical significance (a).

	Alanine Aminotransferase	Aspartate Aminotransferase	Gamma Glutamyltransferase	Creatinine Phosphokinase	Cholesterol	Glucose	Blood Urea Nitrogen	Creatinine	Bun / Creatinine ratio	Lactose Dehydrogenase	Amylase	Lipase	Tetra Iodothyronine
Captive versus Wild	0.02	0.19	0.88	0.20	<0.01	0.48	<0.01	<0.01	1.00	No data	<0.01	0.48	<0.01
Captive versus Captive-Release	0.21	0.85	0.44	0.67	No data	<0.00	0.07	0.02	0.73	No data	No data	No data	No data
Wild versus Captive-Release	0.26	0.06	0.21	0.42	No data	0.02	0.33	0.25	0.64	No data	No data	No data	No data
Un-implanted versus Implanted	0.91	0.94	0.96	0.97	0.99	0.83	0.89	0.93	0.93	No data	0.90	0.73	0.97
Un-implanted versus Non-representative	0.96	0.86	0.93	0.99	0.90	0.96	0.99	0.95	0.97	0.81	0.90	0.90	0.87

(a) Potential clinical significance was considered if the magnitude of the difference in the parameter comparison had practical relevance with respect to physiological or clinical state.

DISCUSSION

In this project, reference intervals for 16 hematology and 30 serum biochemistry parameters were calculated from laboratory data derived from clinically healthy Vancouver Island Marmots over a 23-year period. In addition to providing baseline health data, these parameters were qualitatively compared to values that have been generated from other rodent species. The general characteristics of the VIM reference ranges appeared to be commensurate with values for other small to medium sized rodents.

The hematology and serum biochemistry parameters also were used as a measure of comparison between different management VIM conditions (wild, captive and captive-release, un-implanted and implanted, clinically normal and abnormal). Eleven of the 46 parameters showed no clinical or statistical differences between any of the groups that were compared, including values which were derived from clinically abnormal animals. Twenty-eight of the 46 parameters showed evidence of statistically significant differences between certain groups, but the small magnitude and nature of these differences did not suggest physiological or clinical significance.

Eight of the 46 parameters had biologically plausible and statistically significant differences between a portion of the compared groups. This included 5 leukogram parameters; white blood cells, segmented neutrophils, lymphocytes, monocytes, basophils, and 3 serum biochemistry parameters; total protein, albumin, and globulin. Potentially significant differences in leukogram and protein parameters occurred between the captive and free-ranging groups (both wild and captive-release). Also, leukogram parameters of clinically normal, un-implanted marmots were lower than the clinically-abnormal group. Although the blood samples from the clinically abnormal animals were collected under a range of

different scenarios, at least 9 were obtained from marmots with confirmed infections and elevated white blood cells and neutrophils. In addition to indicating inflammation or infection, white blood cells (including neutrophils, lymphocytes, monocytes, and basophils) and serum proteins also are indicators of general stress and immune status (Mellish *et al*. 2010; Barker & Boonstra, 2005). Although group characteristics for leukograms and serum proteins still fell within the established references ranges and the magnitude of differences between the groups were relatively small, these results suggest that leukogram and protein values may have the potential to differentiate the physiological or immunological status of healthy individuals in the different management groups.

Although hematology and serum biochemistry parameters are commonly advocated as a tool for the evaluation and comparison of health in individuals and populations (Ruykys et al., 2012; Mellish *et al*., 2010; Masello & Quillfeldt, 2004), there are many factors in addition to health status which may have the potential to influence blood values and reference ranges. This includes intrinsic factors such as age, sex, and seasonality (Stannard *et al*., 2013; Barker & Boonstra, 2005) and extrinsic factors such as sample collection, storage and transport, and laboratory methods of analysis (Dimauro, *et al*., 2008; Low *et al*., 2006). The challenge of using this type of data is determining which parameters are restricted to the evaluation of individuals, which vary according to factors like age and sex, which vary due to vagaries associated with sample handling and laboratory analysis, and which represent legitimate differences between individuals or groups under different treatments. Although statistically significant differences often may be identified, it is also important to consider the magnitude of these differences and their clinical, physiological, or biological relevance or implications (Nakagawa & Cuthill, 2007).

In several instances, differences between groups could possibly be attributable to extrinsic factors. Analytical methodologies may have varied between laboratories (Low *et al*., 2006) and techniques may have altered over time. Due to the cost of analyses, it is also possible that institutions were somewhat selective in terms of which individuals they sampled, focusing on genetically important or older individuals. Pre-analytical factors, such as sample collection, handling and storage, may account for higher variation in results than analytical techniques (Maceda-Veiga, *et al*., 2015). Although attempts were made to standardize the conditions under which blood was collected, stored, and transported, it is possible that some of the samples obtained from free-ranging marmots were artifactually compromised (Low *et al*. 2006). The measurement of lactose dehydrogenase, sodium, potassium, chloride, phosphorous, blood urea nitrogen, creatinine and glucose have all been shown to be influenced by temperature and storage interval (Monden *et al*. 2008; Reese *et al*. 2006). Under field conditions, some marmot samples may have been exposed to logistically imposed variations in ambient temperature and transport delays prior to laboratory analysis. This might represent a consideration in interpreting comparisons between free-ranging and captive marmots.

It is important for ongoing species recovery and future decision-making to better understand if perturbations or artificial manipulation, including captive management and implant surgeries, are having an observable impact on the health of the species. In this project (i) the reference values for clinically healthy Vancouver Island Marmots are comparable to those of other related mammal species, (ii) the parameters of marmots released from captivity are comparable to their wild counterparts, (iii) the parameters of marmots that have been surgically implanted with abdominal radio-transmitters do not differ in any clinically significant way from healthy marmots without transmitters, (iv) healthy captive marmots

demonstrate higher, statistically significant leukogram and serum protein parameters, compared to healthy free-ranging individuals, which is possibly suggestive of increased chronic stress or subclinical health influences (Mellish *et al*. 2010; Barker & Boonstra, 2005), and finally, (v) white blood cell and protein metrics, in conjunction with other clinical data, may present a simple, efficient, and cost-effective parameter for monitoring differences between management groups.

Hematology and serum biochemistry have great utility for assessing health and identifying disease in individuals. Recognising abnormality or dysfunction in these parameters requires an appreciation of normal and this is facilitated by the establishment of baseline measurements. Although the utility of these measurements for assessing group or population effects are not as straightforward, monitoring these parameters over time may allow for the identification of increased individual dysfunction, for ongoing comparisons between the different management groups, and for continued surveillance of the marmots' overall health status. Recognition of future alterations in hematology or serum biochemestry characteristics, in concert with other clinical data, may help to signal changes in health. This in turn may serve to identify emerging health risks that could influence the species' overall health and recovery.

LITERATURE CITED

Armitage, K. B. (2014). *Marmot Biology: Sociality, Individual Fitness, and Population Dynamics.* Cambridge: Cambridge University Press.

Barker, J. M., & Boonstra, R. (2005). Preparing for winter: Divergence in the summer-autumn hematological profiles from representative species of the squirrel family. *Comparative Biochemestry and Physiology Part A 142*, 32-42.

Bellezza, C. A., Sexton, S., Curtin, L. I., Concannon, P. W., Baldwin, B. H., Graham, L. A., . . . Tennant, B. C. (2015). The Laboratory Woodchuck (*Marmota monax*). In *Laboratory Animal Medicine, Third Edition* (pp. 351-386). Elsevier Inc.

Bryant, A. A. (1998). *Metapopulation ecology of Vancouver Island marmots (Marmota vancouverensis), PhD thesis.* Victoria, B.C.: University of Victoria.

Bryant, A. A., & Page, R. E. (2005). Timing and causes of mortality in the endangered Vancouver Island marmot (*Marmota vancouverensis*). *Canadian Journal of Zoology (83)*, 674-682.

Cabezas, S., Blas, J., Marchant, T. A., & Moreno, S. (2007). Physiological stress levels predict survival probabilities in wild rabbits. *Hormones and Behavior (51)*, 313-320.

Cooper, S. T., Richters, K. E., Melin, T. E., Liu, Z.-j., Hordyk, P. J., Benrud, R. R., . . . Sola-Visner, M. C. (2012). The hibernating 13-lined ground squirrel as a model organism for potential cold storage of platelets. *American Journal of Regulatory, Integrative and Comparative Physiology 302 (10)*, 1202-1208.

Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2001). Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Tropica 78*, 103-116.

de With, N. I., Stephen, C., Ribble, R., Schwantje, H., & McAdie, M. (1999). *The potential role of Yersinia spp. in the decline of the Vancouver Island marmot (*Marmota vancouverensis*).* Nanaimo: Centre for Coastal Health.

Deem, S. L., Parker, P. G., & Miller, R. E. (2008). Building bridges: connecting the health and conservation professions. *The Journal of Tropical Biology and Conservation*, 662-665.

Delahay, R. J. et al. (eds). (2009). *Management of Disease in Wild Mammals.* New York: Springer. Ebenhard, T. (1995). Conservation breeding as a tool for saving species from extinction. *Tree 10 (11)*, 438-443.

Dimauro, C., Bonelli, P., Nicolussi, P., Rassu, S. P., Cappio-Borlino, A., & Pulina, G. (2008). Estimating clinical chemistry reference values based on an existing data set of unselected animals. *The Veterinary Journal 178*, 278-281.

Feoktistova, N. Y., Kuznetsova, E. V., Naidenko, S. V., Surov, A. V., & Tikhonova, N. B. (2016). Seasonal changes in blood cells and Biochemical Parameters in the Monglian Hamster (*Allocricetulus curtatus*). *Biology Bulletin 43 (4)*, 344 - 349.

Hanisch, S. L., Riley, S. J., & Nelson, M. P. (2012). Promoting wildlife health or fighting disease: insights from history, philosophy, and science. *Wildlife Society Bulletin 36 (3)*, 477-482.

Harkness, J. E., & Wagner, J. E. (1995). *The Biology and Medicine of Rabbits and Rodents.* Baltimore: Williams and Wilkins.

Jackson, C.; Baker, A.; Doyle, D.; Franke, M.; Jackson, V.; Lloyd, N.; McAdie, M.; Stephens, T.; Traylor-Holzer, K.;. (2015). *Vancouver Island Marmot Population and Habitat Viability Assessment Workshop Final Report.* Apple Valley, MN: IUCN SSC Conservation Breeding Specialist Group.

Janz, D. W., Bryant, A. A., Dawe, N. K., Schwantje, H., Harper, B., Nagorsen, D., . . . Simmons, R. (2000). *National Recovery Plan for the Vancouver Island Marmot (*Marmota vancouverensis*) 2000 Update: RENEW Report No.19.* Ottawa, Ontario: Environment Canada.

Low, M., Eason, D., Elliot, G., McInnes, K., & Paul-Murphy, J. (2006). Hematological and biochemical reference ranges for the kapapo (*Strigops habroptilus*). *Journal of Avian Medicine and Surgery 20 (2)*, 80-88.

Maceda-Veiga, A., Figuerola, J., Martinez-Silvestre, A., Viscor, G., Ferrari, N., & Pacheco, M. (2015). Inside the redbox: Applications of haemtaology in wildlife monitoring and ecosystem health assessment. *Science of the Total Environment 514*, 322-332.

Masello, J. F., & Quillfeldt, P. (2004). Are haematological parameters related to body condition, ornamentation and breeding success in wild burrowing parrots (*Cyanoliseus patagonus*)? *Journal of Avian Biology 35 (5)*, 445-454.

Mathews, F., Moro, D., Strachan, R., Gelling, M., & Buller, N. (2006). Health surveillance in wildlife reintroductions. *Biological Conservation 131*, 338-347.

Mellish, J. E., Hindle, A. G., & Horning, M. (2010). A preliminary assessment of the impact of disturbance and handling on Weddell seals of McMurdo Sound, Antarctica. *Antarctic Science 22 (1)*, 25-29.

Miller, P. S. (2007). Tools and techniques for disease risk assessment in threatened wildlife conservation programmes. *International Zoo Yearbook 41*, 38-51.

Monden, S., Tanaka, M., & Takahashi. (2008). Pre-test blood samples at clinical laboratories - effects of temperature and duration of blood sample storage on measurements. *The Japanese Jounal of Clinical Pathology 56(3)*, 239-42.

Moorhouse, T. P., Gelling, M., McLaren, G. W., Milan, R., & Macdonald, D. W. (2006). Physiological consequences of captive conditions in water voles (*Arvicola terrestris*). *Journal of Zoology 271*, 19-26.

Nakagawa, S., & Cuthill, I. C. (2007). Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological Reviews 82*, 591-605.

Peig, J., & Green, A. J. (2009). New perspectives for estimating body condition from mass / length data: the scaled mass index as an alternative method. *Oikos 118*, 1883-1891.

Probst, R. J., Lim, J. M., Bird, D. N., Pole, G. L., Sato, A. K., & Claybaugh, J. R. (2006). Gender differences in the blood volume of conscious Sprague-Dawley rats. *Journal of the American Association of Lab Animal Science 45 (2)*, 49 - 52.

Reese, W. O., Tramper, D., & Koehler, K. J. (2006). Effects of storage time and temperature after blood sampling from turkeys on plasma concentrations of potassium, sodium, and chloride. *Poultry Science 85*, 1095-97.

Robert, K. A., & Schwanz, L. E. (2013). Monitoring the health status of free-ranging Tammar wallabies using hematology, serum biochemestry, and parasite loads. *The Journal of Wildlife management 77 (6)*, 1232-1243.

Ruykys, L., Rich, B., & McCarthy, P. (2012). Haematology and biochemistry of warru (*Petrogale lateralis* MacDonnell Ranges race) in captivity and the wild. *Australian Veterinary Journal 90 (9)*, 331-340.

Ryser-Degiorgis, M.-P. (2013). Wildlife health investigations: needs, challenges and recommendations. *BMC Veterinary Research 9 9223)*.

Snyder, N. F., Derrickson, S. R., Bessinger, S. R., Wiley, J. W., Smith, T. B., Toone, W. D., & Miller, B. (1996). Limitations of captive breeding in endangered species recovery. *Conservation Biology 10 (2)*, 338-348.

Stannard, H. J., Young, L. J., & Old, J. M. (2013). Further investigation of the blood characteristics of Australian quoll (*Dasyurus* spp.) species. *Veterinary Clinical Pathology 42(4)*, 476-482.

Stevenson, R. D., & Woods, Jr., W. A. (2006). Condition indices for conservation: new uses for evolving tools. *Integrative and Comparative Biology 46 (6)*, 1169-1190.

Swarth, H. S. (1911). Two new species of marmots from North America. *Univ. Calif. Publ. Zool. (7)*, 201-204.

Swarth, H. S. (1912). Report on a collection of birds and mammals from Vancouver Island. *Univ. Calif. Publ. Zool. (10)*, 1-124.

Teare, J. A. (2002). *International Species Information System Physiological Data Reference Values.* Apple Valley, MN: International Species Information System (ISIS).

Vitali, S., Reiss, A., & Eden, P. (2011). Conservation medicine in and through zoos. *International Zoo Yearbook 45*, 160-167.

Wisely, S. M., Santymire, R. M., Livieri, T. M., Marinari, P. E., Kreeger, J. S., Widlt, D. E., & Howard, J. (2005). Environment influences morphology and development for in situ and ex situ populations of black-footed ferret (Mustela nigripes). *Animal Conservation 8*, 321-328.

CHAPTER 3

SURVEILLANCE OF VANCOUVER ISLAND MARMOT (*MARMOTA VANCOUVERENSIS*) HEALTH USING CLINICAL AND POST MORTEM EXAMINATIONS, AND FIELD MORTALITY DETERMINATION

INTRODUCTION

Guidelines produced by the International Union for Conservation of Nature (IUCN) on the management of *ex-situ* populations, reintroductions, and other conservation translocations, have identified health and disease monitoring as an essential component in an effective conservation program (IUCN, 2013; IUCN, 2002). Monitoring helps to identify ongoing or novell threats and allows for the adaptation of management, release, and surveillence regimes. The establishment of characteristic health parameters in captive, captive-release and wild populations delineates what is normal or expected under each treatment and how these differ between groups (Deem *et al.* 2001). Differences in parameters may reflect a response to disparate conditions or they may signal a potential health challenge associated with management or emerging stressors.

Despite their widely acknowledged potential to influence success in conservation programs, the effects of animal health and disease are infrequently reported (Mathews *et al*. 2005). Monitoring health and disease in *ex situ* and *in situ* populations presents a significant challenge. Determining the spectrum of influence that captive conditions have on the health of an endangered species is time-consuming and complicated and monitoring free-ranging populations is limited by the challenges associated with observing, examining, sampling and tracking individuals under field conditions. The health data obtained from these respective populations often is of disparate quality and this makes comparisons problematic.

Identifying potential health threats requires the organization and collation of data from a variety of sources. Grouping or clustering health data may have the potential to facilitate comparisons and allow timely detection of potential trends. Syndromic surveillance involves the systematic collection and evaluation of a wide range of health data from multiple sources to identify, characterize, and predict potential patterns, clusters, or syndromes as they are occurring. This allows for the detection of non-specific trends and for the tracking of specific syndromes using incomplete or imperfect data. This type of surveillance facilitates the filtering and efficient transfer of health or disease information so that risks can be identified and further investigated, and so that timely, informed decisions can be made. Although syndromic surveillance has been most commonly used to filter and identify syndromes in large, complex datasets, it may also present a conceptual framework for the detection of health and disease patterns in *ex situ* and *in situ* populations of endangered species, which may only be signaled by incomplete data from a relatively small number of individuals or events. This allows for the timely detection, comparison, investigation, and management of health risks before definitive diagnoses can be fully realized.

