THE EFFECTS OF A COMPLEX TROPHIC STRUCTURE OF MAMMALIAN HOST SPECIES ON THE ECOLOGY OF EMERGING INFECTIOUS DISEASES

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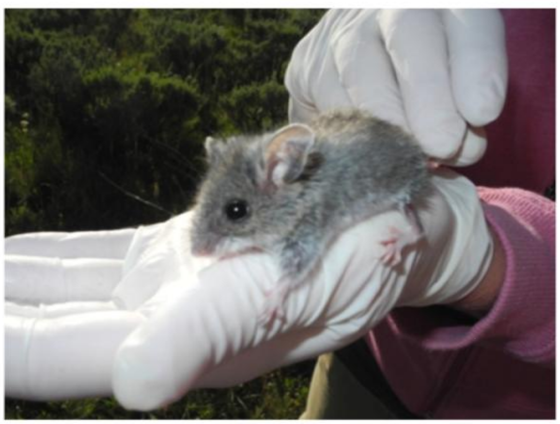

✦ ABSTRACT

Wildlife-parasite interactions among both ectoparasites and haemoparasites and their hosts are not well known among North American mammals, particularly in the case of relatively intact and complex communities of mammals that include top-level predators, large herbivores and a wide variety of rodent species. Understanding the distribution of haemoparasites among potential mammalian hosts can indicate links between hosts, biological vectors, disease agents, and human disease risk. This study examines the role and effects of a complex community of mammalian host species in maintaining the overall health of the ecosystem. Thereby, it explores the indirect and direct effects of wildlife in preventing the emergence of human infectious diseases depending upon land-use change/vegetation cover and host species richness.

Rodents were captured and screened for blood parasites and ectoparasites in spring and summer 2011 within Grand Teton National Park (Figure 1). Sites were chosen by land-use /vegetation cover. Small blood samples from trapped individuals were collected and kept in lysis buffer/FTA cards. All the animals were released unharmed after blood sampling and ectoparasite collection. Collaborative efforts lead to collection of blood/tick samples from large predators, mesocarnivores and ungulates. Parasite DNA isolated from mammalian blood samples is being analyzed using the polymerase chain reaction and reverse line blot. DNA sequencing will be carried out to identify *Plasmodium*, *Rickettsia Babesia*, *Borrelia*, *Ehrlichia*, *Hepatozoon*, *Anaplasma*, and *Theileria* haemoparasites in the blood and in tick/flea samples.

✦ INTRODUCTION

This survey of haemoparasites will provide a first look at the haemoparasite status of mammal populations in western US. This might elucidate possible predator-prey parasitic interactions through a detailed characterization of the trophic network, and form the basis for investigating the community ecology of zoonotic mammalian infectious diseases. Empirically examining the potential protective role of the trophic complexity of mammalian communities is a novel field, which just has been proposed theoretically. However its real effects on the ecosystem and in human health have yet to be documented.

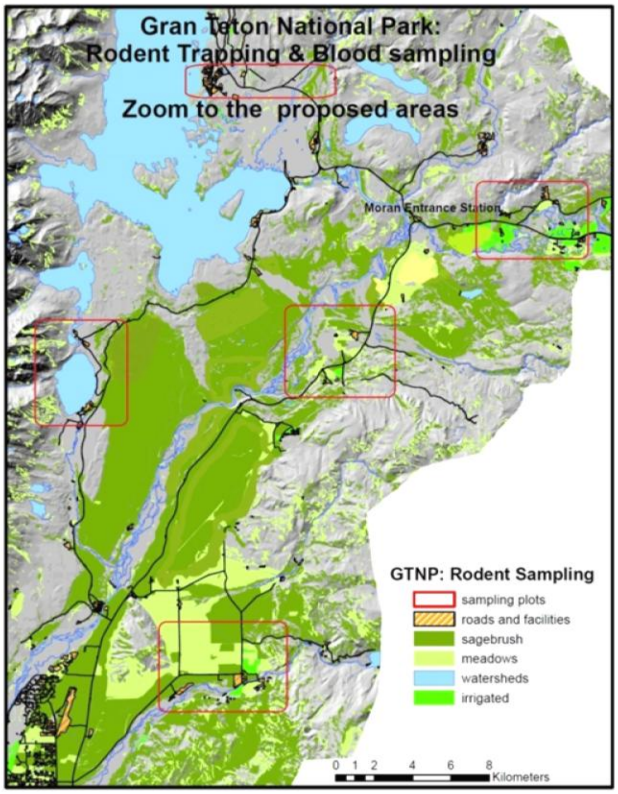

Figure 1.Map of sites used in this study.

