PLANT TOXINS INFLUENCE DIET SELECTION AND INTESTINAL PARASITES IN A SPECIALIST HERBIVORE

by

Marcella Rene Fremgen

A thesis

submitted in partial fulfillment

of the requirements for the degree of

Master of Science in Biology

Boise State University

December 2015

BOISE STATE UNIVERSITY GRADUATE COLLEGE

DEFENSE COMMITTEE AND FINAL READING APPROVALS

of the thesis submitted by

Marcella Rene Fremgen

Thesis Title: Plant Toxins Influence Diet Selection and Intestinal Parasites in a Specialist Herbivore

Date of Final Oral Examination: 15 October 2015

The following individuals read and discussed the thesis submitted by student Marcella Rene Fremgen, and they evaluated her presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

Jennifer S. Forbey, Ph.D.	Chair, Supervisory Committee
John W. Connelly, Ph.D.	Member, Supervisory Committee
James R. Belthoff, Ph.D.	Member, Supervisory Committee

The final reading approval of the thesis was granted by Jennifer S. Forbey, Ph.D., Chair of the Supervisory Committee. The thesis was approved for the Graduate College by John R. Pelton, Ph.D., Dean of the Graduate College.

DEDICATION

For my family, and especially my Papa Joe. You have always inspired me, and are greatly missed.

ACKNOWLEDGEMENTS

This graduate work was made possible by many individuals and agencies, including committee members, funders, field and lab technicians and volunteers, and family. Special thanks to my supervisory committee: Dr. Jen Forbey, Dr. Jack Connelly, and Dr. Jim Belthoff. Without their support and guidance, this research would not have been possible. Thanks especially to Jen for challenging me and encouraging me to think broadly and pursue new ideas. Your enthusiasm and work ethic are great inspirations! I appreciate all the support, mentoring, advice, and time you have put into this project and into me personally. You have been wonderful both to learn from and to spend time with in the field and along the way. Thanks to Jack for your guidance on study design, methods, and writing. You are a wonderful resource for sage-grouse researchers and managers, and are an invaluable source of knowledge. Your expertise is greatly valued, and I have learned a lot from you and enjoyed your company in the field! Thanks to Dr. Belthoff for your guidance on quantitative analysis, behavioral ecology, and your great attention to detail. You have improved my graduate experience and my technical skills.

Funding and equipment were provided primarily by Idaho Fish and Game (Pittman-Robertson funds), with additional funding provided by the National Science Foundation (NSF DEB-1146194 and NSF-IOS-1258217), and Sigma Xi Grants-In-Aid. Idaho Fish and Game has provided extensive logistical support, and special thanks go out to Dr. Jack Connelly, Dave Musil, Lisa Cross, Ross Winton, Ann Moser, and Regan Berkley. I appreciate the opportunity to track and study radio-marked birds, as well as all

the equipment and assistance. Additionally, thanks to Castle Rocks and Lake Walcott State Parks for their willingness to provide comfortable and warm winter housing.

I also want to thank Dr. Gail Patricelli and Dr. Alan Krakauer from University of California – Davis, for your interest and guidance. You both provided many insights and opportunities to pursue great ideas, while also sharing knowledge and providing a great deal of assistance in methods and analysis. The vocalization work would not have been possible without you. I am very thankful for my opportunity to have worked with you and learned from you, and have enjoyed my field experiences at your site!

Also, I would like to extend my thanks to the numerous technicians and volunteers in both the field and the lab. There are too many volunteers to list here, but those that have provided the most support include: Lisa Cross, Chelsea Merriman, Nicole Benson, Brecken Robb, Karli Graski, Kayla Luke, Kaisha Young, Brittainy Pendleton, Jessica Higley, Brieana Goods, Jacque Pena, Joel Velasco, Michael Tarantino, Christopher Ellis, and Raynell Jones. Many thanks to all those that came out to sample sagebrush with me during winter in Idaho, and thanks to those who stayed in the lab, spending hours and hours processing all those samples! Additionally, thanks to Amy Ulappa and Graham Frye for your protocol development, samples you saved, and assistance you provided along the way. Thanks to other current Forbey lab graduate students, especially Jordan Nobler, and also Zoe Tinkle and Dan Melody.

Thanks to Dr. Patrick Magee at Western State College for introducing me to grouse research. I will always be thankful for the opportunities and support that you provided! Finally, thanks to my family and friends for all of your support during this time. Thanks to Aleshia Fremgen, who also studies sage-grouse and therefore provided

excellent support and understanding. Thanks for coming to the field with me, keeping me company through all my writing and analysis, and for helping me track down references. To my parents, Ann Fremgen and Tom Fremgen, thanks for your support and interest. I am looking forward to being home for the holidays now! And thanks to Mike Tarantino, who came to the field, worked in the lab, and provided unwavering support throughout the entire process. You all have been encouraging, motivating, and supportive throughout this process. Thanks for being there every step of the way!

ABSTRACT

Herbivores select plants and patches that generally maximize nutrient intake and minimize intake of plant secondary metabolites (PSMs). Protein is important for growth, reproduction and maintenance, but maximizing intake of protein is often limited by concentrations of PSMs that are potentially toxic to herbivores and energetically expensive to process. However, the consequences of ingesting PSM are often dose-dependent. At high doses, PSMs generally have negative physiological effects and are avoided, but some PSMs can be therapeutic against parasites at low doses and could therefore be selected. We used Greater Sage-grouse (*Centrocercus urophasianus,* hereafter, sage-grouse) to test how PSMs influence diet selection and parasite loads in a free-ranging avian herbivore. Specifically, we examined selective foraging by sage-grouse and how foraging patterns influence habitat use throughout winter at a mixed sagebrush site. We found that selective foraging did not influence landscape-scale habitat selection between two species of sagebrush. However, more fine-scale selection was influenced by PSMs and structural characteristics within a species. We also examined how selective foraging may influence parasite loads in sage-grouse. We tested the relationship between intake of PSMs, intestinal exposure of parasites to PSMs, and parasite loads. Parasite loads in sage-grouse were correlated with higher concentrations of PSMs, suggesting that PSMs may make sage-grouse more susceptible to parasites, or that parasites are resistant to sagebrush PSMs. This research informs basic science on foraging ecology, parasitology, and habitat use by an avian herbivore. Additionally, it

provides information to managers about factors that influence diet selection and potential health consequences of ingested PSMs by wildlife.

AUTOBIOGRAPHICAL SKETCH OF AUTHOR

Marcella Fremgen was born and raised in Golden, Colorado. She attended Western State College of Colorado (now Western State Colorado University) in Gunnison, and graduated with a Bachelor of Arts in May 2011 in Biology. It was at Western that Marcella was introduced to Gunnison Sage-grouse, a species she continued to work with until 2013, when she joined Boise State University. Marcella also worked for the United States Forest Service, Colorado Parks and Wildlife, and Oregon State University. In addition, she is a member of Tri Beta Biological Honorary Society and The Wildlife Society.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

PSM	Plant Secondary Metabolite
AUC	Area Under the Curve (on a chromatogram)
AIC_c	Akaike's Information Criterion, with sample size adjustment
DW	Dry Weight

GENERAL INTRODUCTION

Herbivores have abundant food supplies of varying nutritional quality, and must select the highest quality resources from those available (Sinclair et al. 1982). For example, herbivores select plants and patches that generally maximize nutrient intake and minimize intake of plant secondary metabolites, or PSMs (Guglielmo et al. 1996, Stolter et al. 2005, Frye et al. 2013). Intake of PSMs is regulated because PSMs are potentially toxic to herbivores and processing ingested PSMs can be energetically expensive (Sorensen and Dearing 2006, Au et al. 2013, Forbey et al. 2013). Therefore, animals regulate exposure to PSMs via behavioral (Wiggins et al. 2003) and physiological mechanisms (Sorensen and Dearing 2006, Estell 2010). Additionally, because protein is important for growth, reproduction and maintenance, herbivores generally select for high protein food resources. (Chastel et al. 1995, DeGabriel et al. 2009). Acquisition of protein can be limited by dietary PSMs, further increasing the importance of minimizing intake of PSMs (Jakubas et al. 1993, Guglielmo et al. 1996, DeGabriel et al. 2009, Au et al. 2013).

However, side effects associated with PSM consumption are dose-dependent. At high doses, PSMs generally have negative physiological effects, but some PSMs may be therapeutic at low doses (Forbey et al. 2009). For example, some PSMs have anti-parasitic properties (Zhu et al. 2013). While generalist herbivores occasionally exploit PSMs for their therapeutic properties (Huffman and Seifu 1989, Huffman 1993, Huffman 1997, McLennan and Huffman 2012, Su et al. 2013), specialist herbivores may not be

able to exploit this resource. Specialist herbivores face a number of challenges that may limit their ability to self-medicate for parasites, including energy constraints and PSM-resistant parasites. Self-medication, to my knowledge, has not been evaluated in specialist herbivores, and is an important aspect of wildlife health.

Greater Sage-grouse (*Centrocercus urophasianus*, hereafter, sage-grouse) are specialist avian herbivores that feed almost exclusively on sagebrush (*Artemisia* spp.) during the winter months (Patterson 1952, Remington and Braun 1985, Thacker et al. 2012). Sagebrush synthesizes a suite of PSMs (sesquiterpene lactones, monoterpenes and phenolics) that make the shrubs less palatable. As sagebrush obligates, sage-grouse require intact sagebrush habitats for both cover and forage throughout the year. Sage-grouse habitat is declining rapidly, and this decrease has been associated with population declines throughout the range of the species (Schroeder et al. 2004, Aldridge et al. 2008, Bruce et al. 2011). Currently, sage-grouse occupy approximately half of their estimated pre-settlement range (Schroeder et al. 2004). It is therefore important to understand resource use thoroughly, and factors that influence the health of sage-grouse, to best conserve or restore habitats that maximize the success of sage-grouse.

Selection of sagebrush subspecies by sage-grouse during winter depends upon availability and chemistry (Beck 1977, Vasquez 1999, Frye et al. 2013). Sage-grouse select plants and sagebrush species with the highest protein content (Remington and Braun 1985, Barnett and Crawford 1994, Gregg et al. 2008, Frye et al. 2013) or lowest concentration of PSMs (Remington and Braun 1985, Frye et al. 2013). Sage-grouse foraging patches are often located in areas dominated by “dwarf” species of sagebrush, including *Artemisia nova* and *A. arbuscula* (Dalke et al. 1963, Connelly et al. 2004,

Bruce et al. 2011, Hagen et al. 2011, Arkle et al. 2014), which generally have lower PSMs than big sagebrush species (Frye et al. 2013, Ulappa et al. 2014). However, dwarf sagebrush comprises a relatively small proportion of the landscape in some areas, and a mix of big sagebrush (*A. tridentata*) dominates most habitats throughout the Great Basin (Beck et al. 2009). In addition, species, subspecies, and populations of sagebrush vary in PSMs, both quantitatively and qualitatively. For example, sagebrush taxa can be identified based on unique chemical profiles (Thacker et al. 2012) and the concentrations of each compound (Kelsey et al. 1982). The toxic and potential therapeutic benefit of PSM ingestion against parasites and pathogens is likely to be dependent on the types of compounds, the concentration of individual PSMs, and the mixture of compounds consumed. Moreover, the distribution of sagebrush taxa across the landscape is likely to change. For example, three-tip sagebrush (*A. tripartita*) has a relatively small range (Tirmenstein 1999) compared to big sagebrush (Freeman et al. 1991), but populations are expected to expand by 1.3% for every 1% increase in temperature (Dalgleish et al. 2011). In addition to changes in distribution, the PSM concentrations in sagebrush are expected to increase with increased predicted changes in temperature and atmospheric carbon (Forbey et al. 2013). Climate change is also predicted to reduce physiological tolerance to PSMs by herbivores (Dearing et al. 2008) and increase pathogenicity of parasites (Molnar et al. 2013a; 2013b). These multi-scale changes in the landscape and physiology may alter how sage-grouse interact with sagebrush. Therefore, it is important to understand how sage-grouse select patches and individual plants in an environment with different types of sagebrush and the potential physiological consequences of selecting sagebrush with specific PSM profiles.

In the first chapter, I examined selective foraging by sage-grouse and how foraging patterns influence habitat use throughout winter at a sagebrush site with co-dominant Wyoming big sagebrush (*A.t. wyomingensis*) and three-tip sagebrush (*A. tripartita*). This habitat was of interest because Wyoming big sagebrush is relatively widespread, and the range of three-tip sagebrush is expected to expand (Baker 2006, Lesica et al. 2007, Beck et al. 2009, Dalgleish et al. 2011). Selective foraging can influence habitat use at multiple scales (Frye et al. 2013), but did not influence landscape-scale habitat selection between these species of sagebrush at my site. However, more fine-scale selection was influenced by a variety of chemical and structural characteristics for each sagebrush species.

In the second chapter, I examined how the intake of PSMs may influence parasite loads in sage-grouse. Intestinal parasites are common in sage-grouse (Christiansen and Tate 2011) and may influence nutrient acquisition (Nelson 1955) and therefore energy available for other activities, including PSM detoxification. I tested the relationship between intake of PSMs, concentrations of unchanged PSMs in the intestines (indicator of toxin load), and intestinal parasite loads of *Raillietina centrocerci* at four sites in southern Idaho. I also evaluated ecological factors that may contribute to parasite loads, including site, season, host sex, site elevation, and flock size. Across all four sites in a single season, sage-grouse had higher intestinal parasite loads with higher PSM loads, suggesting that PSMs may make sage-grouse more susceptible to parasites, or that these parasites are resistant to sagebrush PSMs. Factors that influenced parasite loads were site, season, bird sex, and both diversity and concentration of individual PSMs.

To perform this research, I used sage-grouse with necklace-style radio-transmitters, which allowed us to track individual animals. Telemetry fundamentals state that transmitters should be placed on individuals that represent the population and display normal demographic and behavioral traits. While transmitters are integral to wildlife research, they may have negative effects on survival, energetics, or behavior. For example, radio-transmitters decreased lek attendance by sage-grouse (Gibson et al. 2013), but for males that do attend leks, necklace-style transmitters (collars) may interfere with the male strut display on leks during spring. Therefore, my third chapter evaluates the vocalization characteristics of male sage-grouse with and without collars. I found that several aspects of the strut vocalization differ between collared and non-collared males, however not all of these characteristics have not previously been linked to reproductive success so the impacts of these differences on reproductive success are unknown.

In the fourth chapter, I evaluated if sagebrush age was related to phytochemistry, and if there is an easy way to estimate plant age in the field. Given the relationship between PSMs and diet selection and parasites, it is important to understand parameters that influence variation in PSMs across the landscape. Age is one factor that can influence PSM concentrations, due to trade-offs plants make between growth and defense (Messina et al. 2002). Specifically, age-dependent PSMs can influence herbivores (Shiojiri et al. 2011). Landscape-scale disturbances (e.g. fire, mowing, restoration) can alter the age distribution of plants, and therefore the dietary quality of sagebrush. It is therefore important to identify how age influences PSMs and develop methods to estimate the age of plants. I measured the circumference of a plant at the base, and found that it was strongly correlated with plant age. This provides a useful field technique to

assess sagebrush recruitment in the field. However, there was no correlation between sagebrush age and any of the phytochemical variables I measured. Therefore, habitat treatments that remove decadent sagebrush are not likely to influence sagebrush forage quality, but will remove cover and potentially have other negative ecological impacts (Davies et al. 2009, Davies et al. 2012).

This research informs basic science on foraging ecology, parasitology, and habitat use by an avian herbivore. Additionally, it provides information to managers about resource selection and potential health consequences for a species of concern. This information could inform habitat conservation and sagebrush restoration efforts to improve habitat quality (Appendix A).

Literature Cited

Aldridge, C. L., S. E. Nielsen, H. L. Beyer, M. S. Boyce, J. W. Connelly, S. T. Knick, and M. A. Schroeder. 2008. Range-wide patterns of Greater Sage-grouse persistence. Diversity and Distributions 14:983-994.

Arkle, R. S., D. S. Pilliod, S. E. Hanser, M. L. Brooks, J. C. Chambers, J. B. Grace, K. C. Knutson, D. A. Pyke, J. L. Welty, and T. A. Wirth. 2014. Quantifying restoration effectiveness using multi-scale habitat models: implications for sage-grouse in the Great Basin. Ecosphere 5:1-32.

Au, J., K. J. Marsh, I. R. Wallis, and W. J. Foley. 2013. Whole-body protein turnover reveals the cost of detoxification of secondary metabolites in a vertebrate browser. Journal of Comparative Physiology B: Biochemical Systemic and Environmental Physiology 183:993-1003.

Baker, W. L. 2006. Fire and restoration of sagebrush ecosystems. Wildlife Society Bulletin 34:177-185.

Barnett, J., and J. Crawford. 1994. Pre-laying nutrition of sage-grouse hens in Oregon. Journal of Range Management 47:114-118.

Beck, J., J. Connelly, and K. Reese. 2009. Recovery of Greater Sage-grouse habitat features in Wyoming big sagebrush following prescribed fire. Restoration Ecology 17:393-403.

Beck, T. 1977. Sage-grouse flock characteristics and habitat selection in winter. Journal of Wildlife Management 41:18-26.

Bruce, J., W. Robinson, S. Petersen, and R. Miller. 2011. Greater Sage-grouse movements and habitat use during winter in central Oregon. Western North American Naturalist 71:418-424.

Chastel, O., H. Weimerskirch, and P. Jouventin. 1995. Influence of body condition on reproductive decision and reproductive success in the Blue Petrel. Auk 112:964-972.

Christiansen, T. J., and C. M. Tate. 2011. Parasites and infectious diseases of Greater Sage-grouse. Pages 113 - 126 *in* S. T. Knick, and J. W. Connelly, editors. Studies in Avian Biology. University of California Press, Berkeley, CA.

Connelly, J. W., S. T. Knick, M. A. Schroeder, and S. J. Stiver. 2004. Conservation Assessment of Greater Sage-grouse and Sagebrush Habitats. *in* Western Association of Fish and Wildlife Agencies, Cheyenne, Wyoming, USA.

Dalgleish, H. J., D. N. Koons, M. B. Hooten, C. A. Moffet, and P. B. Adler. 2011. Climate influences the demography of three dominant sagebrush steppe plants. Ecology 92:75-85.

Dalke, P. D., D. B. Pyrah, D. C. Stanton, J. E. Crawford, and E. F. Schlatterer. 1963. Ecology, productivity, and management of sage-grouse in Idaho. Journal of Wildlife Management 27:810-841.

Davies, K. W., J. D. Bates, D. D. Johnson, and A. M. Nafus. 2009. Influence of Mowing Artemisia tridentata ssp wyomingensis on Winter Habitat for Wildlife. Environmental Management 44:84-92.

Davies, K. W., J. D. Bates, and A. M. Nafus. 2012. Mowing Wyoming Big Sagebrush Communities With Degraded Herbaceous Understories: Has a Threshold Been Crossed? Rangeland Ecology & Management 65:498-505.

Dearing, M., J. Forbey, J. McLister, and L. Santos. 2008. Ambient temperature influences diet selection and physiology of an herbivorous mammal, *Neotoma albigula*. Physiological and Biochemical Zoology 81:891-897.

DeGabriel, J., B. Moore, W. Foley, and C. Johnson. 2009. The effects of plant defensive chemistry on nutrient availability predict reproductive success in a mammal. Ecology 90:711-719.

Estell, R. 2010. Coping with shrub secondary metabolites by ruminants. Small Ruminant Research 94:1-9.

Forbey, J. S., A. L. Harvey, M. A. Huffman, F. D. Provenza, R. Sullivan, and D. Tasdemir. 2009. Exploitation of secondary metabolites by animals: a response to homeostatic challenges. Integrative and Comparative Biology 49:314-328.

Forbey, J. S., N. L. Wiggins, G. G. Frye, and J. W. Connelly. 2013. Hungry grouse in a warming world: emerging risks from plant chemical defenses and climate change. Wildlife Biology 19:374-381.

Freeman, D. C., W. A. Turner, E. D. McArthur, and J. H. Graham. 1991. Characterization of a narrow hybrid zone between two subspecies of big sagebrush (*Artemisia tridentata*, Asteraceae). American Journal of Botany 78:805-815.

Frye, G. G., J. W. Connelly, D. D. Musil, and J. S. Forbey. 2013. Phytochemistry predicts habitat selection by an avian herbivore at multiple spatial scales. Ecology 94:308-314.

Gibson, D., E. J. Blomberg, G. L. Patricelli, A. H. Krakauer, M. T. Atamian, and J. S. Sedinger. 2013. Effects of radio collars on survival and lekking behavior of male Greater Sage-grouse. Condor 115:769-776.

Gregg, M., J. Barnett, and J. Crawford. 2008. Temporal variation in diet and nutrition of preincubating Greater Sage-grouse. Rangeland Ecology and Management 61:535-542.

Guglielmo, C., W. Karasov, and W. Jakubas. 1996. Nutritional costs of a plant secondary metabolite explain selective foraging by Ruffed Grouse. Ecology 77:1103-1115.

Hagen, C., M. Willis, E. Glenn, and R. Anthony. 2011. Habitat selection by Greater Sage-grouse during winter in southeastern Oregon. Western North American Naturalist 71:529-538.

Huffman, M. 1993. Further observations on the use of the medicinal plant, *Vernonia amygdalina* (Del), by a wild chimpanzee, its possible effect on parasite load, and its phytochemistry. African Study Monographs 14:227-240.

Huffman, M. A. 1997. Current evidence for self-medication in primates: A multidisciplinary perspective. Yearbook of Physical Anthropology 40:171-200.

Huffman, M. A., and M. Seifu. 1989. Observations on the illness and consumption of a possibly medicinal plant *Vernonia amygdalina* by a wild chimpanzee in the Mahale Mountains National Park, Tanzania. Primates 30:51-63.

Jakubas, W., W. Karasov, and C. Guglielmo. 1993. Coniferyl benzoate in quaking aspen (*Populus tremuloides*) - Its effect on energy and nitrogen digestion and retention in Ruffed Grouse (*Bonasa umbellus*). Physiological Zoology 66:580-601.

Kelsey, R. G., J. R. Stephens, and F. Shafizadeh. 1982. The chemical constituents of sagebrush foliage and their isolation. Journal of Range Management 35:617-622.

Lesica, P., S. V. Cooper, and G. Kudray. 2007. Recovery of big sagebrush following fire in southwest Montana. Rangeland Ecology and Management 60:261-269.

McLennan, M. R., and M. A. Huffman. 2012. High frequency of leaf swallowing and its relationship to intestinal parasite expulsion in "village" chimpanzees at Bulindi, Uganda. American Journal of Primatology 74:642-650.

Messina, F. J., S. L. Durham, J. H. Richards, and E. D. McArthur. 2002. Trade-off between plant growth and defense? A comparison of sagebrush populations. Oecologia 131:43-51.

Molnar, P. K., A. P. Dobson, and S. J. Kutz. 2013a. Gimme shelter - the relative sensitivity of parasitic nematodes with direct and indirect life cycles to climate change. Global Change Biology 19:3291-3305.

Molnar, P. K., S. J. Kutz, B. M. Hoar, and A. P. Dobson. 2013b. Metabolic approaches to understanding climate change impacts on seasonal host-macroparasite dynamics. Ecology Letters 16:9-21.

Nelson, O. C. 1955. A field study of the sage-grouse in southeastern Oregon with special reference to reproduction and survival. Thesis, Oregon State College, Corvallis, USA.

Patterson, R. L. 1952. The Sage Grouse in Wyoming. Sage Books, Inc., Denver, CO, USA.

Remington, T., and C. Braun. 1985. Sage-grouse food selection in winter, North Park, Colorado. Journal of Wildlife Management 49:1055-1061.

Schroeder, M. A., C. L. Aldridge, A. D. Apa, J. R. Bohne, C. E. Braun, S. D. Bunnell, J. W. Connelly, P. A. Deibert, S. C. Gardner, M. A. Hilliard, G. D. Kobriger, S. M. McAdam, C. W. McCarthy, J. J. McCarthy, D. L. Mitchell, E. V. Rickerson, and S. J. Stiver. 2004. Distribution of Sage-Grouse in North America. Condor 106:363-376.

Shiojiri, K., R. Karban, and S. Ishizaki. 2011. Plant age, seasonality, and plant communication in sagebrush. Journal of Plant Interactions 6:85-88.

Sinclair, A., C. Krebs, and J. Smith. 1982. Diet quality and food limitation in herbivores - the case of the snowshoe hare. Canadian Journal of Zoology 60:889-897.

Sorensen, J. S., and D. M. Dearing. 2006. Efflux transporters as a novel herbivore countermechanism to plant chemical defenses. Journal of Chemical Ecology 32:1181-1196.

Stolter, C., J. Ball, R. Julkunen-Tiitto, R. Lieberei, and J. Ganzhorn. 2005. Winter browsing of moose on two different willow species: food selection in relation to plant chemistry and plant response. Canadian Journal of Zoology 83:807-819.

Su, H. H., Y. C. Su, and M. A. Huffman. 2013. Leaf swallowing and parasitic infection of the Chinese lesser civet *Viverricula indica* in northeastern Taiwan. Zoological Studies 52:8.

Thacker, E., D. Gardner, T. Messmer, M. Guttery, and D. Dahlgren. 2012. Using gas chromatography to determine winter diets of Greater Sage-grouse in Utah. Journal of Wildlife Management 76:588-592.

Tirmenstein, D. 1999. *Artemisia tripartita*. *in* Fire Effects Information System. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Library, <http://www.fs.fed.us/database/feis/plants/shrub/arttrp/all.html>. Accessed 23 May 2015.

Ulappa, A. C., R. G. Kelsey, G. G. Frye, J. L. Rachlow, L. A. Shipley, L. Bond, X. Z. Pu, and J. S. Forbey. 2014. Plant protein and secondary metabolites influence diet selection in a mammalian specialist herbivore. Journal of Mammalogy 95:834-842.

Vasquez, M. G. 1999. Determination of winter use of sagebrush subspecies by Gunnison Sage-grouse on Flat Top Mountain: Assessment for prescribed fire planning. Thesis, Western State College of Colorado, Gunnison, USA.

Wiggins, N., C. McArthur, S. McLean, and R. Boyle. 2003. Effects of two plant secondary metabolites, cineole and gallic acid, on nightly feeding patterns of the common brushtail possum. Journal of Chemical Ecology 29:1447-1464.

Zhu, L., J. L. Dai, L. Yang, and J. Qiu. 2013. In vitro ovicidal and larvicidal activity of the essential oil of *Artemisia lancea* against *Haemonchus contortus* (Strongylida). Veterinary Parasitology 195:112-117.

CHAPTER ONE: DIET SELECTION BY GREATER SAGE-GROUSE IN POST-FIRE HABITATS DOMINATED BY THREE-TIP AND WYOMING BIG SAGEBRUSH

Abstract

Diet quality influences habitat use, movement, and reproductive success for free-ranging herbivores. Herbivores attempt to consume sufficient nutrients while avoiding plant secondary metabolites (PSMs) that act as chemical defenses. PSMs can have harmful effects on herbivores, and they are abundant in sagebrush plants (*Artemisia* spp.). Species of sagebrush have uniquely identifiable chemical profiles, which may influence overall diet quality and selection by herbivores. Three-tip sagebrush (*A. tripartita*) is a dominant or co-dominant shrub species in parts of the Great Basin. Several studies have identified its potential to expand range in post-fire environments because of its ability to re-sprout, which will be increasingly important in landscapes faced with more frequent fire regimes. Despite the current and future distribution of this plant, its importance to wildlife as a forage resource has been understudied. I evaluated the dietary quality of three-tip sagebrush relative to Wyoming big sagebrush for wintering Greater Sage-grouse (*Centrocercus urophasianus*) in south-central Idaho. I identified winter foraging sites of sage-grouse, and then analyzed structural characteristics of plants, crude protein content, phenolic concentrations, and monoterpene concentrations in browsed and non-browsed plants at these sites. Three-tip sagebrush had a different chemical profile than the sympatric Wyoming big sagebrush (*A. tridentata wyomingensis*) at foraging patches. Three-tip sagebrush had relatively lower protein content, higher monoterpene content,

and fewer individual monoterpenes compared to Wyoming big sagebrush. Browsed plants had higher crude protein but similar total monoterpene concentration compared to non-browsed plants for both species of sagebrush. Structural (plant height) and dietary (individual monoterpenes and protein) parameters influenced grouse use of both species of sagebrush. At the landscape scale, the different chemistry of three-tip did not influence habitat selection by sage-grouse, as both Wyoming and three-tip sagebrush were browsed relative to their availability. Therefore, three-tip sagebrush may provide a food source that is equivalent to Wyoming sagebrush for sage-grouse in post-fire landscapes where other species of sagebrush have not yet recovered. However, three-tip and Wyoming big sagebrush may both be less palatable than other species of sagebrush and the consequences of relying on three-tip as a dominant forage for sage-grouse or other wildlife should be further evaluated.

Introduction

Foraging resources available to herbivores vary in nutritional quality. Thus, natural selection likely operates on individuals to seek and use high quality resources among those available (Sinclair et al. 1982). Forage quality helps explain fine-scale and large-scale habitat use since not all plants provide the same nutritional benefit (Anderson et al. 2010). Herbivores generally select plants and patches to maximize nutrient intake and minimize intake of plant secondary metabolites, or PSMs (Guglielmo et al. 1996, Stolter et al. 2005, Frye et al. 2013). Additionally, herbivores choose structural characteristics that may help herbivores avoid predation, among other factors. PSM intake is regulated because PSMs are potentially toxic to herbivores and processing ingested PSMs can be energetically expensive (Sorensen and Dearing 2006, Forbey et al.

2013). Protein is important for growth, reproduction, and maintenance (Chastel et al. 1995, DeGabriel et al. 2009) and PSMs can inhibit the digestion of protein (Guglielmo et al. 1996, DeGabriel et al. 2009, Au et al. 2013, Kohl et al. 2015). Diet selection can influence habitat selection at larger spatial scales as herbivores select areas where they can acquire high quality food resources (Moore et al. 2010, Youngentob et al. 2011, Frye et al. 2013, Ulappa et al. 2014).

Greater Sage-grouse (*Centrocercus urophasianus*, hereafter, sage-grouse) are avian herbivores that specialize almost exclusively on sagebrush (*Artemisia* sp.) during the winter months (Patterson 1952, Klebenow and Gray 1968, Frye et al. 2013). Sagebrush synthesizes a suite of PSMs (sesquiterpene lactones, monoterpenes, and phenolics) that deter browsing by vertebrate herbivores (Welch and McArthur 1981, Remington and Braun 1985, Frye et al. 2013, Ulappa et al. 2014). Sage-grouse rely on intact sagebrush habitat for both cover and forage throughout the year, but sage-grouse habitat is declining rapidly in both quantity and quality. Loss of habitat has been associated with population declines of sage-grouse around 70% prior to 1985, with continued 2% annual range wide declines (Connelly et al. 2000, Aldridge et al. 2008, Bruce et al. 2011, Garton et al. 2011). Currently, sage-grouse occupy approximately 56% of their pre-settlement range (Schroeder et al. 2004), and their conservation has been a concern for almost a century (Hornaday 1916, Connelly et al. 2000, Connelly et al. 2004). It is therefore important to understand resource use thoroughly, from structure to chemistry, for future conservation of habitats that maximize the success of sage-grouse.

Consumption of sagebrush taxa during winter by sage-grouse likely depends upon availability (Vasquez 1999), snow cover (Beck 1977, Remington and Braun 1985), and

chemistry (Remington and Braun 1985, Frye et al. 2013). Sage-grouse select plants, patches, and sagebrush species with the highest nitrogen content (Remington and Braun 1985, Barnett and Crawford 1994, Gregg et al. 2008, Frye et al. 2013) or lowest concentration of PSMs (Remington and Braun 1985, Frye et al. 2013). Foraging patches for wintering sage-grouse are often located in areas dominated by dwarf species of sagebrush (Dalke et al. 1963, Bruce et al. 2011, Hagen et al. 2011, Arkle et al. 2014). These taxa generally have lower concentrations of PSMs and are more palatable than big sagebrush species (Rosenreter 2004, Frye et al. 2013, Arkle et al. 2014). While dwarf sagebrush, including low (*A. arbuscula*) and black sagebrush (*A. nova*), might not provide adequate cover from predators, these species nonetheless appear important for foraging in winter.

However, dwarf sagebrush species comprise a relatively small proportion of the land cover in sagebrush landscapes. Low sagebrush covers approximately 11.3 million hectares, and black sagebrush dominates 11,200 hectares throughout the West (Steinberg 2002, Fryer 2009), while big sagebrush (*Artemisia tridentata* spp.) covers approximately 55.1 million hectares (Freeman et al. 1991, Schroeder et al. 2004). A mix of big sagebrush dominates most habitats throughout the Great Basin (Beck et al. 2009). Additionally, restoration projects often focus on big sagebrush habitats, with approximately 2.2 million hectares of restoration efforts in the Great Basin between 1990 and 2014 occurring primarily in big sagebrush (Arkle et al. 2014). Although three-tip sagebrush (*A. tripartita*) has a relatively small range (Tirmenstein 1999) compared to big sagebrush (Freeman et al. 1991), populations of three-tip are expected to expand by 1.3% for every 1% increase in temperature (Dalgleish et al. 2011). Additionally, three-tip

sagebrush recovers twice as fast as big sagebrush after fires (Beck et al. 2009) and plants can re-sprout instead of reestablishing from seed (Passey and Hugie 1962, Lesica et al. 2007), which may contribute to range expansions as the fire return interval decreases throughout the West (Baker 2006).

Use of three-tip sagebrush by wildlife has been understudied. Although sage-grouse will use three-tip for nesting cover, they used it less than expected based on availability in south-central Idaho (Lowe et al. 2009). Moreover, hens that did nest under three-tip sagebrush had lower nesting success. As a food resource, domestic sheep (*Ovis aries*) will eat three-tip sagebrush when offered supplemental alfalfa and hay (Fraker-Marble et al. 2007). Mule deer (*Odocoileus hemionus*) used areas with three-tip sagebrush and Wyoming big sagebrush (*A. tridentata wyomingensis*) in proportion to their availability, and selection of three-tip sagebrush for food varied year to year (Wambolt 2001). However, diet quality and phytochemistry of three-tip sagebrush remains largely understudied, and selection of three-tip sagebrush for food has not been evaluated in other herbivores, including sage-grouse.

I examined diet selection by sage-grouse inhabiting a landscape dominated by three-tip sagebrush and Wyoming big sagebrush, to evaluate how grouse select between and within these species in a post-fire environment. I predicted that sage-grouse would select patches and plants of sagebrush with the highest crude protein and lowest concentrations of PSMs in habitats dominated by three-tip and Wyoming sagebrush. In addition, selection can be influenced by structural components, such as cover and topography, which are considered important for sage-grouse winter habitat use (Beck 1977, Connelly et al. 2000). I tested three main hypotheses:

Hypothesis 1: Sage-grouse select sagebrush at several spatial scales (plant, patch, and habitat) based on concentrations of PSMs.

Hypothesis 2: Sage-grouse select sagebrush at several spatial scales (plant, patch, and habitat) based on crude protein content.

Hypothesis 3: Sage-grouse select sagebrush at several spatial scales (plant, patch, and habitat) based on structural habitat characteristics (height, density, and cover).

These hypotheses are not mutually exclusive and all three factors (PSMs, crude protein content, and structural habitat characteristics) may contribute to selection at each scale. Additionally, certain characteristics may drive selection at different spatial scales depending on dietary and structural requirements necessary to meet both long-term and immediate needs (including dietary, cover, and thermal needs) for an individual. Additionally, other studies (Frye et al. 2013, Arkle et al. 2014) have shown that sage-grouse diet selection is driven by different plant characteristics (PSMs, protein, and plant species) at different scales.

Methods

Study Site

All fieldwork was conducted at one site in south-central Idaho during winter 2013-2014. Craters (42.958690 N, -113.398059 W) is located in Power, Blaine, and Minidoka counties, with the majority of work concentrated in Minidoka County. The site is dominated by Wyoming big sagebrush and three-tip sagebrush. The site had relatively sparse sagebrush cover (average ± SEM: 7.8 ± 6.3%) following an extensive fire history (Figure 1.1). Elevations range from 1,300 m to 1,650 m. The local climate had average

summer temperatures at 30°C and average winter temperatures between -11°C to 4°C. Average annual precipitation was 24 cm, with most precipitation falling as snow. However, average snow depth when I visited the site did not exceed 6 cm. There were 22 VHF radio-transmitters on sage-grouse at this site from November 2013 - March 2014.

Field Methods

Idaho Department of Fish and Game (IDFG) captured and marked sage-grouse with radio-transmitters and leg bands using standard capture and marking techniques (Geisen et al. 1982, Wakkinen et al. 1992). Grouse were trapped February through April 2013 using spotlights and a long-handled net. Birds were weighed, measured, and fitted with aluminum leg bands and 14-15 g necklace-style VHF radio-transmitter collars designed for sage-grouse. Birds were released at the site they were captured.

During winter 2013-2014, sage-grouse were flushed from occupied patches during daylight hours by locating a radio-marked bird. Birds were flushed during mid-winter (16 December to 15 February), after sage-grouse switch to their winter diet of sagebrush (Connelly et al. 1988). Radio-marked birds were not flushed within three weeks of a prior flushing event. After birds were flushed, I located the foraging patch using tracks (if snow was present) and fresh fecal pellets to identify the patch boundary, and then located plants within the patch boundaries that were fed on by the flock that was flushed. Patch boundaries were determined based on the distribution of fresh pellets and browse, and a 10 by 10 m square grid was placed on the center of the patch, from which samples were collected. Foraging patches are identifiable because sage-grouse bite leaves, leaving bright green meristem tissue visible (Figure 1.2).

Leaves were collected from three browsed and three non-browsed plants, from

various size classes, within a patch and pooled to form one browsed sample and one non-browsed sample for each size class. Used patches were defined as a group of sagebrush plants with evidence of recent sage-grouse visitation. Browsed plants had a minimum of ten fresh bite marks by sage-grouse. Non-browsed plants were those with no more than one browse mark by sage-grouse, and evidence of sage-grouse presence (tracks, droppings, or other browsed plants) within 1 m of the plant (Frye et al. 2013). These criteria were established to ensure that non-browsed plants were encountered by sage-grouse but not selected. Sagebrush species were identified in the field using morphological characteristics, and identification was verified using monoterpene profiles (Thacker et al. 2012). Leaf samples were kept on ice in the field and transferred to a -20° C freezer in the laboratory to prevent volatilization of monoterpenes.

Average snow depth was recorded at each patch at the time of collection of leaf samples, as snow cover can influence resource availability. I measured snow depth at 5 random points within the patch boundary. Canopy cover, height and plant density were measured along two perpendicular 20 m transects at each patch (Canfield 1941, Wambolt et al. 2006). Slope, aspect, and elevation were recorded using a clinometer, compass, and GPS unit, respectively.

I also generated a set of random coordinates within the study area boundary using ArcGIS (Environmental Systems Research Inc., Redlands, California) to compare diet quality and structural characteristics between used patches and available patches. Coordinates were constrained by the boundary for known sage-grouse winter use in the study area, determined from flight locations collected by IDFG. For every flush site (used patch) where samples were collected, samples were also collected at a random site

that same day. At each random coordinate, the researcher searched for plants with fresh browse. If browse was present (n = 1 patch), three samples were collected from browsed plants and pooled to form a composite, and three randomly selected non-browsed plants were collected and pooled to form a second composite. If no browse was present, the researcher collected sagebrush clippings from three randomly selected sagebrush plants of each species and pooled collections into one composite sample per species. Additionally, the snow depth and transect data were collected at random patches.

Laboratory Methods

Because grouse pluck leaves instead of eating whole stems (Remington and Braun 1985, Frye et al. 2013), I removed leaves from woody biomass for laboratory analysis. Leaves were removed by dipping samples into liquid nitrogen and brushing leaves off the stems into a separate container. Forceps were used to remove additional debris and dead leaves from the leaf material (Frye 2012). Samples were ground with a mortar and pestle in liquid nitrogen, homogenized to a sample size of approximately 2 mm, and weighed into separate vials for analysis. All weighed samples were stored at -20° C until chemical analysis.

I used headspace gas chromatography to detect monoterpenes in leaf samples, using a gas chromatograph (Agilent 6890N) with a headspace auto-sampler (Hewlett-Packard HP7694). A 100 mg subsample of ground leaf matter was measured immediately after grinding into a 20 ml gas chromatography headspace vial. Compounds were identified using a cocktail of monoterpene standards to generate reference retention times. However, not all compounds could be identified and unknown compounds were labeled based on retention times (min). Retention times and peak areas (area under the

curve, AUC) were calculated using HP ChemStation version B.01.00 (Santa Clara, California, USA). Headspace and gas chromatograph settings and operating conditions are detailed in Appendix B.

Coumarin and total phenolic content were assessed using colorimetric assays using the same extract. Samples (50 mg wet weight) were extracted for two 3-min periods in 1.0 ml GC-grade methanol in a sonicating water bath and filtered through glass wool. For the coumarin assay, 50 µl subsamples of extracts were pipetted into a 96-well plate in triplicate. Color intensity of the extract was measured using a BioTek Synergy MX multi-mode plate reader (BioTek, Winooski, Vermont, USA) at an absorbance of 350 nm excitation and 460 nm emission at room temperature. Scopoletin (# 5995-86-8, Acros Organics) diluted in methanol was used as a standard (0 to 80 µM). To measure total phenolics, an adapted Folin-Ciocalteau assay (Ainsworth and Gillespie 2007) was used, where samples were diluted with methanol to fit within the standard curve. Gallic acid (# 92-6-15, Acros Organics) diluted in methanol was used as a standard (0 to 2900 µM). For each sample extract and standard, 20 µl of the dilution was pipetted in triplicate into 96 well plates. Next, 100 µl of 10% Folin-Ciocalteau reagent was added to each well, mixed gently, and 80 µl of 700 mM (7.5%) sodium carbonate was added and mixed. Plates were allowed to incubate at room temperature for 2 hours, and then were shaken on the plate reader for 60 seconds before reading. Color intensity was measured using a BioTek Synergy MX multi-mode plate reader at an absorbance of 765 nm at room temperature.

Protein analysis was completed using 1.5 g (wet weight) from each homogenized and ground sample. Samples were dried in an oven at 60° C for 24 hours, and scanned

for reflectance across all wavelengths in the near infrared and visible range using an ASD FieldSpec Pro, using default settings. The ASD scans will be used to develop predictive equations for protein using near infrared wavelengths, for future analysis of sagebrush samples (Boegh et al. 2002, Tamburini et al. 2015). Crude protein (% of dry matter) was determined using combustion methods (Dairy One Forage Laboratory, in Ithaca, New York).