The Vancouver Island Marmot (*Marmota vancouverensis)* is a critically endangered sciurid endemic to the mountains of central Vancouver Island (Swarth, 1912; Swarth, 1911). In the early to mid-1980s there were indications that the marmot's population was stable or increasing, with approximately 300 to 350 individuals occupying at least 30 colony sites. However, in the late 1980s and throughout the 1990s, the Vancouver Island Marmot (VIM) demonstrated precipitous declines. By 1998 the wild population had dropped below 100 animals and by 2003 it was reduced to less than 30 individuals at 5 colonies, making it

Canada's most endangered mammal (Jackson *et al.* 2015; Nagorsen, 2005; Janz *et al.* 2000).

The impending threat of species extinction led to the initiation of an intensive captive breeding program in 1997. Since its inception, this program has involved the participation of three Canadian zoological institutions, the Toronto Zoo (TZ), the Calgary Zoo (CZ), and the Mountain View Conservation and Breeding Society in Langley, British Columbia (MV) and the construction of a dedicated marmot facility at Mount Washington on Vancouver Island, the Tony Barrett Mount Washington Marmot Recovery Centre (MRC). Although there has been significant recovery of the wild population, the VIM continues to be managed by an intensive program of captive breeding, reintroduction, and translocations. Further details on the history of the VIM recovery project are provided in Chapter 1.

In situ and *ex situ* management has subjected the VIM to a unique and dynamic assemblage of natural and artificial influences. The ability to identify and track these influences with respect to marmot health is vital for effective long-term management and for vigilent recognition, ellucidation, and mitigation of potential risks that may jeoprodise the recovery of this species. The marmot recovery project has generated a great range of qualitative and quantitative, health-related data from both free-ranging and captive marmots. These have originated from a multitude of sources, and include clinical, pathological, morphological, physiological, demographic, epidemiological, and environmental data. Because of its disparate, individualistic, and heterogeneous nature, the utility of these data for elucidating marmot health patterns has not been fully realized.

Clinical evaluations conducted by veterinarians, necropsies performed by veterinary pathologists, and properly vetted observations made by field staff monitoring free-ranging marmots through radio-telemetry, have the potential to

offer reliable and robust health-related data. Although this information has been typically used to evaluate individuals or singular events, it also has the potential to be extrapolated more broadly. In this chapter I use syndromic surveillence of disparate, historical data from physical examinations, post mortem examinations, and field evaluation of marmot mortalities, to identify possible trends and risk factors relating to the health of captive, captive-release and wild populations of VIM, and to evaluate the utility of this approach in informing future marmot health management and species recovery.

MATERIALS AND METHODS

Classification of marmot management groups

Captive marmots were defined as any captive-born marmot originating from the TZ (Toronto, Ontario), the CZ's Devonian Wildlife Conservation Centre (De Winton, Alberta), the MV (Langley, British Columbia) and the MRC (Mount Washington, British Columbia) or any wild-born marmot that was maintained at one of these facilities for more than a single, active season for the purpose of captive breeding. Four wild-born marmots that died during their initial 30-day quarantine period following capture were intended to be part of the captive breeding program and were defined as captive marmots even though they were not maintained beyond one active season. Wild-born marmots that were held for brief intervals at the MRC (periods of time that ranged from hours to weeks), and then re-released during the same active season, were classified as wild. These individuals were captured and held to simplify translocations and were never intended to be part of the captive breeding program. This latter group did not suffer any mortalities during their limited time in captivity.

From 1997 to 2016, a total of 639 individuals were maintained in captivity, including 61 wild-born marmots originally captured from the wild and 578 marmots (from 170 litters) born and weaned in captivity. This number does not include marmots from at least 11 unsuccessful, captive litters (6.1% of total litters) in which all pups failed to survive to weaning age (at approximately 28 to 30 days) or individual pups that were part of successfully weaned captive litters that failed to survive to weaning age.

Captive-release marmots were defined as those animals released to the wild from captivity, including those originally born in captivity and any wild-born marmot that had been maintained in captivity for more than a single active season prior to release. From 2003 to 2016 a total of 490 captive marmots were released to the wild including 482 captive-born individuals (103 born and weaned at TZ, 116 at CZ, 98 at MV and 165 at MRC) and 8 wild-born individuals (time in captivity ranged from 394 to 4350 days, average = 2091 days). Two of these wild-born, captive-release marmots were recaptured and permanently returned to captivity.

All 490 captive marmots that were released to the wild were maintained at the MRC for a minimum of 30 days prior to release, as a way of verifying their health and minimizing the potential for introduction of novel pathogens into the extant free-ranging populations. From 2003 to 2012, all 373 of the captive non-pups released to the wild spent at least one hibernation at the MRC. The average interval that these marmots spent at the MRC prior to release was 450 days (range 207 to 2271 days). From 2008 to 2011, 35 pups born at the MRC were released to the wild in their first summer (average age 112 days, range 93 to 123 days). From 2013 to 2016 a total of 82 captive marmots, originating from the other captive facilities, were released to the wild during the same year in which they arrived at the MRC. The average interval of time that these marmots spent at the MRC prior to release, was

58 days (range 42 to 77 days). Overall, 50.3% of the releases from captivity occurred in July, 27.8% in August, 14.0% in September, 7.4% in June, 0.4% in May, and 0.2% in April.

Pre-conditioned marmots were defined as captive-release individuals that were translocated to a new site after successfully surviving at least one hibernation in the wild. This treatment primarily represented an attempt to mitigate the poor survival of captive-release marmots introduced directly into Strathcona Park. Captive marmots were released at Mount Washington, where survival was consistently high, and then subsequently recaptured and moved after at least one winter in the wild. In 2008 one captive-release individual was opportunistically relocated to Mount Cain after successfully surviving its first wild hibernation at Mount Washington and from 2012 to 2016, a total of 32 pre-conditioned marmots were trapped and translocated from Mount Washington to Strathcona Park. The interval between initial release from captivity and subsequent relocation ranged from 316 to 1042 days (average = 402 days). Between 2003 and 2009, four captive-release marmots that had moved into unsuitable habitat shortly after their initial release were recaptured and returned to their original release site or to a new site and were not considered to be pre-conditioned.

Wild marmots were defined as any wild-born individual that had never been maintained in a captive facility or any wild-born marmot temporarily held at the MRC for less than a single, active season and then returned to the wild.

Translocation marmots were wild marmots that were deliberately moved from one site to another. From 1996 to 2015, fourteen wild marmots were translocated to various sites in the Nanaimo Lakes region. From 2012 to 2016 a total of 58 wild marmots were translocated from Mount Washington to Strathcona Park.

Captive-release, pre-conditioned, wild, and translocation marmots were collectively defined as 'free-ranging'.

Marmot ages were categorized as young-of-the-year (pups or juveniles in their first summer), yearling (individuals in their second summer), two-year old (third summer), and adults (fourth or subsequent summers). The age of all captive-born individuals was known with certainty. The age categories of wild-born marmots were known or extrapolated from previously described pelage characteristics (Bryant 1998) and relative size and appearance. In the field, age categories could be determined with relative certainty, except in the case of some individuals classified as two year-olds, that exhibited variable characteristics which overlapped those of yearlings and adults.

Monitoring of free-ranging marmots

Free-ranging marmots were monitored in the field with surgically implanted, intra-abdominal VHF (very high frequency) radio-transmitters. Internal transmitters were used because the dramatic seasonal mass changes that individuals demonstrate during hibernation made conventional neck collars impractical. From 1992 to 2016 a total of 898 implant surgeries were performed on VIM including 96 replacement surgeries. Techniques for surgical implantation of intraperitoneal transmitters have been previously described in a range of wild species (Van Vuren, 1989; Ranheim *et al.* 2004; Soto-Azat *et al.* 2008) and the specific details concerning transmitters and handling, anesthetic and surgery techniques used on this project are described in more detail in Appendix D, E and F. Because implantation required a surgical procedure and an adequate capacity for convalescence, marmots were not implanted close to hibernation (i.e. early in the field season following emergence, when body condition and food resources were low and when female marmots might be pregnant or late in the field season close to immergence when the marmots'

metabolic rate was starting to decline and healing capacity might be reduced). Most surgeries were performed between the middle of June and the end of August. During the course of this project, temperature-responsive transmitters from Custom Telemetry ® (Watkinsville, Georgia), Telonics ® (Mesa, Arizona), Advanced Telemetry Systems ® (Isanti, Minnesota) and Holohil Systems Limited ® (Carp, Ontario) were used. The pulse rate and temperature response of the transmitters was configured to maximize functionality and prolong battery life. A reduced pulse rate associated with lowered body temperature helped to prolong battery life during hibernation and during the active season a low transmitter pulse rate was used to signal potential marmot mortalities.

Four hundred and eighty-four of the 490 captive-release marmots were surgically implanted with an abdominal radio transmitter prior to release and then afforded a period of convalescence in captivity. Intervals between captive surgery and release ranged from 11 to 93 days (average = 28 days). Three implanted marmots were held until the following year due to transient problems or injuries that precluded same-year release and one individual died prior to release due to transmitter complications. One marmot was recaptured and surgically implanted 50 days after being initially released without a transmitter and 32 individuals were later recaptured and had their transmitters replaced. One captive-release marmot was recaptured for a second transmitter replacement.

From 1992 to 2016 a total of 379 implant surgeries were performed on wild marmots including 63 replacement surgeries. Wild marmots being intentionally prepared for translocation were surgically implanted with radio-transmitters and then allowed to convalesce at their familiar colony sites for a period which ranged from 21 to 37 days (average = 30 days). In five instances, wild marmots were not recaptured and translocated until the year following their surgery. Four marmots

that were captured at aberrant locations including Bamfield, Nanaimo (2), and Nanoose on Vancouver Island were surgically implanted and translocated to suitable habitat within days of initial capture. All four marmots survived for at least 350 days (range 350 to 1511 days), indicating that these marmots were not adversely affected by prompt relocation following surgery.

Physical Examinations

Comprehensive physical examinations were conducted on both captive and free-ranging VIM. These examinations were conducted by a veterinarian and involved an evaluation of the cardiovascular, respiratory, musculoskeletal, nervous, integumentary, and urogenital systems, and an assessment of the marmot's body condition and weight. Individuals with previously implanted radio-transmitters were routinely palpated to ensure that the units were mobile or free-floating within the abdominal cavity. Marmots that did not display identifiable physical abnormalities were classified as clinically normal or healthy at the time of examination. Prior to physical examination, most marmots were immobilized with an intramuscular injection of ketamine hydrochloride (10 mg/kg) and midazolam hydrochloride (0.25 mg/kg), and then maintained on inhaled isoflurane. In some instances, marmots were mask induced with isoflurane without receiving any injectable immobilization agents.

Physical examinations of VIM were conducted under the following conditions:

1) Captive marmots:
    i) In addition to examinations initiated during quarantine (total = 55) or in response to specific health concerns (61), all captive marmots maintained at the TZ, CZ, MV and MRC received routine physical examinations on an annual or biennial basis. These examinations were conducted late in the

active season in anticipation of hibernation or prior to transfer to another captive facility.

ii) Captive-release, pre-release - 485 of the 490 marmots released from captivity were surgically implanted with radio-transmitters and were given pre-operative examinations at MRC to confirm their suitability for surgery. There was also a pre-release examination which followed the post-operative, convalescence period. This second examination occurred on the day of release or on the day that preceded it. The 5 un-implanted marmots also received pre-release exams.

2) Free-ranging marmots:

i) Captive-release, post-release - marmots were examined if they were recaptured for transmitter replacement surgery.

ii) Captive-release, pre-conditioned - following recapture, these marmots were typically held for a short interval (hours to weeks) at the MRC and then examined on the day of their re-release to a new site or on the day that preceded it.

iii) Wild marmots receiving transmitters were given pre-operative examinations to confirm their suitability for surgery.

iv) Wild marmots also received examinations if they were re-trapped for transmitter replacement surgery.

v) Thirty-eight out of 61 wild marmots were examined shortly after capture but prior to being transferred to the captive breeding program.

vi) Wild marmots, translocation - these marmots were recaptured after an *in situ* convalescent period and typically held for a short interval (hours to days) at the MRC. These marmots were re-examined on the day of their translocation or on the day that preceded it.

Post Mortem Examination / Causes of mortality

All marmots found dead in the wild or in captivity underwent a standardized post mortem examination. Necropsies were performed by pathologists at the Province of British Columbia's Animal Health Centre (Abbotsford, BC), the Province of British Columbia's Wildlife Branch (Victoria, British Columbia), the Calgary Zoo (Calgary, Alberta) and the Ontario Veterinary College (University of Guelph, Guelph, Ontario). Wherever possible, the same pathologists were recruited to perform or review post mortem examinations, so that these individuals developed increased experience in recognizing, comparing and interpreting lesions and abnormalities, and were better able to identify potential patterns. The attending pathologist had the discretion to determine the thoroughness of the post mortem examination and to perform ancillary diagnostic procedures, as they felt necessary.

The thoroughness of a post mortem examination also was determined by the circumstances under which a marmot was recovered. Captive VIM that died or were euthanized during the active season were examined within hours or days of death. This facilitated gross, histological and microbiological examination. Captive marmots that died during the hibernation period were most commonly recovered during routine nest-box checks, conducted at weekly to monthly intervals, and their carcasses demonstrated varying degrees of autolysis. Deterioration of the carcass was somewhat delayed by the cooler ambient temperatures (5 to 7° C) that characterized hibernation management. Typically, the tissues of these marmots maintained sufficient integrity for a macroscopic post mortem examination, but the potential for histological and microbiological examination was greatly reduced.

Free-ranging VIM could not be monitored with the same day-to-day intensity as their captive counterparts, and this complicated the field recovery of intact, dead marmots. Although field workers occasionally encountered the remains of

unidentified marmots of unknown origin, most of the documented mortalities in free-ranging marmots involved individuals that were previously implanted with radio-transmitters and located by telemetry. Hibernation mortalities were identified in those marmots that failed to emerge from their burrow in the spring and whose transmitter remained underground on a slow pulse rate. If there were no indications as to cause of death following site investigation or if a slow transmitter pulse was detected remotely by aerial or ground telemetry, the cause of death was listed as "unknown".

The interval between death and recovery of free-ranging marmot remains was quite variable and ranged from days to years. The recovery of free-ranging marmots which had died during hibernation occurred on only two occasions and necessitated digging the dead marmots out of their hibernaculum. Decomposition of these recovered carcasses was probably delayed by the cooler and more stable, ambient temperatures that characterize natural burrows.

The integrity of remains from dead, free-ranging marmots also could be influenced by consumption and disruption by predators or scavengers, moisture (rain or snow), sunlight and ambient temperatures. In most instances, the death of a free-ranging marmot resulted in the recovery of incomplete remains, which ranged from an isolated transmitter to a partial body. In the absence of a complete carcass, the proximate causes of a marmot mortality were determined by site investigation and the characteristics of the marmot remains.

Classification of predator mortalities

VIM are susceptible to a suite of predators (cougars, wolves, eagles, and bears), and each species typically left a characteristic presentation of the remains following a predation event.

Cougars (*Puma concolor*) are solitary hunters. Once a cougar successfully killed a non-juvenile marmot, it typically delayed feeding and relocated the carcass to a sheltered spot under the cover of dense vegetation (Naughton, 2012). Often these sites were situated well away from locations typically frequented by marmots. Cougars fastidiously prepared the marmot's body prior to consumption. They used their incisors to barber or pluck the marmot's fur, leaving an encircling perimeter or matt of hair. They typically removed and rejected the stomach and gastrointestinal tract of their prey (Wild, 2013) and where applicable, the implanted abdominal transmitter. Cougars also disarticulated the carcass during feeding and in many instances, they left the more robust bones (skull, pelvis, and long bones) and the poorly fleshed portions of the body (feet and tail).

Grey Wolves (*Canis lupus*) are pack hunters and display competitive behavior following a predation event. They typically consumed marmots at the location of the kill, and ingested most of the carcass, including the bones, gastrointestinal tract, and skin. They would only leave small remnants such as the upper incisors mounted in a portion of the premaxillary bone, and a portion of the distal tail. Wolves would not consume abdominal transmitters, but they would frequently leave bite marks on the surface of units, particularly those that were encapsulated with wax. In addition, wolves often showed what appeared to be a gastro-colic response, and tell-tale feces often were found in close proximity to their marmot kills.

The preferred prey size for Golden Eagles (*Aquila chrysaetos*) is 0.5 to 4.0 kilograms (Watson, 1997) and most free-ranging VIM fall within this range. In many instances, Golden Eagle predation on full-size marmots left indications of an initial struggle, characterized by disturbance of low-lying vegetation, and feather and body tracks on the ground or in the snow. The eagle's presence often was evidenced by down and contour feathers, and by large splashes of urates. A female Golden Eagle

has difficulty flying with a prey item that matches her own body weight (approximately 6.0 kilograms), and under most conditions, she can only manage to carry about half of this amount (Watson, 1997). Therefore, Golden Eagles often began consuming marmots at the kill site, leaving partial remains, including the skeleton and some viscera, surrounded by irregular patches of plucked hair. In a few instances, field workers flushed Golden Eagles off their marmot kills. On three occasions Golden Eagles were observed carrying small marmots (pups or yearlings) away from colony sites (Bryant, 1998) and at least one marmot radio-transmitter was used to locate a Golden Eagle nest (J. MacDermott, personal communication).