This project combines the fields of disease ecology, epidemiology, and conservation. Exploring how preserving high species diversity in mammal communities, along with a strong complexity in all its trophic levels, can have an intrinsic measurable value to human health, it will be used to address conservation policies. Emerging infectious disease (EID) risk models will directly benefit the human populations, bringing together new land-use management strategies that will benefit humans as well as protect wildlife.

✦ PROJECT DESCRIPTION

Grand Teton National Park (GTNP) and Yellowstone National Park (YNP) are the largest protected areas within the Greater Yellowstone Ecosystem (GYE) and, at the same time, they are the largest semi-intact northern temperate zone ecosystem on Earth. In fact, this area has substantially more large carnivores and large ungulates that, although endemic to the area, were reintroduced to YNP after the gradual loss of habitat and deliberate extermination programs that led to their demise throughout most of the United States in the early 1900s. By 1926, when the National Park Service ended its predator control efforts, there were no gray wolf packs left in YNP, no cougars, no wolverines and almost no grizzly bears, etc. In the decades that followed, the importance of gray wolves, for example, as part of a functioning ecosystem came to be better understood (Ripple and Beschta 2004, Schmitz et al 2008.), and the gray wolf was eventually listed as an endangered species throughout its traditional range except Alaska. Research after its devastation, and on the recovery and restitution of the Great Yellowstone area, has shown the value of diversity for the performance and complex trophic structure of this ecosystem (Ripple and Beschta 2004, Schmitz et al 2008.).

However, other aspects of the beneficial value of a complex community of mammals in terms of a healthy ecosystem and therefore lower emerging infectious disease (EID) risk has not been deeply explored yet. Mechanisms of disease transmission are determined by the capabilities of the pathogens, by the immune system of the host, role of vectors, interactions between haemoparasites as well as interactions among the species making up the host network. The diversity and distribution of haemoparasites in any large mammal fauna, including the flagship GYE, are virtually unknown, outside of a few diseases having economic importance for livestock and public health (e.g. brucellosis, tuberculosis, chronic wasting disease). However, many diseases are endemic to North America but are not well understood in terms of transmission factors, prevalence, and contagiousness. Many of these pathogens nowadays are considered to be potential EIDs that could spread to adjacent areas with climate change, land-use shift and the expansion of distributions of the natural vectors of such haemoparasites. This study will be focused in rodent-borne diseases/tick-borne diseases and mosquito-borne malaria that have a high value for human public health as zoonotic diseases as well as for the unknown natural history of the mammalian community network of the GYE. Pathogens from the following genera will be targeted: *Plasmodium*, *Rickettsia*, *Babesia*, *Borrelia*, *Ehrlichia*, *Hepatozoon*, *Anaplasma*, *Theileria*, *Francisella* and *Trypanosoma*.

To accomplish this project, small rodent species were captured and screened for blood parasites and ectoparasites in spring and summer of 2011 within the GYE. Sites were chosen by land-use/vegetation cover. Small blood samples from trapped individuals were collected and kept in lysis buffer/FTA cards. All the animals were released unharmed after blood sampling and ectoparasite collection. Collaborative efforts lead to collection of blood/tick samples from large predators, mesocarnivores and ungulates. Parasite DNA isolated from mammalian blood samples is being analyzed using the polymerase chain reaction (PCR) and reverse line blot (RLB). DNA sequencing, as well as visually examination of blood smears, will be carried out to identify the haemoparasites, mentioned above, in the blood and in ticks/fleas samples.

SCIENTIFIC SIGNIFICANCE

No comprehensive study has been undertaken of the pathogen community in the GYE using PCR, RLB and DNA sequencing to screen for mammalian haemoparasites. To date, studies have been limited to eimerias, coccidias, and general gastrointestinal parasites of small rodents, ungulates and some carnivores. Some viruses, such as distemper, rabies, and canine parvovirus have been detected in wolves (Smith and Almberg 2007) and brucellosis has been studied in ungulates (Treanor et al. 2007). Mammalian haemoparasite status, zoonosis transmission, parasite sharing among hosts, and biological vectors are poorly known among mammals of western North America in general. The influence of predators on haemoparasite diversity and infection levels in large herbivores and small mammals also is poorly understood (Cross et al. 2009). At this point, a crucial step towards understanding the influence of parasites on mammal populations and EID risk is simply to describe the distribution of parasites within the entire mammal community and characterize the interconnections between pathogens, ectoparasite vectors, hosts species and land-use.

Finally, the field work described in this brief repot will provide the basis for the second, third and fourth chapters of the graduate student’s dissertation, which will be an integrative study of mammal species that are connected through trophic and parasitic networks. The GYE, which is the last example of a well-conserved temperate ecosystem in the northern hemisphere, will be a first step towards characterizing the haemoparasite community in a mammal community with a full complement of top predators. Hopefully, this work will help to clarify the favorable influence of carnivores and large herbivores and other less known interactions on parasite infections dynamics. Moreover, one of these benefits might be decreasing the risk of human EIDs transmitted by rodents which would be expected to reduce the negative perception of the local community toward these animals.

