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Woodpecker Forage Availability within Disturbed Habitat Types in the Black Hills. Brian E. Dickerson[1], A. K. Ambourn[2], M. A. Rumble[1], K. K. Allen[2], and C. P. Lehman[3]. *[1]U.S. Forest Service, Rocky Mountain Research Station, Rapid City, SD 57702. [2]U.S. Forest Service, Forest Health Management, Rapid City, SD 57702. [3]South Dakota Department of Game Fish and Parks, Custer State Park, 13329 US Highway 16A, Custer, South Dakota 57730.*

In-row and Between-row Weed Control in an Organic Crop Production System. Mauricio Erazo-Barradas[1], Sharon Clay[1], and Frank Forcella[2]. *[1]Plant Science Department, South Dakota State University, Brookings, SD 57007. [2]USDA-ARS North Central Soil Cons. Res. Lab, Morris, MN 56267.*

Enhanced Conversion of Lignocellulose to Biofuels. Rajesh Sani. *Department of Chemical and Biological Engineering, South Dakota School of Mines and Technology, Rapid City, SD 57701.*

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The Non-Target Effects of Thiamethoxam on *Coleomegilla maculata*. Insecticide Transmission via *Rhopalosiphum padi* Consuming Treated Wheat Plants. Mike Bredeson[1], Jonathan Lundgren[2], and Neil Reese[1]. *[1]South Dakota State University, USDA-ARS, Brooking, SD 57007. [2]USDA-ARS, Brookings, SD 57007.*

Plant Community Patterns on the Signal Hill Research Natural Area. J. L. Butler and B. E. Dickerson. *US Forest Service, Rocky Mountain Research Station, Forest and Grassland Research Laboratory. Rapid City, SD 57702.*

In Silico Analysis of Disease Resistance Genes in Cultivated Barley (*Hordeum vulgare* L.: Poaceae). E. J. Anderson, E. A. Opitz, B.V. Benson, and M. P. Nepal. *Department of Biology & Microbiology, South Dakota State University, Brookings, SD 57007.*

In Silico Analysis of Disease Resistance Genes in Foxtail Millet (*Setaria italica*: Poaceae). E. A. Opitz, E. J. Anderson, B.V. Benson, and M. P. Nepal. *Department of Biology & Microbiology, South Dakota State University, Brookings, SD 57007.*

Genetic Structure of Endangered Populations of Native Mulberry in the Upper Midwest USA. S. J. Schreier. S. Piya, and M.P. Nepal. *Department of Biology & Microbiology, South Dakota State University, Brookings, SD 57007.*

What Makes Weather Events Memorable? K. J. Haselhorst. *Department of Atmospheric Sciences, South Dakota School of Mines and Technology, Rapid City, SD 57701.*

Identifying a Productive Rhythm in the Chemistry Laboratory using Observational Data of an Innovative First- and Second-year Instructional Laboratory Sequence. Matthew L. Miller, David P. Cartrette, and Angelica Reyes. *South Dakota State University, Brookings, SD 57007.*

The Flipped Classroom Experience in Chemistry 106. Melody Jewell. *Department of Chemistry & Biochemistry, South Dakota State University-University Center Campus, Sioux Falls, SD 57107.*

Vegetation Trends of Sagebrush Habitat near Greater Sage Grouse Leks in Northwestern South Dakota after 20 Years. Daryl E. Mergen[1], Carin J. Corley[1], Charles D. Bonham[2], Shelly Deisch[1], Dave Ode[2], and Samantha L. Nichols[1]. *[1]Mergen Ecological Delineations, Inc., 1835 Parkview Boulevard, Colorado Springs, CO 80905.UniScale Corporation, Loveland, CO 80537. [3]South Dakota Game Fish & Parks Department, Pierre, SD 57501.*

COMBINED MINUTES OF THE 99TH ANNUAL MEETING OF THE SOUTH DAKOTA ACADEMY OF SCIENCE FRIDAY AND SATURDAY 28 AND 29 MARCH 2014 HOSTED BY SOUTH DAKOTA SCHOOL OF MINES AND TECHNOLOGY BEST WESTERN RAMKOTA HOTEL, RAPID CITY, SD

EXECUTIVE COMMITTEE MEETING

President Gary Larson opened the meeting 12:30 am, Friday 28 April 2014, and welcomed members of the Executive Council to the 99th South Dakota Academy of Science Annual Meeting at the Best Western Ramkota Hotel hosted by the South Dakota School of Mines and Technology and noted that a quorum was present. Gary noted that because the 98th meeting was cancelled in response to a severe ice storm in Sioux Falls, the 99th meeting was the first meeting after the 97th in 2012.

Reports

The Secretary's Report, Treasurer's Report and Proceedings Editors Report were given by Donna Hazelwood, Jeff Palmer, and Bob Tatina, respectively. Members moved and seconded acceptance of the respective reports.

Old Business

Fellows 2012

Fellows Elected to the South Dakota Academy of Science for 2012 and honored at the Banquet are Nels Granholm, SDSU, the late Audrey Gabel, BHSU, Mark Gabel, BHSU, and Robert (Bob) Tatina, DWU. No fellows were elected 2013.

SDAS web page

The SDAS website http//www.sdaos.org is hosted by Miner Solutions owned by Shane Miner. Although Shane has moved to a position with WGBH in Boston, the Executive Council voted to continue utilizing Miner solutions to host the webpage. Payment is made from the Proceedings Account.

New Business

Fellows 2015

A call for nomination of Fellows for 2015 will be sent. Brian Patrick has agreed to head the nomination process.

Invitation to all Fellows to attend the SDAS Centennial meeting 2015

The Executive Council passed a motion by Brian Patrick and seconded by Vicki Geiser to formally invite the Fellows to attend the centennial meeting as guests of the Executive Council.

The Centennial meeting of the South Dakota Academy of Science

The meeting will be 10-11 April 2015 at Cedar Shores Resort, Oacoma, SD.

Bob Tatina has been in contact with Dr. Michael J. Mullin, Professor of History at Augustana College who is in the process of writing a history of the first 100 years of the Academy. Dr. Mullin has also been invited to serve as Keynote speaker. Bob has read a draft of the first portion of the manuscript and is looking forward with enthusiasm to the final draft. A portion of the manuscript has been presented by Mullin to a history conference and was well received.

Committee Report

Proceedings by Robert Tatina

The Proceedings Account is solvent. Abstracts are included in the Proceedings. Complimentary copies are provided to libraries. Proceedings for the 2012 meeting have been uploaded to the website. All volumes dating back to 1915 have been digitized and are available in a searchable format on website.

Bob plans to have a special centennial edition of the Proceedings with Michael Mullin's history of the Academy available at the Centennial meeting. Toward that end, articles submitted to the 2014 Proceedings will need to be forwarded to Bob in final format by 15 July 2014.

Committees

Neil Reese has kindly agreed to serve as the Resolutions Committee. Auditing Committee are Mark Gabel and Donna Hazelwood. AAAS Undergraduate Poster Judges are Dave Bergman, Jim Sorenson, and George Mwangi. Judges for the first SDAS paper presentations are Vicki Geiser and Brian Patrick.

BUSINESS MEETING SATURDAY 29 MARCH 2014

Passing of the Gavel

The gavel was passed from outgoing President Gary Larson, SDSU, to Chun Wu, Mount Marty College.

Presidential Address

The Presidential address on the timely topic "Always Room for You in Science" was presented by incoming President Chun Wu.

Committee Reports

Audit Committee

Mark Gabel and Donna Hazelwood audited the 2012-13-14 account for Treasurer Jeff Palmer. Mark gave the report and commended Jeff on the most organized and put together report that he has seen in his many years of serving on the auditing committee.

Proceedings Editor Report

Proceedings Editor Robert Tatina reported that the proceedings account is solvent. A total of 40 print copies have been made and distributed to SD libraries, Abstracting Services and Thompson Scientific. The remaining copies join the complete set that Bob has stored at his residence in Michigan.

For the Centennial Proceedings, Mike Mullin, historian at Augustana College, has been contracted to write a history of the Academy. The paper weaves the history of the Academy with that of South Dakota and of the USA. Bob issued a call for help with articles, artifacts, and photographs for the centennial edition. He suggested that a glossy photograph would be appropriate for the cover.

Resolutions Committee

Neil Reese presented the following resolutions:

The Members of the Academy thank the following: 1)The Local Planning Committee: Andrew Detwiler and Thomas Montoya; 2) the local registration committee headed by Amy L. Montoya and the members of Eta Kappa Nu for working the registration desk: Lindsey Evans, Shaun Gruenig, Dustin Moser, Zachary Norstedt, Benjamin Dykstra, Joshua Leland, Lance Baum, Mason Cover, Preston Cook, and Dana Jensen; 3) the Rapid City Convention & Visitors Bureau for providing area maps, visitor guides/pamphlets, and name badges; 4) Dr. Richard Gowen and his wife Nancy for supporting the SDAS; 5) Dr. Dick Gowen for his stimulating address, "Trusting Autonomous Systems"; 7) special thanks to Bob Tatina for his continued excellence and dedication as editor of the Proceedings of the SDAS; 8) special recognition to Gary Larson for serving two terms as SDAS President (2012-2013 and 2013-2014); 9) thanks to the ever-faithful Donna Hazelwood, Secretary, and the well-organized Jeff Palmer, Treasurer; 10) thanks to Uriel Buitrago-Suarez for his past service as Webmaster and to Tim Mullican for assuming these duties; 11) we thank Brian Patrick, Vicki Geiser, and Gary Larson for judging the first student Oral Presentations Contest, and Dave Bergmann, Jim Sorenson and George Mwangi for judging the Undergraduate Student Poster Contest; 12) we thank the organizers of the Symposium Committees: Bioinformatics by Vicki Geiser, Paleontology by Clint Boyd, and Science Education by Matt Miller; and lastly 13) we thank the Best Western Ramkota Inn for providing an excellent venue for the 2014 meeting.

Oral Presentation Awards

Prizes awarded are 1st place, $100.00, 2nd place, $75.00, and 3rd place, $50.00. The winning presentations are listed in sequential order and the names of the winners are in boldface type.

First Place: "Variability in micro-computed tomography images for non-destructive evaluation of friction-stir weld quality." **Alex Wulff**, William Cross, Michael West, Antonette Logar, and Edward Corwin. Department of Mathematics and Computer Science, South Dakota School of Mines and Technology.

Second Place: "DNA methylation used to identify candidate loci associated with drought induced trans-generational plasticity that may inhibit range expansion in *Boechera stricta*." **J. Alsdurf**, C. Anderson, and D. Siemens. Integrative Genetics Program, Black Hills State University.

Third Place: "Interpreting native upland tallgrass prairie communities of South Dakota's prairie coteau using multivariate methods." **Diane M. Narem**[1], Lan Xu[1], Gary E. Larson[1], and Dave J. Ode[2]. [1]Natural Resource Management, South Dakota State University. [2] South Dakota Game Fish and Parks Department.

Poster Competition Awards

The winners receive a one-year subscription to Science compliments of the American Association for the Advancement of Science.

"Movement Patterns and Hibernation of Ornate Box Turtles in South Dakota." **C. Griffith** and A. Higa**.** Department of Math, Science, & Technology, Oglala Lakota College

"Genetic Structure of Endangered Populations of Native Red Mulberry in the Upper Midwest USA." **S. J. Schreier**. S. Piya and M.P. Nepal. Department of Biology & Microbiology, South Dakota State University.

Elections

The Executive Council provided for election the following slate of nominees:

Second Vice-President:	Vicki Geiser, MMC
Web Master:	Tim Mullican, DWU
Member-at-Large 2014-2016	Arvid Boe, SDSU
	Xu Lan, SDSU
	Matt Miller, SDSU
	Thomas Montoya, SDSM&T

The floor was opened for nominations, but none were forthcoming. Brian Patrick moved and Dave Berman seconded that the slate of officers provided by the Executive Council be accepted. The motion passed by acclimation.

Upcoming meetings

100th Annual Meeting hosted by SDAS Executive Council, at Cedar Shore, 10 and 11 April 2015

101th Annual Meeting hosted by University of Sioux Falls 2016.

102th Annual Meeting hosted by Dakota Wesleyan University 2017.
103rd Annual Meeting hosted by South Dakota State University 2018.
104th Annual Meeting hosted by Mount Marty College 2019.
105th Annual Meeting hosted by Northern State University 2020.
Executive Council Fall Meeting 2014: Al's Oasis, Oacoma, SD, Saturday, 6 September 2014

Recap of the 99th Annual meeting of the South Dakota Academy of Science

Forty papers were presented at the contributed paper sessions Friday afternoon and Saturday morning, and 25 posters were presented Friday afternoon and Saturday morning. There were 82 registrations for the spring meeting.

Adjournment

Brian Patrick moved and Mark Gabel seconded the adjournment of the 99th- Annual SDAS Meeting.

Respectfully submitted,
Donna Hazelwood
Secretary, SDAS

TREASURER'S REPORT—2014

01 April 2013	**Checking Account Balance**	**12,519.72**
	Certificate of Deposit	8,577.50
	Petty Cash	0.00
	Total Assets	21,097.22

	Income	***Expenses***
Spring 2013 Meeting Refunds		2895.00
Spring 2013 Meeting Expenses		1081.43
Stamps for Mailing Refunds		46.00
Spring 2013 Meeting Deposits	2865.00	
2013 Membership Payments	275.00	
Fall 2013 Meeting Expenses & Lunch		262.15
2014 Science Fair Awards		640.00
Cash for Cashbox 2014 Meeting		200.00
TOTALS	**6050.00**	**5134.58**

31 March 2014	**Checking Account Balance**	**13,435.14**
	Certificate of Deposit	**8,628.96**
	Petty Cash	**0.00**
	Total Assets	**22,064.10**

Respectfully Submitted,
Jeffrey S. Palmer

PROCEEDINGS EDITOR'S REPORT—2014

Report for the year from April 1, 2013 to March 31, 2014.

1. The following libraries were mailed a complimentary paper copy: AC, BHSU, DSU, DWU, MMC, NSU, OLC, SDSMT, SDSU, UND, UND, Am. Mus. Nat. Hist., U of WA and the Booth Fish Hatchery
2. The following indexing/ abstracting services and institutions were mailed complimentary paper copies: Chemical Abstract Services, AcadSci., Inc. , Cambridge Scientific Abstracts, GeoRef Library, Baywood Publishing (Anthropology), Thompson Scientific (Biosis)
3. The following non-member institution purchased paper copies: Linda Hall Library (Kansas City, MO) 1 copy; Curran and Associates (Red Hook, MA) 2 copies
4. Electronic files of the 2013 Proceedings have been sent to Miner Solutions for publishing to the SDAOS website.
5. For the 2013 Proceedings:
   No. of paper copies printed = 40
   No. of pages = 268
   No. of full papers published = 14
   No. of abstracts published = 61
   No. of titles only published = 45
   No. paper copies distributed to libraries, abstracting services, individuals, etc.= 29
   Electronic files of were provided to Shane Miner of Miner Solutions for uploading to the SDAS website.
6. First call for papers sent out early February; second call sent out 1 March.
7. 2014 Schedule of Events was produced and distributed
8. Proceedings Ledger

Beginning Balance (4/1/2013)	**$9707.94**	
INCOME		
Page Charges	3850.00	(Invoiced $4280.00, 100% paid on 4/7/2013)
Reprints	300.00	(Invoiced $400.00)
Subscriptions	30.00	(Invoiced $20.00)
2012 page charges	600.00	
Total Income	**$5740.00**	
Expenses		
Postage	296.06	
Supplies	160.14	
Formatting	1410.00	
Printing	1529.66	
Total Expenses	**$6851.94**	
Ending Balance (3/31/2014)	**$8596.00**	

Note: As of 4/29/2014, 100% of charges for the 2014 Proceedings were paid.

Submitted by
Robert Tatina, Editor

FELLOWS OF THE SOUTH DAKOTA ACADEMY OF SCIENCE

Kenneth F. Higgins 1998
Chuck Estee1999
No nominations 2000
Carroll Hanten 2001
Emil F. Knapp 2001
No nominations 2002
Clyde Brashier 2003
Milton Hanson 2003
S. Laeticia Kiltzer 2003
Robert Looyenga 2003
Arlen Viste 2003
Everett White 2003
Perry H. Rahn 2004
Robert Stoner 2004
No nominations 2005
No nominations 2006
No Nominations 2007
No Nominations 2008
No Nominations 2009
Maureen Diggins 2011
Gary Earl 2011
H.L. Hutcheson 2011
Waldemar "Wally" Klawiter Jr. 2011
Nels Grandholm 2012
the late Audrey Gabel 2012
Mark Gabel 2012
Robert (Bob) Tatina 2012
No Nominations 2013
No Nominations 2014

2014 MEMBERSHIP LIST

SENIOR MEMBERS

Baird, Nancy	
Barnes, Michael E.	SDGFP
Bergmann, David J.	BHSU
Butler, Jack L.	USFS
Clay, Sharon A.	SDSU
Corwin, Ed	SDSMT
Dash, Ilchgerel	SDSMT
Detwiler, Andy	SDSMT
Erazo-Barradas, Mauricio	
Ezrailson, Cathy M.	USD
Gabel, Mark	BHSU
Geiser, Vicki	MMC
Hansen, Stephanie	SDSU
Hazelwood, Donna	DSU
Higgins, Kenneth	SDSU
Hoyer, Ross	SDSMT
Jewell, Melody	SDSU
Johnson, Gary D.	SMU
Juntti, Tom	Sierra Club
Krack, Kaitlynn	SDSU
Kunza, Lisa	SDSMT
Laganiere, Dean	SDSMT
LaGarry, Hannan	OLC
Larson, Gary	SDSU
Logar, Antonette	SDSMT
Mergen, Daryl E.	Mergen Ecological Delineations, Inc
Meyer, Justin	SDSMT
Miller, Matthew L.	SDSU
Montoya, Thomas P.	SDSMT
Mullican, Tim	DWU
Mwangi, George K.	USF
Myer, Heidi	SDSU
Nam, Soonkie	SDSMT
Nichols, Samantha	SDGFP
Pagnac, Darrin	SDSMT
Palmer, Jeffrey	DSU
Parris, David C.	New Jersey State Museum
Patrick, L. Brian	DWU
Rahn, Perry H.	SDSMT
Reese, Neil	SDSU
Roggenthen, William	SDSMT
Rumble, Mark A.	U.S. Forest Service
Sani, Rajesh K.	SDSMT
Sarah, Anwar J.	SDSU
Shelton, Sully	SDSMT
Sorenson, James C.	MMC
Swanson, David	USD
Szczerbinska, Barbara	DSU
Tatina, Robert	DWU
Uresk, Daniel W.	USDA
Viste, Arlen E.	AC
Welsh, Ed Badlands	NP
Wilkins, Wu Justin	Mammoth Site
Wulff, Alex	SDSMT
Xu, Lan	SDSU

STUDENT MEMBERS

Alsdurf, Jake	BHSU
Andersen, Ethan	SDSU
Becket, Kristen	SDGFP
Benson, Benjamin	SDSU
Bredeson, Mike	SDSU
Deutscher, Tyrel	SDSU
Dolan, Lucas	DSU
Meiers, Lindsey	SDSU
Mergen, Zach	BHSU
Mosco, Taylor	DSU
Zimmerman, Sarah	SDGFP

PRESIDENTIAL ADDRESS

THERE IS ALWAYS ROOM FOR YOU IN SCIENCE

Address to the South Dakota Academy of Science
Rapid City Ramkota Inn, Rapid City, SD
April 4, 2014

Presented by Chun Wu
Mount Marty College
Yankton, SD 57078

ABSTRACT

The number of scientific discoveries seems more 100 years ago than today simply because there were so many unknown areas. As time advanced and many blanks were filled, scientific discoveries seemed to slow down, which tends to discourage potential researchers. The author started the speech with two questions: "Is there still room in sciences?" "Do we have a chance to make a big difference?" After that, the author presented one example to demonstrate how everyday research work can make a difference in science. In cancer treatment, we used to have only two options after surgery: radiation therapy and chemotherapy. Now, the patients hear the third FDA approved option: targeted drug therapy. The primary targets for those targeted drugs are specific proteins, which, when overexpressed, allow abnormal cancer cells to live and multiply. The targeted drugs inhibit the overexpression of those protein targets. Identification of those protein targets was a critical step. Where were those targets identified in the first place? In labs of basic health sciences. That was where students played important roles decades ago. Students usually complain about sitting in the lab doing tedious work day after day, seemingly going nowhere. That work pays off today. The author also pointed out that science develops continuously. During different stages in history, the theoretical form and focus of concern changed. For instance, in Mechanics, there were Aristotle mechanics, Newtonian mechanics and quantum mechanics. Medical science follows a similar trend. Microbiology established by Louis Pasteur could only explain infectious diseases. Endocrinology established by Arnold Berthold described "hormone imbalance", providing a reasonable explanation for slow growth, liver and kidney failure, sexuality recession and obesity. In 1949, Dr. Linus Pauling raised the concept of "molecular diseases", providing a scientific explanation for cancers, cardiovascular diseases and autoimmune diseases.

With respect to the question "Are there still unknown fields?", the author presented a few examples in protein science. There are some proteins. We know how important they are, and how they regulate certain biological functions. But we have not identified them. Once identified, they will have major effects in those fields. One example is the module or pathway controlling the size and

shape of organs. The second question is how exactly homologous recombination occur in meiosis? And which amino acid transporter is the upstream regulator of the mammalian target of the rapamycin pathway, in other words, can we measure extracellular amino acid levels then induce the mammalian target of rapamycin response. What are the cytosolic receptors of gas molecules such as H_2, and H_2S, and what is the molecular identity of the mitochondrial Ca uptake protein? Those questions sound simple, but they are unanswered. Here are some other new frontiers in medical science: DNA methylation plus epigenetics; brain mapping; genome 3D structure; promoter and intron region sequencing; metabolism: isocitrate dehydrogenase mutation, and function of noncoding DNA, etc.

In closing, the author left two final questions to the audience. “Where is your position in sciences?” “How can you fill in the gap in the field your interest?”

KEYNOTE ADDRESS

AUTONOMOUS SYSTEMS

Richard J. Gowen, PhD, FIEEE
President and CEO, Dakota Power

Presented to the South Dakota Academy of Science
Rapid City Ramkota Inn, Rapid City, SD
April 4, 2014

ABSTRACT

Much of science is directed to increasing our understanding of the world's most advanced autonomous system—the human. This evening I invite you to join me on a journey to consider the question: Can we trust the world's most advanced autonomous system?

We have lived intimately associated with a most advanced autonomous system--ourselves. May I pose some questions about ourselves: what do we know about the ability to create and communicate thoughts; share emotions; monitor the integrity of our mind and body; organize to achieve goals; and if you've experienced it, the drive to reproduce another human. While science strives to understand these processes and many more that are the core of our human system, our experiences help give us confidence of what we can trust our autonomous system to accomplish.

Consider the process you are using to trust being in this room. Our human autonomous system has learned to assess our environment and compare it to our previous experiences. We trust such continual evaluation of our experiences to guide our actions.

The action that occurred in response to the need to take immediate action to avoid an atmospheric discharge at the Three Mile Island nuclear power plant illustrates the question of trusting an autonomous system. The operation of a nuclear power plant is complex and demanding. The need to take action occurred just after the transition from a manual to a computer controlled operating system. While the computer system correctly told the operators what should be done, the operators did not trust what they perceived to be just a simple computer system and chose to manually override the computer. Their manual actions led to a major loss in trust in our ability to safely operate nuclear power systems. The lack of trust in the integrity of providing nuclear power led to the critical loss of the investments necessary to continue the growth of nuclear power in America. Fortunately, the science and technology communities cooperated to develop the Probabilistic Risk Assessment Process to assure the integrity and trust of our nuclear power systems.

The human autonomous system is continuously adapting to its environment. However there are incidents in which the human system is unable to respond

without a loss in the integrity of the system. Science is continuing the development of innovative applications of technology to develop alternatives to replace those functions.

There is a great need to understand the ability of our mind to draw upon memories to condition current actions. The return of veterans has brought greater emphasis on understanding and treating Post-Traumatic Stress Disorders (PTSD). Differing from obvious physical changes, we are coming to better appreciate the complex and often unchartered world of the mind.

Increasingly we see examples of how individuals with paraplegic, quadriplegic, and whole body challenges are able to adapt their autonomous systems to regain functionality. Impressive progress has been made with the integration of implants to regain the ability to control functionality with the mind. Researchers advocate developing Cyborgs to be super human autonomous systems by physically linking the mind with access to global information.

While science continues to provide better understanding of the human autonomous system, it is important to remember that we are inherently driven to avoid those things that would harm us, obtain adequate nourishment, and strive for an acceptable quality of life. We are experiencing challenges with the increasing global population by over 80 million people a year. The now 7 billion people live in areas of great population density with environmental and economic disparity.

The increasing diversity in the levels of cultural, political, and religious extremism bring new meaning to the question: Can we trust the world's most advanced autonomous system?

Senior Research and Symposium Papers

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PERMEABILITY OF THE INYAN KARA GROUP IN THE BLACK HILLS AREA AND ITS RELEVANCE TO A PROPOSED IN-SITU LEACH URANIUM MINE

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Department of Geology & Geological Engineering
Rapid City, SD 57701
perry.rahn@sdsmt.edu

ABSTRACT

Fluvial sandstone channels in the Cretaceous Inyan Kara Group in western South Dakota have variable thickness and texture, causing variability of the hydraulic conductivity. Pumping tests in two 120-ft thick sandstones at a proposed in-situ leach uranium mine in the Dewey/Burdock area provide hydraulic conductivity data. The hydraulic conductivity in the upper sandstone (Fall River Formation) is approximately 0.45 ft/day and the lower sandstone (Chilson Member of the Lakota Formation) is approximately 1.56 ft/day. These data, along with the prevailing gradient of the potentiometric surface, yield an average groundwater velocity for these two sandstones in the Inyan Kara Group of approximately 66 ft/year.

A groundwater velocity determination of 5,480 ft/year in the Inyan Kara Group near the Dewey/Burdock site was based on 1963 tritium data (Gott et al. 1974). This value seems very high, and contradicts the velocity based on hydraulic conductivity, but if valid, indicates fast groundwater movement through very permeable units or through fractures.

An important environmental consideration following the abandonment of this proposed uranium mine is that the groundwater will migrate down gradient and may contain a high concentration of dissolved uranium (with daughter products radium and radon) and selenium. The rate of movement of these elements would be less than the groundwater velocity because of retardation associated with geochemical reactions related to changes of pH and oxidation/reduction potential.

Keywords

Permeability, groundwater, Inyan Kara Group, uranium mine

INTRODUCTION

This paper contains information about the hydrogeology and permeability of a Cretaceous sandstone aquifer in South Dakota called the Inyan Kara Group. Particular attention is focused on the southwestern Black Hills area where an in-

Figure 1. Photograph of the Rapid Creek water gap in Rapid City, as seen from the top of the Alex Johnson hotel. The "M" is on the Chilson Member of the Lakota Formation. The closer sandstone is the Fall River Formation. The two Cretaceous sandstones dip easterly at approximately 20 degrees.

situ leach (ISL) uranium mine is planned. The purpose of this paper is to evaluate the permeability of the Inyan Kara Group and to ascertain the groundwater velocity and potential for contaminant transport from this mine.

The Inyan Kara aquifer, originally called the Dakota aquifer, underlies much of South Dakota and is one of the most famous aquifers in the United States. In the Black Hills area the Inyan Kara Group contains two prominent sandstone strata, the Chilson Member of the Lakota Formation and the overlying Fall River Formation (Figure 1).

Darton (1909) conducted some of the earliest hydrogeologic studies of this aquifer. Gries (1958) developed a stratigraphic model showing that the Dakota Sandstone in eastern South Dakota is roughly equivalent to the Newcastle Formation and the Inyan Kara Group in western South Dakota. Schoon (1971) studied facies within the Inyan Kara Group and showed that the sandstone units, such as the Chilson Member, are fluvial channel units in the Lakota Formation. Keene (1973) studied the Inyan Kara aquifer in Fall River County and found the potentiometric surface in western Fall River County slopes southerly. Strobel et al. (2000) showed the potentiometric surface elevation for the Inyan Kara Group in the southern Black Hills. Bredehoeft et al. (1983) examined alternate theories for regional recharge to this aquifer and concluded that much recharge occurs

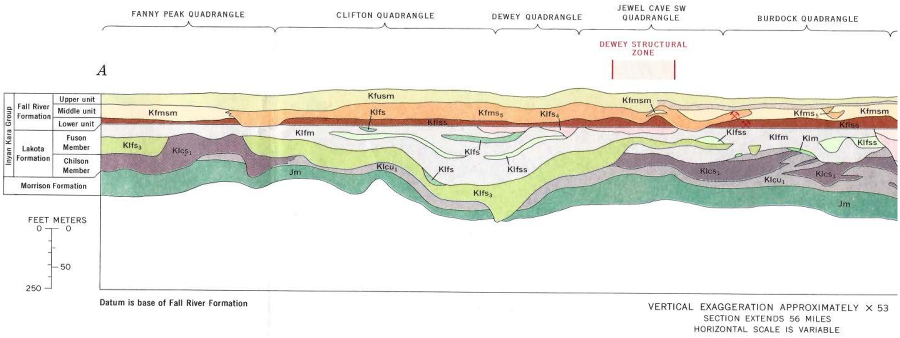

Figure 2. Diagrammatic geologic cross section showing stratigraphy of the Inyan Kara Group in the southern Black Hills (from Gott et al. 1974). The Fall River Sandstone is shown as Kfms$_5$, and the Chilson sandstone member of the Lakota Formation is shown as Klcs$_1$. The two main sandstone units show great variability in thickness from the Wyoming border through the Dewey, Burdock and Edgemont areas.

from leakage from overlying shale. Case (1984) and Carter et al. (2003) showed typical permeability values for the Inyan Kara aquifer.

Detailed geologic mapping in the southern Black Hills was conducted by the U.S. Geological Survey (USGS) in the 1950s and 60s for the purpose of evaluating uranium resources. Figure 2 is a cross section of the Inyan Kara Group in the southwestern flank of the uplift showing stratigraphic facies and the variable thickness of this unit.

HYDRAULIC CONDUCTIVITY DETERMINATION

Several published reports quantitatively document the hydraulic conductivity (K) of the Inyan Kara Group (Table 1). Information concerning the data shown in Table 1 and other studies is given below.

Table 1. Permeability data for the Inyan Kara Group (Driscoll et al. 2002)

	Hydraulic Conductivity (ft/day)	**Transmissivity (ft^2/day)**
Niven (1967)	0 – 100	—
Miller and Rahn (1974)	0.712	178
Gries et al. (1976)	1.26	—
Bredehoeft et al. (1983)	8.3	—
Kyllonen and Peter (1987)	—	0.86 – 6,000

Niven (1967) measured the permeability of cored samples from outcrops in the Black Hills and found the mean hydraulic conductivity of the Lakota Formation is 22.5 ft/day and the Fall River Formation is 4.8 ft/day. Bredehoeft et al.

(1983) pointed out that "one would expect the outcrop to yield higher hydraulic conductivities than exist in the subsurface". Using a best-fit model of western South Dakota, Bredehoeft et al. (1983) determined a horizontal hydraulic conductivity of 8.29 ft/day in the Inyan Kara Group sandstone units.

Based on a recovery test for a 2,300-ft well into the Fall River and Lakota Formations at Box Elder, Miller and Rahn (1974) found the transmissivity (T) to be 1,333 gpd/ft (equivalent to 178 ft^2/day). Because the thickness (b) of the two sandstone units at Box Elder is 250 ft, the hydraulic conductivity (K) is 0.712 ft/day.

Three observation wells were used at Wall, South Dakota, where a pumping test showed anisotropic transmissivity (Rahn 1992). The 3,300-ft deep wells are open to the Lakota Formation, and the sandstone thickness is approximately 200 ft. The transmissivity averages 2,400 gpd/ft (321 ft^2/day) and storativity is 0.000,027. The average hydraulic conductivity is: $K = T/b = 321$ ft^2/day/200 ft = 1.60 ft/day. The principal transmissivity direction was found to be N 35° W, interpreted as the paleodirection of Cretaceous rivers.

An 11-day constant discharge pumping test involving 13 observation wells was conducted in the Lakota aquifer at TVA's proposed underground uranium mine near Dewey, South Dakota (Boggs 1983). In this area the Cretaceous Inyan Kara Group dips approximately 5 degrees towards the west-southwest off the Black Hills uplift and consists of two sandstone aquifers, the Fall River Formation, typically 120 ft of fine-grained sandstone, underlain by the Chilson Member of the Lakota Formation, 120 ft of fine-to-coarse grained sandstone (Boggs and Jenkins 1980; Boggs 1983). Figure 3 shows these two units. The pumping tests indicate the Lakota at this location is exceptionally permeable, having a transmissivity of 4,400 gpd/ft (587 ft^2/day). Boggs (1983) noted that this test site is in an area where the Lakota is composed of a thick, exceptionally coarse-grained sandstone.