Black Bears (*Ursus americanus*) were random, opportunistic predators of marmots and this was reflected in the apparently haphazard nature of carcass consumption. Bears would typically consume most of the bones and soft tissues and leave behind a twisted hide with irregular remnants of the subcutaneous fat and panniculus muscles, which was coated with detritus such as leaves, dirt, and broken twigs. In cases of bear predation there was also proximal evidence of the bear's presence, such as piles of feces or a bedding area.

Because of their small size, most marmot pups were probably carried away or consumed in their entirety after being killed. Implanted pups left negligible remains except for occasional clumps of hair, skin, and their transmitter.

RESULTS

Physical Examination

From August 1992 to September 2016, a total of 3,174 veterinary examinations were conducted on captive and free-ranging VIM. This includes examination of 632 captive individuals and 353 wild individuals. Accounting for marmots that overlapped the two populations, a total of 944 individual marmots were examined. The circumstances of these examinations are summarized in Table 3.1 and the ages of wild and captive marmots are compared in Figure 3.1.

On average, captive marmots were examined more frequently than wild marmots (an average of 4.1 examinations per captive marmot compared to 1.4 examinations per wild marmot). Physical evaluation of captive marmots resulted in the identification of a number of health events or clusters that were not seen in free-ranging marmots (Table 3.2). Although seven weaned pups died before receiving physical examinations, all of the remaining 571 captive-born pups were evaluated prior to their first hibernation. This compares to 63 wild pups who were either examined before being transferred into captivity (27) or prior to implant surgery (36). Captive-born pups exhibited a number of congenital disorders that were not identified in wild pups including a patent foramen ovale, bilateral cataracts, dental malocclusion, and bilateral aplasia of the hind feet. One wild-born pup that was brought into captivity possessed an atrial septal defect and his full sibling (born after the wild-born parents had been taken into captivity) exhibited stunting, micro-opthalmia, and scoliosis. The greater longevity of captive marmots allowed for observation and physical examination of many older marmots, compared to their wild counterparts (Figure 3.1) and this may have allowed for the identification of additional age-related health events or syndromes within the captive population. Although cardiovascular disease and neoplasia often were diagnosed at the time of

necropsy following acute death in captive marmots, some of these cases were preceeded by observable clinical signs. Marmots with chronic heart disease exhibited murmurs or weight loss that were detected clinically and initial signs of neoplasia included emaciation, depression, inappetance, facial assymetry, epistaxis, and unilateral exopthlamos. Two mature, captive males exhibited acute hind end paralysis that resulted from degeneration and prolapse of their invertebral discs. Cardiovascular disease, neoplasia and disc degeneration were not identified in any of the free-ranging marmots that were given physical examinations.

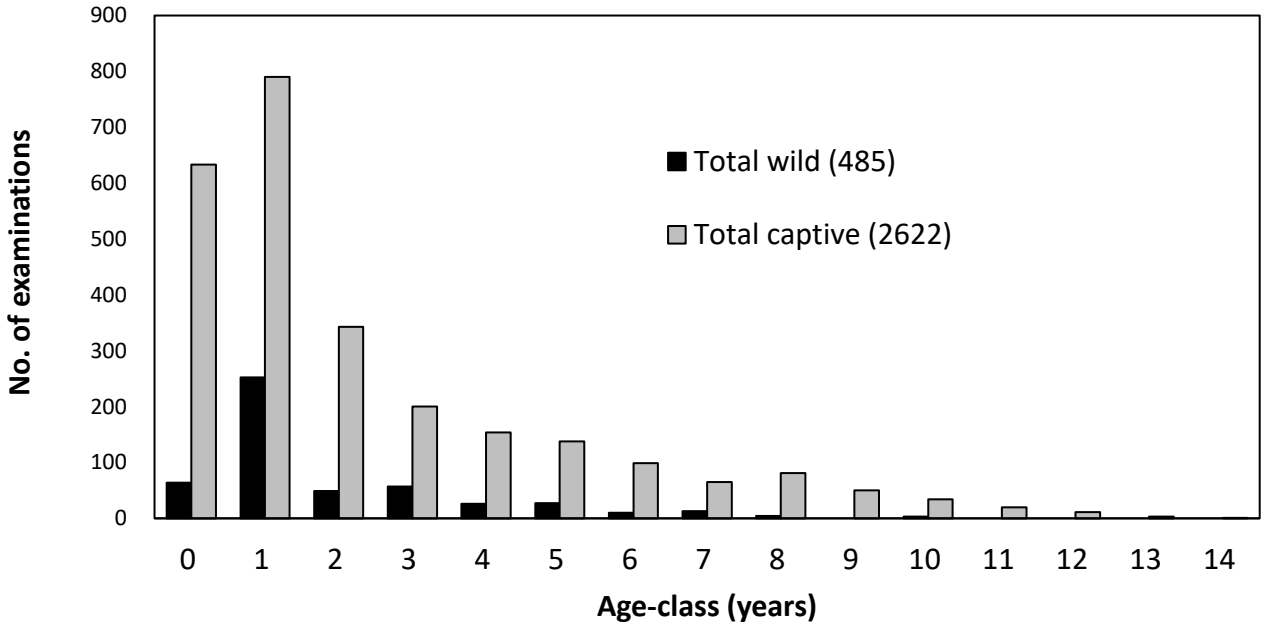
One condition that was exclusive to wild-born marmots was a syndrome of chronic, generalized, perifollicular dermatitis that was widespread in the original, extant colony at Mount Washington and was attributed to an unclassified, intrafollicular mite (Janz *et al*., 2000). This condition persisted in those individuals that were taken into the captive program, but it did not visibly affect their progeny or cage-mates.

Table 3.1. Circumstances of 3,174 physical examinations conducted on captive and free-ranging Vancouver Island Marmots (*Marmota vancouverensis*).

Captive (2622)				Free-ranging							
				Captive-release (67)			Wild (485)				
Annual / biennial exam	Pre-surgical (a)	Pre-release exam (b)	Other (c)	Pre-surgical (a) (d)	Implant replacement	Translocation (pre-conditioned)	Pre-surgical (a)	Implant replacement	Translocation	Captivity (e)	Other (f)
1513	486	507	116	1	33	33	316	63	61	38	6

a) Examination prior to surgical implantation of abdominal radio-transmitter
b) Some marmots had more than one pre-release exam due to delays in their release or concerns about transient injuries. One implanted marmot died prior to release due to complications associated with its transmitter
c) Examinations of wild-born marmots conducted in the initial captive quarantine period or exams initiated in response to specific health concerns
d) One individual was surgically implanted 50 days after being released from captivity without a transmitter
e) Physical examinations conducted on captured wild-born marmots prior to being placed in the captive program
f) Miscellaneous examinations associated with ear-tag replacement or misidentified marmots that already had pre-existing transmitters

Figure 3.1. Age comparison of 3,107 physical examinations conducted on wild and captive Vancouver Island Marmots (*Marmota vancouverensis*).



Post Mortem Examination / Causes of Mortality

Mortality in Captive Marmots: As of January 2017, 109 marmot deaths had occurred in captivity (49 wild-born and 60 captive-born). A post mortem examination was performed on 106 or 97.22% of the 109 captive mortalities. Examinations were not performed on two recently weaned pups whose bodies were not recovered. The body of a third pup that died from known conspecific trauma, was too badly mutilated for a necropsy. In one adult post mortem, advanced autolysis of the carcass (recovered during hibernation) precluded a diagnosis. A specific cause of death was identified in 106 of the mortalities, and the diagnostic categories are summarized in table 3.1. The sex-age distribution of the mortalities is summarized in figure 3.2. The average male age was 6.37 years (range 0.1 to 11.5) and the average female age was 8.17 years (range 0.1 to 14.6). Of the 78 marmots that survived to adulthood at 3 years of age, the average age for males was 8.9 years (n = 46) and the average age for females was 10.4 years (n = 32).

Cardiovascular disease accounted for the highest number of captive marmot mortalities, and included 16 cases of dilatative or dilated cardiomyopathy, 6 cases of myocarditis, 1 case of endocardiosis, 1 case of cerebral hemorrhage, 1 case of atherosclerosis, 4 cases in which the pathologist diagnosed non-specific heart disease, and 3 cases which were described as congestive heart failure. One five-year old, captive-born male suffered cardiac arrest during anesthesia and a captive-born male died acutely of myocardial fibrosis at 1.4 years of age. Including two additional congenital cases (atrial septal defect, patent foramen ovale), 27 captive males died from cardiovascular disease compared to 9 females. Twenty-four males died of acquired cardiovascular disease in adulthood and their average age was 9.5 years (range 5 to 11.5). In the 9 females, the average age was 11.8 years (range 10.3 to 13.1).

Neoplasia occurred in combination with 5 of the 34 cardiovascular cases (3 males, 2 females) and was identified in 21 additional mortalities (8 males, 13 females). The anatomical categorization of these cases is presented in Table 3.4. The average age for the 11 males with neoplasia was 9.0 years (range 6.4 to 11.5) and for the 15 females it was 10.4 years (range 8.2 to 14.6).

A total of 25 (22.9%) of the captive deaths were attributed to infections or inflammation. This include 6 cases with multisystemic involvement, 6 pulmonary, 5 hepatic, 2 pancreatic, 2 gastrointestinal, 2 neurological, 1 urogenital, and 1 integumentary. There were two instances in which captive pups exhibited severe neurological symptoms and were ultimately euthanized. At post mortem, both pups were diagnosed with meningoencephalitis. The etiological agent in one case was a protozoan, possibly *Sarcocystis neurona* and in the other it was the microsporidia, *Encephalitozoon cuniculi.*

Of the 109 marmot mortalities that occurred in captivity, 17 (15.6%) were attributed to management or captive conditions. Six weaned pups were fatally attacked by older, non-related marmots after they escaped through the mesh of their natal enclosures. Four of the 61 wild-caught marmots died in quarantine during the intial transition to captive conditions. Three of these deaths were attributed to bacterial infections and one resulted from a cecal perforation arising from the microbial alterations associated with a new, captive diet. One marmot died from trauma associated with a fall in its enclosure and one was euthanized following elbow trauma of unknown origin. A captive pup scheduled for release experienced an extreme, chronic inflammatory reaction to its implanted transmitter, resulting in significant encapsulation and visceral adhesions which could not be surgically resolved. Two captive marmots died of hypothermia after being exposed to hibernation temperatures of -7° C while occupying an outdoor nest-box.

From the winter of 1997/98 to the winter of 2015/16 there were a total of 1651 individual marmot hibernations in captivity with 26 mortalities, indicating a success rate of 98.4%. Over the course of these 19 winters there were 547 pup hibernations (first winter) and 264 yearling hibernations (second winter) in captivity, with only 1 pup mortality. There was an additional adult mortality in December 2016, bringing the total number of captive hibernation mortalities to 27. Underlying pathology was identified in 24 of these hibernation deaths. Mortality occurring during captive hibernation most typically involved older marmots with identifiable health problems. This included 12 cases of cardiovascular disease, 4 cases of neoplasia, and 1 case of cardiovascular disease in combination with neoplasia. Three older marmots also succumbed to infections or inflammation during hibernation. The hibernation deaths of 4 younger captive marmots also were attributed to infection or inflammation. At least some of these mortalities appear to be bacterial or fungal in origin. However, most hibernation mortalities were not immediately recovered, and therefore, microbiology results should be interpreted with caution (de With *et al*. 1999).

Table 3.2. Clinical conditions initially identified from 3,174 Vancouver Island Marmot (*Marmota vancouverensis*) physical examinations. A total of 944 marmots were examined, including 632 captive individuals and 353 wild individuals. Sixty-two marmots were examined following release from captivity.

Category	Diagnosis	Occurrence in free-ranging marmots (wild & captive release)	Occurrence in captive marmots	Comments
cardiovascular	persistent tachycardia	0	1	heart rate consistently above 300 bpm
cardiovascular	acquired cardiac disease	0	28+	cardiac murmur
congenital / early onset	atrial septal defect	0	1	identified in wild-caught, captive yearling exhibiting un-thriftiness
congenital / early onset	patent foramen ovale	0	1	cardiac murmur
congenital / early onset	dental malocclusions	0	2	overgrowth of upper and lower incisors (both cases were litter-mates)
congenital / early onset	post weaning un-thriftiness	2 wild	2	significantly smaller than litter-mates
congenital / early onset	unilateral anopthalmis / scoliosis / stunting	0	1	captive-born marmot which was the progeny of wild-caught parents
congenital / early onset	hind foot aplasia	0	1	
congenital / early onset	bilateral, congenital cataracts	0	2	
congenital / early onset	unilateral, narrowed palpebral fissure	0	1	
iatrogenic / management	transmitter reaction	0	1	large abdominal mass
infectious / inflammatory	menigoencephalomyelitis	0	2	depression, ataxia
infectious / inflammatory	facial abscess	1? wild	7+	unilateral facial swelling
hibernation	post hibernation emaciation	1 captive-release	0	extreme emaciation and depression in captive-release marmot
miscellaneous	abdominal hernia	0	1	palpation of fluctuant peri-abdominal mass
miscellaneous	degeneration and herniation of intervertebral disc(s)	0	2	acute hind-end paralysis
miscellaneous	paraphimosis	0	1	possibly associated with neurological symptoms
neoplasia	facial neoplasia	0	7	epistaxis, facial assymetry, unilateral exopthalmus
neoplasia	other neoplasia	0	3	emaciation
parasitic	mites	25 wild	8	poor hair coat and hair loss, only occurred in wild-born marmots originally from Mount Washington
parasitic	cutaneous myiasis	0	1	bot fly larvae infesting skin of ventral abdomen

Table 3.2. (cont.) Clinical conditions initially identified from 3,174 Vancouver Island Marmot (*Marmota vancouverensis*) physical examinations. A total of 944 marmots were examined, including 632 captive individuals and 353 wild individuals. Sixty-two marmots were examined following release from captivity.

Category	Diagnosis	Occurrence in free-ranging marmots (wild & captive release)	Occurrence in captive marmots	Comments
traumatic	unilateral / bilateral fractures of incisors	0	2	impaired regrowth of incisors following trauma
traumatic	fracture of carpal bones	1 wild	0	lameness
traumatic	chronic fracture of right olecranon	0	1	lameness
traumatic	head trauma	0	1	head tilt
traumatic?	resorption of head and neck of femur	0	1	lameness
traumatic?	unilateral, corneal opacity	1 wild	1	

Table 3.3. Categories of mortality observed in 109 captive Vancouver Island Marmots (*Marmota vancouverensis*), 1997 to 2016.

Diagnostic category	No. Cases	%
cardiovascular	29	26.6
infectious / inflammation	25	22.9
neoplasia	21	19.2
iatrogenic / management (a)	13	11.9
congenital / early onset (b)	6	5.5
cardiovascular & neoplasia	5	4.6
quarantine (a)	4	3.7
unknown	3	2.8
intervertebral disc degeneration	2	1.8
mesenteric torsion	1	0.9

(a) Iatrogenic / management was defined as any mortality directly related to the marmots being manipulated or confined in captivity. This included conspecific trauma, falls in enclosures, inappropriate hibernation temperatures and transmitter reactions. Death during the quarantine period was also attributed to captive confinement.

(b) Early onset was defined as any condition diagnosed at the time of first examination either ante-mortem or post-mortem.

Figure 3.2. Sex-age distribution of 109 mortalities in captive, weaned Vancouver Island Marmots (*Marmota vancouverensis*), 1997 to 2016.

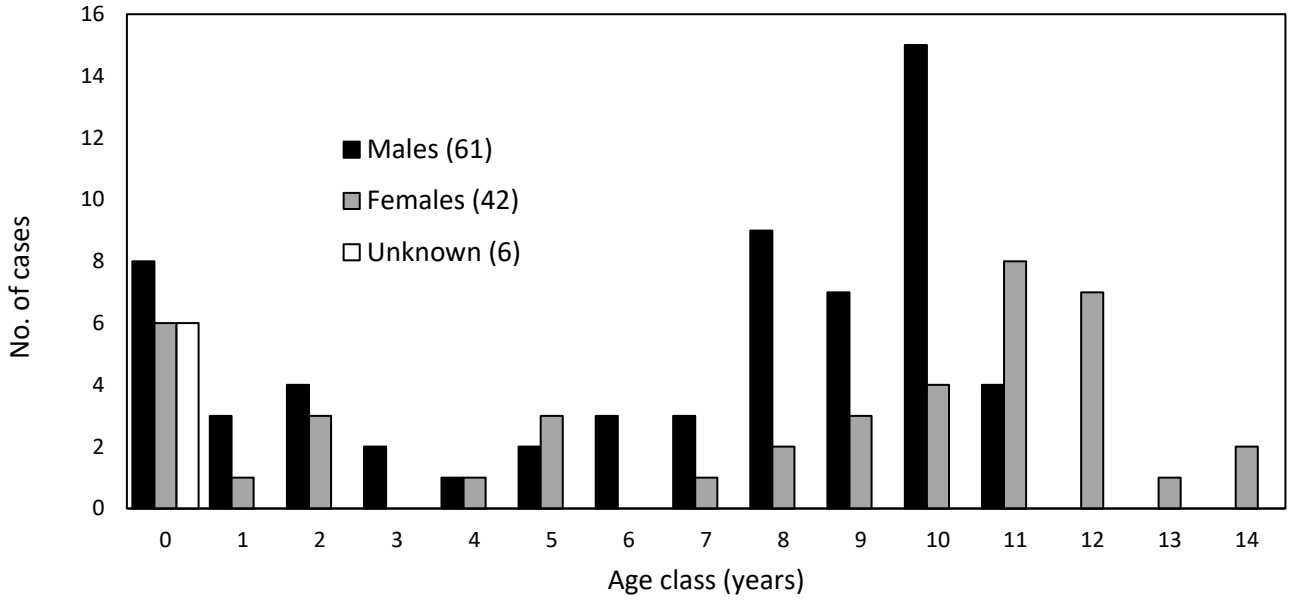


Figure 3.3. Sex-age distribution of 518 mortalities in free-ranging Vancouver Island Marmots (*Marmota vancouverensis*), 1992 to 2016.