Statistical Methods

All statistical analysis used JMP Pro 11.0 (SAS Institute Inc. 2013) and R version 3.2.0 (R Foundation for Statistical Computing 2015). First, the dietary quality of each species was evaluated through non-parametric univariate comparisons. I compared total monoterpene concentration (AUC/ 100 μg dry weight, DW), individual monoterpene concentrations (see Table 1.1 for compound names, concentrations were in AUC/ 100 μg DW), the total number of monoterpenes detected at >1% of total AUC and present in > 70% of samples for that species, percent crude protein (% of DW), coumarin concentration (μmol of scopoletin equivalents/ g DW), and total phenolic (μmol of gallic acid equivalents/ g DW) concentration among all size classes of *A. tridentata wyomingensis* and *A. tripartita*, averaged by patch. Preliminary analyses showed no difference within a species based on plant size, so all further analyses averaged size classes for each patch. Additionally, I compared plant height (cm) between species using ANOVA, and the number of bite marks on each plant for each species using a non-parametric univariate comparison.

I used contingency analyses to assess habitat selection at the landscape-scale by comparing the availability of each species of sagebrush at used and random patches.

Random patches were considered to be the expected (available) proportions, and used patches were considered the observed frequency of patch use.

Diet selection at the plant scale and patch scale were evaluated separately for each species of sagebrush present at the site, because each species had unique monoterpene profiles. To address issues with multicollinearity, I tested individual monoterpenes, phenolics, coumarins, protein, and plant height for correlations. I removed correlated variables ($|r| > 0.7$) for each species (Table 1.1; Appendix C), and remaining variables were used to build models. Variables were selected if they represented a unique chemical class (e.g. protein, phenolics, coumarins, monoterpenes), were present in both species of sagebrush (to allow comparison between species), were chemicals of known identity, or those that had higher concentrations than correlated variables.

Diet selection at the patch scale was determined by averaging the diet quality values for all plants within each patch, for each species. This provided a patch average of browsed and non-browsed plants together to compare the average patch value, or quality, between used and random patches. When present, browsed plants at random patches were included, although this only occurred at one (6.25%) random patch. By comparing patches with an average of both browsed and non-browsed plants, this provides a comparison of overall patch quality. Additionally, I had high detection of browsed plants (Appendix D), giving me confidence that there was no bias in the patch quality at random patches by collecting only non-browsed plants, and also reduced bias by including browsed plants, when present, in the patch average at the random patch to best represent the overall patch quality.

Habitat selection at the patch scale was evaluated using a logistic regression where patch type (used or random) was the binary response and continuous predictors included nutrients (protein), PSMs (individual monoterpenes, total phenolics, and coumarins), and structure (height, percent cover, and density). Models were compared to one another and to a null (intercept-only) model using information-theoretic methods (Burnham and Anderson 2002), for each species separately. I used Akaike's Information Criterion values with a sample size bias-adjustment (AIC_c) for each predictor variable. Models that ranked below the null (i.e. higher AIC_c value) were removed from further analysis, and models within 2 AIC_c units from the top model (i.e. $\Delta AIC_c < 2$) were considered to be the top models. For models within 2 AIC_c units from the top model odds, ratios were calculated to predict odds of patch use.

Diet selection at the plant scale was evaluated with conditional logistic regressions (Hosmer and Lemeshow 1985), where plant type (browsed or non-browsed) was the binary response and the continuous predictors were nutrients, PSMs, and structural variables. Models were stratified by patch, with paired used and random patches. Temporal pairs for used patches allowed me to control for seasonal variation in monoterpene content (Kelsey et al. 1982). Models were compared to one another and to a null (intercept-only) model using information-theoretic methods, for each species separately. Model comparison and final analysis were the same for plant use as they were for patch use.

To address selection of plants occurring at finer scales, I evaluated whether biomass gained or PSMs consumed per bite differed between species of sagebrush at our site. For ten plants for each species of sagebrush, I clipped leaves off each plant to mimic

browsing by sage-grouse. Clipped leaves were weighed with an analytical balance to assess the average amount of biomass consumed per bite. This may indicate which plant provides the greatest benefit (or cost) per bite, based on the biomass available in each bite. I estimated the concentration of PSMs and crude protein consumed per bite for each species as the product of biomass per bite and the average concentration of monoterpenes (AUC/100 μg dry weight) or protein concentration, respectively, for each species.

Selection thresholds were explored using a generalized additive model (GAM) and smoothing parameters, using data from both sagebrush species together. Top parameters from plant-scale analysis (Δ $AIC_c \leq 2$) that best predicted browse were modeled independently. These predictors included plant height, crude protein, number of monoterpene compounds, and one individual monoterpene (Unknown 21.5). Parameters (protein, number of compounds) with confidence intervals that overlap 1.0 (Table 1.9) do not produce regressions with reliable confidence intervals. The average value for each parameter within the patch was calculated by averaging browsed and non-browsed plant values within the used patch. Selection was determined by the difference between the average parameter values between browsed and non-browsed plants within the used patch. Positive differences (higher values in browsed plants than non-browsed plants) were considered to theoretically indicate selection for a parameter, while negative differences (lower values in browsed plants than non-browsed plants) indicated theoretical selection against a parameter. Values of zero indicated no selection. This analysis allowed me to determine if there was a particular threshold across the range of average values for each parameter within the patch where selection occurred. Models were plotted with 95% Bayesian confidence intervals using the package {mgcv} in R.

Results

Diet Quality and Structure Comparison by Species

The phytochemistry of three-tip sagebrush differed from Wyoming big sagebrush (Table 1.2, Figure 1.3), and both species had uniquely identifiable monoterpene profiles (Appendix E). Briefly, three-tip sagebrush had almost 1.5 times higher concentrations of total monoterpenes, and higher concentrations of camphene and monoterpene Unknown 21.0 than Wyoming big sagebrush (Figure 1.4). Wyoming big sagebrush had higher concentrations of β-pinene, 1,8-cineole, and monoterpene Unknown 21.5 than three-tip sagebrush. Wyoming big sagebrush had 1.5 times as many individual monoterpenes as three-tip sagebrush. Three-tip sagebrush had lower crude protein, lower total phenolic concentrations, and higher coumarin concentrations than Wyoming big sagebrush. Three-tip sagebrush (mean ± SE: 30.92 ± 2.50 cm) was shorter than Wyoming big sagebrush (mean ± SE: 52.72 ± 3.90 cm; ANOVA: $F_{1,41} = 13.6518$, $P = 0.001$). Despite these chemical and structural differences, the number of bite marks by sage-grouse per plant did not differ between species ($Z_{41} = -0.53765$, $P = 0.5908$).

Winter Habitat Selection at the Landscape Scale

Sage-grouse selection of foraging sites was not influenced by the presence of either Wyoming big sagebrush or three-tip sagebrush (Table 1.3; Chi-squared: $\chi^2 = 1.286$, $P = 0.526$), as grouse used both species in proportion to their availability. Availability varied for each patch type: Wyoming big sagebrush was available at 50% of patches, three-tip at 6% of patches, and the remaining patches (44%) had both species of sagebrush present ("mixed"; Table 1.3). Mixed patches could have any ratio of three-tip sagebrush to Wyoming big sagebrush.

Sagebrush cover was nearly two times greater at random patches (mean ± SEM: 10.0 ± 1.8%) than at used patches (5.6 ± 4.6%; ANOVA: $F_{1,30} = 4.3282$, $P = 0.046$), however sagebrush density did not differ between random (0.89 ± 0.22 plants/m^2) and used patches (0.67 ± 0.16 plants/m^2; ANOVA: $F_{1,30} = 0.705$, $P = 0.408$). The percent cover for each species of sagebrush (as opposed to total shrub cover) did not differ between used and random patches. Additionally, average sagebrush height for the patch was not significantly taller at random patches (53.1 ± 23.2 cm) than at used patches (38.9 ± 22.6 cm; ANOVA: $F_{1,30} = 2.689$, $P = 0.111$).

Winter Habitat Selection at the Patch Scale

Habitat selection at the patch scale was analyzed for each sagebrush species using logistic regression and AIC_c model selection. For Wyoming big sagebrush, selection of patches was most influenced by average plant height (Table 1.4; Figure 1.5). Odds of patch use declined by a factor of 0.92 for every 1 cm increase in plant height. Percent cover for Wyoming sagebrush plants and the concentration (AUC/ 100 μg dry weight) of monoterpene Unknown 21.0 were the only other model parameters that performed better than the null model, although neither model fell within 2 Δ AIC_c units of the top model. The 85% confidence interval overlapped 1.0 for the odds ratio for percent cover, and is therefore not a reliable predictor of use. Odds of patch use declined by a factor of 0.94 for every 1 AUC/ 100 μg dry weight (DW) increase in monoterpene Unknown 21.0.

The top model for three-tip sagebrush was the average concentration of phenolics (μmol/g dry weight) of plants within the patch, followed by average concentration of β-pinene (AUC/ 100 μg DW; Table 1.5, Figure 1.5). However, 85% confidence intervals for these parameters overlapped 1, indicating models were unreliable for predicting odds

of use. Although it did not fall within 2 Δ AIC_c units of the top model, the average height of three-tip plants in the patch was the only other model parameter that performed better than the null model. Odds of patch use decreased by a factor of 0.82 for every 1 cm increase in plant height. Odds ratio confidence intervals at the 85% level are reported in Table 1.6 for both Wyoming and three-tip sagebrush. Models with 85% confidence intervals that do not overlap 1 are the parameters for monoterpene Unknown 21.0 in Wyoming big sagebrush and height for both species of sagebrush.

Winter Habitat Selection at the Plant Scale

Monoterpene Unknown 21.5 and height were the strongest predictors of diet selection at the plant scale for Wyoming big sagebrush, and fit data better than the null model (Table 1.7). The odds of plant use decreased by a factor of 0.16 for every 1 AUC/ 100 μg dry weight (DW) increase in monoterpene Unknown 21.5. The model for plant height did not fall within 2 Δ AIC_c units of the top model, but was greater than 10% of the top model weight. The odds of plant use decreased by a factor of 0.96 for every 1 cm increase in plant height. Browsed plants were shorter than non-browsed plants and had lower concentrations of monoterpene Unknown 21.5 in Wyoming big sagebrush (Figure 1.6).

For three-tip sagebrush, the best predictors of plant use were the total number of major monoterpene compounds (compounds with an AUC > 1% of the total AUC in > 70% of samples, and retention time < 24 minutes), and percent crude protein (Table 1.8). However, 85% confidence intervals for these parameters overlapped 1, indicating models were unreliable for predicting odds of use. Browsed plants had a higher number of monoterpene compounds than non-browsed plants and had higher concentrations of crude

protein in three-tip sagebrush (Figure 1.6). Odds ratio confidence intervals at the 85% level are reported in Table 1.9 for both Wyoming and three-tip sagebrush. Only parameters for Wyoming sagebrush (plant height, monoterpene Unknown 21.5) had 85% confidence intervals not overlapping 1.0, and were the models selected for GAM analysis.

Diet Selection at the Bite Scale

The approximate biomass per bite of three-tip sagebrush (mean ± SEM; 0.0201 ± 0.0008 g/bite) was smaller than Wyoming sagebrush (mean 0.0290 ± 0.00016 g/bite; ANOVA: $F_{1,35} = 27.167$, $P < 0.001$). Therefore, Wyoming big sagebrush provides greater biomass intake per bite than three-tip. However, there was no difference in the average concentrations of PSM per bite between species (ANOVA: $F_{1,35} = 0.0925$, $P = 0.763$), due to the relatively small bite size and high PSM concentration per gram for three-tip sagebrush, and a relatively large bite size and low PSM concentration per gram for Wyoming big sagebrush. Average crude protein per bite was higher for Wyoming big sagebrush (mean ± SEM: 0.3851 ± 0.0212 % crude protein per bite) than for three-tip sagebrush (0.2065 ± 0.0085 % protein per bite; ANOVA: $F_{1,35} = 68.772$, $P < 0.001$).

Thresholds of Selection

Generalized additive models (GAMs) were used to explore the threshold of selection for the two best-performing parameters at the plant scale for each sagebrush species: plant height, monoterpene Unknown 21.5, crude protein, and number of monoterpene compounds. For plant height, plant scale selection drastically declined around 55 cm (Figure 1.7). For monoterpene Unknown 21.5, plant scale selection declined steadily at concentrations around 8 AUC/ 100 μg dry weight (Figure 1.8). The

GAMs for crude protein and number of monoterpene compounds did not show any relationships between the patch average and the difference between browsed and non-browsed plants, and had wide confidence intervals. Thus, the GAMs did not help to identify any meaningful threshold of selection for top parameters from modeling top diet selection parameters from three-tip sagebrush model selection. Modeling GAMs with both sagebrush species together and independently did not improve model confidence intervals for these two parameters.

Discussion

Phytochemistry differs between three-tip sagebrush and Wyoming big sagebrush, however sage-grouse did not appear to selectively forage on either species at a landscape scale. I documented different chemical profiles for the two species of sagebrush examined. The chemical profiles for three-tip sagebrush and Wyoming big sagebrush at Craters are unique and individual compound concentrations are significantly different between species. To our knowledge, the chemistry (besides protein) of three-tip sagebrush has not previously been documented. The concentration of protein for Wyoming big sagebrush fell within the range documented previously. Because gas chromatograph detectors vary in their ability to detect compounds, and retention times may shift over years, I could not accurately compare monoterpene concentrations among existing studies without using the same standards for comparison. Therefore, I focused on comparing protein content documented in other studies.

Wyoming big sagebrush plants at Craters had similar crude protein content to plants at Brown's Bench (mean ± SE: Craters 10.32 ± 0.31 %; Brown's Bench 10.58 ± 0.15%, from Frye et al. 2013). The crude protein detected in three-tip sagebrush at

Craters (10.32 ± 0.31%) is within the range of protein found in other species of sagebrush (Table 1.10; range of 9.3 to 16.2% for *A. tridentata* spp., *A. nova*, and *A. arbuscula*), and slightly above crude protein of *A. tripartita* from Dubois, Idaho (8.4 ± 0.1%, from Fraker-Marble et al. 2007).

Despite relatively higher concentrations of PSMs and relatively lower protein concentrations in three-tip compared to Wyoming, sage-grouse browsed on three-tip sagebrush at our site relative to availability across the landscape. However, I did not evaluate the relationship between biomass availability (volume of foliage) and plant selection within a patch, which may address habitat or diet selection at smaller scales. There was also no difference the number of bite marks per plant for each species, and no difference in PSMs consumed per bite. However, Wyoming big sagebrush had higher crude protein content per bite than three-tip sagebrush. Because the concentration of PSMs was equal per bite for each species, neither species provides a low PSM per bite resource, assuming equal bites per plant for each species. However, the higher crude protein per bite of Wyoming big sagebrush suggests that Wyoming big sagebrush is a foraging choice that may be more nutrient efficient. However, I did not find evidence for grouse selecting Wyoming big sagebrush more than it is available (e.g. selectively foraging) at the species-level or in the number of bites taken per plant. This apparent lack of species-level selection was unexpected, since previous literature has documented that herbivores select plants with relatively lower PSM concentrations and higher protein concentrations (Stolter et al. 2005, DeGabriel et al. 2009, Youngentob et al. 2011, Frye et al. 2013, Ulappa et al. 2014), which suggests that Wyoming big sagebrush should be a more valuable food resource.

This unexpected lack of selection for particular species may be due to trade-offs among phytochemicals, since effects of consuming PSMs are dose-dependent and only certain monoterpenes may have negative effects on physiology. While Wyoming big sagebrush had higher crude protein, it also had higher total phenolics, a greater diversity of monoterpenes, and higher concentrations of 12 individual monoterpenes than three-tip. However, three-tip had a higher overall concentration of monoterpenes driven by five individual monoterpenes that were higher than in Wyoming sagebrush. A specific chemical, concentration, or even particular mixture of chemicals may be a deterrent. For example, 1,8-cineole and camphor, but not α-pinene, β-pinene, or camphene inhibited digestive enzymes in sage-grouse, which may influence selection behavior (Kohl et al. 2015). It is possible that consuming both species of sagebrush allows sage-grouse to diversify the PSMs consumed, which may minimize overloading any one detoxification pathway (Marsh et al. 2006). For example, once the threshold for a particular PSM in Wyoming big sagebrush is reached (e.g. monoterpene Unknown 21.5), sage-grouse may benefit from consuming three-tip that has a lower concentrations of that chemical. The benefit of higher protein content in Wyoming big sagebrush may be offset by some of the unique chemicals or higher concentrations of particular monoterpenes, which can be mitigated by consuming three-tip. Captive feeding trials, like those recently conducted on another sagebrush specialist, the pygmy rabbit (*Brachylagus idahoensis*, Camp et al. 2015) are required to test tradeoffs among phytochemicals and other plant characteristics (e.g. cover). These choice trials can complement diet selection studies on free-ranging herbivores by providing necessary ranking of parameters that best predict diet selection.

The unexpected lack of selection for particular species may also be due to overall low shrub availability. Arkle et al. (2014) found that 10-20% dwarf sagebrush cover and 10-15% Wyoming big sagebrush cover for a combined 20-35% cover best predicted sage-grouse occupancy. For comparison with other diet selection studies, Brown's Bench, Idaho (Frye et al. 2013) had mean sagebrush canopy cover for live plants at 17.6% ± 4.0% (n = 110), whereas mean live sagebrush cover at Craters was 7.8% ± 6.3% (n = 32), less than half the cover available at Brown's Bench (Wilcoxon test: Z = -6.814, $P < 0.001$). The canopy cover for Brown's Bench falls within the recommended guidelines for sage-grouse winter habitat (10-30% canopy cover; Connelly et al. 2000), but Craters falls below the lower recommended limit. Similarly, sagebrush cover was higher at foraging sites in North Park, Colorado than at Craters, but cover was highly variable (45 – 87% cover; Remington and Braun 1985). This suggests that grouse habitat at Craters meets some of the fundamental niche requirements for grouse (e.g. food present, cover present), but may be sub-optimal habitat. Given the low cover, forage is a limited resource and may therefore be selected based on availability of shrubs rather than on structural or dietary quality of those shrubs.

Shrub height is important to herbivores because moderately sized plants allow herbivores to see approaching predators, while remaining relatively difficult to be seen. Therefore, grouse may not be using patches with cover that falls within recommended guidelines since sagebrush height exceeded the recommended winter guidelines (25-35 cm; Connelly et al. 2000) substantially at random sites. This pattern was also observed by Frye et al. (2013), in which plant height at used patches (33.3 ± 7.9 cm) was lower than random (42.7 ± 15.4 cm), which was driven by differences in species composition

between used and random sites. Additionally, Arkle et al. (2014) found that sage-grouse occupancy was lower at sites with very short or very tall plants, and that plant height between 40-55 cm best predicted occupancy. The recommended guidelines for winter habitat suggest sagebrush heights between 25 and 35 cm above snow are ideal for sage grouse (Connelly et al. 2000). Although three-tip sagebrush is less available (30% of shrubs at random patches, 35% of shrubs at used patches) than Wyoming big sagebrush (70% of shrubs at random patches, 65% of shrubs at used patches), it was within the recommended shrub height, whereas Wyoming big sagebrush was taller than recommended. The GAM analysis showed plant selection declined drastically at heights greater than 55 cm, which is above the recommended winter heights. Thirty-three percent of Wyoming big sagebrush plants were above 55 cm tall and only four percent of three-tip sagebrush plants were above 55 cm tall.

Grouse did not select habitat based on the presence of either species of sagebrush, however grouse did select for particular phytochemical and structural characteristics at smaller scales. At the patch-scale, used patches with Wyoming big sagebrush, plants had lower concentrations of monoterpene Unknown 21.0 and were shorter than at random patches. For patches with three-tip sagebrush, selected patches had shorter plants and relatively higher concentrations of phenolics and β-pinene than random patches. Total phenolics and β-pinene were not correlated with any other parameters that I measured, however they may be negatively correlated with other compounds (e.g. individual phenolics, sesquiterpene lactones) that were not measured, but may nonetheless influence foraging behavior more than parameters we did measure. The relationships between three-tip chemistry and use by grouse (both phenolics and β-pinene) were weak (odds of

use ratios overlapped 1.0 at the 85% confidence interval), indicating that those factors may not be good predictors of selection at the patch scale. If only strong relationships (85% confidence intervals for odds of use ratios not overlapping 1, and Δ $AIC_c < 2$) are considered, then the parameters influencing habitat use at the patch scale follow patterns previously documented in the literature. I documented grouse selecting patches with lower concentrations of monoterpenes (Unknown 21.0) and shorter plant height that was within habitat guidelines. Other studies have found that grouse selected patches with low PSM concentrations (Remington and Braun 1985) and selected shorter plants at used patches than random because the selected food at those sites was a dwarf sagebrush species (Frye et al. 2013).

Consistent with the patch scale, the best predictors for use of Wyoming big sagebrush at the plant scale were lower concentrations of monoterpenes (Unknown 21.5) and shorter plants. For three-tip sagebrush, higher protein and higher numbers of monoterpene compounds were the best predictors for use at the plant scale. Again, the relationship between plant selection and predictive parameters for three-tip sagebrush were weak. For strong parameters (odds ratio 85% confidence intervals do not overlap 1.0 for models, and Δ $AIC_c < 2$), selection matched previous literature on grouse diet selection, with grouse selecting plants with lower PSMs (monoterpene Unknown 21.5), higher nutrient concentration (protein), and moderate plant heights (Remington and Braun 1985, Frye et al. 2013). In contrast, grouse selected for higher diversity of monoterpenes in three-tip sagebrush, although the relationship was weak, and total number of compounds in three-tip was significantly lower than the number in Wyoming big sagebrush. It may be beneficial for animals to decrease exposure to any single

compound by selecting for a greater diversity of PSMs (Dearing and Cork 1999, Marsh et al. 2006). Total monoterpene concentrations were weakly negatively correlated with PSM diversity for Wyoming big sagebrush (r = -0.5718) and three-tip (r = -0.1762). This relationship supports studies in captive herbivores showing that detoxification pathways are less likely to be overloaded by consuming lower concentrations of a higher number of individual PSMs (Marsh et al. 2006).

This is the first formal documentation of sage-grouse eating three-tip sagebrush. Although Lowe et al. (2009) found that sage-grouse hens do not select three-tip for nest cover, my study found that three-tip may be an acceptable food resource for sage-grouse during winter. However, acceptable food does not always translate to optimal food or optimal habitats. Future studies are necessary to determine if consumption of three-tip sagebrush impacts population parameters, such as reproductive success. While reproductive parameters have not been evaluated at this site yet, winter flock sizes at Craters are smaller than flock sizes at other sites in Idaho with current (unpublished data) or previous studies (Frye et al. 2013) on diet selection (mean ± SEM: 4.3 ± 0.7 birds per flock at Craters compared to 19.9 ± 2.4 at Brown's Bench, 32.4 ± 9.0 at Owyhee Mountains, 12.6 ± 2.1 at Raft River; ANOVA: $F_{3,161} = 7.195$, $P < 0.001$) where dwarf species of sagebrush were available. This may indicate that large flock sizes are unable to persist in current conditions because grouse at Craters are occupying sub-optimal habitat. The availability and use of three-tip as forage may become increasingly important because three-tip sagebrush can re-sprout after fire (Passey and Hugie 1962, Lesica et al. 2007), therefore allowing it to re-establish more quickly after fires than big sagebrush (Beck et al. 2009). With a warming climate and projected increases in the fire

frequency across the Great Basin, three-tip sagebrush may expand its range (Baker 2006, Dalgleish et al. 2011). Although sage-grouse do consume three-tip, my study did not test whether three-tip can replace other species of sagebrush for sage-grouse or other species reliant on sagebrush for food. Additional studies are needed to understand how wildlife may use or select three-tip sagebrush for food and cover relative to other species across its range, and how dietary quality influences fitness for herbivores.

Literature Cited

Ainsworth, E., and K. Gillespie. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nature Protocols 2:875-877.

Aldridge, C. L., S. E. Nielsen, H. L. Beyer, M. S. Boyce, J. W. Connelly, S. T. Knick, and M. A. Schroeder. 2008. Range-wide patterns of Greater Sage-grouse persistence. Diversity and Distributions 14:983-994.

Anderson, T. M., J. G. C. Hopcraft, S. Eby, M. Ritchie, J. B. Grace, and H. Olff. 2010. Landscape-scale analyses suggest both nutrient and anti-predator advantages to Serengeti herbivore hotspots. Ecology 91:1519-1529.

Arkle, R. S., D. S. Pilliod, S. E. Hanser, M. L. Brooks, J. C. Chambers, J. B. Grace, K. C. Knutson, D. A. Pyke, J. L. Welty, and T. A. Wirth. 2014. Quantifying restoration effectiveness using multi-scale habitat models: implications for sage-grouse in the Great Basin. Ecosphere 5:1-32.

Au, J., K. J. Marsh, I. R. Wallis, and W. J. Foley. 2013. Whole-body protein turnover reveals the cost of detoxification of secondary metabolites in a vertebrate browser. Journal of Comparative Physiology B: Biochemical Systemic and Environmental Physiology 183:993-1003.

Baker, W. L. 2006. Fire and restoration of sagebrush ecosystems. Wildlife Society Bulletin 34:177-185.

Barnett, J., and J. Crawford. 1994. Pre-laying nutrition of sage-grouse hens in Oregon. Journal of Range Management 47:114-118.

Beck, J., J. Connelly, and K. Reese. 2009. Recovery of Greater Sage-grouse habitat features in Wyoming big sagebrush following prescribed fire. Restoration Ecology 17:393-403.

Beck, T. 1977. Sage-grouse flock characteristics and habitat selection in winter. Journal of Wildlife Management 41:18-26.

Boegh, E., H. Soegaard, N. Broge, C. B. Hasager, N. O. Jensen, K. Schelde, and A. Thomsen. 2002. Airborne multispectral data for quantifying leaf area index, nitrogen concentration, and photosynthetic efficiency in agriculture. Remote Sensing of Environment 81:179-193.

Bruce, J., W. Robinson, S. Petersen, and R. Miller. 2011. Greater Sage-grouse movements and habitat use during winter in central Oregon. Western North American Naturalist 71:418-424.

Burnham, K.P., and D.R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York, USA.

Camp, M. J., L. A. Shipley, T.R. Johnson, J. S. Forbey, J. L. Rachlow, and M. M. Crowell. 2015. Modeling trade-offs between plant fiber and toxins: a framework for quantifying risks perceived by foraging herbivores. Ecology 92:3292-3302.

Canfield, R. H. 1941. Application of the line interception method in sampling range vegetation. Journal of Forestry 39:388-394.

Chastel, O., H. Weimerskirch, and P. Jouventin. 1995. Influence of body condition on reproductive decision and reproductive success in the Blue Petrel. Auk 112:964-972.

Connelly, J., H. Browers, and R. Gates. 1988. Seasonal movements of sage-grouse in southeastern Idaho. Journal of Wildlife Management 52:116-122.

Connelly, J., M. Schroeder, A. Sands, and C. Braun. 2000. Guidelines to manage sage-grouse populations and their habitats. Wildlife Society Bulletin 28:967-985.

Connelly, J. W., S. T. Knick, M. A. Schroeder, and S. J. Stiver. 2004. Conservation Assessment of Greater Sage-grouse and Sagebrush Habitats. *in* Western Association of Fish and Wildlife Agencies, Cheyenne, Wyoming, USA.

Dalgleish, H. J., D. N. Koons, M. B. Hooten, C. A. Moffet, and P. B. Adler. 2011. Climate influences the demography of three dominant sagebrush steppe plants. Ecology 92:75-85.

Dalke, P. D., D. B. Pyrah, D. C. Stanton, J. E. Crawford, and E. F. Schlatterer. 1963. Ecology, productivity, and management of Sage-grouse in Idaho. Journal of Wildlife Management 27:810-841.

Dearing, M. D., and S. Cork. 1999. Role of detoxification of plant secondary compounds on diet breadth in a mammalian herbivore, *Trichosurus vulpecula*. Journal of Chemical Ecology 25:1205-1219.

DeGabriel, J., B. Moore, W. Foley, and C. Johnson. 2009. The effects of plant defensive chemistry on nutrient availability predict reproductive success in a mammal. Ecology 90:711-719.

Forbey, J. S., N. L. Wiggins, G. G. Frye, and J. W. Connelly. 2013. Hungry grouse in a warming world: emerging risks from plant chemical defenses and climate change. Wildlife Biology 19:374-381.

Fraker-Marble, M. J., K. L. Launcbbaugh, and J. W. Walker. 2007. Differences in food ingestion and digestion among sheep classified as high or low sagebrush consumers. Rangeland Ecology and Management 60:191-194.

Freeman, D. C., W. A. Turner, E. D. McArthur, and J. H. Graham. 1991. Characterization of a narrow hybrid zone between 2 subspecies of big sagebrush (*Artemisia tridentata*, Asteraceae). American Journal of Botany 78:805-815.

Frye, G. G. 2012. Phytochemical ecology of an avian herbivore, the Greater Sage-grouse: implications for behavior, physiology, and conservation. Thesis, Boise State University, Boise, USA.

Frye, G. G., J.W. Connelly, D. D. Musil, and J. S. Forbey. 2013. Phytochemistry predicts habitat selection by an avian herbivore at multiple spatial scales. Ecology 94:308-314.

Fryer, J. L. 2009. *Artemisia nova*. *in* Fire Effects Information System. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory. <http://www.fs.fed.us/database/feis/plants/shrub/artnov/all.html>. Accessed 23 May 2015.

Garton, E. O., J. W. Connelly, J. S. Horne, C. A. Hagen, A. Moser, and M. A. Schoeder. 2011. Greater Sage-grouse population dynamics and probability of persistence. *in* S. T. Knick, and J.W. Connelly, editors. Greater Sage-grouse: ecology and conservation of a landscape species and its habitats. Studies in Avian Biology. University of California Press, Berkeley, CA.

Geisen, K. M., T. J. Schoenberg, and C. E. Braun. 1982. Methods for trapping sage-grouse in Colorado. Wildlife Society Bulletin 20:425-426.

Gregg, M., J. Barnett, and J. Crawford. 2008. Temporal variation in diet and nutrition of pre-incubating Greater Sage-grouse. Rangeland Ecology and Management 61:535-542.

Guglielmo, C., W. Karasov, and W. Jakubas. 1996. Nutritional costs of a plant secondary metabolite explain selective foraging by Ruffed Grouse. Ecology 77:1103-1115.

Hagen, C., M. Willis, E. Glenn, and R. Anthony. 2011. Habitat selection by Greater Sage-grouse during winter in southeastern Oregon. Western North American Naturalist 71:529-538.

Hornaday, W. T. 1916. Save the Sage Grouse from Extinction: A Demand from Civilization to the Western States. Volume 5. Permanent Wild Life Protection Fund: New York Zoological Park, New York City, USA.

Hosmer, D. W., and S. Lemeshow. 1985. Goodness-of-fit tests for the logistic-regression model for matched case-control studies. Biometrical Journal 27:511-520.

Kelsey, R. G., J. R. Stephens, and F. Shafizadeh. 1982. The chemical constituents of sagebrush foliage and their isolation. Journal of Range Management 35:617-622.

Klebenow, D. A., and G. M. Gray. 1968. Food habits of juvenile sage-grouse. Journal of Range Management 21:80-83.

Kohl, K. D., E. Pitman, B. C. Robb, J. W. Connelly, M. D. Dearing, and J. S. Forbey. 2015. Monoterpenes as inhibitors of digestive enzymes and counter-adaptations in a specialist avian herbivore. Journal of Comparative Physiology B: Biochemical Systemic and Environmental Physiology 185:425-434.

Lesica, P., S. V. Cooper, and G. Kudray. 2007. Recovery of big sagebrush following fire in southwest Montana. Rangeland Ecology and Management 60:261-269.

Lowe, B., D. Delehanty, and J. Connelly. 2009. Greater Sage-grouse (*Centrocercus urophasianus*) use of threetip sagebrush relative to big sagebrush in south-central Idaho. Wildlife Biology 15:229-236.

Marsh, K. J., I. R. Wallis, R. L. Andrew, and W. J. Foley. 2006. The detoxification limitation hypothesis: Where did it come from and where is it going? Journal of Chemical Ecology 32:1247-1266.

Moore, B., I. Lawler, I. Wallis, C. Beale, and W. Foley. 2010. Palatability mapping: a koala's eye view of spatial variation in habitat quality. Ecology 91:3165-3176.

Passey, H. B., and V. K. Hugie. 1962. Sagebrush on Relict Ranges in the Snake River Plains and Northern Great Basin. Journal of Range Management 15:273-278.

Patterson, R. L. 1952. The Sage Grouse in Wyoming. Sage Books, Inc., Denver, CO, USA.

Remington, T., and C. Braun. 1985. Sage-grouse food selection in winter, North Park, Colorado. Journal of Wildlife Management 49:1055-1061.

Rosentreter, R. 2004. Sagebrush identification, ecology, and palatability relative to sage-grouse. Pages 1-14 *in* UDSA Forest Service Proceedings RMRS - P - 000.

Rosentreter, R. and R. G. Kelsey. 1991. Xeric big sagebrush, a new subspecies in the *Artemisia tridentata* complex. Journal of Range Management 44:330-335.

Schroeder, M. A., C. L. Aldridge, A. D. Apa, J. R. Bohne, C. E. Braun, S. D. Bunnell, J. W. Connelly, P. A. Deibert, S. C. Gardner, M. A. Hilliard, G. D. Kobriger, S. M.

McAdam, C. W. McCarthy, J. J. McCarthy, D. L. Mitchell, E. V. Rickerson, and S. J. Stiver. 2004. Distribution of Sage-grouse in North America. Condor 106:363-376.

Sinclair, A., C. Krebs, and J. Smith. 1982. Diet quality and food limitation in herbivores - the case of the snowshoe hare. Canadian Journal of Zoology 60:889-897.

Sorensen, J., and M. Dearing. 2006. Efflux transporters as a novel herbivore countermechanism to plant chemical defenses. Journal of Chemical Ecology 32:1181-1196.

Steinberg, P. D. 2002. *Artemisia arbuscula. in* U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory, <http://www.fs.fed.us/database/feis/>. Accessed 23 May 2015.

Stolter, C., J. Ball, R. Julkunen-Tiitto, R. Lieberei, and J. Ganzhorn. 2005. Winter browsing of moose on two different willow species: food selection in relation to plant chemistry and plant response. Canadian Journal of Zoology 83:807-819.

Tamburini, E., G. Ferrari, M. G. Marchetti, P. Pedrini, and S. Ferro. 2015. Development of FT-NIR models for the simultaneous estimation of chlorophyll and nitrogen content in fresh apple (*Malus domestica*) leaves. Sensors 15:2662-2679.

Thacker, E., D. Gardner, T. Messmer, M. Guttery, and D. Dahlgren. 2012. Using gas chromatography to determine winter diets of Greater Sage-grouse in Utah. Journal of Wildlife Management 76:588-592.

Tirmenstein, D. 1999. *Artemisia tripartita*. *in* Fire Effects Information System. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Library, <http://www.fs.fed.us/database/feis/plants/shrub/arttrp/all.html>. Accessed 23 May 2015.

Ulappa, A. C., R. G. Kelsey, G. G. Frye, J. L. Rachlow, L. A. Shipley, L. Bond, X. Z. Pu, and J. S. Forbey. 2014. Plant protein and secondary metabolites influence diet selection in a mammalian specialist herbivore. Journal of Mammalogy 95:834-842.

Vasquez, M. G. 1999. Determination of winter use of sagebrush subspecies by Gunnison Sage-grouse on Flat Top Mountain: Assessment for prescribed fire planning. Western State College of Colorado, Gunnison, Colorado, USA.

Wakkinen, W., K. Reese, J. Connelly, and R. Fischer. 1992. An improved spotlighting technique for capturing sage-grouse. Wildlife Society Bulletin 20:425-426.

Wambolt, C., M. Frisina, S. Knapp, and R. Frisina. 2006. Effect of method, site, and taxon on line-intercept estimates of sagebrush cover. Wildlife Society Bulletin 34:440-445.

Wambolt, C. L. 2001. Mule deer foraging preference among five sagebrush (*Artemisia* L.) taxa. Western North American Naturalist 61:490-494.

Welch, B. L., and D. McArthur. 1979. Variation in winter levels of crude protein among *Artemisia tridentata* subspecies grown in a uniform garden. Journal of Range Management 32:467-469.

Welch, B. L., and D. McArthur. 1981. Variation of monoterpenoid content among subspecies and accessions of *Artemisia tridentata* grown in a uniform garden. Journal of Range Management 34:380-384.

Youngentob, K., I. Wallis, D. Lindenmayer, J. Wood, M. Pope, and W. Foley. 2011. Foliage chemistry influences tree choice and landscape use of a gliding marsupial folivore. Journal of Chemical Ecology 37:71-84.

Tables

Table 1.1 Monoterpenes present at greater than 1% total AUC present in > 70% of samples (retention times < 24 minutes) for each species of sagebrush browsed by Greater Sage-grouse (*Centrocercus urophasianus*) at Craters, Idaho, USA in winter 2013-2014. Species present included Wyoming big sagebrush (*Artemisia tridentata wyomingensis*; ATW) and three-tip (*A. tripartita*; AT). Compounds were identified based on retention times (minutes) and co-chromatography with standards. Asterisks (*) indicate major compounds used in diet selection modeling at the plant and patch scale, for each species of sagebrush (see Methods for selection criteria).

Monoterpene	Approximate Retention Time (minutes)	Sagebrush Species
Unknown 3.2	3.20	AT, ATW
Unknown 3.6	3.65	ATW*
Unknown 11.9	11.88	ATW
Unknown 12.4	12.45	AT, ATW
α-pinene	12.95	AT, ATW
Camphene	13.50	AT*, ATW*
β-pinene	14.57	AT*, ATW*
α-phellendrine	15.61	ATW*
ρ-cymene	16.39	AT, ATW*
1,8-cineole	16.73	AT*, ATW
Unknown 18.2	18.28	ATW
Unknown 18.6	18.66	AT, ATW
Unknown 20.5	20.57	ATW
Camphor	20.74	ATW*
Unknown 21.0	21.08	AT*, ATW
Unknown 21.5	21.55	AT*, ATW*
Unknown 23.5	23.55	ATW

Table 1.2 Mean (95% confidence interval) concentrations for plant secondary metabolite (total and individual monoterpenes, total phenolics, and coumarins), nutrient content (% crude protein), structure (height), and use (number of bite marks per plant) in Wyoming big sagebrush (*Artemisia tridentata wyomingensis*, ATW) and three-tip (*Artemisia tripartita*, AT) at Craters, Idaho, USA. Use referred to browse by Greater Sage-grouse (*Centrocercus urophasianus*) during winter 2013-2014. Mean values, 95% confidence, and results from nonparametric univariate comparison (Kruskal-Wallis 2-sample test with normal approximation) tests are shown for each compound compared between species of sagebrush.

Parameter	Value in ATW	Difference	Value in AT	p-value	Z
*Total monoterpenes[1]	67.69 (63.56 – 71.83)	<	99.45 (91.67 – 107.42)	< 0.001	4.820
*Unknown 3.2[1]	27.02 (24.77 – 29.26)	>	15.18 (10.36 – 20.00)	< 0.001	-5.548
Unknown 3.6[1]	7.30 (6.22 – 8.37)	>	---[7]		
Unknown 11.9[1]	8.00 (5.44 – 10.56)	>	---[7]		
*Unknown 12.4[1]	2.13 (1.45 – 2.80)	<	10.45 (9.27 – 11.63)	< 0.001	6.960
*α-pinene[1]	0.48 (0.21 – 0.77)	<	6.64 (5.59 – 7.68)	< 0.001	5.198
*Camphene[1]	2.57 (2.01 – 3.41)	<	16.87 (14.35 – 19.39)	< 0.001	5.249
*β-pinene[1]	4.89 (3.47 – 6.31)	>	2.37 (0.72 – 4.00)	0.006	-2.760
*ρ-cymene[1]	5.30(3.91 – 6.68)	<	35.34 (31.68 – 39.00)	< 0.001	7.592
α-phellendrine[1]	2.81 (2.33 – 3.27)	>	---[7]		
*1,8-cineole[1]	3.71 (2.45 – 4.95)	>	0.97 (0.49 – 1.45)	< 0.001	-4.044
Unknown 18.2[1]	5.34 (4.47 – 6.02)	>	---[7]		
*Unknown 18.6[1]	12.17 (9.97 – 14.38)	>	4.67 (0.23 – 9.10)	< 0.001	-5.486
Unknown 20.5[1]	1.74 (1.13 – 2.36)	>	---[7]		
Camphor[1]	7.88 (6.61 – 9.16)	>	---[7]		
*Unknown 21.0[1]	1.76 (1.48 – 2.04)	<	54.28 (43.80 – 64.76)	< 0.001	4.497
*Unknown 21.5[1]	9.62(6.71 – 12.52)	>	1.58 (1.30 – 1.86)	< 0.001	-4.368
Unknown 23.5[1]	6.63 (4.12 – 9.14)	>	---[7]		
*Number compounds[2]	13.03 (12.33 – 13.72)	>	8.50 (7.62 – 9.37)	< 0.001	-4.968
*Total phenolics[3]	2975 (2595 – 3356)	>	2128 (1703 – 2553)	0.002	-3.178

Total coumarins[3]	3.16 (1.75 – 4.56)	=	4.48 (0.79 – 8.17)	0.294	1.500
*Crude protein[4]	13.26 (12.83 – 13.71)	>	10.31 (9.65 – 10.97)	< 0.001	-4.796
*Height[5]	52.22 (45.77 – 58.67)	>	29.09 (24.06 – 34.11)	< 0.001	-3.331
Use[6]	11.52 (6.60 – 16.44)	=	14.31 (3.97 – 26.65)	0.591	-0.538

***** Characteristic significantly different between species, $\alpha = 0.05$.

[1] Monoterpenes, concentration in AUC/ 100 µg dry weight (DW), numbers following "Unknown" refer to retention time in the chromatogram for the unknown compound and are identifying characteristics for each compound

[2] Total number of monoterpenes with retention times < 24 minutes, > 1% total AUC, and present in > 70% of samples for each taxa (Appendix C)

[3] Phenolics and coumarins, concentrations in µmol of scopoletin (coumarins) or gallic acid (phenolics) equivalents/ g DW

[4] Crude protein, % DW

[5] Height (cm)

[6] Use (number of bite marks by Greater Sage-grouse per plant)

[7] --- indicates a monoterpene that was below the limit of detection for that species

Table 1.3 Dominant cover types at foraging sites (Used) used by Greater Sage-grouse (*Centrocercus urophasianus*) and randomly-selected available sites (Random) during winter 2013-2014 at the Craters, Idaho, USA. Sagebrush species present included Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) and three-tip sagebrush (*A. tripartita*). Mixed sagebrush habitats included both Wyoming big sagebrush and three-tip sagebrush.