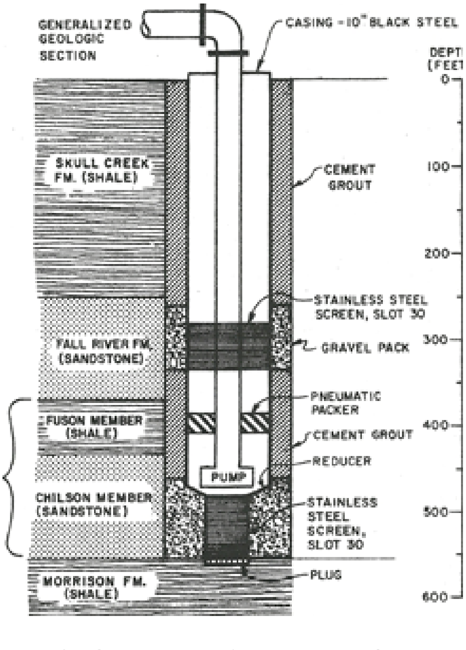

Figure 3. Geologic log of Burdock well (Boggs and Jenkins 1980).

A pumping test in the Chilson Member of the Lakota Formation for the proposed Tennessee Valley Authority (TVA) underground mine at Burdock, South Dakota, by Boggs and Jenkins (1980) indicated the transmissivity is approximately 1,400 gpd/ft (187 ft^2/day). A contour map of the drawdown (Boggs and Jenkins, 1980, Figure 19) shows a slight elongation in the northeasterly direction [Note: Boggs and Jenkins (1980) showed this map but mistakenly reported this

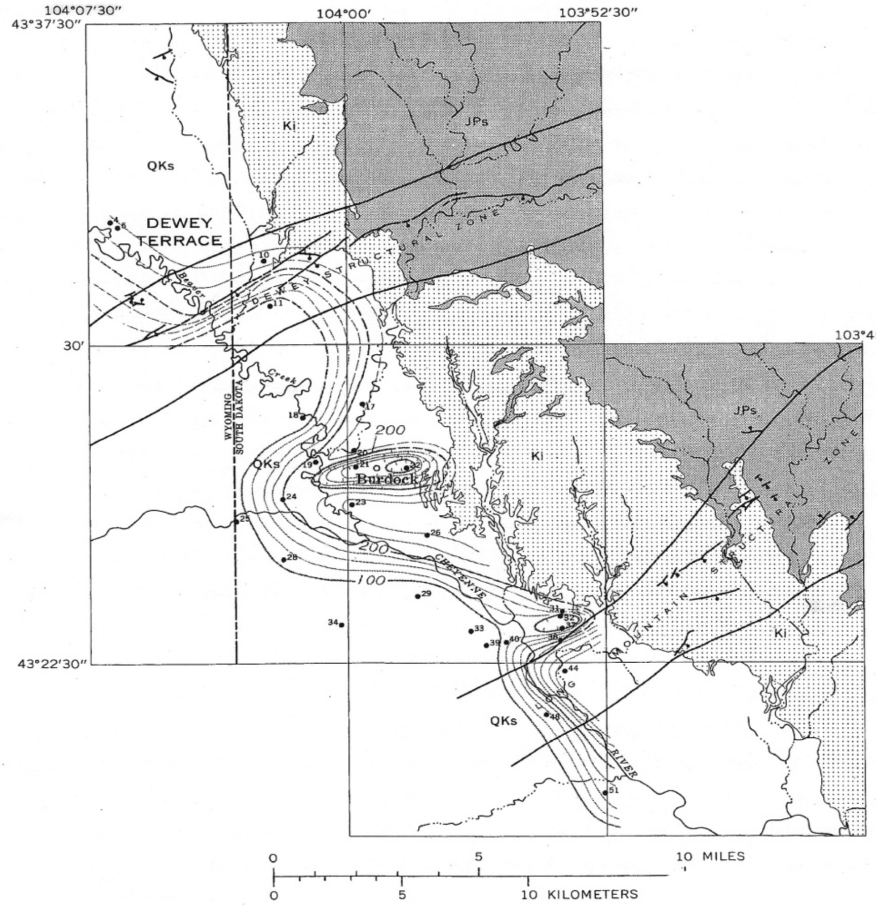

Figure 4. Map showing tritium distribution in groundwater in the Inyan Kara Group (from Gott et al. 1974).

elongation is "…in a northwesterly direction".] The greater permeability in the northeast-southwest direction most likely reflects the orientation of the Cretaceous stream channels. The Chilson Member consists of 120 ft of fine-grained sandstone and siltstone; hence the hydraulic conductivity is 11.7 gpd/ft (1.56 ft/day). A second test on the Fall River Formation found the transmissivity to be 400 gpd/ft (53.5 ft^2/day). The Fall River Formation consists of approximately 120 ft of interbedded fine-grained sandstone, siltstone, and carbonaceous shale; hence the hydraulic conductivity is 3.33 gpd/ft (0.45 ft/day). Subsequent pumping tests for the proposed Powertech ISL mine in the Dewey/Burdock area (Knight-Piesold 2008; Powertech 2009a, 2009b) indicate similar transmissivity values to those determined by the TVA investigations.

TRITIUM

An unusual insight into the permeability and groundwater velocity was provided by Gott et al. (1974) who reported the presence of tritium in groundwater in the Inyan Kara Group near Burdock, South Dakota (Figure 4). The tritium originated from the 1963 Pacific hydrogen bomb fallout. From the location of the tritiated groundwater, Gott et al. (1974) determined that "…near the confluence of Beaver Creek and the Cheyenne River, a flow of 15 feet per day is required to transmit tritium rain-out of the year 1963 from the recharge area at the Inyan Kara outcrop to the positions of the large tritium concentration…" No supporting groundwater velocity calculations were provided by Gott et al. (1974), and this velocity seems quite high. It is possible that there was a tritium sampling error. If, in fact, the true velocity (V_t) is 15 ft/day (equivalent to 5,480 ft/year), two unlikely scenarios would be manifest:

> 1. **Hydraulic conductivity.** The Darcy velocity (V_d), also called the "specific discharge", through an aquifer is equal to the true velocity (V_t) times the porosity (here assumed to be 10%). Therefore: V_d = V_t (porosity) = 15 ft/day (0.1) = 1.5 ft/day. Near Burdock the potentiometric surface in the Inyan Kara Group slopes south-southwesterly (Boggs and Jenkins 1980). TVA (1979) reported a hydraulic gradient of 9.8 m/km in the Lakota Formation at the Burdock site. The spacing of the equipotential contour lines varies locally, and in the Dewey/Burdock area a head loss from 3700 ft to 3600 ft elevation occurs over a distance of approximately 2.4 miles. Therefore, H/L = 100 ft/12,672 ft = 0.0079. From "Darcy's Law" (Rahn, 1996): V_d = K (H/L); therefore the hydraulic conductivity (K) = V_d /H/L = 1.50 ft/day/0.0079 = 190 ft/day. This value seems unrealistically high. For example, Boggs and Jenkins (1980) show the hydraulic conductivity of the Fall River Formation at Burdock is only 0.45 ft/day.
>
> 2. **Recharge rate.** Consider the discharge (Q) of groundwater through 1 ft^2 cross section of the Inyan Kara aquifer having V_t = 15 ft/day. With 10% porosity, the effective cross-sectional area is 0.1 ft^2, and the discharge through this cross section would be: Q = V_t A = 15 ft/day (0.1 ft^2) = 1.5 ft^3/day. Adequate recharge from precipitation on Inyan Kara outcrops is necessary to sustain this discharge. Brobst (1961) shows the Inyan Kara Group in this area dips approximately 500 ft/mile (equivalent to 5.4 degrees); thus the discharge through a horizontally-oriented recharge area required to service a 1 ft^2 cross section of sandstone = 1.5 ft^3/day (sin 5.4 degrees) = 1.5 (0.09427) = 0.1414 ft^3/day. Sustaining this discharge would require 365 (0.1414) = 51.6 ft of precipitation recharging annually on the outcrops. Obviously this is not possible.

Gott et al. (1974) could have over-estimated the groundwater velocity because they underestimated the distance of transport from the suspected recharge area to the sampling site. Figure 4 shows the locations of wells and springs sampled in 1967 for tritium. The distance to the Inyan Kara outcrops ranges from approximately one to five miles away. Gott et al. (1974) do not show calculations supporting their velocity determination of 15 ft/day, but most likely they assumed a slug flow of tritiated water moved from the recharge area to the sampled well or spring site from rainout that fell in 1963. [Note: tritiated precipitation peaked in 1963 (Freeze and Cherry 1979); Back et al. (1983) show tritiated rain fell in the Black Hills area as early as 1953.] From Figure 4, fifteen feet per day appears to be the maximum velocity.

Another possible explanation for the presence of tritium in the sampled wells is that it entered the aquifer quite close to the sampling locality. It may have leaked downward through overlying shale (from Figure 3 the Fall River Formation is only 250 ft depth). This explanation would support the model by Bredehoeft et al. (1983) suggesting recharge to the Inyan Kara Group occurs through the overlying shale. Another possible explanation is that tritiated water may have leaked down along the casing of the sampled well. However this seems unlikely since many of the sampled sites are artesian wells or springs.

A study by Johnson (2012) shows recent tritium concentration data in this area of up to 15.3 tritium units. Considering that the half-life of tritium is 12.3 years, these data may essentially represent the same water as 1967 except that the tritium has undergone radioactive decay. This indicates the water is not moving through the aquifer at 15 ft/day.

Another possible explanation for the presence of tritium as shown in Figure 4 is that in 1963 it recharged the outcrops as visualized by Gott et al. (1974) but traveled along extremely permeable pathways. These could be fractures in the sandstone (Figure 5) or very permeable conglomerate channels. Gott et al. (1974) point out that cross-bedded sandstone beds contain many intertonguing lenses that vary from fine-grained sandstone to conglomerate with pebbles greater than three inches in diameter. The direction of dip of the crossbeds within the sandstones indicates the paleostreams flowed northwesterly. Figure 4 shows the “Dewey Fracture Zone”. This zone is shown on geologic maps as the Dewey Fault by Brobst (1961) and the Dewey Fault and Structural Zone by DeWitt et al. (1989). Schnabel (1963) describes well-defined vertical joint systems in the Burdock quadrangle that typically strike N 75-85 degrees E and N 35-45 degrees E. The isograms of tritium content on Figure 4 seem to bend in a manner suggestive that high tritium is associated with the fractures. While the 15 ft/day groundwater velocity posited by Gott et al. (1974) seems enigmatic, a possible explanation is that the tritiated water followed fractures, and did not travel through the sandstone interstices.

Hydrogeologists recognize that at a specific local site groundwater may move faster than the general regional flow. For example, in another Black Hills aquifer, the karstic Madison Limestone, there are diffuse flow components as well as solution-enlarged conduits that transport groundwater very rapidly (Long and Putnam 2004; Long et al. 2007). Back et al. (1983) used ^{14}C to determine the age of groundwater over a large area of western South Dakota. At Midland, for example, they found that the water was recharged 20,100 years ago, and using 10% porosity and the 260-km (162 mile) distance to the recharge area, they determined that the velocity was 12.9 m/yr (42.3 ft/year). The hydraulic gradient

Figure 5. Photograph of "Hangman's Hill" in Rapid City showing the cross-bedded sandstone (Chilson Member of the Lakota Formation) and a pronounced fracture.

in the Midland area is 1.7 X10^{-3}, and the hydraulic conductivity was determined to be 24 X 10^{-6} m/sec (0.63 ft/day). This hydraulic conductivity is close to that determined from pumping tests in Rapid City (Greene and Rahn 1995). Despite these determinations of hydraulic conductivity and velocity, locally there are conduits in the Madison Limestone that permit exceptionally fast movement. For example, dye introduced into sinkholes along Boxelder Creek was traced to Gravel Spring in 68 minutes (Rahn and Gries 1973). The distance is 2,200 ft, so the groundwater traveled at 47,000 ft/day. The dye was traced to City Spring in Rapid City in 34 days (Rahn and Gries 1973; Greene and Rahn 1995). This distance is 7.5 miles, so the groundwater traveled at 1170 ft/day. Unlike the Madison Limestone, the Inyan Kara Group is not a karst aquifer; nevertheless local high groundwater velocity may occur.

In spite of the seemingly enigmatic tritium data, this paper shows a groundwater velocity determination through the two sandstones at the Dewey/Burdock area in the conventional manner using hydraulic conductivity data from pumping tests. These calculations assume that no fractures or extremely permeable conglomerate units are present, and that groundwater flows through the sandstone in a manner predicted by Darcy's Law.

APPLICATION OF PERMEABILITY DATA TO THE PROPOSED DEWEY/BURDOCK MINE

The impact of the withdrawal of groundwater is an important part of any environmental assessment. The South Dakota Department of Environment and

Natural Resources (SD DENR) approved the withdrawals for this ISL mine but is currently involved in litigation over Powertech's proposed withdrawal of 8,500 gallons per minute (gpm) from the Inyan Kara aquifer and 551 gpm from the Madison aquifer. Most of this water is pumped back into the aquifer and is used to introduce oxidizing chemicals necessary for the solution of the uranium minerals. Actual water consumed ("make-up water") is estimated by Powertech to be only 170 gpm. The predicted drawdown from groundwater withdrawals has been discussed in the Water Rights Section of the SD DENR website and is not included in this paper. Rather, the emphasis of this paper is to assess the natural groundwater velocity in this aquifer since this is a critical factor for evaluating the long-term impacts of introducing chemicals ("lixiviants") into this aquifer.

A major concern of ISL uranium mining is the chemistry of the residual groundwater after mining ceases, the rate of groundwater movement downgradient through this aquifer, and the possibility of mixing with water in other aquifers. Studies of existing ISL mines have shown that dissolved uranium and selenium are of particular concern (Borch et al. 2012). In order to assess the environmental impact of residual groundwater following mining at the proposed Dewey/Burdock project area, one can use permeability data to predict the groundwater velocity in the channel sandstones of the Inyan Kara Group.

For the purpose of this paper, pumping test data and the conventional use of hydraulic conductivity with Darcy's Law were applied to determine the groundwater velocity. Table 2 summarizes aquifer parameters that are most relevant to the proposed Dewey/Burdock in-situ leach uranium project. Figure 3 is a geologic section of the Burdock well used in a pumping test by Boggs and Jenkins (1980). The top of the Fall River Formation lies at 250 ft depth and the top of the Chilson Member of the Lakota Formation lies at 430 ft depth. From Table 2, transmissivity averages 365 ft^2/d at the three Lakota Formation (Chilson Sandstone Member) sites. The range of values, from 187 to 587 ft^2/d, is caused, by some degree, to the variable thickness of the sandstone. The coarseness of the sandstone is certainly another reason for transmissivity variability; for example, the largest value, 587 ft^2/d, occurs where coarse-grained sandstone is reportedly present.

Table 2. Relevant aquifer data (modified from Boggs and Jenkins 1980)

Site	Unit studied	Hydr. Cond. (ft/d)	Thickness (ft)	Transmissivity (ft^2/d)
Dewey	Lakota Fm. (Chilson)	4.92	120	587
Burdock	Lakota Fm. (Chilson)	1.56	120	187
Burdock	Fall River Fm.	0.45	120	53.5

Groundwater velocity at the Dewey and Burdock area can be estimated by using the data from Table 2. The natural potentiometric surface slopes approximately WSW at a gradient (H/L) of approximately 0.0079. The effective porosity has been reported at 17% for the sandstones in the Inyan Kara Group (Rahn

1981), but this may be high since hydrogeologists using Darcy Law calculations for other aquifers in the Black Hills area have used 10% effective porosities (Back et al. 1983). For simplicity, in this paper, if one assumes that the effective porosity is 10%, then the groundwater velocity can be determined as follows:

- Dewey area (Lakota Fm): $V_t = V_d/10\% = K\ (H/L)/0.1 = 4.92\ (0.0079)/0.1 = 0.389$ ft/day (equivalent to 142 ft/year).
- Burdock area (Lakota Fm): $V_t = 1.56\ (0.0079)/0.1 = 0.123$ ft/day (equivalent to 44.9 ft/year).
- Burdock area (Fall River Fm): $V_t = 0.45\ (0.0079)/0.1 = 0.0316$ ft/day (equivalent to 11.5 ft/year).

An average groundwater velocity within the Inyan Kara Group at the Dewey/Burdock area is assumed to be the average of these three velocities, i.e. 0.18 ft/day (equivalent to 66 ft/year). [Note: this velocity is greater than the "maximum natural groundwater velocity" of approximately 12 ft/year estimated by Powertech (2009a).]

As shown in Figure 2, the Inyan Kara Group in the southern Black Hills has complex stratigraphy and hence has considerable permeability variability. The Inyan Kara Group includes permeable sandstone channels that could carry groundwater faster than an average value of 66 ft/year. At some places the sandstone channels are not present. A crude estimate of the permeability variability can be made by comparing the hydraulic conductivity values from western South Dakota pumping tests described in the above references. These values include: 0.94 (Miller and Rahn 1974); 1.52, 1.47, and 1.85 (Rahn 1992); 1.85, 1.73, 1.70, 1.53, 1.98, 0.17, and 0.17 ft/d (Boggs and Jenkins 1980). Also using 8.3 ft/d (from Bredehoeft et al., 1983) yields twelve values. These values have an approximate log-normal distribution, and hence the largest 10% hydraulic conductivity value would be approximately 9 ft/d. For the velocity determinations of the three sites described above, average hydraulic conductivity is 2.31 ft/d. Therefore, there is a 10% probability that the hydraulic conductivity would be approximately 9/2.31 = 4 times that value. Another factor that plays an important part of any sensitivity analysis is the effective porosity, which in this paper is assumed to be 10%.

RELEVANT HYDROGEOLOGIC CONDITIONS IN FALL RIVER COUNTY

Figure 6 is a geologic cross section near Edgemont, South Dakota, illustrating the typical geology of much of southern Fall River County. At Igloo, for example, the Inyan Kara Group was encountered at 1,085 ft depth and the Madison Limestone was encountered at 3,590 ft. The potentiometric surface of the Madison at the Igloo site is 3,700 ft elevation (Strobel et al. 2000). This is above the land surface, and creates a flowing artesian well. The potentiometric surface of the Inyan Kara, on the other hand, is only 3,250 ft; this elevation is below the land surface. Under these conditions, it is very unlikely that groundwater could

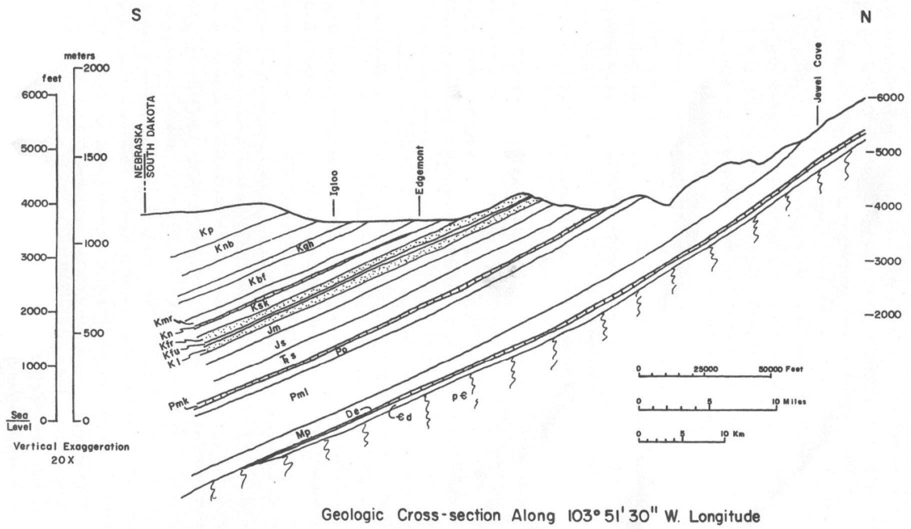

Figure 6. Geologic cross section near Edgemont, South Dakota (from Roggenthen et al. 1985). The sedimentary rocks include the Fall River Formation (K_{fr}) ,the Lakota Formation (K_l) and the Pahasapa Limestone (M_p).

move from the Inyan Kara aquifer down to the Madison Limestone in most of southern Fall River County.

A relevant groundwater quality issue involves Powertech's plan to drill a waste disposal well into the Minnelusa or Deadwood Formation (Nuclear Regulatory Commission 2012). Rahn and Gries (1973) and Greene (1999) showed that there is a hydraulic connection between the Minnelusa Formation and the Madison Limestone at places. There is the possibility that the Madison aquifer could become contaminated with this waste.

DISSOLVED VS SOLID URANIUM IN A SANDSTONE AQUIFER

The ISL mining process will dissolve solid uranium minerals that occur in the sandstone. A consideration relevant for a pre-mining environmental assessment of an uranium ISL site is a comparison between the amount of uranium that is naturally dissolved in groundwater and the amount of uranium found as solid minerals in the aquifer matrix.

For the purpose of these calculations, a hypothetical uraniferous sandstone aquifer simulating the Dewey/Burdock area in the southwestern Black Hills is used, i.e., the 120 ft (36.6 m) thick Chilson Member of the Lakota Formation. An area underlying 10 acres (4.047 ha) is used for these calculations. The sandstone at this site is nearly horizontal; hence the volume can be approximated as: 36.6 m (4.047×10^4 m^2) = 1.48×10^6 m^3.

Solid uranium

According to the U.S. Senate (1972), as of October 1, 1963, uranium mining in the Black Hills totaled 367,497 tons (3.3339 X 10^8 kg) of ore; this ore contained 1,352,000 lbs (6.1327 X 10^5 kg) of U_3O_8. The actual amount of uranium within U_3O_8 ("yellowcake") can be determined from the atomic weights of uranium (238) and oxygen (16) as follows: 3 (238)/3 (238) + 8 (16) = 0.848 = 84.8%. Therefore, from the historic mining data, the amount of uranium mined in the southern Black Hills is 84.8% (6.1327 X 10^5 kg) = 5.2039 X 10^5 kg.

TVA (1979) reported that at the Dewey/Burdock area "…the ore minerals coat sand grains and fill interstices of complexly cross-stratified sandstone along solution fronts similar to 'roll' type deposits…" For this paper, the proportion of uranium in the sandstone at the Dewey/Burdock site is assumed to be the same as the uranium in the ore historically mined in the southern Black Hills. This proportion equals 5.2039 X 10^5 kg/ 3.3339 X 10^8 kg = 0.0015609 = 0.1561%. Assuming the sandstone weighs 2.20 gm/cm^3, the mass of the sandstone at the Dewey/Burdock site would be 2.2 X 10^4 kg/m^3 (1.480 X 10^6 m^3) = 3.257 X 10^{10} kg. Assuming the ore is distributed throughout the sandstone, the mass of uranium in solid form within this sandstone is 0.00156 (3.257 X 10^{10} kg) = 5.080 X 10^7 kg. [Interestingly, this is equivalent to approximately 15% of all the uranium historically mined in the southern Black Hills. Also of interest is that at the Dewey and Burdock site Powertech Uranium Corporation (2010) estimates the total amount of U_3O_8 to be 10,813,000 lb (4.905 X 10^6 kg.]

Dissolved uranium

Williamson and Carter (2001) showed data on dissolved uranium in groundwater in the Black Hills. In the Edgemont area, the highest concentration is approximately 30 micrograms per liter (μg/L), the same as U.S. Environmental Protection Agency's maximum permissible concentration for drinking water. In the southern Black Hills, Oak Ridge Gaseous Diffusion Plant (1980) showed uranium concentrations in groundwater; 340 samples averaged approximately 10 μg/L, although values up to 200 μg/L were found. Groundwater in Cretaceous rocks typically had high values. TVA (1979) reported that in 1977 the Burdock Well #1 had dissolved uranium ranging from 0.10 to 9.50 μg/L and dissolved ^{224}Ra ranging from 111.4 to 230.1 ρCi/L. For this paper it is assumed that dissolved uranium in the groundwater at this site has a concentration of 30 μg/L (equivalent to 3 X 10^{-5} kg/m^3). Assuming 10% porosity, the ten-acre sandstone unit used for these calculations contains 0.10 (1.48 X 10^6 m^3) = 1.480 X 10^5 m^3 of groundwater. Therefore the amount of dissolved uranium at this site is 1.48 X 10^6 m^3 (3 X 10^{-5} kg/m^3) = 44.4 kg.

> **Comparison**
>
> From above, the mass of the solid uranium under the ten acre site is 50.8 X 10^6 kg, and the mass of the liquid uranium under this site is 44.4 kg. The ratio is 1.14 X 10^6 : 1. Solid uranium (in the form of minerals such as carnotite, coffinite, uraninite, etc.) vastly exceeds the uranium dissolved in groundwater. This indicates that ISL uranium mining has the potential to dissolve vast amounts of uranium from its natural solid form.

The originally proposed method of mining by TVA at the Dewey/Burdock site was to be a conventional underground mining method (TVA, 1979). The proposed method of mining by Powertech (2009a, 2009b) is "solution mining", known as "in-situ leach", also called "in-situ recovery". This method will utilize chemicals pumped into the sandstone that dissolve the solid uranium minerals. Given the vast quantities of solid uranium that could be dissolved at this site, after mining ceases there will most likely still be a high concentration of dissolved uranium (as well as other elements) in the groundwater. The Nuclear Regulatory Commission (2012) notes "at the end of the uranium recovery process, constituents that were mobilized by the lixivients remain in the production aquifer. The NRC requires that groundwater quality be restored to a concentration limit established by the NRC." This water will migrate down gradient. Dissolved uranium generally precipitates in fully reduced zones, but uranium sorption also depends on the presence of iron hydroxides (Johnson and Tutu 2013, who concluded "…the resulting uncertainty of uranium sorption is quite high."). Although chemicals could be introduced to mitigate high concentrations of dissolved uranium, its ultimate concentration is unknown.

ENVIRONMENTAL IMPACTS FROM ISL URANIUM MINES

A major concern of conventional or ISL uranium mines is the fate of radioactive elements that remain in the area after mining ceases. Environmental impacts from conventional uranium mines in the Edgemont area are described by Rahn and Hall (1982).

A draft Environmental Impact Statement (DEIS) for the proposed Dewey/Burdock ISL uranium mine was prepared by the Nuclear Regulatory Commission (2012). [The NRC refers to the in-situ-leach (ISL) method of solution mining as in-situ recovery (ISR) method.] The DEIS, available "on line", contains a wealth of background environmental data but has no post-mining groundwater velocity determination such as given in this paper. Rather, the impacts envisioned by this project are discussed in terms of tables showing "ratings". For instance, NRC states the overall groundwater impact will be "small to moderate" and the "proposed mitigation measures will eliminate or substantially lessen potential adverse environmental impacts."

Following mining, the residual fluids in the project area will begin migrating through the aquifer. A major concern about ISL uranium mines is that no dissolved minerals or chemicals introduced into the mined zone should escape the project area. Mining companies assure the public that this will not happen because, during operation, recovery wells surround the injection wells. Presently there are six active in-situ leach (ISL) uranium recovery mines in the U.S. (Borch et al. 2012). Hall (2009) studied two ISL uranium mines in Texas and found that dissolved uranium and selenium are the two elements most likely to be above restoration objectives following termination of ISL operations.

In nearby Crawford, Nebraska, the Crow Butte ISL uranium mine has been in operation by Cameco Resources for 21 years. Coatings of coffinite, carnotite, and uraninite occur in basal Chadron conglomerate beds 65 -135 m (213 – 443 ft) below the ground surface (Spalding et al. 1984). The Crow Butte mine includes a system of 4,500 wells that use a solution of water, oxygen, and bicarbonate to dissolve uranium minerals and bring the liquid to a processing plant on the surface. A system of monitoring wells on the perimeter of the mined area is used to detect any excursions from the mine area. Dave Carlson, retired from the Nebraska Department of Environmental Quality, commented that the project has not negatively affected the surrounding aquifers (Woster 2012). A website for the SD DENR states that the most commonly reported problems at the Crow Butte mine are spills from production fluids and leaks in pond liners. They also report that "other problems include excursions for process fluids beyond the limits of the production zone".

Commenting on efforts to restore groundwater at an ISL mine at the Christensen Ranch area near Pumpkin Buttes, Wyoming, Lustgarten (2012) stated that a Nuclear Regulatory Commission report "… concluded that restoring water to baseline levels was 'not attainable' for many of the contaminants, including uranium".

SUMMARY

The chemistry of groundwater at an abandoned ISL uranium mine will be changed from its pre-mine condition. The amount of chemical change and the groundwater velocity downgradient from the mined site are important for any environmental assessment. The chemistry of this water will be greatly altered. Elements such as uranium, radium, and selenium will be dissolved by chemicals during the mining operation. These elements originally were bound up within the Inyan Kara aquifer as solid minerals. Solution mining will set them free as dissolved constituents in the groundwater. Their concentration and mobility within the aquifer is uncertain.

The ultimate fate of groundwater contaminants from an ISL uranium mine depends on the groundwater velocity and the natural attenuation that could immobilize contaminants such as uranium and selenium. In this paper, the natural groundwater velocity through the Inyan Kara aquifer in the Dewey/Burdock area is determined to be approximately 66 ft/year. If this relatively slow rate is representative, the average migration of contaminants from the proposed Dewey/Burdock ISL uranium mine would not appear to be a great concern to landowners who live miles away. The 1963 tritium data showing much faster velocity is an unresolved issue. It is not known if the data are accurate; but if they are, there could be fractures or permeable conglomerate channels that transmit groundwater much faster than the velocity calculated by traditional Darcy Law as presented in this paper.

Future research should include detailed hydrogeologic study of the permeability of fractures and the coarse-grained facies of the Inyan Kara Group as well as quantification of the chemical changes that could retard contaminant transport in groundwater from an ISL uranium mine.

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TOPOGRAPHIC, GEOLOGIC, AND DENSITY DISTRIBUTION MODELING IN SUPPORT OF PHYSICS EXPERIMENTS AT THE SANFORD UNDERGROUND RESEARCH FACILITY (SURF)

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ABSTRACT

The Sanford Underground Research Facility (SURF) has constructed laboratories on the 4850 Level in the underground. These laboratories are hosting particle physics investigations, such as those associated with neutrino physics and dark matter searches. The laboratories are located in the underground to take advantage of the overlying rock that shields the experiments from the effects of cosmic radiation. The efficiency of the shielding typically is evaluated in terms of water equivalent depth, which is the thickness and density of rock that would be the equivalent depth of water. In order to provide a basis for evaluation of the effects of the shielding rock, we developed a geologic model using available data and densities assigned to the rocks making up a cone of material centered over the Davis Campus on the 4850 Level. This information is presented in a series of cones that are contoured in terms of water equivalent depths. Paths are shortest in the area directly over the laboratory and longest at shallow angles that penetrate greater columns of rock. The analysis of the density cones shows that the most important effect is due to variations in topography. Although variations in the density of the rock affect the water equivalent depths, particularly in the western area of the site, the effects are secondary. The influence of stopes or mined out areas are not significant due to backfilling of most of the voids and the generally small mined volumes in relation to the much larger volume of the geologic cone of analysis.

Keywords

Sanford Laboratory, attenuation, density

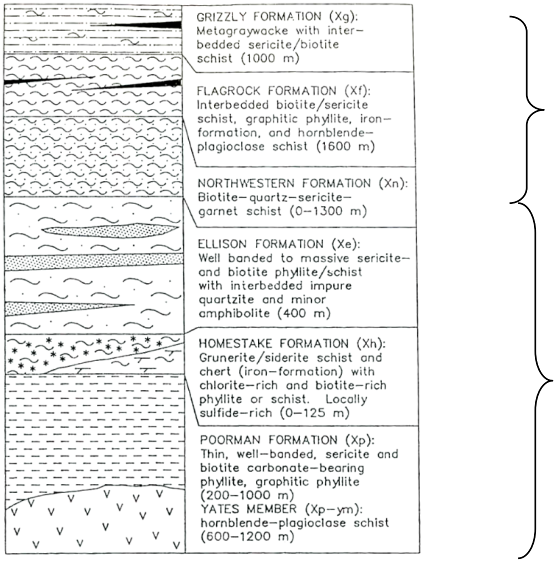
INTRODUCTION

The mission of the Sanford Underground Research Facility (SURF), located in the Black Hills of South Dakota, relies upon relationships involving physics, geology, and mining. This interplay became evident during the preliminary planning and construction phase of the laboratory (Lesko et al. 2011), when issues of excavation, rock stability, and attenuation of cosmic radiation by the overlying rock were investigated. Particle physics laboratories have been and are being constructed at SURF to support studies of neutrino physics and searches for dark matter. The primary reason for location of these laboratories underground is to shield the experiments from the effects of cosmic radiation that would add unacceptably high background signals if they were located at the surface (Lesko et al. 2011). Although neither the postulated dark matter particles nor neutrinos are significantly impeded by the rock, the overburden attenuates the muon flux, thus providing an effective filter for particle physics studies. Similarly, the shielding effect of the rock is important in detectors that are searching for very rare events associated with local sources of radioactive decay, such as those experiments searching for neutrinoless double-beta decay. The rock above the laboratories provides crucial shielding for the detector systems at depth for both of these types of studies.