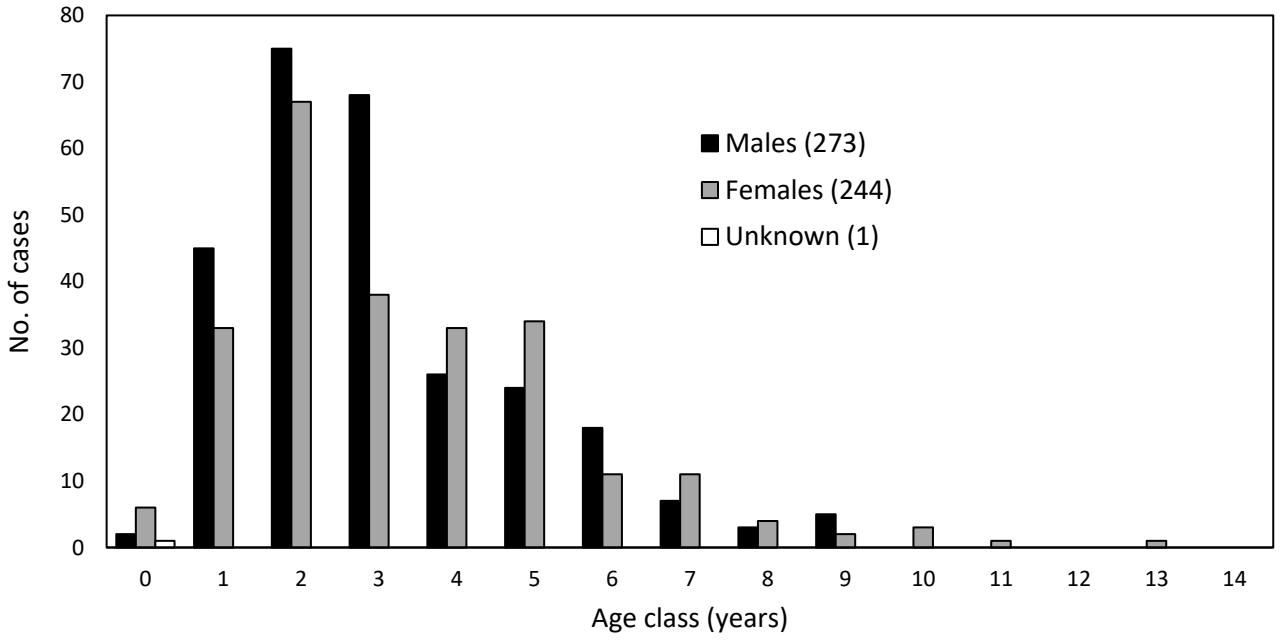


Table 3.4. Anatomical description of neoplasia in 26 captive Vancouver Island Marmots (*Marmota vancouverensis*) 1997 to 2016.

Anatomical description	No. Cases	%
pulmonary	6	23.1
hepatic	6	23.1
facial - oral	3	11.5
facial - periorbital	3	11.5
fascial - nasal	1	3.8
splenic	2	7.7
abdominal	1	3.8
adrenal	1	3.8
mammary	1	3.8
uterus	1	3.8
multi-systemic	1	3.8

Mortality in Free-ranging Marmots: From 1992 to the completion of the 2016 field season, a total of 533 confirmed mortality records were generated from free-ranging marmots. This included records for 183 wild marmots (including 44 translocated marmots) and 350 captive-release marmots (including 20 pre-conditioned marmots). Implanted marmots accounted for 515 (96.6%) of the mortality records. There were also 15 records in which field workers encountered the remains of unidentified marmots. In 5 of these cases, old or desiccated marmot remains (mostly small numbers of disarticulated bones and clumps of hair) were found in proximity to burrow entrances and were presumably pushed out of the burrow by other marmots. Although it is probably reasonable to assume that these marmots died underground, the timing of their deaths could not be reliably determined, and therefore the cause of death in these marmots was categorized as 'unknown'.

Causes of mortality of free-ranging marmots are summarized in table 3.5 and the sex-age distribution of these mortalities is summarized in Figure 3.3. The average age of males was 3.28 years (range 0.2 to 9.5) and the average female age was 3.57 years (range 0.2 to 13.1).

Wild marmots

From 1992 to 2016 a total of 379 implant surgeries were performed on wild marmots, including 63 replacement surgeries (total = 316 individuals). Mortality was confirmed in 167 or 52.8% of these implanted wild marmots. Only 7 (2.1%) of these telemetered marmots (4 wild and 3 translocated) were recovered in a condition which was suitable for a post mortem examination. The necropsy results for these 7 marmots (and one un-telemetered individual) are presented in Table 3.7.

Four of these 7 wild marmots died following manipulation, including one individual which died from acute hyperthermia following handling and field surgery in 1992, and three translocated individuals which were recovered from their

hibernaculum in the spring of 1997 after a failed translocation attempt. A fourth marmot mortality from this same group could not be retrieved. These translocated marmots died during hibernation despite exhibiting good body stores and a definitive cause of death was not identified despite intensive investigation (de With *et al*. 1999). In addition to these four related mortalities, there was one other record of a translocated marmot that failed to emerge in the spring and its transmitter remained underground on a slow pulse.

The intact carcass of a wild, telemetered marmot was recovered in May 2005 and post-mortem examination indicated that although it had been actively feeding aboveground (as evidenced by ingesta in its gastrointestinal tract) it had probably died of post-emergence emaciation. In 7 other instances, wild (non-translocated) telemetered marmots failed to emerge from their burrows in the spring, and their transmitters remained underground on a slow pulse rate. Only 1 of these 8 hibernation mortalities involving wild marmots occurred in the winter that followed implant surgery, with the remainder dying during subsequent winters.

Captive-release

From 2003 to 2016 a total of 490 captive marmots were released to the wild (annual numbers summarized in Figure 1.2 and Appendix A), including 482 captive-born individuals and 8 wild-born individuals. A total of 485 of the captive-release marmots were surgically implanted with an abdominal radio transmitter for post release tracking and 33 of these individuals were later recaptured and had their transmitters replaced. Two of the 490 released marmots (one implanted, one not implanted) were recaptured and permanently returned to the captive breeding program. Mortality was confirmed in 349 (72.1%) of the 484 marmots fitted with radio transmitters that remained in the wild.

Only 22 or 4.55% of the 484 telemetered marmots were recovered in a condition that was suitably intact for a post mortem examination. In addition, a single un-telemetered, post-release marmot was recovered after drowning in a reservoir. No pre-conditioned marmots were recovered for necropsy. The necropsy results for these 23 intact, post-release marmots are presented in Table 3.8.

Ten of the 23 post-release marmots presented for necropsy were diagnosed with post-emergent emaciation as the proximate cause of death. These marmots were discovered in May or early June. Eight of these marmots were found aboveground and two were dug out of a shallow hibernaculum. Telemetry on these latter two marmots indicated that their body temperatures were still cycling into periods of euthermy late into the spring before they died in their burrow. Another post-emergent marmot was still alive when it was recovered by field staff but perished while being transported into care. Extreme weight loss and loss of fat stores in a post-emergent, adult male may have resulted in an abdominal radio-transmitter becoming lodged in its pelvic canal, resulting in a fatal impaction of the gastrointestinal tract. At least one of the recently emerged marmots was found dead at the hibernaculum entrance, and it is possible that burrowing out through deep or frozen snow placed an additional burden on the marmot's limited energy stores.

In addition to the 10 cases where an intact carcass was recovered, there were 75 additional records (70 captive-release, 5 pre-conditioned) where post-release marmots died during hibernation. In these cases, a marmot failed to emerge from hibernation and its radio-transmitter continued to transmit a slow-pulse (i.e. low temperature) signal from the hibernaculum. Two marmots appeared to have emerged from hibernation and then died in their burrows shortly afterward. In at least two instances, a whole group of marmots failed to emerge from hibernation, and in at least two others, an individual marmot survived, whereas its burrow-

mates did not. Of the 80 hibernation mortalities documented in post-release marmots (excluding pre-conditioned marmots), 76 occurred in the first winter following release from captivity and 4 died during subsequent winters. Death during hibernation accounted for 44.4% of the captive-release mortalities for which there was an identified cause (N = 171), compared to 9.5% for unmanipulated (non-translocated), wild marmots (N = 84). Predation accounted for 166 or 31.1% of the 533 mortalities in free-ranging marmots (Table 3.6). Mortalities attributed to predators are summarized according to year in Figure 3.4.

Table 3.5. Categories of mortality for wild, translocated, captive-release and pre-conditioned Vancouver Island Marmots (*Marmota vancouverensis*) including post mortem reports and mortality records, *N* = 533.

Wild (n = 139)	**No. Cases**	**%**
Predation	73	52.5
Hibernation	8	5.8
Other	3	2.2
Unknown	55 (a)	39.6
Translocated (n = 44)	**No. Cases**	**%**
Predation	5	11.4
Hibernation	5	11.4
Other	0	0.0
Unknown	34	77.3
Captive-release (n = 330)	**No. Cases**	**%**
Predation	86	26.1
Hibernation	80	24.2
Other	5	1.5
Unknown	159	48.2
Pre-conditioned (n = 20)	**No. Cases**	**%**
Predation	2	10.0
Hibernation	5	25.0
Other	0	0.0
Unknown	13	65.0

(a) This includes 16 records involving the remains of unidentified wild marmots encountered by field staff.

Table 3.6. Predator mortality for wild, translocated, captive-release and pre-conditioned Vancouver Island Marmots (*Marmota vancouverensis*) from 1992 to 2016 (total = 166).

	Cougar	Golden Eagle	Grey Wolf	Black Bear	Avian (a)	Dog	Unknown
Wild (n = 73)	38	8	9	0	1	1	16
Translocated (n = 5)	0	1	2	0	0	0	2
Captive-release (n = 86)	38	25	8	1	0	0	14
Pre-conditioned (n = 2)	0	1	0	1	0	0	0
Total	**76**	**35**	**19**	**2**	**1**	**1**	**32**

(a) Marmot pup killed by avian predator, possibly Northern Goshawk (*Accipiter gentilis*)

Figure 3.4. Yearly predation mortalities of free-ranging Vancouver Island Marmots (*Marmota vancouverensis*) from 1992 to 2016 (total = 166).

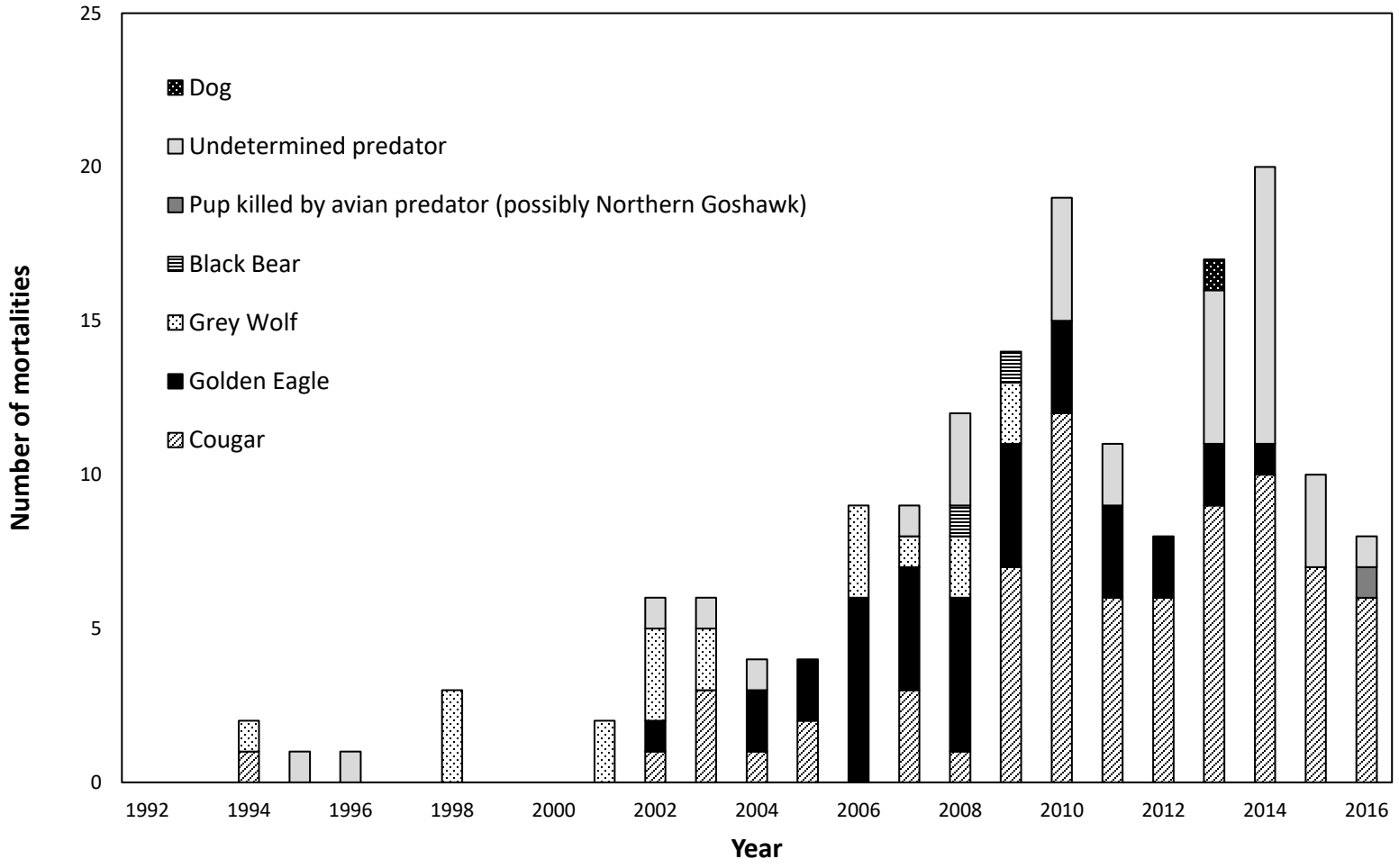


Table 3.7. Post mortem diagnosis in 8 (7 telemetered, 1 non-telemetered) wild Vancouver Island Marmots (*Marmota vancouverensis*) 1992 – 2016.

Diagnostic category	Comments	No. Cases
hibernation	group of 4 died in hibernaculum after failed translocation, 3 carcasses recovered (2 telemetered, 1 un-telemetered), etiology not determined	3
hibernation	post-emergent emaciation found aboveground	1
iatrogenic	hyperthermia from handling & surgery	1
infectious	hepatic abscess in a ten-year old female	1
predation	suspect domestic dog	1
unknown	post mortem inconclusive	1

Table 3.8 Post mortem diagnosis in 23 (22 telemetered, 1 non-telemetered) captive-release Vancouver Island Marmots (*Marmota vancouverensis*) 2003 – 2016.

Diagnostic category	Comments	No. Cases	%
hibernation (total =10)	post-emergent emaciation, found aboveground	8	34.8
	group of 2 recovered in shallow hibernaculum	2	8.7
predation (4)	Golden Eagle	3	13.0
	Cougar	1	4.3
drowning	fell into reservoir	2	8.7
trauma	fall from cliff	1	4.3
suspected trauma	lesions suggested traumatic injury	1	4.3
iatrogenic	transmitter impaction in pelvis	1	4.3
unknown	post mortem inconclusive	3	13.0

DISCUSSION

The Vancouver Island Marmot recovery project has collected extensive information on both free-ranging and captive marmots, and from these data it is apparent that captive, wild and captive-release marmots are subjected to disparate assemblages of health determinants and exhibit different spectrums of health outcomes. In addition, there are significant differences in the capacity to collect health data and conduct surveillance on these populations.