Habitat Type	Used	Random
Wyoming big sagebrush	6	8
Three-tip sagebrush	3	1
Mixed sagebrush	7	7
Total	**16**	**16**

Table 1.4 Model components, log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias-adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the selection models for Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) patches used by Greater Sage-grouse (*Centrocercus urophasianus*) flocks at Craters in southern Idaho, USA during winter 2013-2014. Patch use (used/random) was the binary response for each model. Top models, with < 2 ΔAIC_c from the top model for each predictor category and with an AIC_c value lower than the null model, are shown in **bold**. "Unknown" compounds are monoterpenes, identified by retention time.

Predictor category	Model	Log Likelihood	Number of Parameters (K)	AIC_c	ΔAIC_c	Akaike weight (w_i)
PSM	**Unknown 21.0**[1]	-18.74	2	41.94	0.00	0.16
	NULL	-20.08	1	42.32	0.38	0.13
	α-phellendrine[1]	-19.01	2	42.49	0.55	0.12
	Camphor[1]	-19.20	2	42.86	0.92	0.10
	Camphene[1]	-19.29	2	43.04	1.10	0.09
	Unknown 21.5[1]	-19.72	2	43.93	1.96	0.06
	Unknown 3.6[1]	-19.79	2	44.05	2.11	0.06
	Coumarins[2]	-19.92	2	44.30	2.36	0.05
	Total phenolics[2]	-19.94	2	44.34	2.40	0.05
	α-pinene[1]	-19.98	2	44.41	2.47	0.05
	β-pinene [1]	-20.08	2	44.62	2.68	0.04
	Cineole[1]	-20.08	2	44.62	2.68	0.04
	Number of monoterpenes[3]	-20.08	2	44.62	2.68	0.04
Nutrient	NULL	-20.08	1	42.32	0.00	0.76
	Protein[4]	-20.07	2	44.60	2.28	0.24
Structure	**Height**[5]	-13.64	2	31.74	0.00	0.97
	Percent cover	-18.48	2	41.42	9.68	0.01
	NULL	-20.08	1	42.32	10.58	0.00

[1] PSM: monoterpene compounds, AUC/ 100 μg dry weight (DW)

[2] PSM: total phenolics and coumarins (subclass of phenolics), μmol scopoletin equivalents or gallic acid equivalents/ g DW

[3] PSM: total number of monoterpenes with retention times < 24 min, > 1% total AUC, and present in > 70% of samples

[4] Nutrient: crude protein, %

[5] Structural variable: height, cm

Table 1.5 Model components, log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias-adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the selection models for three-tip sagebrush (*Artemisia tripartita*) patches used by Greater Sage-grouse (*Centrocercus urophasianus*) flocks at Craters in southern Idaho, USA during winter 2013-2014. Patch use (used/random) was the binary response for each model. Top models, with $< 2\ \Delta AIC_c$ from the top model for each predictor category, are shown in **bold**.

Predictor category	Model	Log Likelihood	Number of Parameters (K)	AIC_c	ΔAIC_c	Akaike weight (w_i)
PSM	**Total phenolics**[1]	-4.12	2	13.32	0.00	0.59
	β-pinene[2]	-4.49	2	14.18	0.85	0.38
	NULL	-9.56	1	21.45	7.38	0.01
	Number of monoterpenes[3]	-8.40	2	21.90	7.83	0.01
	Coumarins[1]	-9.28	2	23.66	9.59	0.00
	Camphene[2]	-9.33	2	23.76	9.69	0.00
	Cineole[2]	-9.56	2	24.20	10.13	0.00
Nutrient	NULL	-9.56	1	21.45	0.00	0.69
	Protein[4]	-9.00	2	23.10	1.64	0.31
Structure	**Height**[5]	-6.49	2	18.07	0.00	0.62
	NULL	-9.56	1	21.45	3.38	0.12

[1] PSM: total phenolics and coumarins (subclass of phenolics), μmol scopoletin equivalents or gallic acid equivalents/ g DW

[2] PSM: monoterpene compounds, AUC/ 100 μg dry weight (DW)

[3] PSM: total number of monoterpenes with retention times $\leq$ 24 min, > 1% total AUC, and present in $\geq$ 70% of samples

[4] Nutrient: crude protein, %

[5] Structural variable: height, cm

Table 1.6 The 95% (and 85%) confidence intervals for odds ratios predicting patch use by Greater Sage-grouse (*Centrocercus urophasianus*) foraging on Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) and three-tip sagebrush (*Artemisia tripartita*). Confidence intervals that overlap 1.0 are reported in *italics*, indicating parameters that do not predict odds of use reliably. Parameters in **bold** are top models from AIC_c model selection (models with $< 2\ \Delta\ AIC_c$ from the top model, with AIC_c values lower than the null model). Predictor variables are listed in order of increasing AIC_c value. "Unknown" compounds are monoterpenes, identified by retention time.

Species	Predictor Variable	Odds Ratio	85% Confidence Interval
Wyoming big sagebrush	**Height**	0.92	-0.13 to -0.04
	Unknown 21.0	0.94	-0.19 to 0.06
	Percent Cover	0.999	-25.24 to -1.67
Three-tip sagebrush	**Total Phenolics**	152.34	*-0.47 to 9.58*
	β-pinene	11.13	*0.41 to 4.40*
	Height	0.82	-0.34 to -0.05

Table 1.7 Model components, log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias-adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the diet selection models for Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) plants browsed by Greater Sage-grouse (*Centrocercus urophasianus*) at Craters in southern Idaho, USA during winter 2013-2014. Plant use (browsed/non-browsed) was the binary response for each model. Top models, with < 2 ΔAIC_c from the top model for each predictor category, are shown in **bold**. "Unknown" compounds are monoterpenes, identified by retention time.

Model	Log Likelihood	Number of Parameters (K)	AIC_c	ΔAIC_c	Akaike weight (w_i)
Unknown 21.5[1]	-10.02	1	22.10	0.00	0.31
Height[2]	-11.16	1	24.39	2.29	0.10
Unknown 3.6[1]	-11.17	1	24.41	2.31	0.10
Cineole[1]	-11.42	1	24.91	2.81	0.08
Protein[3]	-11.46	1	25.00	2.89	0.07
Number of monoterpenes[4]	-11.64	1	25.35	3.24	0.06
α-Phellendrine[1]	-11.67	1	25.41	3.31	0.06
Camphor[1]	-11.79	1	25.65	3.55	0.05
β-pinene[1]	-11.88	1	25.82	3.72	0.05
Total phenolics[5]	-11.94	1	25.95	3.85	0.04
Camphene[1]	-11.98	1	26.03	3.92	0.04
Coumarins[5]	-12.01	1	26.09	3.98	0.04
Null	-14.14	0	28.32	6.22	0.00

[1] PSM: monoterpene compounds, AUC/ 100 μg dry weight (DW)

[2] Structural variable: height, cm

[3] Nutrient: crude protein, %

[4] PSM: total number of monoterpenes with retention times ≤ 24 min, > 1% total AUC, and present in ≥ 70% of samples

[5] PSM: total phenolics and coumarins (subclass of phenolics), μmol scopoletin equivalents or gallic acid equivalents/ g DW

Table 1.8 Model components, log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias-adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the diet selection models for three-tip sagebrush (*Artemisia tripartita*) plants browsed by Greater Sage-grouse (*Centrocercus urophasianus*) at Craters in southern Idaho, USA during winter 2013-2014. Plant use (browsed/non-browsed) was the binary response for each model. Top models, with $< 2\ \Delta AIC_c$ from the top model for each predictor category, are shown in **bold**. "Unknown" compounds are monoterpenes, identified by retention time.

Model	Log Likelihood	Number of Parameters (K)	AIC_c	ΔAIC_c	Akaike weight (w_i)
Number of monoterpenes[1]	-5.55	1	13.27	0.00	0.29
Crude Protein[2]	-5.67	1	13.51	0.23	0.26
Unknown 21.5[3]	-6.97	1	16.11	2.83	0.07
Camphene[3]	-7.10	1	16.35	3.08	0.06
Coumarins[4]	-7.15	1	16.46	3.19	0.06
Unknown 21.0[3]	-7.17	1	16.51	3.23	0.06
β-pinene[3]	-7.29	1	16.75	3.47	0.05
Cineole[3]	-7.35	1	16.85	3.58	0.05
Height[5]	-7.44	1	17.05	3.77	0.04
Total phenolics[4]	-7.44	1	17.05	3.77	0.04
Null	-24.38	0	48.81	35.54	0.00

[1] PSM: total number of monoterpenes with retention times $\leq$ 24 min, > 1% total AUC, and present in $\geq$ 0% of samples

[2] Nutrient: crude protein, %

[3] PSM: monoterpene compounds, AUC/ 100 μg dry weight (DW)

[4] PSM: total phenolics and coumarins (subclass of phenolics), μmol scopoletin equivalents or gallic acid equivalents/ g DW

[5] Structural variable: height, cm

Table 1.9 The 95% (and 85%) confidence intervals for odds ratios predicting plant use by Greater Sage-grouse (*Centrocercus urophasianus*) foraging on Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) and three-tip sagebrush (*Artemisia tripartita*). Confidence intervals that overlap 1.0 are reported in *italics*, indicating parameters that do not predict odds of use reliably. Parameters in **bold** are top models from AIC_c model selection (models with $< 2\ \Delta\ AIC_c$ from the top model). Predictor variables are listed in order of increasing AIC_c value. "Unknown" compounds are monoterpenes, identified by retention time.

Species	Predictor Variable	Odds Ratio	85% Confidence Interval
Wyoming big sagebrush	**Unknown 21.5**	0.89	-0.22 to -0.02
	Height	0.96	-0.079 to 0.006
Three-tip sagebrush	**Number of compounds**	3.88	*-0.13 to 2.84*
	Protein	5.63	*0.15 to 3.31*

Table 1.10 Mean protein content (SEM) for sagebrush (*Artemisia*) species that have been previously evaluated for dietary quality. For Ulappa et al. (2014) data, high browse and low browse ranges are shown for each study site, comparing browse for pygmy rabbits (*Brachylagus idahoensis*). Values with * indicate that the range is a 95% confidence interval rather than SEM. Values with ** indicate that the resource did not list data in text or in tables, but instead displayed data graphically. Therefore, mean and SEM were estimated from summary graphs within the paper for these sources.

Sagebrush Taxa	Location	Percent Crude Protein (% dry weight)	Source
A. tripartita	Craters, Idaho	10.32 (0.31)	---
	Dubois, Idaho	8.4 (0.1)	Fraker-Marble et al. 2007
A. tridentata wyomingensis	Magic, Idaho	High browse: 11.61 (0.18)	Ulappa et al. 2014
		Low browse: 11.23 (0.21)	
	Lemhi, Idaho	High browse: 13.47 (0.26)	
		Low browse: 12.49 (0.22)	
	Southern Idaho (4 study sites)	10.86 (0.69)*	Frye 2012 (thesis)
	North Park, Colorado	14.2 (4.5)*	Remington and Braun 1985 **
	Common garden, Ephraim, Utah	11.8	Welch and McArthur 1979
	Harney County, Oregon	15.9 (0.43)	Barnett and Crawford 1994
	West-central Montana (Perma)	12.5	Kelsey et al. 1982
A. tridentata wyomingensis (continued)	Wyoming (Carmody, Cedar Rim)	12.9 (0.18)	Unpublished data
	Lander, Wyoming	Browsed: 17.4 (0.79)	Unpublished data
		Non-browsed: 17.0 (0.51)	
A. tridentata tridentata	West-central Montana (Ramsay)	13.1	Kelsey et al. 1982
	Common garden, Ephraim, Utah	14.5	Welch and McArthur 1979
A. tridentata vaseyana	Common garden, Ephraim, Utah	11.1	Welch and McArthur 1979
	North Park, Colorado	11.0 (2.0)*	Remington and Braun 1985 **
	Southern Idaho (4 study sites)	11.42 (0.69)*	Frye 2012 (thesis)
	West-central Montana (Missoula)	14.0	Kelsey et al. 1982

	Washington and Gem Counties, Idaho	9.3 (0.9)	Rosentreter and Kelsey 1991
	Southeastern Oregon (Lakeview)	10.44 (0.11)	Unpublished data
A.t. xericensis	Washington and Gem Counties, Idaho	10.4 (0.7)	Rosentreter and Kelsey 1991
A. arbuscula	Harney County, Oregon	14.2 (0.47)	Barnett and Crawford 1994
	Southern Idaho (4 study sites)	10.02 (0.24)*	Frye 2012 (thesis)
	Southeastern Oregon and northwestern Nevada	2002: 16.2 (0.5)	Gregg et al. 2008
		2003: 12.0 (0.1)	
	Southeastern Oregon (Lakeview)	9.97 (0.09)	Unpublished data
A. nova	Southern Idaho (4 study sites)	10.39 (0.44)*	Frye 2012 (thesis)

Figures

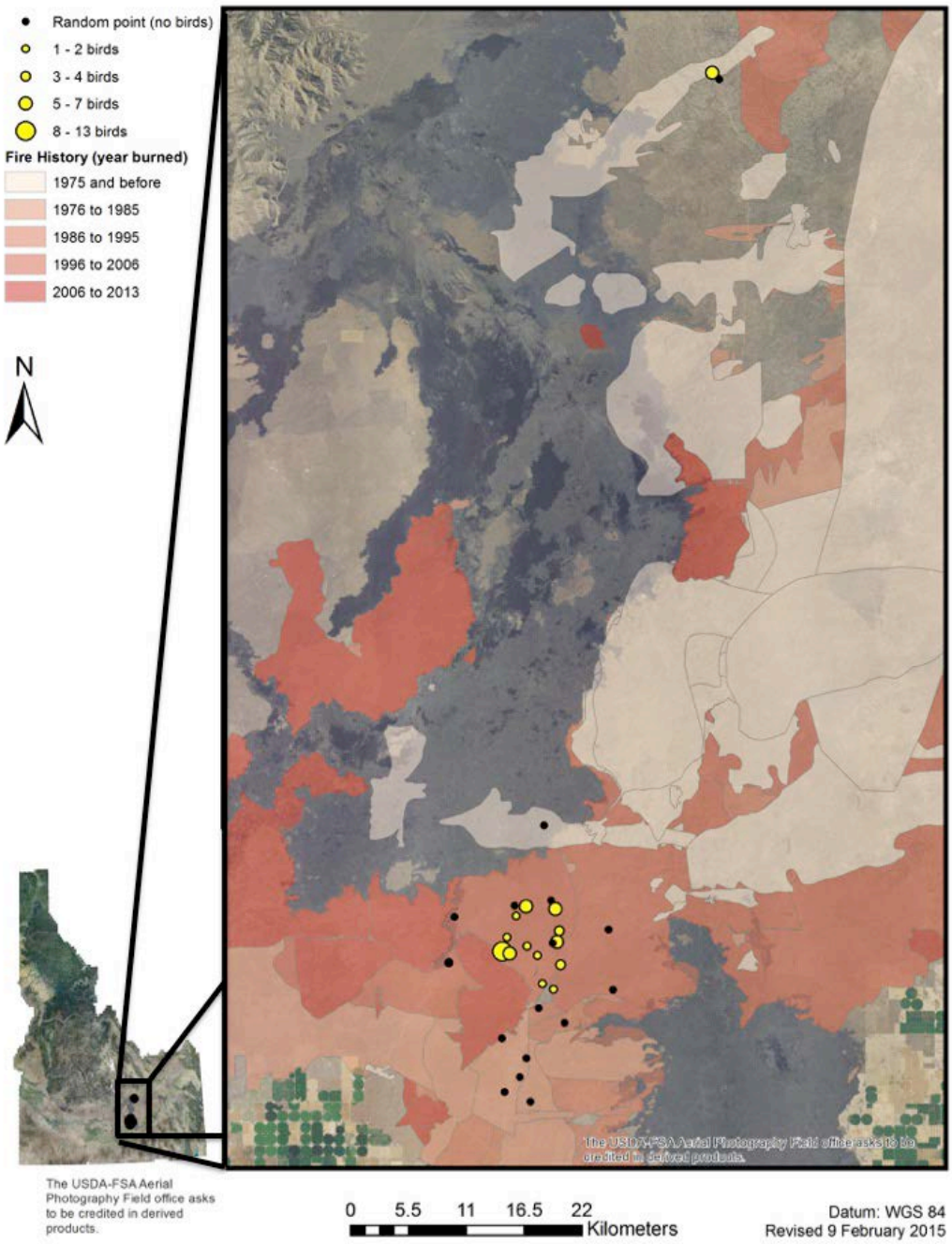


Figure 1.1 The Craters study site (42.958690 N, -113.398059 W), in central Idaho, USA, has an extensive fire history. Fire history data provided by Bureau of Land Management Burley Field Office (2015). Point size indicates the number of birds flushed from each foraging patch.

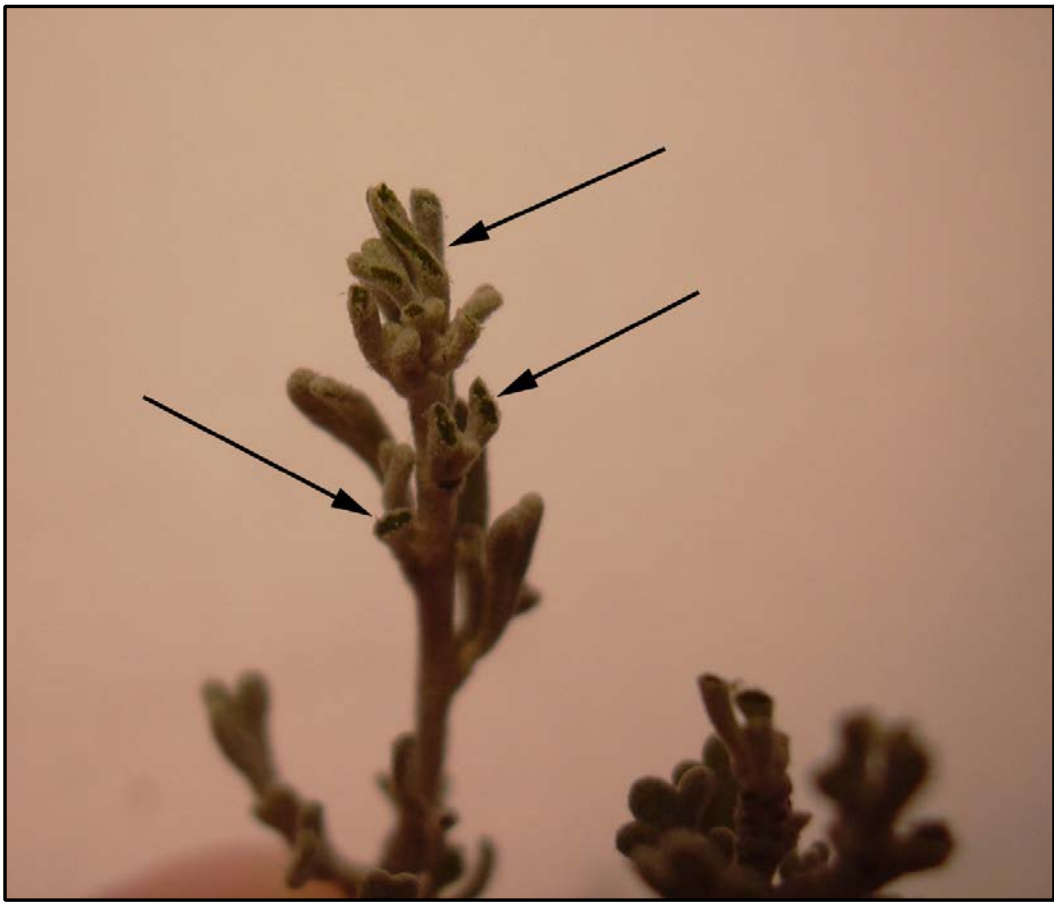


Figure 1.2 Fresh sage-grouse bite marks. The dark green leaf tissue indicates fresh browsing. Old browse turns brown after several days. Photo by Graham Frye (2012).

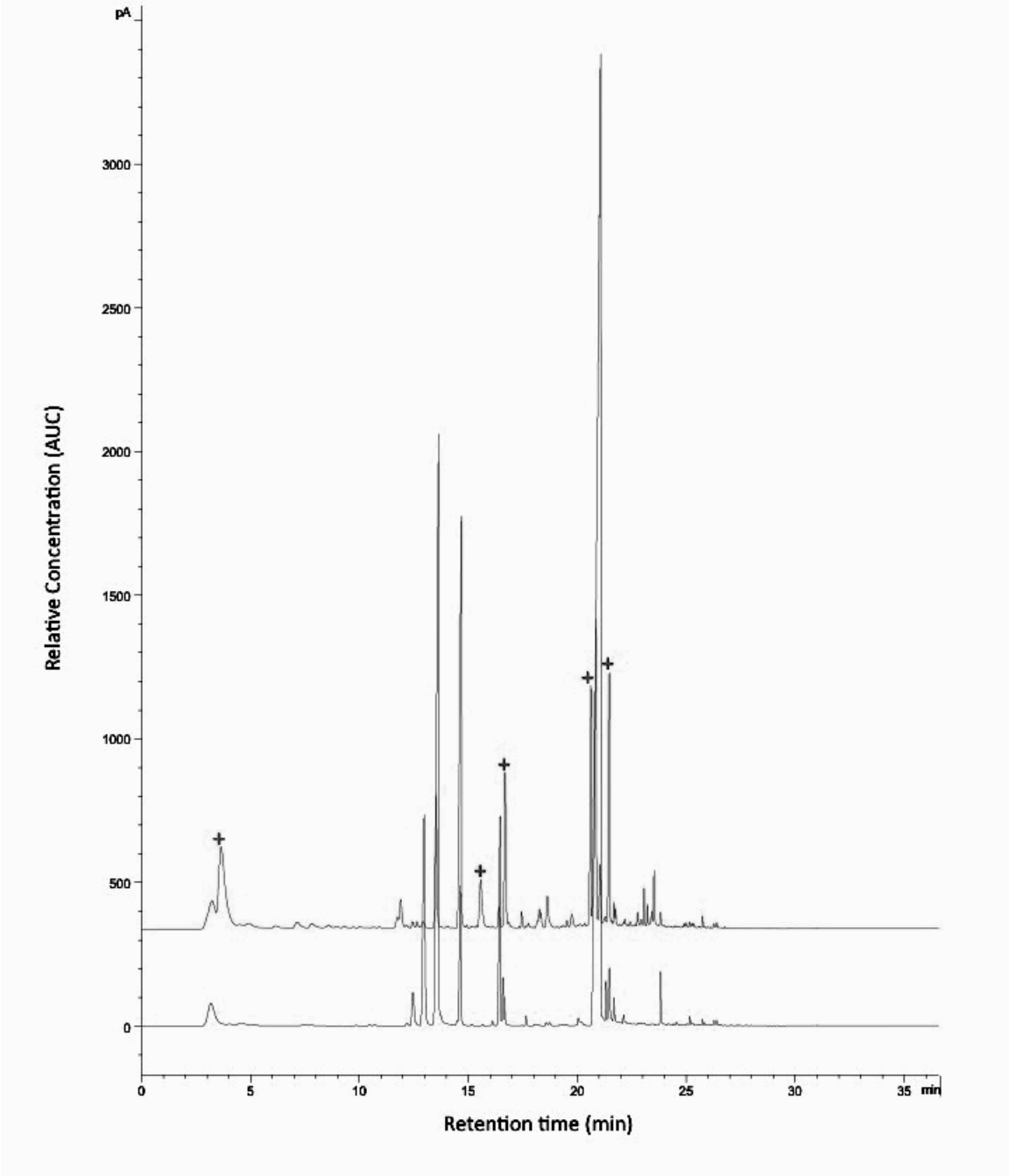


Figure 1.3 Representative monoterpene profiles for three-tip (bottom line; *Artemisia tripartita*) and Wyoming big sagebrush (top line; *A. tridentata wyomingensis*) from Craters, Idaho, USA. Peaks show individual compounds, with the height of the peak indicating relative abundance of the compound. Plus signs ($^{+}$) indicate compounds found only in Wyoming big sagebrush. There were no compounds in three-tip sagebrush that were not present in Wyoming big sagebrush.

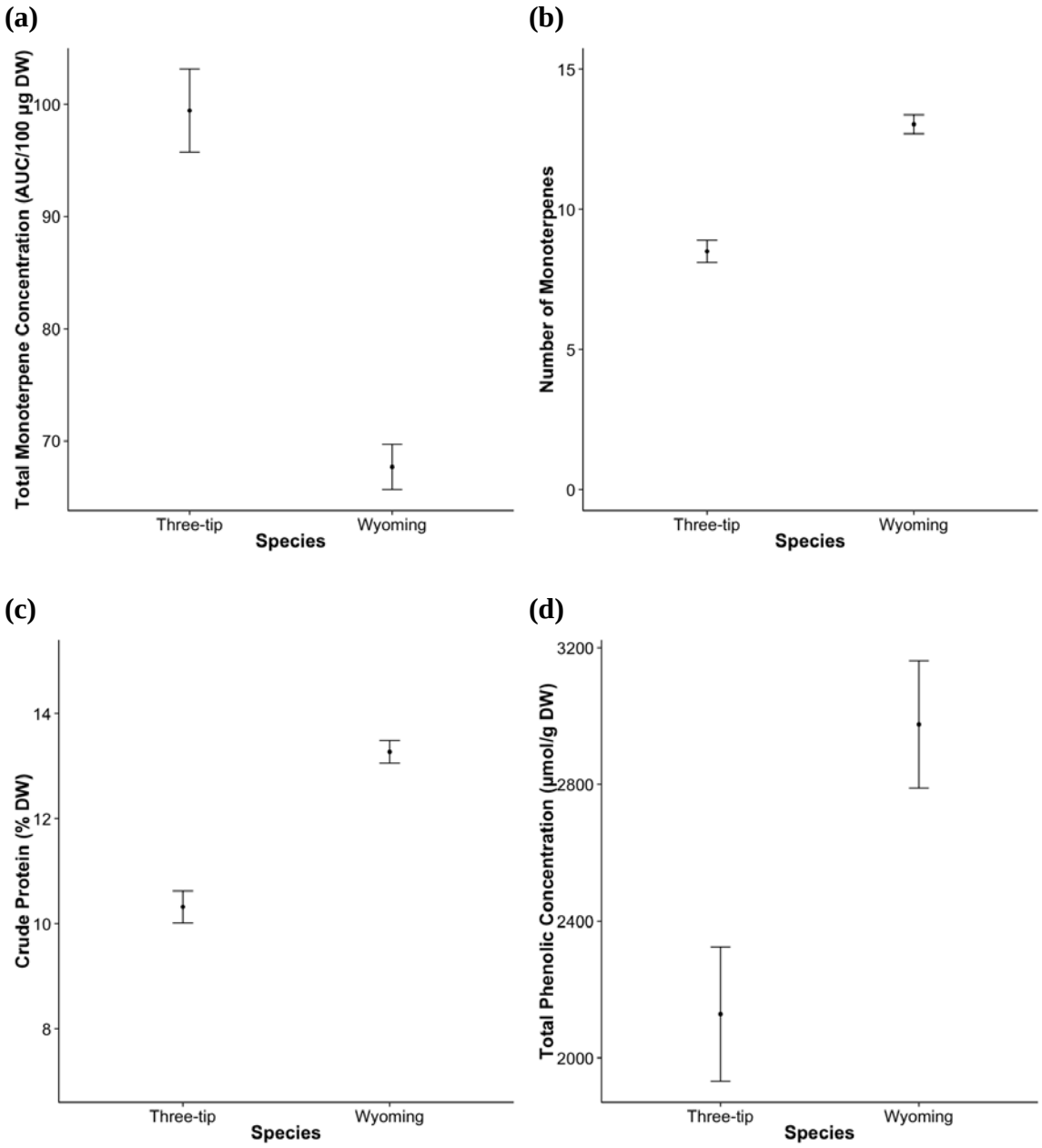


Figure 1.4 Mean ± SEM phytochemical characteristics for sagebrush samples collected at Greater Sage-grouse (*Centrocercus urophasianus*) foraging patches at Craters, Idaho. Samples were collected in winter 2013-2014 for Wyoming big sagebrush (*Artemisia tridentata wyomingensis*, n = 63) and three-tip sagebrush (*Artemisia tripartita*, n = 27). Chemical characteristics include: (a) total monoterpene concentrations (AUC/ 100 µg dry weight [DW]), (b) number of monoterpene compounds with retention times < 24 minutes and AUC > 1% of total AUC, (c) crude protein (%), and (d) total phenolic concentrations (µmol gallic acid equivalents/g DW).

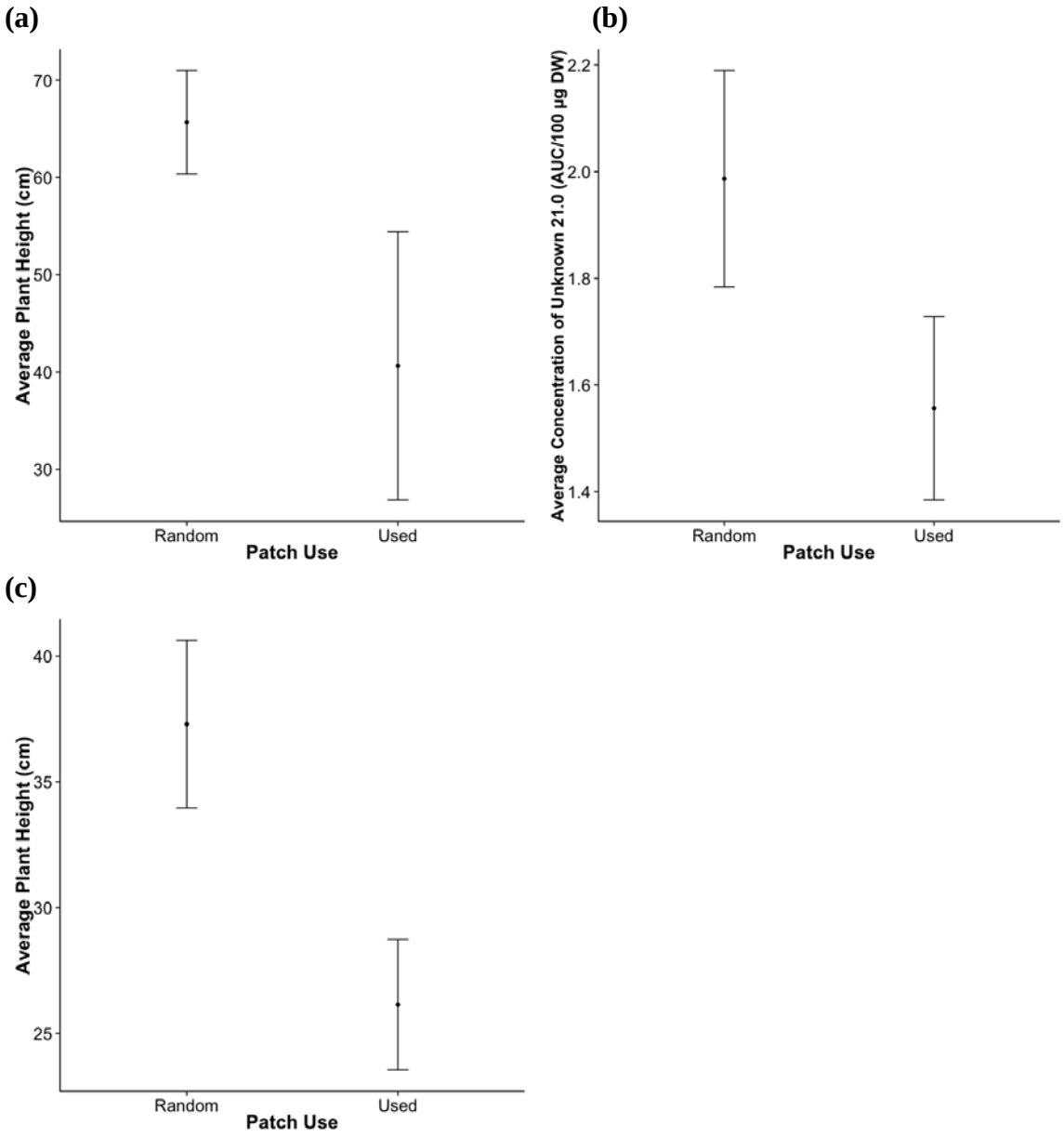


Figure 1.5 Plant height (cm; a) and concentration (AUC/ 100 µg dry weight) of monoterpene Unknown 21.0 (b) had the strongest influence on patch-scale selection for Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) by Greater Sage-grouse (*Centrocercus urophasianus*). Plant height (c) had the strongest influence on plant-scale selection for three-tip sagebrush (*Artemisia tripartita*) by Greater Sage-grouse (*Centrocercus urophasianus*). All graphs show mean ± SEM.

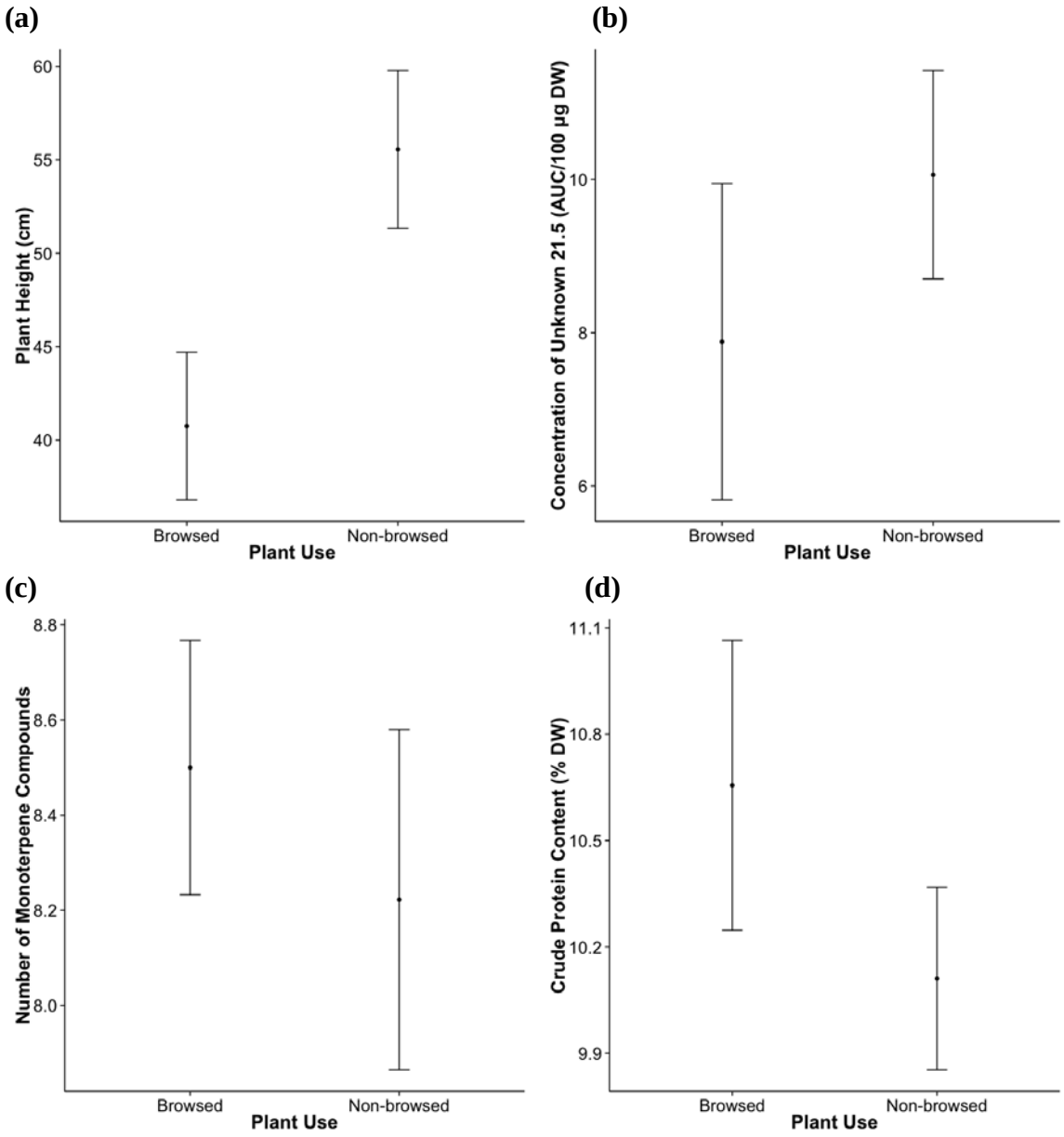


Figure 1.6 Plant height (cm; a) and concentration (AUC/ 100 µg dry weight) of monoterpene Unknown 21.5 (b) had the strongest influence on plant-scale selection for Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) by Greater Sage-grouse (*Centrocercus urophasianus*). The number of monoterpene compounds present (c), and percent crude protein (d) had the strongest influence on plant-scale selection for three-tip sagebrush (*Artemisia tripartita*) by Greater Sage-grouse (*Centrocercus urophasianus*). All graphs show mean ± SEM.

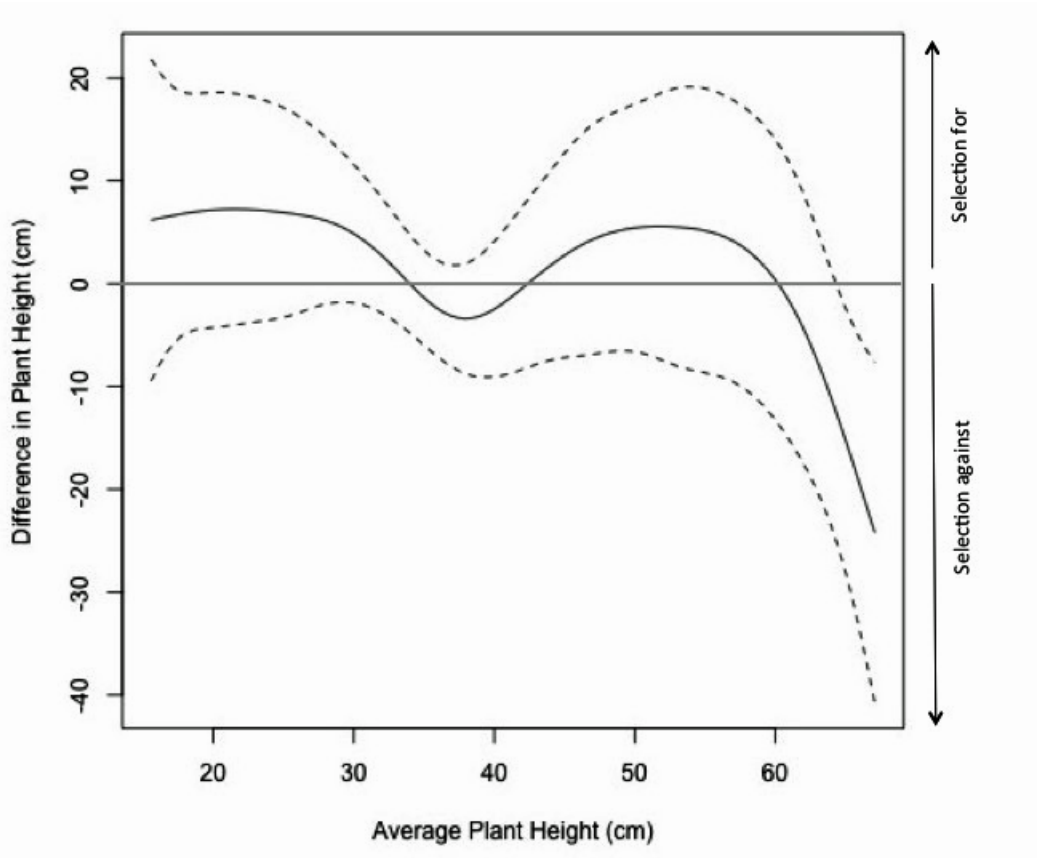


Figure 1.7 The difference in plant height (cm) between paired browsed and non-browsed sagebrush samples (n = 90) from Craters, Idaho, as a function of mean plant height (cm) for that patch (n = 16 used patches). Samples included both Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) and three-tip sagebrush (*A. tripartita*) browsed by Greater Sage-grouse (*Centrocercus urophasianus*). Values above zero are theoretically selected for, and indicate shorter browsed plants than non-browsed, while values below zero are selected against and indicate taller browsed plants. The gray line shows 0.0 on the y-axis, where no selection occurs. The solid black line shows the smoothed fit for the generalized additive model, and dashed lines show 95% confidence bands derived from the model.