✦ MATERIALS AND METHODS

Study area:

The field study was located in the GYE, in Grand Teton National Park (43°44`0``N 110°48`12``W). Field sites were specifically located at: AMK ranch, Two Ocean Lake, Triangle X Ranch, and pastures close by the Moran entrance.

Mammal trapping:

To collect blood samples from small mammals, we used 110 Sherman live traps placed at ~10-meter intervals in 10 transect lines of 100m length.

Traps were located with respect to previous evidence of small rodent trails, feces, or rodent activity. Seven parcels were chosen for this purpose, divided by anthropogenic disturbance and land-use/vegetation cover: 1 grazing plot, 1 horse ranch, and 1 settlement as part of the highly disturbed areas lacking large mammals and 1 pristine wet meadows, 1 pristine meadow, 1 pristine sagebrush plot, and 1 pine/willow forest as part of the undisturbed areas where a complex trophic structure in the mammalian community is expected to occur. This effort was achieved by one doctoral graduate student as project leader, one undergraduate field assistant, and one additional field assistant with vast veterinary technician training. Oats, sunflower seeds and dry raisins were used as bait and occasionally mixed with peanut butter. Traps were baited at sunset and checked the following morning at sunrise. Traps were maintained closed during the day and at night they were provided cotton balls to avoid hypothermia. Trapping was done during 5 consecutive nights on average. All traps were numbered; all sites were marked using colorful flagging and also were GPS georeferenced. All individuals captured were identified with numbered ear tags, weighed, sexed and released unharmed (see appendix for the details on rodents trapped).

Blood sample collection:

When an animal was captured, the Sherman live trap was placed for 3 minutes inside of a chamber composed of a clear plastic box (58x43x35 cm) with a snap-on lid and a petri dish containing 5 cotton balls soaked with 15ml of Isoflurane. Otherwise, Isoflurane was administrated using a nose cone made of a 50 ml falcon tube (30 x 115 mm polypropylene) with 3 cotton balls soaked with 5 ml of Isoflurane, which induces anesthesia in about 0.5 min.

Mammals easily recovered from the anesthesia in 3-5 min (Parker 2008). From each anesthetized small (<100 g) mammal, we collected blood in a 40-µl capillary tube from the sub-mandibular vein or saphenous vein, and as an alternative a retro-orbital plexus puncture (Table 1). Part of the sample was preserved in Longmire's lysis buffer/FTA cards and part was used to make a smear. This blood collection protocol was chosen since this method is the least invasive among others described for small size mammals (Van Herck 2001).

Table 1: Rodent blood samples collected at GTNP.

Species	***Tamias amoenus***	***Tamias minimus***	***Microtus montanus***	***Peromyscus maniculatus***	***Spermophilus armatus***	***Zapus princeps***	***Sorex vagrans***
Number collected	3	18	20	20	12	10	1

Blood sample aliquots:

Biological samples from large mammals were obtained through collaboration with established research groups that already have collected blood samples from wolves, elks, bison, snowshoe hares, jackrabbits, wolverines, grizzly bears (Appendix 1).

From those blood samples, were taken aliquots of up to 3 drops of either: whole fresh/frozen blood, or the same volume of buffy coat and red blood coagulate; or whole blood with EDTA, into of each cryotube filled with Longmire`s lysis buffer. Then, the mixture was shaken well by inverting, thus the mix was homogenized. The new sample was stored in a -20Cº or -80Cº freezer at UMSL. Information regarding mammal species, date of collection, sex, age, weight, GPS coordinates and blood source type (whole, frozen, EDTA, etc) was recorded.

Collaborative agreements were established during 2011 with the National Park Service–YNP, USGS-NOROCK, US Forest Service, Montana Fish, Wildlife & Parks, and with the Montana State University. The agreements consist solely in sharing of a small subsample of mammalian blood already collected by each research group for their own particular projects. These collaborations will continue as long as the researchers capture mammals in the future, or if they are able to share blood samples collected in the past.

Blood smears:

Blood smears were made for the majority of the captured small mammals. Each blood smear was made using whole fresh blood starting from ½ blood drop, making a thin layer of red blood cells. After this critical procedure, the blood smears were dried at room temperature and, immediately after that, the blood smears were fixed in methanol 100% for 3 minutes, submerging the slides inside a coplin jar filled with methanol. Then, the samples were dried again at room temperature, and finally they were stored. Blood smears were stained at UW-NPS Research Center's lab with Giemsa stain to identify and quantify parasitemias of haemoparasites. Haemoparasite identification will be done using an optical microscope, immersion oil, and 1000X magnification. Measurements of the parasites will be made using an ocular micrometer (Anderson, 1990, Davidson and Calpin 1976).