The attenuation of muons is a function of the depth, surface topography, and rock densities being traversed by the cosmic radiation (Lesko et al. 2011). Mei and Hime (2006) showed that the expected decrease in muon intensity is exponential and is a function of the overburden density. The common measure that combines these two attributes is water equivalent depth, which is the distance travelled by a muon through water corrected for the difference in density between water and the density of the material. Therefore, the attenuation of cosmogenic muons is due to both the distance traversed by the muons and the density of the rock through which they travel. Use of the average density of the rocks is a good first approximation in conjunction with the observed topographic relief, which causes the thickness of the rock shield to change. However, the Sanford Laboratory is constructed in a complexly folded metasedimentary and metaigneous terrain with some variation in rock densities among the geologic units. Therefore, the goal of this project was to evaluate the effect of those variations in rock density based upon the best available geologic model of the volume surrounding the laboratories and the topography in the vicinity of the facility.

SURF Stratigraphy and Structural Geology—SURF, which was the site of the former Homestake Gold Mine, is located in the northern end of the Black Hills of South Dakota. The site is characterized by a youthful topography with canyons that are narrow and steep sided. The average elevation in the vicinity of the laboratory site is ~1,640 m above sea level and has a maximum relief of ~220 m in the area investigated.

The southern portion of the Black Hills is a structural dome consisting of a core of intensely folded Precambrian metamorphic schists and phyllites (Redden and Lisenbee 1990). The Precambrian rocks are overlain unconformably by Paleozoic sedimentary rocks that dip gently away from the core of the dome. The northern Black Hills, where the Sanford Laboratory is located, were created primarily by the intrusion of Tertiary-age rhyolites, trachytes, and phonolites. These intrusive rocks have domed the overlying Phanerozoic rocks and exposed the Precambrian crystalline rocks at the site, and sedimentary rocks are absent in the immediate vicinity of the laboratory. The laboratory is developed within



Formations occurring at the surface and in cores acquired during exploration activities.

Formations occurring in the underground workings at Sanford site.

Figure 1. ***Precambrian stratigraphy at and near Sanford Laboratory (after Bachman and Caddey 1990). The Grizzly and Flagrock Fm. are not exposed by the underground workings, and only the lowermost portion of the Northwestern Fm. is present in the underground drifts.***

a thick series of Precambrian metasedimentary and metaigneous rocks that were deposited during the Precambrian at approximately 2,000 Ma based on dating of a tuffaceous unit in the Ellison Fm. (1,974 ± 8 Ma [Redden et al. 1990]). The sediments were fine-grained sands, silts, and muds with lesser amounts of chemical precipitates, and the protolith of the igneous rocks was basalt (Dodge 1942). The sedimentary and igneous rocks were metamorphosed subsequently to become the phyllites, schists, and amphibolites present at the site. The Precambrian units that make up the stratigraphy of the SURF site are shown in Figure 1.

The geologic structure of the Homestake Mine was summarized by Morelli et al. (2010) who listed five deformational events, many of which were associated with large tectonic movements related to the assembly of this part of North America during the Proterozoic. The net result of these events was to produce complexly folded metasedimentary units cored by the mafic Yates Member amphibolite (Caddey et al. 1991).

Goals and Approach—The approach for calculating the effects of density differences in the geologic units consisted of constructing a geologic model of the structure based upon available information, estimating the densities along selected virtual drill holes, applying the average density and length of the virtual drill hole in the rock to convert the results to a depth equivalent attenuation model. A virtual drill hole is defined for the purpose of this study as being the predicted geology and associated densities if a hole were drilled at a selected location and orientation. In order to assess the effect of geology on the cosmic radiation attenuation, we created a family of paths that intersect the

Davis Campus through the cone of geology being considered. An example of two such paths is shown in Figure 2 (dotted lines). This information was then contoured to display the depth equivalent values.

Geologic Model--Calculation of the density distribution required the production of a geologic model for the subsurface in the vicinity of the site. The model was developed using the Maptek Vulcan mine design software package along with numerous other data sources. These included diamond drill core logs, review of the historical underground geology mapping (both the rough field mapping and the finalized inked maps available at the Homestake Adams Research and Cultural Center [HARCC], Deadwood, SD), underground on-site review of the mapping, and surface mapping (Redden et al. 2010). Other published sources that were particularly useful for the geological analysis included Dodge (1935; 1942), Nutsch (1989), and Caddey et al. (1991). Once the geologic data were assembled, reviewed and adjusted, geologic cross-sections were developed using the Vulcan software. Those portions of the geologic model that fit within a 45° cone centered on the Davis Campus were evaluated for the effect of density and topography (Figure 2).

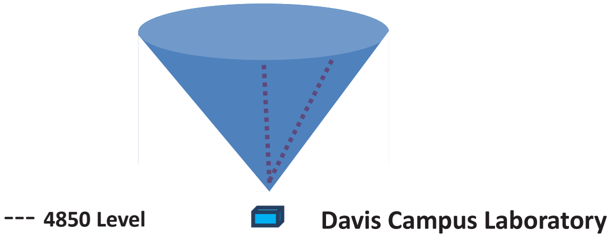


Figure 2. ***Diagrammatic sketch showing the cone centered over the Davis Campus at the 4850 Level. This is the cone that will contain the geologic model. Also shown are two virtual drill holes that were used to generate the water equivalent model.***

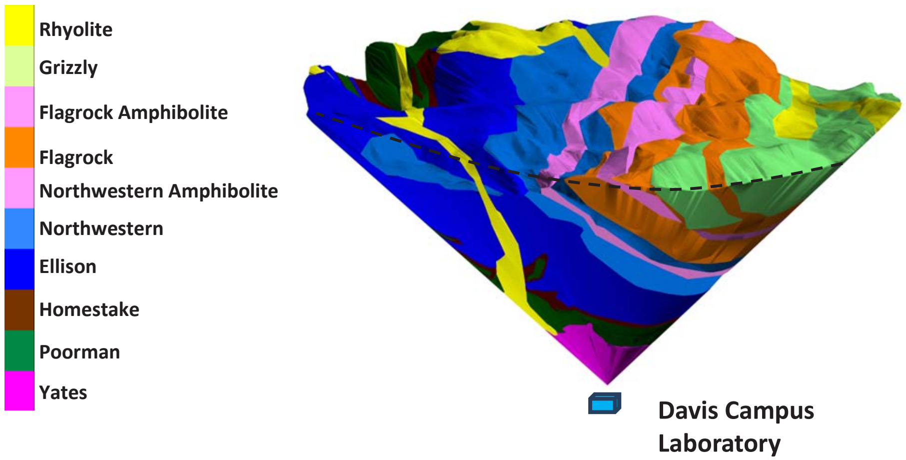


Figure 3. Geologic model within the 45° cone centered on the Davis Campus located at the 4850 Level.

Based upon the geologic cross sections and model, the cone of Figure 2 was populated with the developed geologic model (Figure 3). As in Figure 2, the cone is centered over the Davis Campus that is constructed on the 4850 Level of SURF. The cross-cutting relationship of the rhyolite (yellow) is displayed in the western part of the area.

Estimation of Densities**—**Although the geologic formations are well-defined mapping units, acquisition of representative samples for the determination of densities may be complicated by variations in lithologies within those geologic units. With the exception of the amphibolite units of the Yates Member of the Poorman Formation, the other metasedimentary units typically have varying proportions of schists, phyllites, and quartzites. The lack of matrix porosity simplifies the density modeling, however, and overall fracture porosity is limited as well. Jones (2010) reported that the rock on the 4850 Level trapped large bubbles of air in the roof of that level even though it was flooded to a depth of 97.5 m (320 ft) for over 16 months. This indicated that permeabilities, and, by inference porosities, were very small. Therefore, the densities are predominately due to the individual mineral densities and are not a function of porosity.

Table 1 lists the densities used to calculate the water equivalent depths. Nutsch (1989) measured 32 samples for density measurements and also referenced internal Homestake studies including 24 samples by Sumner (1965, HMC Report), and 18 samples by Mathisrud (1947, HMC Report). Density measurements were also acquired as part of the preliminary design for development of the laboratory (Lesko et al. 2011), which included primarily the Yates Unit, the upper part of the Poorman Formation, and rhyolites intruding the sequence on the 4850 Level. These are identified as RESPEC RSI in Table 1. All density values were given the same weight in this investigation even though the data were collected and determined by various authors over time.

Because no published values were available for the Northwestern, Flag Rock, or Grizzly Formations, density measurements were made as part of this study. Hole 17462 from the Homestake Core Archive was drilled from the 6800 Level in a generally southerly direction and penetrated all three of these units. Ten samples from this drill hole were measured from each of these three units and their average densities are included in Table 1. Surficial weathering and thin Cambrian sandstones that cover a small portion of the site were not considered due to their minor contributions.

In order to construct a representative volume based on a cone centered in the SURF underground based upon density distribution of lithologies and topography (Figure 2), we created virtual drill holes using the Vulcan software environment. These virtual drill holes consist of the lithologies and expected densities that would be encountered if the drill hole were drilled in the specified location and orientation. A family of virtual drill holes was created every 22.5° around the center overlying the Davis Campus. Within each of the 22.5° families a drill hole was created every 5° vertically. The average densities for each drill hole were then plotted at the point of intersection between the virtual drill hole and the horizontal plane. These values then were contoured as water equivalent depths (Fig. 4).

Table 1. ***Densities used for the calculation of water equivalent depths. Standard deviations are shown as ± for densities determined during this study (HMC refers to Homestake Mining Company reports and RSI refers to Report of Site Investigations).***

Formation	Density	References
Grizzly Formation	2.78 ± 0.06 g/cm^3	This study
Flagrock Formation	2.98 ± 0.15 g/cm^3	This study
Flagrock Amphibolite	2.98g/cm^3	Assumed to be same as Northwestern Amphibolite
Northwestern Formation	2.84 ± 0.05 g/cm^3	This study
Northwestern Amphibolite	2.98g/cm^3	Nutsch (1989)
Ellison Formation	2.73 g/cm^3	Mathisrud and Sumner (1967, HMC Report); Nutsch (1989); Bachman and Marlowe, HMC Memo (1991, HMC Report)
Homestake Formation	3.26 g/cm^3	Mathisrud and Sumner (1967, HMC Report)
Poorman Formation	2.86 g/cm^3	Mathisrud and Sumner (1967, HMC Report); Nutsch (1989); Bachman and Marlowe, HMC Memo (1991, HMC Report)
Yates Amphibolite	2.93 g/cm^3	Nutsch (1989); RESPEC RSI. May 2010; RESPEC RSI. June 2010
Rhyolite Dikes	2.54 g/cm^3	Nutsch (1989); RESPEC RSI. May 2010; RESPEC RSI. June 2010

Figure 4 shows the distribution of densities derived from the analysis of the virtual drill holes and the effect of density on the water equivalent depth. The most obvious feature in the plot of the densities in Figure 4a is a density low in the western area that is associated with a rhyolite dike swarm. Figure 4b shows the water equivalent depths using a density of 2.84 g/cm^3, which is the average value for rocks at the site weighted for volume and their respective densities. Because the density does not vary in this plot, the plot shows only the effects of topography. Figure 4c used the densities for the rock including the geologic model developed as part of this study. Figure 4d shows the difference between the contoured plots of Figure 4b (using an average density of 2.84 g/cm^3) and Figure 4b (using densities derived in this study).

Mining operations during gold production excavated extensive volumes of rock, and the resulting stopes and workings are well documented in the Vulcan database for the Laboratory. Although the total amount of the stoped volumes that were backfilled cannot be determined unambiguously, most were backfilled. The effects of the mined areas are included in the model of Figure 4c assuming a reduction of 22 percent from the average density of 2.84 g/cm^3. However, the volumes of rocks removed as part of the mining process affect only 0.5 percent of the volume of the cone, and the stoped volumes do not affect the plots significantly.

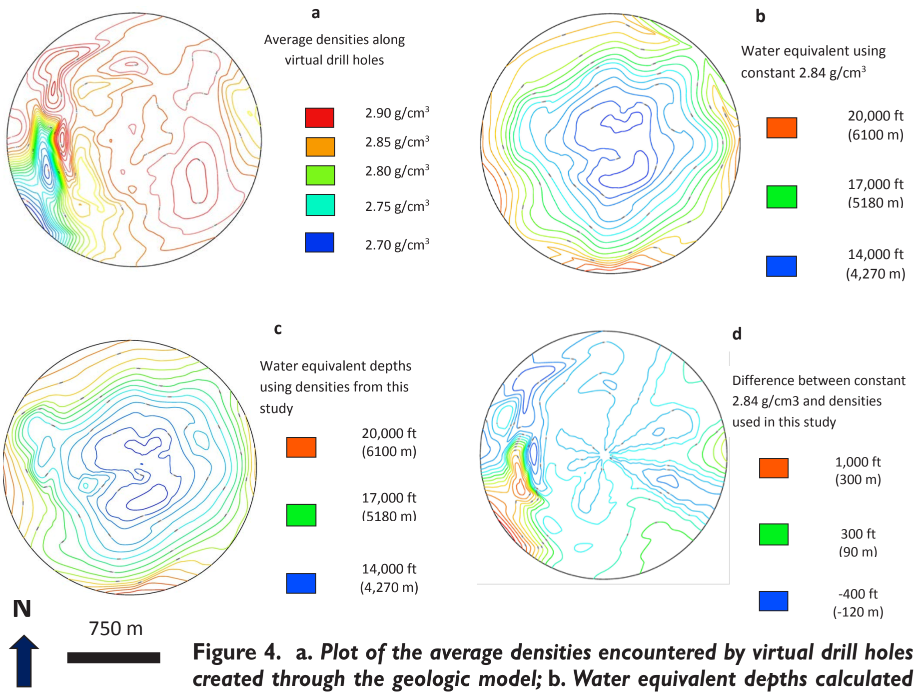


Figure 4. a. ***Plot of the average densities encountered by virtual drill holes created through the geologic model;*** **b.** ***Water equivalent depths calculated using an average density of 2.84 g/cm^3;*** **c.** ***Water equivalent depths calculated using densities derived during this study;*** **d.** ***Plot of the difference between the plots using an overall average density of 2.84 g/cm^3 and the densities used in this study.***

DISCUSSION

The geologic model from which the water equivalent depths were calculated relies heavily upon the availability of subsurface data such as mapping in the drifts and exploration drill holes. At greater distances from the underground workings direct information becomes less abundant and, therefore, the geologic model is more dependent upon surface mapping, such as the geologic map of the Lead Quadrangle by Redden et al. (2010), as well as cores and logging acquired as part of the exploration activities of the Homestake Mine. The dike swarms, for this model are assumed to be one hundred percent rhyolite in composition. However, these dike zones are not 100% rhyolite, but rather are a mixture of rhyolite and intruded host rock, which reduces the effects of the lower density of the rhyolite. Some smaller rhyolites may not be included in the model due to their apparently lesser extents and insufficient data to determine their locations accurately. These include the dike swarm within the west wall of the 4850 Davis Campus, the dike swarm east of the present 4850 Davis Campus, the dike swarm cutting through the Northwestern, and a dike swarm cutting through the Flag Rock. Additionally, the surface exposure of a rhyolite sill east of the Open Cut is not part of the model. The relatively thin phonolite intrusives that are oriented east west and which dip to the north also are not addressed in the model.

In addition to topography, the analysis presented here takes variations in rock density into account along with the angle at which incident muons would arrive at a laboratory located at the 4850 Level at SURF. The composition appears to be reasonably consistent within individual metasedimentary formations, which allows a limited number of density measurements to reflect the average density of the units. The one exception to this observation occurs in Flagrock Formation, which has more pyrite-rich intervals resulting in higher densities in those short sections. Table 1 shows that the standard deviation for the Flagrock is over twice that of the adjacent Grizzly and Northwestern Formations as a result of this variation in composition. Metamorphic grade changes within the limits of the mined underground which may also have some effect on the overall density pattern.

Figure 4a shows the plot of the average densities encountered by the virtual drill holes. In general, the densities are uniform in the eastern portion of the area with greater differences in the west. This is due primarily to the influence of the rhyolite dike system. Figures 4b, 4c, and 4d show that the greatest effect on the water equivalent depth is due to variations in topography. This point is emphasized by Figure 4d which shows the difference between using an overall average density of 2.84 g/cm^3 and the use of identified formation densities along with the geologic structure. Differences do exist but their effects are not pronounced with the possible exception of the rhyolite intrusions in the western portion of the plot. These results are consistent with the observation that, in general, large variations in density in the Precambrian crystalline rocks do not occur with the exception of the Homestake Fm. (Table 1), which has a significantly greater density than the other crystalline rocks. This unit, however, is volumetrically small and does not appear to have a significant effect. Although some areas of the Homestake underground hosted large stopes where ore was mined, most were backfilled. They are volumetrically insignificant (< 0.5 percent of the volume of the cone) and affect the water equivalent calculations only slightly.

The geologic model shown in the cone centered on the Davis Laboratory is the most detailed approximation of the geology in the vicinity of SURF currently available. As shown in Table 1, in general, the rocks at SURF tend to have a density slightly less than three times that of water. Based upon the water equivalent contoured values, topography is the primary factor affecting the water equivalent depths for different paths. The rhyolite intrusions have the next most important effect although their influence is limited to the western area of the cone of investigation.

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THE FIRST RECORD OF *OSBORNODON* (CARNIVORA: CANIDAE) FROM THE ORELLAN OF SOUTH DAKOTA

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ABSTRACT

During the 2013 summer season, a visitor at Badlands National Park discovered an *in situ* fossil dog skull, and reported the location to park rangers, following in correspondence with the park's Visitor Site Report program. The skull was fortuitously left at its locality so the contextual data could be properly collected. The discovery came from a fairly fossiliferous limestone in the upper Scenic Member of the Brule Formation, Jackson County, South Dakota. Unofficially known as the Abbey mudstone, this unit lies within the *Merycoidodon bullatus* zone and is thus within the fourth and latest subdivision of the Orellan North American Land Mammal Age. After preparation, the specimen was identified as the rare hesperocyonine canid, *Osbornodon renjiei*. This diagnosis is based primarily on the elongated molars and a ridged entoconid forming a basined talonid on the m1. This specimen of *O. renjiei* possesses the most complete dentition of any specimen yet reported in the literature. Previously known occurrences of *O. renjiei* comprise four specimens from the Orellan of North Dakota, a single Whitneyan occurrence in Nebraska, and two specimens, previously attributed to the closely related taxon *Cynodictis* (synonymized with "*Mesocyon*") *temnodon* in the Whitneyan "*Protoceras* channels" of South Dakota. The Badlands National Park specimen herein represents a new stratigraphic record in South Dakota, contributing a new faunal constituent to the local Late Orellan fauna as well as further understanding of the biochronology of the South Dakota Big Badlands paleobiogeographic region.

Keywords

Badlands, Brule, Canidae, Orellan, Osbornodon

INTRODUCTION

The White River Badlands of the Great Plains are some of the richest fossil bearing units in North America. Significant paleontological discoveries have been made in South Dakota since the mid-1800's and continue today (e.g. Leidy 1856; Boyd and Welsh 2014). The unique geology and rich fossil resources were major justifications for the establishment of Badlands National Monument in 1939 and accession of the South Unit for Badlands National Park in 1978. Badlands National Park has received around 900,000 visitors annually since 2005. Many of these visitors exercise the park's open hiking policy, which facilitates nearly complete access to all areas within the park. This policy results in visitors directly interacting with geologic and paleontological resources. Because of this, the Visitor Site Report (VSR) program was established. VSR's are an invaluable resource to educate the public on how fossil resources are managed in the park as well as to provide opportunities for visitor participation in the science through discovery. These VSRs have resulted in several significant fossil discoveries in the park, and has proved to be a vital tool in the management of fossil resources.

One particular VSR from the early part of the summer 2013 visitor season resulted in the collection of a small caniform carnivore skull within the upper portion of the Scenic Member, Brule Formation. On-site inspection showed that the skull was too large for *Hesperocyon*, but also too small for the next most common caniform, *Daphoenus* (Amphicyonidae). Significance was evaluated on the potential of this specimen being a poorly known canid, such as *Mesocyon*, or a small amphicyonid, exemplified by *Daphoenictus* or *Paradaphoenus*. The fossil was found as float in a frequently visited area, so the threat of potentially destructive impact or theft provided further justification to collect. After collection and subsequent preparation at the on-site preparation laboratory, the specimen was identified as a new geographic and stratigraphic occurrence of the rare canid *Osbornodon renjiei*.

Previous taxonomic understandings of White River Group Canidae has been somewhat convoluted, particularly with *Hesperocyon* (*see* Wang 1994). Recent studies, grounded in modern techniques, of fossil and modern canids have provided major resolution to historically confusing taxonomy (Wang 1994; Wang et al. 1999; Tedford et al. 2009).

The oldest occurrence of canids begins with *Hesperocyon gregarius* in the mid-Duchesnian Lac Pelletier Lower Fauna, Cypress Hills Formation, Saskatchewan (Bryant 1992). Though Wang (1994) refers to *Prohesperocyon* as the only co-occurring canid with *H. gregarius* in the Chadronian, and possible progenitor to Canidae, later phylogenetic analysis placed *Prohesperocyon* within the Miacidae (Wesley-Hunt and Flynn 2005; Wesley-Hunt and Werdelin 2005). Hesperocyonines diversified slightly through the Orellan and Whitneyan, but *H. gregarius* remains the dominant canid component of the White River Chronofauna (Wang 1994). Three subfamilies occur throughout the White River Chronofauna: Hesperocyoninae, Borophaginae, and Caninae. Borophagines and canines are collectively known from only three specimens in correlative units containing Orellan faunas (Wang et al. 1999; Tedford et al. 2009). Hesperocyonines greatly diversified throughout the Arikareean, then were reduced to extinction in the early Barstovian (Wang 1994). The Borophaginae appeared in the Orellan, diversified dramatically in the Arikareean and throughout the Miocene until their extinction in the Blancan (Wang et al. 1999). The third subfamily, the Caninae, with the only White River Group occurrence in the Orellan, held a low diversity through the rest of the Oligocene and Early Miocene, followed by increasing diversification starting at the Middle Miocene and continuing into the Recent (Tedford et al. 2009).

In the Scenic Member of the Brule Formation of South Dakota, typically attributed to Orellan faunas, the canid record is dominated by *Hesperocyon*. Several hundred specimens found throughout the Great Plains region are held in various museum collections (see Appendix I in Wang 1994). Occurrences of other canids during this lithologic interval and biostratigraphic zonation in South Dakota are limited to a single occurrence of another hesperocyonine, "*Mesocyon*" *temnodon* (SDSM 2653). Elsewhere, in coeval sites, non-*Hesperocyon* specimens are still limited to a partial dentary of the canine *Leptocyon* from Nebraska, four specimens of *Osbornodon renjiei* from North Dakota, and thirteen specimens of "*Hesperocyon*" *coloradensis* from Colorado (Tedford et al. 2009; Wang 1994). Any additional information into the early diversification and dispersals of canids would be beneficial, given the limited diversity previously recorded.

GEOLOGIC AND BIOCHRONOLOGIC SETTING

The specimen described herein originated in the upper Scenic Member of the Brule Formation, preserved in a carbonate nodule from the informally designated Abbey mudstones in the upper-middle Scenic sandstone interval (Figure 1A; Evanoff et al. 2010).

Another locality, also situated in the Abbey mudstones, provides further insight on biostratigraphic zonation. BADL-LOC-00593, also known as the "Saber-site", has produced a multitaxic fauna of the Late Orellan. Although most taxa recovered have somewhat expansive ranges in the White River Group, the presence of *Merycoidodon bullatus* (BADL 16877; Artiodactyla: Merycoidodontidae) and a yet to be determined species *Ischyromys* (BADL 59711; Rodentia: Ischyromidae) mark the fourth and latest Orellan subdivision. The "*Merycoidodon bullatus*" zone (Prothero and Emry 2004), or Or4 (Janis et al. 2008), is characterized by the first appearance of *M. bullatus* and the last appearance of *Ischyromys*, specifically *I. typus* (Prothero and Emry 2004). However, Heaton (1993) and Simpson (1985) collectively identify 5 specimens of *Ischyromys* from the lowermost Poleslide and Whitney members of South Dakota and Nebraska, which are typically associated with Whitneyan faunas. These Whitneyan occurrences of *Ischyromys* were not recognized in succeeding publications characterizing biostratigraphic subdivisions (Prothero and Whitlessey 1998; Prothero and Emry 2004). The Whitneyan occurrences were not explicitly invalidated by Prothero and Whitlessey (1998), but Anderson (2008) recognizes early and late Whitneyan occurrences of the genus. Considering that *Ischyromys* likely occurs in the Whitneyan, a Whitneyan fauna has yet to be confirmed within any horizon within the Scenic Member. Though the biochronologic assessment is tentative and requires further biostratigraphic study, it is most likely that this new record of *Osbornodon* is within the "*Merycoidodon*" *bullatus* zone (Or4).

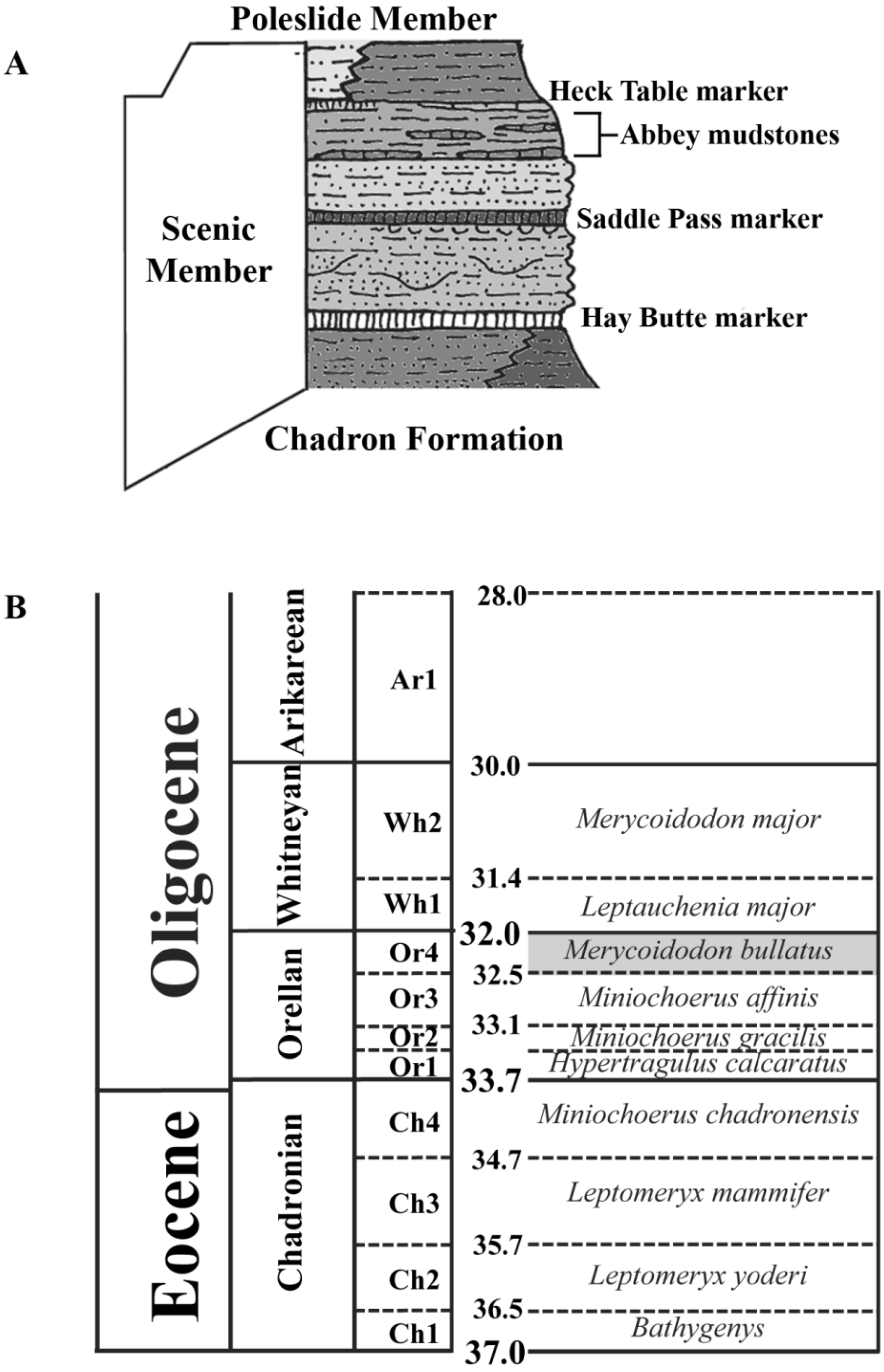

Figure 1. Diagram displaying the stratigraphic (A; modified from Evanoff et al, 2010) and biostratigraphic (B) zones of BADL 63382.

INSTITUTIONAL ABBREVIATIONS

AMNH, American Museum of Natural History; F:AM, Frick collection, American Museum of Natural History; BADL, Badlands National Park; SDSM, South Dakota School of Mines and Technology Museum of Geology.

SYSTEMATIC PALEONTOLOGY

Class MAMMALIA Linnaeus 1785
Order CARNIVORA Bowdich 1821
Suborder CANIFORMIA Kretzoi 1943
Family CANIDAE Gray 1821
Subfamily HESPEROCYONINAE Tedford 1978
Osbornodon Wang 1994
Osbornodon renjiei Wang 1994
?*Cynodictis* sp. maj. Matthew 1899: 54 (AMNH 1382 and 1386)
?*Cynodictis temnodon* Wortman and Matthew 1899: 130 (in part as above)
Cynodictis temnodon (Wortman and Matthew) Matthew 1901: 370 (in part as above)
Procynodictis temnodon (Wortman and Matthew) Matthew 1918: 189 (in part as above)
Pseudocynodictis sp. Hough 1948a: 97
Osbornodon renjiei Wang 1994: 114
Osbornodon renjiei Wang and Tedford 1996: 441

Referred Specimen—BADL 63382, slightly crushed anterior portion of skull with complete upper dentition excluding the right P1, left and right dentaries with complete lower dentition excluding the incisors and left p1 (Table 1, Figure 2).

Locality and Horizon—North Unit Badlands National Park, Jackson County, South Dakota (Figure 3). Abbey Mudstone, Scenic Member, Brule Formation, White River Group (Orellan). Specific site information is protected under federal law. Researchers who have an appropriate purpose to inquire further should contact the on-staff Paleontologist at Badlands National Park.

Description—The skull of BADL 63382 is slightly crushed, showing no distortion. The most diagnostic features of *Osbornodon* are in the basicranial region, but part of the cranium is missing in BADL 63382. However, taxonomic assessment is possible due to the specimen's near complete dentition. Two of the distinguishing synapomorphies of *Osbornodon*, provided by Wang (1994), are present in this specimen: 1) The molars are more quadrate and the M2/m2 are enlarged just slightly more than what is observed in *Mesocyon*; 2) the talonids of m1-m2 are basined.

The fracturing of the skull makes it difficult to determine the profile of the premaxilla, maxilla, and frontals. It is also difficult to gauge frontal inflation, if any. However, the muzzle is not elongated as in more derived species of *Osbornodon* and more similar to basal Hesperocyoninae, such as *Hesperocyon* (see Wang 1994). This character is also shared with *O. sesnoni* (Wang 1994). The infraorbital foramina are also primitively rounded, as is also observed in *O. sesnoni* (Wang 1994).