In captivity, VIM can be observed on a regular basis, and any suspected abnormalities in behaviour, appetite, excretion, or physical appearance (body condition, symmetry, posture, locomotion, hair coat, etc.) can be promptly recognized, evaluated, and monitored. In addition, captive marmots were routinely examined on an annual or biennial basis. Ninety-six per cent of the captive mortalities were efficiently identified and extensively investigated. Intensive observation, clinical evaluation, and post mortem examination of captive marmots resulted in the description of a number of health conditions. Although many of these conditions would present grossly observable signs, most were not identified in the numerous physical examinations that were conducted on free-ranging VIM (Appendix G). However, it is important to note that physical examinations were only conducted on those free-ranging individuals that could be successfully live-trapped and this may represent an important distinction in sampling between the captive and free-ranging populations. It is always possible that the health of some free-ranging marmots may have been compromised, and that the adaptive behavioral responses associated with "sickness behavior" precluded these marmots from being active and trappable above-ground (Hart, 1988). Predation, scavenging, ambient conditions, and delays in recovery reduced the effectiveness of mortality investigation in free-ranging marmots. Site accessibility also represented a

significant challenge in determining the occurrence, timing and causes of mortality in the field, and this varied greatly between locations. It is possible that the small number of intact carcasses that were recovered in the field for post mortem examination (31) also reflected the low number of non-predatory deaths that occur in the free-ranging population.

One of the original objectives of the captive program was to establish a safeguard population, so that marmots could be maintained under controlled conditions without being exposed to the multiple threats that exist in the wild. However, captivity is not entirely benign and artificial conditions appear to have imposed a new set of risk factors for Vancouver Island Marmots. Cardiovascular disease was quite prevelent in captive male marmots and its onset was earlier than in females. Cardiomyopathy has been documented in other rodent species including woodchucks (*Marmota monax*), Norway rats (*Rattus norvegicus*) and black rats (*Rattus rattus*) (Roth & King, 1986). In laboratory rats, cardiomyopathy is most prevelent in older males and the severity and age of onset are influenced by a number of factors including stress, nutrition, and ambient conditions (Rothenburger *et al.* 2014). Obesity has also been recognised as an important risk factor for cardiomyopathy in a number of other species (Wong & Marwick, 2007). In captivity, Vancouver Island Marmots exhibit a shorter hibernation period, receive less exercise, are subject to increased hygiene and parasite control, and are fed a more protracted, uniform, and nutritionally-concentrated diet than their wild counterparts. A comparison between *in situ* and *ex situ* marmots shows that body condition indices were consistently and significantly higher in captive animals, indicating a higher level of adiposity which may be increasing their suseptibility to cardiomyopathy (Appendix H).

Hibernation mortalities occurred infrequently in captivity and most typically involved older marmots with identifiable health problems. Mortality records from this project support previous research indicating that death during hibernation also is an uncommon occurrence in wild, unmanipulated VIM (Bryant & Page, 2005). Low hibernation mortality also has been documented for other species, including the Olympic marmot (*Marmota olympus*) (Griffin *et al*. 2008). Differentiating between hibernation mortality and the mortality that occurs shortly before or after hibernation is difficult, and verification of high winter survival in both Vancouver Island and Olympic marmots required intensive monitoring using radiotelemetry.

Conversely, hibernation mortality appeared to be significant in marmots released from captivity. Records from this project indicated that 76 of the 80 hibernation mortalities in captive-release marmots occurred during the first winter following release. Reduced overwinter survival during the first hibernation may represent a significant limiting factor for the marmot reintroduction program (Jackson, 2005). Captive VIM typically have shorter hibernations than wild marmots, and the difference in duration can be several weeks or longer (Bryant & McAdie, 2003). Although marmot life cycles are controlled by endogenous, circannual rhythms, these can be readily altered by certain exogenous factors, such as temperature, photoperiod, and food availability. Modification of endogenous cycles has the potential to disrupt growth, patterns of food consumption, reproduction and hibernation and these asynchronous cycles can be entrained for an extended period of time (Concannon *et al.* 1997). It is possible that captive-release marmots are released with aberrant endogenous cycles and as a result have disrupted hibernation patterns that compromise their first hibernation in the wild.

For wild and captive-release VIM, predation represented the most significant cause of mortality. Predation has been implicated as an important factor in the

original population declines of this species (Bryant & Page, 2005; Janz *et al.* 2000) and as a significant cause of mortality in captive-release marmots (Aaltonen *et al.* 2009). During the original population declines that occurred in the late 1980s and 1990s, Wolves, cougars, and eagles were thought to represent important predators of VIM (Janz, *et al.* 2000). Aaltonen, *et al.* 2009 concluded that cougars and wolves were the most important predator for wild marmots and that Golden Eagles represented the most significant predator for captive-release marmots. In recent years, the influence of the respective predators appears to have changed. No wolf predations of telemetered marmots have been recorded since 2009 and no mortalities from Golden Eagles have been documented since 2014. During the 1990s, adult and juvenile Golden Eagles were routinely observed at active and extirpated marmot colonies, but sightings by field staff in recent years have become quite uncommon (Marmot Recovery Foundation inventory records). Over the last eight years, cougars appear to represent the biggest predation threat to be detected in wild and captive-release marmots (Figure 4).

Physical examinations conducted by experienced veterinarians, necropsies performed by veterinary pathologists, and observations made by field staff offer reliable and robust health-related data. Based upon this study, the systematic collection and evaluation of these data allows for the identification and characterization of potential patterns, clusters, or syndromes and the recognition of potential health and health risk patterns in the VIM. Although the captive population was characterized by increased longevity, it also exhibited a greater number of clinical syndromes, not all of which were age-related. Intensive management and support in captivity may allow some affected animals to survive for a longer period of time than their wild counterparts, and potentially reproduce. If any of these conditions have a genetic basis, there is a potential risk that these

characteristics will be perpetuated within the captive population. Captive-release marmots exhibited poor survival in their first wild hibernation. More rigorous management of food availability, light and temperature regimes in captivity (that more closely mimic natural cycles) may help to normalize hibernation patterns and increase post-release survival.

There was very little evidence of clinical disease in the free-ranging marmots that were examined following capture for implant surgery or translocation. However, this population had very few older individuals and opportunities to evaluate them through physical and post mortem examinations were very limited. Despite this limitation, site investigations were able to identify potential causes of mortality in free-ranging marmots that could be influencing marmot recovery. Predation continued to represent the most significant cause of mortality in free-ranging marmots and recent declines in the wild population may indicate that it is still too small to overcome ongoing predation pressure. Predator-pits have been described in other species including lemmings and rabbits (Calvete *et al.* 1997; Krebs, 1996) and it is possible that enhanced augmentation or predator management may be necessary for the VIM to overcome the current effects of predation.

With endangered species like the VIM, it is possible that health events with significant implications may only be expressed in a small number of individuals and may therefore lack numerical or statistical robustness. In addition, case documentation often involves the collection of incomplete or discrepant data. Syndromic surveillance appears to provide a conceptual framework for the ongoing characterization and comparison of these case limited or incomplete health events, and facilitates detection, evaluation and tracking of patterns or clusters as they are occurring. This allows for filtering and efficient transfer of health information so that ongoing risks can be identified and further investigated, and so that timely,

evidence-based decisions can be made. It is important to recognize that for this approach to be effective in the long-term, the monitoring process needs to be continual and dynamic in nature, so that new data originating from the multitude of sources are properly integrated, contextualized, and adapted with respect to previously identified events and patterns. The range of potential influences and risks elucidated in this study support the importance of continued surveillance with respect to the health management and recovery of the Vancouver Island Marmot.

LITERATURE CITED

Aaltonen, K., Bryant, A. A., Hostetler, J. A., & Oli, M. K. (2009). Reintroducing endangered Vancouver Island Marmots: survival and cause-specific mortality rates of captive-born versus wild-born individuals. *Biological Conservation 142*, 2181-2190.

Bryant, A. A. (1998). *Metapopulation ecology of Vancouver Island Marmots (*Marmota vancouverensis*).* Victoria: University of Victoria.

Bryant, A. A., & McAdie, M. L. (2003). Hibernation ecology of wild and captive Vancouver Island Marmots (*Marmota vancouverensis*). *Adaptive Strategies and Diversity in Marmots* (pp. 149-156). Montreux: International Marmot Network.

Bryant, A. A., & Page, R. E. (2005). Timing and causes of mortality in the endangered Vancouver Island Marmot (Marmota vancouverensis). *Canadian Journal of Zoology (83)*, 674-682.

Calvete, C., Villafuerte, R., Lucientes, J., & Osacar, J. J. (1997). Effectiveness of traditional wild rabbit restocking in Spain. *Journal of Zoology 241 (2)*, 271-277.

Concannon, P., Roberts, P., Baldwin, B., & Tennant, B. (1997). Long-term entrainment of circannual reproductive and metabolic cycles by northern and southern hemisphere photoperiods in Woodchucks (*Marmota monax*). *Biology of Reproduction 57*, 1008-1015.

de With, N. I., Stephen, C., Ribble, R., Schwantje, H., & McAdie, M. (1999). *The potential role of Yersinia spp. in the decline of the Vancouver Island Marmot (*Marmota vancouverensis*).* Nanaimo: Centre for Coastal Health.

Deem, S. L., Parker, P. G., & Miller, R. E. (2008). Building bridges: connecting the health and conservation professions. *The Journal of Tropical Biology and Conservation*, 662-665.

Griffin, S. C., Taper, M. L., Hoffman, R., & Mills, L. S. (2008). The case of the missing marmots: are metapopulation dynamics or range-wide declines responsible? *Biological Conservation 141*, 1293-1309.

Hart, B. L. (1988). Biological basis of the behavior of sick animals. *Neuroscience & Biobehavioral Reviews 12 (2)*, 123-127.

IUCN/SSC Guidelines for Reintroductionsand Other Conservation Translocations. Version 1.0. Gland, Switzerland: IUCN Species Survival Commission

IUCN Technical Guidelines on the Management of Ex-situ Populations for Conservation. (2002). Retrieved from ICUN: http://www.iucn.org/about/work/programmes/species/publications/iucn_guidelines_and__policy__statements/

Jackson, C. L. (2005). *First year fidelity and survival in reintroduced captive-bred Vancouver Island Marmots (*Marmota vancouverensis*).* Victoria: University of Victoria.

Jackson, C.; Baker, A.; Doyle, D.; Franke, M.; Jackson, V.; Lloyd, N.; McAdie, M.; Stephens, T.; Traylor-Holzer, K.;. (2015). *Vancouver Island Marmot Population and Habitat Viability Assessment Workshop Final Report.* Apple Valley, MN: IUCN SSC Conservation Breeding Specialist Group.

Janz, D. W., Bryant, A. A., Dawe, N. K., Schwantje, H., Harper, B., Nagorsen, D., . . . Simmons, R. (2000). *National Recovery Plan for the Vancouver Island Marmot (*Marmota vancouverensis*) 2000 Update: RENEW Report No.19.* Ottawa, Ontario: Environment Canada.

Krebs, C. J. (1996). Population cycles revisited. *Journal of Mammalogy 77(1)*, 8-24.

Matthews, F., Orros, M., McLaren, G., Gelling, M., & Foster, R. (2005). Keeping fit on the ark: assessing the suitability of captive-bred animals for release. *Biological Conservation 121*, 569-577.

Naughton, D. (2012). *The Natural History of Canadian Mammals*. Canadian Museum of Nature and University of Toronto Press, Toronto, Ontario.

Ranheim, B., Rosell, F., Haga, H. A., & Arnemo, J. M. (2004). Field anaesthetic and surgical techniques for implantation of intraperitoneal radio transmitters in Eurasian beavers (*Castor fiber*). *Wildlife Biology 10 (1)*, 11-15.

Raverty, S., & Black, S. (2001). Causes of death in captive Vanvouver Island Marmots (*Marmota vancouverensis*) including presumptive mycoplasmosis. *Canadian Veterinary Journal 42*, 386-387.

Roth, L., & King, J. M. (1986). Congestive cardiomyopathy in the Woodchuck (*Marmota monax*). *Journal of Wildlife Disease 22 (4)*, 553-537.

Rothenburger, J. L., Himsworth, C. G., Treuting, P. M., & Leighton, F. A. (2014). Survey of cardiovascular pathology in wild urban *Rattus norvegicus* and *Rattus rattus*. *Veterinary Pathology Online*.

Soto-Azat, C., Boher, F., Fabry, M., Medina-Vogel, G., & Pascual, P. (2008). Surgical implantation of intra-abdominal radio-transmitters in Marine Otters (*Lontra felina*) in Central Chile. *Journal of Wildlife Diseases 44 (4)*, 979-982.

Swarth, H. S. (1911). Two new species of marmots from North America. *University of California Publications in Zoology (7)*, 201-204.

Swarth, H. S. (1912). Report on a collection of birds and mammals from Vancouver Island. *University of California Publications in Zoology (10)*, 1-124.

Van Vuren, D. (1989). Effects of intraperitoneal implants on yellow-bellied marmots. *Journal of Wildlife Management. 53 (2)*, 320-323.

Watson, J. (1997). *The Golden Eagle.* London: T & A D Poyser.

Wild, P. (2013). *The Cougar: Beautiful, Wild and Dangerous.* Madeira Park: Douglas and McIntyre.

Wong, C., & Marwick, T. H. (2007). Obesity cardiomyopathy: pathogenesis and pathophysiology. *Nature Reviews Cardiology 4*, 436-443.

CHAPTER 4

CONCLUSION

The overarching goal of this thesis was to expand upon our understanding of individual and population health in the critically endangered Vancouver Island Marmot (*Marmota vancouverensis*). My approach was to describe and evaluate select clinical and pathological data originating from a variety of sources. This involved the analysis of 1,106 Vancouver Island Marmot (VIM) blood profiles, 3,174 physical examinations, 140 necropsies, and 533 field mortality records. In Chapter 2, I calculated hematology and serum biochemistry reference ranges as a baseline metric for assessing VIM health. I also determined that VIM blood values are qualitatively comparable to those that have been calculated for other rodent species and that certain leukogram and plasma protein values may have potential utility as a quantitative measure for comparing VIM management groups. In Chapter 3, I use physical examinations, post mortem examinations, and field evaluation of marmot mortalities, to identify health trends in captive, captive-release and wild VIM. These analyses sought to determine what is expected for this species under different conditions (wild, captive and captive-release) and to identify potential risk factors that may be influencing the species' capacity to achieve or maintain health and to meet defined recovery objectives.

DEFINING HEALTH IN THE VANCOUVER ISLAND MARMOT

The relative state of health of a wildlife population is not necessarily defined by a discrete set of biological characteristics but by human expectations and constructs (Stephen, 2014) which seek to identify an ideal standard of population well-being (Hanisch, *et al.* 2012). With the formulation of officially recognized

conservation strategies, the VIM's health status is determined by the species' relative capacity to fulfill discrete recovery objectives with specific numerical, spatial, and temporal components. Evaluating the population's health includes the identification and assessment of determinants and outcomes which are compatible with, or which compromise, the species' capacity for achieving normative recovery goals. VIM health is contingent upon many factors including the attributes of the animals, their environment, the presence or absence of tangible, effective recovery actions, and the logistical and socio-economic feasibility of implementing these actions.

THERE IS NOT ONE HEALTH FOR VIM

It is important to recognize that health in the Vancouver Island Marmot cannot be characterized by a singular, uniform state. Recovery efforts for VIM have involved an intensive program of captive breeding and conservation translocations, requiring the monitoring and management of several groups or subpopulations. These include; (A) the original *in situ* population that was under threat and demonstrating dramatic declines, (B) the captive founder population consisting of wild-born individuals captured and transitioned to the artificial conditions of captivity, (C) the *ex situ* population comprised of captive-born animals raised under the artificial conditions of captivity, (D) the captive-release population consisting of captive individuals transitioning back to the wild, and (E) the free-ranging recovery population consisting of successful captive-release marmots, their progeny and the descendents of the original wild marmots. Each of these groups has been subject to a unique set of natural and artificial determinants which have influenced health outcomes and expectations. There are also significant differences in the extent to which each of these groups can be monitored and manipulated. However, from a conservation and management perspective, the relative state of health of the

subpopulations will collectively influence the species' capacity to achieve recovery objectives. If the health of one group is compromised, it has the potential to jeopardise the integrity of the other groups and affect species recovery overall.

The original *in situ* population

In the 1980s and 1990s, there was dramatic destabilization of the VIM population and it suffered severe declines. As a result, the wild population was no longer autonomous and self sustaining, and it was deemed to be incapable of independently achieving recovery targets (Janz, et al., 2000). The original population declines had been attributed to demographic and ecosystem effects (Janz, et al., 2000), but there was insufficient historical data for this analysis to consider whether changes to marmot health may also have been a contributing factor.

The *ex situ* population

Captivity is not entirely benign and artificial conditions posed a novel set of risk factors for VIM, particularly as management and husbandry practices were in their initial stages of development. Regular observation, routine clinical evaluation, and thorough post mortem examination of captive individuals resulted in the description of a number of health conditions not identified in any free-ranging marmots. For example, intensive management and protection in captivity appears to have led to increased marmot longevity, allowing for the appearance of age-related problems such as acquired cardiovascular disease and neoplasia, which collectively accounted for approximately 50% of captive mortalities. As captive marmots age, it is possible that obesity may represent an increased risk factor for these disorders. Although there were congenital defects identified in the captive population, they did not occur with enough frequency or predictability to suggest a progressive increase in the number of genetically compromised individuals or in the level of inbreeding (Pimm *et al*., 2006; Raikkonen *et al*., 2006). None of the individuals

displaying congenital disorders were allowed to breed in captivity and since 2011 no congenital problems have been been identified in the captive population. It is possible that captive support may have prolonged the survival of young marmots suffering from these abnormalities.

Overall, the captive population has proven effective in meeting its defined objectives, which includes acting as a long-term safeguard, a genetic reservoir, and a provider of marmots for release. The captive program's future health, and its capacity for continuing to achieve recovery targets, appears to be dependent upon the continued maintenance of effective biosecurity measures and a suitable captive population size, which allows for the proper maintenance of genetic integrity. A viable captive program is dependent upon political will and adequate resources.