Ectoparasites collection:

Each trapped mammal was checked through direct visual inspection and ectoparasites were collected with anatomical fine tweezers. Ectoparasites (ticks and flies) were kept in Eppendorf vials for 24 hrs in order to let the blood meals with the host DNA be thoroughly digested (Schall and Smith, 2006). The parasites were then preserved in 1 ml 70% ethanol (Beveridge et al. 1985), frozen and subsequently lyophilized. Hence, it was possible to preserve the ectoparasite DNA until it can be processed in Dr. Ricklefs' lab.

Molecular analysis:

To identify haemoparasitic organisms, DNA extracted from the collected samples (blood and ectoparasites) were subjected first to RLB assay which was carried out to assess in a cost-effective way the identification of bacterial and piroplasma haemoparasites from the collected samples. DNA of the pathogens was amplified by multiplex PCR using biotin-labeled general primers for eubacteria and piroplasma. 5'-end amino-link species-specific probes were blotted in lines using Miniblotter 45 (Immunetic, Cambridge, MA). Results were detected by using a chemiluminiscent substrate in x-ray film (Allan et al. 2010, Pichon et al. 2003). This assay has the advantage of screening 43 samples against 44 different probes, therefore it is an excellent screening tool for the first stage of the molecular analysis. In addition, PCR will be carried out using taxon-specific primers. In the case of positive infections, 18S rDNA, 16S rDNA, mitochondrial cytochrome b, or other specific genetic markers will be sequenced for parasite identification. According to objectives established in this project, we will identify *Plasmodium*, *Rickettsia*, *Babesia*, *Borrelia*, *Ehrlichia*, *Hepatozoon*, *Anaplasma*, *Theileria*, *Francisella*, and *Trypanosoma* haemoparasites. Thus, we will use specific primers to these haemoparasites. DNA sequencing will be done by the Genomics Core Facility- PennState.

✦ NEW DATA

The field season proposed for 2012 will provide data from 24 new sampling sites and as a result about 1,300 new rodent samples from the GYE area. These sites will be distributed in a block design, having 4 blocks containing the treatment plots. Accordingly, the same lab analysis will be performed on the new blood and ectoparasite samples. Blood samples obtained in 2012-2013 from collaboration will be analyzed by the same methodology described above.

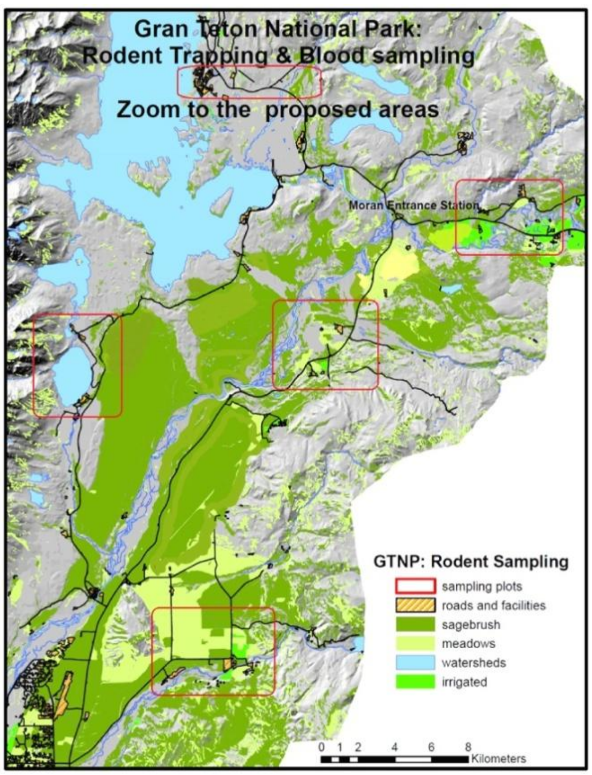

Figure 2. Map of proposed 2012 field season.

✦ PENDING ANALYSIS

Lab analysis: RLB, PCR and DNA sequencing.

1) Phylogenetic analysis of the pathogen's DNA sequences obtained. Published pathogen sequences in GenBank will be used as alignment template; MEGA 5 will be used for this purpose. Phylogenetic tree building will be made for each pathogen species found using Geneious. Phylogenetic tree visualization will be corrected with FigTree.

2) Statistical analysis: Logistic regression will be use to find a set of independent variables such as sex and age that might be responsible for haemoparasites species prevalence differences within host species. Student's t-test will be performed to determine if there are significant differences between the mammalian host parasitemias of highly disturbed areas compared with pristine areas (land-use/vegetation cover).

3) Spatial relationships using GIS will analyze in a visual fashion the risk of EIDs depending upon rodent species abundance and richness, haemoparasites prevalence, plot location in relation with human settlements, individual specific location and geographic characterization.