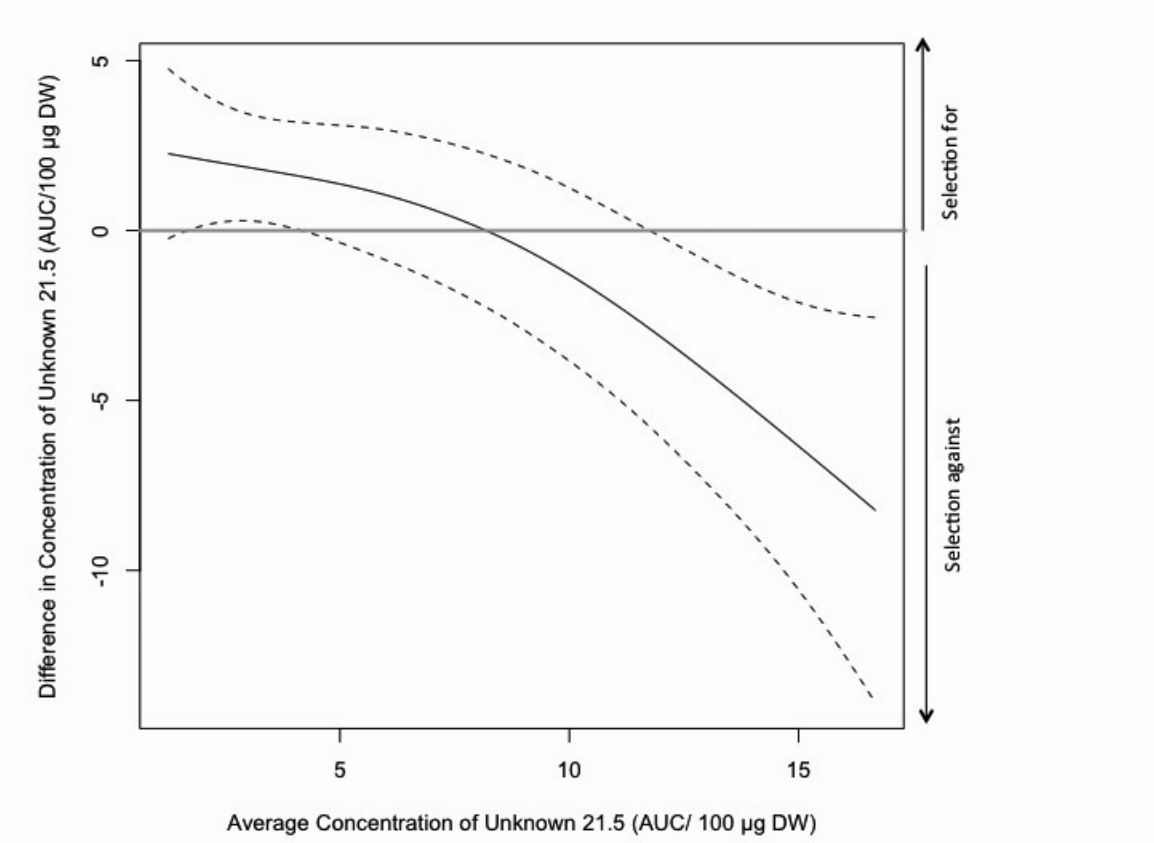


Figure 1.8 The difference in concentrations of monoterpene Unknown 21.5 (AUC/ 100 µg DW) between paired browsed and non-browsed sagebrush samples (n = 90) from Craters, Idaho, as a function of mean concentration of Unknown 21.5 (AUC/ 100 µg DW) for that patch (n = 16 used patches). Samples included both Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) and three-tip sagebrush (*A. tripartita*) browsed by Greater Sage-grouse (*Centrocercus urophasianus*). Values above zero are theoretically selected for, and indicate higher phenolic concentrations in browsed plants than non-browsed, while values below zero are selected against and have lower monoterpene (Unknown 21.5) concentrations in browsed plants. The gray line shows 0.0 on the y-axis, where no selection occurs. The solid black line shows the smoothed fit for the generalized additive model, and dashed lines show 95% confidence intervals derived from the model.

CHAPTER TWO: RELATIONSHIPS BETWEEN PLANT SECONDARY METABOLITES (PSMS) AND AN INTESTINAL PARASITE IN GREATER SAGE-GROUSE

Abstract

Herbivores are challenged with finding high quality food from available plants. Herbivores generally attempt to consume sufficient nutrients and avoid plant secondary metabolites (PSMs) that are potentially toxic for consumers and energetically expensive to detoxify. However, the effects of PSMs may be dose-dependent. For example, PSMs in high doses may make herbivores more susceptible to parasites by increasing energy allocation towards detoxification and excretion rather than immune function, but the same compounds may have therapeutic effects against parasites at low doses. Therefore, I predicted high intake and absorption of PSMs would be positively correlated with parasite loads in herbivores. Alternatively, I predicted that ingested PSMs that are not absorbed (i.e. excreted unchanged) would be negatively correlated with intestinal parasite loads in herbivores. To test these predictions, I analyzed PSMs in browsed sagebrush, fecal excretion of unchanged PSMs, and parasite loads in free-ranging Greater Sage-grouse (*Centrocercus urophasianus*) consuming sagebrush during the winter. I used gas chromatography to quantify monoterpenes (a major class of PSMs) in sagebrush and fecal samples. I used the McMaster egg counting technique to quantify parasite loads of a tapeworm (*Raillietina centrocerci*) in fecal samples of sage-grouse. *Raillietina centrocerci* is the only known endoparasite found in sage-grouse in Idaho. I compared parasite loads among sites, seasons, and between sexes, and evaluated how parasites

related to PSMs in browsed sagebrush, PSMs in fecal pellets, and ecological characteristics from foraging sites. There was significant geographic variation in parasite loads throughout southern Idaho and a trend for lower parasite loads in winter than in fall. Animals excreting higher concentrations of monoterpenes in feces exhibited higher parasite loads. Fecal cineole, α-phellendrine, and camphor had the strongest positive correlations with parasite loads. Results suggest that intestinal exposure to PSMs may make sage-grouse more susceptible to endoparasites, or that parasites are resistant to PSMs regularly consumed by specialist herbivores. The interactions between PSMs and parasite loads may have profound ecological consequences because parasite loads and PSMs can both decrease body condition and fitness in wildlife.

Introduction

Plants are relatively abundant food resources, but they are often defended against herbivore attack with plant secondary metabolites (PSMs). PSMs are commonly associated with negative side effects for the herbivore (Appendix F). PSMs limit food intake, constrain available energy, and alter energy budgets because detoxification of PSMs is metabolically costly (Guglielmo et al. 1996, Wiggins et al. 2003, Mangione et al. 2004, Sorensen et al. 2005b, Au et al. 2013). The costs associated with PSM detoxification generally result in selective foraging behavior by herbivores whereby they consume plants that maximize energy and nutrient intake while limiting PSM exposure (Youngentob et al. 2011, Frye et al. 2013, Ulappa et al. 2014).

Energetic constraints related to PSM detoxification may limit the energy available for other activities, including reproduction (Jakubas et al. 1993, DeGabriel et al. 2009), locomotion (Sorensen et al. 2005b), and immune function (Smilanich et al. 2009).

Weakened immune function and reduced energy budgets may allow parasites and pathogens to proliferate. The interaction between PSM intake and parasite resistance may stem from competing use of energy for both detoxification and immune function (Delahay et al. 1995, Martin et al. 2003, Stenkewitz et al. 2015).

Despite the high costs often associated with consuming PSMs, negative side effects are dose dependent, and low doses of PSMs may be therapeutic. For example, some PSMs have anti-helminthic properties (Appendix F, Table F.2), and PSMs may also be effective for treating parasites and pathogens (Forbey et al. 2009). Some animals consume plants rich in PSMs when they are severely infected with intestinal parasites, employing a strategy called self-medication (Appendix F). Self-medication has been studied in insects (Singer et al. 2009, Singer et al. 2014, Gowler et al. 2015), as well as a variety of mammals, including domestic animals (Villalba and Provenza 2007, Landau et al. 2010, Amit et al. 2013), civets (Su et al. 2013), and primates (Huffman and Seifu 1989, Huffman 1993, McLennan and Huffman 2012). For example, domestic sheep (*Ovis aries*) eat foods higher in tannins when their parasite burdens are high, but sheep discontinue their selection of tannin-rich plants after being treated with anti-parasitic drugs, thereby avoiding negative consequences of PSMs once the parasite loads no longer have a high cost to the host (Villalba et al. 2010). There has been no previous quantitative documentation of self-medication in vertebrate species with specialized diets that regularly consume PSMs (hereafter, specialists).

The ability to self-regulate parasite loads may have profound ecological benefits because parasite loads can decrease body condition, fitness, or survival in free-ranging wildlife (Boyce 1990, Holmstad et al. 2005, Singer et al. 2009, see also Gibson 1990,

Tsuji and DeIuliis 2003). Increases in some individual parasites and in the overall parasite community can impact breeding, survival, body mass, and population growth of willow ptarmigan (*Lagopus lagopus lagopus*; Holmstad et al. 2005). In Greater Sage-grouse (*Centrocercus urophasianus*; hereafter, sage-grouse), malaria-infected males and males with hematomas produced by lice visit breeding grounds less often and have lower reproductive success than non-infected males (Boyce 1990). Additionally, female sage-grouse chose males treated with antibiotics more than males without antibiotics. Similarly, female guppies (*Poecilia reticulata)* select males with fewer parasites (McMinn 1990).

Both being infested with parasites and the detoxification of PSMs constrain energy budgets of herbivores. This is an important trade-off for free-ranging herbivores, and the balance between parasites and PSMs depends on relative cost for each alternative (Forbey et al. 2009, Singer et al. 2009, Landau et al. 2010). Parasites can directly alter energy budgets by limiting nutrient acquisition (e.g. Cestodes, Nelson 1955). Additionally, parasites may indirectly alter energy budgets by increasing energy devoted to the immune response (Martin et al. 2003). Infected individuals can increase their food intake to offset both direct and indirect energetic costs associated with intestinal parasites (Ponton et al. 2011). However, compensatory feeding is not always possible for animals consuming diets containing PSMs (Wiggins et al. 2003).

Dietary specialists and generalists may differ in how PSMs and parasites interact and influence foraging. Although herbivores that specialize can generally consume higher concentrations of PSMs than generalists, many specialists rely on a variety of behavioral and physiological mechanisms to limit their exposure to PSMs (Wiggins et al. 2003,

Sorensen et al. 2004, Wiggins et al. 2006, Kohl et al. 2015). Specifically, some specialist herbivores (including sage-grouse) excrete PSMs unchanged (Sorensen et al. 2004, Frye 2012), which limits their exposure to PSMs and reduces some negative effects, while maximizing exposure of intestinal parasites to PSMs. Additionally, specialist herbivores consume higher concentrations of PSMs than most generalists, but due to their narrow diet, specialists are exposed to a lower diversity of PSMs. As a consequence of this narrow diet, specialists are also less able to consume novel PSMs (Sorensen et al. 2005a, Torregrossa et al. 2012), which may be necessary for self-medication (Huffman and Seifu 1989). Furthermore, intestinal parasites in specialist herbivores are routinely exposed to the compounds found in the host diet, and therefore may have evolved resistance mechanisms to these compounds (von Samson-Himmelstjerna 2012). Also, the physiological mechanisms employed by specialist herbivores, such as efflux transporters and detoxification enzymes, are energetically expensive mechanisms for detoxification (Sorensen and Dearing 2006), and may constrain energy budgets. Therefore, specialists may be unable to increase their PSM load or eat novel PSMs for therapeutic benefits.

Sage-grouse are specialists on sagebrush (*Artemisia* spp.), from which they ingest a variety of PSMs throughout the year. The diet of sage-grouse during the winter months is entirely sagebrush (Patterson 1952). During spring and summer, adult grouse shift their diet to eat about 60% sagebrush and include forbs (Nelson 1955, Gregg et al. 2008). As a result, grouse consume much lower concentrations of PSMs during summer, which potentially leaves them less defended from intestinal parasites. The winter diet of sagebrush is typically resumed in October (Connelly et al. 1988, Connelly et al. 2000).

The high PSM diet of a specialist herbivore is ideal for evaluating the energetic trade-off between PSM detoxification and parasite regulation.

Sagebrush PSMs (phenolics and monoterpenes) have anti-parasite properties *in vitro* and *in vivo* against coccidian parasites that can occur in grouse, including *Eimeria* (Allen et al. 1997, Allen et al. 1998). A high proportion of monoterpenes pass directly through the digestive tract in grouse and are excreted unchanged in the feces (Frye 2012, Thacker et al. 2012). Therefore, intestinal parasites of sage-grouse would be exposed to the same PSMs consumed by the host. Although regulated absorption minimizes systemic exposure and therefore the toxic consequences of PSMs in hosts, the mechanisms of regulated absorption (efflux transporters) come at an energetic cost (Sorensen et al. 2005b, Sorensen and Dearing 2006).

Sage-grouse have a diverse array of parasites including over thirty species of arthropods, helminthes, and microparasites (Patterson 1952, Boyce 1990, Christiansen and Tate 2011). The *Raillietina centrocerci* tapeworm (Figure 2.1) is the most widespread and abundant intestinal macroparasite of sage-grouse (Simon 1940, Nelson 1955, Christiansen and Tate 2011), but infection is not known to be fatal. *R. centrocerci* is the only tapeworm previously documented in sage-grouse of Idaho (Simon 1937, Simon 1940, Christiansen and Tate 2011). Tapeworm infestations occur in the small intestine, and heavy parasite loads potentially block the passage of food particles and prevent nutrient acquisition, therefore limiting host vitality and possibly fitness (Nelson 1955). Therefore, sage-grouse with high parasite loads may experience energy deficits that limit their ability to detoxify PSMs. Alternatively, winter diets with high PSM concentrations that have anti-helmenthic properties may reduce intestinal parasite loads

in sage-grouse.

I evaluated how intake and fecal excretion of PSMs by sage-grouse is related to *Raillietina centrocerci* loads. I predicted that excreted PSMs would decrease loads of this endoparasite in sage-grouse due to high intestinal exposure of parasites to PSMs. Alternatively, because the mechanisms used to regulate the absorption of PSMs can compromise energy budgets and immune function, it is possible that intake and excretion of PSMs would increase parasite loads (Figure 2.2). Also, intestinal parasites in sage-grouse are regularly exposed to PSMs from sagebrush and may have evolved resistance against PSMs in the intestines.

Methods

Study Sites

Sage-grouse pellet samples were collected from four study sites throughout southern Idaho during three different winters. All contained stands of Wyoming big sagebrush (*A. tridentata wyomingensis*) but differed in the other species of sagebrush occurring on the sites.

The Owyhee site (42° 38’ N, 116° 03’ W) is located in the southwestern part of Idaho in Owyhee County (Figure 2.2). The dominant vegetation includes Wyoming big sagebrush and low sagebrush (*A. arbuscula*) stands. Elevations range from 1590 m to 1820 m. Average annual precipitation is approximately 23 cm, with a maximum snow depth of 8 cm during collections in winter 2011-2012.

The Brown’s Bench site (42° 11’ N, 114° 46’ W) is located along the southern border of Idaho in Twin Falls County (Figure 2.2). The study site has a mosaic of black sagebrush (*Artemisia nova*) and Wyoming big sagebrush stands. Elevations range from

1,550 m to 1,750 m. Average annual precipitation is approximately 26 cm. Snowpack was 8 cm when pellets were collected in winter 2011-2012 (Frye 2012).

The Craters of the Moon site (hereafter, Craters; 42° 57’ N, 113° 23’ W) is in Power, Blaine and Minidoka Counties (Figure 2.2). The site is dominated by sparse Wyoming big sagebrush and sparse three-tip sagebrush (*A. tripartita*), and has an extensive fire history. Elevations range from 1,300 m to 1,650 m. Average annual precipitation is 24 cm, with most precipitation falling as snow. Snow depth during winter did not exceed 6 cm during sample collection in winter 2013-2014.

The Raft River site (42° 9’ N, 113° 24’ W) is in Cassia County, south and east of Jim Sage Mountain (Figure 2.2). Low sagebrush and Wyoming big sagebrush dominate the site. Other sagebrush species present include black sagebrush, basin big sagebrush (*A. t. tridentata*), and mountain big sagebrush (*A. t. vaseyana*). Elevations range from 1,380 m to 2,140 m. Average annual precipitation is 33 cm, with maximum snow depths of 5 cm in December and January when sage-grouse pellets were collected. Pellets were collected during fall 2014, winter 2013-2014, spring 2014, and winter 2014-2015.

Field Methods

Idaho Department of Fish and Game captured sage-grouse using standard spotlighting techniques (Geisen et al. 1982, Wakkinen et al. 1992) at all four study sites during the spring preceding my sample collection. Grouse were weighed, fitted with aluminum leg bands, and 14-15 gram VHF transmitters designed for sage-grouse. Birds were released at the site of capture.

Sage-grouse were flushed from foraging patches by locating radio-marked birds using telemetry. Trained observers identified bird sex and counted flock size as birds

flushed. Birds were flushed during late fall (1 November to 15 December), mid-winter (1 January to 15 February), and spring (1 March to 15 April). Radio-marked birds were flushed no more than one time during each sampling period. Tracks and pellets were used to identify the patch boundary, then researchers located plants within the patch that were fed on by the flock. Grouse bite leaves, leaving clear evidence of foraging (Remington and Braun 1985, Frye 2012). Leaf clippings were taken from three browsed plants at each patch and were pooled to form a single browsed sample (Frye et al. 2013). A composite of fresh fecal droppings were collected from each flush site, representing the whole flock at the foraging site. Both pellet and leaf samples were stored separately on ice while in the field. Samples were transferred to a -20° C freezer as soon as possible to minimize volatilization of monoterpenes, because monoterpene emission rates increase with increasing ambient temperature (Tingey et al. 1980). All work complied with Institutional Animal Care and Use Committee (IACUC) protocol 006-AC13-010.

Laboratory Methods

I used the McMaster egg counting technique (Gordon and Whitlock 1939, Cringoli et al. 2004, Ballweber et al. 2014) to quantify intestinal parasite loads within fecal pellets of sage-grouse. McMaster egg counts are correlated with adult tapeworm abundance in Red Grouse (*Lagopus lagopus scotius*; Moss et al. 1990, Seivwright et al. 2004). The length of pellets was measured with calipers and mass of each pellet was weighed on an analytical balance. Samples were homogenized, and partitioned into two samples: 2 g wet weight was used for the McMaster technique, and 0.5 g wet weight was ground in liquid nitrogen and a 100 mg subsample was transferred into 20 ml gas

chromatography headspace vial for monoterpene identification and quantification. All weighed samples were stored at -20° C until chemical analysis.

Monoterpenes were detected in pellets (i.e. fecal monoterpenes) and sagebrush (e.g. plant monoterpenes) using headspace gas chromatography. Grouse pluck leaves instead of eating whole stems (Remington and Braun 1985, Frye et al. 2013). Therefore leaves were separated from woody biomass for chemical analysis. Leaves were removed by dipping samples into liquid nitrogen and brushing leaf matter off into a separate container. Dead leaves and debris were removed with forceps (Frye 2012). Sagebrush samples were ground in a mortar and pestle with liquid nitrogen, and a 100 mg subsample was weighed into 20 ml headspace vials, and used to assess monoterpene concentrations with the gas chromatograph (e.g. plant monoterpenes). Similarly, the fecal sample was ground using the same method and a 100 mg subsample was used for monoterpene quantification (e.g. fecal monoterpenes).

Monoterpene standards were included to provide reference retention times for compound identification in both fecal and plant samples (Table 2.1). Samples were analyzed using a gas chromatograph (Agilent 6890N) with a headspace auto-sampler (Hewlett-Packard HP7694). Co-chromatography with a standard cocktail was used to identify compounds, although it was not possible to identify all compounds. Retention times (minutes) and peak areas (Area Under the Curve, AUC) were calculated using HP ChemStation version B.01.00 (Santa Clara, California, USA). Headspace and gas chromatograph settings are detailed in Appendix B. Samples were dried for 24-48 hours at 60° C and re-weighed to obtain sample dry weights, which were used to standardize concentrations of compounds. Chemical diversity was calculated using a Shannon index

for all compounds present at greater than 1% of the total AUC (area under the curve) for fecal samples. I did not calculate chemical diversity of ingested PSMs because the amount of plant matter consumed for each chemical profile was unknown.

Additionally, protein was assayed in sagebrush samples, because it can be limiting for specialist herbivores (Mattson 1980, Au et al. 2013). Protein is essential for the formation and maintenance of enzymes, which may be used in detoxification or excretion of PSMs, immune function, or general physiology (Robbins 1983). Additionally, protein mediates the trade-off between growth and immunological defense in some systems (Scriber and Slansky 1981, Cotter et al. 2011). Sagebrush samples were ground and sent to Dairy One Forage Laboratory (Ithaca, New York). Their laboratory uses the combustion method to quantify percent crude protein.

The McMaster egg counting technique (Appendix G) was used to obtain quantitative estimates of the number of oocytes (eggs) per gram biomass of feces using etched counting chambers (Gordon and Whitlock 1939). Parasite species have not been identified by a parasitologist or using genetic methods, and therefore there may be different species present in the grouse feces we measured. Pellets (2 g wet weight) were placed in 28 ml of a saturated salt and sugar solution (400 g sodium chloride and 500 g table sugar dissolved in 1000 ml of tap water). Pellets were allowed to defrost at room temperature and stirred vigorously to suspend the fecal matter into the salt-sugar solution. The solution was filtered through folded, pre-weighed cheesecloth and funneled into test tubes. From there, two 0.15 mL subsamples were pipetted into McMaster slide chambers. Eggs were counted under a microscope at 100x magnification by trained

observers. The cheesecloth and sample was dried in a 60° C oven for one week, and re-weighed to obtain sample dry weights, which were used to standardize the egg counts.

Sample Collection

I compared *Raillietina centrocerci* parasite loads among different years, seasons, sites, flock sexes, flock sizes, and diet variables (including protein, plant monoterpenes, and fecal monoterpenes) for Greater Sage-grouse for a total of 79 samples from four sites in southern Idaho from 2011 to 2015 (Brown's Bench n =10, Craters n = 13, Owyhee n = 8, Raft River n = 48). Samples were all collected during winter, with the exception of 13 samples at Raft River collected in fall 2013. An additional 5 samples from Raft River during spring 2014 were included for temporal analysis, but no monoterpene data were collected on that subset. Analyses among seasons and between years used only samples from Raft River, giving a total of 48 samples for the temporal analysis.

Statistical Methods

All statistical analysis used JMP Pro 11.0 (SAS Institute Inc. 2013) and R version 3.2.0 (R Foundation for Statistical Computing 2015). All analyses used the logarithm of eggs per gram dry weight (DW) as the metric for parasite load as the response variable, because the logarithm of eggs per gram DW has a normal distribution (Moss et al. 1990, Arneberg et al. 1998, Arneberg 2001, Seivwright et al. 2004, Mougeot et al. 2006).

Initially, I tested the effect of year on parasite load, because previous literature indicated storage decreases parasite detectability in samples that are stored in a refrigerator or freezer for one to three weeks (van Wyk and van Wyk 2002, Cringoli et al. 2011, Rinaldi et al. 2011). Because I collected samples at different sites and in different years, sample storage times varied. To account for potential storage effects, I assessed if

parasite load varied by year, which would preclude any comparison among sites. Specifically, I compared winter samples within a single site, Raft River, from 2013-2014 (stored 1.5 years at -20° C) and 2014-2015 (stored 0.5 years at -20° C) using ANOVA. Because samples were collected at multiple sites and from different years, I also tested for an interaction effect (2-way ANOVA) between site and year using all samples collected during winter. Additionally, to test if parasite loads changed with season, I used ANOVA to compare samples from Raft River during fall, winter and spring. For all ANOVA tests with significant differences between groups, a post-hoc Tukey-HSD test was used to evaluate which groups differed from one another.

Pearson correlation analyses were used to eliminate correlated variables ($|r| > 0.7$) for ecological and chemical predictor variables, including fecal monoterpenes, plant monoterpenes, and protein. Compounds that were present at all sites were selected over compounds only present at a subset of sites, compounds with known identity were selected over unknown compounds, and compounds with higher concentrations were selected over those found at low concentrations. When correlated with one another, fecal monoterpenes were selected over plant monoterpenes because intestinal parasites would be exposed to fecal PSMs (excreted PSMs), rather than the concentrations in the plant (ingested PSMs can be absorbed or excreted, therefore only exposing parasites to a subset of these concentrations). Of all plant monoterpene parameters, only total plant monoterpenes qualified for analysis through this parameter selection process, and the remaining chemical variables assessed were fecal monoterpenes.

Two-stage modeling using the information theoretic approach (Franklin et al. 2000, Washburn et al. 2004, Bonnot et al. 2011) was used to evaluate the best predictors

of parasite loads from the remaining variables. This approach was repeated, modeling parasite loads among all sites during winter (regional) and only at Raft River across seasons. Season and site varied (Figures 2.3 and 2.4) and were included as covariates for models. In the first stage of modeling, ecological variables (site or season, bird sex, flock size, and patch elevation) were compared to one another using Akaike's Information Criterion adjusted for small sample size (hereafter, AIC_c; Burnham and Anderson 2002, and diet variables (patch protein, total monoterpenes, and individual compounds) in both pellets and plants were compared to one another. In the second stage of modeling, ecological and chemical variables with AIC_c weights greater than 10% of the top model's weight were combined to assess which variables were the strongest predictors of parasite loads. For the top models ($\Delta AIC_c < 2$), linear regression graphs were used to illustrate trends for each predictor variable.

Results

Temporal and Geographic Variation in Parasite Loads

At Raft River there was no difference in parasite loads between years (winter 2013-2014 n=15, winter 2014-2015 n=15; ANOVA $F_{1,31} = 1.643$, P= 0.209). There was also no interaction between collection site and year (2-way ANOVA: $F_{5,55} = 0.699$, P = 0.407), therefore any differences between samples from different sites were driven by either site or year. Variation in parasite loads among all three seasons [fall (n = 13), winter (n = 30), and spring (n = 5)] at Raft River was not significant (ANOVA: $F_{1,41}$ = 2.875, P = 0.098), however parasite loads in fall were almost twice as high as winter (Figure 2.4). There was a significant effect of site on parasite loads (Figure 2.3; $F_{3,55}$ = 4.8768, P = 0.0042), but parasite loads did not differ between years ($F_{1,55}$ = 0.941, P =

0.336). The Tukey-HSD test showed that birds at Raft River had higher parasite loads than Brown's Bench ($P = 0.022$) and Craters ($P = 0.050$), and that birds at Owyhee had slightly but not significantly higher parasite loads than Brown's Bench ($P = 0.054$) and Craters ($P = 0.111$).

Parasite Load Model Selection

To analyze parasite loads, I used site and season as covariates. When analyzing all sites together (regional scale), I excluded any data that did not fall within the early winter time frame (1 January to 15 February), and used site as a covariate. In the first stage of model selection, the top models (AIC_c w_i > 10% top model) at all sites (regional scale) included site, sex, elevation, fecal α-phellendrine, and fecal camphor (Table 2.2). For modeling at Raft River only, season was included as a covariate. At Raft River, the top models included season, fecal cineole, fecal camphor, total fecal monoterpenes and fecal compound diversity (Table 2.3).

Patterns of Parasite Loads at a Regional Scale

The final regional scale models with $\Delta AIC_c < 2$ included two ecological variables (sex and site) and two PSM predictors (fecal α-phellendrine and fecal camphor; Table 2.4). Male sage-grouse had higher parasite loads than females (Figure 2.6a; ANOVA: $F_{1,68} = 4.426$, $P = 0.039$), and there was substantial geographic variation (Figure 2.3). Overall, grouse with high fecal α-phellendrine had lower parasite loads (Figure 2.6b), however there is also significant geographic variation in the concentrations of α-phellendrine in plants at each site (Wilcoxon: $Z = 4.83463$, $P < 0.001$) as some sites do not have plants producing α-phellendrine (Brown's Bench, n=10; Owyhee, n=7) while other sites do (Craters, n=13; Raft River, n=25). Sites with high parasite loads (e.g. Raft

River) also had low concentrations of α-phellendrine. Grouse with high fecal camphor had higher parasite loads than birds with low fecal camphor (Figure 2.6c). Models and AIC_c information are shown in Table 2.4.

Patterns in Parasite Loads Within a Site (Raft River)

The top models at Raft River with $\Delta AIC_c < 2$ included one ecological variable (season), and three PSM variables (fecal cineole, total fecal monoterpenes, and fecal compound diversity; Table 2.5). Parasite loads were higher in birds with high concentrations of fecal cineole, total fecal monoterpenes, and high fecal compound diversity (Figures 2.7a, 2.7b, and 2.7c, respectively). Outliers did not influence the order of top models.

Discussion

I compared *Raillietina centrocerci* parasite loads across different years, seasons, sites, flock sexes, flock sizes, quality of diet (protein, monoterpenes) and fecal excretion of PSMs for Greater Sage-grouse. There was geographic and seasonal variation in parasite loads, but no annual variation. Additionally, several dietary components explained variation in parasite loads. Flock sex was also an important factor in parasite loads, likely because different levels of hormones can influence parasite abundance (Ezenwa et al. 2012).

Although there was no significant difference in parasite loads among seasons, sage-grouse parasite loads were almost twice as high in fall than winter. This pattern may be due to either annual variation in the parasite life cycle, or from changing to a winter diet. There was higher variation in fall parasite loads than winter, possibly because young birds in fall can have higher parasite loads (Nelson 1955) compared to

older birds, which maintain lower parasite loads through winter. As young birds age and they shift from consuming insects (an intermediate host) to sagebrush (Patterson 1952, Nelson 1955, Klebenow and Gray 1968, Peterson 1970), their parasite loads may stabilize at levels similar to other adult birds, decreasing the variation within the population. Because our analysis occurred at the flock level rather than for individuals, we were unable to test differences in parasite loads between adult and juvenile birds. Finally, the higher variability in parasite loads in spring than winter may be due to the hormonal variation in males during that time. The immunocompetence handicap hypothesis suggests that there is a physiological trade-off between testosterone and immunity (Folstad and Karter 1992). For example, hormones increase abundance of some parasites in Grant's gazelle, and suppress others (Ezenwa et al. 2012).

Parasites did not differ across years. Despite the steady parasite loads during the two consecutive years sampled at Raft River, further collection over longer time periods could be used to understand any cyclical variation in parasite loads, and all counts should be conducted as soon as possible after collection to minimize potential error associated with storage time. Interannual variation in parasite loads could be used to evaluate how parasite cycles influence population regulation, as with other long-term population dynamics studies in grouse (Roberts and Dobson 1994, Hudson et al. 1998, Formenti et al. 2013, Dunham et al. 2014, Martinez-Padilla et al. 2014). Dense populations can have higher disease transmission risk (Arneberg et al. 1998, Arneberg 2001, Holmstad et al. 2005, Cross et al. 2010). However, flock size, which ranged from single birds to flocks over 100, did not appear in any of the top ecological models in our first stage of modeling, suggesting that parasite loads in sage-grouse are not regulated by population

density at current host densities, or are regulated by population density during other times of year than during our sample collection period.

Fecal α-phellendrine, fecal camphor, fecal cineole, fecal total monoterpenes, and compound diversity of fecal monoterpenes were the top chemical variables that explained parasite loads. For α-phellendrine, higher PSM concentrations were associated with lower intestinal parasite loads (Figure 2.6), which would be predicted by the self-medication hypothesis. However, this relationship is likely site-driven due to substantial geographic variation in α-phellendrine that paralleled geographic variation in parasite loads. For fecal cineole, camphor, and total monoterpenes, higher PSM concentrations were associated with higher intestinal parasite loads (Figures 2.6 and 2.7), even though both cineole and camphor have anti-helminthic properties (Zhu et al. 2013, Oliveira et al. 2014). This relationship suggests that parasite loads were not affected by PSM concentrations in the host's diet in this system. It is possible that hosts eating diets rich in PSMs are immunocompromised due to the energetic costs of PSMs, and are therefore unable to defend against parasites. An alternative explanation is that parasites that have co-evolved with sagebrush specialist herbivores are resistant to PSMs in sagebrush that may have anti-helminthic action against generalist parasites. *In vitro* tests of sagebrush compounds on parasites from both specialist and generalist herbivores are needed to evaluate parasite resistance to PSMs.

Another important chemical variable was fecal monoterpene diversity, which was positively correlated with parasite loads. Herbivores that consume diverse PSMs likely decrease the maximum dose of any single chemical, which may minimize negative physiological effects on the consumer (Freeland and Janzen 1974, Dearing and Cork

1999, Marsh et al. 2006, McLean and Duncan 2006, Wiggins et al. 2006). This strategy benefits the host by preventing the saturation of any detoxification pathway, and it increases the number of potentially bioactive compounds that come in contact with parasites (Provenza et al. 2007, Villalba and Provenza 2007, Forbey et al. 2009). The suite of PSMs an animal consumes from a mixed diet may be more useful to treat diverse parasite loads than any single PSM, because parasites would be more likely to develop resistance against a single PSM than a suite of compounds (Waller 2006, Forbey et al. 2009). However, I documented the opposite relationship, with high parasite loads associated with high monoterpene diversity, which may be related to the evolution of drug resistance by intestinal parasites that are routinely exposed to relatively low doses of PSMs (Waller 2006, Sengupta et al. 2013) rather than exposure to a high dose of a few chemicals.

The PSMs in sagebrush are known to regulate parasite loads in other systems. Some endoparasite species have been controlled using extracts and individual compounds from *Artemisia* plants (Allen et al. 1997, Allen et al. 1998), and other PSMs in sagebrush have similar effects (Dasgupta and Roy 2010, Landau et al. 2010, Zhu et al. 2013, Oliveira et al. 2014). Previous work has demonstrated that cineole and camphor are both capable of decreasing parasite survival (Zhu et al. 2013, Oliveira et al. 2014). However, these studies used lab culture assays to test a parasite typically found in generalist hosts (*Haemonchus contortus* from domestic *Ovis aries*), while I evaluated responses of parasites in free-ranging specialist hosts. The doses used in Zhu et al. and Oliveira et al. were similar to the concentrations found in the intestines of sage-grouse (Kohl et al. 2015) that intestinal parasites would actually experience, and similar to the

concentrations in sagebrush at our study sites (unpublished data, Frye et al. 2013). Theoretically, heavily parasitized sage-grouse could self-medicate by consuming plants with high PSM concentrations to regulate their intestinal parasites. However, I found sage-grouse that excreted higher concentrations of PSMs also had higher parasite loads. This suggests that (a) parasite loads of *Raillietina centrocerci* are not detrimental and therefore do no select for self-medication behavior, (b) specialist herbivores cannot self-medicate due to already high consumption of PSMs and energy constraints, or (c) parasites are resistant to PSMs in sagebrush.

Self-medication balances the costs of consuming PSMs with costs of parasite loads. Therefore, the cost incurred by the parasite must exceed the cost of the PSM load for self-medication to be a beneficial strategy for a host to employ. For low-cost immune challenges, costs and toxicity of PSMs may be more detrimental to the host than the impacts of the immune challenge or parasite (Forbey et al. 2009). However, if the host has a high or costly parasite load, then there is a greater probability the host will exploit PSMs for therapeutic benefits (see Figure 1 in Forbey at al. 2009). In these instances, a more toxic PSM with a narrow therapeutic index[1] may be necessary to achieve the benefits of regulating parasite loads. Sage-grouse with high loads of *Raillietina centrocerci* incur relatively low costs (Thorne 1969), and therefore the probability that sage-grouse will exploit PSMs for therapeutic benefit is relatively low.

Alternatively, specialist herbivores may already be at the upper threshold of PSM consumption, and increased PSM consumption for self-medication may be limited by energetic constraints. Herbivores may face a resource-mediated trade-off between

[1] Therapeutic index: compares the amount of a therapeutic agent (drug) that causes a therapeutic effect to the amount that causes toxicity. A wide therapeutic index means that a substance is relatively "safe" for use, having relatively low toxicity.

detoxification of PSMs and immune function (Schmid-Hempel 2003, Cotter et al. 2011), and even specialist herbivores experience detrimental effects of PSMs on immune function from PSMs in their host plant (Smilanich et al. 2009). Specialists consume relatively high concentrations of PSMs, which require energy to detoxify or excrete (Sorensen and Dearing 2006). This may limits the energy available for immune function. It is energetically costly to maintain and deploy immune defenses (Martin et al. 2003, Schmid-Hempel 2003), which may result in higher parasite loads if energy is allocated away from immune function.

Another possibility is that parasites in specialist herbivores may become resistant to PSMs ingested by their host over evolutionary time. Drug resistance can occur when parasites are exposed to the same treatment method (e.g. drug, compound, PSM) repeatedly (Waller 2006), alone, or in low doses (Sengupta et al. 2013). Through extensive exposure, intestinal parasites in sage-grouse digestive systems may have adapted to resist the negative effects of PSMs found in sagebrush. However, like co-administration of multiple drugs (Debabov 2013), a mixed diet may be beneficial for host immunocompetence (Muller et al. 2015), possibly because the diet exposes parasites to novel compounds that may regulate parasite loads better.

Sage-grouse infected with *Raillietina centrocerci* appear to be in good physical condition (Thorne 1969), but heavy tapeworm loads can cause intestinal blockages or low nutrient assimilation (Nelson 1955), in turn causing reduced body mass, reduced vigor, and increased susceptibility to other parasites (Cole and Friend 1999). Also, the current host-parasite relationship might be altered by climate change. Climate warming will likely influence aspects of host-parasite interactions, including pathogen life history,

pathogen survival, disease transmission, increased host susceptibility, and increased frequency and severity of disease outbreaks (Harvell et al. 2002, Molnar et al. 2013b). Parasites with intermediate hosts, like *Raillietina centrocerci*, are likely to persist in a warming climate and to show altered host-parasite relationships (Molnar et al. 2013a).

Previous research has shown that self-medication occurs in multiple taxa as a method to regulate endoparasite loads. However, the documented taxa (e.g. domestic livestock, primates) are not specialist herbivores and do not normally consume diets high in PSMs. Sage-grouse are sagebrush obligate herbivores and consume high concentrations of PSMs, and diverse PSMs, in their diet. In sage-grouse, high intestinal parasite loads were generally associated with higher PSM loads, which suggests that the host-parasite relationships in this specialist herbivore may not be regulated through diet selection or self-medication. Despite these results, sage-grouse provide a model organism to further test the relative trade-offs between costs of parasites and costs of PSMs as well as investigate mechanism of resistance to PSMs in hosts and parasites.

Literature Cited

Allen, P. C., H. D. Danforth, and P. C. Augustine. 1998. Dietary modulation of avian coccidiosis. International Journal for Parasitology 28:1131-1140.

Allen, P. C., J. Lydon, and H. D. Danforth. 1997. Effects of components of *Artemisia annua* on coccidia infections in chickens. Poultry Science 76:1156-1163.

Amit, M., I. Cohen, A. Marcovics, H. Muklada, T. A. Glasser, E. D. Ungar, and S. Y. Landau. 2013. Self-medication with tannin-rich browse in goats infected with gastro-intestinal nematodes. Veterinary Parasitology 198:305-311.

Arneberg, P. 2001. An ecological law and its macroecological consequences as revealed by studies of relationships between host densities and parasite prevalence. Ecography 24:352-358.

Arneberg, P., A. Skorping, B. Grenfell, and A. F. Read. 1998. Host densities as determinants of abundance in parasite communities. Proceedings of the Royal Society B: Biological Sciences 265:1283-1289.

Au, J., K. J. Marsh, I. R. Wallis, and W. J. Foley. 2013. Whole-body protein turnover reveals the cost of detoxification of secondary metabolites in a vertebrate browser. Journal of Comparative Physiology B: Biochemical Systemic and Environmental Physiology 183:993-1003.

Ballweber, L. R., F. Beugnet, A. A. Marchiondo, and P. A. Payne. 2014. American Association of Veterinary Parasitologists' review of veterinary fecal flotation methods and factors influencing their accuracy and use - is there really one best technique? Veterinary Parasitology 204:73-80.

Bonnot, T. W., M. L. Wildhaber, J. J. Millspaugh, A. J. DeLonay, R. B. Jacobson, and J. L. Bryan. 2011. Discrete choice modeling of shovelnose sturgeon habitat selection in the Lower Missouri River. Journal of Applied Ichthyology 27:291-300.

Boyce, M. 1990. The Red Queen visits sage-grouse leks. American Zoologist 30:263-270.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York, USA.

Christiansen, T. J., and C. M. Tate. 2011. Parasites and infectious diseases of Greater Sage-grouse. Pages 113 - 126 *in* S. T. Knick, and J. W. Connelly, editors. Studies in Avian Biology. University of California Press, Berkeley, CA.

Cole, R. A., and M. Friend. 1999. Miscellaneous parasitic diseases. USGS Biological Resources Division.

Connelly, J., H. Browers, and R. Gates. 1988. Seasonal movements of sage-grouse in southeastern Idaho. Journal of Wildlife Management 52:116-122.

Connelly, J., M. Schroeder, A. Sands, and C. Braun. 2000. Guidelines to manage sage-grouse populations and their habitats. Wildlife Society Bulletin 28:967-985.

Cotter, S. C., S. J. Simpson, D. Raubenheimer, and K. Wilson. 2011. Macronutrient balance mediates trade-offs between immune function and life history traits. Functional Ecology 25:186-198.

Cringoli, G., L. Rinaldi, M. P. Maurelli, M. E. Morgoglione, V. Musella, and J. Utzinger. 2011. *Ancylostoma caninum:* Calibration and comparison of diagnostic accuracy of flotation in tube, McMaster and FLOTAC in faecal samples of dogs. Experimental Parasitology 128:32-37.

Cringoli, G., L. Rinaldi, V. Veneziano, G. Capelli, and A. Scala. 2004. The influence of flotation solution, sample dilution and the choice of McMaster slide area (volume) on the reliability of the McMaster technique in estimating the faecal egg counts of gastrointestinal strongyles and *Dicrocoelium dendriticum* in sheep. Veterinary Parasitology 123:121-131.

Cross, P. C., E. K. Cole, A. P. Dobson, W. H. Edwards, K. L. Hamlin, G. Luikart, A. D. Middleton, B. M. Scurlock, and P. J. White. 2010. Probable causes of increasing brucellosis in free-ranging elk of the Greater Yellowstone Ecosystem. Ecological Applications 20:278-288.

Dasgupta, S., and B. Roy. 2010. Antiparasitic activity of methanolic extract of *Acacia oxyphylla* (Leguminosae) against *Raillietina echinobothrida*. Journal of Parasitic Diseases 34:14-19.

Dearing, M. D., and S. Cork. 1999. Role of detoxification of plant secondary compounds on diet breadth in a mammalian herbivore, *Trichosurus vulpecula*. Journal of Chemical Ecology 25:1205-1219.

Debabov, D. 2013. Antibiotic resistance: origins, mechanisms, approaches to counter. Applied Biochemistry and Microbiology 49:665-671.

DeGabriel, J., B. Moore, W. Foley, and C. Johnson. 2009. The effects of plant defensive chemistry on nutrient availability predict reproductive success in a mammal. Ecology 90:711-719.

Delahay, R. J., J. R. Speakman, and R. Moss. 1995. The energetic consequences of parasitism - effects of a developing infection of *Trichostrongylus tenuis*

(Nematoda) on Red Grouse (*Lagopus lagopus scoticus*) energy balance, body weight, and condition. Parasitology 110:473-482.

Dunham, N. R., S. T. Peper, C. E. Baxter, and R. J. Kendall. 2014. The parasitic eyeworm *Oxyspirura petrowi* as a possible cause of decline in the threatened Lesser Prairie-chicken (*Tympanuchus pallidicinctus*). Plos One 9:5.

Ezenwa, V. O., L. S. Ekernas, and S. Creel. 2012. Unravelling complex associations between testosterone and parasite infection in the wild. Functional Ecology 26:123-133.

Folstad, I., and A. J. Karter. 1992. Parasites, bright males, and the immunocompetence handicap. American Naturalist 139:603-622.

Forbey, J. S., A. L. Harvey, M. A. Huffman, F. D. Provenza, R. Sullivan, and D. Tasdemir. 2009. Exploitation of secondary metabolites by animals: A response to homeostatic challenges. Integrative and Comparative Biology 49:314-328.