BADL 63382 is the only described specimen of *Osbornodon* with incisors preserved. Both I1-2 have central cusps with two lateral accessory cusps. I3 is much larger than the other incisors, with a medial accessory cusp; there is a bridge on the posterior margin of the I3 with a slight bulge along the base of the bridge,

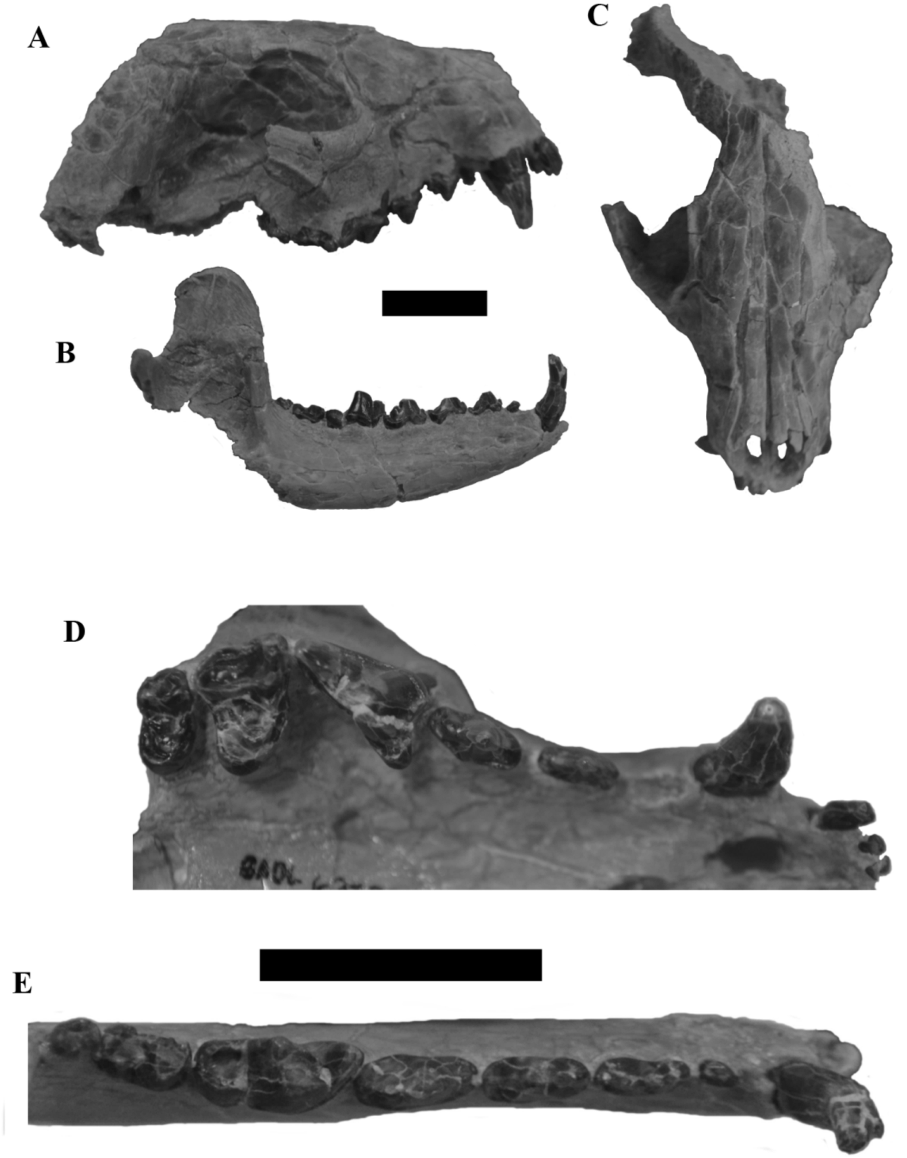

Figure 2. Views of BADL 63382: Skull in right lateral view (A); dentary in right lateral view (B); skull in dorsal view (C); right maxillary tooth row in occlusal view (D); right dentary tooth row in occlusal view (E). Scale bars equal 2 cm.

Table 1. Measurements of BADL 63382 (all measurements are in mm).

	I1	**I2**	**I3**	**C**	**P1**	**P2**	**P3**	**P4**	**M1**	**M2**
Length	2.09	2.18	2.45	6.44	3.4	6.4	7.66	10.78	7.93	4.68
Width	1.2	1.34	3.22	4.16	2.1	2.5	3.68	7.59	11.37	8.2
				Height=13.48						

	i1	**i2**	**i3**	**c**	**p1**	**p2**	**p3**	**p4**	**m1**
Length	N/A	N/A	N/A	5.9	2.69	6.57	7.55	8.53	11.7
Width	N/A	N/A	N/A	4.2	1.88	2.94	3.31	3.81	5.19
				Height=12.41					

	m1 tgd	**m1 tld**	**m2**	**m2 tgd**	**m2 tld**	**m3**	**m3 tgd**	**m3 tld**
	6.81	4.51	6.6	3.3	3.54	3.4	1.65	1.55
	5.41	4.74	3.95	3.9	3.77	2.84	2.62	2.59

Length at Nasals	48.93	Depth of ramus at m1	15.52
Width at infraorbital foramen	27.86	Depth of ramus at m2	16.27
Depth of jugal at the orbit	9.83	Depth at angular process	48.8
Width at frontal process	28.76	Width of coronoid	17.15
Length C-M2	49.26	Diamter of the orbit (dorso-ventral)	19.65
Length I-M2	57.94	Length of incisors to anterior orbit	42.89
Length c-m2	51.88	Width across C's	22.7
Length c-m3	56.16	Width across I's	13.32
		Width across upper carnassials	39.57

but no noticeable presence of a lingual cuspule. C is somewhat conical with anteromedial and posterior ridges. P1 is single rooted and cusped. P2 and P3 contain an incipient posterior cusp on the cingulum. The P4 has a large and anteriorly positioned protocone, comparable to what has been observed in Orellan specimens of *O. renjiei* from North Dakota and unlike Whitneyan occurrences of the species (Wang 1994). The molars are antero-posteriorly lengthened, particularly along the margin of the protocone to metaconule, and have become more quadrate with a wider trigon on the M1. The M2 is slightly larger than what has been measured in "*Mesocyon*" *temnodon*.

The lower canine contains anterior and posterior ridges that are directed medially. The p1 is simple and single rooted. The p2 has anterior and posterior cingular cusps. The p3 has a vague posterior accessory cusp, more similar to what has been observed in F:AM 63963 (see Wang 1994). The p4 has a well-developed posterior accessory cusp. The m1 trigonid possesses a basined talonid, which is formed from a ridged entoconid. The entoconid rises to the height of the hypoconid. The hypoconid is notably larger than the entoconid, as is characterized in other hesperocyonine canids. The m1 hypoconid is also moderately

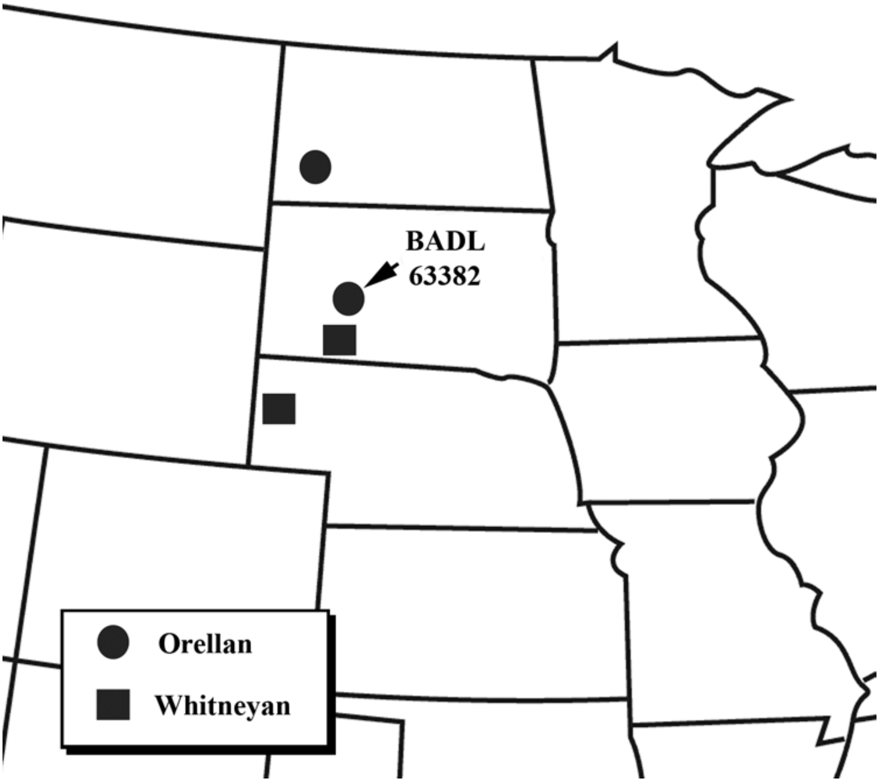

Figure 3. The known occurrences of Osbornodon renjiei, with legend marking biostratigraphic occurrences. New occurrence from this report (BADL 63382) is marked above. It should also be noted that the known occurrences of O. sesnoni are within the same vicinity as the Whitneyan occurrence of O. renjiei in South Dakota.

worn, suggesting that the hypoconid was originally slightly taller than the entoconid. This differs from borophagines and canines, which have cuspate entoconid enlarged to matching size of the hypoconid, and a transverse ridge between the hypoconid and entoconid, forming a basined talonid. The m2 contains an anteriorly positioned protoconid-metaconid ridge with a reduced paraconid. The m3 still retains the protoconid and metaconid with a faint transverse ridge between the two. The m3 trigonid is missing the paraconid, and only contains an anterior ridge. The m3 talonid is enlarged, relative to a reduced trigonid.

CONCLUSION

BADL 63382 represents a new occurrence of *Osbornodon renjiei* in the Latest Orellan "*Merycoidodon bullatus* zone" (Or4) of South Dakota, supported by a correlative fauna in the same lithologic unit. This study serves as a report of a new record which happens to adhere to the current biostratigraphic and biogeographic information provided by Wang (1994). This new record demonstrates a slightly wider biogeographic range during the Latest Orellan, before its subsequent dispersal further south in the Great Plains.

Osbornodon is the longest lived genera within the Hesperocyoninae, spanning approximately 18 million years from the Late Orellan to the Early Barstovian. This would also be true within the Canidae, with the exception of the Orellan to Clarendonian range (~29 million years) of the canine *Leptocyon* (Tedford et al. 2009). The record of *Osbornodon* begins with *O. renjiei* from the late Orellan Fitterer Ranch Locality of North Dakota (Wang 1994). The current record extends the Orellan range of *O. renjiei* in South Dakota. *Osbornodon renjiei* and *O. sesnoni* have been defined in Whitneyan localities in South Dakota and Nebraska, inferring the earliest emigration and diversification of *Osbornodon* (Wang 1994). Both species disappeared in the Whitneyan, in near correspondence with the overturn of most components of the White River Chronofauna of the Whitneyan-Arikareean transition. There is a brief apparent absence of *Osbornodon* in the Early early Arikareean (Tedford et al. 2004; [Ar1] Janis et al. 2008), then a sudden appearance of *O. wangi* from the middle of the Late early Arikareean (Tedford et al. 2004; [Ar2] Janis et al. 2008) Brooksville 2 Local Fauna, Florida (Hayes 2000). There is a near 8 million year hiatus of the taxon's fossil record from the Late early Arikareean until the genus reappears in the Late Arikareean with *O. iamonensis* and *O. brachypus* from Nebraska and Wyoming respectively, with *O. iamonensis* continuing into the Hemingfordian while extending its range to Florida. Dispersal and diversity continue with *O. scitulus* in the Early Hemingfordian of Nebraska, Texas, and Florida (Wang 2003). The last occurrence of the genus, *O. fricki*, is from Early Barstovian sites in Nebraska, California, and New Mexico (Wang 1994).

ACKNOWLEDGEMENTS

I thank Rachel Benton and Megan Cherry at Badlands National Park for access to specimens as well as providing the permission to collect this new *Osbornodon*. I also thank Levi Moxness, one of the fellow seasonal staff, who helped me collect the specimen and associated field data. Gratitude is also extended to Mindy Householder, who did a wonderful job preparing the specimen. Many thanks to Emmett Evanoff (University of Norther Colorado, Greeley) who has done tremendous work and has provided a better understanding of the White River Group. Thanks again to Xiaoming Wang (Los Angeles County Museum of Natural History) and Andy Farke (Raymond M. Alf Museum of Paleontology) for their useful comments and advice. Last, but not least, I give my utmost appreciation to the visitors who report their fossil discoveries while maintaining a respectful attitude for site preservation.

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A CLARENDONIAN OCCURRENCE OF THE FOSSIL INSECTIVORE, *PLESIOSOREX* (MAMMALIA), FROM WASHINGTON

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ABSTRACT

A specimen of *Plesiosorex* was recently discovered by Kevin Meeks from the Granger Clay Pit, Yakima County, Washington, from sediments of the Ellensburg Formation which were deposited during the Clarendonian North American Land Mammal Age (NALMA). This unusual insectivore had been found previously from Miocene rocks, but herein, is recorded its first known occurrence from Washington, based on a right lower jaw fragment with the first molar. The specimen is larger than the known Clarendonian species, *Plesiosorex latidens*, but smaller than the Barstovian species, *Plesiosorex donroosai*.

Keywords

Plesiosorex, Insectivore, Fossil, Clarendonian, Washington

INTRODUCTION

Plesiosorex was recently discussed (Martin, 2012), and three North American species from the Miocene were recognized: *P. coloradensis*, *P. donroosai*, and *P. latidens*. In North America, *Plesiosorex* appeared during the early Hemingfordian NALMA and persisted into the late Hemphillian NALMA (Martin 2012). Its distribution is widespread with the Hemingfordian species, *P. coloradensis*, having been found in Colorado (Wilson 1960), South Dakota (Martin 1976), Idaho (Tedrow and Martin 1988), Nebraska (Bailey 2004), and Delaware (Emry and Eshelman 1998). Occurrences of *Plesiosorex*, *P. donroosai*, have been described from Barstovian deposits in South Dakota (Green 1977; Martin 1987, 2012; Pagnac 2012), Nebraska (Voorhies 1990), and Oregon (Shotwell 1968; Martin 2012). The youngest recognized species, *P. latidens* (originally termed *Meterix latidens*), has been recovered from Clarendonian rocks in Nevada (Hall 1929; Green 1977), Nebraska (Voorhies 1969; Martin 2012), and Oregon (Shotwell 1968; Martin 2012). Hemphillian specimens from Nebraska (Voorhies 1990) have not yet been assigned to species. Based upon the new discovery of the specimen discussed in this contribution, the range of *Plesiosorex* is extended northwesterly into the state of Washington.

This specimen was derived from the Granger Clay Pit, a locality in the Ellensburg Formation of central Washington. The Ellensburg Formation at this locality occurs suprajacent to the Elephant Mountain Basalt, which has been dated at 10.5 Ma (See Madin 2009; Smith et al. 2013). The Ellensburg Formation at the Granger Clay Pit was dated as *ca.* 10.3 Ma by Smith (1988). These dates are consistent with correlative dates based on mammals from the Clarendonian NALMA (Martin and Mallory 2011).

Lipotyphla Haeckel, 1866
Plesiosoricidae Winge, 1917
Plesiosorex Pomel, 1848
Plesiosorex latidens (Hall 1929)
Plesiosorex sp. cf. *P. latidens*

Referred Specimen: University of Louisiana Geology Museum, Lafayette (ULGM) V3485, right dentary with m1 from ULGM locality V-19.

Description: ULGM V3485 (Fig. 1) consists of a right dentary, broken through the alveoli of p4 and just anterior to the coronoid process. The dentary is shallow (3.35 mm below the anterior root of m1) and slender (2.5 mm wide below the anterior root of m1). The posterior end of the symphyseal scar indicates the symphysis extended posteriorly to a point below the anterior portion of the m1 as is typical of *Plesiosorex*. A centrally positioned mental foramen lies below the posterior moiety of the m1. A robust anterior margin of the coronoid process remains, although the remainder of the ascending ramus is missing. The only tooth remaining in the dentary is the m1, which is large (length=4.75 mm; maximum width=2.75 mm) and unworn with very tall, slender, cusps. The trigonid is higher than the talonid, delicately constructed with pointed cusps connected by sharp crests. The paraconid and metaconid are conical, and a small parastylid-like cuspule lies at the anterior base of the paraconid that would interlock with the posterior edge of the p4. The paraconid-protoconid crest exhibits a distinct notch that extends down a third of the crown length. The crest from the protoconid to the metaconid is much higher, but also notched. The trigonid is deeply basined and widely open lingually to the same depth as the talonid valley between the metaconid and entoconid. A small cuspule occurs at the posterior base of the metaconid. The conical entoconid lies at the posterolingual tooth corner and is higher than the hypoconid. The posterior crest connecting to the hypoconid is also deeply notched. A minute hypoconulid lies medially on the crest. The hypoconid, like the protoconid, is V-shaped, and the cristid obliqua angles anteriorly to a point half the height of the protoconid. No cingulids occur on m1, and the labial enamel faces of the protoconid and hypoconid exhibit slight crenulations. Two alveoli each remain for the m2-m3, and the posterior alveolus of p4 is large, indicating that this tooth was likewise large and robust. The alveolar length of m1-m3 is 9.55 mm, intermediate between the measurements by Green (1977) of *P. coloradensis* (alveolar length = 8.5 mm) and *P. donroosai* (alveolar length = 11.8 mm); most measurements of *P. latidens* are also intermediate (See Green 1977).

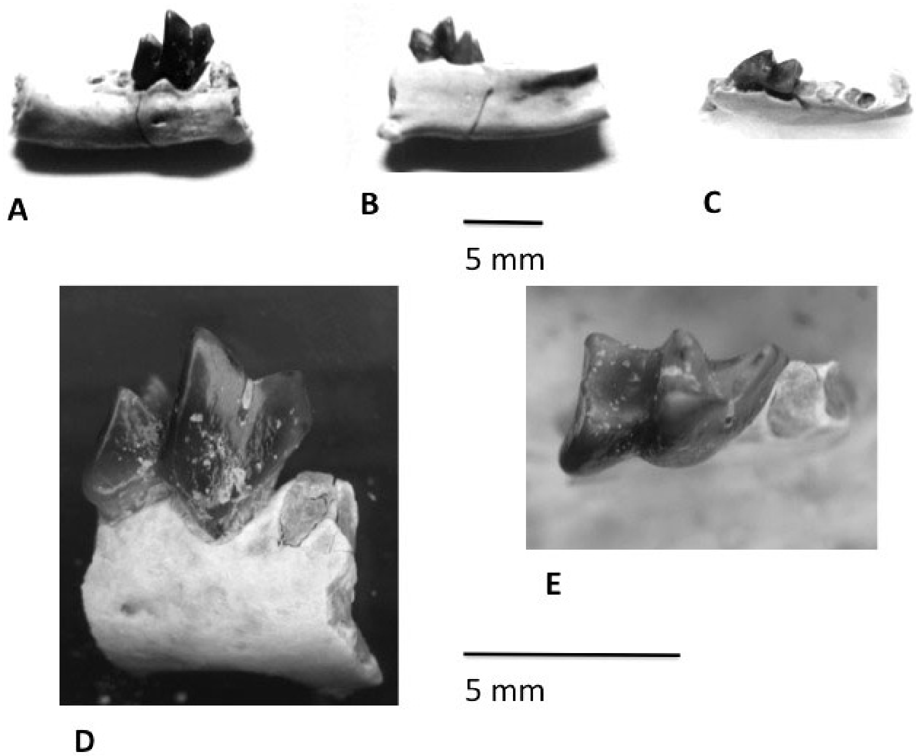

Figure 1. **Plesiosorex** ***sp. cf.*** **P. latidens,** ***ULGM 3485 A) right dentary, buccal view; B) lingual view; C) occlusal view; D) m1, buccal view; E) occlusal view.***

DISCUSSION

ULGM 3485 is slightly larger than most specimens of *Plesiosorex latidens* but distinctly smaller than *Plesiosorex donroosai.* Based on a single specimen, the insectivore from the Granger Clay Pit is provisionally referred to *P. latidens.*

ULGM 3485 represents the first known occurrence of the enigmatic insectivore, *Plesiosorex*, from the state of Washington. The specimen was recovered from the Granger Clay Pit, from sediments deposited during the Clarendonian NALMA (Martin and Mallory 2011; Smith et al. 2013). The occurrence in Washington extends the known paleogeographic range of *Plesiosorex* and represents its most northerly known occurrence in North America.

ACKNOWLEDGMENTS

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VARIABILITY IN MICRO-COMPUTED TOMOGRAPHY IMAGES FOR NON-DESTRUCTIVE EVALUATION OF FRICTION-STIR WELD QUALITY

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ABSTRACT

Micro-computed tomography (MCT) provides a tool for creating three-dimensional images of a sample by combining a series of two-dimensional "slices" of the object. This capability has led to an increasing number of applications of MCT data in research, including non-destructive evaluation and three-dimensional reconstructions of objects. However, a lack of standardization in the imaging process may lead to inconsistent results if the researcher is not aware of the factors which impact the resolution, detail, and density accuracy of the scan. This work addresses the variability present in the Xradia MCT-400 data and suggests techniques for controlling that variability. Sample preparation, including size, placement, and composition, imaging parameters such as threshold values, x-ray sample counts, scan resolution, and conversion of the output format for subsequent use of the data are discussed in the context of non-destructive evaluation of weld quality for friction stir welding of aluminum, but are universal for all applications. This work was performed in part during the Research Experience for Undergraduates (REU) hosted at SDSM&T in the summer of 2013.

Keywords

Micro-computed tomography, X-Ray reconstruction, Friction stir weld evaluation

INTRODUCTION

Research utilizing micro-computed tomography (MCT) has become extensive and diverse in recent years. Applications of these systems can be found in biomedical, computational, materials and geologic sciences, but all have encountered the same problem: lack of standardization regarding setup, imaging, and post-processing. The unfortunate result of this is that research conducted at different locations typically generates different results and reduces or eliminates the chances of repeatable accurate imaging.

To assist in a multi-disciplinary usage standard for the research conducted with MCT, we address the key components of the MCT process: mechanism of imaging, post-imaging / postrendering editing, and reconstruction of images while discussing the variabilities that exist at each stage of this process.

Non-destructive evaluation of friction stir weld samples is a very important result of this standardized technique [2]. The process itself includes moving a rotating pin-tool between two pieces of materials to create a very high quality bond or joint [2]. Friction stir welding can produce hidden defects within a weld, called wormholes. A structurally unsound joint can go undetected and result in dangerous consequences should it ever be employed in high-strain applications [2]. The results of a defect-free weld yield some of the highest strength bonds in homogeneous and heterogeneous materials fusing.

METHODS AND RESULTS

Imaging—Understanding the parameters that are essential to the imaging stage is the simplest way to create a standard for research that is repeatable for any imaging process. Each of the following parameters comprises a piece of the full tomographical recipe. By adhering to precise measurements while setting up a recipe, different researchers and facilities will be able to duplicate images and validate measurements.

The main imaging component behind MCT is the X-ray system which consists of the X-ray source and the image-processing detector (see Figure 1 as taken from South Dakota School of Mines and Technology's (SDSM&T) Xradia Micro-CT 400). As with all X-ray images, materials being imaged are rendered as a variance of relative densities within either the material or the surrounding air. The image is subject to detail changes depending on the distances of the source to the

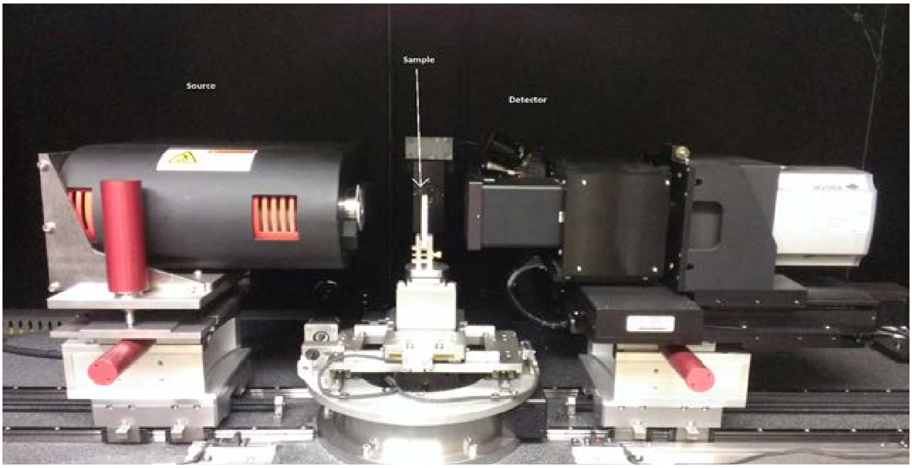

Figure 1. Xradia XCT-400 components.

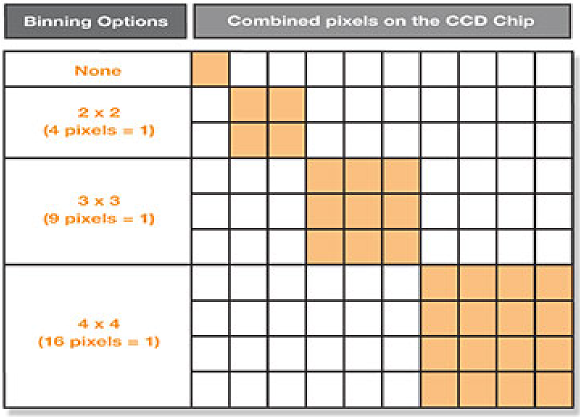

Figure 2. Example of binning and resolution impact [4].

sample and the sample to the detector; the behavior is analogous to a flashlight shining on any object and the resulting shadow being cast against some flat surface. This is due to the conic and divergent beam emitted from the X-ray source and the distance to the detector. As the distance from the source (focused with a filter or otherwise) to the sample is increased, a more precise representation of the material is attained as most divergent X-rays miss the detector. As the detector's distance from the sample is increased, the sample becomes artificially enlarged which will reduce resolution but can provide a more detailed view of fields of interest. The particular sample in Figure 1 is a piece of a friction stir weld. Larger samples can be run intact to avoid unnecessary damage to a finished joint.

Nearly all MCT devices will have different size capacities, resolutions, and distance options for placement of source, detector and sample. After an image has been calibrated for tomography on any particular machine, the manufacturers of MCT devices propose that the standard for resulting calibration be concisely defined by voxel size, or cubic pixel measurement.

An additional parameter of standard X-ray imaging lies in the use of binning. Binning is a technique used with difficult-to-scan materials such as highly-dense alloys or large samples with defractive properties that can obscure the detection. It works by artificially boosting the count of X-ray particles detected in the imaging process by doing neighborhood sampling and averaging of surrounding pixels in the image with the current pixel (illustrated in Figure 2) [4]. This results in a less-clear image and reduced resolution but is sometimes needed for otherwise un-scanable materials.

MCT differs from standard X-ray imaging in that the sample is not statically fixed once the source and detector have been configured. With MCT, as with medical CT-scans, the source and detector are rotated about the sample as seen in Figure 3. With most non-medical MCT machines, such as SDSM&T's, the sample is rotated throughout the scan.

This results in two-dimensional images, called slices and, when taken at small enough rotational adjustments, can be fed through a back-projection algorithm to reconstruct three-dimensional digital representations of the material [3].

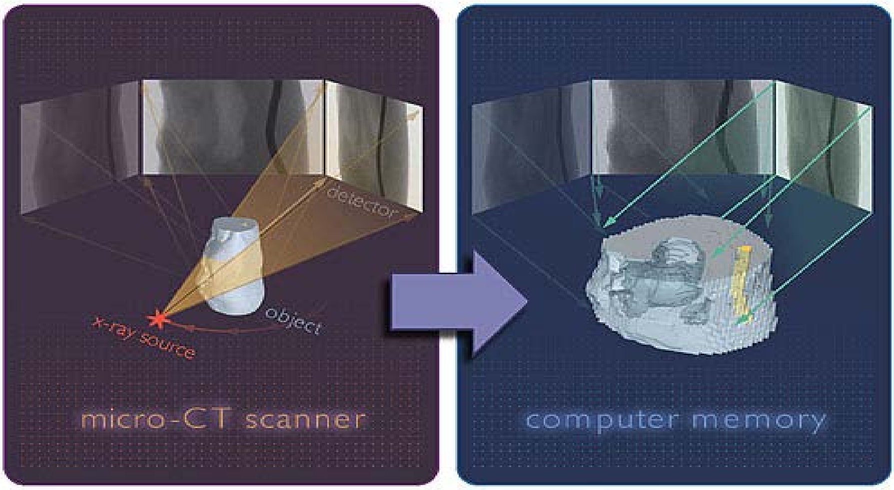

Figure 3. Imaging and projection of MCT [3].

Further, linear slice traversals through the material at each unit of resolution can be produced by layering the transposed images. Back-projection starts with a masked area to be determined either by system or user to specify the material of interest and omit all other data. This process is then used to construct a histogram of each image as captured by the MCT while rotating the sample and normalizing the changes. Finally, the images are correlated based on the rotation and distance information known at the time of imaging; this results in a finished tomography [3]. At the stage of creating a tomograph it is crucial to report the source's exposure time length, power settings, filters being used, number of reference images (pictures of no sample during the tomography for calibration during the sample scan), angles rotated and number of images.

Without consideration for power, distance, exposure and angle configuration, it is nearly impossible to produce the same image from the same sample. An example of faulty results can be found in the research done on behalf of a third-party consultant at SDSM&T who wanted to investigate talc dispersion across a polymer surface. The XCT-400 was built with an anticipated resolution of 20 μm for a voxel, but was teased to 0.26 μm by adjusting the distances between source and detector. When computing the average size of talc particles and volume percentage dispersed across a sample of polymer, we found that varying these parameters could yield discrepancies of up to ten times each other's measurements.

Another example of poor calibration can be seen in friction stir welding wormhole defect analysis. In such a case, using the incorrect power settings and filters can produce large ringing artifacts that obscure the image. Though nearly any quality of scan can be used to detect the presence of large defects, smaller defects and their characteristics can go unnoticed or misrepresented when X-ray diffractions are captured by the image processor. In most cases these defects (easily seen along the side of Figures 4 and 5) can help fill in the pores or wormholes caused by a bad friction stir process.

Most Micro-Computed Tomography systems provide a way to save a digital copy of the recipe for later use. We recommend securing a digital recipe and manually validating voxel size before proceeding to any post rendering.

Post-Imaging and Touch-up Rendering—Different MCT machines have different standard output tomography data formats. We recommend that the output be specified as either the format made by Digital Imaging and Communications in Medicine (DICOM) or as layered png images. This will enable more cross-platform cooperation; Avizo Fire 8 by Visualization Science Group, which is the software of choice for SDSM&T, will handle both of these formats as well as the proprietary txm and txm-exm formats from the XCT-400. When selecting the data format to be evaluated, the operator should be aware that some are compressed, such as the txm-exm format, and may lose image quality and any measurements kept by the MCT machine during tomography renderings. Furthermore, many detailed and multiple-framed tomographies will be quite large. The larger data set, without any software support for formats larger than the rendering computer's memory, will result in very poor rendering, editing and analysis performance. Indeed, it is not uncommon to have tomographies of 40GB or larger in the non-compressed formats.

After the data set has been imported into Avizo, the first stage for analysis is to define the region or regions of interest of the tomography. This can be done by creating an Ortho-Slice module in the project view located along the right side. From here, artifact avoidance can be done by selecting the area of material that is of interest using a Sub-Volume module. This removes the blooming as seen in Figure 4 and will yield the material as selected in the bounded regions.

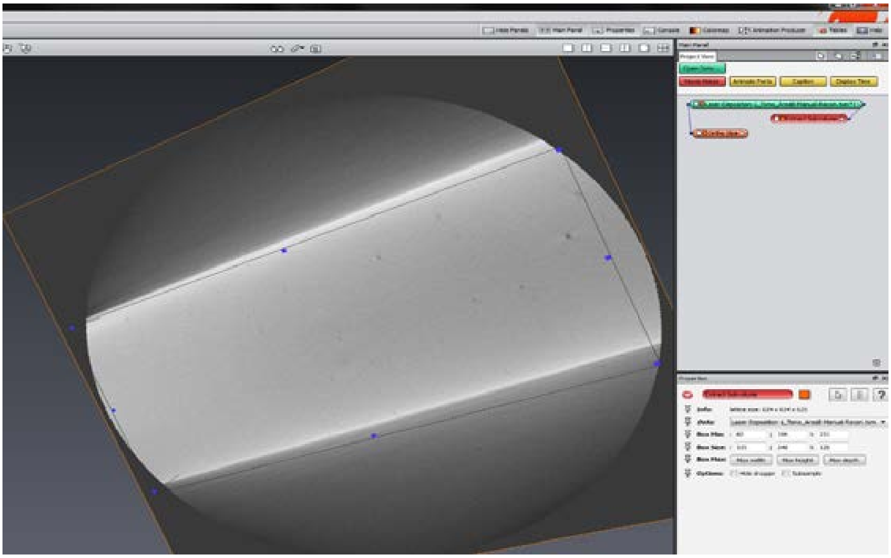

Figure 4. Avizo Fire Ortho-Slice and Sub-Volume modules.

The Sub-Volume module is configured by slice count in each axis as recorded by the MCT during processing. As a result, the real measurements of the selected material can be calculated by multiplying the voxel size by each of the number of slices selected in this selection operation. After selection, the material extracted can sometimes artificially re-expand to fill the resolution of the full tomography and will greatly reduce the image quality. If the software being used supports resampling the data, as Avizo does, it is advisable to do so against the original tomography to restore the lost detail.

After all necessary volume operations have been finished, or if the artifacting is not sufficient to interfere with material selection, an Interactive-Thresholding module can be attached for improved clarity. Thresholding is the ability to select the material by its density (or in the X-ray image its grey-scale value) by selecting upper and lower limits on a histogram of the tomography. The results can be very good and simple to produce if no severe artifacts are in the scan. Figure 5 illustrates a selected section of a friction stir weld joint with a defect or air-pocket inside of it. By thresholding the high end of the histogram to the material while keeping the low end where the sample becomes air, the material can be instantly transferred out of the artifacts and air. Avizo will show what is selected by highlighting the area.

Thresholding is inherently an inaccurate stage of the post-rendering process. To accurately identify structures more complex than the binary rendering of air and material illustrated in Figure 5, someone who understands the material needs to estimate the density that correlates to each material. Given the variabilities present in the imaging stage, in some research, the thresholding was done in such a way that the resulting materials constituted the volume fraction anticipated in fiber fatigue testing. This particular step, though producing variable results, can be repeatedly tested and adjusted if the densities thresholded against are recorded [1].