The captive-release and free-ranging recovery population

Captive-release marmots have been documented to have lower survival than their wild-born counterparts, and this is most apparent in their first year following release from captivity (Aaltonen *et al*., 2009). The first hibernation in the wild represented a significant cause of mortality and it is possible that captive marmots are released with shortened or abberent endogenous cycles which may compromise their initial hibernation. There are limited data on the fall body mass of captive-release marmots, but it is also possible that poor body condition at the time of immergence or emergence could also jeopardise overwinter survival. Captive-release marmots that survive their first winter in the wild exhibit subsequent hibernation survival that is comparable to their wild counterparts. Although marmots released from captivity do survive and facilitate growth of the free-ranging population (Jackson *et al.,* 2015), it is possible that this contribution could be significantly enhanced by increasing survival during their first hibernation.

Although captive-release marmots were not routinely trapped and examined during their first year following release, they were opportunistically captured, examined, and sampled in subsequent years. In terms of physical examinations, body condition, bloodwork and occurance of morbidity, these surviving captive-release marmots were clinically comparable to their wild-born counterparts. Overall, there was a paucity of health conditions identified in free-ranging marmots (both wild and captive-release) compared to their captive counterparts. This could be due to a fundamental lack of disease, or limited opportunities to observe or examine compromised marmots due to their reclusive behaviors or poor survival. In addition to not being observed or evaluated with the same intensity, *in situ* marmots did not appear to exhibit the same longevity as *ex situ* animals, thereby limiting the appearance of age-related disorders.

For wild and captive-release Vancouver Island marmots, predation represented the most common cause of mortality. In each of these two groups, predation accounted for at least 95% of the non-hibernation deaths for which a specific cause was identified. Predation has been implicated as an important factor in the original population declines of the species (Bryant & Page, 2005; Janz *et al.* 2000) and as a significant cause of mortality in captive-release marmots (Aaltonen *et al.* 2009). Mortality records current to the end of the 2017 field season indicate that predation continues to be a limiting factor in marmot population growth and recovery. Even though the wild population has shown encouraging growth associated with reintroductions and translocations, it may still be too small to overcome the inhibiting effects of ongoing predation pressure. Similar situations have been described for a range of species including lemmings and rabbits (Calvete *et al.* 1997; Krebs, 1996). Like the original *in situ* population, the current recovery

population lacks autonomous sustainability and remains dependent upon captive augmentation to achieve defined recovery goals.

INFECTIOUS DISEASE

Infectious agents have been identified as an important cause of declines in many endangered populations (Biedrzycka & Kloch, 2016) and have been suggested as a potential threat to VIM (Jackson *et al.* 2015; Janz *et al.* 2000; Bryant, 1998). There are many examples of rare species being affected by infectious disease, including chytridiomycosis in amphibians, canine distemper in Serengeti lions (*Panthera leo*), Ethiopian wolves (*Canis simensis*) and black-footed ferrets (*Mustela nigripes*), feline leukemia virus in Iberian Lynx (*Lynx pardinus*) and *Plasmodium* sp. in Hawaiin land birds (Gordon, *et al*., 2015; Roznik & Alford, 2015; Ewan *et al*., 2012; Cleaveland, 2009; Thorne & Williams, 1998). With respect to VIM, this analysis of clinical and pathological data did not identify any specific infectious agents in captive or free-ranging marmots that represented a generalized population threat. However, there is always the ongoing risk of future exposure to a novel pathogen. Zoological facilities maintain eclectic collections of multiple taxa from a diversity of sources, each with their own spectrum of infectious agents (Snyder, et al., 1996). Captive facilities may also inadvertently harbour pest species which provide an additional reservoir for introduction of exotic pathogens. Although captive VIM are maintained under strict biosecurity protocols, quarantine procedures may be compromised whenever marmots are transferred between facilities or translocated. Also, infectious agents may emerge or be maintained in sympatric or introduced species on Vancouver Island. The threat of novel infectious agents cannot be quantified because it is impossible to delineate the full spectrum of VIM's suseptibility to infectious agents. This susceptibilty could potentially increase if

existing biosecurity measures are relaxed, or if the population experiences additional loss of genetic variation and immunocompetence due to a decrease in population size (Biedrzycka & Kloch, 2016).

RECOMMENDATIONS

- Although most of the current threats to the VIM population appear to be associated with anthropogenic or ecological factors, artificial management, low genetic diversity, and small numbers place the species at additional risk for introduction of novel infectious agents and genetic deterioration. Although this risk cannot be quantified, continued health surveillance of the captive and free-ranging populations, using the data from this thesis as a baseline for comparison, will help to recognize future changes and identify potential threats.
- It is possible that existing biosecurity measures in captivity may have acted as an effective safeguard against the introduction of novel infectious diseases. These precautions are well integrated into existing management protocols and should be continued.
- Obesity may increase the risk for age-related disorders in captive marmots, including cardiovascular disease and neoplasia. Management practices, including diet composition and seasonal duration of feeding, and how they influence body condition and hibernation, should be reviewed.
- It is possible that congenital problems and mange may be indicative of genetic compromise or inbreeding in VIM. Although the occurrence of these disorders appears to have abated over time, marmots should be carefully monitored for their reappearance. In general, marmots with congenital problems should not be allowed to breed and marmots exhibiting mange should not be allowed to breed to closely related individuals.

- The first wild hibernation results in significant mortality of captive-release marmots. Although marmots are confirmed to have good body reserves at the time of their release from captivity, it is possible that they may lose condition as they transition to a wild environment. This loss may compromise their ability to survive their first hibernation. There is a need for further research which compares the hibernation characteristics of captive, captive-release, and wild marmots and investigates the effects of entrainment of endogenous cycles in captivity, marmot body condition at immergence and emergence, hibernacula characteristics and the benefits of pre and post-hibernation supplementation.
- From 1992 to 2016 a total of 898 implant surgeries were performed on VIM including 96 replacement surgeries. Two fatal complications (0.22% of total surgeries) have arisen directly from implanted transmitters. In one case, the radio-transmitter become lodged in the pelvic canal of an emaciated post-emergent male, resulting in a fatal impaction of the gastrointestinal tract. In the second case, the outer surface of the transmitter caused an extreme, chronic inflammatory reaction with significant encapsulation and visceral adhesions. These problems were subsequently mitigated by having the manufacturer increase the diameter of the transmitters and by having the transmitters encapsulated in a hard, biologically inert resin. Although there were focal adhesions to the greater omentum associated with the early use of wax-coated transmitters (1992 to 2010, Appendix D), there was no corrosion, leakage or overt breakdown of the radio-transmitters as has been reported in other species (Arnemo *et al*., 2018). Based upon bloodwork, physical examinations (including post-surgical evaluations) and necropsies, the implantation of abdominal radio-transmitters does not appear to impact marmot health, and these units should continue to be used to monitor free-ranging marmots. They represent the most

effective method for monitoring survival and mortalities in the free-ranging population.

- Although mortalities in free-ranging marmots are generally characterized by limited remains and forensic clues, these events should continue to be rigorously investigated as part of an expanding data set.
- Although recovery of intact marmot carcasses from the field is a rare occurrence, these events represent significant clinical data and should be comprehensively evaluated as opportunities present themselves.

THESIS SIGNIFICANCE

My thesis extended our understanding of health in the Vancouver Island Marmot by collating select clinical and pathological data from multiple sources. It described characteristics for blood values, morbidity, and mortality under different management treatments (wild, captive and captive-release) and identified potential threats to both captive and free-ranging marmots. Although these results are important, they are by no means definitive in nature. This analysis is not meant to represent an exhaustive or conclusive treatment of VIM health data. The greatest utility of this thesis is that it can serve as a template for ongoing comparisons and for recognizing change. Marmot health and its influences are complex and dynamic in nature, and therefore continued health surveillance and evaluation of existing and new data represent important keys for effective management and species recovery.

LITERATURE CITED

Arnemo, J., Ytrehus, B., Madslien, K., Maimsten, J., Brunberg, S., Segerstrom, P., Evans, A. L., & Swenson, J. E. (2018). Long-term safety of intraperitoneal radio transmitter implants in brown bears (*Ursus arctos*). *Frontiers in Veterinary Science* 5: 252.

Aaltonen, K., Bryant, A. A., Hostetler, J. A., & Oli, M. K. (2009). Reintroducing endangered Vancouver Island marmots: survival and cause-specific mortality rates of captive-born versus wild-born individuals. *Biological Conservation 142*, 2181-2190.

Biedrzycka, A., & Kloch, A. (2016). Development of novel associations between MHC alleles and susceptibility to parasitic infections in an isolated population of an endangered mammal. *Infection, Genetics and Evolution 44*, 210-217.

Bryant, A. A., & Page, R. E. (2005). Timing and causes of mortality in the endangered Vancouver Island marmot (*Marmota vancouverensis*). *Canadian Journal of Zoology (83)*, 674-682.

Bryant, A. A. (1998). *Metapopulation ecology of Vancouver Island marmots* (Marmota vancouverensis), PhD thesis. Victoria, B.C.: University of Victoria.

Calvete, C., Villafuerte, R., Lucientes, J., & Osacar, J. J. (1997). Effectiveness of traditional wild rabbit restocking in Spain. *Journal of Zoology 241 (2)*, 271-277.

Cleaveland, S. (2009). Viral threats and vaccination: disease management of endangered species. *Animal Conservation 12*, 187-189.

Ewan, J. C., Armstrong, D. P., Parker, P. A., & Seddon, P. J. (2012). *Reintroduction Biology: Integrating Science and Management.* Wiley- Blackwell.

Gordon, C. H., Banyard, A. C., Hussein, A., Laurenson, M. K., Malcolm, J. R., Marino, J., . . . Sillero-Zubiri, C. (2015). Canine distemper in endangered Ethiopian wolves. *Emerging Infectious Diseases 21 (5)*, 824-832.

Jackson, C.; Baker, A.; Doyle, D.; Franke, M.; Jackson, V.; Lloyd, N.; McAdie, M.; Stephens, T.; Traylor-Holzer, K.;. (2015). *Vancouver Island Marmot Population and Habitat Viability Assessment Workshop Final Report.* Apple Valley, MN: IUCN SSC Conservation Breeding Specialist Group.

Janz, D. W., Bryant, A. A., Dawe, N. K., Schwantje, H., Harper, B., Nagorsen, D., . . . Simmons, R. (2000). *National Recovery Plan for the Vancouver Island Marmot (Marmota vancouverensis) 2000 Update: RENEW Report No.19.* Ottawa, Ontario: Environment Canada.

Krebs, C. J. (1996). Population cycles revisited. *Journal of Mammalogy 77(1)*, 8-24.

Pimm, S. L., Dollar, L., & Bass Jr., O. L. (2006). The genetic rescue of the Florida pather. *Animal Conservation 9*, 115-122.

Raikkonen, J., Bignert, A., Mortensen, P., & Fernholm, B. (2006). Congenital defects in a highly inbred wild wolf population (*Canis lupus*). *Mammalian Biology 71 (2)*, 65-73.

Roznik, E. A., & Alford, R. A. (2015). Seasonal ecology and behavior of an endangered rainforest frog (*Litoria rheocola*) threatened by disease. *PloS One 10(5): e127851.doi:10.137/journal*, 1-17.

Snyder, N. F., Derrickson, S. R., Bessinger, S. R., Wiley, J. W., Smith, T. B., Toone, W. D., & Miller, B. (1996). Limitations of captive breeding in endangered species recovery. *Conservation Biology 10 (2)*, 338-348.

Stephen, C. (2014). Toward a modernized defintion of wildlife health. *Journal of Wildlife Diseases 5 (3)*, 427-430.

Thorne, E. T., & Williams, E. S. (1998). Disease and endangered species: the black-footed ferret as a recent example. *Conservation Biology 2(1)*, 66-74.

Appendix A. Vancouver Island Marmot (*Marmota vancouverensis*) captive population numbers from 1997 to 2018.

Year	Wild marmot captures excluding pups (26)	Wild pup captures (45)	Total wild captures (71)	Weaned captive litters (180)	Breeding Pairs (from 2005 to 2018)	Proportion of Pairs Breeding (2005 to 18)	Weaned captive pups (612)	Weaned males (56%)	Weaned females (44%)	Unknown	Average weaned litter size	Captive Mortalities (113)	Releases of captive marmots (515)	Recaptures (2)	Captive Total (58)
1997	4	2	6	0			0	0	0	0	0.00	0	0	0	6
1998	5	3	8	0			0	0	0	0	0.00	2	0	0	12
1999	9	10	19	0			0	0	0	0	0.00	4	0	0	27
2000	1	4	5	2			8	5	3	0	4.00	1	0	0	39
2001	2	5	7	1			2	1	1	0	2.00	1	0	0	47
2002	2	4	6	5			13	8	5	0	2.60	3	0	0	63
2003	1	2	3	7			18	10	8	0	2.57	4	4	1	77
2004	0	1	1	8			26	19	7	0	3.25	2	9	0	93
2005	0	0	0	13	26	0.50	48	31	17	0	3.69	5	15	0	121
2006	0	0	0	14	30	0.47	55	27	28	0	4.00	4	31	1	142
2007	0	0	0	15	37	0.41	60	33	27	0	4.00	3	37	0	162
2008	0	0	0	23	46	0.50	85	41	40	4	3.70	11	59	0	177
2009	0	0	0	20	48	0.42	71	33	38	0	3.55	9	68	0	171
2010	0	0	0	18	49	0.37	55	33	22	0	3.06	11	85	0	130
2011	0	0	0	18	36	0.50	51	26	24	1	2.83	8	66	0	107
2012	0	0	0	6	26	0.23	22	11	11	0	3.66	9	34	0	86
2013	0	0	0	6	25	0.24	18	11	7	0	3.00	6	16	0	82
2014	0	0	0	6	19	0.36	18	10	8	0	2.83	16	29	0	55
2015	0	0	0	5	12	0.42	15	9	6	0	3.00	1	24	0	45
2016	1	5	6	3	14	0.21	13	8	5	0	4.33	9	13	0	42
2017	0	2	2	5	11	0.45	19	12	7	0	3.80	3	11	0	49
2018	1	8	9	5	12	0.42	15	11	4	0	3.00	1	14	0	58
Total	**26**	**46**	**72**	**180**	**391**	**0.40**	**612**	**339**	**268**	**5**	**3.40**	**113**	**515**	**2**	**58**

Appendix B. Descriptive statistics for hematological variables from clinically normal or healthy Vancouver Island Marmots (*Marmota vancouverensis*) before & after removal of outliers. Used in calculation of reference values in accordance with guidelines established by Species 360, formerly the International Species Information System (Teare, 2002).