4) Trophic network modeling will incorporate mammalian host-haemoparasite-vector interactions and disease transmission dynamics in a metanetwork, which will shed light on the relationships between diversity of pathogens (e.g. pathogens aggregation) and the complexity of the mammalian community.

✦ INTERPRETATION

1) High richness of haemoparasites, but in low prevalence in the mammalian community, would be interpreted as a proxy for ecosystem healthiness and for low EID risk.

2) Low richness of haemoparasites, but high prevalence of the most pathogenic species, in the host species would be interpreted as low ecosystem healthiness and high EID risk.

3) EIDs would be also studied by location, proximity to human settlement, level of disturbance (land-use) and abundance of rodent species. Therefore, it would be possible to infer the dynamic patterns of disease transmission and risk.

4) Identification of the pathogens present in the rodent mammalian community would provide the possible reservoir of each studied disease.

The data generated with this project will enhance the knowledge of the ecology of infectious diseases of the North American mammalian fauna, uncertain until now. Conclusive data regarding the distribution and dynamics of these pathogens associated with the trophic structure of certain areas can lead to a better understanding of disease risk and spread. This work also will provide a direct insight in the role of carnivores and large herbivores as modulators of disease transmission and hopefully the interpretation of the results will be useful to conservation policy makers and for promoting the study of the ecosystem health/functioning as a cohesive unit.

✦ DETAIL OF THE BROADER IMPACTS OF THE PROPOSED ACTIVITIES

This project addresses a major gap in current knowledge and understanding of EID dynamic patterns and risk factors through an empirical study of the semi-pristine area of the GYE. This comprehensive in-situ study integrates a diverse array of disciplines such as disease ecology, epidemiology, and conservation, which will lead to increase understanding of the role of a complex trophic structure in disease dynamics.

Exploring how preserving high species diversity of a mammalian community, along with a strong complexity in all its trophic levels, can have an intrinsic measurable value to human health, which will be used to address conservation policies and management. EID risk models will directly benefit the human populations, bringing together new land-use management that will benefit humans as well as protect wildlife.

This project currently promotes, and it will continue to do so throughout its extent, collaboration among several governmental agencies such as YNP-National Park Service, Northern Rocky Mountain Science Center-U.S. Geological Survey, U.S. Forest Service, Montana Fish, Wildlife & Parks and Ecology Department of the Montana State University. In addition, the interdisciplinary nature of this research provides many opportunities for undergraduate students to be involved in hands-on field and lab research as part of an undergraduate mentoring program. In addition, this research project supports the doctoral dissertation research of a graduate student. Outreach will be focused in the local communities, general public and tourists of GYE, as well as through the broad dissemination through scientific publications and presentations in scientific conferences.

✦ ACKNOWLEDGEMENTS

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APPENDIX

Appendix 1

COLLABORATIONS

Principal Investigator	Institution	Type of Involvement	Type of sample	Material provided in 2011
Dr. Mark Haroldson	Northern Rocky Mountain Science Center-USGS	Access to biological material	Grizzly bear blood aliquot	48 samples
Dr. Paul Cross	Northern Rocky Mountain Science Center-USGS	Access to biological material	Elk blood aliquot	30 samples
Dr. Dan Tyers	U.S. Forest Service	Access to biological material	Wolverine & snowshoe hare blood aliquot	2 samples 20 samples
Dr. Neil Anderson	Montana Fish, Wildlife & Parks	Access to biological material	Elk blood aliquot	41 samples
Dr. Douglas Smith	YNP- National Park Service	Access to biological material	Wolf blood aliquot	222 samples
Dr. Rick Wallen	YNP- National Park Service	Access to biological material	Bison blood aliquot	28 samples
Dr. John Treanor	YNP- National Park Service	Access to biological material	Elk blood aliquot	30 samples
Dr. Robert Garrott	Ecology Department Montana State University	Access to biological material	Jackrabbit & elk blood aliquot	4 samples 2 samples