By working this procedure in reverse in conjunction with reconstruction, we have found that details of the nature of friction stir weld defects become more apparent than before. These results can be seen in Figure 6, with the axes enabled

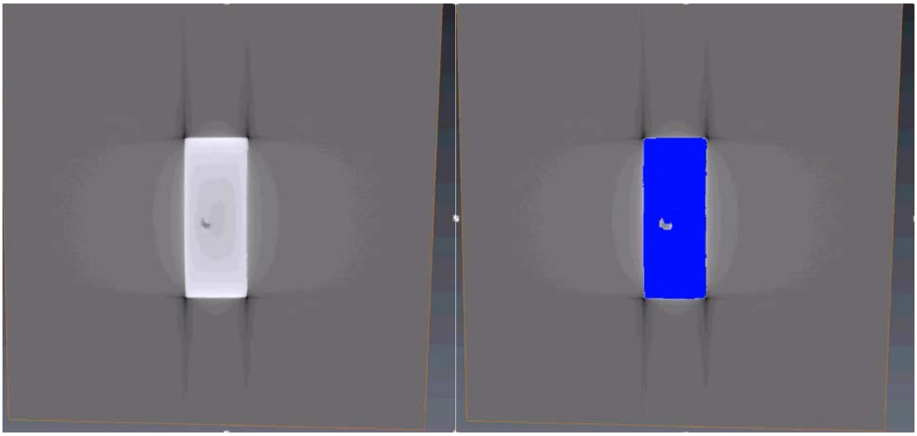

Figure 5. Avizo Fire Ortho-Slice (left) and the thresholded data(right).

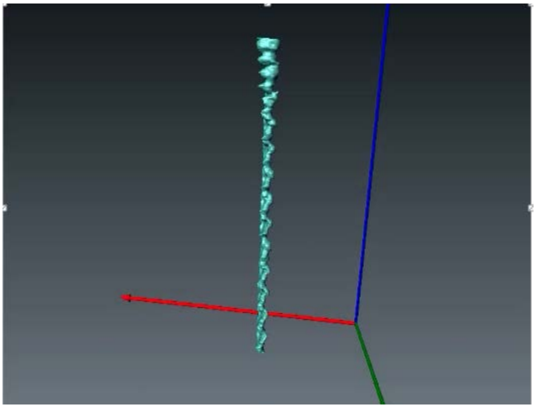

Figure 6. Avizo Fire wormhole defect rendering. For perspective, the three axes are shown.

for perspective. Such data have become paramount to research to remove these defects at weld run time [2].

Reconstruction—Once a thresholded area has been selected and applied, a new data set will be produced with only the selected materials present with no variability in density or gray-scale. This rendered material can be transformed easily into a surface using the Generate-Surface module. While generating a surface from the thresholded material, one must be aware that the smoothing configurations will remove data from the material scanned before to make a more natural surface. If using a scan of materials that is nearly at the limit for resolution of the MCT, one must consider turning off all smoothing operations.

The results of this module can be seen in Figure 6 and can be exported to 3D printing solutions like MakerBot, movies for analysis, or as the surface itself to 3D-pdf documents. Mathematical analysis is also possible and can be done with Avizo's internal systems or exported to Matlab or Abaqus for further evaluation. For assistance in the evaluation of wormhole defects, the surface renderings can be simplified to triangles and then the normal vectors of each can be computationally mapped to better understand the curvature at each selected elevation (Figure 7).

Using the built-in Label-Analysis and Volume-Fraction modules, one can quickly calculate each separate particle in the surface and generate metrics on surface area and volume or the ratio between the empty and the thresholded data. These results are also heavily dependent upon whether any smoothing had occurred previously as the surface's structure can change substantially.

Comparison—To illustrate the differences in quality these steps can make, we ran an experiment using a section of friction stir weld joint. In Figure 8, the image on the left was constructed using a binning factor of two to approach five-thousand X-ray counts at six seconds of exposure and being scanned at 65 kV. The scan on the right has been constructed using no binning factors and at the power setting of 85 kV for 10 seconds of exposure. Both images were produced using a source distance of 100 mm and a detector distance of 80mm and both were run using the same low-energy filter (LE-5 by XRadia). It should be noted

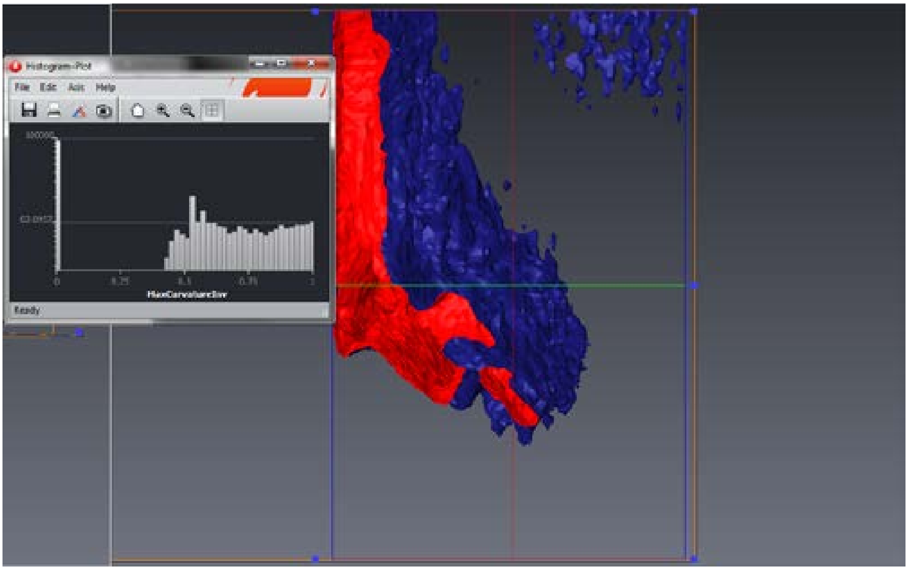

Figure 7. Avizo Fire wormhole defect analysis using Matlab's curvature evaluation via plugin.

Figure 8. XCT binning and power settings effect on defect detail. The image on the left looks clearer, but does not contain the same blur because the binning has balanced out the curvature on this sample.

that though the image on the left looks clearer, it does not contain the same blur as the binning has balanced out the curvature on this sample.

The improved defect detail of the image on the right will facilitate better post-imaging evaluation and a more realistic estimation of the defect's behavior. These scans were both modified using a Sub-Volume module to remove air and excess material. After completion, resampling was conducted on each tomography to ensure that all clarity possible persisted to the thresholding stage. The results after all thresholding and rendering can be seen in Figure 9 and the profile of the image in Figure 10. The red material is a common defect found between samples, and the blue region is unique to the 85 kV sample (the right image in Figure 8).

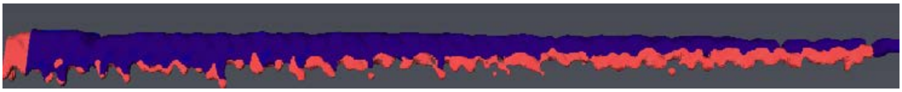

Figure 9. Comparison of surface renderings given imaging differences.

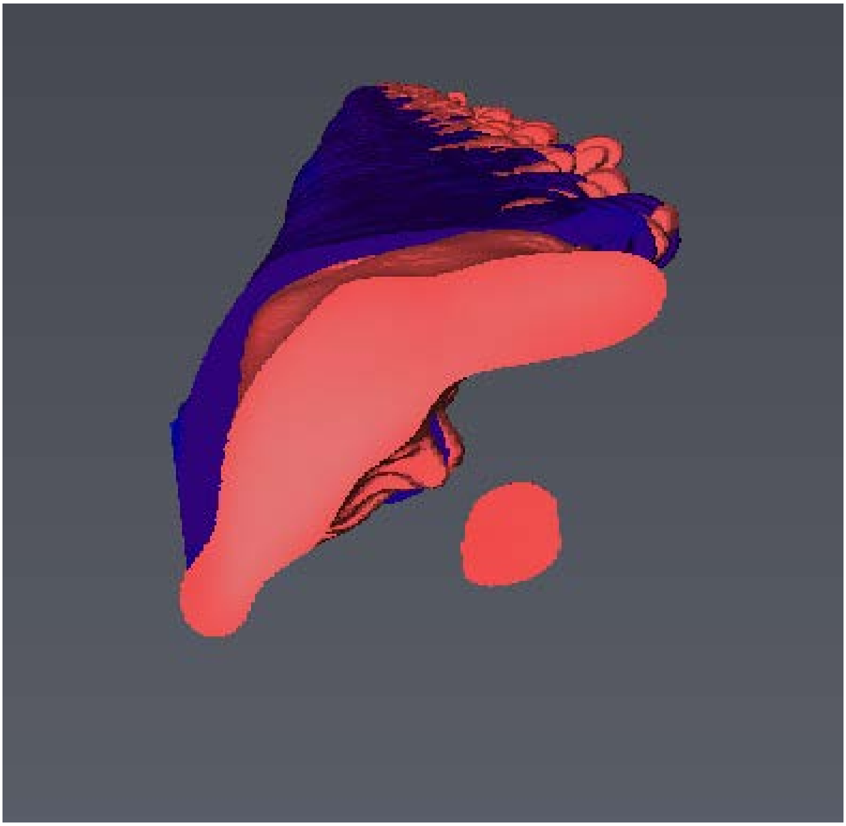

Figure 10. A profile image of Figure 9.

DISCUSSION

While MCT use in research is rapidly becoming a frequently-used tool, failure to report settings and properly use the technologies associated with this process generally precludes repeatable experimentation. Creating awareness of correct sample preparation, imaging and post-processing configurations can mitigate many uncertainties regarding resolution, detail, and density measurements. The accuracy in non-destructive evaluation of friction stir welding is paramount as the joints made are among the highest strength bonds currently produced.

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USE OF A COMMERCIAL FEEDING STIMULANT DURING MCCONAUGHY RAINBOW TROUT REARING

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ABSTRACT

This study examined the use of a commercial feeding stimulant, Bioflake (Bio-Oregon, Longview, WA), during McConaughy strain rainbow trout (*Oncorhynchus mykiss*) rearing in two trials. The first trial began at the onset of feeding and lasted for 28 days, with the trout receiving either a commercial starter diet, or the same diet supplemented with 5% Bioflake for the first five days of feeding. Mean tank ending weights after 27 days of feeding were 112 g for the starter diet and 131 g for the Bioflake supplemented diet, but these means were not significantly different. There were also no significant differences between the diets in weight gain, feed conversion ratio, individual fish length and individual fish weight. A second trial occurred from rearing days 28 to 56, with trout fed either a commercial diet of #1 granules or the same diet with 5% Bioflake added during days 28 through 32. For this trial, mean tank ending weights were 267 g and 312 g for the #1 granule diet and the Bioflake supplemented diet respectively, but these means exhibited considerable variation and were not significantly different. There were also no significant differences between the diets in weight gain, feed conversion ratio, individual fish length and individual fish weight. While the results from these trials indicate no statistically-beneficial effects from the use of Bioflake supplementation during McConaughy strain rainbow trout feeding, interpretation of the data is hampered by considerable variation and small sample sizes.

Keywords

Rainbow trout, feeding stimulant, diet, krill, *Oncorhynchus mykiss*

INTRODUCTION

Many species and strains of fish have difficulty accepting artificial feed at the onset of feeding and when transitioning from one feed size to another (De Silva and Anderson 1995; Barnes et al. 2006). Attempts to counteract such feeding difficulties have used a variety of natural and synthetic ingredients as feed attractants or stimulants (NRC 1993). Nucleotide (AMP) and nucleoside (inosine) components have been identified as chemo-attractants for aquatic animals (Mackie 1973; Ramadan and Atef 1991; Kubitza et al. 1997) and may be potential feeding stimulants in fish (Mackie and Andron 1978; Kubitza et al. 1997) to enhance feed intake. Amino acids also are potent attractants (Carr et al. 1996). Krill (*Euphausia* spp.) meal contains many amino acids that stimulate fish feeding (Shimizu et al. 1990) and is the most commonly used feed attractant in aquaculture (Yoshitomi 2004; Liu et al. 2012).

Bioflake (Bio-Oregon, Longview, WA) is a commercially-produced feeding stimulant based on krill. The effectiveness of Bioflake has not been evaluated previously during rainbow trout (*Oncorhynchus mykiss*) rearing. Thus, the objective of this study was to determine the effects of using Bioflake during initial feeding and during feed size transition in McConaughy strain rainbow trout. This strain can be difficult to feed train and culture (Barnes et al. 2006).

METHODS

Two trials were conducted at McNenny State Fish Hatchery, Spearfish, SD. Well water at a constant temperature of 11°C (total hardness as $CaCO_3$, 360 mg/L; alkalinity as $CaCO_3$, 210 mg/L; pH 7.6; total dissolved solids, 390 mg/L) was used throughout rearing.

For the first trial, a common pool of approximately 1,800 McConaughy strain rainbow trout (600 g) was divided into groups of 300 fish (100 g) which were placed into each of six, 100-L cylindrical tanks. Flows in each tank were set at 12 L/min. Tanks were cleaned as needed to eliminate excess food and fish waste, and mortalities were removed and recorded daily. Twenty fish were weighed to the nearest 0.01 g and measured to the nearest 0.1 mm from the common pool at the start of each trial.

Three control tanks received a commercial starter feed (Table 1) for 27 days, while three experimental tanks received the commercial starter mixed with 5% Bio-Flake for the first five days of feeding, followed by just starter for the remain-

Table 1. Manufacturer's ingredients list for the commercial diets and Bio-Flake. Ingredients are listed in the order that they appear on the feed label.

Starter	#1
Fish Meal	Fish Meal
Wheat Flour	Wheat Flour
Fish Oil	Fish Oil
Feather Meal	Feather Meal
Poultry by-product Meal	Brewer Yeast
Brewer Yeast	Squid Meal
Squid Meal	Poultry By-product Meal
Choline Chloride	L-Ascorbyl-2-Polyphosphate (Vit C)
L-Ascorbyl-2-Polphosphate	Choline Chloride
Vitamin/Mineral Premix	Vitamin/Mineral Premix

der of the experiment. Daily feed rations were dispensed hourly from 0800 to 1600 using automatic feeders (Sweeney Enterprises, Inc., Boerne, TX). After 27 days, total tank weights were recorded to the nearest 0.01g, and five fish per tank were weighed to the nearest 0.01 g and measured to the nearest 0.1 mm.

Constant water temperature allowed the use of the hatchery constant (HC) method to determine feeding levels for the tanks, with a planned feed conversion of 1.1 (Buterbaugh and Willoughby 1967). Feeding rates were based on McConaughty strain protocols defined in Barnes et al. (2006). HC was 3.3 for the first five days of feeding to prevent buildup of wasted feed in the tanks while the fish were learning to eat. HC was then increased to 5.28 for four days, 6.6 for five days and maintained at 7.92 for the remainder of the experiment. Daily feed amounts were weighed to the nearest 0.1 g.

At the end of the first trial, the fish were mixed into a common pool and redistributed among the six tanks. Feeding commenced on rearing day 28. Three control tanks received #1 granules (Table 1) and three experimental tanks received starter mixed with 5% Bioflake added for the first five days of the trial. After the first five days, all of the fish received #1 granules until the end of the trial. Total tank weights and individual fish weights and lengths were recorded as in the first trial. For both trials, total tank weight gain was determined by subtracting end weight from initial weight and feed conversion ratio was determined by dividing the amount of feed fed by weight gained.

Data were analyzed using the T test with the SPSS (9.0) statistical analysis program (SPSS 1999). Because of the small sample sizes and preliminary aspect of the trail, significance was set at $P < 0.10$. All percentage data were arcsine transformed to stabilize the variances (Kuehl 2000). Individual fish data were analyzed as per sacrificial pseudoreplication (Hurlbert 1984).

RESULTS

At the end of the first trial, mean tank ending weights were 112 g and 131 g after 27 days of feeding for the starter diet and the Bioflake supplemented diet respectively, but these means were not significantly different (Table 2). There were also no significant differences between the diets in weight gain, feed conversion ratio, individual fish length and individual fish weight (Table 3). A second trial occurred from rearing days 28 to 56, with trout fed either a commercial diet of #1 granules or the same diet with 5% Bioflake added during days 28 through 32. For this trial, mean tank ending weights were 267 g and 312 g for the #1 granule diet and the Bioflake supplemented diet respectively, but these means exhibited considerable variation and were not significantly different. There were also no significant differences between the diets in weight gain, feed conversion ratio, individual fish length and individual fish weight.

Table 2. Mean (+/- SE) rearing data, including feed conversion ratio (FCR[a]) from tanks of McConaughy strain rainbow trout fry fed one of two diets in two different trials (N = 3).

	Control	**BioFlake**
Trial 1 – rearing days 0 to 27		
Start weight (g)	100	100
End weight (g)	112.3 + 12.4	131.3 + 11.0
Gain (g)	12.0 + 12.4	31.3 + 11.0
Food fed (g)	90.4	90.4
FCR	3.82 + 0.71	3.60 + 1.05
Trial 2 – rearing days 28 to 56		
Start weight (g)	121	121
End weight (g)	267.3 + 25.2	312.7 + 14.7
Gain (g)	146.3 + 25.2	191.7 + 14.7
Food fed (g)	109.4	109.4
FCR[a]	0.80 + 0.17	0.58 + 0.04

[a] Feed Conversion Ratio (FCR) = food fed / gain.

Table 3. Mean (+/- SE) individual fish lengths and weights from tanks of McConaughy strain rainbow trout fry fed one of two diets in two different trials.

	Control Tanks	BioFlake Tanks
Trial 1		
Start length (mm)	20.5 + 0.2	20.5 + 0.2
End length (mm)	37.4 + 0.2	38.4 + 0.6
Start weight (g)	0.08 + 0.01	0.08 + 0.01
End weight (g)	0.46 + 0.01	0.50 + 0.02
Trial 2		
Start length (mm)	37.9 + 0.1	37.9 + 0.1
End length (mm)	54.1 + 0.9	54.2 + 0.9
Start weight (g)	0.48 + 0.02	0.48 + 0.02
End weight (g)	1.42 + 0.06	1.47 + 0.09

DISCUSSION

Interpreting the results from this study is difficult because of the large amount of variation and small sample sizes. The low statistical power resulting from small sample sizes is particularly problematic (Curtis et al. 1991). The McConaughy strain has historically shown a large amount of variation in fish size during hatchery rearing (Barnes and Durben 2010). In addition, the relatively short duration of this study may also have precluded any determination of statistically-significant results. A long-term study may be better able to describe the effect of a feeding stimulant mixture in a production hatchery (Papatryphon and Soares 1999). There is considerable variation in length of studies examining feed additives, with most studies lasting longer than 60 days (Aubin et al. 2005; Sealey et al. 2007). However, Staykov et al. (2007) noted improved rainbow trout growth and feed conversion after only 42 days of feeding a dietary supplement.

Similar to fish meal and shrimp meal, krill meal is highly palatable (Barrows 2000). It has been used successfully to replace fish meal in the diets of rainbow trout and other species (Olsen et al. 2006; Yoshitomi et al. 2006; Yoshitomi et al. 2007; Moren et al. 2007; Querol et al. 2012) and has been shown to be a feed attractant for fish (Kolkovski et al. 2000). Krill in particular has been shown to have positive effects during the first feeding of salmonid fry (Akiyama et al. 1984). Even though this study showed no statistical improvement with the use of krill meal to start trout on feed and to transition between feed sizes, the data suggest possible positive effects from krill use. Further experimentation with large samples sizes and additional replication would be desirable.

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AN AGE-STRUCTURED MODEL FOR THE AMERICAN DIPPER POPULATION IN THE BLACK HILLS OF SOUTH DAKOTA

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ABSTRACT

Historically, the American Dipper, *Cinclus mexicanus*, nested along many streams throughout the Black Hills of South Dakota. Presently, the breeding range has contracted to Spearfish and Whitewood creeks in the northern Black Hills. This population is genetically distinct from other populations in western North America and is considered threatened in the state. We examined long-term survival of the Black Hills American Dipper population using a discrete-time, age-structured model. An annual population survival rate of 0.59 per year was calculated using an exponential curve fit to data from a 2002 color-banded cohort tracked through six years. Comparison of one-year, post-capture survival rates of juveniles (7/12 = 0.58) and adults (11/13 = 0.85) suggests that juvenile survival may be lower than that of adults, however, results were not statistically significant. A net population reproductive rate of 1.40 fledged young per adult female was estimated from annual nesting season survey data collected from 2004-2009. The Black Hills population of the American Dipper has a higher survival rate and a lower reproductive rate than those reported from other populations. When age-structure is neglected, estimated model parameters predict an annual growth of less than 1% in the population. When age-structured survival is incorporated, the model predicts a 4% annual population decline, and simulations incorporating demographic stochasticity about the estimated parameter values suggest an 80% probability of a 50% population reduction over the next 25 years. We recommend that conservation strategies for the Black Hills American Dipper population focus on actions to increase reproductive rates.

Keywords

American Dipper, survival estimate, reproductive rate, population model

INTRODUCTION

A truly aquatic songbird, the American Dipper, *Cinclus mexicanus*, is found along mountain streams in western North America from Alaska to Panama (Willson and Kingery 2011). Strongly attached to fast-flowing streams, they depend upon permanent, clean, cold water that remains unfrozen in winter. They rarely stray more than a few yards from water and even in flight tend to follow the meandering path of the stream (Backlund 2009). American Dippers are not migratory, but short-range dispersal and movement to lower elevations in winter does occur (Price and Bock 1983). Out-of-range records are rare (Willson and Kingery 2011) most likely due to the species' aversion to flying over land. Overall population size and current population trends are uncertain (Willson and Kingery 2011), but because of its restrictive habitat requirements, numbers cannot be large.

The American Dipper occupies the eastern edge of its overall range in the Black Hills of South Dakota (Willson and Kingery 2011). The Black Hills of western South Dakota and northeastern Wyoming form an isolated mountain range within the Great Plains. They are separated from the Big Horn Mountains in Wyoming and other mountain ranges to the west and southwest by over 240 kilometers of dry grassland and sagebrush. It is hypothesized that the dispersal of dippers to the Black Hills resulted from water connections between the Black Hills and the Rocky Mountains during the last ice age (Backlund 2009). The Black Hills population of American Dipper is now believed to be isolated from Rocky Mountain populations as a result of extensive intervening grasslands and a lack of water connections between the Black Hills and Rocky Mountains (Backlund 2009). The Black Hills population is genetically distinct from other populations to the west (Anderson et al. 2007) further supporting the hypothesis that the population is isolated. American Dippers historically inhabited several permanent streams in the Black Hills. Presently, breeding populations are found only on Spearfish and Whitewood creeks. The species was listed as state threatened in 1996 (Backlund 2009). Dippers are important indicators of water quality, and population declines may signal potential environmental problems such as stream pollution and degradation, sedimentation, reduced stream flows, and a lack of available nest sites (Feck and Hall 2004).

In this study, we consider a discrete-time, age-structured model for the American Dipper population in the Black Hills of South Dakota. Annual survival and reproductive rates are estimated from data from this population (see for example Lovett 2008) and the results are used to explore the potential persistence or decline of the population.

Model development—We now develop a discrete-time, age-structured population model for the American Dipper in the Black Hills. Consider a closed (no emigration or immigration) population consisting of two age-classes, juveniles (inexperienced breeders) and adults (experienced breeders). Let J_n and A_n be the number of juvenile and adult females at time n respectively. We assume here that there is a 1:1 ratio of males to females in the population or, more realistically, that there are at least a sufficient number of males to pair and mate with all available females. Since American Dippers are known to be polygamous (Price and Bock, 1983), this seems to be a reasonable simplifying assumption. The basic discrete-time model described here incorporates differential annual survival and reproductive rates within the two classes. Let λ_j and λ_a denote the reproductive rates (number of female offspring per female per year) and s_j and s_a the annual survival rates of juveniles and adults, respectively. If population size is measured each year immediately prior to reproduction, we obtain the first-order, linear, recursive system given by

$$J_{n+1} = s_j(\lambda_j J_n + \lambda_a A_n)$$
$$A_{n+1} = s_a(J_n + A_n).$$

Population growth occurs if the dominant eigenvalue of this system is greater than 1. The magnitude of this eigenvalue then represents the long-term growth rate of the population and the corresponding eigenvector gives the steady-state age distribution of the population. In the special case where there is no difference between juvenile and adult reproductive rates, we denote them both by λ and this requirement for population growth is given by

$$s_j\lambda + s_a > 1.$$

If we further assume that the annual survival rates of juveniles and adults are equal and denoted by s, our condition for growth of the population further simplifies to

$$s(1+\lambda) > 1.$$

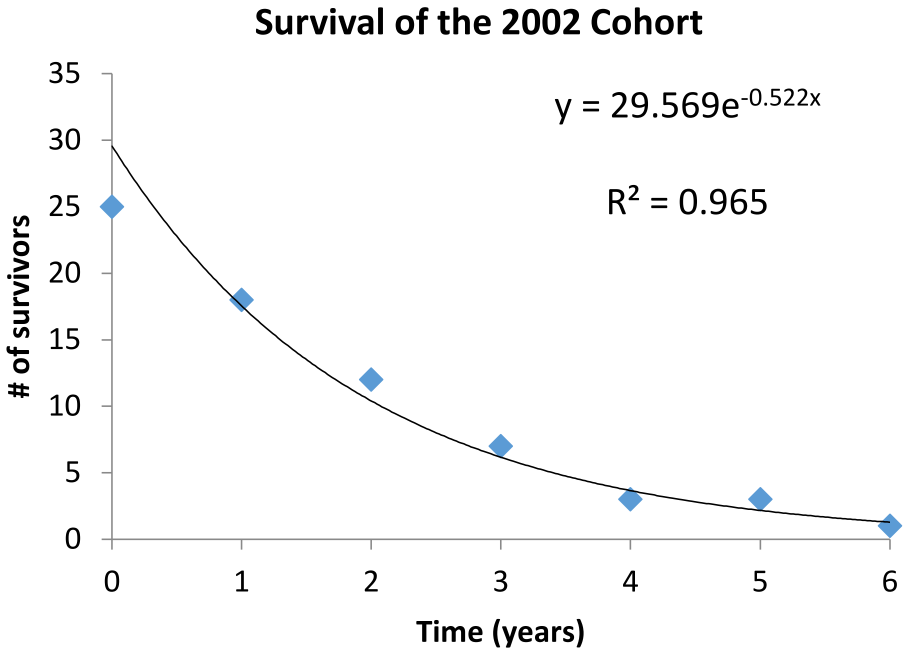

Figure 1. *An exponential curve fit to the number of surviving American Dippers from the 2002 color-banded cohort is used to estimate the annual survival rate as* $s = e^{-0.522} \approx 0.593$

Table 1. ***The survival rate, s, of American Dippers in the Black Hills, lower and upper limits of the 95% confidence interval, and the standard error (SE) of the regression on the semi-log transformed data is calculated for various subgroups within each cohort. Sample size is N and Years indicates the number of years included in the regression. Also given is the proportion of each subgroup that survived the year-after-banding (YAB).***

2002 Cohort	N	Years	YAB	s	Lower 95%	Upper 95%	SE
All Individuals	25	7	0.7200	0.5931	0.5294	0.6644	0.2338
Adult Females	4	4	0.7500	0.6335	0.4785	0.8388	0.1458
Adult Males	8	7	1.0000	0.6931	0.6154	0.7807	0.2449
Adult Unknown	1	1	0.0000				
Adults Combined	13	7	0.8462	0.6374	0.5696	0.7133	0.2314
Juveniles Combined	12		0.5833				
2003 Cohort	**N**	**Years**	**YAB**	**s**	**Lower 95%**	**Upper 95%**	**SE**
All Individuals	10	7	0.5000	0.6697	0.5806	0.7725	0.2939
Adult Females	1	4	1.0000				
Juveniles Combined	9		0.4444				
2004 Cohort	**N**	**Years**	**YAB**	**s**	**Lower 95%**	**Upper 95%**	**SE**
All Individuals	9	3	0.3333	0.3333	0.3333	0.3333	0.0000
Adult Males	3	3	0.6667	0.5774	0.2010	1.6585	0.1174
Adult Unknown	1	1	0.0000				
Adults Combined	4	3	0.5000	0.5000	0.5000	0.5000	0.0000
Juveniles Combined	5	2	0.2000				
2005 Cohort	**N**	**Years**	**YAB**	**s**	**Lower 95%**	**Upper 95%**	**SE**
All Individuals	8	5	0.5000	0.7364	0.6003	0.9033	0.2031
Adult Females	5	5	0.6000	0.6960	0.5836	0.8300	0.1750
Adult Males	3	5	0.3333	0.8027	0.5361	1.2020	0.4012

Estimation of annual survival—Between 2002 and 2005, Backlund (unpublished data) captured and uniquely color-banded four cohorts of American Dippers along Spearfish and Whitewood creeks in the northern Black Hills of South Dakota. When possible, the age, (juvenile or adult), and sex of each bird were identified. This population was intensively surveyed (48 sites along 28 km of Spearfish Creek and 9 sites along 7 km of Whitewood Creek) by Lovett (see Lovett 2008) during the spring and summer months each year from 2003 through 2009 and all sightings of color-banded birds were recorded. The annual survival rate of the Black Hills population of the American Dipper was estimated for all individuals (pooled juveniles and adults), adult females, adult males, and all adults (male and female combined) in each cohort (Table 1) by fitting an exponential decay curve to these data (Figure 1).

Table 2. ***The net reproductive rate, λ, of American Dippers in the Black Hills defined as the average number of offspring per adult female, is estimated as the number of nestlings per adult female, N/A, and as the number of fledglings per adult female, F/A. The annual count was based on combined data from the two observational sites, Spearfish Creek and Whitewood Creek, as well as from the first and second broods.***

Year	Adult Females (A)	Nestlings (N)	N / A	Fledglings (F)	F / A
2004	35	63	1.800	44	1.257
2005	34	55	1.618	52	1.529
2006	32	65	2.031	41	1.281
2007	35	74	2.114	63	1.800
2008	36	47	1.306	42	1.167
2009	36	64	1.778	48	1.333
	Average		1.774		1.395
	Std. Dev.		0.292		0.232
	Lower 95%		1.468		1.151
	Upper 95%		2.081		1.638

Comparing the one-year, post-capture survival rates of juveniles (7/12 = 0.583) and adults (11/13 = 0.846) from the 2002 cohort group, we find that juvenile survival may be lower than that of adults. A modified two-tailed comparison of the two population proportions (Agresti and Coffo 2000) showed no significant difference ($P = 0.184$) between these rates, most likely due to the relatively small sample sizes. The one-year, post-capture survival rates of juveniles and adults in the other three cohorts could not be compared due to insufficient numbers of juveniles (0 in 2005) or adults (1 in 2003 and 0 in 2004) in the cohort. Juvenile survival in the 2003 and 2004 cohorts was 4/9 (0.444) and 1/5 (0.200) respectively. Both are lower than the one-year, post-capture rate observed for adults in the 2002 cohort, further suggesting that juvenile survival may be lower than that of adults. The 95% confidence interval for the pooled estimate of all juveniles gives $s_j = 0.4615 \pm 0.1917$.

Estimation of the reproductive rate—The reproductive rate, λ, was estimated (Table 2) using data on the dipper population obtained from annual surveys by Lovett of known nest sites along Spearfish and Whitewood creeks from 2004 through 2009 (see Lovett 2008). The number of nesting attempts, as well as the number of observed young in each nest and the number of fledged young observed at each nest site, were recorded for both first and second broods at each site. Double brooding at nest sites along Spearfish Creek ranged from a low of 19% in 2008 to a high of 52% in 2007 (see Lovett 2008). Insufficient data regarding the age of individuals at the time of nesting precluded estimation of separate reproductive rates for adults (experienced breeders) and juveniles who were nesting for the first time. Net reproduction was calculated as both the ratio of observed young in each nest to adult females and also as the ratio of fledged young at each nest site to adult females. American Dippers typically leave the nest at age 24-26 days and remain with the adults in close proximity to the nest site for several days (Willson and Kingery 2011) during which time fledglings can be counted. To determine the number of adult females we counted the number of distinct nest sites for all first broods each year, the assumption being that each involved a distinct female. In a few cases individual color-banded females were known to have initiated multiple broods - presumably because of a failed first nesting attempt. If this was known, this female was only counted once. Some females that were not color-banded were potentially counted more than once in our total, thus we have no estimate of the number of potential non-nesting females present in the population. Observed young in the nest and fledged young at the nest were determined by adding the totals of the first and second (if applicable) broods at each site. The number of observed young in the nest probably overestimates the reproductive rate since not all birds will survive to fledging. Using the number of fledged young probably underestimates the reproductive rate since in some cases dispersal away from the nest site may have occurred preventing a full count of the fledglings. For simulation in the model, we assume a 1:1 ratio of males to females both in the nest and after fledging and divide each ratio by two since we are here tracking only the female population.

Simulation results—*Stella* (version 9.0.2, iseesystems, Lebanon, NH) was used to run all simulations of the model. To introduce a stochastic element, we allowed both the reproductive rate and the annual survival rate to vary annually according a normal distribution. The simulation results presented below are based on the average values obtained from 10,000 runs of the model over a 50-year time interval using the specified parameters and an initial population of 25 juvenile and 35 adult individuals. These initial conditions closely approximate the average number of adult females and fledged juvenile females (assuming a 1:1 sex ratio among fledglings) observed in the population (see Lovett 2008). Since we are primarily interested in the persistence of the population, the simulation results presented give the probability of the population being reduced by 50% and 75% from its initial size.