Parameter		Units	Mean	Min	Max	*N*	S.D.	3 x S.D.	minus 3 x S.D.	plus 3 x S.D.	Number of outliers	Recalculated Mean	Min	Max	*N*	S.D.
WHITE BLOOD CELLS																
White blood cells	WBC	x 10^9/L	5.1	0.6	35.9	1030	2.4	7.1	-2.0	12.2	12.0	5.0	0.6	12.2	1018	1.9
Segmented neutrophils	Neutro	x 10^9/L	2.7	0.3	26.6	1027	1.6	4.8	-2.1	7.6	10.0	2.6	0.3	7.6	1017	1.2
Band neutrophils	Bands	x 10^9/L	0.0	0.0	0.5	1027	0.0	0.1	-0.1	0.1	15.0	0.0	0.0	0.0	1012	0.0
Lymphocytes	Lympho	x 10^9/L	2.0	0.0	12.0	1027	1.2	3.7	-1.7	5.6	9.0	1.9	0.0	5.5	1018	1.1
Monocytes	Mono	x 10^9/L	0.3	0.0	6.1	1027	0.4	1.1	-0.7	1.4	15.0	0.3	0.0	1.4	1012	0.3
Eosinophils	Eosino	x 10^9/L	0.0	0.0	1.1	1027	0.1	0.2	-0.2	0.3	16.0	0.0	0.0	0.2	1011	0.0
Basophils	Baso	x 10^9/L	0.0	0.0	0.3	1027	0.0	0.1	-0.1	0.1	24.0	0.0	0.0	0.1	1003	0.0

Appendix B (cont.) Descriptive statistics for hematological variables from clinically normal or healthy Vancouver Island Marmots (*Marmota vancouverensis*) before & after removal of outliers. Used in calculation of reference values in accordance with guidelines established by Species 360, formerly the International Species Information System. (Teare, 2002)

Parameter		Units	Mean	Min	Max	*N*	S.D.	3 x S.D.	minus 3 x S.D.	plus 3 x S.D.	Number of outliers	Recalculated Mean	Min	Max	*N*	S.D.
ERYTHROCYTES																
Red blood cells	RBC	x 10^{12}/L	6.4	2.8	8.9	1025	0.7	2.2	4.3	8.6	15.0	6.4	4.2	8.5	1010	0.6
Hemoglobin	Hb	g/L	144.4	15.0	198.0	1016	16.2	48.7	95.7	193.0	12.0	145.1	98.0	187.0	1004	14.3
Hematocrit	Hmt	L/L	0.4	0.2	0.6	1030	0.0	0.1	0.3	0.6	9.0	0.4	0.3	0.6	1021	0.0
Mean corpuscular volume	MCV	fl	65.8	5.1	92.7	992	5.6	16.7	49.1	82.5	15.0	65.9	50.0	82.3	977	3.5
Mean corpuscular hemoglobin	MCH	pg	22.4	16.3	33.1	986	1.6	4.8	17.6	27.2	0.0	22.4	16.3	33.1	986	1.6
Mean corpuscular hemoglobin concentration	MCHC	g/L	339.5	253.0	493.0	996	15.3	46.0	293.6	385.5	13.0	339.3	294.0	384.0	983	12.3
Red cell distribution width	RCDW	%CV	14.3	5.2	30.3	904	2.1	6.4	7.9	20.7	27.0	14.4	10.1	20.3	877	1.5
HEMOSTASIS																
Platelet count		x 10^9/L	323.0	37.0	1295.0	767	104.9	314.6	8.3	637.6	6.0	318.7	37.0	625.0	761	91.7
Mean platelet volume		fl	8.9	0.0	16.9	704	1.4	4.3	4.6	13.2	10.0	8.9	6.7	14.0	694	1.3

Appendix B (cont.) Descriptive statistics for hematological variables from clinically normal or healthy Vancouver Island Marmots (*Marmota vancouverensis*) before & after removal of outliers. Used in calculation of reference values in accordance with guidelines established by Species 360, formerly the International Species Information System. (Teare, 2002)

Parameter	Units	Mean	Min	Max	*N*	S.D.	3 x S.D.	minus 3 x S.D.	plus 3 x S.D.	Number of outliers	Recalculated Mean	Min	Max	*N*	S.D.
ELECTROLYTES AND ACID-BASE															
Sodium	mmol/L	143.7	120.0	164.0	532	4.6	13.9	129.8	157.7	7.0	143.8	130.0	155.8	525	4.0
Potassium	mmol/L	5.6	3.3	50.0	531	2.8	8.5	-2.9	14.1	9.0	5.4	3.3	13.3	522	1.4
Sodium / Potassium ratio	ratio	27.9	3.0	43.6	531	6.5	19.4	8.5	47.3	6.0	28.1	8.6	43.6	525	6.1
Chloride	mmol/L	102.0	18.0	112.0	529	5.5	16.5	85.5	118.5	1.0	102.1	88.0	112.0	528	4.1
Bicarbonate	mmol/L	26.4	9.0	39.0	147	5.9	17.6	8.7	44.0	0.0	26.4	9.0	39.0	147	5.9
Carbon Dioxide	mmol/L	26.4	5.0	38.0	357	5.6	16.8	9.6	43.2	0.0	26.4	5.0	38.0	357	5.6
Anion Gap		20.9	6.0	75.9	515	7.1	21.4	-0.5	42.3	3.0	20.7	6.0	41.0	512	6.6
Calcium	mmol/L	2.4	1.4	22.0	601	0.9	2.7	-0.2	5.1	3.0	2.4	1.4	3.3	598	0.2
Phosphorous	mmol/L	1.9	0.1	5.8	602	0.5	1.6	0.2	3.5	8.0	1.8	0.7	3.5	594	0.5
Calcium / Phosphorous ratio	ratio	1.5	0.4	28.3	576	1.3	3.9	-2.5	5.4	3.0	1.4	0.4	4.3	573	0.4
Calculated Osmolality	mmol/kg	297.5	265.0	355.0	514	7.7	23.1	274.5	320.6	6.0	297.3	276.0	319.0	508	6.7

Appendix C. Descriptive statistics for serological variables from clinically normal or healthy Vancouver Island Marmots (*Marmota vancouverensis*) before & after removal of outliers. Used in calculation of reference values in accordance with guidelines established by Species 360, formerly the International Species Information System. (Teare, 2002)

Parameter		Units	Mean	Min	Max	*N*	S.D.	3 x S.D.	minus 3 x S.D.	plus 3 x S.D.	Number of outliers	Recalculated Mean	Min	Max	*N*	S.D.
PROTEINS																
Total Protein		g/L	62.2	6.0	107.0	652	10.2	30.7	31.6	92.9	9.0	62.0	42.0	90.0	643	9.0
Albumin		g/L	27.8	17.0	84.0	566	5.2	15.6	12.2	43.3	11.0	27.3	17.0	43.0	555	3.6
Globulin		g/L	33.3	18.6	75.0	561	6.9	20.8	12.4	54.1	8.0	32.9	18.6	53.0	553	6.0
Albumin / Globulin ratio	A / G ratio	ratio	0.9	0.3	10.0	563	0.5	1.5	-0.6	2.4	2.0	0.9	0.3	1.9	561	0.2
LIVER AND MUSCLE																
Total Bilirubin		µmol/L	2.8	0.0	15.0	435	1.7	5.1	-2.3	7.9	7.0	2.6	0.0	7.0	428	1.4
Alkaline Phosphatase	ALP	IU/L	77.2	5.0	1101.0	564	68.7	206.2	-129.1	283.4	8.0	71.6	5.0	266.0	556	41.3
Alanine Aminotransferase	ALT	IU/L	23.1	1.0	314.0	526	33.4	100.3	-77.1	123.4	14.0	18.6	1.0	117.0	512	17.0
Aspartate Aminotransferase	AST	IU/L	43.9	1.0	601.0	458	61.3	183.9	-139.9	227.8	9.0	37.1	1.0	222.0	449	34.5
Gamma Glutamyltransferase	GGT	IU/L	4.5	0.0	55.0	526	5.1	15.3	-10.8	19.8	10.0	4.0	0.0	18.0	516	3.0
Creatinine Phosphokinase	CPK	IU/L	640.5	56.0	10748.0	595	924.8	2774.5	-2134.0	3414.9	12.0	526.2	56.0	2970.0	583	366.2
Cholesterol		mmol/L	7.2	3.7	13.1	72	2.0	5.9	1.3	13.2	0.0	7.2	3.7	13.1	72	2.0
Glucose	Gluc	mmol/L	8.8	1.2	18.3	637	2.4	7.3	1.5	16.1	7.0	8.8	2.2	15.8	630	2.3

Appendix C (cont.) Descriptive statistics for serological variables from clinically normal or healthy Vancouver Island Marmots (*Marmota vancouverensis*) before & after removal of outliers. Used in calculation of reference values in accordance with guidelines established by Species 360, formerly the International Species Information System. (Teare, 2002)

Parameter		Units	Mean	Min	Max	*N*	S.D.	3 x S.D.	minus 3 x S.D.	plus 3 x S.D.	Number of outliers	Recalculated Mean	Min	Max	*N*	S.D.
RENAL FUNCTION																
Blood Urea Nitrogen	BUN	mmol/L	11.1	3.1	70.0	638	3.8	11.4	-0.3	22.6	6.0	10.9	3.1	22.2	632	2.6
Creatinine	Creat	µmol/L	82.5	7.0	224.0	623	23.6	70.7	11.8	153.3	6.0	81.9	18.0	150.0	617	21.8
Bun / Creatinine ratio		ratio	0.1	0.0	1.0	623	0.1	0.2	-0.1	0.4	10.0	0.1	0.0	0.4	613	0.1
OTHER																
Lactose Dehydrogenase	LDH	IU/L	1785.0	865.0	4068.0	30	822.3	2466.9	-681.9	4251.8	0.0	1785.0	865.0	4068.0	30	822.3
Amylase		IU/L	878.3	147.0	2373.0	79	481.8	1445.3	-567.0	2323.5	2.0	839.8	147.0	2086.0	77	423.0
Lipase		IU/L	191.8	60.0	856.0	73	137.2	411.7	-219.9	603.5	0.0	191.8	60.0	856.0	73	137.2
Tetra Iodothyronine		nmol/L	57.5	20.0	112.0	152	17.3	52.0	5.5	109.6	1.0	57.2	20.0	91.0	151	16.8

Appendix D. Characteristics of very high frequency (VHF) implantable radio-transmitter used in Vancouver Island Marmots (*Marmota vancouverensis*) from 1992 to 2018.

Manufacturer	Model	Diameter (mm)	Length (mm)	Weight (grams)	Maximum Longevity (days)	Age Category	Encapsulation Material	Preparation	Duty Cycle	Temperature Responsive Pulse Rate	Years
Custom Telemetry®	Not recorded	25	90	35	Not recorded	Pups and non-pups	Wax	Disinfected povidone-iodine solution	None	Continuous temperature response	1992 - 1993
Telonics®	IMP/300/L	23	85	40	1095	Non-pups	Wax	Disinfected povidone-iodine solution	9 hours on 15 hours off	Temperature triggered response (35 ppm above 30°C / 25 ppm below 30°C)	1994 - 2009
Telonics®	IMP/325/L	23	100	50	1095	Non-pups	Wax	Disinfected povidone-iodine solution	9 hours on 15 hours off	Temperature triggered response (35 ppm above 30°C / 25 ppm below 30°C)	2010
Telonics®	IMP/200/L	23	61	25	540	Pups	Wax	Disinfected povidone-iodine solution	9 hours on 15 hours off	Temperature triggered response (35 ppm above 30°C / 25 ppm below 30°C)	2008 - 2010
Advanced Telemetry Systems®	M1215T	12	64	13	294	Pups	Resin	Sterilized ethylene oxide gas	None	Continuous temperature response	2011 - 2013
Advanced Telemetry Systems®	M1230T	18	68	25	728	Pups	Resin	Sterilized ethylene oxide gas	None	Continuous temperature response	2011 - 2013
Advanced Telemetry Systems®	M1240T	20	78	40	1226	Non-pups	Resin	Sterilized ethylene oxide gas	None	Continuous temperature response	2011 - 2013
Holohil Systems®	A1-2TH	19	79	35	1095	Non-pups	Plasti Dip® (butyl rubber)	Disinfected povidone-iodine solution	None	Continuous temperature response	2006 - 2009
Holohil Systems®	A1-2TH	25	90	55	1800	Non-pups	Plasti Dip® (butyl rubber)	Sterilized ethylene oxide gas	None	Continuous temperature response	2009 - 2011
Holohil Systems®	A1-2TH	25	90	50	1800	Non-pups	Resin	Sterilized ethylene oxide gas	None	Continuous temperature response	2012 - 2018

Appendix D. (cont.) Characteristics of very high frequency (VHF) implantable radio-transmitter used in Vancouver Island Marmots (*Marmota vancouverensis*) from 1992 to 2018.

Manufacturer	Model	Diameter (mm)	Length (mm)	Weight (grams)	Maximum Longevity (days)	Age Category	Encapsulation Material	Preparation	Duty Cycle	Temperature Responsive Pulse Rate	Years
Holohil Systems®	S1-2TH	15	65	18	540	Pups	Plasti Dip® (butyl rubber)	Sterilized ethylene oxide gas	None	Continuous temperature response	2007 - 2011
Holohil Systems®	S1-2TH	15	65	18	540	Pups	Resin	Sterilized ethylene oxide gas	None	Continuous temperature response	2012 - 2013

Appendix E. Summary of Vancouver Island Marmot (*Marmota vancouverensis*) implant surgeries, 1992 to 2018.

Year	Wild marmots					Captive-release marmots		Annual Total
	new surgeries	replacement surgeries	total (wild & translocation)	wild population estimate	% of total wild population	new surgeries (pre-release)	replacement surgeries (post release)	
1992	7	0	7	211	3.3	0	0	7
1993	11	2	13	189	6.9	0	0	13
1994	6	3	9	233	3.9	0	0	9
1995	4	0	4	139	2.9	0	0	4
1996	4	0	4	122	3.3	0	0	4
1997	2	0	2	118	1.7	0	0	2
1998	4	0	4	74	5.4	0	0	4
1999	3	0	3	60	5.0	0	0	3
2000	7	0	7	51	13.7	0	0	7
2001	12	2	14	43	32.6	0	0	14
2002	7	1	8	41	19.5	1	0	9
2003	3	6	9	31	29.0	3	0	13
2004	1	6	7	32	21.9	9	0	17
2005	8	3	11	40	27.5	15	0	26
2006	3	0	3	60	5.0	30	2	35
2007	9	0	9	68	13.2	37	1	47
2008	3	4	7	100	7.0	59	2	68
2009	18	3	21	160	13.1	68	4	93
2010	30	3	33	280	11.8	85	5	123
2011	12	1	13	350	3.7	67	6	86
2012	19	11	30	375	8.0	32	11	73
2013	57	8	65	313	20.8	16	0	81
2014	35	2	37	245	15.1	27	0	64
2015	26	3	29	240	12.1	24	1	54
2016	23	7	30	166	18.1	13	1	44
2017	13	4	17	150	11.3	11	33	28
2018	24	5	29	145	20.0	14	0	43
Total	353	72	425	149 (average)	12.4 (average)	511	33	969

Appendix F. Trapping, handling, anesthesia & implant surgery techniques in Vancouver Island Marmots (*Marmota vancouverensis*)

Trapping

Free-ranging marmots were captured using live-traps (*Havahart*® model # 1079, dimensions 32" x 10" x 12") which were baited with a trail of peanut butter or set up so that they represented the marmot's only option for egress from a burrow. Traps set for marmots were continuously monitored or checked frequently. Because of their susceptibility to hyperthermia (Ken Langelier, personal communication, 1992), marmots were not typically handled or immobilized if ambient temperatures exceeded 20°C. Following capture, marmots held in live traps were covered with a fitted trap-cover to provide shade, minimize stress, and to act as a visual barrier to reduce self-trauma associated with hitting the sides of the wire trap. Marmots undergoing implantation were transported to a central field site for surgery. The distance to this site was minimized so that transport was as quick and efficient as logistically possible. Hunter pack-frames were often used to carry the trapped marmot to the handling location. In hot weather, a sealed plastic bag containing flattened snow (if available) or an ice-pack were placed beneath a portion of the trap on the pack-frame's shelf to facilitate cooling during transport. Marmots trapped at Mount Washington were often transported by foot or by vehicle to the Tony Barrett Mount Washing Marmot Recovery Centre (MRC) for implant surgery or for examination and staging prior to translocation. Wild marmots captured for the captive breeding program were trapped, examined, and then directly transported to the Toronto Zoo (total = 26), Calgary Zoo (25) by vehicle and commercial airline, or to the MRC (10) by vehicle.

Handling

For most procedures involving sampling, marking or examination of captive or free-ranging marmots, chemical restraint was required. Excessive physical restraint of the marmots was avoided because of the potential for traumatic injuries including diaphragmatic hernias which have occurred in woodchucks at Cornell University following physical restraint, (Tennant, personal communication, 2001) and hyperthermia. The marmots were run into a tapered, cloth handling bag which fit snugly around the end of the trap or against the doorway of their nest-box. Any obstacle such as rocks or branches lying in the "run-way" area of the cloth bag was removed beforehand to avoid injury to the marmot. In captivity, the concrete floor was padded by pulling additional shavings into the "run-way" area. Once the marmot had reached the apex of the bag, the bag was gripped or twisted behind the animal to provide better restraint and prevent its turning or egress, taking care to not grab its tail.

Immobilization / Anesthesia

Once they were manually restrained in the handling bag the marmots received an intramuscular injection of ketamine hydrochloride (10 mg/kg) and midazolam hydrochloride (0.25 mg/kg). Ketamine hydrochloride (100 mg/ml) was combined with midazolam hydrochloride (5 mg/ml) in a 2:1 ratio and administered at an approximate volume of 0.15 ml / kg into the epaxial or lumbar muscles, which were palpated and isolated through the wall of the bag. Following injection, care was taken so that the marmot was not excessively wrapped or physically restrained within the bag, which could increase its struggling, elevate its body temperature, or interfere with its breathing. Marmots were monitored for a suitable drug response through the wall of the bag. This included reduced struggling, progressive muscle relaxation, loss of balance or a righting response, reduced response to tactile stimuli

and slower, deeper respirations. They were held in the bag until they were sufficiently tractable or immobilized for safe handling. Typically, younger marmots (pups and yearlings) were found to be less responsive to the dosage regime than adults. The drugs also appeared to have a more profound effect on the marmots later in the active season (i.e. more pronounced in September and October).

Once the marmot was sufficiently tractable, it was removed from the handling bag and further induced with inhaled isoflurane. The marmots were given a titrated gas mixture consisting of oxygen (delivered at approximately 1 liter per minute from a "click" style oxygen flow-meter / regulator) fed through a calibrated, Tech 3 isoflurane vaporizer, typically set at an initial level between 2.5 and 3.5%. The gas mixture was delivered to the animal through a modified Jackson Rees non-rebreathing circuit mated to a clear plastic anesthesia mask. The anesthesia mask was fitted with a flexible diaphragm (made from a fenestrated surgical glove taped to its rim) which formed an effective seal around the marmot's head to minimize gas leakage. Due to the short duration of most procedures, the difficulty of visualizing the glottis and epiglottis, the potential for oropharyngeal trauma, and marmot anatomical characteristics (obligate nasal breathers and limited capacity for regurgitation and gastric reflux) marmots were not routinely intubated during anesthesia. In some instances, pups were manually restrained in the handling bag and mask induced without receiving any injectable immobilization agents.