Appendix 2

7/6/2011	AMK ranch	124	Zapus princeps	Western Jumping Mouse	male	adult	21	
7/15/2011	Sagebrush (Two Ocean Lake)	125	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	adult	21.6	
7/15/2011	Sagebrush (Two Ocean Lake)	126	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	adult	25.4	
7/15/2011	Sagebrush (Two Ocean Lake)	128	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	adult	27.5	
7/15/2011	Sagebrush (Two Ocean Lake)	129	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	juvenile	8	
7/15/2011	Sagebrush (Two Ocean Lake)	130	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	adult	20.7	
7/15/2011	Meadow (Two Ocean Lake)		Sorex vagrans	Vagrant Shrew	female	juvenile	4.8	nymphs
7/15/2011	Meadow (Two Ocean Lake)	131	Zapus princeps	Western Jumping Mouse	female	adult	30	pregnant
7/15/2011	Meadow (Two Ocean Lake)	132	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	adult	20.6	
7/16/2011	Sagebrush (Two Ocean Lake)	126	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	adult		**deceased upon discovery**
7/16/2011	Sagebrush (Two Ocean Lake)	128	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	adult		recapture
7/16/2011	Sagebrush (Two Ocean Lake)	125	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	adult		recapture
7/16/2011	Sagebrush (Two Ocean Lake)	133	Microtus montanus	Montane Vole	male	juvenile	21	
7/16/2011	Sagebrush (Two Ocean Lake)	130	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	adult		recapture
7/16/2011	Sagebrush (Two Ocean Lake)	134	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	juvenile	11.2	
7/16/2011	Sagebrush (Two Ocean Lake)	135	Microtus montanus	Montane Vole	male	juvenile	19.2	
7/16/2011	Meadow (Two Ocean Lake)	136	Zapus princeps	Western Jumping Mouse	female	adult	27.9	
7/16/2011	Meadow (Two Ocean Lake)	137	Microtus montanus	Montane Vole	male	adult	25.1	
7/16/2011	Meadow (Two Ocean Lake)	138	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	juvenile	10.4	
7/16/2011	Meadow (Two Ocean Lake)	139	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	juvenile	10	
7/16/2011	Meadow (Two Ocean Lake)	140	Microtus montanus	Montane Vole	female	juvenile	15.5	
7/17/2011	Sagebrush (Two Ocean Lake)	141	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	juvenile	12.2	
7/17/2011	Sagebrush (Two Ocean Lake)	142	Microtus montanus	Montane Vole	male	adult	22.8	
7/17/2011	Sagebrush (Two Ocean Lake)	128	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	adult		recapture
7/17/2011	Sagebrush (Two Ocean Lake)	143	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	juvenile	11	
7/17/2011	Sagebrush (Two Ocean Lake)	125	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	adult		recapture
7/17/2011	Meadow (Two Ocean Lake)		Zapus princeps	Western Jumping Mouse				scaped
7/17/2011	Meadow (Two Ocean Lake)	131	Zapus princeps	Western Jumping Mouse	female	adult		recapture
7/17/2011	Meadow (Two Ocean Lake)	144	Microtus montanus	Montane Vole	female	adult	43	pregnant
7/17/2011	Meadow (Two Ocean Lake)	132	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	adult		recapture
7/17/2011	Meadow (Two Ocean Lake)	145	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	juvenile	12.7	
7/17/2011	Meadow (Two Ocean Lake)		Sorex vagrans	Vagrant Shrew	female	adult	5.9	**deceased upon discovery**
7/17/2011	Meadow (Two Ocean Lake)	146	Microtus montanus	Montane Vole	male	juvenile	18.3	

Appendix 2 (Continued)

7/22/2011	Triangle X Ranch	180	Tamias minimus	Least Chipmunk	female	adult	49	lactating + tail
7/22/2011	Triangle X Ranch	177	Tamias minimus	Least Chipmunk	male	adult	40.1	
7/22/2011	Triangle X Ranch	178	Tamias minimus	Least Chipmunk	female	adult	44	lactating
7/22/2011	Triangle X Ranch	181	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	juvenile	14.4	
7/22/2011	Triangle X Ranch	185	Tamias minimus	Least Chipmunk	female	adult	39.9	lactating
7/22/2011	Triangle X Ranch	183	Tamias minimus	Least Chipmunk	male	adult	36.1	
7/22/2011	Triangle X Ranch		Tamias minimus	Least Chipmunk				scaped
7/22/2011	Triangle X Ranch	184	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	juvenile	15.4	
7/23/2011	Triangle X Ranch	171	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	juvenile		recapture
7/23/2011	Triangle X Ranch	184	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	juvenile		recapture
7/23/2011	Triangle X Ranch	170	Tamias minimus	Least Chipmunk	female	adult		recapture
7/23/2011	Triangle X Ranch	182	Tamias minimus	Least Chipmunk	male	adult		recapture
7/23/2011	Triangle X Ranch	172	Tamias minimus	Least Chipmunk	male	adult		recapture
7/23/2011	Triangle X Ranch	177	Tamias minimus	Least Chipmunk	male	adult		recapture
7/23/2011	Triangle X Ranch	173	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	adult		recapture
7/23/2011	Triangle X Ranch	180	Tamias minimus	Least Chipmunk	female	adult		recapture
7/23/2011	Triangle X Ranch	198	Spermophilus armatus	Uinta Ground Squirrel	male	adult	385	same trap with chipmunk ID 197
7/23/2011	Triangle X Ranch	197	Tamias minimus	Least Chipmunk	male	adult	40.1	no blood
7/23/2011	Triangle X Ranch	189	Microtus montanus	Montane Vole	female	adult	43.5	
7/23/2011	Triangle X Ranch	200	Tamias minimus	Least Chipmunk	male	juvenile	31.8	
7/23/2011	Triangle X Ranch	195	Tamias minimus	Least Chipmunk	female	adult	44	lactating
7/23/2011	Triangle X Ranch		Tamias minimus	Least Chipmunk				scaped
7/23/2011	Triangle X Ranch	190	Microtus montanus	Montane Vole	male	adult	26.2	
7/23/2011	Triangle X Ranch	193	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	adult	20.5	**deceased**
7/23/2011	Triangle X Ranch	191	Microtus montanus	Montane Vole	male	juvenile	17.8	
7/23/2011	Triangle X Ranch	199	Tamias minimus	Least Chipmunk	male	adult	56.7	
7/23/2011	Triangle X Ranch		Tamias minimus	Least Chipmunk				scaped
7/23/2011	Triangle X Ranch	196	Tamias minimus	Least Chipmunk	male	juvenile	30.7	
7/23/2011	Triangle X Ranch	192	Tamias minimus	Least Chipmunk	female	adult	45.4	lactating
TOTAL	**88 bleeded individuals.**							
NOTE: DECEASED ANIMALS WERE SENT TO Dr. Bob Oakleaf C/O Martin ON 08/02/2011 (Wyoming Game & Fish Department)								