We first consider the special case $\lambda_j = \lambda_a = \lambda$ and $s_j = s_a = s$ (i.e. that there is no age-structured difference in either the reproductive or survival rates). Under this assumption, the model exhibits population growth provided the dominant eigenvalue $s(1+\lambda) > 1$. Using the estimated annual survival rate of $s = 0.5931$ (calculated from all individuals in the 2002 cohort) and our estimated annual reproductive rate of $\lambda = 0.6973$ produces the dominant eigenvalue $s\ (1 + \lambda)$ of 1.0067, which indicates a less than 1% annual growth in the population. Calculation of the corresponding eigenvector indicates a stable age-structure in the population of 41% juveniles and 59% adults (Figure 2).

Since juvenile survival may be lower than that of adults (see Estimation of Survival), we next consider the case where $s_j \neq s_a$. Population growth then requires that $s_j\lambda + s_a > 1$. Using the estimated annual survival rate of $s_j = 0.4615$ (pooled estimate for all juveniles) and $s_a = 0.6374$ (adults from the 2002 cohort) and $\lambda = 0.6973$ yields the dominant eigenvalue of $s_j\lambda + s_a = 0.9592$. This indicates an approximate 4% annual decay in the population. Calculation of the corresponding eigenvector indicates a stable age-structure consisting of 34% juveniles and 66% adults (Figure 3).

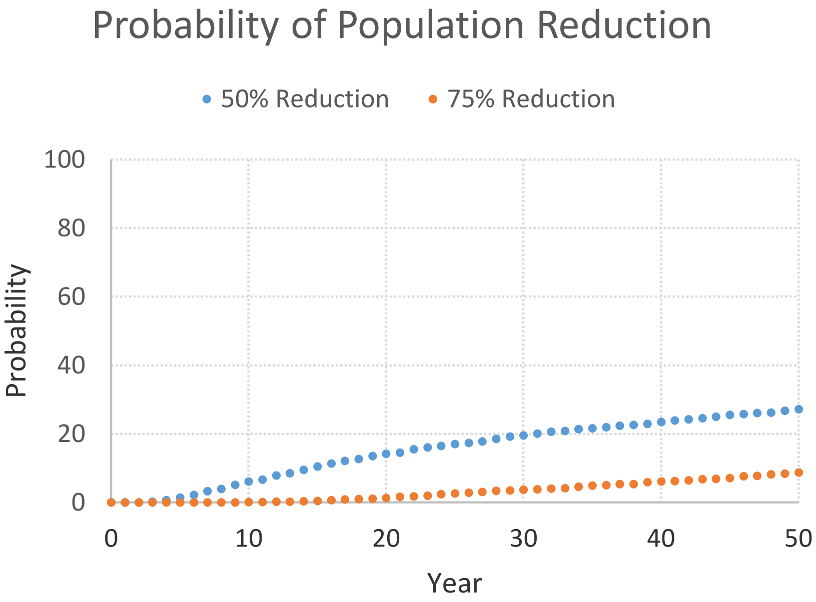

Figure 2. ***The probability of a 50% (blue) and 75% (pink) reduction in overall American Dipper population size by year with no difference between juvenile and adult survival or reproduction. Results are based on 10,000 runs of the model with initial conditions $J_0 = 25$, $A_0 = 35$ and parameters λ = Normal (0.6973, 0.1162) and s = Normal (0.5931, 0.1).***

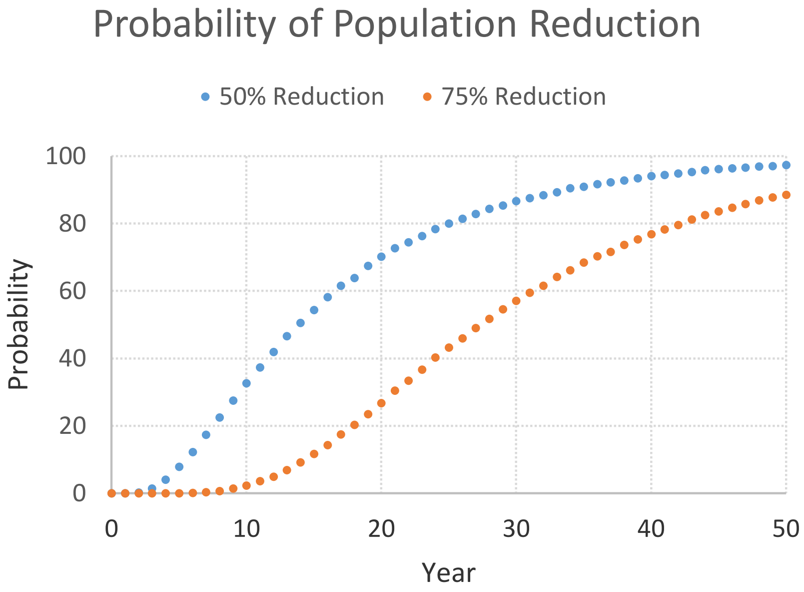

Figure 3. ***The probability of a 50% (blue) and 75% (pink) reduction in overall American Dipper population size by year with age-structured survival rates and no difference between juvenile and adult reproduction. Results are based on 10,000 runs of the model with initial conditions $J_0 = 25$, $A_0 = 35$ and parameters λ = Normal (0.6973, 0.1162), s_j = Normal (0.6973, 0.1162) and s_a = Normal (0.6374, 0.1).***

DISCUSSION

Intensive surveys of the dipper population in the northern Black Hills from 2004-2009 seem to indicate a relatively stable (over the short term), but small, population size. Breeding season estimates of the number of adult birds ranged from 57-66 during the six years of survey work (see Lovett 2008). We have here investigated an age-structured, discrete-time model to assess the long-term prospects for the persistence or decline of this population using estimates of the annual reproductive and survival rates from color-banded cohorts and nesting surveys.

Price and Bock (1983) estimated adult survival (during two years) of dippers in Colorado as 50% and 39% under the conservative assumption that breeders that did not return had died (some birds may have simply dispersed to new areas). Ealey (1977) found the one-year survival rate of 32 banded dippers in Alberta to be 44%. Our estimates are considerably higher, ranging from 50% (2004 cohort) to 74% (2005 cohort). It is not clear if this is due simply to the lack of dispersal in the Black Hills dipper population (i.e. that the counting is better), or if it could also indicate a more stable food supply, or the availability of higher quality winter habitat. We found that juvenile survival seemed to be lower than that of adult birds. This is consistent with the results presented by Price and Bock (1983), although again our estimated juvenile survival rate (46%) is higher than their more conservative calculation (33% and 23% during two years). Since most mortality occurs during the winter months (Price and Bock 1983), it may be that the Black Hills population of the American Dipper is not as severely limited by the availability of winter stream habitat or food supplies as are other populations.

We estimated the net reproductive rate as 1.395 fledged offspring per adult female and as 1.774 hatched nestlings per adult female. Previously reported breeding success rates (see Willson and Kingery 2011) were 47 to 69% fledged young per egg and 57 to 86% hatchlings per egg. Assuming an average of 4.1 eggs per clutch would result in approximately 1.9-2.8 fledged young per adult female and 2.3-3.5 hatched nestlings per adult female. Since some females produce second broods, these estimates are almost certainly on the low side. Hence, it seems that the net reproductive rate of dippers in the Black Hills is considerably lower than the range of values reported elsewhere. Additional study of this population is needed to determine if this lower reproductive rate is due to a limited availability of suitable nest sites, limited food supply during the breeding season, disturbance, low genetic variability (i.e., inbreeding) resulting from low population size, or other factors.

Our calculations suggest that the Black Hills population of the American Dipper has higher survival rates and lower reproductive rates than those from other populations. We have presented a simplified age-structured model to investigate the potential persistence or decline of this small, isolated population. When we neglect age-structured differences between juveniles and adults, our estimates of the annual survival and reproductive rates suggest a less than 1% annual growth rate in the Black Hills population of the American Dipper. Simulations of the model in this case indicated an approximately 17% probability of a 50% reduction in the population over the next 25 years. When age-structured survival was taken into account, our estimates result in a 4% annual decline in the population and model simulations indicated an 80% probability of a 50% population decline over the next 25 years. These simulations should, however, be considered illustrative only. The parameter estimates are certainly not robust enough to make definitive conclusions. The best case and worst case scenarios that would be obtained using the 95% confidence intervals for the parameter estimates cover the range from a less than 1% chance of population decline to a greater than 99% probability of decline. From 2004-2009, the number of nesting adult females in the population remained relatively stable (see Table 2) suggesting that the model neglecting age structure may be a better fit to observed trends than is the model incorporating age structure. Recall that our estimate of juvenile survival was not statistically significantly different from adult survival, however, sample sizes were very small. Also, as shown in Table 1, year-after-banding survival of juveniles varied considerably from year to year. Hence, further study is needed to better estimate the juvenile survival rate and its impact on the dipper population dynamics. The average estimates calculated here appear to indicate a population that is right on the edge between sustained growth and decline. Because of this, the relatively small size of the population and given its very limited habitat in the Black Hills, the population may be particularly susceptible to random catastrophic events such as flooding or extreme and prolonged winter stream freezing.

To better predict the long-term trend in the dipper population in the Black Hills will require , additional study. There are a number of potentially important factors that we have not included in our simplified age-structured model presented here. More complex models might include: adding in additional age classes (we here considered only two), sex-structure (we neglected males here), and dispersal between adjacent subpopulations (Spearfish Creek and Whitewood Creek). More detailed data would be needed to estimate the parameters of these more complex models. Data on the age of individuals at the time of nesting are needed to better estimate more detailed age-structured reproductive rates, and larger cohorts of color-banded birds would allow for estimation of age-structured survival rates. Additionally, these data need to be collected over a sufficiently long time period in order to develop estimates for the range of the annual variation in the model parameters.

Perhaps, most importantly, density dependence in the reproductive rate needs to be considered, since nest site availability has been suggested as the most significant limiting factor for American Dipper populations (Price and Bock 1983). Only 19 of the 48 nest sites monitored by Lovett occurred at natural sites – the other 29 were man-made sites such as bridges or nest boxes. This is consistent with nest site availability being a significant contributing factor to the lower net reproductive rates observed for the dipper population in the Black Hills. We recommend that conservation strategies for the Black Hills American Dipper population focus on actions such as placing artificial nesting structures to increase nest site availability and limiting disturbance around known nesting sites to increase reproductive rates.

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POPULATION ESTIMATES AND HABITAT ASSOCIATIONS OF THE BEAR LODGE MEADOW JUMPING MOUSE IN THE BLACK HILLS OF SOUTH DAKOTA

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ABSTRACT

The Bear Lodge meadow jumping mouse (*Zapus hudsonius campestris*) is commonly reported from the Black Hills of South Dakota and the adjacent Bear Lodge Mountains of Wyoming, however little information is available regarding its population densities and habitat associations. The purpose of this study was to determine the present distribution, population estimates, and habitat affinities of *Z. hudsonius campestris* in the Black Hills of South Dakota and compare its current distribution to the historical record. Live trapping methods were used to estimate the population density of this species. Capture rates ranged from 0 to 4.7 individuals per 100 trap nights, with a mean ± 1 SD of 1.7 ± 1.6. The Jolly-Seber population estimate per km of stream ($\bar{x}$ ± 1 SD) was 31 ± 26, and was significantly different from the minimum-number known alive estimate ($\bar{x}$ ± 1 SD) of 18 ± 14 per km of stream ($t = -3.65$, df = 15, $P = 0.002$). The relative abundance of jumping mice ranged from 7 to 78% of the total number of small mammals. On 4 of the 16 streamside transects, the jumping mouse was the most numerous species, and was overall very common. Jumping mice were significantly correlated with percent litter cover ($r = 0.673$, df = 14, $P = 0.006$), percent moss cover ($r = 0.58$, df = 14, $P = 0.039$), and negatively correlated with percent grass cover ($r = -0.546$, df = 14, $P = 0.035$). Captures occurred at elevations ranging from 1279 to 1890 m. Jumping mice were captured throughout the range of historical records, and were even collected in Meade County, despite a lack of reports of meadow jumping mice from that county in the past.

Keywords

Bear Lodge, meadow jumping mouse, *Zapus hudsonius campestris*, Dipodidae, habitat

INTRODUCTION

The Bear Lodge meadow jumping mouse (*Zapus hudsonius campestris*) is endemic to the Black Hills of South Dakota, the adjacent Bear Lodge Mountains of northeastern Wyoming, and northwestern South Dakota (Krutzsch 1954; Hall 1981). Turner (1974) summarized the work of 46 researchers from 1947 to 1967, and reported 207 specimens of *Z. hudsonius campestris* from the Black Hills of South Dakota and the adjacent Bear Lodge Mountains, but reported no specimens from Meade and Fall River counties. No information was provided on population densities or relative abundance however. More recently, Duckwitz (2001) captured 7.2 meadow jumping mice per 1000 trap nights in Wind Cave National Park in Custer County during the summers of 1999 to 2000. Between 1977 and 1985, Easton and Wrenn (1988) reported capturing a total of 70 *Z. hudsonius campestris* in Spearfish Canyon, Lawrence County, between 1977 and 1985, but no catch per unit effort or relative abundance was reported.

Beauvais (2000) and Hafner and Yensen (1998) characterized *Z. hudsonius campestris* as rare, but did not conduct any systematic studies to determine the relative abundance and habitat affinities of this species. Cryan (2004) described the information concerning *Z. hudsonius* on the Northern Great Plains as "limited and biased, and there are no long-term data with which to evaluate population trends in the region." Therefore, the purpose of this study is to: 1) determine the present distribution, population sizes, and habitat affinity of *Zapus hudsonius campestris* in the Black Hills of South Dakota, and 2) compare the present distribution and abundance with historical records of this species.

METHODS

To determine population estimates and the distribution of the Bear Lodge meadow jumping mouse in the Black Hills, I sampled 16 study sites along small streams between June 2010 and July 2011, and another two control areas between June and July 2012 along dry ravines. Twenty-five Sherman Live Traps® were placed every 10 m along each side of the drainage. At the midpoint of these primary transects, two perpendicular transects with 25 traps spaced at 10-m intervals were established on opposite sides of each drainage to determine how far this species ranges from the water's edge. Occasionally, the perpendicular transects were established at opposite ends of the streamside transects because of steep terrain which precluded the placement of traps. Thus, a total of 100 live traps at each study site were set for three trap nights, and GPS coordinates were recorded at each trap location.

Traps were baited with rolled oats, corn, and barley with molasses (as used by Meaney et al. (2003) and Frey and Malaney (2009) in studies of other subspecies of *Z. hudsonius*), and supplied with cotton/polyester bedding material. Trainor et al. (2007) stated that this species is primarily nocturnal; therefore traps were checked between 0800 and 1000 h each morning for three consecutive days. Small mammals were weighed and marked with either a passive integrated transponder (PIT) tag inserted between the scapulae or an ear tag. The relative age (adult, subadult, or juvenile) and sex were also recorded. Additional information recorded for males included whether the testes were abdominal or scrotal, and the relative size of the nipples was recorded for females. Voucher specimens were collected from incidental trap mortalities or animals captured during the last night of the trapping period and were deposited in the mammalogy collection at Dakota Wesleyan University. All methods followed the guidelines of the American Society of Mammalogists for the use of wild mammals in research (Gannon et al. 2007) and recommendations of the Dakota Wesleyan University's Institutional Animal Care and Use Committee.

From my capture/recapture data, I determined the minimum-number known alive (MNA) for the jumping mice on each study site (Krebs 1999). I also calculated Jolly-Seber population estimates using a Java applet (http://people.hws.edu/ryan/Ryan/Pages/Jolly.html). Populations were assumed to be located along the linear streamside transects based on the small number of individuals captured along the perpendicular transects. Therefore, population estimates were converted to the number of individuals per km by extrapolation from the effective length of the streamside transects. To determine the effective length of the transects, I added the average distance between recaptures to the actual length of the transect. This was based on the assumption that an animal would encounter the transect if at least half of the length of its home range came into contact with the transect at either end.

To determine the habitat affinities of this subspecies, I measured several habitat variables along one streamside transect and one perpendicular transect at 20-m intervals at each study site. Abiotic measurements included the elevation of the study site, soil compaction and soil moisture. Soil compaction was measured with a DICKY-john Soil Compaction Tester (DICKY-john Corporation, 5200 Dickey John Road, Auburn, Illinois, 62615, USA) at 10 cm and 20 cm below the ground surface. Soil moisture was measured at 10 cm depth with a model number DSMM500 Digital Soil Moisture Meter (General Tools and Instruments, 80 White Street, New York, NY 10013-3567). Canopy cover (Leaf Area Index – LAI) was measured with a WinSCANOPY digital image analyzer (Regent Instruments, Inc., 21 Antares Drive, Nepean, Ontario K2E 7T8) at 1 meter above the ground surface. Percent cover of grasses, forbs, shrubs, rock, moss, litter, and fallen timber was estimated at ground level using the method of Daubenmire (1959).

RESULTS

A total of 88 *Z. hudsonius campestris* was captured on 14 of 16 trapping sites located in riparian zones in Lawrence, Meade, Pennington, and Custer Counties in the Black Hills of South Dakota (Figure 1, Appendix A). No jumping mice were captured in the two control areas located at xeric sites. There was no significant difference in the number of jumping mice captured between the 2010 and 2011 trapping seasons ($t = -1.27$, df = 14, $P = 0.224$). The number of jumping mice captured per 100 trap nights ranged from 0 to 4.7, with a mean ± 1 SD of 1.7 ± 1.6 (Table 1). The Jolly-Seber population estimate per km of stream ($\bar{x}$ ± 1 SD) was 31 ± 26, and was significantly different from the MNA estimate ($\bar{x}$ ± 1 SD) of 18 ± 14 per km of stream (t = -3.65, df = 15, p = 0.002). The relative abundance of jumping mice relative to other species of small mammals captured ranged from 0 to 78% with a mean % ± 1 SD of 23 ± 22. Other species captured included the deer mouse (*Peromyscus maniculatus*), white-footed mouse (*Peromyscus leucopus*), meadow vole (*Microtus pennsylvanicus*), long-tailed vole (*Microtus longicaudus*), prairie vole (*Microtus ochrogaster*), Southern red-backed vole (*Clethrionomys gapperi*), least chipmunk (*Tamias minimus*), red squirrel (*Tamiasciurus hudsonicus*), northern flying squirrel (*Glaucomys sabrinus*), masked shrew (*Sorex cinereus*), short-tailed weasel (*Mustela erminea*), and the least weasel (*Mustela nivalis*).

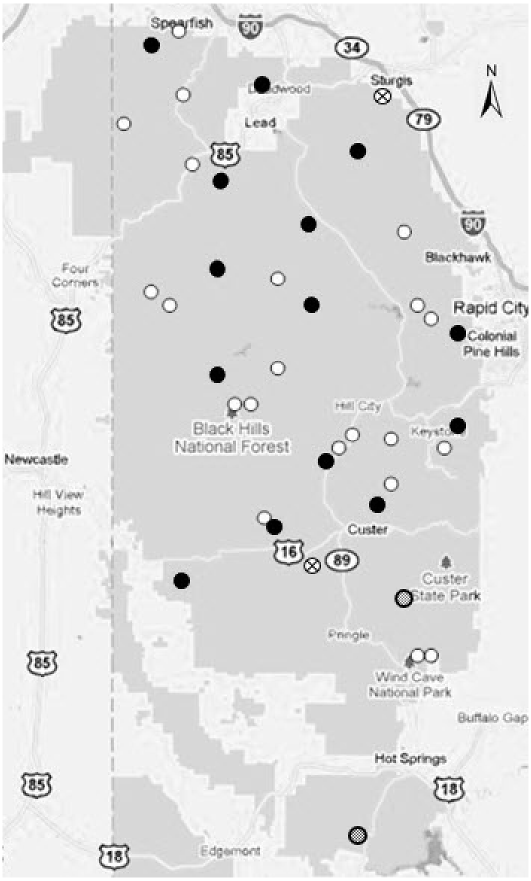

Figure 1. Locations of jumping mouse in this study (solid circles) and absent (shaded circles) in this study, along with historical localities (clear circles). Locations of two xeric control areas where jumping mice were absent are shown as circles with an X.

Jumping mice were nearly always captured near water, with 92% of the captures occurring along the streamside transects, with a mean distance between individual recaptures of 69 m. This species was captured along the perpendicular transects extending away from the stream on only two of the study sites. Two individuals were captured at distances of 58 and 108 meters from Castle Creek in 2010 (see Table 1 for locality). In addition, four individuals were captured on a steep slope covered with scattered shrubs at a mean distance of 130 m from City Creek in 2011 (see Table 1 for locality). No jumping mice were captured on either of the two xeric control sites that were trapped in 2012.

Capture locations of jumping mice ranged in elevation from 1279 to 1890 m (Figure 2). No jumping mice were captured at the two lowest elevations at 1042 and 1235 m.

Habitat associations—Jumping mice were not restricted to any particular riparian association, but appeared to be limited by the need for a perennial water

Table 1. Locations, trapping dates, number of jumping mice captured per 100 trap nights, and Jolly-Seber estimates of Bear Lodge meadow jumping mice in the Black Hills of South Dakota from 2010-2012.

Study Site	County	GPS Coordinates (WGS 84 map datum)	Trapping Dates	No/100 Trap Nights	Jolly-Seber Estimate/km stream	Elevation (m)
Spring Cr.	Pennington	13T 0610247 UTM 4857568	30 June-2 July 2010	2.7	58	1635
Tepee Gulch	Pennington	13T 0629694 UTM 4863824	28-30 July 2010	1	16	1305
Castle Creek	Pennington	13T 0605295 UTM 4880344	4-6 August 2010	0.6	10	1543
S. Fork Castle Cr.	Pennington	13T 0591223 UTM 4870367	12-14 July 2011	3.7	55	1890
Rhoads Fork Cr.	Pennington	13T 0591628 UTM 4887884	15-17 July 2011	3.3	45	1816
Elk Creek	Meade	13T 0615020 UTM 4905661	20-22 July 2010	0.6	26	1438
Marcotte GPA	Meade	13T 0615660 UTM 4916567	20-22 June 2012	0	0	1235
Higgins Gulch	Lawrence	13T 0583705 UTM 4924187	16-18 June 2010	4	94	1279
Hanna Creek	Lawrence	13T 0592048 UTM 4902302	23-25 June 2010	2.7	32	1726
Victoria Creek	Pennington	13T 0628963 UTM 4975249	14-16 June 2011	1.7	36	1298
City Creek	Lawrence	13T 0599453 UTM 4915598	22-24 June 2011	4.7	65	1550
N Fk Boxelder Cr.	Lawrence	13T 0604320 UTM 4897492	6-8 July 2011	2.3	24	1718
Hell Canyon	Custer	13T 0594277 UTM 4841133	28-30 June 2011	0.6	13	1578
French Cr.	Custer	13T 0604902 UTM 4847447	21-23 July 2011	0.67	6	1718
Willow Cr.	Custer	13T 0613719 UTM 4853409	26-28 July 2011	1.3	16	1747
FR336	Custer	13T 0617456 UTM 4833850	7-9 July 2010	0	0	1548
Harry Mills Rd	Custer	13T 0602168 UTM 4842021	27-29 July 2012	0	0	1682
Whitney Preserve	Fall River	13T 0617117 UTM 4799533	14-16 July 2010	0	0	1042
Mean				**1.7**	**31**	
SD				**1.6**	**26**	

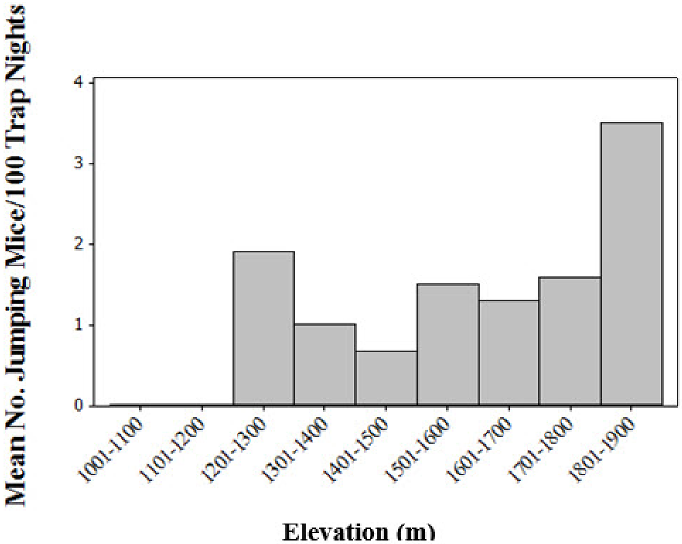

Figure 2. Number of jumping mice captured per 100 trap nights at various study site elevations.

source. Riparian vegetation ranged from areas dominated by ponderosa pine (*Pinus ponderosa*), birch species (*Betula* sp.), and willow (*Salix* sp.) to Black Hills spruce (*Picea glauca*), willow (*Salix* sp.), and red osier dogwood (*Cornus sericea*).

Because the variances of the number of jumping mice captured in 2010 and 2011 were not significantly different (F = 0.91, df = 7, P = 0.907), data were pooled from 2010 and 2011 for all correlation and multiple regression analyses. After one outlier was eliminated from the data set, the total number of *Z. hudsonius campestris* captured on each area was positively correlated with percent moss cover (r = 0.58, df = 14, P = 0.039) and percent cover of litter (r = 0.673, df = 14, P = 0.006). Additionally, the number of jumping mice was negatively correlated with percent grass cover (r = -0.546, df = 14, P = 0.035).

A comparison of the distribution of the Bear Lodge meadow jumping mice captured in this study to historical records revealed no major reduction in the range of this species in the Black Hills of South Dakota (Figure 1). There were a few marginal records within the last 25 years beyond the borders of this study (Duckwitz 2001, Easton and Wrenn 1988), but this was a result of differences in study site locations rather than a contraction in the distribution of this species.

DISCUSSION

Although there are no comparable studies that have specifically targeted the Bear Lodge meadow jumping mouse, the number of individuals captured per 100 trap nights was considerably higher than reported by Duckwitz (2001). Even more revealing is the relative abundance of this species across the study sites (Table 1). On average, the Bear Lodge meadow jumping mouse made up almost one-fourth of the small mammal community in the northern and central Black Hills of South Dakota. This species comprised about 50% of the small mammal community at several study sites, with a high of 78% in the City Creek drainage near Deadwood, SD. Thus, the fact that *Z. hudsonius campestris* is the dominant small mammal species in riparian zones of higher altitudes suggests that it is very common in suitable habitats in the Black Hills of South Dakota, despite the many disturbances that this area has experienced.

Despite the capture of several jumping mice over 100 meters from water, this species is extremely dependent on free-flowing streams in the Black Hills. This also suggests that buffer zones are needed at least 200 meters along streams near logging operations. An active logging operation was being conducted near the North Boxelder Creek study site, but the areas along the stream where jumping mice frequent were undisturbed and higher than average numbers of jumping mice were captured on this site. Forest managers should still be concerned with protecting watersheds from run-off associated with logging operations to protect aquatic and riparian habitat for other species.

Grazing activity was also observed on the Elk Creek and South Fork Castle Creek study areas. Jumping mice were found on both of these areas, with Elk Creek having a below average capture rate and the South Fork Castle Creek an above average capture rate. The small number of grazing sites in this study precludes any conclusion regarding the effect of grazing on jumping mice however.

The Black Hills has a long history of small-stream flooding. Flooding is probably normal for this area with its steep elevation gradients. In 1972, over 38 cm of rain in a 6-h period resulted in 238 deaths and $160 million in damage as a result of flooding of Rapid Creek near Rapid City, SD (Carter et al. 2002). Driscoll et al. (2012) concluded that other drainages, including Boxelder Creek, Elk Creek and Spring Creek have experienced paleoflooding at intensities exceeding the 1972 Rapid City flood. It is possible that flash flooding could drown young jumping mice while they are still in the nest. However, the effect of flooding on the Bear Lodge meadow jumping mouse has not been documented. Further studies concerning the effects of environmental disturbances are warranted to document the effects on this species.

The vegetation analysis conducted in this study suggests that the Bear Lodge meadow jumping mouse is associated with streams with banks covered with abundant moss, litter, and a mixture of grasses and forbs. Turner (1974) reported specimens from 1275 to 1975 m elevation between 1947 and 1967, which is comparable to the range of elevations in which jumping mice were found in this study. Thus, it appears that elevations below 1250 m are insufficient to support Bear Lodge meadow jumping mouse populations. Due to their strong association with perennial streams, any factor that reduces stream flow to zero is likely to result in local extinction of Bear Lodge meadow jumping mouse populations.

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Appendix A. Capture locations for each Bear Lodge meadow jumping mouse captured from June 2010 through July 2011 in the Black Hills of South Dakota.

Study site	Sex	County	GPS Coordinates (UTM)	Elevation (m)
Higgins Gulch	female	Lawrence	13T 0583707 4924150	1283
Higgins Gulch	male	Lawrence	13T 0583705 4924165	1279
Higgins Gulch	female	Lawrence	13T 0583697 4924234	1275
Higgins Gulch	male	Lawrence	13T 0583715 4924263	1267
Higgins Gulch	male	Lawrence	13T 0583704 4924180	1285
Higgins Gulch	male	Lawrence	13T 0583706 4924157	1281
Higgins Gulch	female	Lawrence	13T 0583707 4924150	1283
Higgins Gulch	male	Lawrence	13T 0583704 4924180	1280
Higgins Gulch	female	Lawrence	13T 0583710 4924218	1286
Higgins Gulch	female	Lawrence	13T 0583707 4924151	1299
Higgins Gulch	female	Lawrence	13T 0583690 4924131	1303
Higgins Gulch	female	Lawrence	13T 0583700 4924140	1308
Hanna Creek	female	Lawrence	13T 0592029 4902263	1712
Hanna Creek	unknown	Lawrence	13T 0592046 4902263	1716
Hanna Creek	male	Lawrence	13T 0592047 4902360	1740
Hanna Creek	male	Lawrence	13T 0592059 4902343	1726
Hanna Creek	male	Lawrence	13T 0592049 4902310	1726
Hanna Creek	male	Lawrence	13T 0592029 4902263	1712
Spring Creek	male	Pennington	13T 0610111 4857540	1651
Spring Creek	female	Pennington	13T 0610194 4857542	1630
Spring Creek	female	Pennington	13T 0610262 4857576	1631
Spring Creek	male	Pennington	13T 0610093 4857598	1635
Spring Creek	male	Pennington	13T 0610162 4857538	1636

Spring Creek	male	Pennington	13T 0610298	4857594	1643
Spring Creek	male	Pennington	13T 0610158	4857535	1637
Spring Creek	male	Pennington	13T 0610093	4857598	1635
Elk Creek	male	Meade	13T 0614860	4905644	1438
Elk Creek	female	Meade	13T 0614017	4905665	1441
Tepee Gulch	female	Pennington	13T 0629731	4863808	1310
Tepee Gulch	male	Pennington	13T 0629721	4863811	1316
Tepee Gulch	female	Pennington	13T 0629664	4863835	1316
Castle Creek	male	Pennington	13T 0605206	4880187	1303
Castle Creek	female	Pennington	13T 0605167	4880164	1314
Victoria Creek	male	Pennington	13T 0628905	4975176	1291
Victoria Creek	male	Pennington	13T 0628963	4975313	1281
Victoria Creek	male	Pennington	13T 0628966	4975268	1285
Victoria Creek	male	Pennington	13T 0628965	4975261	1285
Victoria Creek	male	Pennington	13T 0628966	4975268	1285
City Creek	male	Lawrence	13T 0599432	4915518	1546
City Creek	male	Lawrence	13T 0599526	4915574	
City Creek	male	Lawrence	13T 0599442	4915546	1548
City Creek	female	Lawrence	13T 0599463	4915680	1560
City Creek	male	Lawrence	13T 0599467	4915686	1557
City Creek	female	Lawrence	13T 0599453	4915588	1545
City Creek	male	Lawrence	13T 0599455	4915666	1554
City Creek	female	Lawrence	13T 0599512	4915567	1582
City Creek	male	Lawrence	13T 0599531	4915573	1583
City Creek	female	Lawrence	13T 0599455	4915666	1554
City Creek	female	Lawrence	13T 0599456	4915650	1552
City Creek	female	Lawrence	13T 0599454	4915618	1549
City Creek	female	Lawrence	13T 0599402	4915591	1557
City Creek	male	Lawrence	13T 0599501	4915563	1571
Hell Canyon	female	Custer	13T 0594330	4841109	1566
Hell Canyon	female	Custer	13T 0594251	4841146	1565
N. Fork Boxelder	male	Lawrence	13T 0604280	4897533	1721
N. Fork Boxelder	female	Lawrence	13T 0604352	4897468	1727
N. Fork Boxelder	female	Lawrence	13T 0604331	4897497	1718
N. Fork Boxelder	male	Lawrence	13T 0604261	4897534	1719
N. Fork Boxelder	female	Lawrence	13T 0604367	4897495	1713

N. Fork Boxelder	male	Lawrence	13T 0604358	4897490	1715
N. Fork Boxelder	female	Lawrence	13T 0606341	4897472	1723
S. Fork Castle Creek	male	Pennington	13T 0591256	4870324	1889
S. Fork Castle Creek	female	Pennington	13T 0591242	4870338	1896
S. Fork Castle Creek	female	Pennington	13T 0591201	4870381	1900
S. Fork Castle Creek	male	Pennington	13T 0591240	4870369	1884
S. Fork Castle Creek	female	Pennington	13T 0591234	4870357	1896
S. Fork Castle Creek	male	Pennington	13T 0591277	4870310	1890
S. Fork Castle Creek	female	Pennington	13T 0591245	4870329	1893
S. Fork Castle Creek	male	Pennington	13T 0591235	4870350	1898
S. Fork Castle Creek	female	Pennington	13T 0591198	4870394	1900
S. Fork Castle Creek	male	Pennington	13T 0591187	4870387	1902
S. Fork Castle Creek	female	Pennington	13T 0591215	4870355	1909
Rhoads Fork Creek	female	Pennington	13T 0591650	4887922	1823
Rhoads Fork Creek	female	Pennington	13T 0591638	4887889	1818
Rhoads Fork Creek	female	Pennington	13T 0591605	4887835	1827
Rhoads Fork Creek	male	Pennington	13T 0591626	4887895	1809
Rhoads Fork Creek	male	Pennington	13T 0591667	4887939	1816
Rhoads Fork Creek	female	Pennington	13T 0591585	4887802	1819
Rhoads Fork Creek	male	Pennington	13T 0591644	4887898	1814
Rhoads Fork Creek	female	Pennington	13T 0591639	4887890	1820
Rhoads Fork Creek	male	Pennington	13T 0591585	4887802	1819
Rhoads Fork Creek	female	Pennington	13T 0591644	4887898	1814
French Creek	male	Custer	13T 0604933	4847546	1697
French Creek	female	Custer	13T 0604872	4847432	1736
Willow Creek	female	Custer	13T 0613723	4853388	1739
Willow Creek	female	Custer	13T 0613732	4853424	1740
Willow Creek	female	Custer	13T 0613725	4853419	1743
Willow Creek	female	Custer	13T 0613730	4853426	1740

THE FLORA OF HARDING COUNTY: A CENTURY OF BOTANY IN NORTHWESTERN SOUTH DAKOTA, USA

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ABSTRACT

We report the current knowledge of the vascular plant flora of Harding County, South Dakota, which includes 724 species. Introduced species have increased from five percent of the flora to 13% over the last century. Relatively small changes have occurred in plant species duration, species habit or major groups over 100 years. Mean coefficients of conservatism varied from 4.3 to 6.2 in eight areas across the county, with the lowest values in riparian areas and highest values in pine-dominated buttes.