Formenti, N., R. Vigano, L. Rotelli, N. Ferrari, M. C. Cerutti, and P. Lanfranchi. 2013. Effect of suboptimal environment and host age on helminth community of Black Grouse (*Tetrao tetrix*). European Journal of Wildlife Research 59:351-358.

Franklin, A. B., D. R. Anderson, R. J. Gutierrez, and K. P. Burnham. 2000. Climate, habitat quality, and fitness in Northern Spotted Owl populations in northwestern California. Ecological Monographs 70:539-590.

Freeland, W. J., and D. H. Janzen. 1974. Strategies in herbivory by mammals: role of plant secondary compounds. American Naturalist 108:269-289.

Frye, G. G. 2012. Phytochemical ecology of an avian herbivore, the Greater Sage-grouse: implications for behavior, physiology, and conservation. Boise State University.

Frye, G. G., J. W. Connelly, D. D. Musil, and J. S. Forbey. 2013. Phytochemistry predicts habitat selection by an avian herbivore at multiple spatial scales. Ecology 94:308-314.

Geisen, K. M., T. J. Schoenberg, and C. E. Braun. 1982. Methods for trapping sage-grouse in Colorado. Wildlife Society Bulletin 20:425-426.

Gibson, R. 1990. Relationships between blood parasites, mating success and phenotypic cues in male sage grouse (*Centrocercus urophasianus*). American Zoologist 30:271-278.

Gordon, H. M., and H. V. Whitlock. 1939. A new technique for counting nematode eggs in sheep faeces. Journal of the Council for Scientific and Industrial Research 12:50-52.

Gowler, C. D., K. E. Leon, M. D. Hunter, and J. C. de Roode. 2015. Secondary defense chemicals in milkweed reduce parasite infection in monarch butterflies, *Danaus plexippus*. Journal of Chemical Ecology 41:520-523.

Gregg, M., J. Barnett, and J. Crawford. 2008. Temporal variation in diet and nutrition of pre-incubating Greater Sage-grouse. Rangeland Ecology and Management 61:535-542.

Guglielmo, C., W. Karasov, and W. Jakubas. 1996. Nutritional costs of a plant secondary metabolite explain selective foraging by Ruffed Grouse. Ecology 77:1103-1115.

Harvell, C. D., C. E. Mitchell, J. R. Ward, S. Altizer, A. P. Dobson, R. S. Ostfeld, and M. D. Samuel. 2002. Climate warming and disease risks for terrestrial and marine biota. Science 296:2158-2162.

Holmstad, P. R., P. J. Hudson, and A. Skorping. 2005. The influence of a parasite community on the dynamics of a host population: a longitudinal study on Willow Ptarmigan and their parasites. Oikos 111:377-391.

Hudson, P. J., A. P. Dobson, and D. Newborn. 1998. Prevention of population cycles by parasite removal. Science 282:2256-2258.

Huffman, M. 1993. Further observations on the use of the medicinal plant, *Vernonia amygdalina* (Del), by a wild chimpanzee, its possible effect on parasite load, and its phytochemistry. African Study Monographs 14:227-240.

Huffman, M. A., and M. Seifu. 1989. Observations on the illness and consumption of a possibly medicinal plant *Vernonia amygdalina* (Del), by a wild chimpanzee in the Mahale Mountains National Park, Tanzania. Primates 30:51-63.

Jakubas, W. J., B. C. Wentworth, and W. H. Karasov. 1993. Physiological and behavioral effects of coniferyl benzoate on avian reproduction. Journal of Chemical Ecology 19:2353-2377.

Klebenow, D. A., and G. M. Gray. 1968. Food habits of juvenile sage-grouse. Journal of Range Management 21:80-83.

Kohl, K. D., E. Pitman, B. C. Robb, J. W. Connelly, M. D. Dearing, and J. S. Forbey. 2015. Monoterpenes as inhibitors of digestive enzymes and counter-adaptations in a specialist avian herbivore. Journal of Comparative Physiology B: Biochemical Systemic and Environmental Physiology 185:425-434.

Landau, S., H. Azaizeh, H. Muklada, T. Glasser, E. D. Ungar, H. Baram, N. Abbas, and A. Markovics. 2010. Anthelmintic activity of *Pistacia lentiscus* foliage in two Middle Eastern breeds of goats differing in their propensity to consume tannin-rich browse. Veterinary Parasitology 173:280-286.

Mangione, A. M., M. D. Dearing, and W. H. Karasov. 2004. Creosote bush (*Larrea tridentata*) resin increases water demands and reduces energy availability in desert woodrats (*Neotoma lepida*). Journal of Chemical Ecology 30:1409-1429.

Marsh, K. J., I. R. Wallis, R. L. Andrew, and W. J. Foley. 2006. The detoxification limitation hypothesis: where did it come from and where is it going? Journal of Chemical Ecology 32:1247-1266.

Martin, L. B., A. Scheuerlein, and M. Wikelski. 2003. Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? Proceedings of the Royal Society B: Biological Sciences 270:153-158.

Martinez-Padilla, J., S. M. Redpath, M. Zeineddine, and F. Mougeot. 2014. Insights into population ecology from long-term studies of Red Grouse *Lagopus lagopus scoticus*. Journal of Animal Ecology 83:85-98.

Mattson, W. J. 1980. Herbivory in relation to plant nitrogen content. Annual Review of Ecology and Systematics 11:119-161.

McLean, S., and A. J. Duncan. 2006. Pharmacological perspectives on the detoxification of plant secondary metabolites: Implications for ingestive behavior of herbivores. Journal of Chemical Ecology 32:1213-1228.

McLennan, M. R., and M. A. Huffman. 2012. High frequency of leaf swallowing and its relationship to intestinal parasite expulsion in "village" chimpanzees at Bulindi, Uganda. American Journal of Primatology 74:642-650.

McMinn, H. 1990. Effects of the nematode parasite *Camallanus cotti* on sexual and non-sexual behavior in the guppy (*Poecilia reticulata*). American Zoologist 30:245-249.

Molnar, P. K., A. P. Dobson, and S. J. Kutz. 2013a. Gimme shelter - the relative sensitivity of parasitic nematodes with direct and indirect life cycles to climate change. Global Change Biology 19:3291-3305.

Molnar, P. K., S. J. Kutz, B. M. Hoar, and A. P. Dobson. 2013b. Metabolic approaches to understanding climate change impacts on seasonal host-macroparasite dynamics. Ecology Letters 16:9-21.

Moss, R., I. B. Trenholm, A. Watson, and R. Parr. 1990. Parasitism, predation and survival of hen Red Grouse (*Lagopus lagopus scoticus*) in spring. Journal of Animal Ecology 59:631-642.

Mougeot, F., S. M. Redpath, and S. B. Piertney. 2006. Elevated spring testosterone increases parasite intensity in male Red Grouse. Behavioral Ecology 17:117-125.

Muller, K., F. Vogelweith, D. Thiery, Y. Moret, and J. Moreau. 2015. Immune benefits from alternative host plants could maintain polyphagy in a phytophagous insect. Oecologia 177:467-475.

Nelson, O. C. 1955. A field study of the Sage-grouse in southeastern Oregon with special reference to reproduction and survival. Thesis, Oregon State University, Corvallis, USA.

Oliveira, G. L., T. M. Vieira, V. F. Nunes, M. D. Ruas, E. R. Duarte, D. D. Moreira, M. A. C. Kaplan, and E. R. Martins. 2014. Chemical composition and efficacy in the

egg-hatching inhibition of essential oil of *Piper aduncum* against *Haemonchus contortus* from sheep. Brazilian Journal of Pharmacognosy 24:288-292.

Patterson, R. L. 1952. The Sage Grouse in Wyoming. Sage Books, Inc., Denver, CO, USA.

Peterson, J. G. 1970. The food habits and summer distribution of juvenile sage-grouse in Central Montana. The Journal of Wildlife Management 34:147 - 155.

Ponton, F., F. Lalubin, C. Fromont, K. Wilson, C. Behm, and S. J. Simpson. 2011. Hosts use altered macronutrient intake to circumvent parasite-induced reduction in fecundity. International Journal for Parasitology 41:43-50.

Provenza, F. D., J. J. Villalba, J. Haskell, J. W. MacAdam, T. C. Griggs, and R. D. Wiedmeier. 2007. The value to herbivores of plant physical and chemical diversity in time and space. Crop Science 47:382-398.

Remington, T., and C. Braun. 1985. Sage-grouse food selection in winter, North Park, Colorado. Journal of Wildlife Management 49:1055-1061.

Rinaldi, L., G. C. Coles, M. P. Maurelli, V. Musella, and G. Cringoli. 2011. Calibration and diagnostic accuracy of simple flotation, McMaster and FLOTAC for parasite egg counts in sheep. Veterinary Parasitology 177:345-352.

Robbins, C. T. 1983. Wildlife feeding and nutrition. Academic Press, New York, USA.

Roberts, M. G., and A. P. Dobson. 1994. The population dynamics of parasitic helminths. Mathematical Biosciences 126:191-214.

Schmid-Hempel, P. 2003. Variation in immune defence as a question of evolutionary ecology. Proceedings of the Royal Society B: Biological Sciences 270:357-366.

Scriber, J. M., and F. Slansky. 1981. The nutritional ecology of immature insects. Annual Review of Entomology 26:183-211.

Seivwright, L. J., S. M. Redpath, F. Mougeot, L. Watt, and P. J. Hudson. 2004. Faecal egg counts provide a reliable measure of *Trichostrongylus tenuis* intensities in free-living Red Grouse *Lagopus lagopus scoticus*. Journal of Helminthology 78:69-76.

Sengupta, S., M. K. Chattopadhyay, and H. P. Grossart. 2013. The multifaceted roles of antibiotics and antibiotic resistance in nature. Frontiers in Microbiology 4:1-13.

Simon, F. 1937. A new cestode, *Raillietina centrocerci*, from the Sage-grouse *Centrocercus urophasianus*. Transactions of the American Microscopical Society 56:340-343.

_____. 1940. The parasites of the sage-grouse (*Centrocercus urophasianus*). Pages 77-100 *in* University of Wyoming Publications, Laramie, WY, USA.

Singer, M., K. Mace, and E. Bernays. 2009. Self-medication as adaptive plasticity: increased ingestion of plant toxins by parasitized caterpillars. Plos One 4:1-8.

Singer, M. S., P. A. Mason, and A. M. Smilanich. 2014. Ecological immunology mediated by diet in herbivorous insects. Integrative and Comparative Biology 54:913-921.

Smilanich, A. M., L. A. Dyer, J. Q. Chambers, and M. D. Bowers. 2009. Immunological cost of chemical defence and the evolution of herbivore diet breadth. Ecology Letters 12:612-621.

Sorensen, J. S., and M. D. Dearing. 2006. Efflux transporters as a novel herbivore countermechanism to plant chemical defenses. Journal of Chemical Ecology 32:1181-1196.

Sorensen, J. S., J. D. McLister, and M. D. Dearing. 2005a. Novel plant secondary metabolites impact dietary specialists more than generalists (*Neotoma* spp.). Ecology 86:140-154.

_____. 2005b. Plant secondary metabolites compromise the energy budgets of specialist and generalist mammalian herbivores. Ecology 86:125-139.

Sorensen, J. S., C. A. Turnbull, and M. D. Dearing. 2004. A specialist herbivore (*Neotoma stephensi*) absorbs fewer plant toxins than does a generalist (*Neotoma albigula*). Physiological and Biochemical Zoology 77:139-148.

Stenkewitz, U., O. K. Nielsen, K. Skirnisson, and G. Stefansson. 2015. The relationship between parasites and spleen and bursa mass in the Icelandic Rock Ptarmigan *Lagopus muta*. Journal of Ornithology 156:429-440.

Su, H. H., Y. C. Su, and M. A. Huffman. 2013. Leaf swallowing and parasitic infection of the Chinese lesser civet *Viverricula indica* in northeastern Taiwan. Zoological Studies 52:8.

Thacker, E., D. Gardner, T. Messmer, M. Guttery, and D. Dahlgren. 2012. Using gas chromatography to determine winter diets of Greater Sage-grouse in Utah. Journal of Wildlife Management 76:588-592.

Thorne, T. 1969. Diseases in Wyoming Sage-Grouse. Western States Sage-grouse Workshop, Sage-Grouse Committee.

Tingey, D. T., M. Manning, L. C. Grothaus, and W. F. Burns. 1980. Influence of light and temperature on monoterpene emission rates from slash pine. Plant Physiology 65:797-801.

Torregrossa, A. M., A. V. Azzara, and M. D. Dearing. 2012. Testing the diet-breadth trade-off hypothesis: differential regulation of novel plant secondary compounds by a specialist and a generalist herbivore. Oecologia 168:711-718.

Tsuji, L., and G. DeIuliis. 2003. Endoparasites of Northern sharp-tailed grouse, *Tympanuchus phasianellus phasianellus*, as related to age and position on the lek. Arctic 56:266-270.

Ulappa, A. C., R. G. Kelsey, G. G. Frye, J. L. Rachlow, L. A. Shipley, L. Bond, X. Z. Pu, and J. S. Forbey. 2014. Plant protein and secondary metabolites influence diet selection in a mammalian specialist herbivore. Journal of Mammalogy 95:834-842.

van Wyk, J. A., and L. van Wyk. 2002. Freezing of sheep faeces invalidates *Haemonchus contortus* faecal egg counts by the McMaster technique. Onderstepoort Journal of Veterinary Research 69:299-304.

Villalba, J., and F. Provenza. 2007. Self-medication and homeostatic behaviour in herbivores: learning about the benefits of nature's pharmacy. Animal 1:1360-1370.

Villalba, J. J., F. D. Provenza, J. O. Hall, and L. D. Lisonbee. 2010. Selection of tannins by sheep in response to gastrointestinal nematode infection. Journal of Animal Science 88:2189-2198.

von Samson-Himmelstjerna, G. 2012. Anthelmintic resistance in equine parasites - detection, potential clinical relevance and implications for control. Veterinary Parasitology 185:2-8.

Wakkinen, W., K. Reese, J. Connelly, and R. Fischer. 1992. An improved spotlighting technique for capturing sage-grouse. Wildlife Society Bulletin 20:425-426.

Waller, P. J. 2006. Sustainable nematode parasite control strategies for ruminant livestock by grazing management and biological control. Animal Feed Science and Technology 126:277-289.

Washburn, B. E., D. J. Tempel, J. J. Millspaugh, R. J. Gutierrez, and M. E. Seamans. 2004. Factors related to fecal estrogens and fecal testosterone in California Spotted Owls. Condor 106:567-579.

Wiggins, N., C. McArthur, N. Davies, and S. McLean. 2006. Behavioral responses of a generalist mammalian folivore to the physiological constraints of a chemically defended diet. Journal of Chemical Ecology 32:1133-1147.

Wiggins, N., C. McArthur, S. McLean, and R. Boyle. 2003. Effects of two plant secondary metabolites, cineole and gallic acid, on nightly feeding patterns of the common brushtail possum. Journal of Chemical Ecology 29:1447-1464.

Youngentob, K., I. Wallis, D. Lindenmayer, J. Wood, M. Pope, and W. Foley. 2011. Foliage chemistry influences tree choice and landscape use of a gliding marsupial folivore. Journal of Chemical Ecology 37:71-84.

Zhu, L., J. L. Dai, L. Yang, and J. Qiu. 2013. In vitro ovicidal and larvicidal activity of the essential oil of *Artemisia lancea* against *Haemonchus contortus* (Strongylida). Veterinary Parasitology 195:112-117.

Tables

Table 2.1 Retention times of monoterpene compounds in sagebrush generated using headspace gas chromatography. Sagebrush (*Artemisia* sp.) samples were collected in southern Idaho, USA, at Greater Sage-grouse (*Centrocercus urophasianus*) foraging sites. Fremgen 2013-2015 compound names and retention times are shown first, with names and retention times from Frye 2011-2012 samples shown second. Compounds were identified using known standards and co-chromatography.

Monoterpene Name Fremgen, Frye	**Retention Time (minutes)** Fremgen, Frye
Unknown 3.2 min, Unknown #1	3.28, 3.2
Unknown 12.2 min, Unknown #2	11.85, 12.2
α-pinene	12.87, 13.2
Camphene	13.45, 13.9
β-pinene	14.57, 15. 0
α-phellendrine	15.60, NA
Cymene	16.39, 16.9
1,8-Cineole	16.73, 17.2
Camphor	20.74, 21.1
Unknown 21.5 min, Unknown #8	21.56, 21.8

Table 2.2 Model components, log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias-adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the first stage of Greater Sage-grouse (*Centrocercus urophasianus*) parasite load model selection at all sites (Brown's Bench, Craters, Owyhee and Raft River) in southern Idaho, USA during winter. Models with greater than 10% of the top AIC_c weight (w_i) were selected for the final models (in **bold**). "Unknown" compounds are monoterpenes, identified by retention time.

Scale	Predictor Category	Model	Log Likelihood	Number of Parameters (K)	AIC_c	ΔAIC_c	Akaike weight (w_i)
All sites (winter only)	Ecological	**Sex + Site**	1.27	9	18.99	0.00	0.63
		Elevation + Site	-3.63	6	20.90	1.91	0.24
		Site	-5.75	5	22.58	3.59	0.10
		Flock Size + Site	-5.85	6	25.29	6.30	0.03
		Null	-12.75	2	29.71	10.72	0.00
	Dietary	**Fecal α-phellendrine[1] + Site**	-4.69	4	18.44	0.00	0.48
		Fecal Camphor[1] + Site	-2.66	7	18.91	0.47	0.38
		Fecal Compound Diversity[2] + Site	-4.84	6	23.23	4.79	0.04
		Fecal Cineole[1] + Site	-5.43	6	24.45	6.01	0.02
		Total Fecal Monoterpenes[1] + Site	-5.94	6	25.47	7.03	0.01
		Fecal α-phellendrine[1] + Site	-6.09	6	25.77	7.33	0.01
		Fecal Unknown 3.2[1] + Site	-6.14	6	25.86	7.42	0.01
		Protein[3] + Season + Site	-5.86	6	25.87	7.43	0.01
		Fecal Compound Diversity[2] + Total Fecal Monoterpenes[1] + Site	-5.06	7	26.28	7.84	0.01
		Total Fecal Monoterpenes[1] + Site	-6.47	6	27.09	8.65	0.01
		Null	-12.75	2	29.71	11.27	0.00

[1] PSM: monoterpenes (area under the curve/ 100 μg dry weight)

[2] Compound diversity (calculated as a Shannon diversity index from fecal monoterpenes > 1% of the total AUC for sample)

[3] Nutrient: crude protein (% dry weight)

Table 2.3 Model components, log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias-adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the first stage of Greater Sage-grouse (*Centrocercus urophasianus*) parasite load model selection at Raft River in southern Idaho, USA. Models with greater than 10% of the top AIC_c weight (w_i) were selected for the final models (in **bold**). Flock sex was male or female.

Scale	Predictor Category	Model	Log Likelihood	Number of Parameters (K)	AIC_c	ΔAIC_c	Akaike weight (w_i)
Raft River	Ecological	**Season**	-10.55	3	27.71	0.00	0.36
		Null	-12.00	2	28.31	0.60	0.26
		Elevation + Season	-10.14	4	29.33	1.62	0.16
		Flock Size + Season	-10.17	4	29.39	1.68	0.15
		Flock Sex + Season	-9.74	5	31.11	3.40	0.07
	Dietary	**Fecal Cineole[1] + Season**	-5.11	4	19.28	0.00	0.32
		Fecal Total Monoterpenes[1] + Season	-5.35	4	19.78	0.50	0.25
		Fecal Total Monoterpenes[1] + Fecal Compound Diversity[2] + Season	-4.76	5	21.23	1.95	0.12
		Fecal Cineole[1] + Fecal Camphor[1] + Season	-4.88	5	21.38	2.09	0.11
		Fecal Cineole[1] + Fecal Compound Diversity[2] + Season	-5.30	5	22.26	2.98	0.07
		Fecal Camphor[1] + Season	-7.13	4	23.31	4.02	0.04
		Fecal Cineole[1] + Fecal Camphor[1] + Total Fecal Monoterpenes[1] + Fecal Compound Diversity[2]	-4.55	6	23.57	4.29	0.04
		Protein[3] + Season	-7.30	4	24.42	5.14	0.02
		Season	-10.55	3	27.71	8.43	0.00
		Null	-12.00	2	28.31	9.03	0.00

[1] PSM: monoterpenes (area under the curve/ 100 μg dry weight)

[2] Compound diversity (calculated as a Shannon diversity index from fecal monoterpenes > 1% of the total AUC for sample)

[3] Nutrient: crude protein (% dry weight)

Table 2.4 Model components, log-likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the final stage of parasite load modeling at all field sites during winter only. Linear models with log-transformed parasite load response were assessed using data from Brown's Bench, Craters, Owyhee and Raft River, Idaho, USA. Flock sex was male or female.

Model	Log Likelihood	Number of Parameters (K)	AIC_c	ΔAIC_c	Akaike weight (w_i)
Fecal Camphor[1] + Sex + Site	2.94	8	12.94	0.00	0.43
Fecal α-phellendrine[1] + Sex + Site	0.12	6	14.09	1.16	0.24
Sex + Site	-0.03	7	16.17	3.24	0.09
Elevation[2] + Sex + Site	0.99	8	16.97	4.03	0.06
Fecal α-phellendrine[1] + Elevation[2] + Site	-2.70	5	17.02	4.08	0.06
Fecal α-phellendrine[1] + Fecal Camphor + Sex + Site	-2.84	5	17.31	4.37	0.05
Fecal α-phellendrine[1] + Site	-4.69	4	18.44	5.50	0.03
Fecal Camphor[1] + Site	-2.66	6	18.91	5.97	0.02
Fecal Camphor[1] + Elevation[2] + Site	-1.40	7	19.08	6.25	0.02
Elevation[2] + Site	-3.63	6	20.90	7.97	0.01
Site	-5.75	5	22.58	9.65	0.00
Null	-12.75	2	29.71	16.78	0.00

[1] PSM: monoterpenes (area under the curve/ 100 μg dry weight)

[2] Compound diversity (calculated as a Shannon diversity index from fecal monoterpenes > 1% of the total AUC for sample)

Table 2.5 Model components, log-likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the final stage of parasite load modeling at Raft River. Linear models with log-transformed parasite load response were assessed using data from Raft River, Idaho, USA.

Model	Log Likelihood	Number of Parameters (K)	AIC_c	ΔAIC_c	Akaike weight (w_i)
Fecal Cineole[1] + Season	-5.11	4	19.28	0.00	0.33
Fecal Total[1] + Season	-5.35	4	19.78	0.50	0.26
Fecal Total[1] + Compound Diversity[2] + Season	-4.76	5	21.23	1.95	0.13
Fecal Cineole[1] + Fecal Camphor[1] + Season	-4.88	5	21.38	2.09	0.12
Fecal Cineole[1] + Compound Diversity[2] + Season	-5.30	5	22.26	2.98	0.07
Fecal Camphor[1] + Season	-7.13	4	23.31	4.02	0.04
Fecal Total[1] + Fecal Cineole[1] + Fecal Camphor[1] + Compound Diversity[2]	-4.55	6	23.57	4.29	0.04
Season	-10.55	3	27.71	8.43	0.00
Null	-12.00	2	28.31	9.03	0.00

[1] PSM: monoterpenes (area under the curve/ 100 μg dry weight)

[2] Compound diversity (calculated as a Shannon diversity index from fecal monoterpenes > 1% of the total AUC for sample)

Figures

(a)

(b)

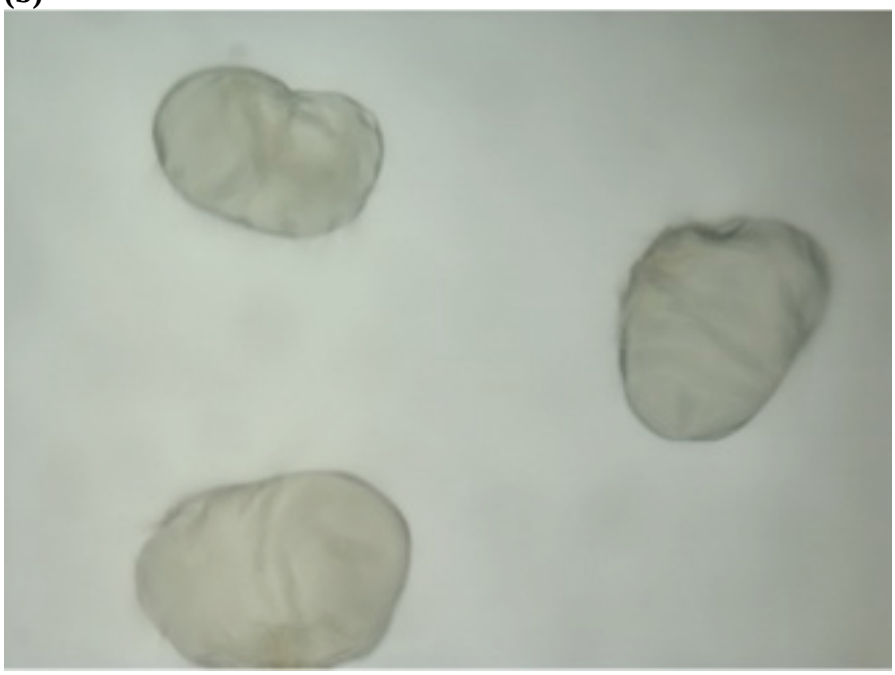


Figure 2.1 *Raillietina centrocerci* tapeworm (a) and eggs (b) isolated from Greater sage-grouse (*Centrocercus urophasianus*) pellets collected in southern Idaho, USA. Photograph by Joel Velasco 200x magnification.

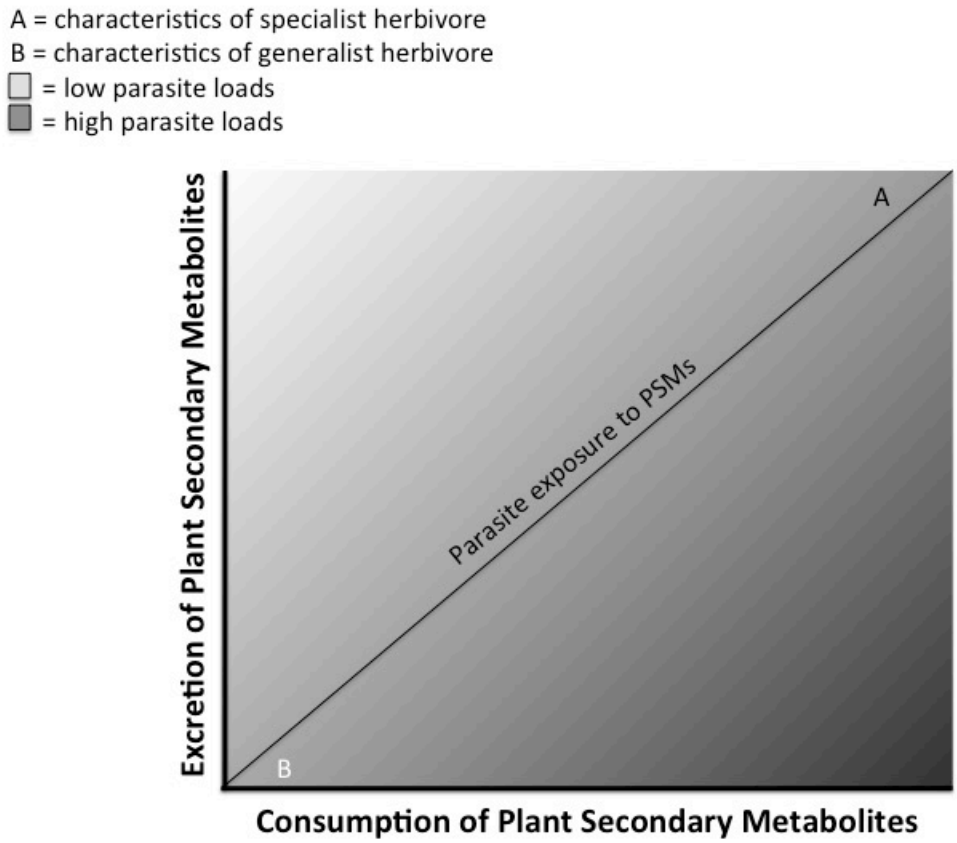


Figure 2.2 Conceptual diagram depicting how consumption and excretion of plant secondary metabolites (PSMs) by host animals may influence parasite loads. When hosts consume high concentrations of PSMs and excrete, rather than absorb, high concentrations of PSMs, parasites in the intestines will be exposed to PSMs more than parasites with hosts that consume fewer PSMs or absorb more PSMs (excrete less). Typical PSM consumption and physiological capacity to excrete (rather than absorb) PSMs for specialist herbivores (A) and generalist herbivores (B) are shown.

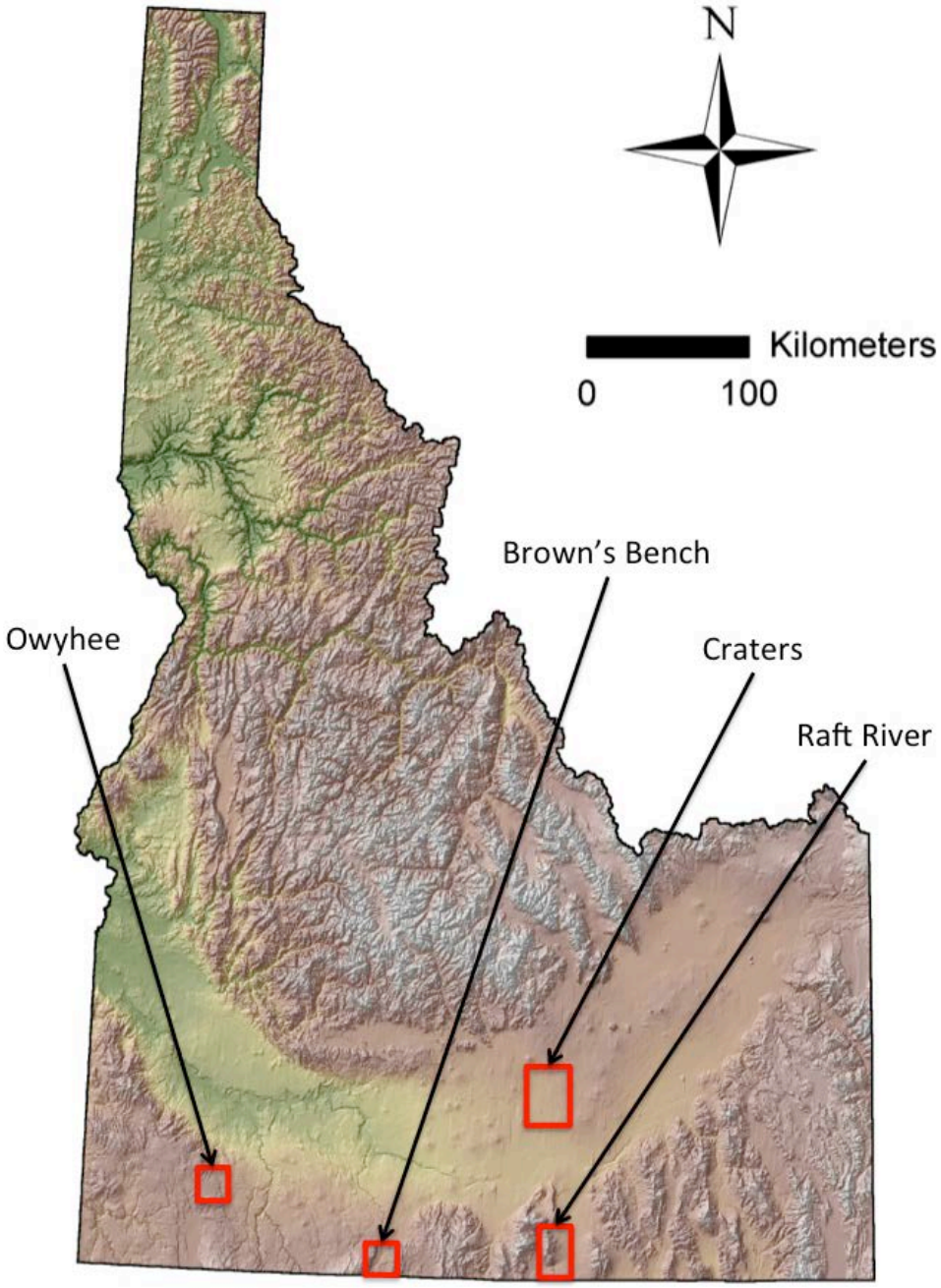


Figure 2.3 Sagebrush and fecal pellets of Greater Sage-grouse (*Centrocercus urophasianus*) were collected at foraging sites from four sites across southern Idaho, including Brown's Bench, Craters, Owyhee, and Raft River. Sagebrush and fecal pellets were collected at foraging sites in winter 2011 - 2012 (Owyhee and Brown's Bench), winter 2013 - 2014 (Craters and Raft River) and winter 2015 (Raft River).

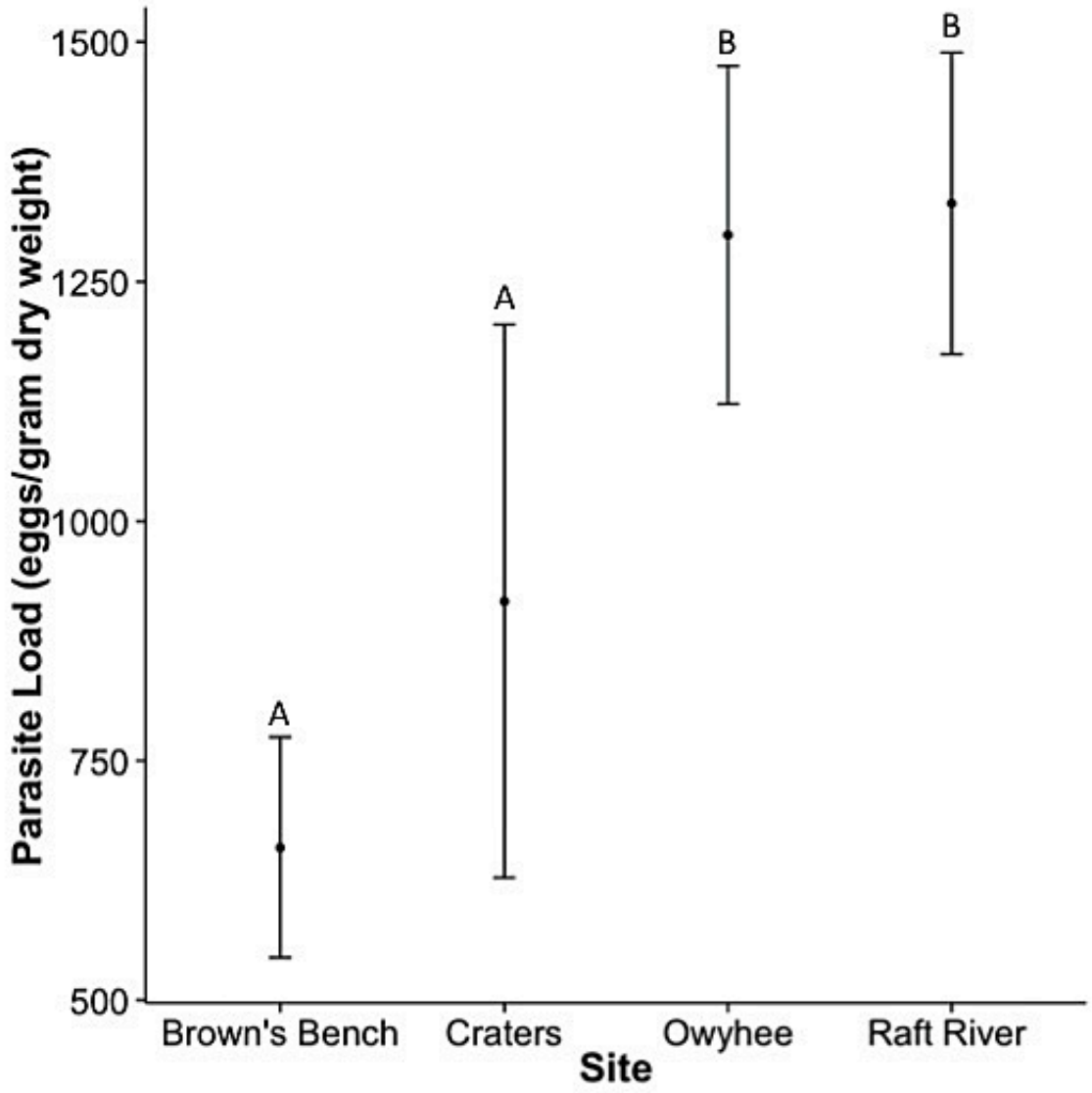


Figure 2.4 Mean ± SEM for parasite loads (eggs/gram dry weight) of *Raillietina centrocerci* tapeworms in Greater Sage-grouse (*Centrocercus urophasianus*) fecal pellets in southern Idaho, USA, by study site. Fecal pellets were collected from sites in early winter (1 January to 15 February) in 2011 through 2015, and parasite loads were quantified using the McMaster egg counting technique. Grouse at Brown's Bench and Craters had significantly lower parasite loads than grouse at Owyhee and Raft River.

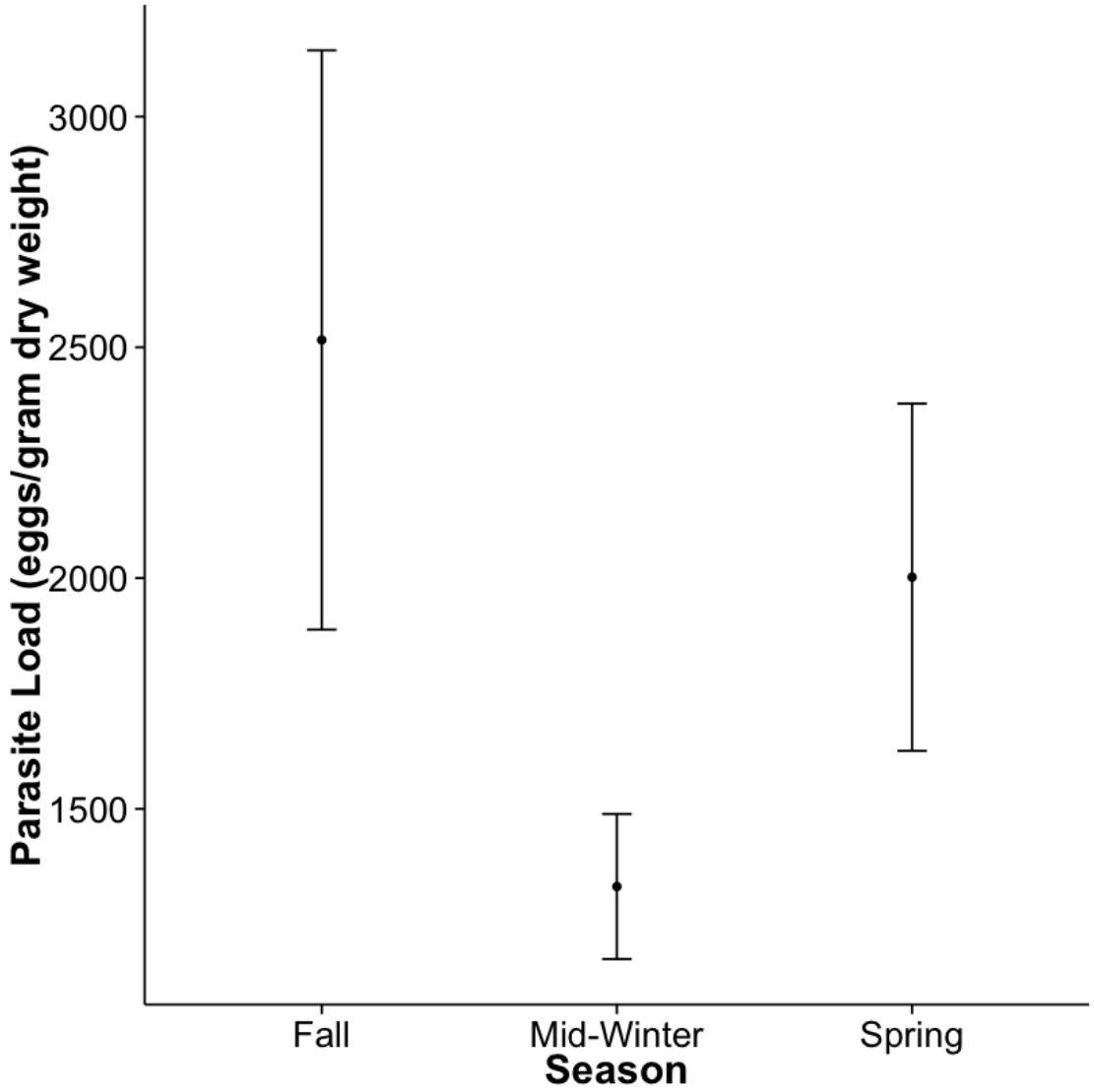


Figure 2.5 Mean ± SEM for parasite loads (eggs/gram dry weight) of *Raillietina centrocerci* tapeworms in fecal pellets of Greater Sage-grouse (*Centrocercus urophasianus*) at Raft River, Idaho, USA, by season. Fecal pellets were collected from sites in late fall (1 November to 15 December, n = 13), mid-winter (1 January to 15 February, n = 30) and spring (1 March to 1 April, n = 5) in 2013, 2014, and 2015, and parasite loads were quantified using the McMaster egg counting technique.