Appendix 2 (Continued)

7/17/2011	Meadow (Two Ocean Lake)	140	Microtus montanus	Montane Vole	female	juvenile		recapture
7/18/2011	Grazing plot (Elk ranch refuge)	147	Microtus montanus	Montane Vole	female	adult	22.5	
7/19/2011	Grazing plot (Elk ranch refuge)	148	Microtus montanus	Montane Vole	male	adult	27.8	
7/19/2011	Grazing plot (Elk ranch refuge)	149	Spermophilus armatus	Uinta Ground Squirrel	male	juvenile	180	
7/19/2011	Grazing plot (Elk ranch refuge)	150	Spermophilus armatus	Uinta Ground Squirrel	female	juvenile	190	
7/19/2011	Grazing plot (Elk ranch refuge)	151	Spermophilus armatus	Uinta Ground Squirrel	female	juvenile	165	
7/19/2011	Grazing plot (Elk ranch refuge)	153	Spermophilus armatus	Uinta Ground Squirrel	male	juvenile	205	
7/19/2011	Grazing plot (Elk ranch refuge)	154	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	juvenile	14.5	
7/20/2011	Grazing plot (Elk ranch refuge)	155	Zapus princeps	Western Jumping Mouse	male	adult	23	
7/20/2011	Grazing plot (Elk ranch refuge)	150	Spermophilus armatus	Uinta Ground Squirrel	female	juvenile		recapture
7/20/2011	Grazing plot (Elk ranch refuge)	149	Spermophilus armatus	Uinta Ground Squirrel	male	juvenile		recapture
7/20/2011	Grazing plot (Elk ranch refuge)	154	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	juvenile		recapture
7/20/2011	Grazing plot (Elk ranch refuge)	156	Spermophilus armatus	Uinta Ground Squirrel	male	juvenile	185	
7/20/2011	Grazing plot (Elk ranch refuge)	157	Spermophilus armatus	Uinta Ground Squirrel	male	juvenile	205	
7/20/2011	Grazing plot (Elk ranch refuge)	158	Spermophilus armatus	Uinta Ground Squirrel	male	juvenile	150	
7/20/2011	Grazing plot (Elk ranch refuge)	159	Spermophilus armatus	Uinta Ground Squirrel	male	juvenile	215	
7/20/2011	Grazing plot (Elk ranch refuge)	160	Spermophilus armatus	Uinta Ground Squirrel	female	adult	300	lactating
7/20/2011	Grazing plot (Elk ranch refuge)	188	Tamias amoenus	Yellow Pine Chipmunk	male	juvenile	38.8	no blood sample
7/20/2011	Grazing plot (Elk ranch refuge)	161	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	adult	29.1	**deceased**
7/20/2011	Grazing plot (Elk ranch refuge)	162	Zapus princeps	Western Jumping Mouse	male	adult	20.9	
7/20/2011	Grazing plot (Elk ranch refuge)	164	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	juvenile	18.9	
7/20/2011	Grazing plot (Elk ranch refuge)	166	Zapus princeps	Western Jumping Mouse	female	adult	22.3	lactating
7/20/2011	Grazing plot (Elk ranch refuge)	167	Zapus princeps	Western Jumping Mouse	male	adult	26	
7/20/2011	Grazing plot (Elk ranch refuge)	165	Microtus montanus	Montane Vole	male	adult	39.6	
7/20/2011	Grazing plot (Elk ranch refuge)	168	Microtus montanus	Montane Vole	male	adult	28.6	
7/22/2011	Triangle X Ranch	169	Tamias minimus	Least Chipmunk	male	adult	35.4	
7/22/2011	Triangle X Ranch	170	Tamias minimus	Least Chipmunk	female	adult	39.8	lactating
7/22/2011	Triangle X Ranch	174	Tamias minimus	Least Chipmunk	female	adult	40.4	lactating
7/22/2011	Triangle X Ranch	173	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	adult	23.5	
7/22/2011	Triangle X Ranch	175	Tamias minimus	Least Chipmunk	male	juvenile	30	
7/22/2011	Triangle X Ranch		Tamias minimus	Least Chipmunk				scaped
7/22/2011	Triangle X Ranch	171	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	juvenile	14.3	
7/22/2011	Triangle X Ranch	172	Tamias minimus	Least Chipmunk	male	adult	37.5	
7/22/2011	Triangle X Ranch	182	Tamias minimus	Least Chipmunk	male	adult	39.6	scaped + tail