Keywords

floristics, grassland, Northern Great Plains, South Dakota, vegetation

INTRODUCTION

This report documents vascular plant species from Harding County, South Dakota, from historical and recent plant collections. Literature describing the flora of Harding County is lacking. The most notable publication is by Visher (1914) who completed a species list for the county. Visher's publication was based upon collections made by him in 1910-1912, and collections made by Rowley in 1909, Fromme in 1910, and Over in 1911. Heidel and Dueholm (1995) reported on "sensitive" species in Custer National Forest in Carter County, Montana, and Harding County. Gabel and Simonson (2008) reported the vegetation present on sand dunes and adjacent areas in Harding and Perkins Counties.

Hansen (1985) and Hansen and Hoffman (1988) described land classifications based on potential natural vegetation for the study area, resulting in 26 designations of habitat types including steppe, shrub-steppe, woodland and forest habitats. Studies in areas adjacent to Harding County include a study of the flora of the Grand River National Grassland (Kopp 2004) in Perkins County, SD. Mincemoyer (2006) reported on the plant resources including "species of concern" for south-central and southeast Montana. Harding County was also included in floristic studies by the Great Plains Flora Association (1986), Van Bruggen (1996) and the Flora of North America Editorial Committee (1993+). Our study objective is to report vascular plant species collected in Harding County through 2014, and to compare the current floristic makeup to that reported by Visher (1914) a century ago.

STUDY AREA

Harding County is the northwestern most county in South Dakota (Figure 1) with a land area of 691,786 ha and a population of 1353 (South Dakota Association of County Commissioners 2014). Land ownership includes about 12,092 ha managed by the Bureau of Land Management, and 29,756 ha managed by the U. S. Forest Service. The state of South Dakota owns about 102,325 ha (South Dakota Office of School and Public Lands 2014) and the remainder is privately owned. The Little Missouri River drains most of the western part of the county. The North Fork of the Moreau River drains the southern part of the county while the central and north central parts of the county are drained primarily by the South Fork of the Grand River (Johnson 1988). About 2,700 ha of the county is water. A diverse topography of the area includes gentle rolling plains to steep slopes, badlands, sand dunes, and numerous large buttes with sandstone cap rock that provide areas with greatest elevations (Hogan and Hogan Fouberg 2001). Sand dune soils represent about 17,300 ha of the county. The largest buttes include the North and South Cave Hills, the East and West Short Pines and the Slim Buttes, which are covered with ponderosa pine (*Pinus ponderosa*) forest. The elevation ranges from 817 m in the southeast corner of the county near the Moreau River to 1224 m in the East Short Pines. Much of the surface area of the county is composed of sagebrush steppe and native and non-native grasslands with 88% used for livestock grazing (Johnson 1988).

The long-term average annual precipitation is 37 cm most of which falls in spring and as early summer thunder storms (Johnson 1988). Annual precipitation was 5 cm below the long-term average 32% of time the since 1894 and was 5 cm above the long-term average 26% of the years (High Plains Regional Climate Center 2014).

METHODS

Data for this project were obtained from vascular plant specimens collected during routine U.S. Forest Service activities, from a project at RM (acronyms follow Thiers 2014) in 2009-2010, and projects at BHSC from 2002-2013 (Gabel and Simonson 2008). Additional data were obtained from electronic databases queried for previous plant collections. Eleven databases were queried, and from those, four were found to contain significant information for this study. The four databases include Kartesz (2013), USDA NRCS (2014), Hartman et al. (2009), and the Black Hills State University Herbarium (2014). The USDA

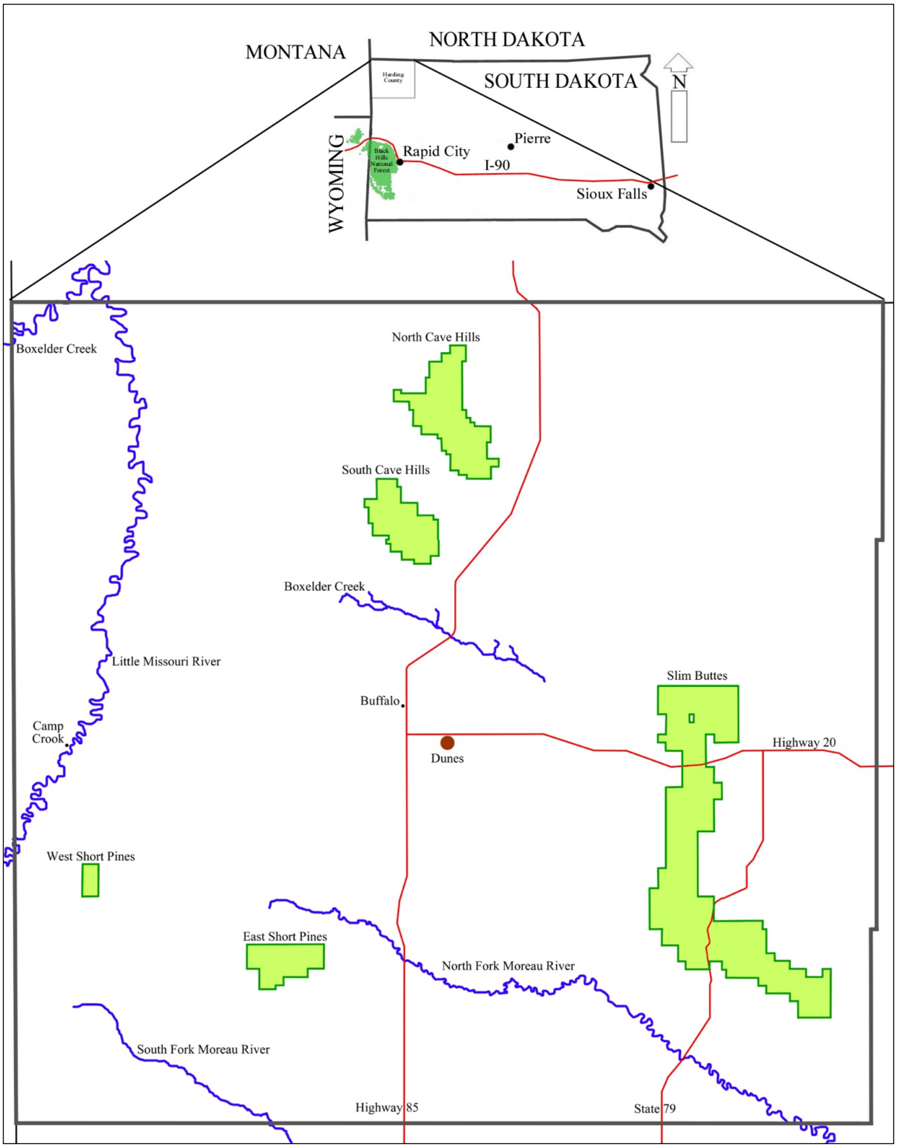

Figure 1. Location of project area and major areas sampled within Harding County, South Dakota.

NRCS and Kartesz sites often include county level information for plant species distributions. Of greater value were databases at RM, (which include specimens from USFS) and BHSC (which include data from DWU, GFND, NDA, NEB, NPWRC, SDC and SDU).

Visher collections were verified in several herbaria, primarily SDU and RM. Additional collections were made using the meander search method (Alberta Native Plant Council 2000). Plant names follow the Flora of North America (Flora of North America Editorial Committee 1993+) or for families that have not yet been completed, USDA Plants (USDA, NRCS 2014), the Integrated Taxonomic Information System (ITIS 2014) or Tropicos (2014). Synonyms used in previous works were updated for ease of analyses. Comparisons were made between modern records and historical collections.

Coefficients of Conservatism (C values) for most native plant species were obtained from the Northern Great Plains Floristic Quality Assessment Panel (2001). Mean C values ($\overline{C}$) were determined by summing the C values and dividing by the total number of native species present. Values were calculated for the entire county and for eight physiographic areas within the county. Floristic quality index (FQI) values were also calculated by using the formula FQI = $\overline{C}\sqrt{N}$ for eight areas within Harding County and the entire county.

Climate data for Harding County, South Dakota, was compiled from all available recording station records and all available data within and near Harding County, South Dakota. The combined long-term monthly precipitation totals are from January 1893 to May 2014; the long-term average was calculated from all available data from Antelope Range Station SD (3/1/1951 to 1/31/2008), Camp Crook SD (1/1/1893 to 3/31/2013), Ellingson 1 NW SD (6/1/1909 to 8/31/1963), Harding 3 SE SD (12/2/1951 to 3/31/2013), Ludlow SD (3/11/1924 to 3/31/2013), Ralph 3 NW SD (6/1/1941 to 7/31/2003), Redig 9 NE SD (10/13/1914 to 12/31/2011), and Knobs MT (9/5/1951 to 3/31/2013) listed on the High Plains Regional Climate Center website (http://www.hprcc.unl.edu/data/historical/ -- specifically monthly precipitation totals at http://www.hprcc.unl.edu/cgi-bin/cli_perl_lib/cliMAIN.pl?sd0223, sd1294, sd2614, sd3560, sd5048, sd6907, sd7062, and mt4715).

RESULTS

The oldest plant collection seen from Harding County was *Maianthemum racemosum* (L.) Link collected in 1893 by Williams (RM 443764). A list of vascular plant species present in Harding County reported by Visher (1914) contained 432 species names that are considered valid today. Work since that time has added 292 species to the flora of which 230 are native (Appendix 1). The cumulative number of species known from the county by decade is shown in Figure 2. In the intervening century, 63% of the original Visher era plant names have been changed (Flora of North America Editorial Committee 1993+; Integrated Taxonomic Information System 2014; Tropicos 2014).

Perhaps the most obvious change in the vegetation has been the increase of introduced species from five percent to 13% of the flora. The results from the comparisons of growth habit, major plant groups, and plant duration are in Figures 3-5. The data show that less than six percent change has occurred among any group or category within a group in the last 100 years. The largest change observed was a 5% increase in graminoids.

Mean coefficient of conservatism ($\overline{C}$) as determined based only on native species reported by Visher (1914) was 5.3, while $\overline{C}$ including all Visher reported native species plus native species reported in this study was 5.4 in 2014. The eight areas of the county with the most collections (plus data from sand dunes (Gabel and Simonson 2008)) are compared in Table 1. The highest $\overline{C}$ value (6.2) was in the West Short Pines of the Custer National Forest, while the highest FQI value was from the Slim Buttes area, also in the Custer National Forest. The lowest $\overline{C}$ value was calculated from the species in the Moreau River Valley (4.3), while the Shaw Creek plant species produced the lowest FQI value (20.1). Table 2 shows the average of $\overline{C}$ by category for the eight locations of this study (plus sand dune data), indicating that stream valleys are higher in low (0-3) and intermediate (4-6) values, while four of five large pine forested butte areas have greater percentages of higher values (7-10). The East Short Pines have similar percentages for all three categories.

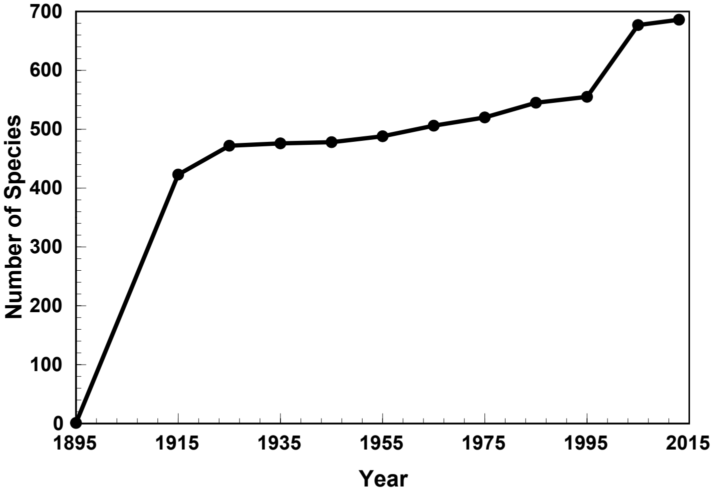

Figure 2. Cumulative number of species known from Harding County by decade.

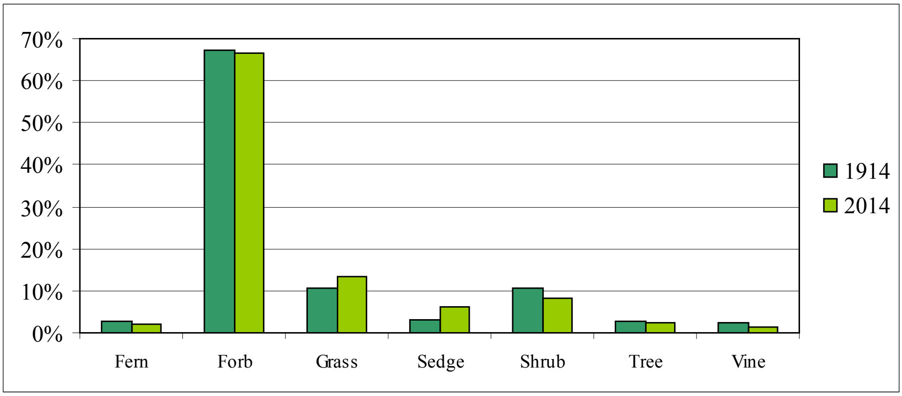

Figure 3. Percents of life form types for vascular plant species from Harding County, SD. Dark green bars indicate percent species reported in 1914. Light green bars indicate percent of species in this study.

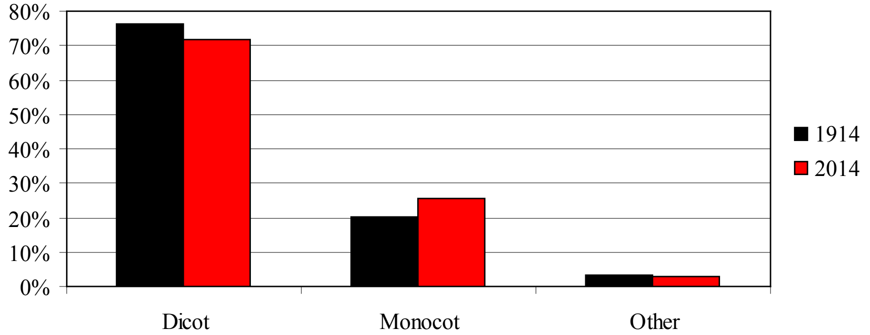

Figure 4. Percents of major vascular plant groups in Harding County, SD. Black bars indicate percent species reported in 1914. Red bars indicate percent of species in this study. "Dicots" include eudicots, and non-monocot paleoherbs. "Other" includes ferns, equisetophytes, lycopodiophytes and conifers.

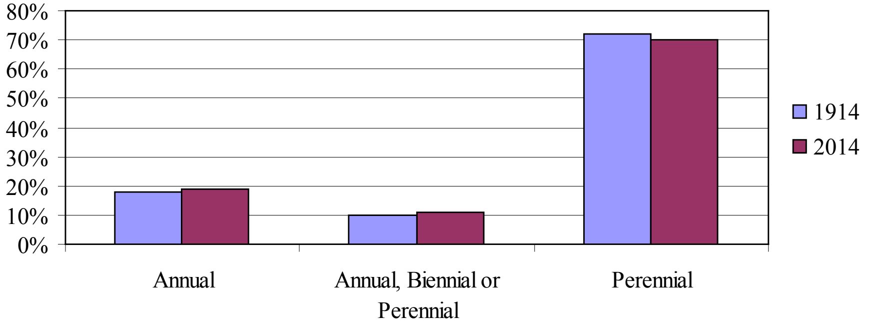

Figure 5. Percent vascular plant species by duration as reported by USDA NRCS Plants (2014). Left most bar of each pair represents species reported by Visher in 1914. Right bar of each pair represents data from this report. Middle pair of bars represents species that are reported as mixed duration by USDA NRCS (2014).

Table 1. Mean Coefficient of Conservatism values and floristic quality index values for eight sites in this study and sand dune values from Gabel and Simonson (2008) in Harding County, SD.

Site	Slim Buttes	N. Cave Hills	S. Cave Hills	E. Short Pines	W. Short Pines	Moreau River	Little Missouri River	Shaw Creek	Sand Dunes
mean C	5.8	5.9	5.9	5.1	6.2	4.3	4.4	4.4	4.5
FQI	81.8	61.4	46.6	35	52.6	24.9	48.8	20.1	38.7

Table 2. Percents of Coefficients of Conservatism (C) by category for eight sites in this study and sand dune values from Gabel and Simonson (2008) in Harding County, SD.

Site	Slim Buttes	N. Cave Hills	S. Cave Hills	E. Short Pines	W. Short Pines	Moreau River	Little Missouri River	Shaw Creek	Sand Dunes
0-3	21	17	16	30	14	38	35	24	32
4-6	36	40	37	34	28	44	41	57	44
7-10	44	43	47	36	58	18	21	19	24

DISCUSSION

Of the 432 names determined to be valid from the Visher (1914) list, 271 of the taxa these names represent now have names different than they did in 1914. The name changes are due to either application of nomenclatural rules (e.g., using the earliest validly published name) or new knowledge that results in a better understanding of the phylogeny of plant groups.

The consistency of values for growth habit, major plant groups, and plant duration (Figures 3-5) was surprising. The greatest change observed (5%) was the increase in number of graminoids, probably a result of more thorough collection and study of grasses and sedges.

Of the species observed in Harding County but not included in Visher's (1914) list, 79% are native while 62 species are introduced (with slightly less than 1% categorized as both by USDA NRCS 2014). The Northern Great Plains Floristic Quality Assessment Panel (2001) reported that 83% of the vascular plant species in the Dakotas were native. We surmised that the majority of the native species not included in Visher's 1914 list have not been introduced since 1914, but were present and overlooked until recently. In contrast, the increase in introduced species was expected given the human disturbance within Harding County over the last 100 years and the invasiveness of many of those species. We noted that the increase to 13% of introduced species countywide was higher than the 8% introduced species in the dune areas of the county (Gabel and Simonson 2008), but less than the 17% recorded for North and South Dakota in 2001 (Northern Great Plains Floristic Quality Assessment Panel 2001).

Mean coefficients of conservatism for the entire county changed little (from 5.3 to 5.4) between 1914 and 2014, however, several areas show a contrast in $\overline{C}$ values. Stream-related habitats were areas of lowest $\overline{C}$ values, perhaps because of the congregation in stream valleys by domesticated grazing mammals (Knight 1994) and wildlife together with disturbance from frequent flooding that favors ruderal species. Other areas with relatively low $\overline{C}$ values are sand dunes and surrounding swales ($\overline{C} = 4.5$) as reported by Gabel and Simonson (2008). The areas with highest $\overline{C}$ values are in more rugged or less accessible areas because of greater topographic relief and great variation of elevation, light, slope, aspect, and soil moisture. The greater $\overline{C}$ values for the five butte areas could also be the result of a greater level of sampling compared to riparian areas. Archaux et al. (2006) found a curvilinear relationship with level of exhaustiveness of sampling based on time. Differences among botanists, intensity of surveys, and number of different habitats investigated all influence the number of species that are recorded (Oredsson 2000). Plot size, plant size, and spatial patterns of individual plants also influence detection and frequency of species within an area (Bonham 2013). Time is another bias in species richness; generally greater time spent exploring an area results in greater species richness (Klimeš et al. 2001). Visher had just a few years to produce his list while the current list was assembled after a century of collection.

The FQI calculation is designed to limit the influence of area alone (Northern Great Plains Floristic Quality Assessment Panel 2001, Swink and Wilhelm 1979). The high FQI values for Slim Buttes could be partially due to the relatively large land area. As an example, the Slim Buttes area at 23,537 ha is approximately 45 times larger than the area sampled in West Short Pines. While the West Short Pines area has a high diversity and high $\overline{C}$ (6.2), the FQI is 52.6. In contrast the Slim Buttes area has $\overline{C}$ = 5.8 and the largest FQI value in the study at 81.8.

Kostel (2006) studied the vascular plant species of the Buffalo Gap National Grassland (241,473 ha) and the Oglala National Grassland (38,235 ha) in southwestern South Dakota and northwestern Nebraska. For the Buffalo Gap National Grassland $\overline{C}$ = 4.4 and FQI = 88.9, and for the Oglala National Grassland $\overline{C}$ = 4.4 and FQI = 76.9.

Kopp (2004) reported 462 species from the Grand River National Grassland in Perkins County, SD (adjacent to Harding County). He also noted that the flora included 14% introduced species. Kopp reported a $\overline{C}$ value of 4.6 for the Grand River National Grassland, with seven areas within the grassland ranging in $\overline{C}$ values from 4.0 to 5.7 and FQI values from 29.4 to 60.1.

It has been reported by Hamlin et al. (2012) and Angelo (2014) that even in some well studied and highly populated areas there is little change in the total number of native species although introduced species increased dramatically. Angelo (2014) warned against assigning causes to vegetation changes without multidisciplinary studies.

Forty-seven Visher specimens represent species not on his 1914 list. Possible explanations for their omission include failure to transmit specimens to Aven Nelson at the Rocky Mountain Herbarium, University of Wyoming, who identified collections for Visher, possibly because they were thought to be duplicate specimens. Possibly the authors of the 1914 list grouped some specimens of closely related species, or in the last 100 years the interpretation of species boundaries has changed for some taxa. Problematic species are listed in Appendix 2.

The consistency of plant species, habitat types, duration and plant groups, the high number of native species and the relatively large values for $\overline{C}$ and FQI lead us to conclude that the vascular flora of Harding County has remained relatively stable since Visher's 1914 work. This is further supported by an only 8% increase in introduced species compared to a 17% increase in exotic species for North and South Dakota during a similar time interval. The unregulated grazing (ca. 1870 and forward) that took place before enactment of the Taylor Grazing Act in 1934 caused unintended damage to soil, vegetation, streams and springs (Bureau of Land Management 2014), thus it is unknown what the vascular flora of Harding County might have lost before Visher's compilation.

Baseline studies, such as the present one, are critical to any understanding of environmental impacts and restoration efforts. While the overall vegetation of Harding County is apparently in good condition based on plant species composition, richness, and coefficients of conservatism, we anticipate greater human impacts in the near future and a resultant increase in the percent of introduced species and habitat degradation. For example, sagebrush habitat, important to sage grouse survival in Harding County, is continually being impacted with an array of anthropogenic disturbances. Single or isolated impacts may pose little risk alone, however, the cumulative impact is reducing, degrading and fragmenting sagebrush habitat (and other vegetation types). As a consequence, sage grouse (*Centrocercus urophasianus*), a species dependent on sagebrush habitat, are being extirpated in western South Dakota (Smith et al. 2004; South Dakota Department of Game, Fish and Parks 2014). In addition, sagebrush habitat in western South Dakota is important for other sagebrush obligate species, including Brewer's sparrow (*Spizella breweri*), sage thrasher (*Oreoscoptes montanus*), sage sparrow (*Amphispiza belli*), short-horned lizard (*Phrynosoa hemandesi*), sagebrush vole (*Lemmiscus curtatus*) and pronghorn (*Antilocapra americana*).

Watching and hunting animals have become important human activities and retaining habitat is important for the continued survival of those organisms. Visher (1914) estimated that within the county there were six species of amphibians, 15 species of reptiles (eight were listed), 50 mammal species and 200 bird species. Kiesow (2006) reported six amphibians and 11 reptiles in Harding County. Higgins et al. (2002) reported 51 species of mammals, and the South Dakota Ornithologist's Union (1991) reported 229 species of birds from the county.

The South Dakota Department of the Environment and Natural Resources (2014) has reported that there are 89,843 leased mineral hectares within Harding County. Lawson et al. (2011) recommended that to limit impacts of oil drilling on birds, noise should be reduced at well sites, vegetation disturbance should be limited especially around drill pads and roads, perching sites for birds should be maintained, and road construction should be limited. We concur with these recommendations. Maintaining high native plant diversity will be key to maintaining high animal diversity, and this is ultimately dependent on ranchers and other land managers continuing to use responsible management practices.

ACKNOWLEDGEMENTS

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Appendix 1. Vascular plant species of Harding County listed alphabetically by modern scientific names. Family names follow Stevens (2014), with abbreviations created by deleting "-aceae" from family names. C of C = Coefficients of Conservatism (Northern Great Plains Floristic Quality Assessment Panel 2001). "Visher 1914" indicates species listed by Visher (1914) with currently accepted name. Visher sp. = Visher specimen seen if value = 1. Source codes for databases: US = USDA NRCS Plants, BO = Biota of North America Project, RM = Rocky Mountain Herbarium, BH = Black Hills State University Herbarium, FS/CC = USDA Forest Service at Camp Crook, SD, SDDA = South Dakota Department of Agriculture. Visher specimens in databases are noted by Vi, while Fromme specimens are indicated by Fr.

Genus	Species	Family	C of C	Native/ Introduced	Visher 1914	Visher sp.	Source
Acer	negundo	Sapind.	1	N	1	1	US, BO, RM, Vi, BH Vi
Achillea	millefolium	Aster.	3	N/I	1	1	US, BO, RM Vi, BH Vi
Achnatherum	hymenoides	Po.	5	N	1	1	US, BO, RM Vi, BH Vi
Agastache	foeniculum	Lami.	7	N	1	1	US, BO, RM Vi, BH Vi, Fr
Agoseris	glauca	Aster.	8	N	1	1	US, BO, RM Vi, BH
Agrimonia	gryposepala	Rosaceae	5	N	0	1	BO, RM Vi, BH
Agrimonia	striata	Rosaceae	5	N	1	1	BO, RM, BH, Fr, Vi
Agropyron	cristatum	Poaceae	x	I	0	0	US, BO, RM, BH
Agrostemma	githago	Caryophyll.	x	I	1	0	US, BO,
Agrostis	gigantea	Po.	x	I	0	0	RM, BH
Agrostis	scabra	Po.	1	N	0	1	US, BO, RM Vi, BH Fr, Vi
Agrostis	stolonifera	Po.	x	I	0	0	BO,RM, BH
Agrostis	exarata	Po.	10	N	0	0	RM
Alisma	gramineum	Alismat.	2	N	0	0	BO, BH
Alisma	triviale	Alismat.	2	N	1	1	US, BO, RM, Vi, BH, Fr
Allium	geyeri	Alli.	10	N	1	0	BH
Allium	textile	Alli.	7	N	1	1	US, BO, RM Vi, BH Vi
Almutaster	pauciflorus	Aster.	10	N	1	1	US, BO, RM Vi, BH Vi
Alopecurus	aequalis	Po.	2	N	1	1	US, BO, RM Vi, BH Vi
Alopecurus	arundinaceus	Po.	x	I	0	0	BO, BH
Alopecurus	carolinianus	Po.	0	N	0	1	US, BO, RM, BH Vi
Alyssum	alyssoides	Brassic.	x	I	0	0	BO, RM, BH
Alyssum	desertorum	Brassic.	x	I	0	0	RM, BH
Amaranthus	albus	Amaranth.	x	I	1	1	BO, RM Vi, BH Vi
Amaranthus	retroflexus	Amaranth.	0	N	1	1	US, BO, RM Vi, BH Vi
Ambrosia	acanthicarpa	Aster.	0	N	1	1	BO, RM Vi, BH
Ambrosia	artemisiifolia	Aster.	0	N	1	1	BO, RM Vi
Ambrosia	psilostachya	Aster.	2	N	1	1	US, BO, RM Vi, BH Vi
Ambrosia	trifida	Aster.	0	N	1	1	US, BO, BH Vi, Fr
Amelanchier	alnifolia	Ros.	6	N	1	1	US, BO, RM Vi, BH Vi
Amelanchier	humilis	Ros.	6	N	0	0	US - Not Seen

Amelanchier	utahensis	Ros.	?	N	0	0	BO, RM
Amorpha	canescens	Fab.	9	N	1	0	BO, RM
Anagallis	minima	Myrsin.	?	N	0	0	RM
Andropogon	gerardii	Po.	5	N	1	1	US, BO, RM Vi, BH Vi, Fr
Andropogon	hallii	Po.	5	N	1	1	US, BO, RM Vi, BH Fr, Vi
Androsace	occidentalis	Primul.	5	N	1	0	US, BO, RM, BH
Androsace	septentrionalis	Primul.	5	N	0	0	BO, BH
Anemone	cylindrica	Ranuncul.	7	N	1	1	US, BO, RM Vi, BH Vi
Antennaria	corymbosa	Aster.	?	N	0	0	BH
Antennaria	howellii	Aster.	5	N	0	0	BO, RM, BH
Antennaria	microphylla	Aster.	7	N	0	0	US, BO, RM, BH
Antennaria	neglecta	Aster.	5	N	0	0	BO, RM, BH
Antennaria	parvifolia	Aster.	6	N	1	1	US, BO, RM Vi, BH
Antennaria	rosea	Aster.	?	N	1	1	RM, BH Fr
Apocynum	androsaemifolium	Apocyn.	6	N	1	1	US, BO, RM Vi, BH Fr
Apocynum	cannabinum	Apocyn.	4	N	0	1	US, BO, BH Fr
Arabis	pycnocarpa	Brassic.	7	N	1	0	US, BO, RM Vi
Arctium	minus	Aster.	x	I	0	0	BO - Not Seen
Arctostaphylos	uva-ursi	Eric.	8	N	1	1	US, BO, RM Vi, BH Vi
Aristida	purpurea	Po.	4	N	1	1	US, BO, RM Vi, BH Vi, Fr
Arnica	fulgens	Aster.	10	N	0	0	US, BO, RM, BH
Arnica	sororia	Aster.	10	N	0	0	BH
Artemisia	absinthium	Aster.	x	I	0	0	BO, BH
Artemisia	biennis	Aster.	0	N/I	1	1	US, BO, RM Vi, BH Vi
Artemisia	campestris	Aster.	5	N	1	1	US, BO, RM Vi, BH Vi, Fr
Artemisia	cana	Aster.	7	N	1	1	US, BO, RM Vi, BH Fr
Artemisia	dracunculus	Aster.	4	N	1	0	US, BO, RM, BH
Artemisia	frigida	Aster.	4	N	1	1	US, BO, RM Vi, BH Vi, Fr
Artemisia	longifolia	Aster.	7	N	1	1	US, BO, RM Vi, BH Vi
Artemisia	ludoviciana	Aster.	3	N	1	1	US, BO, RM Vi, BH Vi, Fr
Artemisia	tridentata	Aster.	7	N	1	1	US, BO, RM Vi, BH Vi
Asclepias	pumila	Apocyn.	7	N	1	1	US, BO, RM Vi, BH Vi, Fr
Asclepias	speciosa	Apocyn.	4	N	1	1	US, BO, RM Vi, BH Vi
Asclepias	verticillata	Apocyn.	3	N	1	0	US, BO, BH
Asclepias	viridiflora	Apocyn.	8	N	0	1	US, BO, RM Vi, BH
Asparagus	officinalis	Asparag.	x	I	1	1	BO, RM Vi
Astragalus	agrestis	Fab.	6	N	0	0	US, BO, RM, BH
Astragalus	alpinus	Fab.	?	N	0	0	BH
Astragalus	australis	Fab.	9	N	0	0	US, BO, RM, BH
Astragalus	bisulcatus	Fab.	5	N	1	1	US, BO, RM Vi, BH Vi