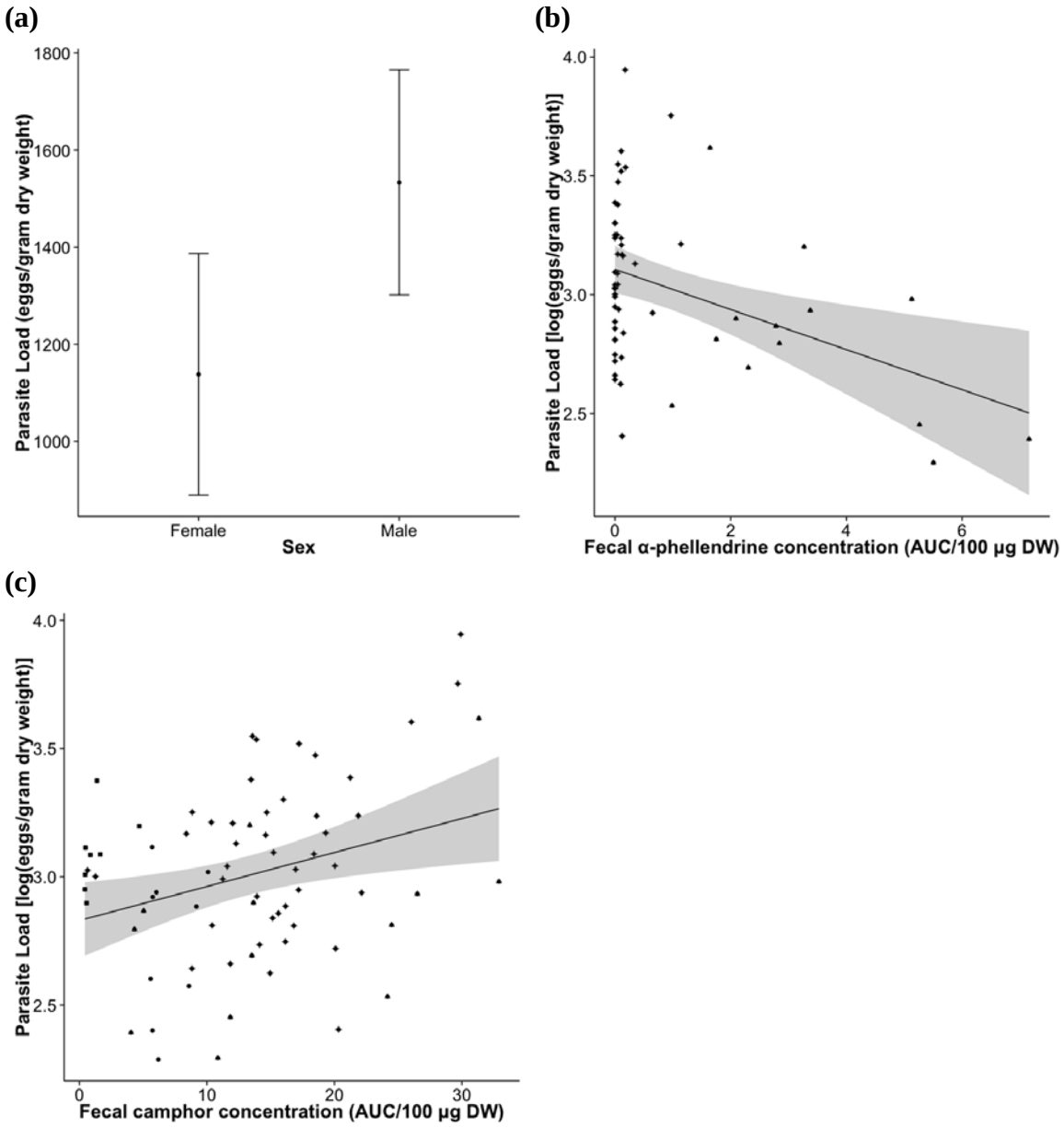


Figure 2.6 Relationships between (a) bird sex, (b) fecal α-phellendrine (AUC/ 100 µg dry weight), and (c) fecal camphor (AUC/ 100 µg dry weight) and *Raillietina centrocerci* parasite loads in Greater Sage-grouse (*Centrocercus urophasianus*) in southern Idaho, USA. Study sites included: Brown's Bench (●), Craters (▲), Owyhee (■), and Raft River (+). Confidence intervals (95%) are depicted with gray shaded error bars. For the linear regression of α-phellendrine compared to parasite loads, $n = 61$, $P = 0.002$, $r^2 = 0.215$. For the linear regression of fecal camphor compared to parasite loads, $n = 61$, $P = 0.002$, $r^2 = 0.3484$.

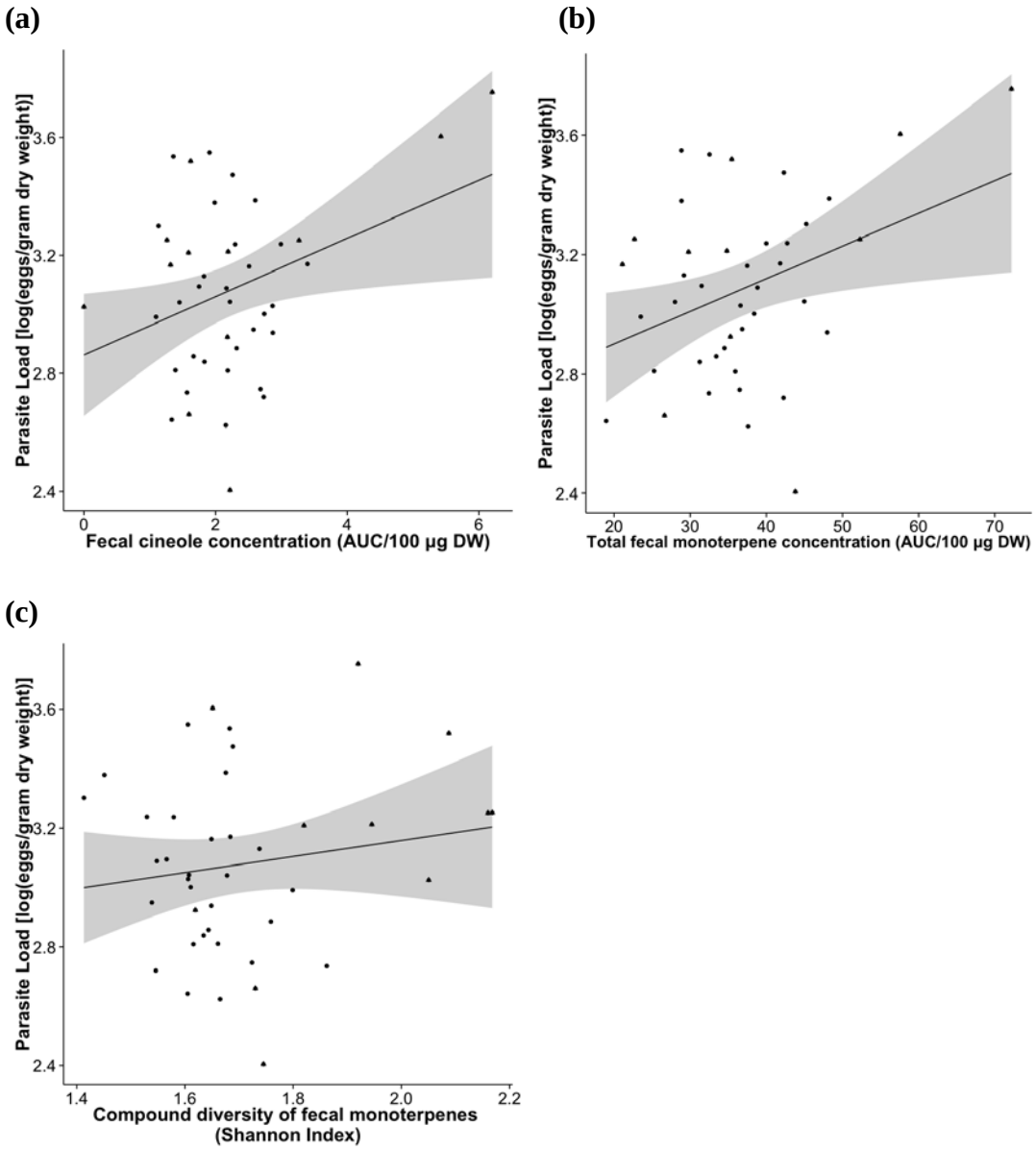


Figure 2.7 Linear model of (a) fecal cineole (AUC/ 100 µg dry weight), (b) total fecal monoterpene content (AUC/ 100 µg dry weight), and (c) compound diversity of fecal monoterpenes (measured with a Shannon Index) as predictors of *Raillietina centrocerci* parasite loads in Greater Sage-grouse (*Centrocercus urophasianus*) at Raft River in southern Idaho, USA. Samples were collected in fall (●) and winter (▲). Confidence intervals (95%) are depicted with gray shaded error bars. One outlier was excluded, with no effect on the final model ranks. For the linear regression of fecal cineole compared to parasite loads, $n = 48$, $P = 0.001$, $r^2 = 0.274$. For the linear regression of fecal total monoterpenes compared to parasite loads, $n = 48$, $P = 0.001$, $r^2 = 0.278$. For the linear regression of compound diversity of fecal monoterpenes compared to parasite loads, $n = 48$, $P = 0.050$, $r^2 = 0.092$.

CHAPTER THREE: NECKLACE-STYLE RADIO TRANSMITTERS ARE ASSOCIATED WITH CHANGES IN DISPLAY VOCALIZATIONS OF MALE GREATER SAGE-GROUSE

Abstract

Radio-transmitters are used widely in wildlife research, which allows researchers to track individual animals and monitor activity. To provide accurate information about a population, transmitters must be deployed on a representative sample of animals, and the transmitter must not alter the behavior or demographics of the individuals. Greater Sage-grouse (*Centrocercus urophasianus*), a species of concern, has been studied intensely using radio-transmitters for the last several decades. Males fitted with radio-transmitters are less likely to attend leks than those without transmitters. Necklace-style transmitters may also interfere with the vocalizations of sage-grouse during their strut displays on the lek. Certain vocalization characteristics have been linked to mating success. Therefore, I investigated whether radio-transmitters altered vocalization quality of male sage-grouse. I recorded vocalizations from paired (strutting on the same day on the same lek) collared (n=6) and non-collared (n=7) adult male sage-grouse on three leks in south-central Idaho. I evaluated 13 characteristics of vocalizations, and found that four characteristics differed between collared and non-collared males. Collared males had a narrower bandwidth for the primary whistle (lower maximum frequency and higher minimum frequency), a shorter primary whistle, and a shorter secondary coo than non-collared males. These

vocalization characteristics have not previously been linked to reproductive success, but demonstrate that collars may alter the production of normal breeding vocalizations. Additionally, primary whistle frequencies produced by collared birds fell outside the normal range of variation for non-collared males throughout the range of sage-grouse. It is important to consider the impacts of collars on all aspects of grouse behavior when designing and implementing studies.

Introduction

Radio transmitters are commonly used in wildlife studies, allowing researchers to track individual animals and remotely monitor certain activities. The fundamental assumptions to telemetry are that radio-transmitters are attached to a representative proportion of the population and that the transmitters do not influence behavior or demographics of marked individuals. It is important to test this assumption, since a number of studies on various taxa have documented effects of transmitters on survival (Theuerkauf et al. 2007, Venturato et al. 2009, Fabian et al. 2015). However, comparatively few studies have evaluated whether transmitters cause behavioral changes. Previous research into impacts of transmitters has identified detrimental effects on energetics and activity budgets (Zenzal et al. 2014), reproduction (Ward and Flint 1995), and lek attendance (Gibson et al. 2013). In some instances, the attachment method or antenna cause these differences in behavior or survival rather than the transmitter itself (Millspaugh et al. 2012). Understanding the impacts of monitoring techniques is important when studying sensitive species, or species of management concern, as

negative impacts on behavior may contribute to population level declines in an already compromised species.

Greater Sage-grouse (*Centrocercus urophasianus*; hereafter, sage-grouse) are a species of concern throughout western North America due to long-term population declines (Aldridge et al. 2008, Garton et al. 2011) and range contractions (Schroeder et al. 2004). The conservation concern for sage-grouse has spurred a large number of demographic and habitat use studies involving radio-collared birds across the western United States and Canada. Despite widespread use of radio-transmitters, there are a limited number of studies evaluating impacts of transmitters on sage-grouse. Initially, Pyrah (1970) expressed concern over the use of collar "poncho-markers" on males because their design interfered with the birds' breeding displays (also see Amstrup 1980). While poncho-markers are not the same as modern transmitters, the attachment method (collar around the neck) is similar to current designs. Later research suggested that modern necklace-style transmitters were not detrimental for hens to wear (Caizergues and Ellison 1998). Recent work has shown that necklace-style radio-transmitters did not impact the flush order of sage-grouse (Frye et al. 2014), suggesting that necklace-style transmitters do not significantly affect predator-escape behavior. However, necklaces were reported to decrease male lek attendance and sightability at leks at some sites (Gibson et al. 2013). Males with necklaces do appear on leks at other sites (Baumgardt 2011, Fremgen et al. 2015). However, although the latter studies documented lek attendance, they did not compare attendance rates or other measures of behavior between collared and non-collared birds.

I evaluated the effects of necklace-style radio transmitters on the strut vocalizations of male sage-grouse on leks. During the spring breeding season, male and female sage-grouse gather on leks, where males perform strut displays and females assess male displays to choose a mate (Patterson 1952, Wiley 1973). The rate and acoustic quality of the strut display is linked to male reproductive success (Gibson and Bradbury 1985, Gibson et al. 1991, Gibson 1996, Patricelli and Krakauer 2010). The potential mechanisms of interference by collars on male strutting are varied. Male strutting includes rapid inflation and movement of an esophageal air sac (Dantzker et al. 1999, Krakauer et al. 2009), which may be constricted by radio-collars. Male display movements are integrally linked to sound production (Koch et al. 2015), which may be altered by collars. The male strut display is also very energetically costly (Vehrencamp et al. 1989) and the added weight or stress associated with a radio-transmitter may result in increased energy expenditure and altered activity budgets. These impacts may in turn influence reproductive behavior and success. Given the movement-intensive display performed by male sage-grouse during courtship, I tested the possibility that radio-collars placed near the esophageal air sac could interfere with the acoustic properties of the strut display of males. I compared the vocalizations of six male sage-grouse fitted with necklace-style radio transmitters and seven paired control males from the same leks on the same days to determine whether collars affect male performance of strut vocalizations on the lek.

Methods

Adult male sage-grouse were fitted with necklace-style radio-transmitters in south-central Idaho (42° 9’ N, 113° 24’ W) in spring 2013. Vocalizations were recorded from six radio-collared males and seven non-collared males for six days on three different leks between 24 March and 14 April 2014, after males had been allowed to adjust to their transmitter for approximately one year. During early lekking season, males are predominantly from two social classes, including dominant and subdominant birds, but juveniles are present in low numbers (Jenni and Hartzler 1978, Walsh et al. 2004). Therefore, recordings from non-collared birds that are displaying are likely to fall within the top two social classes. It was important to select socially equivalent grouse, so I tested for differences in the average inter-strut interval (ISI) for each bird using ANOVA, because ISI is correlated with mating success and social ranking (Gibson and Bradbury 1985, Gibson 1996, Patricelli and Krakauer 2010). All collared birds were adult males, as verified by examination of the plumage during capture the previous year, and males were allowed to adjust to their collars for one year. Non-collared and collared male were recorded on the same day, within several minutes of one another, on the same lek. I recorded vocalizations for several minutes for each focal bird, which provided an average of 9 ± 4.5 vocalizations to analyze per male. Audio was recorded from a blind near the lek with a Marantz PMD670 portable audio recorder (16 bit, 48 KHz linear PCM), with Sennheiser microphone (K6 with omnidirectional ME62 capsule) and a 22-inch Telinga Pro parabola. Vocalizations were assigned to the focal male by comparing the timing of struts observed on videos of male lek behavior that were paired with audio

recordings of the same bird. I recorded vocalizations from males that were within 15 m of one another on the lek, so that paired males were similar distances from the microphone. Additionally, I verified that there were no obstructions (e.g. plants, rocks) between the recording equipment and the grouse with the video recordings, and removed any measurements recorded with obstructions that may have blocked sound transmission.

Vocalizations were visualized as spectrograms (FFT size 512; Hann window) and measured in Raven Pro 1.4 (Cornell laboratory of Ornithology, Ithaca, NY U.S.A.) by a single experienced observer. I measured characteristics of the six vocally produced notes from each call: three "coo" notes, two "pop" notes and the primary "whistle" (Figures 3.1 and 3.2). For the coos, I measured the duration and maximum frequency (i.e. highest pitch) of each note. The second coo note is longer and was found most often in the recordings so only this note is considered for analysis. For the pops, I measured the "inter-pop interval" (IPI), which is the time delay between the two pop notes. For the whistle, which occurs during the IPI, I measured the duration and the maximum and minimum frequency of the primary whistle. The primary whistle rises, falls and rises again in frequency; the maximum frequency is the highest pitch of the first rise and the minimum is the lowest pitch of the trough (Figure 3.1). From these measures, I calculated the ratio of primary whistle duration to inter-pop interval, which indicates the fraction the inter-pop interval that is taken up by the whistle. Measurements from individual notes were discarded when overlapped by other sounds, such as calls from songbirds. Most male sage-grouse show a secondary whistle that is lower in amplitude and less frequency-modulated than the primary whistle (Krakauer et al. 2009). These secondary whistles

were too quiet on our recordings to measure reliably and so were excluded from this analysis. I also tested for differences in vocalizations among collars fitted by different trapping personnel, to see if the individual (n=3 trappers) fitting the collar impacted the vocalization characteristics, using ANOVA. For all analyses, a Student's T-test was used to compare the average value of each vocalization characteristic for each collared and non-collared male. Analyses were performed in JMP 11.0 Pro (SAS Institute Inc. 2013).

Results

Four characteristics were significantly different between males with and without necklace-style radio collars (Figure 3.3). Whistles in non-collared males had a higher maximum frequency ($t = 4.854$, $df = 12$, $p = 0.003$), a lower minimum frequency (t = 2.539, df = 12, p = 0.031), and a longer duration ($t = 2.288$, $df = 12$, $p = 0.042$) than whistles in collared males. Non-collared males also had longer second coos than collared males ($t = 3.004$, $df = 11$, $p = 0.019$). The inter-pop interval ($t = -1.699$, $df = 11$, $p = 0.134$), ratio of primary whistle duration to inter-pop interval ($t = 2.006$, $df = 11$, $p = 0.073$) and maximum frequency of second coo ($t = 1.735$, $df = 11$, $p = 0.154$) did not differ between males with and without collars. There is no significant difference between the collared and non-collared birds in the estimated distance between the bird and the microphone during recording ($t = 0.218$ $df = 12$, $p = 0.828$), suggesting that differences in vocal features between the collar groups are not an artifact of differences in distance causing differences in transmission of the sounds. I used strut frequency data to evaluate if males came from socially equivalent groups (e.g. dominant versus subdominant versus juvenile). I found no difference in inter-strut intervals between all males recorded, using

video data for each of the 13 recorded birds, for multiple mornings when data was available (ANOVA: $F_{12,21} = 0.4767$, $P = 0.9065$). Also, no birds mated on mornings that I recorded vocalizations, or on separate observation mornings, suggesting that none of the males I recorded was a dominant individual. Finally, vocalization characteristics did not differ depending on the person that fitted the collar (ANOVA: primary whistle maximum frequency $F_{2,3} = 0.572$, $P = 0.616$; primary whistle minimum frequency $F_{2,3} = 3.11$, $P = 0.185$; primary whistle duration $F_{2,3} = 0.451$, $P = 0.674$; secondary coo duration $F_{2,2} = 0.984$, $P = 0.504$), but sample sizes for each trapper were low and may therefore prevent adequate testing of this effect.

Discussion

Previous studies have found that the rate and acoustic quality of the strut display is critical in determining which males are chosen as mates by females (Wiley 1973, Gibson and Bradbury 1985, Gibson et al. 1991, Gibson 1996, Patricelli and Krakauer 2010). The inter-pop interval (IPI, see Methods) is the most consistent acoustic correlate of male mating success in studies of sage-grouse from the California Mono Lake Basin population, with females preferring males that produce an IPI with a longer duration (Gibson and Bradbury 1985, Gibson et al. 1991, Gibson 1996). These previous studies also suggested that the amplitude of the whistle may be positively correlated with the IPI and that the amplitude of the whistle may be more important than IPI *per se* (Bradbury 1985, unpublished data, Gibson 1996, Dantzker et al. 1999). I did not find a difference in IPI between collared and non-collared males in the Idaho population. However, I found that collared males produced shorter whistles, which may be due to a lower whistle

amplitude (i.e. the end of the whistle drops below detectible amplitude sooner, thus appearing shorter). Similarly, the shorter duration of the coo notes in collared males may be due to a lower amplitude of these notes. Further studies of vocalizations calibrated for amplitude would be needed to test this possibility.

Previous studies on sage-grouse also found positive correlations between male mating success and the maximum frequency of the whistle as well as the difference between the maximum and minimum frequency of the whistle (i.e. the whistle bandwidth) in some, but not all, years and leks (Gibson and Bradbury 1985, Gibson et al. 1991, Gibson 1996). In my study, collared males produced whistles with a lower maximum frequency and a narrower bandwidth than non-collared males. If these display characteristics are important to females in this Idaho population, then collared males may be less attractive to females and therefore less likely to reproduce. The average maximum whistle frequency among collared males in this study was 2,219 Hz compared to 2,611 Hz in non-collared males. Among the six collared males, one male produced a whistle with a maximum whistle frequency within the range produced by non-collared males (2,545 Hz), but the other five males produced whistles with maximum frequencies more than 200 Hz below the range found in the non-collared males (the range of these five collared males was 1,967-2,279 Hz and the range of all non-collared males I measured in Idaho (n=7 birds) was 2,507-2,756 Hz). The frequency of whistles of collared males were highly unusual not only for the Idaho population, but for populations throughout the range of the sage-grouse. Four of the six collared males from my study produced maximum whistle frequencies below that of 350 calls from non-collared males

(n=350 birds) across seven populations of sage-grouse (range = 2,053-2837 Hz, mean = 2,413 Hz; Krakauer et al. 2009). Because the present study had a small sample size, the average maximum whistle frequency found here may not accurately represent the vocalizations of a larger sample of collared males. However, this study suggests that collaring can have a large effect on some males, causing their fitness-relevant acoustic signals to be outside the normal range of variation in this species and outside of the range of non-collared males within the same population.

Results suggest that some collared males may have difficulty producing normal breeding vocalizations. Displaying males inflate their esophageal air sac by exhaling air from their lungs and directing it into their esophagus (Clarke et al. 1942). The strut display is produced by the rapid distension and inversion of the inflated esophagus behind a pair of pliable apterygia on the breast (i.e. the vocal sacs). This produces a visual display and increases the amplitude of the acoustic signal by resonating sound energy and coupling the sounds to the surrounding air (Dantzker et al. 1999, Krakauer et al. 2009). I propose that necklace collars that encircle the esophagus may interfere with inflation or movement of the vocal sac during display. This interference may increase the costs of an already costly behavior (Vehrencamp et al. 1989), and potentially decrease male reproductive success by decreasing the effectiveness of these vocalizations for attracting females and competing with other males for breeding territories.

Male mating success is also strongly correlated with the rate of strut displays by males and male lek attendance (Wiley 1973, Gibson and Bradbury 1985, Gibson et al. 1991, Gibson 1996, Patricelli and Krakauer 2010). Analysis of the rate of strut displays

between collared and non-collared males is currently underway to address this possibility. While sage-grouse and sharp-tail grouse with radio-collars have been reported to attend leks in several studies, the lek attendance rates for collared birds were not compared to those of non-collared birds (Baumgardt 2011, Drummer et al. 2001). This is an important distinction, but is costly and difficult to measure the difference in attendance between collared and non-collared birds effectively, requiring intensive monitoring efforts to compare birds marked with color bands only and birds with radio-transmitters. However, previous study of radio-collar effects in a population of sage-grouse in Nevada did compare collared birds to non-collared birds, and found dramatic decreases in lek attendance by collared males when compared to non-collared males (Gibson et al. 2013). Taken together, these results suggest necklace collars may impact male fitness by reducing male attendance and their display quality when they do attend leks. Additional studies on behavior, energetics, and activity budgets should be initiated to evaluate relative costs associated with different radio-transmitter styles of attachment.

Literature Cited

Aldridge, C. L., S. E. Nielsen, H. L. Beyer, M. S. Boyce, J. W. Connelly, S. T. Knick, and M. A. Schroeder. 2008. Range-wide patterns of Greater Sage-grouse persistence. Diversity and Distributions 14:983-994.

Amstrup, S. C. 1980. A radio-collar for game birds. Journal of Wildlife Management 44:214–217.

Baumgardt, J. A. 2011. Probability of attendance and sightability of Greater Sage-grouse on leks: relating lek-based indices to population abundance. Thesis, University of Idaho, Moscow, USA.

Caizergues, A. and L. N. Ellison. 1998. Impact of radio-tracking on Black Grouse (*Tetrao tetrix*) reproductive success in the French Alps. Wildlife biology 4:205-212.

Clarke, L. F., H. Rahn, and M. D. Martin. 1942. Sage grouse studies part II: Seasonal and sexual dimorphic variation in the so-called "air sacs" region of the sage-grouse. Wyoming Game and Fish Bulletin 2:13-27.

Dantzker, M. S., G. B. Deane, and J. W. Bradbury. 1999. Directional acoustic radiation in the strut display of male sage-grouse, *Centrocercus urophasianus*. Journal of Experimental Biology 202:2893-2909.

Drummer, T. D., R. G. Corace III, and S. J. Sjogren. 2011. Sharp-tailed Grouse lek attendance and fidelity in Upper Michigan. Journal of Wildlife Management 73: 311-318.

Fabian, C., A. Benitez-Lopez, J. T. Garcia, C. A. Martin, J. Vinuela, and F. Mougeot. 2015. Assessing the short-term effects of capture, handling and tagging of Sandgrouse. Ibis 157:115-124.

Fremgen, A. L., C. P. Hansen, M. A. Rumble, R. S. Gamo, J. J. Millspaugh. 2015. Male Greater Sage-grouse detectability on leks. Journal of Wildlife Management < http://onlinelibrary.wiley.com/enhanced/doi/10.1002/jwmg.1001> Accessed 24 October 2015.

Frye, G. G., J. W. Connelly, D. D. Musil, C. Cardinal, L. Cross, and J. S. Forbey. 2014. Do necklace-style radiotransmitters influence flushing behavior of Greater Sage-grouse? The Wildlife Society Bulletin 38:433-438.

Garton, E. O., J. W. Connelly, J. S. Horne, C. A. Hagen, A. Moser, and M. A. Schoeder. 2011. Greater Sage-grouse population dynamics and probability of persistence. *in* S. T. Knick, and J.W. Connelly, editors. Greater Sage-grouse: ecology and conservation of a landscape species and its habitats. Studies in Avian Biology. University of California Press, Berkeley, CA.

Gibson, R. M. 1996 Female choice in sage-grouse: the roles of attraction and active comparison. Behavioral Ecology and Sociobiology 39:55-59.

Gibson, R. M., and J. W. Bradbury. 1985. Sexual selection in lekking grouse: phenotypic correlates of male strutting success. Behavioral Ecology Sociobiology 18:117-123.

Gibson, R. M., J. W. Bradbury, and S. L. Vehrencamp. 1991. Mate choice in lekking sage-grouse revisited: the roles of vocal display, female site fidelity, and copying. Behavioral Ecology 2:165-180.

Gibson, D., E. J. Blomberg, G. L. Patricelli, A. H. Krakauer, M. T. Atamian, and J. S. Sedinger. 2013. Effects of radio collars on survival and lekking behavior of male Greater Sage-grouse. The Condor 5:769-776.

Jenni, D. A., and J. E. Hartzler. 1978. Attendance at a sage-grouse lek - implications for spring censuses. Journal of Wildlife Management 42:46-52.

Koch, R. E., A. H. Krakauer, and G. L. Patricelli. 2015. Investigating female mate choice for mechanical sounds in the male Greater Sage-Grouse. Auk 132:349-358.

Krakauer, A. H., M. Tyrell, K. Lehmann, N. Losin, F. Goller, and G. L. Patricelli. 2009. Vocal and anatomical evidence for a two-voiced syrinx in the Greater Sage-grouse *Centrocercus urophasianus*. Journal of Experimental Biology. 212:3719-3727

Millspaugh, J. J., D. C. Kesler, R. W. Kays, R. A. Gitzen, J. H. Schultz, C. T. Rota, C. M. Bodinof, J. L. Belant, and B. J. Keller. 2012. Wildlife Radiotelemetry and Remote Monitoring. Pages 258-283 *in* N. J. Silvy, editor. The Wildlife Techniques Manual 7th edition, Volume 1. The Johns Hopkins University Press, Baltimore, Maryland.

Patterson, R. L. 1952. The Sage Grouse in Wyoming. The Wyoming Game and Fish Commission and Sage Books, Denver, CO.

Patricelli, G. L., and A. H. Krakauer. 2010 Tactical allocation of effort among multiple signals in sage-grouse: an experiment with a robotic female. Behavioral Ecology 21:97-106.

Pyrah, D. 1970. Poncho markers for game birds. Journal of Wildlife Management 34:466-467.

Schroeder, M. A., C. L. Aldridge, A. D. Apa, J. R. Bohne, C. E. Braun, S. D. Bunnell, J. W. Connelly, P. A. Deibert, S. C. Gardner, M. A. Hilliard, G. D. Kobriger, S. M. McAdam, C. W. McCarthy, J. J. McCarthy, D. L. Mitchell, E. V. Rickerson, and S. J. Stiver. 2004. Distribution of Sage-grouse in North America. Condor 106:363-376.

Theuerkauf, J., S. Rouys, and C. Chatreau. 2007. Mortality of radio-tracked wild rats in relation to transmitter weight and resilience of transmitters in relation to their design. Journal of the Royal Society of New Zealand 37:85-90.

Vehrencamp, S. L., J. W. Bradbury, and R. M. Gibson. 1989. The energetic cost of display in male sage-grouse. Animal behavior: 38: 885–896.

Venturato, E., P. Cavallini, P. Banti, and F. Dessi-Fulgheri. 2009. Do radio collars influence mortality and reproduction? A case with Ring-necked Pheasants (*Pasianus colchicus*) in Central Italy. Eurasian Journal of Wildlife Research 55:547-551.

Walsh, D. P., G. C. White, T. E. Remington, and D. C. Bowden. 2004. Evaluation of the lek-count index for greater sage-grouse. Wildlife Society Bulletin 32:56-68.

Ward, D. H., and P. L. Flint. 1995. Effects of harness-attached transmitters on pre-migration and reproduction of brant. Journal of Wildlife Management 59:39-46.

Wiley, R. H. 1973. Territoriality and non-random mating in sage-grouse *Centrocercus urophasianus*. Animal Behaviour Monographs 6: 85-169.

Zenzal, T. J., R.H. Diehl, and F. R. Moore. 2014. The impact of radio-tags on Ruby-throated Hummingbirds (*Archilochus colubris*). The Condor 116: 518-526.

Figures

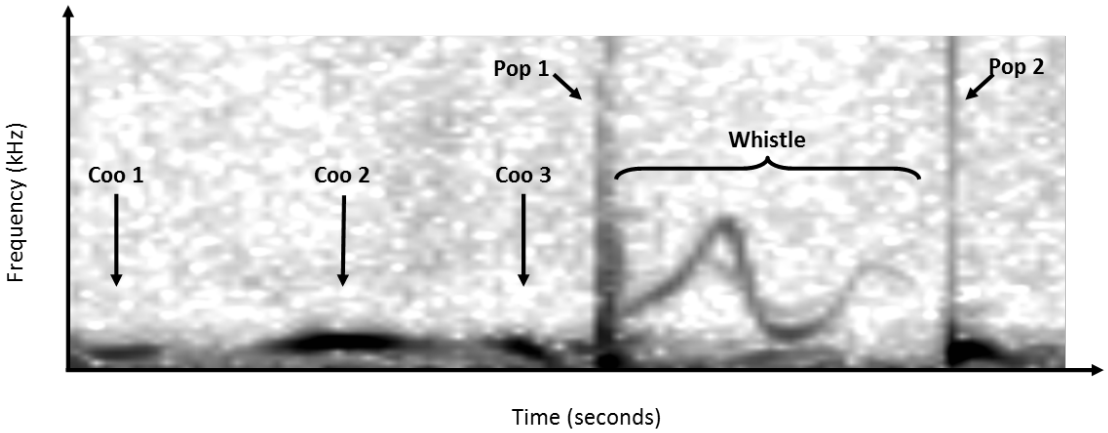


Figure 3.1 Example of a vocalization from a typical male Greater Sage-grouse (*Centrocercus urophasianus*) from a lek in Fremont County, Wyoming. This recording was captured using an on-lek microphone array (Krakauer et al. 2009) instead of the more distant single-microphone recording technique used in this study. This microphone set-up illustrates the full suite of vocal characteristics, some of which are not visible in the more distant single microphone used in this study.

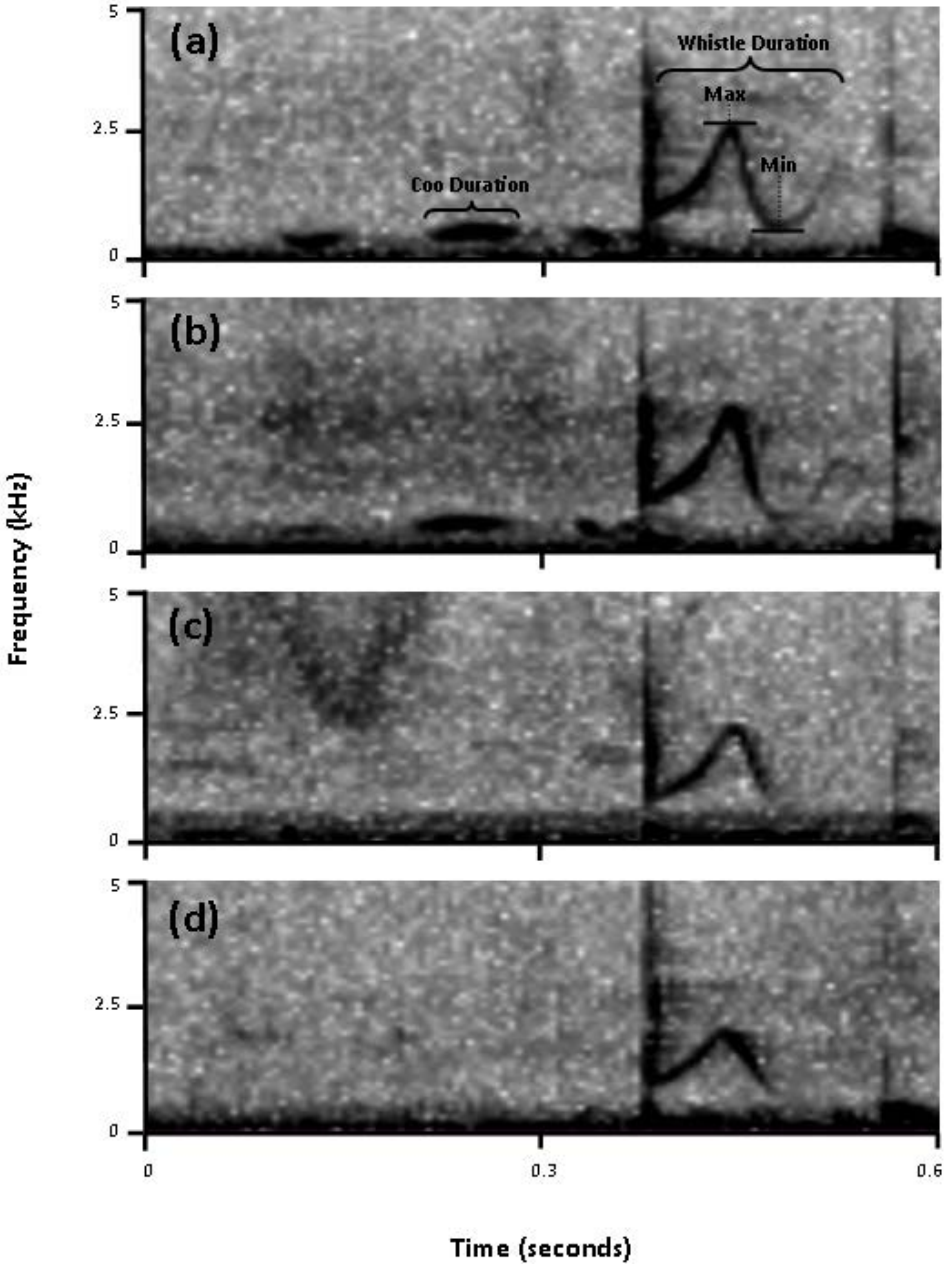


Figure 3.2 Strut vocalizations recorded from two non-collared (a and b) and two collared (c and d) male Greater Sage-grouse (*Centrocercus urophasianus*). The vocalization characteristics found to be significantly different between these groups are labeled in (a): "max" = maximum frequency of the primary whistle (kHz), "min" = minimum frequency of the primary whistle (kHz), "whistle duration" = duration of the primary whistle (s), and "coo duration" = duration of the second coo (s).

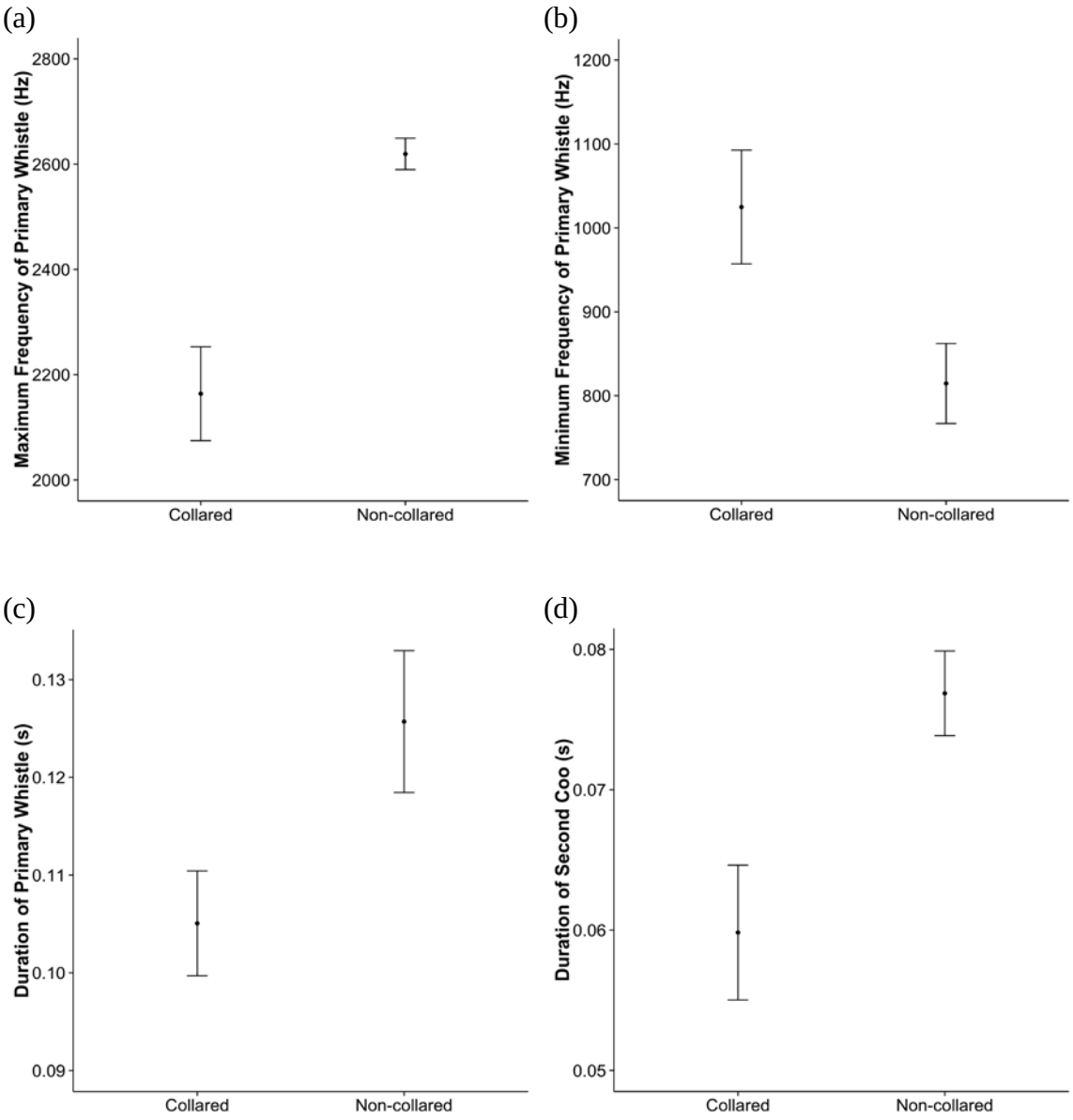


Figure 3.3 Significant differences in vocal characteristics included (a) maximum frequency of primary whistle (Hz), (b) minimum frequency of primary whistle (Hz), (c) whistle duration (s), and (d) duration of second coo (s). All graphs show mean ± SEM comparing the average value for each vocalization characteristic for six collared and seven non-collared birds (except (d), where there were five collared and seven non-collared because the secondary coo was not visible for one collared male).

CHAPTER 4: NON-DESTRUCTIVE SAMPLING METHODS TO DETERMINE SAGEBRUSH AGE AND RELATIONSHIPS BETWEEN AGE AND PLANT PALATABILITY

Abstract

Palatability of plants is an important factor that influences habitat use by herbivores at multiple spatial scales. Additionally, consumption of high quality food resources improves reproductive success for herbivores. Proper management of herbivore habitat requires that managers identify and protect high quality forage resources that can help maximize herbivore fitness. Therefore, managers should identify characteristics of plants that are related with palatability. For Greater Sage-grouse (*Centrocercus urophasianus*) foraging in sagebrush (*Artemisia* sp.) habitats, it is important for managers to identify characteristics of plants that provide high-quality forage. I hypothesized that plant age was related to palatability, and that age could be assessed using non-destructive methods. Sagebrush treatments on federal and private lands throughout the range of sage-grouse have been designed to remove decadent sagebrush to improve forage quality. However, our research showed that there was no difference in plant chemistry (e.g. palatability) between old (decadent) plants and younger seedlings, suggesting that habitat treatments that result in younger stands, such as brush mowing, may not improve forage quality of sagebrush. In addition, I determined that the circumference measured at the base of plants could predict the age of

sagebrush. This method has potential to help managers assess the age of stands and plants following restoration efforts.

Introduction

The defensive chemistry of plants can limit intake by herbivores (Guglielmo et al. 1996, Wiggins et al. 2003). In addition, the spatial and temporal variation of plant chemicals influences habitat use by herbivores (Youngentob et al. 2011, Frye et al. 2013, Ulappa et al. 2014). Therefore, management of herbivores should include proper identification and conservation of the most palatable chemical profiles of plants, or chemotypes. Conservation of palatable plants requires that researchers first identify parameters that influence chemotypes. I hypothesized that the age of a plant is one parameter that influences chemotypes, since plants differentially invest in growth and defense at different developmental points (Karolewski et al. 2011, Liu et al. 2012, Quintero and Bowers 2013, Moreira et al. 2014). Relationships between plant ontogeny and defensive chemistry are complicated and vary by species. In some woody species, such as Scots Pine (*Pinus sylvestris*), the youngest twigs had the lowest concentrations of defensive chemicals (Liu et al. 2012). In contrast, older plants had higher concentrations of defensive chemicals in a variety of perennial grassland species (Elger et al. 2009), as well as in hops (*Humulus lupulus*; Jelinek et al. 2012).