Respiratory rate, heart rate and body temperature were continuously monitored during immobilizations and anaesthesia. Respirations were monitored visually and with a pulse oximeter. Heart rate was monitored using a stethoscope, a pulse oximeter providing pulse rate data and / or a Doppler heart rate monitor with the probe positioned over the medial aspect of the distal tibia (just proximal to the tarsus). Body temperature were monitored using a digital rectal thermometer. In

some instances, particularly early in the active season when fat reserves are low, marmots were provided with a source of supplemental heat such as latex gloves filled with warmed water or an insulated heating pad. To effect cooling on warm days a sealed plastic bag containing flattened snow (if available) or an ice-pack were placed in the areas of greatest heat exchange (inner thigh, abdomen, shoulders, axilla) or the extremities were wetted with isopropyl alcohol.

Implant surgery

As much as possible, surgically implanted, intra-abdominal transmitters were used to monitor free-ranging marmots. Internal transmitters were used because the dramatic seasonal mass changes that are associated with marmot hibernation made conventional collars impractical. Implant surgeries were routinely performed in the dedicated surgery at the TBMWMRC or in the field using high standards of anesthesia and aseptic technique. Because implantation required a surgical procedure and an adequate capacity for convalescence, marmots were not implanted close to hibernation (i.e. early in the field season following emergence when body condition and food resources were low and when female marmots might be pregnant, or late in the field season close to immergence when the marmots' metabolic rate was starting to decline and healing capacity might be reduced). Most surgeries were performed between the middle of June and the end of August. During the course of this project, temperature-responsive transmitters from Custom Telemetry ® (Watkinsville, Georgia), Telonics ® (Mesa, Arizona), Advanced Telemetry Systems ® (Isanti, Minnesota) and Holohil Systems Limited ® (Carp, Ontario) were used. The pulse rate and temperature response of the transmitters was configured to maximize functionality and prolong battery life. Pulse rate declines associated with lowered body temperature helped to prolong battery life

during hibernation and during the active season a low transmitter pulse rate was used to indicate potential marmot mortalities.

From 1992 to 2016 a total of 898 implant surgeries were performed on Vancouver Island marmots including 96 replacement surgeries. Most of the captive marmots being prepared for release were surgically implanted with radio-transmitters and then afforded a period of convalescence in captivity. Intervals between captive surgery and release ranged from 11 to 93 days (mean = 28 days). Three marmots were held until the following year due to transient injuries that precluded same-year release and one individual died due to pre-release complications with its transmitter. 484 of the 490 captive-release marmots were surgically implanted with an abdominal radio transmitter prior to release and 32 of these individuals were later recaptured and had their transmitters replaced. One individual was recaptured a second time for transmitter replacement and one individual was recaptured and surgically implanted 50 days after being initially released without a transmitter.

From 1992 to 2016 a total of 379 implant surgeries were performed on wild marmots (including marmots that were subsequently translocated), including 63 replacement surgeries. Wild marmots being intentionally prepared for translocation were surgically implanted with radio-transmitters and then allowed to convalesce at their familiar colony sites. Although 14 days was deemed to be the minimal interval between field surgery and translocation, this period actually ranged from 21 to 37 days (mean = 30 days) due to challenges associated with recapture, weather, and other field logistics. In five instances, wild marmots were not recaptured and translocated until the following year following surgery. Four marmots which were captured at aberrant locations including Bamfield, Nanaimo and Nanoose on Vancouver Island were surgically implanted and translocated to suitable habitat

within days of capture. All four marmots survived for at least 350 days (range 350 to 1511 days), indicating that these marmots were not adversely affected by prompt relocation following surgery.

All marmots received a comprehensive physical examination prior to surgery. Individuals that were inappropriately sized, compromised by injury, or exhibiting evidence of disease were not implanted with transmitters.

Surgical technique

Following physical examination and placement of the monitoring equipment, marmots were positioned in a v-trough in dorsal recumbency. The fur from a small area of approximately 7 cm long × 5 cm wide was clipped from the ventral abdominal midline. The surgical site was cleaned and disinfected using a routine surgical preparation, consisting of a series of at least three chlorhexidine (or povidone iodine scrub) washes / isopropyl rinses, and finishing with a final application of a povidone iodine solution. To minimize effects on post-surgical thermoregulation, excessive removal or wetting of the fur was avoided. All surgeries were performed using sterile surgical gloves, surgical masks / caps, autoclaved, sterile surgical instruments, sterile occlusive drapes, and aseptic technique. A nonporous, transparent, self-adhesive fenestration drape (Veterinary Specialty Products, Overland Park, KS) was positioned over the surgical site. A longitudinal skin incision approximately 3 cm in length is made along the ventral midline to expose the linea alba, which was then incised after elevation of the body wall. Care was taken so that the underlying, voluminous, thin-walled intestines were not perforated during the initial incision. The incision into the abdominal cavity was extended as necessary using sharp dissection. At an appropriate point the transmitter was removed from its sterile packaging or disinfectant solution. If it had been in a disinfectant solution the unit was well rinsed with warm sterile saline or

lactated Ringer's solution. The functioning and frequency of the transmitter was reconfirmed prior to placement. The transmitter was inserted into the abdominal cavity and positioned laterally and longitudinally, away from the incision site. Bleeding during the procedure was minimal. 3-0 PDS II (polydioxanone) an absorbable, synthetic, monofilament suture (Ethicon, Inc., Somerville, NJ) was used for closure of all tissue layers. Simple, interrupted sutures were used to close the body wall. A simple, continuous pattern was used to close the subcutaneous fat and a subcuticular suture was used to close the skin. Additional simple interrupted skin sutures and a thin layer of surgical tissue adhesive (Vetbond, 3M, St Paul, MN), were also be used to provide additional security of the skin incision. Beginning in 2013, injectable analgesics (Subcutaneous injection of 0.1 to 0.2 mg/kg of Meloxicam, Metacam, Boehringer Ingelheim Vetmedica, Ridgefield, CT) were administered preoperatively. Antibiotics were not routinely administered but were given on occasion at the discretion of the surgeon.

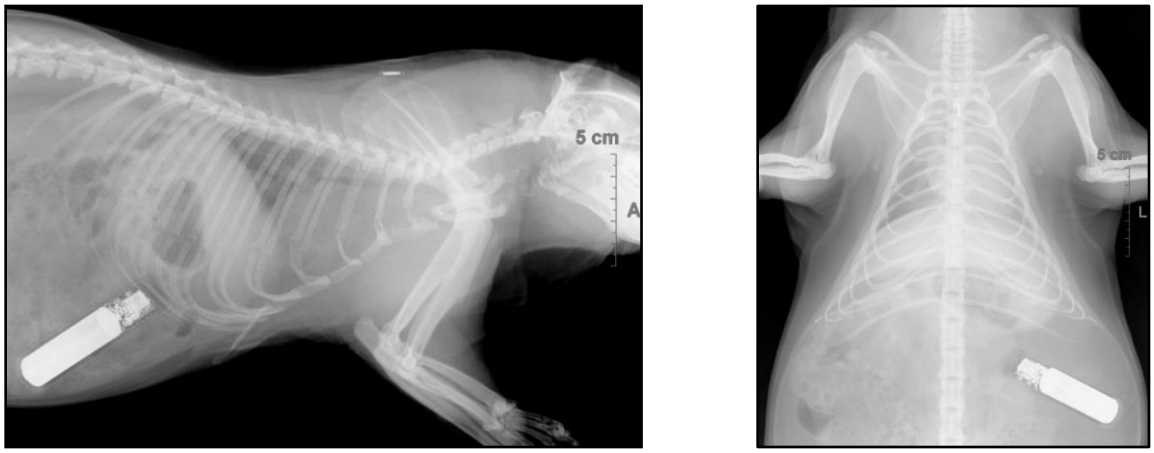
Recovery

Following surgery, measurements and sample collection, the isoflurane was discontinued, and the marmot was given a short interval of pure oxygen. Increased muscle tone and spontaneous postural changes (head up, rising to sternal recumbency) were typically observed within minutes of discontinuing isoflurane. After these initial signs were observed, the marmot was returned to its trap and monitored for further recovery. Once the marmot had attained normal posture and demeanour its trap was covered and placed in a quiet, thermally "neutral" area (not too hot or too cold and out of direct sunlight) and checked periodically. Induction to recovery times were approximately 45 minutes, depending upon additional manipulations (measurements, diagnostics, sampling, etc.) that were being performed. Marmots were held for a minimum of 45 to 60 minutes following

discontinuation of the isoflurane before being returned to their nest-box or into a familiar burrow close to their original capture site. Marmots were not transported until they could effectively maintain their balance within the trap (verified by slight tipping of the trap) in order to reduce the risk of trauma associated with losing their balance while being carried over rough terrain. Mild and transient post-operative imbalance was attributed to the residual effects of ketamine. The duration of the midazolam's sedative effects lasted 60 to 90 minutes (exceeding ketamine's duration of action which is approximately 30 to 45 minutes) and wild marmots typically remained calm during recovery and subsequent transport back to the release site.

Potential complications associated with implantation were mitigated by minimizing animal stress and handling time, employing sound surgical techniques, and by careful selection and handling of the implant units. From 1992 to 2010, transmitters were disinfected by soaking in a povidone-iodine solution for a minimum of 12 hours. These units were subsequently rinsed with Lactated Ringers Solution or physiological saline prior to placement. Beginning in 2011, all transmitters were gas sterilized with ethylene oxide. Transmitters with surface defects were avoided, particularly in the case of "soft" encapsulation materials such as Plasti-dip® and wax. Observations during early replacement surgeries indicated that surface irregularities may have led to the development of focal adhesions between the transmitter and the greater omentum. To date, two fatal complications arising directly from an implanted transmitter have been documented. In one case, extreme post-emergence weight loss in a structurally large, captive-release adult male resulted in the transmitter becoming lodged in its pelvic canal, resulting in a fatal impaction of the gastrointestinal tract. In the second case, involving a captive marmot scheduled for release, the outer surface of the transmitter caused an extreme, chronic inflammatory reaction with significant visceral adhesions which

could not be surgically resolved. These problems were subsequently mitigated by having the manufacturer increase the diameter of the transmitters and by only using transmitters encapsulated in a hard, biologically inert resin.



Lateral (left) and ventrodorsal radiographs of free-floating abdominal radio-transmitter in female Vancouver Island Marmot (*Marmota vancouverensis*). The cylindrical unit was 25 mm x 90 mm and weighed 50 grams.

Appendix G. Summary of morbidity & mortality in captive & free-ranging Vancouver Island Marmots (*Marmota vancouverensis*), 1992 to 2016

System	Diagnosis	Identified or diagnosed by clinical signs or physical examination	Diagnosis requires necropsy or ancillary lab test(s)	Identified in free-ranging marmots	Identified in captive marmots
abdominal	abdominal abscess	N	Y	N	Y
abdominal	peritonitis	N	Y	N	Y
abdominal	colonic impaction due to transmitter	N	Y	Y	N
abdominal	transmitter reaction	Y	confirmed	N	Y
cardiovascular	atrial septal defect	Y	confirmed	N	Y
cardiovascular	persistent tachycardia	Y	confirmed	N	Y
cardiovascular	patent foramen ovale	Y	confirmed	N	Y
cardiovascular	cardiomyopathy	Y	confirmed	N	Y
cardiovascular	endocardiosis	Y	confirmed	N	Y
cardiovascular	cerebral hemorrhage	N	Y	N	Y
cardiovascular	atherlosclerosis	N	Y	N	Y
dental	dental malocclusions	Y	N	N	Y
dental	unilateral / bilateral fractures of upper incisors	Y	N	Y	Y
gastrointestinal	segmented enteritis,	N	Y	N	Y
gastrointestinal	roundworms	Y/N	Y	Y	Y
gastrointestinal	tapeworms	Y/N	Y	Y	Y
gastrointestinal	mesenteric torsion	N	Y	N	Y
gastrointestinal	duodenal ulcer and peritonitis	N	Y	N	Y
gastrointestinal	pancreatitis with peritonitis	N	Y	N	Y
gastrointestinal	perforated ceacal ulcer with peritonitis	N	Y	N	Y
hepatic	cholangiohepatitis	N	Y	N	Y
hepatic	hepatitis	N	Y	N	Y
integumentary	fleas	Y	N	Y	Y
integumentary	ticks	Y	N	Y	N
integumentary	facial abscess	Y	N	Y	Y
integumentary	mites	Y	confirmed	Y	Y
integumentary	cutaneous bots	Y	N	N	Y
multisystemic	stunting, post weaning un-thriftiness	Y	N	Y	Y
multisystemic	septicemia	N	Y	N	Y
multisystemic	capture-related hyperthermia,	Y	N	Y	N
multisystemic	facial neoplasia	Y	confirmed	N	Y
multisystemic	perivasculitis	N	Y	N	Y
multisystemic	malignant histiocytosis	N	Y	N	Y
multisystemic	conspecific trauma	Y	confirmed	Y	Y

Appendix G. (cont.) Summary of morbidity & mortality in captive & free-ranging Vancouver Island Marmots (*Marmota vancouverensis*), 1992 to 2016.

System	Diagnosis	Identified or diagnosed by clinical signs or physical examination	Diagnosis requires necropsy or ancillary lab test(s)	Identified in free-ranging marmots	Identified in captive marmots
multisystemic	neoplasia	Y / N	confirmed	N	Y
musculoskeletal	spondylosis	N	Y	N	Y
musculoskeletal	scoliosis	Y	confirmed	N	Y
musculoskeletal	hind foot aplasia	Y	N	N	Y
musculoskeletal	toe injuries from conspecific trauma	Y	N	Y	Y
musculoskeletal	chronic fracture of right olecranon	Y	confirmed	N	Y
musculoskeletal	fracture of carpal bones	Y	confirmed	Y	N
musculoskeletal	post hibernation emaciation	Y	confirmed	Y	Y
musculoskeletal	abdominal hernia	Y	confirmed	N	Y
musculoskeletal	resorption of head and neck of femur	N	Y	N	Y
musculoskeletal	degeneration and herniation of intervertebral disc(s)	Y	confirmed	N	Y
neurologic	head trauma	Y	confirmed	N	Y
neurological	menigoencephalomyelitis	Y	confirmed	N	Y
ocular	unilateral anopthalmis	Y	N	N	Y
ocular	bilateral, congenital cataracts	Y	confirmed	N	Y
ocular	corneal trauma with opacity	Y	confirmed	Y	Y
ocular	lenticular opacity	Y	N	N	Y
ocular	unilateral, corneal opacity	Y	N	N	Y
ocular	narrowed palpebral fissure	Y	N	N	Y
respiratory	bronchopneumonia	Y/N	confirmed	N	Y
respiratory	pulmonary adenomatosis	N	Y	N	Y
respiratory	laryngeal occlusion	N	Y	N	Y
urogenital	vaginitis, urethritis and cystitis	N	Y	N	Y
urogenital	paraphimosis	Y	N	N	Y

Appendix H. Summary of annual mortality categories in free-ranging Vancouver Island Marmots (*Marmota vancouverensis*) 1992 to 2016.

Year	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Total
Wild	1	0	2	1	0	0	3	0	0	2	6	1	4	1	3	1	0	2	10	7	8	18	30	26	13	139
Predation	0	0	2	1	0	0	3	0	0	2	5	1	3	0	2	0	0	2	6	5	1	13	16	4	7	73
Hibernation	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	2	1	1	4	2	8
Other	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	3
Unknown	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	4	2	5	4	13	17	4	55
Translocated	0	0	0	0	5	0	0	0	0	0	1	2	0	0	0	0	0	1	1	0	1	2	4	10	20	44
Predation	0	0	0	0	1	0	0	0	0	0	1	2	0	0	0	0	0	1	0	0	0	0	0	0	0	5
Hibernation	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	5
Other	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unknown	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2	3	10	20	34
Captive-release	0	0	0	0	0	0	0	0	0	0	0	3	1	9	10	14	26	28	62	47	47	28	16	26	14	330
Predation	0	0	0	0	0	0	0	0	0	0	0	3	1	4	7	9	12	11	13	6	6	4	4	6	2	87
Hibernation	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	2	7	7	27	7	11	3	5	1	3	80
Other	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	1	1	0	0	5
Unknown	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	7	9	22	34	30	20	6	19	9	158
Pre-conditioned	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3	10	4	20
Predation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	2
Hibernation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	0	5
Other	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unknown	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	8	4	13
Total mortalities	1	0	2	1	5	0	3	0	0	2	7	6	5	10	13	15	26	31	73	54	57	49	53	72	48	533

Appendix I. A comparison of common body condition indices in wild & captive male Vancouver Island Marmots (*Marmota vancouverensis*). *Unpublished data.*

	Body mass divided by body length (BM/BL)	**Quetlet's Index** Body mass divided by body length squared (B/L^2)	**Fulton's Index** Body mass divided by body length cubed (B/L^3)	Log-transformed body mass divided by log-transformed body length (log BM/log BL)	**Scaled mass index (gm)** (a)	**Scaled neck index (cm)** (b)
Captive males	89.75397	1.740848	0.03404794	2.137128	4110.01	26.63
Wild males	74.59246	1.520057	0.03149986	2.101056	3816.77	24.05
t	10.384	9.7093	5.761	9.4526	5.42	14.64
df	366.411	382.125	419.318	348.421	420.23	413.15
PV	< 2.2e-16	< 2.2e-16	1.62E-08	< 2.2e-16	9.90E-08	< 2.2e-16

(a) Scaled mass index (sci) comparing body mass of captive and wild marmots according to a standardized body length of 49.45 cm (mean body length for all samples).

(b) Scaled neck index - comparing neck circumference of captive and wild marmots according to a standardized body length of 49.45 cm (mean body length for all samples).