Appendix 2 (Continued)

7/6/2011	AMK ranch	124	Zapus princeps	Western Jumping Mouse	male	adult	21	
7/15/2011	Sagebrush (Two Ocean Lake)	125	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	adult	21.6	
7/15/2011	Sagebrush (Two Ocean Lake)	126	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	adult	25.4	
7/15/2011	Sagebrush (Two Ocean Lake)	128	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	adult	27.5	
7/15/2011	Sagebrush (Two Ocean Lake)	129	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	juvenile	8	
7/15/2011	Sagebrush (Two Ocean Lake)	130	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	adult	20.7	
7/15/2011	Meadow (Two Ocean Lake)		Sorex vagrans	Vagrant Shrew	female	juvenile	4.8	nymphs
7/15/2011	Meadow (Two Ocean Lake)	131	Zapus princeps	Western Jumping Mouse	female	adult	30	pregnant
7/15/2011	Meadow (Two Ocean Lake)	132	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	adult	20.6	
7/16/2011	Sagebrush (Two Ocean Lake)	126	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	adult		**deceased upon discovery**
7/16/2011	Sagebrush (Two Ocean Lake)	128	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	adult		recapture
7/16/2011	Sagebrush (Two Ocean Lake)	125	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	adult		recapture
7/16/2011	Sagebrush (Two Ocean Lake)	133	Microtus montanus	Montane Vole	male	juvenile	21	
7/16/2011	Sagebrush (Two Ocean Lake)	130	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	adult		recapture
7/16/2011	Sagebrush (Two Ocean Lake)	134	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	juvenile	11.2	
7/16/2011	Sagebrush (Two Ocean Lake)	135	Microtus montanus	Montane Vole	male	juvenile	19.2	
7/16/2011	Meadow (Two Ocean Lake)	136	Zapus princeps	Western Jumping Mouse	female	adult	27.9	
7/16/2011	Meadow (Two Ocean Lake)	137	Microtus montanus	Montane Vole	male	adult	25.1	
7/16/2011	Meadow (Two Ocean Lake)	138	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	juvenile	10.4	
7/16/2011	Meadow (Two Ocean Lake)	139	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	juvenile	10	
7/16/2011	Meadow (Two Ocean Lake)	140	Microtus montanus	Montane Vole	female	juvenile	15.5	
7/17/2011	Sagebrush (Two Ocean Lake)	141	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	juvenile	12.2	
7/17/2011	Sagebrush (Two Ocean Lake)	142	Microtus montanus	Montane Vole	male	adult	22.8	
7/17/2011	Sagebrush (Two Ocean Lake)	128	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	adult		recapture
7/17/2011	Sagebrush (Two Ocean Lake)	143	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	juvenile	11	
7/17/2011	Sagebrush (Two Ocean Lake)	125	Peromyscus maniculatus	American Deer Mouse (woodland form)	male	adult		recapture
7/17/2011	Meadow (Two Ocean Lake)		Zapus princeps	Western Jumping Mouse				scaped
7/17/2011	Meadow (Two Ocean Lake)	131	Zapus princeps	Western Jumping Mouse	female	adult		recapture
7/17/2011	Meadow (Two Ocean Lake)	144	Microtus montanus	Montane Vole	female	adult	43	pregnant
7/17/2011	Meadow (Two Ocean Lake)	132	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	adult		recapture
7/17/2011	Meadow (Two Ocean Lake)	145	Peromyscus maniculatus	American Deer Mouse (woodland form)	female	juvenile	12.7	
7/17/2011	Meadow (Two Ocean Lake)		Sorex vagrans	Vagrant Shrew	female	adult	5.9	**deceased upon discovery**
7/17/2011	Meadow (Two Ocean Lake)	146	Microtus montanus	Montane Vole	male	juvenile	18.3	