Defensive compounds, or plant secondary metabolites, influence palatability and thereby diet selection and habitat selection by herbivores. Greater Sage-grouse (*Centrocercus urophasianus*; hereafter, sage-grouse) are sagebrush obligate herbivores, and are a species of concern throughout western North America. Sage-grouse diets are

comprised entirely of sagebrush throughout the winter months (Patterson 1952) and birds also rely on shrubs for cover. Diet quality of sagebrush is influenced partly by concentration of coumarins and monoterpenes (Rosentreter 2004, Frye et al. 2013), and monoterpenes influence habitat use and diet selection by sage-grouse (Remington and Braun 1985, Frye et al. 2013), as well as other herbivores (Ulappa et al. 2014). As the climate warms, plant secondary metabolites (PSMs) are expected to increase in sagebrush, making management of palatable profiles even more important (Revermann et al. 2012, Forbey et al. 2013). Additionally, plant ontogeny influences production of secondary metabolites for some species (Karolewski et al. 2011, Liu et al. 2012), so plant age may be related to palatability. For example, plant age influences induced defense in sagebrush plants, which impacts the amount of damage caused by insect and ungulate herbivores to the plant (Shiojiri et al. 2011). Palatability of plants is therefore partly determined by age, and can therefore influence herbivore behavior.

If age is a parameter that influences plant chemistry, then it could be managed to create grouse habitat with the highest dietary quality. To properly manage for age of sagebrush, managers need a reliable field method to evaluate the age of plants within a sagebrush stand. Because woody plants are often measured using circumference, and tree circumference is correlated with age (Worbes et al. 2003, Nascimbene et al. 2009), I tested whether the circumference at the base of sagebrush plants are correlated with annual ring growth. Correlating age and circumference may yield a simple, nonintrusive method to estimate the age of sagebrush in the field without counting annual rings, which requires destructive sampling. Understanding how age influences palatability of plants is

an important factor in assessing and managing grouse habitat. I hypothesized that plant age would be either positively or negatively correlated with plant defensive chemistry. If younger plants are more palatable, seeding and planting in decadent stands could be effective methods to improve habitat for foraging grouse. Alternatively, if older plants are more palatable, the nutritional consequences of mowing and herbicide could outweigh other potential benefits of these treatments.

Methods

Study Site and Field Methods

Samples were collected from Jim Sage Mountain near Almo, Idaho (42° 9' N, 113° 24' W). This is an arid sage-steppe ecosystem with mostly low sagebrush (*Artemisia arbuscula*) and Wyoming big sagebrush (*A. tridentata wyomingensis*). I used low sagebrush because sage-grouse select dwarf sagebrush species (including low sagebrush) as a foraging resource more than expected based on availability (Frye et al. 2013). Additionally, low sagebrush is a palatable food source for wildlife (Rosentreter 2004). Importantly, the morphology of low sagebrush is appropriate to test these questions because low sagebrush often has a single stem to measure, while other species (e.g. Wyoming big sagebrush) often have split bases, making accurate measurement of annual rings difficult. Low sagebrush plants were selected at randomly generated points, and I only sampled plants with intact stems at the base of the plant to increase accuracy for counting annual growth rings. Plants with split bases cannot be accurately assessed for age. I used destructive sampling methods to collect 53 low sagebrush plants that ranged in size from approximately 5 cm to 55 cm tall to represent a full range of potential

ages for this species. All samples were kept on ice during transport and were transferred to a -20°C freezer as soon as possible.

Sample Processing and Chemical Analysis

Preparation of leaf material for chemical analysis followed procedures outlined in Chapter 1. Chemical analysis for monoterpenes and coumarins also followed procedures outlined in Chapter 1. I focused on monoterpenes and coumarins because monoterpenes are known to influence sage-grouse foraging behavior (Frye et al. 2013) and coumarins are related to plant palatability (Rosentreter 2004). Briefly, I de-wooded and ground leaf samples to a fine powder, then weighed out 0.100 g for monoterpene quantification and 0.050 g for the coumarin assay. Monoterpenes were identified and quantified using headspace gas chromatography, and coumarins were analyzed using a colorimetric assay with a scopoletin standard curve.

Circumference and Age

To determine if circumference can accurately estimate the age of a plant, I cut low sagebrush plants at the base of the plant, using duct tape to hold together the bark on either side of the cut. The circumference of the plant's stem was measured at the base and was recorded in millimeters, to mimic how samples would be measured in the field. Then each plant was brought back to the lab and dipped in baby oil to help intensify the appearance of annual growth rings. The rings were counted, including the center of the stem (Figure 4.1).

Statistical Methods

First, I tested if age was related to the total monoterpene concentration in each plant (AUC/μg dry weight), the number of monoterpene compounds in each plant (number of compounds > 1% total AUC, with retention times earlier than 24 minutes), and the coumarin concentration (μM scopoletin equivalents/g DW). All three types of chemistry were compared to age using Spearman correlation tests. I also tested if age and circumference were related using a Spearman correlation test.

Results

Total monoterpene concentrations were not correlated with age (i.e. annual growth rings), however there was a trend for higher monoterpene content in older plants (Spearman: $rho = 0.189$, $df = 52$, $P = 0.214$). There was no impact of age on the number of individual monoterpene compounds in the plant (Spearman: $rho = 0.117$, $df = 52$, $P = 0.398$). Total coumarin concentrations were not correlated with age (Spearman: $rho = -0.018$, $df = 52$, $P < 0.900$).

I found a strong correlation between the circumference of the base of low sagebrush plants and the annual growth rings of the plant (Figure 4.2; Spearman: $rho=0.995$, $df = 52$, $P < 0.001$). The circumference of low sagebrush plants can be used to estimate the age of the plant using the linear regression formula: age (growth rings) = (0.2087) circumference (mm) + 0.0722.

Discussion

Although total monoterpene and coumarin concentrations were not correlated with the age of low sagebrush plants, concentrations of individual chemicals were not

evaluated in this study and should be evaluated in future research. Many herbivores also select for high protein content (Barnett and Crawford 1994, Gregg et al. 2008, Frye et al. 2013, Ulappa et al. 2014), which was not taken into consideration for this study. Additionally, parameters like habitat quality, plant density, and water accessibility also affect foraging selection by avian herbivores (Jones 2011), possibly more than plant age or chemotypes. Nonetheless, this study is beneficial for managers evaluating the role of the age of plants for wildlife dependent on sagebrush habitats.

We found no evidence that sagebrush age was related to palatability, which suggests that plant age may not influence foraging by herbivores. Sagebrush treatments are typically prescribed with the intent of improving forage for livestock, because decadent sagebrush stands are often considered unproductive. Old plants are generally larger and provide more biomass both for food and cover. These habitat treatment practices remove important cover components, but do not improve forage quality of sagebrush based on protein content (Davies et al. 2009). My results suggest that these treatments may not reduce defensive chemistry and improve palatability, either. Additionally, these practices can have negative ecological impacts (Davies et al. 2012). Therefore, habitat treatments, including brush mowing and defoliation, are unlikely to improve the quality of sagebrush as a foraging resource in sage-grouse habitat.

Using circumference as a measure of annual growth rings is a non-destructive method that allows researchers to assess the age of plants in the field. I validated the use of circumference for estimates of age for *Artemisia arbuscula* in southern Idaho. Further studies will be needed to expand this method to other populations of low sagebrush, other

species of sagebrush, and sagebrush under varying ecological conditions. This tool may also be useful for examining mowing impacts, planting and seeding success, and growth after disturbances.

Literature Cited

Barnett, J., and J. Crawford. 1994. Pre-laying nutrition of sage-grouse hens in Oregon. Journal of Range Management 47:114-118.

Davies, K. W., J. D. Bates, D. D. Johnson, and A. M. Nafus. 2009. Influence of Mowing Artemisia tridentata ssp wyomingensis on Winter Habitat for Wildlife. Environmental Management 44:84-92.

Davies, K. W., J. D. Bates, and A. M. Nafus. 2012. Mowing Wyoming Big Sagebrush Communities With Degraded Herbaceous Understories: Has a Threshold Been Crossed? Rangeland Ecology & Management 65:498-505.

Elger, A., D. G. Lemoine, M. Fenner, and M. E. Hanley. 2009. Plant ontogeny and chemical defence: older seedlings are better defended. Oikos 118:767-773.

Forbey, J. S., N. L. Wiggins, G. G. Frye, and J. W. Connelly. 2013. Hungry grouse in a warming world: emerging risks from plant chemical defense and climate change. Wildlife Biology 19: 374-381.

Frye, G. G., J. W. Connelly, D. D. Musil, and J. S. Forbey. 2013. Phytochemistry predicts habitat selection by an avian herbivore at multiple spatial scales. Ecology 94:308-314.

Gregg, M., J. Barnett, and J. Crawford. 2008. Temporal variation in diet and nutrition of preincubating Greater Sage-grouse. Rangeland Ecology and Management 61:535-542.

Guglielmo, C., W. Karasov, and W. Jakubas. 1996. Nutritional costs of a plant secondary metabolite explain selective foraging by Ruffed Grouse. Ecology 77:1103-1115.

Jelinek, L., M. Doleckova, M. Karabin, T. Hudcova, B. Kotlikova, and P. Dostalek. 2012. Influence of Growing Area, Plant Age, and Virus Infection on the Contents of Hop Secondary Metabolites. Czech Journal of Food Sciences 30:541-547.

Jones, J. 2011. Habitat selection studies in avian ecology: a critical review. The Auk 118: 557-562.

Karolewski, P., A. M. Jagodzinski, and J. Grzebyta. 2011. Influence of tree age, needle age and location in the crown on the phenolic compounds content in needles of young Scots pines. Sylwan 155:797-807.

Liu, R. S., S. A. Gourley, D. L. DeAngelis, and J. P. Bryant. 2012. Modeling the dynamics of woody plant-herbivore interactions with age-dependent toxicity. Journal of Mathematical Biology 65:521-552.

Moreira X., K. A. Mooney, S. Rasmann, W. K. Petry, A. Carrillo-Gavilan, R. Zas, and L. Sampedro. 2014. Trade-offs between constitutive and induced defenses drive geographical and climatic clines in pine chemical defenses. Ecology Letters 17: 537-546.

Nascimbene, J., L. Marini, R. Motta, and P.L. Nimis. 2009. Influence of tree age, tree size, and crown structure on lichen communities in mature alpine spruce forests. Biodiversity Conservation 18: 1509-1522.

Patterson, R. L. 1952. The Sage Grouse in Wyoming. Sage Books, Inc., Denver, CO, USA.

Quintero, C. and M. D. Bowers. 2013. Effects of insect herbivory on induced chemical defenses and compensation during early plant development in *Penstemon virgatus*. Annals of Botany 112: 661-669.

Remington, T., and C. Braun. 1985. Sage-grouse food selection in winter, North Park, Colorado. Journal of Wildlife Management 49:1055-1061.

Revermann, R., H. Schmid, N. Zbinden, R. Spaar, and B. Schroder. 2012. Habitat at the mountain tops: How long can Rock Ptarmigan (*Lagopus muta*) survive rapid

climate change in the Swiss Alps? A multi-scale approach. Journal of Ornithology 153: 891-905.

Rosentreter, R. 2004. Sagebrush identification, ecology, and palatability relative to sage-grouse. USDA Forest Service Proceedings RMRS-P-000.

Shiojiri, K., R. Karban, and S. Ishizaki. 2011. Plant age, seasonality, and plant communication in sagebrush. Journal of Plant Interactions 6:85-88.

Ulappa, A. C., R. G. Kelsey, G. G. Frye, J. L. Rachlow, L. A. Shipley, L. Bond, X. Z. Pu, and J. S. Forbey. 2014. Plant protein and secondary metabolites influence diet selection in a mammalian specialist herbivore. Journal of Mammalogy 95:834-842.

Wiggins, N., C. McArthur, S. McLean, and R. Boyle. 2003. Effects of two plant secondary metabolites, cineole and gallic acid, on nightly feeding patterns of the common brushtail possum. Journal of Chemical Ecology 29:1447-1464.

Worbes, M., R. Staschel, A. Roloff, and W. J. Junk. 2003. Tree ring analysis reveals age structure, dynamics and wood production of a natural forest stand in Cameroon. Forest Ecology and Management 173:105-123.

Youngentob, K., I. Wallis, D. Lindenmayer, J. Wood, M. Pope, and W. Foley. 2011. Foliage Chemistry Influences Tree Choice and Landscape Use of a Gliding Marsupial Folivore. Journal of Chemical Ecology 37:71-84.

Figures

Figure 4.1 A cross-section cut of low sagebrush (*Artemisia arbuscula*) collected at Raft River, Idaho, USA in fall 2015 that was used to assess the relationship between stem circumference and age using annual growth rings. This sample has seven annual growth rings.

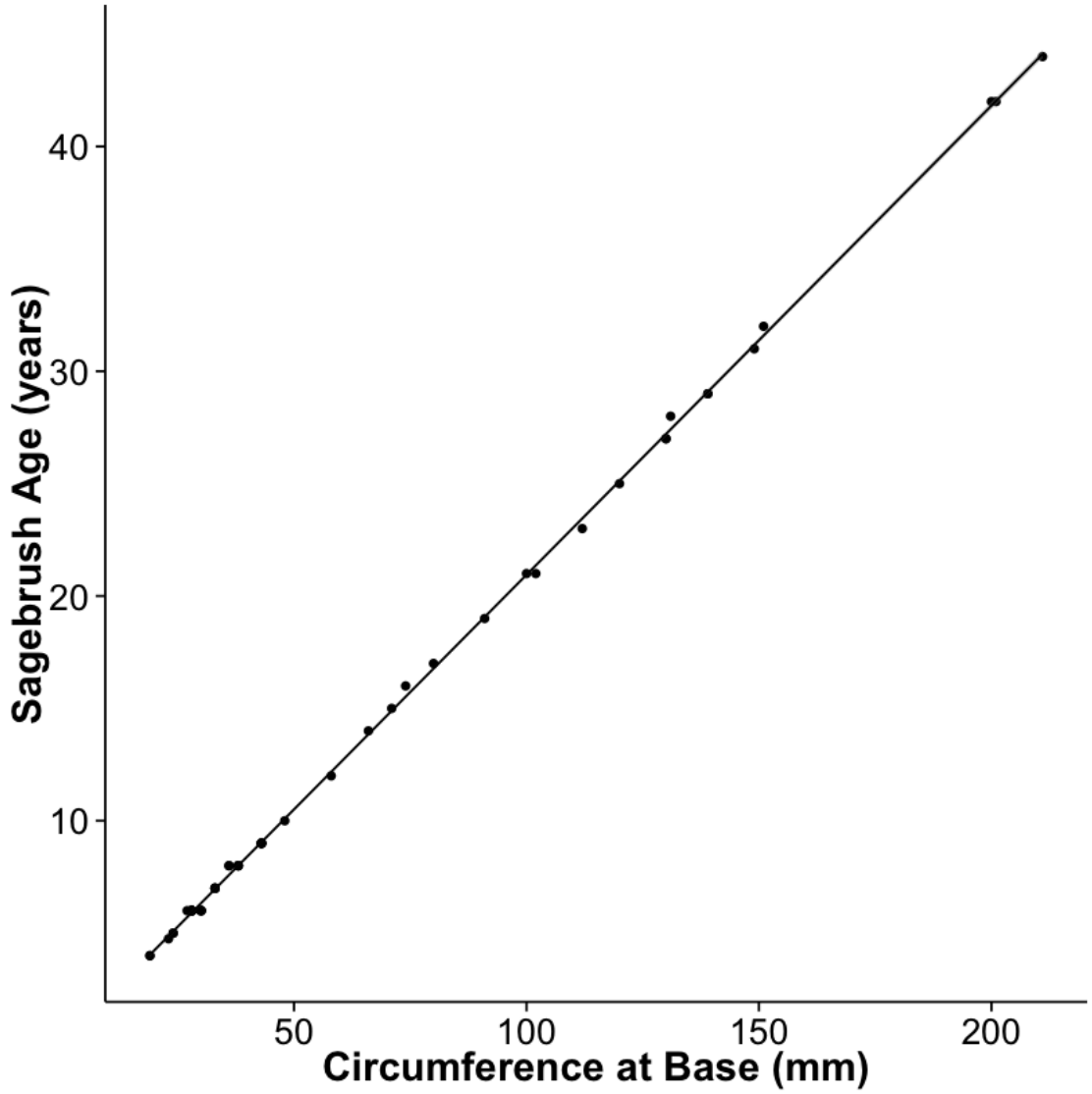


Figure 4.2 Relationship between annual growth rings (age in years) and circumference (mm) of low sagebrush (*Artemisia arbuscula*) plants at Raft River, Idaho (*rho*=0.995, *df* = 52, *P*< 0.001). The formula for the correlation can be used to estimate ages for plants with circumference measures: age (annual growth rings) = (0.2087) circumference (mm) + 0.0722.

APPENDIX A

Management Implications

Management Implications

Chapter One

For herbivores, it is important to consider not just the structural quality of habitat that provides cover, but also the dietary quality of available food resources. When animals forage selectively, this behavior impacts their habitat use (Youngentob et al. 2011, Frye et al. 2013, Ulappa et al. 2014) and movement patterns (Masse and Côte 2013). Additionally, diet quality influences the reproductive success of many animals including brushtail possums (DeGabriel et al. 2009), crickets (Hunt et al. 2004), and birds (Chastel et al. 1995, Gregg et al. 2008). Therefore, it is important maintain high quality food resources to ensure population survival for herbivores.

For Greater Sage-grouse (*Centrocercus urophasianus*) at Craters, I found that grouse may not be selecting specific sagebrush species to eat. At the Craters study site, sage-grouse selected both three-tip (*Artemisia tripartita*) and Wyoming big sagebrush (*A. tridentata wyomingensis*) in proportion to their availability. This site is a post-fire environment with sagebrush cover well below recommended guidelines for winter habitat (Connelly et al. 2000). Therefore, the habitat at Craters may be sub-optimal and grouse may be using acceptable, rather than optimal, food sources. While there was no landscape-scale selection occurring, selection did occur at finer scales for patches and plants with specific structural and phytochemical characteristics, including moderate sized plants, lower plant secondary metabolite (PSM) concentrations, and higher protein concentrations. This suggests that scale is important for habitat selection, and that a diversity of available resources may provide more options for foragers. Diversity may be

important to provide a variety of options for both food and cover. High chemical diversity in foraging resources is important for herbivores because it allows consumers limit intake of any single potentially toxic PSM. Our recommendation is that managers should preserve large, undisturbed tracts of habitat to maintain available diversity in forage resources and should consider the dietary quality of those available resources. Additionally, managers should strive to minimize fire in sagebrush habitats, due to slow recovery times and sparse sagebrush cover following fires (Baker 2006, Beck et al. 2009), thus leading to low forage availability and diversity, and potentially sub-optimal habitat. However, fire is difficult to manage, so post-fire restoration is critical. Three-tip sagebrush provides a post-fire food source that is potentially palatable for wildlife, since it re-establishes more quickly than big sagebrush (Beck et al. 2009). While grouse use three-tip in degraded habitats, how grouse use this species in optimal habitats, and the consequences of consuming a potentially sub-optimal forage plant, is unknown and deserves further attention before management recommendations can be made regarding three-tip sagebrush.

This research highlights the value of conserving diverse sagebrush taxa available because certain species may provide a valuable forage resource during habitat changes (including fire), or at different times of the year. It is important to conserve and restore diverse structural and phytochemical habitats. This creates a landscape better suited for meeting needs of diverse wildlife throughout the year, and when landscapes are altered or disturbed. Restoration efforts, where appropriate, should focus on reseeding with sagebrush plants of high dietary quality (high protein, low PSMs) that were present at the

site prior to disturbance. This requires that managers map and collect seeds from sagebrush species across a wide range to prepare for potential restoration efforts.

Chapter Two

Diet quality was also related to parasite abundance for sage-grouse. Although *Raillietina centrocerci* is not known to be fatal or cause serious negative effects, the parasite may limit nutrient acquisition (Nelson 1955) and energy available for other energetically expensive activities. Additionally, host-parasite dynamics may be altered by climate change (Molnar et al. 2013a, Molnar et al. 2013b), so continued monitoring of this relationship is important. Additionally, host-parasite relationships in other grouse species can drive host population dynamics (Formenti et al. 2013, Dunham et al. 2014, Martinez-Padilla et al. 2014). Therefore, developing a better understanding of the interactions among environmental conditions across space and time, diet quality, parasites and demographics may be important to better predict factors regulating sage-grouse populations.

Chapter Three

Necklace-style radio-transmitters were found to alter some vocalization characteristics of the breeding display performed by male sage-grouse. Collared males had a narrower bandwidth on the primary whistle, and a shorter primary whistle and shorter coo. These characteristics have been linked to breeding success in some years and some populations, although the impact on breeding success in Idaho is unknown (Gibson 1996, Patricelli and Krakauer 2010. However, necklace-style transmitters may alter other aspects of behavior, such as display frequency. Additionally, collared male

sage-grouse do not attend leks as often as males without collars in some locations (Gibson et al. 2013). These studies suggest that alternative methods for transmitter attachment, such as rump-mounted transmitters, should be considered for tracking male Greater Sage-grouse. Moreover, the study underscores the need to consider a broad range of consequences on the immediate behavior (e.g. vocalizations, lek attendance) and long-term fitness (e.g. survival and reproductive success) related to the techniques researchers use to study wildlife.

Chapter Four

I did not find any evidence that plant age is correlated with plant chemistry, however other types of chemistry (e.g. individual phenolics, individual monoterpenes) or crude protein may be correlated with plant age. Therefore, destruction of decadent sagebrush is unlikely to improve forage quality of sagebrush. Circumference was strongly correlated with plant age, providing a relatively easy and rapid technique for managers to assess age of sagebrush in the field. This is useful for managers that wish to assess the success of a seeding project over time, how well a site recovers after a disturbance, or how much recruitment there is in a sagebrush stand.

Literature Cited

Baker, W. L. 2006. Fire and restoration of sagebrush ecosystems. Wildlife Society Bulletin 34:177-185.

Beck, J. L., J. W. Connelly, and K. P. Reese. 2009. Recovery of Greater Sage-grouse habitat features in Wyoming big sagebrush following prescribed fire. Restoration Ecology 17:393-403.

Chastel, O., H. Weimerskirch, and P. Jouventin. 1995. Influence of body condition on reproductive decision and reproductive success in the Blue Petrel. The Auk 112:964-972.

Connelly, J. W., M. Schroeder, A. Sands, and C. Braun. 2000. Guidelines to manage sage-grouse populations and their habitats. Wildlife Society Bulletin 28:967-985.

DeGabriel, J., B. Moore, W. Foley, and C. Johnson. 2009. The effects of plant defensive chemistry on nutrient availability predict reproductive success in a mammal. Ecology 90:711-719.

Dunham, N. R., S. T. Peper, C. E. Baxter, and R. J. Kendall. 2014. The parasitic eyeworm *Oxyspirura petrowi* as a possible cause of decline in the threatened Lesser Prairie-chicken (*Tympanuchus pallidicinctus*). Plos One 9:1-5.

Formenti, N., R. Vigano, L. Rotelli, N. Ferrari, M. C. Cerutti, and P. Lanfranchi. 2013. Effect of suboptimal environment and host age on helminth community of Black Grouse (*Tetrao tetrix*). European Journal of Wildlife Research 59:351-358.

Frye, G. G., J. W. Connelly, D. D. Musil, and J. S. Forbey. 2013. Phytochemistry predicts habitat selection by an avian herbivore at multiple spatial scales. Ecology 94:308-314.

Gibson, R. M. 1996. Female choice in sage-grouse: the roles of attraction and active comparison. Behavioral Ecology and Sociobiology 39: 55–59.

Gibson, D., E. J. Blomberg, G. L. Patricelli, A. H. Krakauer, M. T. Atamian, and J. S. Sedinger. 2013. Effects of radio collars on survival and lekking behavior of male Greater Sage-grouse. The Condor 5:769-776.

Gregg, M., J. Barnett, and J. Crawford. 2008. Temporal variation in diet and nutrition of preincubating Greater Sage-grouse. Rangeland Ecology and Management 61:535-542.

Hunt, J., R. Brooks, M. Jennions, M. Smith, C. Bentsen, and L. Bussiere. 2004. High-quality male field crickets invest heavily in sexual display but die young. Nature 432:1024-1027.

Martinez-Padilla, J., S. M. Redpath, M. Zeineddine, and F. Mougeot. 2014. Insights into population ecology from long-term studies of Red Grouse *Lagopus lagopus scoticus.* Journal of Animal Ecology 83:85-98.

Masse, A., and S. D. Côte. 2013. Spatiotemporal variations in resources affect activity and movement patterns of white-tailed deer (*Odocoileus virginianus*) at high density. Canadian Journal of Zoology 91:252-263.

Molnar, P. K., A. P. Dobson, and S. J. Kutz. 2013a. Gimme shelter - the relative sensitivity of parasitic nematodes with direct and indirect life cycles to climate change. Global Change Biology 19:3291-3305.

Molnar, P. K., S. J. Kutz, B. M. Hoar, and A. P. Dobson. 2013b. Metabolic approaches to understanding climate change impacts on seasonal host-macroparasite dynamics. Ecology Letters 16:9-21.

Nelson, O. C. 1955. A field study of the Sage-grouse in southeastern Oregon with special reference to reproduction and survival. Oregon State College.

Patricelli, G. L., and A. H. Krakauer. 2010. Tactical allocation of effort among multiple signals in sage-grouse: an experiment with a robotic female. Behavioral Ecology 21: 97–106.

Ulappa, A. C., R. G. Kelsey, G. G. Frye, J. L. Rachlow, L. A. Shipley, L. Bond, X. Z. Pu, and J.S. Forbey. 2014. Plant protein and secondary metabolites influence diet selection in a mammalian specialist herbivore. Journal of Mammalogy 95:834-842.

Youngentob, K., I. Wallis, D. Lindenmayer, J. Wood, M. Pope, and W. Foley. 2011. Foliage chemistry influences tree choice and landscape use of a gliding marsupial folivore. Journal of Chemical Ecology 37:71-84.

APPENDIX B

Settings and Sequence Parameters for Monoterpene Quantification Using a Gas Chromatograph and Headspace Auto-sampler

Settings and Sequence Parameters for Monoterpene Quantification Using a Gas Chromatograph and Headspace Auto-sampler

Monoterpene concentrations were quantified using an Agilent 7694 headspace sampler and an Agilent 6890N gas chromatograph. Sagebrush and pellet samples (100 mg) were weighed into 20 mL glass headspace vials. For each sample, 1 ml of headspace gas was injected into a J&W DB-5 capillary column (30m x 250µm x 0.25µm).

Settings for the headspace auto-sampler were:

- Temperatures:
    - Oven temperature at 100°C
    - Loop temperature at 110°C
    - Transfer line temperature at 120°C
- Time Settings:
    - Vial equilibrium time of 20 min
    - Pressurization time of 0.20 min
    - Loop fill time of 0.50 min
    - Loop equilibrium time of 0.20 min
    - Injection time of 0.50 min
- Vial Parameters: no shaking

Settings for the gas chromatograph were:

- Splitless injector at 250°C
- Flame ionization detector at 300°C
- Oven temperature initially at 40°C for 2 min

    - Increased by 3°C/min to 60°C
    - Then increased 5°C/min to 120°C
    - Then increased 20°C/min to 300°C
    - Held at 300°C for 7 min
- Inlet pressure at 80 KPa, flow rate of 1.0 mL/min

The gas chromatograph used nitrogen for the make-up gas, and helium for the carrier gas. The inlet pressure was 80 KPa with a flow rate of 1.0 mL/min.

APPENDIX C

Justification for Selection of Plant Secondary Metabolites for Analysis

Justification for Selection of Plant Secondary Metabolites for Analysis

Plants can produce an incredible number of plant secondary metabolites (PSMs) for defense. Sagebrush taxa (*Artemisia* sp.) are estimated to produce over 100 compounds that may deter herbivory, including monoterpenes, phenolics, and sesquiterpene lactones (Kelsey et al. 1982, Turi et al. 2014). Total numbers of compounds, total concentrations of compounds, presence or concentration of individual compounds, and compound diversity may all drive foraging behavior in herbivores. This leaves a large number of potential model parameters for researchers to evaluate. I chose to evaluate plant secondary metabolites from two major classes, monoterpenes and phenolics.

Monoterpenes are abundant in sagebrush (Kelsey et al. 1982), and individual monoterpenes are known to influence diet selection of wildlife including Greater Sage-grouse (*Centrocercus urophasianus*; Frye et al. 2013). Therefore, I selected monoterpenes for analysis. Previous studies with sage-grouse have found that both total monoterpene content and concentrations of specific monoterpenes may drive foraging behavior of sage-grouse (Remington and Braun 1985, Welch et al. 1988, Frye et al. 2013) Therefore, monoterpenes (both individual compounds and total) were included in the analysis. Additionally, monoterpenes are known to be bio-active (Zhu et al. 2013), and therefore individual and total monoterpenes were analyzed for their impacts on parasite loads. To limit the number of monoterpene compounds analyzed, I selected compounds that were present in greater than 1% of the total AUC (area under the chromatogram curve, or concentration) for the plant, and had to be present at that concentration in 70%

or greater of plants in that taxa. This ensured that compounds analyzed had high enough concentrations to be detected, and were common in the plants analyzed.

Although total phenolic concentration in sagebrush has not yet been associated with diet selection for specialist vertebrate herbivores (Frye et al. 2013, Ulappa et al. 2014), they are abundant in sagebrush (Kelsey et al. 1982). Additionally, phenolics influence diet selection for other taxa of herbivores (Freeland and Janzen 1974), and were therefore included in analysis. Coumarins, a sub-class of phenolics, are related to palatability of sagebrush (Rosentreter 2004) and were therefore included in analysis of diet selection. Although individual phenolics can also have bioactive properties, I did not include phenolics in my analysis of how diet quality is related to parasites. Zhu et al. found that sagebrush extracts (with total monoterpenes and total phenolics) impacted egg hatching of helminthes (2013), therefore suggesting that a class of compounds may inhibit parasites, in addition to individual compounds should be further considered for evaluating self-medication hypotheses. Sesquiterpene lactones may also be effective inhibitors of pathogens (O'Neill et al. 2010), and therefore are an important component of extracts or plant material for evaluating self-medication.

Finally, concentrations of PSMs are often correlated with one another. Therefore, all chemical variables (individual monoterpenes, phenolics, coumarins, and protein) and structural variables (height, cover, density) were assessed, for each species, in a correlation matrix. For correlated variables, I first removed compounds that were found in only one species of sagebrush (this allowed me to compare between species), and those with the lowest concentrations. When deciding between a known compound (e.g.

identified from retention times of known standards) and an unknown compound, the compound with a known identity was retained. Additionally, for the parasite analysis, when a monoterpene in the plant (ingested) was correlated with a monoterpene in the feces, the fecal monoterpene was retained as it represented the concentration of the unchanged PSM that parasites would experience in the intestine.

Literature Cited

Freeland, W. J., and D. H. Janzen. 1974. Strategies in herbivory by mammals: role of plant secondary compounds. American Naturalist 108:269-289.

Frye, G. G., J. W. Connelly, D. D. Musil, and J. S. Forbey. 2013. Phytochemistry predicts habitat selection by an avian herbivore at multiple spatial scales. Ecology 94:308-314.

Kelsey, R. G., J. R. Stephens, and F. Shafizadeh. 1982. The chemical constituents of sagebrush foliage and their isolation. Journal of Range Management 35:617-622.

O'Neill, P. M., V. E. Barton, and S. A. Ward. 2010. The molecular mechanism of action of artemisinin—the debate continues. Molecules 15:1705-1721.

Remington, T., and C. Braun. 1985. Sage-grouse food selection in winter, North Park, Colorado. Journal of Wildlife Management 49:1055-1061.

Rosentreter, R. 2004. Sagebrush identification, ecology, and palatability relative to sage-grouse. Pages 1-14 *in* UDSA Forest Service Proceedings RMRS-P-000.

Turi, C. E., P. R. Shipley, and S. J. Murch. 2014. North American *Artemisia* species from the subgenus *Tridentatae* (Sagebrush): a phytochemical, botanical and pharmacological review. Phytochemistry 98:9-26.

Ulappa, A. C., R. G. Kelsey, G. G. Frye, J. L. Rachlow, L. A. Shipley, L. Bond, X. Z. Pu, and J.S. Forbey. 2014. Plant protein and secondary metabolites influence diet

selection in a mammalian specialist herbivore. Journal of Mammalogy 95:834-842.

Welch, B. L., F. J. Wagstaff, and J. A. Roberson. 1988. Selection of big sagebrush by sage-grouse. Journal of Range Management 44:462-465.

Zhu, L., J. L. Dai, L. Yang, and J. Qiu. 2013. In vitro ovicidal and larvicidal activity of the essential oil of *Artemisia lancea* against *Haemonchus contortus* (Strongylida). Veterinary Parasitology 195:112-117.

APPENDIX D

Browse Detection Surveys

Browse Detection Surveys

Habitat use studies involving a use-available strategy are designed to evaluate how habitat quality differs between resources that an animal uses versus those that are available. Sampling schemes to assess available resources are designed to represent how an animal would use habitat if it followed a random pattern of resource use, rather than selecting resources. Our study design involved comparing structural and dietary quality of a patch between used sites (with browsing) and random sites. At random sites, I did not know if there was recent visitation by Greater Sage-grouse (*Centrocercus urophasianus*). To accurately compare used and random sites, I wanted to limit bias created by omitting random sites that did have browse, since they are likely to be higher quality than sites without browse (Frye et al. 2013). Additionally, I needed to confirm how well I actually detected browse at random sites.

I sampled 16 used sites and 16 random sites at Craters in winter 2013-2014. During this sampling period, I was able to detect browse at all (100%) of the used sites, and found a single browsed plant at one random site (6.25%). The browsed plant was collected separate from non-browsed and was used in patch-level analysis. This minimizes bias by fully representing patch quality at both used and random patches, including both used plants and those that were not browsed. The remaining non-browsed plants at random patches were collected randomly to reduce any additional biases.

In spring 2015, I conducted surveys along transects to determine browse detection and accuracy. The transect was 20 m long and had 15 plants where I used clippers to mimic browsing by sage-grouse. I trained novice observers to identify browsed plants,

and then asked observers to count the number of browse marks on every plant within 1 m of the transect line. The ability of naïve observers (n=3) to recognize browse (presence/absence) and ability to accurately count the number of bite marks was analyzed using basic descriptive statistics. Observers did not know the number of browsed plants on the transect or the location of browsed plants along the transect, and were tested independently from other observers. Plants had between 1 and 50 simulated bites, roughly representing the range of bites found on plants at this site the previous winter. By simulating bite marks of <10 and >10, I could assess our accuracy in classifying plants as either non-browsed (0-1 bites) and browsed (10+ bites).

Overall, the three observers had 97.8% (44/45) success locating browsed plants, as only one observer missed any (one) plants that had simulated browsing. This gives high confidence in our ability to detect browsed plants at random sites. Among all three observers, the average detection of bite marks was 89.2% (181/203), ranging from 86.7 to 93.5%. Observers tended to have the most accurate bite counts when there were less than 25 bites on a single plant, and all observers identified a plant with a single bite mark, demonstrating their ability to detect browse well. Based on this design, no plant, counted by any observer, would have been inaccurately classified as browsed or non-browsed.

Based on these results, I am confident that our use-available design was not biased by lack of detection of browsed plants at random sites. Therefore, for our patch-level analysis, I ran the statistics using the patch average of browsed and non-browsed plants together (for both used patches and random patches) to evaluate differences in overall patch quality. If detection had been lower for browsed plants at random patches,

it would have been necessary to compare non-browsed plants only to account for biased detection. However, this would not accurately represent the patch quality since the use of a patch may be driven by the presence of browsed plants.

Literature Cited

Frye, G. G., J. W. Connelly, D. D. Musil, and J. S. Forbey. 2013. Phytochemistry predicts habitat selection by an avian herbivore at multiple spatial scales. Ecology 94:308-314.

APPENDIX E

Representative Monoterpene Profiles for Sagebrush Species

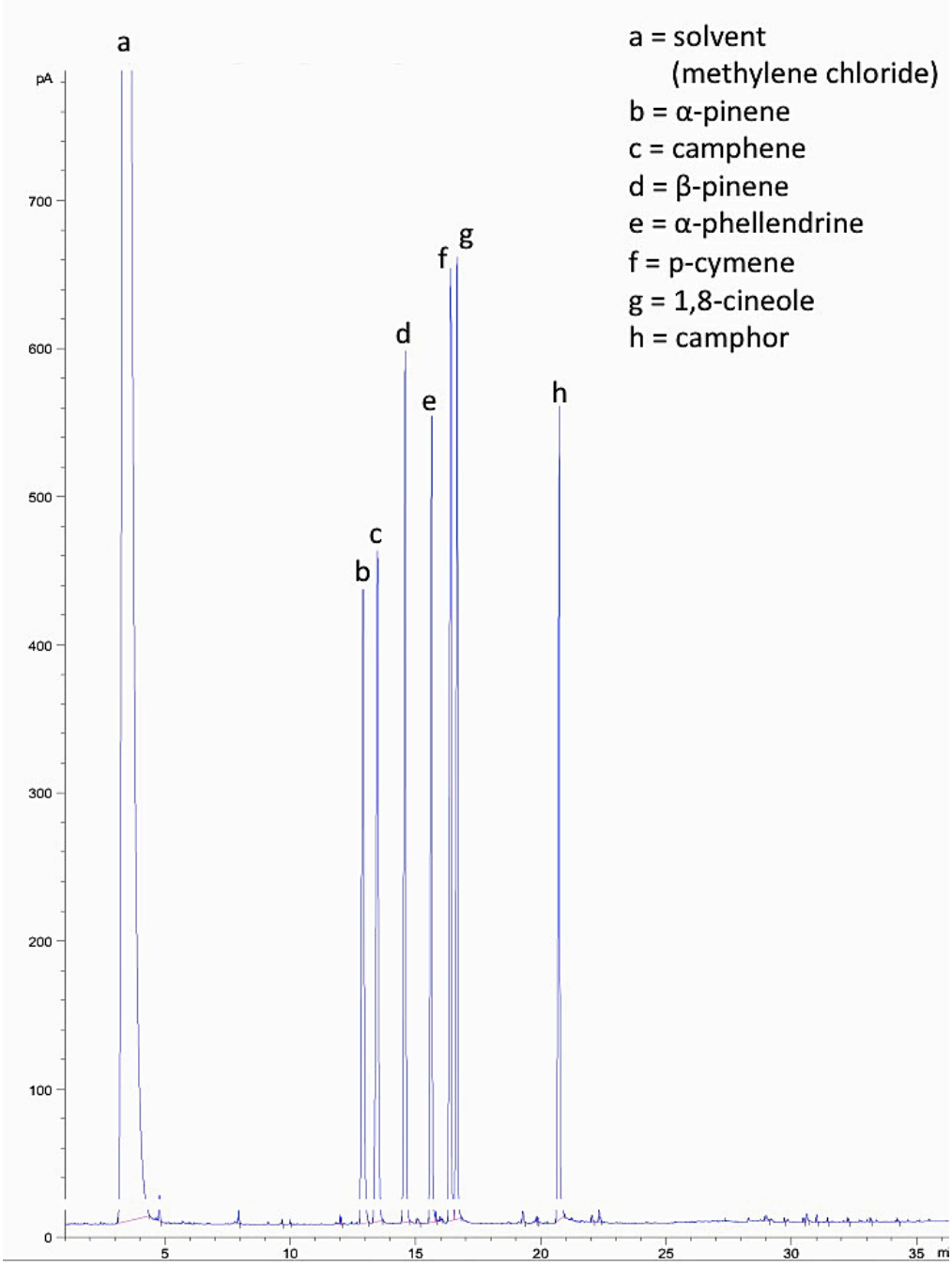


Figure E.1 A representative chromatogram of the standard cocktail used for monoterpene identification. Chromatograms were obtained using headspace gas chromatography (Appendix B) using 5 µL of a 10 mg/mL cocktail, dissolved in methylene chloride. Chromatograms show retention time (compound identification) on the x-axis, and relative concentration (AUC/100 µg dry weight) on the y-axis.

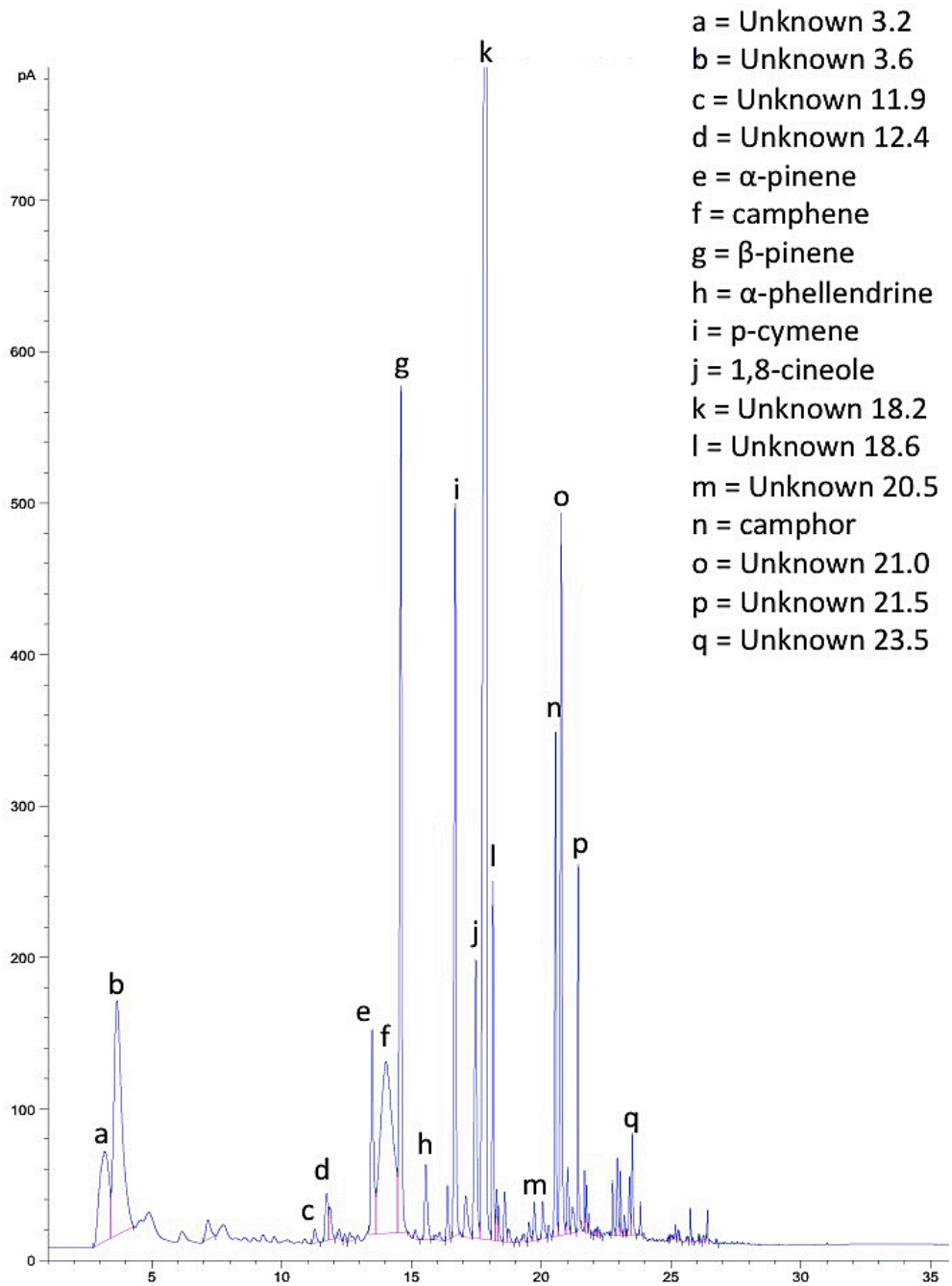


Figure E.2 A representative chromatogram for monoterpenes found in Wyoming big sagebrush (*Artemisia tridentata wyomingensis*). Chromatograms were obtained using headspace gas chromatography (Appendix B) from sagebrush samples collected at Craters, Idaho, USA. Chromatograms show retention time (compound identification) on the x-axis, and relative concentration (AUC/100 µg dry weight) on the y-axis.

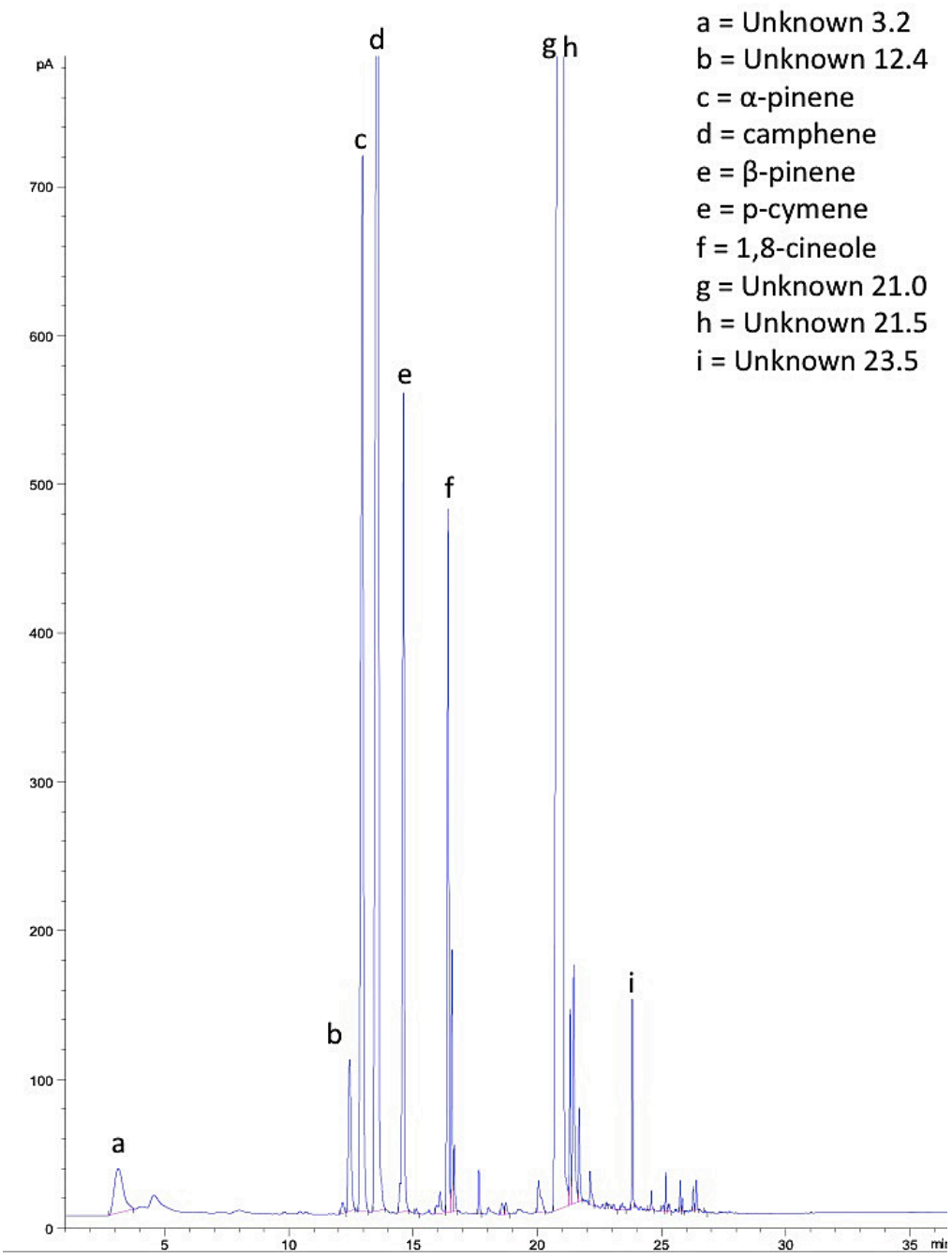


Figure E.3 A representative chromatogram for monoterpenes found in three-tip sagebrush (*Artemisia tripartita*). Chromatograms were obtained using headspace gas chromatography (Appendix B) from sagebrush samples collected at Craters, Idaho, USA. Chromatograms show retention time (compound identification) on the x-axis, and relative concentration (AUC/100 µg dry weight) on the y-axis.

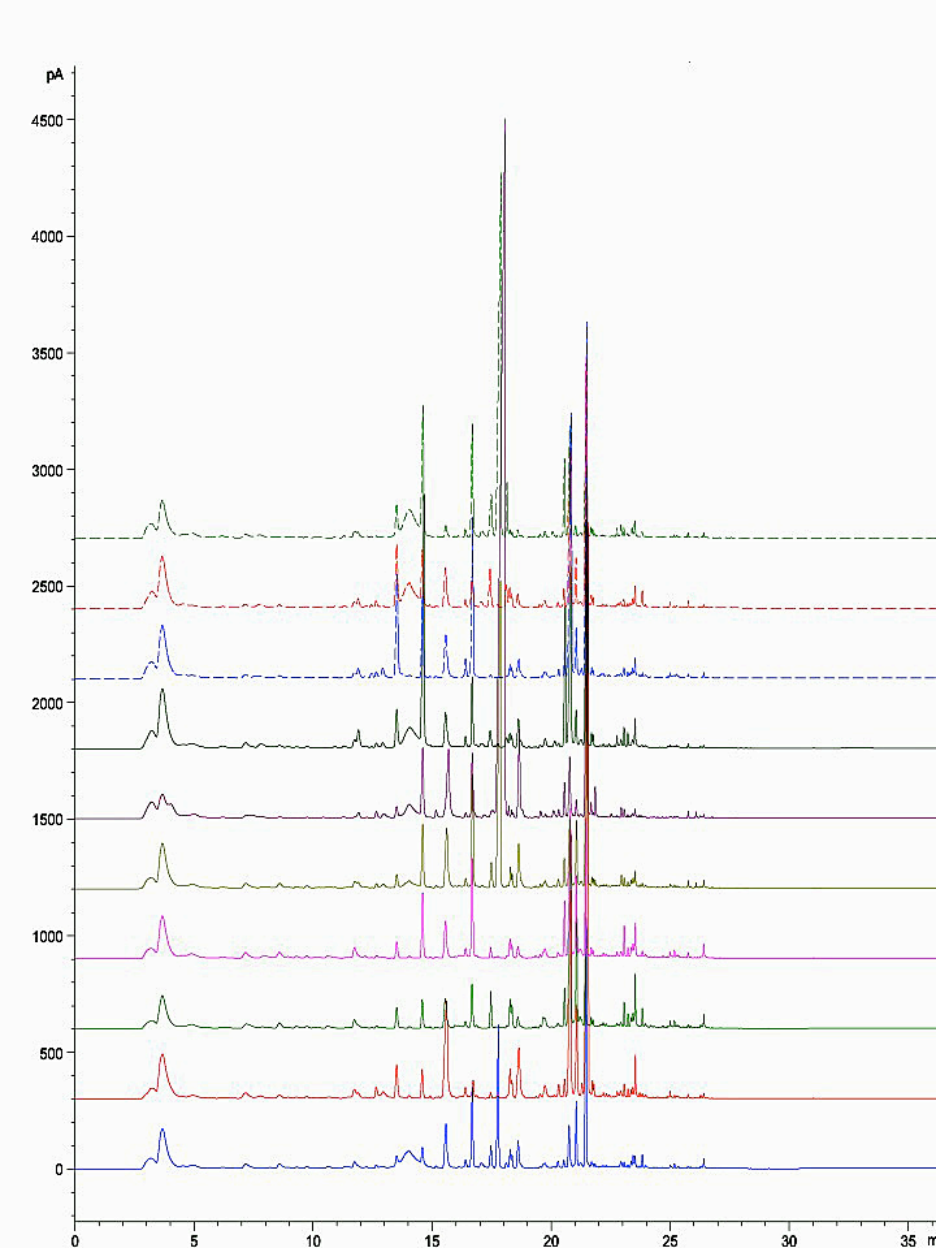


Figure E.4 Ten representative chromatograms for monoterpenes found in Wyoming big sagebrush (*Artemisia tridentata wyomingensis*), showing intraspecific variation. Chromatograms were obtained using headspace gas chromatography (Appendix B) from sagebrush samples collected at Craters, Idaho, USA. Chromatograms show retention time (compound identification) on the x-axis, and relative concentration (AUC/100 µg dry weight) on the y-axis.

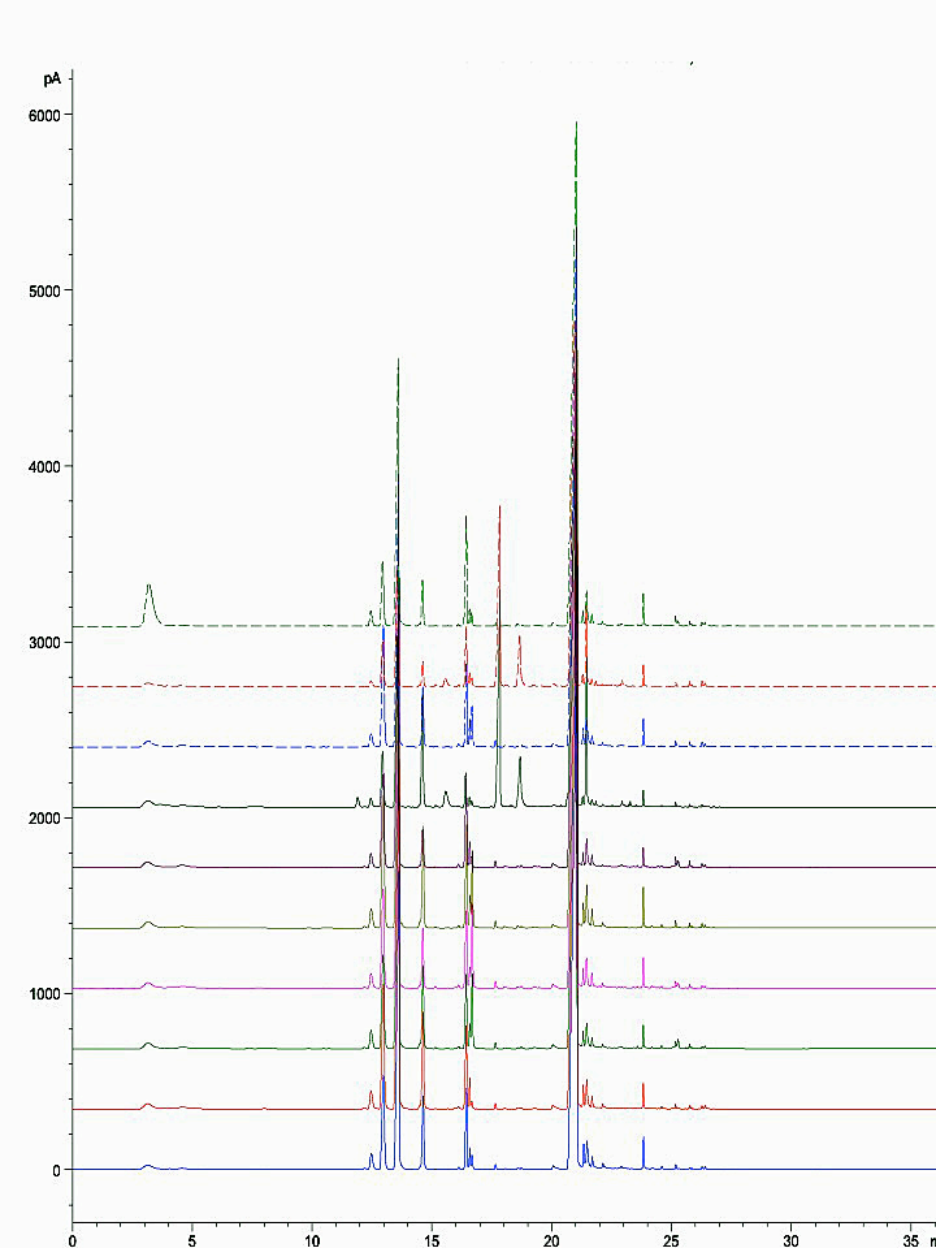


Figure E.5 Ten representative chromatograms for monoterpenes found in three-tip sagebrush (*Artemisia tripartita*), showing intraspecific variation. Chromatograms were obtained using headspace gas chromatography (Appendix B) from sagebrush samples collected at Craters, Idaho, USA. Chromatograms show retention time (compound identification) on the x-axis, and relative concentration (AUC/100 µg dry weight) on the y-axis.

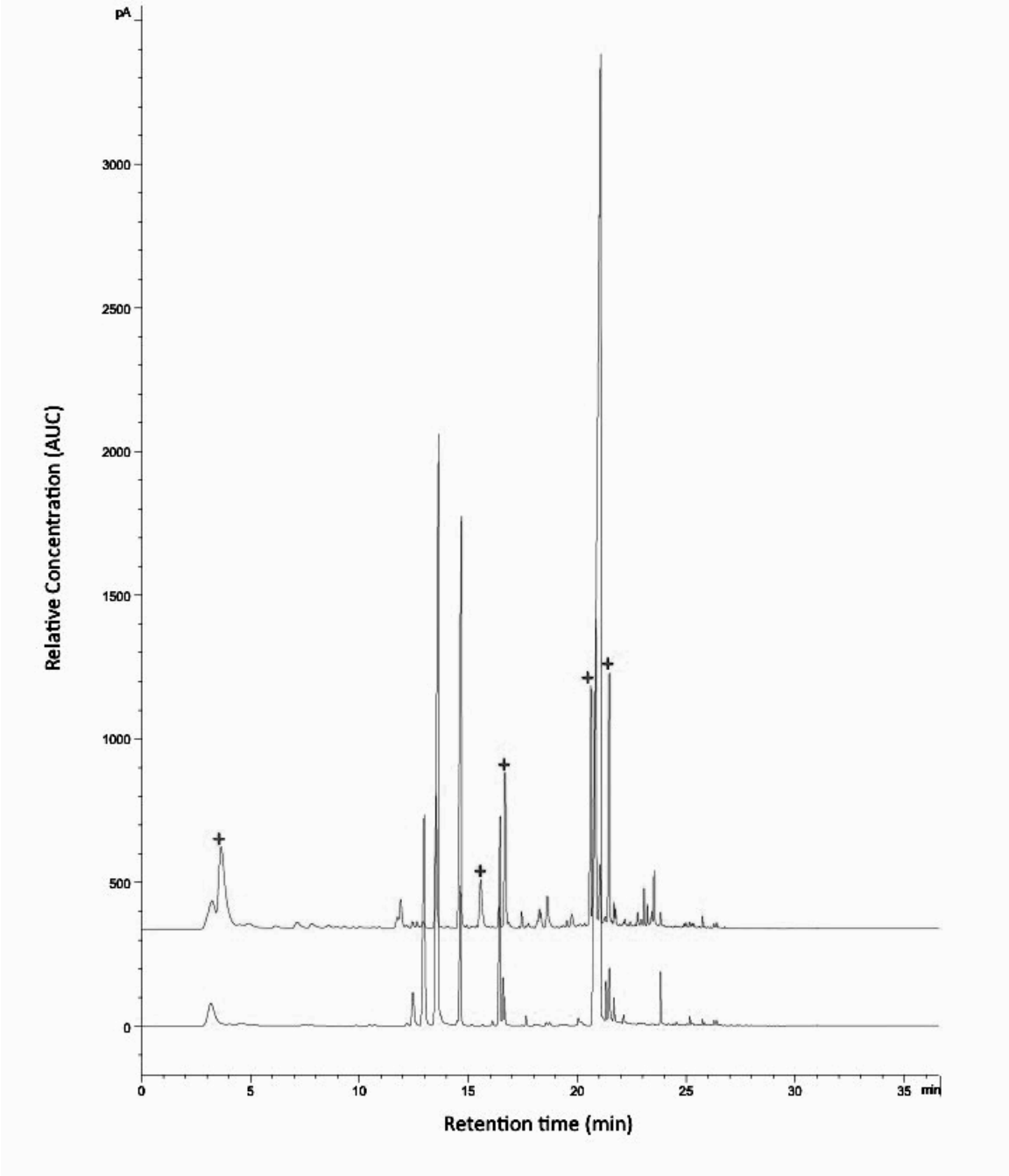


Figure E.6 Representative monoterpene profiles for three-tip (bottom line; *Artemisia tripartita*) and Wyoming big sagebrush (top line; *A. tridentata wyomingensis*) from Craters, Idaho, USA. Peaks show individual compounds, with the height of the peak indicating relative abundance of the compound. Plus signs ($^{+}$) indicate compounds found only in Wyoming big sagebrush. There were no compounds in three-tip sagebrush that were not present in Wyoming big sagebrush.

APPENDIX F

Dose-Dependent Effects of Plant Secondary Metabolite Consumption on Herbivores

Table F.1 Negative physiological side effects are associated with consuming plant secondary metabolites (PSMs). These side effects are dose-dependent, and there may also be dose-dependent therapeutic effects (Table G.2). Behavioral adaptations to these side effects include meal size regulation (Wiggins et al. 2003), habitat selection (Frye et al. 2013), or energy allocation (Sorensen et al. 2005).

Negative Effects	PSM, or Class of Compounds	Details	Study System	Reference
Nausea	Ricin	Vomiting and diarrhea caused by toxin consumption	Ricin[1] ingested by humans	Audi et al. 2005
Altered body temperature	Juniper PSMs	Higher body temperature for animals on PSM-rich diet than on control diet	Woodrats[2] consuming juniper[3] versus control chow	Dearing et al. 2008
Constrain energy budget	Juniper PSMs	Reduced locomotor activity by 25 to 33%	Woodrats[2] consuming juniper[3]	Sorensen et al. 2005
Diuretic	Juniper PSMs	PSM consumption increased urine flow, more diluted urine, decreases blood volume, increased water intake to compensate for water loss through urine	Woodrats[2] consuming juniper[3]	Dearing et al. 2001
Upset pH homeostasis	Coniferyl benzoate	Increased ammonium excretion	Captive Ruffed Grouse[4] consuming aspen buds[5]	Guglielmo et al. 1996
	Eucalyptus PSMs	Increased acidity in urine	Brushtail possums[6] consuming eucalyptus[7]	Wiggins et al. 2006
	α-Pinene	Increased acidity in urine	Woodrats[2]	Dearing et al. 2000
Lower energy assimilation	Coniferyl benzoate	Decreased overall energy assimilation by 24%	Captive Ruffed Grouse[4] consuming aspen buds[5]	Guglielmo et al. 1996
Negative nitrogen balance	Coniferyl benzoate	Ornithine excretion, ammonium excretion, and glucuronic acid conjugation increased nitrogen excretion	Captive Ruffed Grouse[4] consuming aspen buds[5]	Jakubas et al. 1993*a*

Protein turnover	1,8 – cineole, benzoic acid	30% loss of protein from dietary intake, used in detoxification	Captive brushtail possums[6] consuming chow	Au et al. 2013
Energetically expensive to metabolize	Coniferyl benzoate	10% to 14% energetic cost to produce detoxification conjugates (ornithine and glucuronic acid)	Captive Ruffed Grouse[4] consuming aspen buds[5]	Guglielmo et al. 1996
	Juniper PSMs	Higher detoxification conjugate excretion on PSM-rich diet than on control diet	Woodrats[2] consuming juniper[3]	Sorensen et al. 2005
Reduce digestibility of nutrients	Sagebrush terpenoids	Increased in vitro organic matter digestibility with lower crude terpenoids (monoterpenes)	Rumen incocula[8] and sagebrush[9]	Striby et al. 1987
Reduce activity of digestive enzymes	Sagebrush monoterpenes	Some individual monoterpenes decreased enzyme activity in sage-grouse and chicken livers	Greater Sage-grouse[10] and domestic chickens[11]	Kohl et al. 2015
Oxidative stress	Abrin, ricin	Increased reactive oxygen species (ROS) throughout consumer's body	Abrin from *Abrus precatorius* in lab mice[12]	Bhasker et al. 2014
Weight loss [10]	Coniferyl benzoate	Caused weight loss in feeding trials	Captive Ruffed Grouse[4] consuming aspen buds[5]	Jakubas et al. 1993*a* Jakubas et al. 1993*b*
	Juniper PSMs	9% body mass loss on PSM-rich diet compared to control diet	Woodrats[2] consuming juniper[3]	Sorensen et al. 2005
	α-Pinene	Lost 4 to 8% body mass in three days on PSM-rich diet	Woodrats[2] consuming juniper[3]	Dearing et al. 2001 Dearing et al. 2000
Organ failure	Ricin	Toxin ingestion led to liver failure, renal dysfunction, cardiovascular collapse	Ricin[1] ingested by humans	Audi et al. 2005

Death	Various plants	Ingesting certain plants causes fatality due to plant toxins	Many (including ricin[1])	Froberg et al. 2007 Audi et al. 2005

[1] Ricin (castor bean): *Ricinus communis*

[2] Woodrats: *Neotoma stephensi* and *N. albigula*

[3] Juniper: *Juniperus monosperma*

[4] Ruffed Grouse: *Bonsa umbellus*

[5] Aspen: *Populus tremuloides*

[6] Brushtail possum: *Trichosurus vulpecula*

[7] Eucalyptus: *E. globulus, E. regnans*

[8] Rumen inocula: *Odocoileus hemionus, Ovis ammon aries, Bos Taurus*

[9] Sagebrush: *Artemisia* spp. (*A. tridentata wyomingensis, A.t. vaseyana, A.t. tridentata, A. tripartita*)

[10] Greater Sage-grouse: *Centrocercus urophasianus*

[11] Domestic chicken: *Gallus gallus domesticus*

[12] Lab mice: *Mus musculus*

Table F.2 Medicinal effects of certain plant secondary metabolites (PSMs) documented for various taxa. This is not an exhaustive list, but provides information on some bioactive compounds that exist in sagebrush.

Medicinal Effect	PSM	Details	Study System	Reference
Anthelmintic	Sesquiterpene lactones, steroid glucosides	Reduces nematode and gastrointestinal parasite loads, doses (concentration unknown) consumed by wild animals are bioactive	Wild chimpanzees eating *Vernonia amygdalina*	Huffman and Seifu 1989, Huffman 1993; 1997, Koshimizu et al. 1994, Ohigashi et al. 1994
	Tannins	Tannin consumption decreased gastrointestinal nematode loads by 90%, and resulted in lower parasite loads than ivermectin (commercial anthelmintic drug)	Domestic sheep (*Ovis aeries*)	Villalba et al. 2010
	Sesquiterpenes, monoterpenes (α-pinene, β-pinene, 1,8-cineole)	*Piper aduncum* essential oil inhibited nematode hatching. Essential oil was approximately 80% monoterpenes and 14% sesquiterpenes. 1,8-cineole accounted for 56% of the oil volume.	*Haemonchus contortus* nematode in domestic sheep	Oliveira et al. 2014
	1-8-cineole, camphor	1,8-cineole inhibited 77% of larval migration, camphor effects were additive to cineole. Also *Artimesia annua* extracts were effective controlling parasite loads.	*Haemonchus contortus* nematode, *in vitro*	Zhu et al. 2013
	Acacia extracts	Treatment with plant extracts caused paralysis and eventual death in *Raillietina* tapeworms	*Acacia oxyphylla* extracts on *Raillietina*, in vitro	Dasgupta and Roy 2010
Anti-parasitic	Tannins	Anti-parasitic properties in *Pistacia lentiscus*	Goats consuming *Pistacia*	Landau et al. 2010
Antimalarial	Limonoids	Antimalarial activity for consumers	Wild chimpanzees eating *Trichilia*	Krief et al. 2004

	Artemisinin (sesquiterpene lactone)	Antimalarial activity for consumers, as well as anti-cancer activity	*rubescens* Human treatment of malaria	O'Neill et al. 2010
Antibiotic	Methoxypsoralen	Strong antibiotic	Wild chimpanzees eating *Ficus exasperata*	Rodriguez and Wrangham 1993
Anti-coccidial	Monoterpenes: artemisinin, 1,8-cineole, camphor	Chickens treated with single monoterpenes had decreased *Eimeria* loads (effects of each monoterpene were different for each *Eimeria* species)	*In vivo* test of sagebrush (*Artemisia annua*) extracts on *Eimeria* sp. in chickens	Allen et al. 1997, Allen et al. 1998

Literature Cited

Allen, P. C., H. D. Danforth, and P. C. Augustine. 1998. Dietary modulation of avian coccidiosis. International Journal for Parasitology 28:1131-1140.

Allen, P. C., J. Lydon, and H. D. Danforth. 1997. Effects of components of *Artemisia annua* on coccidia infections in chickens. Poultry Science 76:1156-1163.

Au, J., K. J. Marsh, I.R . Wallis, and W. J. Foley. 2013. Whole-body protein turnover reveals the cost of detoxification of secondary metabolites in a vertebrate browser. Journal of Comparative Physiology B: Biochemical Systemic and Environmental Physiology 183:993-1003.

Audi, J., M. Belson, M. Patel, J. Schier, and J. Osterloh. 2005. Ricin poisoning - a comprehensive review. Journal of the American Medical Association 294:2342-2351.

Bhasker, A. S. B., B. Sant, P. Yadav, M. Agrawal, and P. V. L. Rao. 2014. Plant toxin abrin induced oxidative stress mediated neurodegenerative changes in mice. Neurotoxicology 44:194-203.

Dasgupta, S., and B. Roy. 2010. Antiparasitic activity of methanolic extract of *Acacia oxyphylla* (Leguminosae) against *Raillietina echinobothrida*. Journal of Parasitic Diseases 34:14-19.

Dearing, M., J. Forbey, J. McLister, and L. Santos. 2008. Ambient temperature influences diet selection and physiology of an herbivorous mammal, *Neotoma albigula*. Physiological and Biochemical Zoology 81:891-897.

Dearing, M., A. Mangione, and W. Karasov. 2000. Diet breadth of mammalian herbivores: nutrient versus detoxification constraints. Oecologia 123:397-405.

_____. 2001. Plant secondary compounds as diuretics: An overlooked consequence. American Zoologist 41:890-901.

Froberg, B., D. Ibrahim, and R. B. Furbee. 2007. Plant poisoning. Emergency Medicine Clinics of North America 25:375-+.

Guglielmo, C., W. Karasov, and W. Jakubas. 1996. Nutritional costs of a plant secondary metabolite explain selective foraging by Ruffed Grouse. Ecology 77:1103-1115.

Huffman, M. A. 1997. Current evidence for self-medication in primates: A multidisciplinary perspective. Yearbook of Physical Anthropology 40:171-200.

_____. 2003. Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. Proceedings of the Nutrition Society 62:371-381.

Huffman, M. A., and M. Seifu. 1989. Observations on the illness and consumption of a possibly medicinal plant *Vernonia amygdalina* by a wild chimpanzee in the Mahale Mountains National Park, Tanzania. Primates 30:51-63.

Jakubas, W., W. Karasov, and C. Guglielmo. 1993a. Coniferyl benzoate in quaking aspen (*Populus tremuloides*) - its effect on energy and nitrogen digestion and retention in Ruffed Grouse (*Bonasa umbellus*). Physiological Zoology 66:580-601.

Jakubas, W. J., W. H. Karasov, and C. G. Guglielmo. 1993b. Ruffed Grouse tolerance and biotransformation of the plant secondary metabolite coniferyl benzoate. Condor 95:625-640.

Kohl, K. D., E. Pitman, B. C. Robb, J. W. Connelly, M. D. Dearing, and J. S. Forbey. 2015. Monoterpenes as inhibitors of digestive enzymes and counter-adaptations in a specialist avian herbivore. Journal of Comparative Physiology B: Biochemical Systemic and Environmental Physiology 185:425-434.

Koshimizu, K., H. Ohigashi, and M. A. Huffman. 1994. Use of *Vernonia amygdalina* by wild chimpanzee - possible roles of its bitter and related constituents. Physiology and Behavior 56:1209-1216.

Krief, S., M. T. Martin, P. Grellier, J. Kasenene, and T. Sevenet. 2004. Novel antimalarial compounds isolated in a survey of self-medicative behavior of wild chimpanzees in Uganda. Antimicrobial Agents and Chemotherapy 48:3196-3199.

Landau, S., H. Azaizeh, H. Muklada, T. Glasser, E.D. Ungar, H. Baram, N. Abbas, and A. Markovics. 2010. Anthelmintic activity of *Pistacia lentiscus* foliage in two

Middle Eastern breeds of goats differing in their propensity to consume tannin-rich browse. Veterinary Parasitology 173:280-286.

O'Neill, P. M., V. E. Barton, and S. A. Ward. 2010. The molecular mechanism of action of artemisinin—the debate continues. Molecules 15:1705-1721.

Ohigashi, H., M. A. Huffman, D. Izutsu, K. Koshimizu, M. Kawanaka, H. Sugiyama, G. C. Kirby, D. C. Warhurst, D. Allen, C. W. Wright, J. D. Phillipson, P. Timondavid, F. Delmas, R. Elias, and G. Balansard. 1994. Toward the chemical ecology of medicinal plant use in chimpanzees - the case of *Vernonia amygdalina*, a plant used by wild chimpanzees possibly for parasite-related diseases. Journal of Chemical Ecology 20:541-553.

Oliveira, G. L., T. M. Vieira, V. F. Nunes, M. D. Ruas, E. R. Duarte, D. D. Moreira, M. A. C. Kaplan, and E. R. Martins. 2014. Chemical composition and efficacy in the egg-hatching inhibition of essential oil of *Piper aduncum* against *Haemonchus contortus* from sheep. Brazilian Journal of Pharmacognosy 24:288-292.

Rodriguez, E., and R. Wrangham. 1993. Zoopharmacognosy: the use of medicinal plants by animals. *in* K. R. Downum, J. T. Romeo, and H. A. Stafford, editors. Recent advances in phytochemistry: phytochemcial potential of tropical plants. Plenum Press, New York, USA.

Sorensen, J. S., J. D. McLister, and M. D. Dearing. 2005. Plant secondary metabolites compromise the energy budgets of specialist and generalist mammalian herbivores. Ecology 86:125-139.

Striby, K. D., C. L. Wambolt, R. G. Kelsey, and K. M. Havstad. 1987. Crude terpenoid influence on *in vitro* digestibility of sagebrush. Journal of Range Management 40:244-248.

Villalba, J. J., F. D. Provenza, J. O. Hall, and L. D. Lisonbee. 2010. Selection of tannins by sheep in response to gastrointestinal nematode infection. Journal of Animal Science 88:2189-2198.

Wiggins, N., C. McArthur, S. McLean, and R. Boyle. 2003. Effects of two plant secondary metabolites, cineole and gallic acid, on nightly feeding patterns of the common brushtail possum. Journal of Chemical Ecology 29:1447-1464.

Wiggins, N., C. McArthur, N. Davies, and S. McLean. 2006. Behavioral responses of a generalist mammalian folivore to the physiological constraints of a chemically defended diet. Journal of Chemical Ecology 32:1133-1147.

Zhu, L., J. L. Dai, L. Yang, and J. Qiu. 2013. In vitro ovicidal and larvicidal activity of the essential oil of *Artemisia lancea* against *Haemonchus contortus* (Strongylida). Veterinary Parasitology 195:112-117.

APPENDIX G

The McMaster Egg Counting Technique: Quantifying Oocysts in Fecal Pellets

The McMaster Egg Counting Technique: Quantifying Oocysts in Fecal Pellets

I used the McMaster egg counting technique (Gordon and Whitlock 1939) to quantify the number of tapeworm (*Raillietina centrocerci*) oocysts in frozen Greater Sage-grouse (*Centrocercus urophasianus*) fecal pellets. Fresh pellets were collected in the field and stored in a -20° C freezer until analysis. Although storage time in refrigerators can degrade eggs (van Wyk and van Wyk 2002), I found no difference in parasite loads for samples stored different lengths of time at this temperature, however a full analysis of the storage effect is pending. The McMaster method is well established and widespread in veterinary medicine, and was optimized for quantifying parasite loads in this system using the following protocol.

Personal Protective Equiment: lab coat, goggles, rubber gloves, closed toed shoes

Supplies:

- Beakers or plastic containers
- Balance
- Tea strainer, cheesecloth or dental napkin
- Funnel
- Measuring cylinder
- Stirring device (fork, spatula, tongue depressor)
- Pasteur pipettes
- Flotation fluid
- McMaster counting slide
- Compound microscope
- Calipers

Procedure:

1. Prepare fecal pellets by measuring each pellet with calipers and cutting into 0.5 cm long sections. Mix all pellets together in a weigh boat and weigh out following the "decision tree" for pellets.
2. Weigh approximately 2 grams of feces and place into a beaker.
3. Add 28 ml of floatation fluid.
4. Stir the contents of the beaker thoroughly with a tongue depressor or spatula (Figure G.1).
5. Filter the fecal suspension through a tea strainer and layers of cheesecloth into the second container (Figure G.2).
6. Stir the filtrate in the container with a Pasteur pipette.
7. Using the pipette, withdraw a sub-sample as the filtrate is being stirred.
8. Fill the first compartment of the McMaster counting chamber with the sub sample (Figure G.3).

Figure G.1 Stirring fecal material into a saturated salt-sugar solution.

Figure G.2 Filter fecal suspension through cheesecloth and funnel.

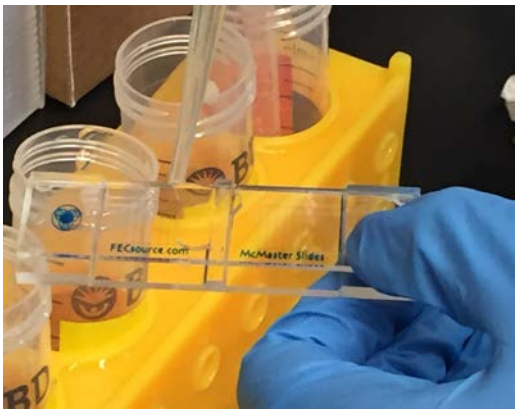


Figure G.3 Fill each chamber of the McMaster slide using a pipette.

9. Stir fluid again and fill second chamber with another sub sample.
10. Allow the counting chamber to stand for 5 minutes.
11. Examine the subsamples of the filtrate under the compound microscope at 10 x 10 magnification (Figure G.4), carefully distinguishing between oocysts and pollen grains (Figure G.5).
12. Identify and count all eggs within the engraved area of both chambers.
13. Dry fecal samples and cheesecloth in an oven at 60° F for 3 days, and re-weigh to measure the sample dry weight.

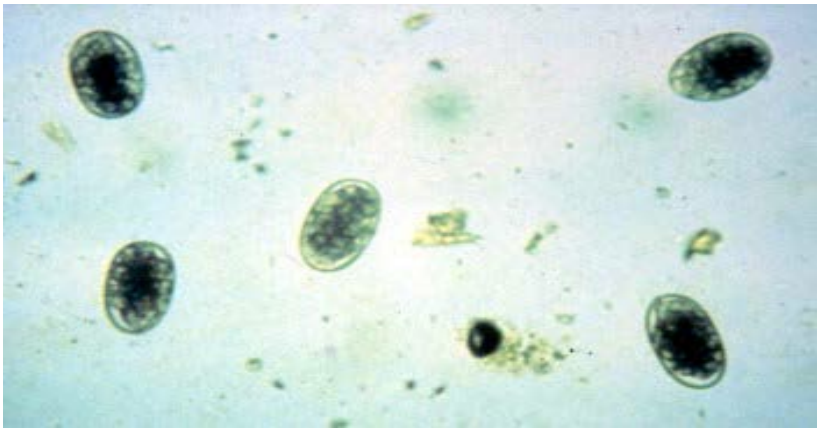


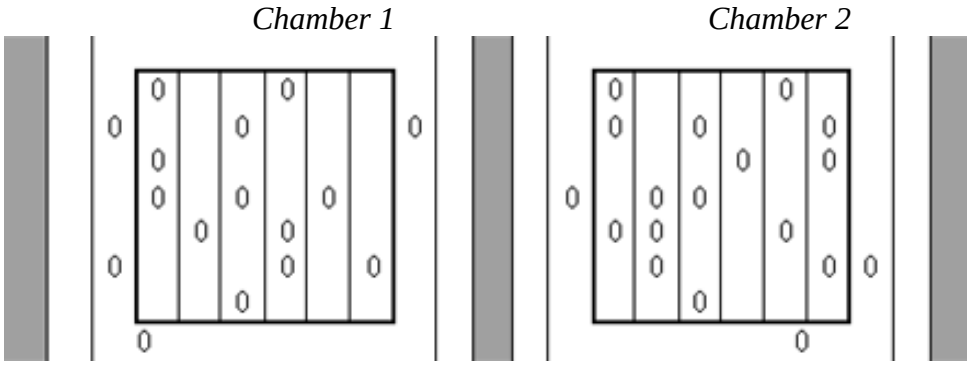
Figure G.4 The McMaster slide chamber under the microscope at 100x. Etched lines are not visible because they are at the edge of the field of view, but 5 oocysts are present in this photo. Photo by Joel Velasco.

Figure G.5 Oocysts and pollen grains can be easily confused. Two pollen grains from *Artemisia* sp. are shown to assist with identification (photo from the USA Pollen Database, 2015).

Calculation of the Results:

- Count the number of eggs within the grid of each chamber, ignoring those outside the etched squares.
- Multiply the total by 50, which estimates the eggs per gram of feces (e.p.g.) Multiply by 50 because: 15 uL per chamber times 2 chambers is a total of 30 uL of solution counted. This is 1/100th of the total sample volume (30 uL; so divide by 100), which contains 2 g (wet) of feces (so multiply by 2).

  For example:



  12 eggs seen in chamber 1 and 15 eggs seen in chamber 2 = (12 +15) x 50 = 1,350 e.p.g.

- Correct for dry weight of sample by dividing e.p.g by the dry weight of the fecal sample (obtained after drying in the oven).

Floatation fluid: salt/sugar solution (specific gravity: 1.28)

1. Dissolve 400 g sodium chloride in 1000 mL tap water to make a saturated salt solution. Beakers can sit on a hot plate to aid in dissolving crystals.
2. Add 500 g sugar to the saturated salt solution.
3. Stir until the sugar is dissolved.

Pellet Weighing "Decision Tree"

First, remove any broken or smashed pellets and place them in a large weigh-boat. Prepare pellets by measuring the length of each pellet on the longest side and record in a lab notebook to evaluate if there is a relationship between size of the pellet and bird sex (Smith et al. 1995). Next, cut each pellet into small pieces (approximately 0.5 cm long) and stir the sample to mix all the pieces together. Weigh all samples into separate Ziploc bags (labeled with "Parasites", "GA", "Monoterpenes" or "Extra". Record the weight (every digit) on the bag and in your lab notebook. Begin weighing samples for analysis:

- 2 g for parasite analysis
    - Weigh into a Ziploc bag labeled "Parasites" with sample information and record weight on bag and in notebook
- 1.5 g for glucuronic acid (GA) analysis
    - Weigh into a Ziploc bag labeled "GA" with sample information and record weight on bag and in notebook
    - Can be anywhere between 1.4 and 1.6 g
- 0.5 g for monoterpene (Appendix B) analysis
    - Weigh into a Ziploc bag labeled "Monoterpenes" with sample information and record weight on bag and in notebook
    - This will eventually be ground with liquid nitrogen and 0.100 g will be weighed into a glass headspace vial. The remainder of the ground sample will go into a glass scintillation vial.
- Remainder as "extra" (place back in original Ziploc and label "extra")

Glucuronic acid (GA) analysis is an important additional measurement because GA is a major metabolic pathway that is related to the amount of PSMs an individual consumes, absorbs, and metabolizes (Guglielmo et al. 1996). Therefore, GA can be used as a biomarker to measure toxicity, or exposure to PSMs. A colorimetric assay (Blumenkrantz and Asboe-Hansen 1973) can be used to quantify the concentration of GA excreted in fecal droppings from avian herbivores, which can be used to estimate toxicity. Relationships between GA, PSMs that were ingested and excreted, and parasite loads can provide insight about the relative costs associated with parasite burdens and PSM detoxification, and can help evaluate potential energetic trade-offs between detoxification and immune function.

Literature Cited

Blumenkrantz, N., and G. Asboe-Hansen. 1973. Effect of amino acids on collagen biosynthesis. *In Vitro* 8: 342-346.

Gordon, H. M., and H. V. Whitlock. 1939. A new technique for counting nematode eggs in sheep faeces. Journal of the Council for Scientific and Industrial Research 12:50-52.

Guglielmo, C., W. Karasov, and W. Jakubas. 1996. Nutritional costs of a plant secondary metabolite explain selective foraging by Ruffed Grouse. Ecology 77:1103-1115.

Smith, F. A., J. L. Betancourt, and J. H. Brown. 1995. Evolution of body-size in the woodrat over the past 25,000 years of climate change. Science 270:2012-2014.

van Wyk, J. A., and L. van Wyk. 2002. Freezing of sheep faeces invalidates *Haemonchus contortus* faecal egg counts by the McMaster technique. Onderstepoort Journal of Veterinary Research 69:299-304.