Astragalus	canadensis	Fab.	5	N	1	1	BO, RM Vi, BH Fr
Astragalus	ceramicus	Fab.	10	N	0	0	US, BO, RM, BH
Astragalus	crassicarpus	Fab.	7	N	1	1	US, BO, RM Vi, BH Vi
Astragalus	flexuosus	Fab.	4	N	0	1	US, BO, RM Vi, BH
Astragalus	gilviflorus	Fab.	7	N	0	0	US, BO, RM, BH
Astragalus	gracilis	Fab.	8	N	0	0	US, BO, RM, BH
Astragalus	laxmannii	Fab.	8	N	0	0	US, BO, BH
Astragalus	lotiflorus	Fab.	6	N	1	1	US, BO, RM Vi, BH
Astragalus	missouriensis	Fab.	7	N	0	0	US, BO, RM, BH
Astragalus	pectinatus	Fab.	8	N	0	0	BH
Astragalus	plattensis	Fab.	10	N	0	0	RM, BH
Astragalus	purshii	Fab.	10	N	1	0	BH
Astragalus	racemosus	Fab.	7	N	0	0	US, BO, BH
Astragalus	simplicifolius	Fab.	?	N	0	1	RM Vi
Astragalus	spatulatus	Fab.	7	N	1	0	US, BO, BH
Astragalus	tenellus	Fab.	8	N	0	0	US, RM, BH
Astragalus	vexilliflexus	Fab.	10	N	1	1	US, BO, RM Vi, BH Fr
Atriplex	argentea	Amaranth.	6	N	1	1	US, BO, RM Vi, BH
Atriplex	canescens	Amaranth.	6	N	1	0	US, BO, RM, BH
Atriplex	dioica	Amaranth.	4	N	0	1	US, BO, RM Vi
Atriplex	gardneri	Amaranth.	6	N	0	1	US, BO, RM Vi, BH
Atriplex	nuttallii	Amaranth.	6	N	0	1	BH Vi, Fr
Atriplex	patula	Amaranth.	x	I	0	0	US, BO, BH Fr
Atriplex	suckleyi	Amaranth.	4	N	0	1	US, RM Vi, BH Vi, Fr
Avena	fatua	Po.	x	I	0	0	BO, BH
Avenula	hookeri	Po.	9	N	0	0	BO, BH
Bacopa	rotundifolia	Plantagin.	3	N	0	0	US, BO, BH
Bassia	scoparia	Amaranth.	x	I	0	0	US, BO, RM, BH
Beckmannia	syzigachne	Po.	1	N	1	1	US, BO, RM Vi, BH Vi, Fr
Berberis	repens	Berberid.	8	N	1	1	US, BO, RM Vi, BH Vi, Fr
Besseya	wyomingensis	Plantagin.	?	N	0	0	BO, BH
Betula	occidentalis	Betul.	8	N	1	1	US, BO, BH Vi
Betula	papyrifera	Betul.	8	N	1	1	US, BO, RM Vi, BH Vi
Bidens	cernua	Aster.	3	N	1	1	BO, BH Vi, Fr
Bidens	vulgata	Aster.	1	N	1	1	US, BO, RM Vi, BH Vi, Fr
Boechera	collinsii	Brassic.	?	N	1	1	RM Vi, BH Vi
Boechera	retrofracta	Brassic.	5	N	0	1	US, RM, BH Vi
Boechera	stricta	Brassic.	8	N	0	0	BH
Bolboschoenus	maritimus	Cyper.	4	N	1	0	BO, RM Vi, BH
Botrychium	virginianum	Ophiogloss.	7	N	1	1	US, BH Vi

Bouteloua	curtipendula	Po.	5	N	1	1	BO, RM Vi, BH Vi, Fr
Bouteloua	dactyloides	Po.	4	N	1	1	US, BO, RM Vi, BH Vi, Fr
Bouteloua	gracilis	Po.	7	N	1	1	US, BO, RM Vi, BH Vi, Fr
Bouteloua	hirsuta	Po.	7	N	0	0	RM
Brickellia	eupatorioides	Aster.	5	N	1	1	US, BO, RM Vi, BH Vi, Fr
Bromus	carinatus	Po.	?	N	0	0	RM
Bromus	ciliatus	Po.	10	N	1	1	US, BO, BH Vi
Bromus	commutatus	Po.	x	I	0	0	RM
Bromus	inermis	Po.	x	I	1	1	BO, RM Vi, BH Fr
Bromus	japonicus	Po.	x	I	0	0	US, BO, RM, BH
Bromus	latiglumis	Po.	8	N	1	0	BH Fr
Bromus	porteri	Po.	8	N	1	1	RM, BH Fr
Bromus	squarrosus	Po.	x	I	0	0	RM, BH
Bromus	tectorum	Po.	x	I	0	0	US, BO, RM, BH
Calamagrostis	montanensis	Po.	8	N	0	0	BO, BH
Calamagrostis	stricta	Po.	5	N	1	0	US, BO, BH
Calamovilfa	longifolia	Po.	5	N	1	1	US, BO, RM Vi, BH Vi, Fr
Callitriche	hermaphroditica	Plantagin.	7	N	1	1	US, BO, RM Vi, BH Vi
Callitriche	heterophylla	Plantagin.	10	N	0	1	US, BO, RM Vi, BH
Callitriche	palustris	Plantagin.	7	N	1	1	RM Vi
Calochortus	nuttallii	Lili.	8	N	1	1	US, BO, RM Vi, BH Vi
Calylophus	serrulatus	Onagr.	7	N	0	1	US, BO, RM Vi, BH Fr
Calystegia	sepium	Convolvul.	x	I	1	1	US, BO, RM Vi
Camelina	microcarpa	Brassic.	x	I	0	0	US, BO, RM, BH
Camelina	sativa	Brassic.	x	I	1	0	US, BO, BH
Campanula	rotundifolia	Campanul.	7	N	1	1	US, BO, RM Vi, BH Vi, Fr
Capsella	bursa-pastoris	Brassic.	x	I	1	0	RM
Carex	atherodes	Cyper.	4	N	0	0	BO, BH
Carex	aurea	Cyper.	8	N	1	1	BO, RM Vi
Carex	backii	Cyper.	10	N	0	0	BH
Carex	brevior	Cyper.	4	N	0	1	US, BO, RM Vi, BH Vi, Fr
Carex	canescens	Cyper.	?	N	0	0	BH
Carex	duriuscula	Cyper.	4	N	0	0	US, BO, BH
Carex	filifolia	Cyper.	7	N	0	0	US, BO, RM, BH
Carex	gravida	Cyper.	5	N	1	1	US, BO, RM, BH
Carex	hoodii	Cyper.	?	N	0	0	RM, BH
Carex	hystericina	Cyper.	7	N	0	1	BO, RM Vi, BH
Carex	inops	Cyper.	?	N	0	0	US, BO, RM, BH
Carex	laeviconica	Cyper.	6	N	0	1	US, BO, RM Vi
Carex	laeviculmis	Cyper.	10	N	0	1	BH Vi

Carex	lasiocarpa	Cyper.	4	N	1	0	BH
Carex	molesta	Cyper.	3	N	0	0	US, BO, BH
Carex	peckii	Cyper.	10	N	0	0	BH
Carex	pellita	Cyper.	4	N	0	1	US, BO, BH Vi
Carex	pensylvanica	Cyper.	8	N	0	1	BH Vi
Carex	praeceptorum	Cyper.	?	N	0	1	BH Vi
Carex	praegracilis	Cyper.	5	N	0	0	US, BO, RM, BH
Carex	rossii	Cyper.	10	N	0	0	BH
Carex	sartwellii	Cyper.	5	N	1	1	BO, RM Vi,
Carex	saximontana	Cyper.	10	N	0	0	BO, RM, BH
Carex	sprengelii	Cyper.	7	N	0	1	US, BO, RM Vi, BH Vi
Carex	torreyi	Cyper.	10	N	1	1	US, BO, RM Vi, BH
Carex	vallicola	Cyper.	10	N	0	0	US, BO, BH
Carex	vesicaria	Cyper.	10	N	1	1	BO - Not Seen
Carex	vulpinoidea	Cyper.	2	N	0	1	US, BO, RM, BH Vi
Carex	xerantica	Cyper.	10	N	0	1	US, BO, RM, BH Fr
Castilleja	sessiliflora	Orobanch.	8	N	1	1	US, BO, RM, BH Vi
Catabrosa	aquatica	Po.	9	N	1	1	US, BO, RM Vi, BH Vi
Celastrus	scandens	Celastr.	5	N	1	1	US, BO, RM Vi, BH Vi
Cenchrus	longispinus	Po.	0	N	0	0	US, BO, BH
Cerastium	arvense	Caryophyll.	2	N/I	1	1	US, BO, RM Vi, BH
Ceratophyllum	demersum	Ceratophyll.	4	N	0	0	BO, BH
Chaenactis	douglasii	Aster.	9	N	1	1	BO, BH Vi
Chamaerhodos	erecta	Ros.	6	N	0	1	US, BO, RM, BH Fr
Chamerion	angustifolium	Onagr.	5	N	1	1	US, BO, RM Vi, BH Vi, Fr
Cheilanthes	feei	Pterid.	10	N	0	0	US, BO, BH
Chenopodium	berlandieri	Amaranth.	0	N	0	0	US, BO, RM Vi, BH Fr
Chenopodium	desiccatum	Amaranth.	5	N	0	1	US, BO, RM Vi, BH Fr
Chenopodium	fremontii	Amaranth.	3	N	1	1	US, BO, RM Vi, BH Vi
Chenopodium	glaucum	Amaranth.	x	I	1	1	US, BO, RM Vi, BH Fr
Chenopodium	leptophyllum	Amaranth.	?	N	1	0	BH
Chenopodium	pratericola	Amaranth.	5	N	0	1	BO, RM Vi, BH Vi
Chenopodium	rubrum	Amaranth.	2	N	1	0	BO, BH
Chenopodium	simplex	Amaranth.	5	N	1	1	US, BO, RM Vi, BH
Chenopodium	subglabrum	Amaranth.	8	N	0	1	BO, BH Fr
Chorispora	tenella	Brassic.	x	I	0	0	RM, BH
Cichorium	intybus	Aster.	x	I	0	0	FS/CC Hansen
Cicuta	maculata	Api.	4	N	1	1	US, BO, RM Vi, BH Vi, Fr
Cirsium	arvense	Aster.	x	I	0	0	US, BO, RM,
Cirsium	canescens	Aster.	8	N	1	1	BO, RM Vi, BH

Cirsium	flodmanii	Aster.	5	N	0	1	US, BO, RM Vi, BH Vi
Cirsium	undulatum	Aster.	7	N	1	1	US, BO, RM Vi, BH Vi, Fr
Cirsium	vulgare	Aster.	x	I	0	0	BO, RM
Clematis	ligusticifolia	Ranuncul.	7	N	1	1	US, BO, BH Fr
Collinsia	parviflora	Plantagin.	10	N	0	0	RM
Collomia	linearis	Polemoni.	5	N	1	1	US, BO, RM Vi, BH Vi, Fr
Comandra	umbellata	Santal.	8	N	1	1	US, BO, RM Vi, BH Vi
Conium	maculatum	Api.	x	I	0	0	US, BO, BH
Convolvulus	arvensis	Convolvul.	x	I	0	0	US, BO, RM
Conyza	canadensis	Aster.	0	N	1	1	US, RM Vi, BH Vi
Corallorhiza	maculata	Orchid.	8	N	0	0	BO, BH
Coreopsis	tinctoria	Aster.	3	N	1	0	US, BO - Not Seen
Corispermum	americanum	Amaranth.	?	N	1	1	US, BO, RM Vi, BH Vi
Cornus	canadensis	Corn.	10	N	1	1	US, BO, RM Vi,
Cornus	sericea	Corn.	5	N	1	1	US, RM Vi, BH Vi, Fr
Corydalis	aurea	Papaver.	4	N	1	1	US, BO - Not Seen
Coryphantha	vivipara	Cact.	10	N	1	1	US, BO, RM Vi,
Crataegus	chrysocarpa	Ros.	6	N	0	1	US, BO, RM, BH Vi
Crataegus	succulenta	Ros.	5	N	0	0	BO, BH
Crepis	occidentalis	Aster.	8	N	0	0	US, BO, RM, BH
Crepis	runcinata	Aster.	8	N	1	1	US, BO, BH Vi
Cryptantha	celosioides	Boragin.	8	N	1	1	US, BO, RM Vi, BH Vi, Fr
Cryptantha	fendleri	Boragin.	4	N	0	0	BO, BH
Cryptantha	thyrsiflora	Boragin.	?	N	0	0	US, RM
Cryptantha	torreyana	Boragin.	6	N	0	0	BH
Cyclachaena	xanthifolia	Aster.	0	N	1	1	BO, RM Vi, BH Vi
Cycloloma	atriplicifolium	Amaranth.	1	N	0	0	BH
Cymopterus	glomeratus	Api.	8	N	0	0	US, BH
Cymopterus	montanus	Api.	8	N	0	0	BH
Cyperus	schweintzii	Cyper.	5	N	0	0	BH
Cystopteris	fragilis	Dryopterid.	8	N	1	1	US, BO, RM Vi, BH
Dalea	candida	Fab.	8	N	1	1	US, BO, RM Vi, BH Fr
Dalea	purpurea	Fab.	8	N	1	1	US, BO, RM Vi, BH Fr
Dalea	villosa	Fab.	8	N	1	1	US, BO, RM Vi, BH Vi, Fr
Dasiphora	fruticosa	Ros.	5	N	1	1	US, BO, RM Vi, BH Vi
Delphinium	bicolor	Ranuncul.	7	N	0	0	BO, RM, BH
Delphinium	nuttallianum	Ranuncul.	?	N	0	0	RM
Descurainia	pinnata	Brassic.	1	N	1	1	US, BO, RM Vi, BH
Dichanthelium	oligosanthes	Po.	6	N	0	0	RM
Dieteria	canescens	Aster.	8	N	1	1	US, BO, RM Vi, BH Fr

Distichlis	spicata	Po.	2	N	1	1	US, BO, RM, BH Vi
Draba	nemorosa	Brassic.	1	N	1	0	US, BO, RM, BH
Draba	reptans	Brassic.	1	N	0	0	RM, BH
Drymocallis	arguta	Ros.	8	N	1	0	US, BO, RM
Drymocallis	glandulosa	Ros.	?	N	0	0	RM
Dryopteris	filix-mas	Dryopterid.	10	N	1	1	US, BO, RM Vi, BH Vi, Fr
Dysphania	ambrosioides	Amaranth.	x	I	1	0	BO, BH
Dyssodia	papposa	Aster.	0	N	0	0	US, BO, RM
Echinacea	angustifolia	Aster.	7	N	1	1	US, BO, RM Vi, BH Fr
Echinochloa	crus-galli	Po.	x	I	1	0	BH
Echinochloa	muricata	Po.	0	N	0	1	BO, RM Vi, BH Vi, Fr
Echinocystis	lobata	Cucurbit.	3	N	1	1	US, BO, RM Vi, BH Vi
Elaeagnus	angustifolia	Elaeagn.	x	I	0	0	RM
Eleocharis	acicularis	Cyper.	3	N	1	1	US, BO, RM Vi, BH Fr
Eleocharis	erythropoda	Cyper.	2	N	0	1	US, RM, BH Fr
Eleocharis	macrostachya	Cyper.	4	N	0	0	US, BH
Eleocharis	palustris	Cyper.	?	N	1	1	US, BO, RM, BH Vi
Eleocharis	parvula	Cyper.	10	N	0	0	BO, BH
Ellisia	nyctelea	Boragin.	0	N	1	1	US, BO, RM Vi, BH Vi
Elyhordeum	macounii	Po.	?	N	1	1	BO, BH Vi
Elymus	canadensis	Po.	3	N	1	1	US, BO, RM Vi, BH Vi, Fr
Elymus	elymoides	Po.	6	N	0	0	US, BO, BH
Elymus	glaucus	Po.	10	N	1	0	US, BO, RM
Elymus	lanceolatus	Po.	7	N	1	1	US, BO, RM, BH Vi
Elymus	repens	Po.	0	I	0	0	BO, BH
Elymus	trachycaulus	Po.	6	N	1	1	US, BO, RM Vi, BH Vi, Fr
Elymus	villosus	Po.	4	N	0	1	BO, RM, BH Fr
Elymus	virginicus	Po.	4	N	1	0	BO, BH Fr
Elymus	wiegandii	Po.	?	N	0	0	BH
Epilobium	brachycarpum	Onagr.	?	N	0	0	RM
Epilobium	ciliatum	Onagr.	3	N	1	1	BO, BH Fr
Epilobium	leptocarpum	Onagr.	?	N	1	1	RM Vi
Equisetum	arvense	Equiset.	4	N	1	1	US, BO, RM Vi, BH Vi
Equisetum	hyemale	Equiset.	3	N	1	1	US, BO, RM Vi, BH Vi
Equisetum	laevigatum	Equiset.	3	N	1	1	US, BO, RM Vi, BH Vi
Equisetum	variegatum	Equiset.	10	N	0	0	RM
Equisetum	x ferrissii	Equiset.	3	N	0	0	RM
Eragrostis	cilianensis	Po.	x	I	0	0	US, BO, RM, BH
Ericameria	nauseosa	Aster.	4	N	1	1	US, BO, RM, BH Vi, Fr
Erigeron	annuus	Aster.	3	N	1	1	US, BO, RM Vi, BH Vi

Erigeron	caespitosus	Aster.	10	N	0	0	BO, RM
Erigeron	compositus	Aster.	10	N	0	0	US, BO, RM, BH
Erigeron	divergens	Aster.	10	N	1	1	US, BO, RM Vi, BH Fr
Erigeron	glabellus	Aster.	7	N	1	1	BO, RM Vi, BH
Erigeron	pumilus	Aster.	8	N	1	0	US, BO - Not Seen
Erigeron	radicatus	Aster.	10	N	0	0	BH
Erigeron	strigosus	Aster.	3	N	0	1	US, BO, RM, BH Fr
Eriogonum	annuum	Polygon.	6	N	1	1	US, BO, RM Vi, BH Fr
Eriogonum	cernuum	Polygon.	9	N	1	1	US, BO, RM Vi, BH Vi
Eriogonum	flavum	Polygon.	7	N	1	1	US, BO, RM, BH Fr
Eriogonum	pauciflorum	Polygon.	5	N	1	1	US, BO, RM Vi, BH Fr
Eriogonum	visheri	Polygon.	8	N	1	1	BO, BH
Eriogonum	x nebraskense	Polygon.	?	N	0	0	RM
Erysimum	asperum	Brassic.	3	N	1	1	BO, RM Vi, BH
Erysimum	cheiranthoides	Brassic.	x	I	1	1	US, BO, RM Vi, BH Vi, Fr
Erysimum	inconspicuum	Brassic.	7	N	1	1	US, BO, RM, BH Fr
Euphorbia	esula	Euphorbi.	x	I	0	0	RM, SDDA
Euphorbia	geyeri	Euphorbi.	1	N	0	0	BH
Euphorbia	glyptosperma	Euphorbi.	0	N	1	0	US, BO, RM Vi, BH Vi, Fr
Euphorbia	hexagona	Euphorbi.	2	N	0	0	BO, BH Vi
Euphorbia	missurica	Euphorbi.	4	N	0	1	US, BO, RM Vi, BH Vi
Euphorbia	serpyllifolia	Euphorbi.	?	N	0	0	RM
Euphorbia	spathulata	Euphorbi.	5	N	1	1	US, BO, BH Vi
Fallopia	convolvulus	Polygon.	x	I	1	1	US, BO, RM Vi, BH Fr
Festuca	saximontana	Po.	8	N	0	0	BH
Fragaria	vesca	Ros.	6	N	1	0	BO, RM
Fragaria	virginiana	Ros.	4	N	0	1	BO, RM Vi, BH
Fraxinus	pennsylvanica	Ole.	5	N	1	1	US, BO, RM Vi, BH Vi
Fritillaria	atropurpurea	Lili.	10	N	1	1	US, BO, RM, BH Vi
Gaillardia	aristata	Aster.	5	N	0	0	BO, BH
Galium	boreale	Rubi.	4	N	1	1	US, BO, RM Vi, BH Fr
Galium	trifidum	Rubi.	8	N	0	1	BO, BH Fr
Galium	triflorum	Rubi.	7	N	1	0	US, BO - Not Seen
Galium	aparine	Rubi.	0	N	0	0	RM
Gayophytum	diffusum	Onagr.	?	N	1	1	BH Fr
Gentiana	affinis	Gentian.	10	N	1	1	BO, RM Vi, BH
Gentianella	amarella	Gentian.	7	N	1	1	US, BO, RM Vi, BH
Geranium	bicknellii	Gerani.	3	N	0	0	BO, BH
Geranium	carolinianum	Gerani.	5	N	0	0	US, BO, RM
Geum	aleppicum	Ros.	4	N	1	1	US, BO, RM Vi, BH Fr

Geum	canadense	Ros.	4	N	1	1	BO, RM, BH Fr
Geum	triflorum	Ros.	8	N	1	1	US, BO, RM Vi, BH Vi
Gleditsia	triacanthos	Fab.	6	N	0	0	RM
Glyceria	grandis	Po.	4	N	0	1	US, BO, RM, BH Vi
Glyceria	striata	Po.	6	N	1	1	US, BO, RM Vi,
Glycyrrhiza	lepidota	Fab.	2	N	1	0	US, BO, RM, BH
Gratiola	neglecta	Plantagin.	0	N	1	1	US, BO, RM Vi, BH Vi
Grindelia	hirsutula	Aster.	?	N	1	1	RM Vi
Grindelia	squarrosa	Aster.	1	N	1	1	US, BO, BH
Gutierrezia	sarothrae	Aster.	6	N	1	1	US, BO, BH Vi, Fr
Hackelia	deflexa	Boragin.	0	N	0	1	US, BO, RM Vi, BH
Hackelia	virginiana	Boragin.	0	N	0	1	BO, BH Fr
Hedeoma	drummondii	Lami.	4	N	1	1	US, BO, RM Vi, BH Fr
Hedeoma	hispida	Lami.	2	N	1	1	BO, BH Fr
Helianthemum	bicknellii	Cist.	10	N	0	0	RM
Helianthus	annuus	Aster.	0	N	1	1	US, BO, RM Vi
Helianthus	maximiliani	Aster.	5	N	1	1	US, BO, RM Vi, BH Vi, Fr
Helianthus	pauciflorus	Aster.	8	N	0	1	US, BO, RM Vi, BH Vi, Fr
Helianthus	petiolaris	Aster.	0	N	1	1	US, BO, RM Vi, BH Vi, Fr
Heracleum	maximum	Api.	3	N	1	1	BO, RM Vi, BH Fr
Hesperis	matronalis	Brassic.	x	I	0	0	RM
Hesperostipa	comata	Po.	6	N	1	1	US, BO, RM Vi, BH Vi
Hesperostipa	spartea	Po.	8	N	0	0	RM, BH
Heterotheca	villosa	Aster.	3	N	1	1	US, BO, RM Vi, BH Vi, Fr
Heuchera	richardsonii	Saxifrag.	8	N	1	1	US, BO, RM Vi, BH Vi
Hieracium	umbellatum	Aster.	6	N	1	1	BO, RM Vi, BH Fr
Hordeum	jubatum	Po.	0	N	1	1	US, BO, RM Vi, BH Vi, Fr
Hordeum	pusillum	Po.	0	N	0	1	US, BO, RM Vi, BH Vi
Hordeum	vulgare	Po.	x	I	0	0	BO, BH
Humulus	lupulus	Cannab.	3	N/I	1	1	US, BO, RM Vi, BH Vi
Hymenopappus	filifolius	Aster.	8	N	1	1	US, BO, RM Vi, BH
Hymenopappus	tenuifolius	Aster.	8	N	0	1	US, BO, BH Fr
Ipomopsis	congesta	Polemoni.	8	N	0	1	US, BO, BH Vi, Fr
Iva	axillaris	Aster.	4	N	0	0	US, BO, RM, BH
Juncus	arcticus	Junc.	5	N	1	1	US, BO, RM Vi, BH
Juncus	bufonius	Junc.	1	N	0	0	US, BO, RM, BH Fr
Juncus	dudleyi	Junc.	4	N	0	0	US, BO, BH
Juncus	interior	Junc.	5	N	0	1	US, BO, RM Vi, BH Vi
Juncus	longistylis	Junc.	10	N	1	1	US, BO, RM Vi, BH Vi
Juncus	nodosus	Junc.	7	N	1	1	US, BO, RM Vi, BH Vi

Juncus	torreyi	Junc.	2	N	1	1	BO, RM Vi, BH Fr
Juniperus	communis	Cupress.	5	N	1	1	US, BO, RM Vi, BH Vi, Fr
Juniperus	horizontalis	Cupress.	6	N	0	1	US, BO, RM Vi, BH Vi
Juniperus	scopulorum	Cupress.	4	N	1	1	BO, RM Vi, BH Vi
Koeleria	macrantha	Po.	7	N	1	1	US, BO, BH Vi, Fr
Krascheninnikovia	lanata	Amaranth.	8	N	1	1	US, BO, BH
Lactuca	canadensis	Aster.	6	N	0	0	RM
Lactuca	serriola	Aster.	x	I	0	0	US, BO, RM,
Ladeania	lanceolata	Fab.	6	N	1	1	US, BO, RM Vi, BH Vi, Fr
Lappula	squarrosa	Boragin.	x	I	0	0	BO, BH
Lappula	occidentalis	Boragin.	2	N	1	1	US, BO, RM, BH
Lemna	minor	Ar.	9	N	1	1	US, BO, RM Vi, BH Vi
Lemna	turionifera	Ar.	1	N	0	0	BO, RM
Lepidium	densiflorum	Brassic.	0	N	0	1	BO, RM Vi, BH Vi, Fr
Lepidium	perfoliatum	Brassic.	x	I	0	0	RM
Lepidium	virginicum	Brassic.	0	N	1	0	US, BO, BH
Leptochloa	fusca	Po.	?	N	0	0	US, BO, BH
Leptosiphon	septentrionalis	Polemoni.	10	N	0	0	US, BO, BH
Leucocrinum	montanum	Asparag.	10	N	1	0	US, BO, BH
Liatris	punctata	Aster.	7	N	1	1	US, BO, RM Vi, BH Fr
Limosella	aquatica	Plantagin.	2	N	0	0	US, BO, RM, BH
Linnaea	borealis	Caprifoli.	10	N	1	0	US, BO - Not Seen
Linum	compactum	Lin.	?	N	0	1	US, BO, RM Vi
Linum	lewisii	Lin.	6	N	1	0	US, BO, RM, BH
Linum	perenne	Lin.	x	I	0	0	BH
Linum	rigidum	Lin.	5	N	1	1	US, BO, RM Vi, BH Fr
Lithospermum	incisum	Boragin.	7	N	1	0	US, BO, RM, BH
Logfia	arvensis	Aster.	x	I	0	0	RM
Lomatium	foeniculaceum	Api.	6	N	0	0	US, BO, RM, BH
Lomatium	macrocarpum	Api.	8	N	1	0	US, BO - Not Seen
Lotus	purshianus	Fab.	3	N	1	1	US, BO, RM Vi, BH Vi, Fr
Lupinus	argenteus	Fab.	8	N	1	1	US, BO, RM Vi, BH Vi
Lupinus	pusillus	Fab.	6	N	1	1	US, BO, RM Vi, BH Fr
Lycopus	americanus	Lami.	4	N	1	1	US, BO, BH Vi
Lycopus	asper	Lami.	4	N	1	1	BO, RM Vi, BH Fr
Lygodesmia	juncea	Aster.	2	N	1	1	US, BO, RM Vi, BH
Lysimachia	ciliata	Myrsin.	6	N	1	1	US, BO, RM Vi, BH Vi, Fr
Madia	glomerata	Aster.	1	N	1	1	BO, RM Vi, BH Vi, Fr
Maianthemum	racemosum	Asparag.	9	N	0	0	BO, RM
Maianthemum	stellatum	Asparag.	5	N	1	1	US, BO, RM Vi, BH Vi

Malva	neglecta	Malv.	x	I	0	0	RM
Malva	pusilla	Malv.	x	I	0	0	RM
Marsilea	vestita	Marsile.	2	N	1	1	US, BO, RM Vi, BH Vi
Matricaria	discoidea	Aster.	x	I	0	0	BO, BH
Medicago	lupulina	Fab.	x	I	0	0	BH
Medicago	sativa	Fab.	x	I	0	0	US, BO, BH
Meliltous	officinalis	Fab.	x	I	1	1	US, BO, RM, BH Fr
Mentha	arvensis	Lami.	3	N	1	1	US, BO, RM Vi, BH Fr
Mentzelia	decapetala	Loas.	5	N	1	1	US, BO, RM Vi, BH Fr
Mentzelia	dispersa	Loas.	2	N	0	0	BO, RM, BH
Mertensia	ciliata	Boragin.	10	N	0	0	BO, BH
Mertensia	lanceolata	Boragin.	9	N	0	0	US, BO, RM Vi, BH Vi
Mertensia	oblongifolia	Boragin.	9	N	0	1	US, BH Vi
Microseris	nutans	Aster.	?	N	0	0	RM, BH
Microsteris	gracilis	Polemoni.	?	N	0	0	RM
Mirabilis	albida	Nyctagin.	6	N	1	0	BO, RM
Mirabilis	linearis	Nyctagin.	7	N	1	1	US, BO, RM Vi, BH Fr
Mirabilis	nyctaginea	Nyctagin.	2	N	1	1	US, BO, RM, BH Fr
Moehringia	lateriflora	Caryophyll.	8	N	0	0	RM
Monarda	fistulosa	Lami.	5	N	1	1	US, BO, RM Vi, BH Vi, Fr
Monolepis	nuttalliana	Amaranth.	3	N	1	1	US, BO, RM Vi, BH
Muhlenbergia	asperifolia	Po.	2	N	1	1	US, BO, RM Vi BH Fr
Muhlenbergia	cuspidata	Po.	8	N	0	1	US, BO, RM Vi, BH Vi, Fr
Muhlenbergia	glomerata	Po.	10	N	0	0	RM
Muhlenbergia	mexicana	Po.	4	N	0	1	BO, RM Vi
Muhlenbergia	racemosa	Po.	4	N	1	1	US, BO, RM, BH Vi, Fr
Mulgedium	pulchellum	Aster.	1	N	1	1	US, BO, BH Fr
Munroa	squarrosa	Po.	0	N	0	0	BO, RM, BH
Musineon	divaricatum	Api.	6	N	0	0	US, BO, RM, BH
Myosurus	minimus	Ranuncul.	2	N	0	0	RM
Nassella	viridula	Po.	5	N	0	0	US, BO, RM, BH
Navarretia	intertexta	Polemoni.	5	N	0	1	US, BH Vi, Fr
Nepeta	cataria	Lami.	x	I	0	0	RM
Nothocalais	cuspidata	Aster.	10	N	1	1	US, RM, BH
Nuttallanthus	texanus	Plantagin.	5	N	0	0	US, BO, RM, BH
Oenothera	albicaulis	Onagr.	5	N	1	1	US, BO, RM Vi, BH Vi
Oenothera	biennis	Onagr.	0	N	1	1	US, BH Vi
Oenothera	cespitosa	Onagr.	8	N	1	1	US, BO, RM Vi, BH
Oenothera	coronopifolia	Onagr.	6	N	1	1	BO, BH Fr
Oenothera	curtiflora	Onagr.	1	N	0	0	US, BO, BH

Oenothera	flava	Onagr.	8	N	1	1	BO, RM Vi, BH
Oenothera	laciniata	Onagr.	1	N	1	0	BO, RM
Oenothera	nuttallii	Onagr.	8	N	1	1	BO, RM Vi, BH Fr
Oenothera	serrulata	Onagr.	7	N	1	1	US, BO, RM Vi, BH Fr
Oenothera	suffrutescens	Onagr.	4	N	1	1	US, BO, RM Vi, BH Fr
Oenothera	villosa	Onagr.	?	N	1	1	US, BO, RM Vi, BH Fr
Oonopsis	multicaulus	Aster.	4	N	0	0	BO, BH
Opuntia	fragilis	Cact.	5	N	1	1	BO, RM Vi, BH Vi
Opuntia	polyacantha	Cact.	3	N	1	1	US, BO, RM Vi, BH
Orobanche	fasciculata	Orobanch.	9	N	0	0	US, BO, BH
Orobanche	ludoviciana	Orobanch.	10	N	1	1	US, BO, BH Vi
Orthocarpus	luteus	Orobanch.	6	N	1	1	US, BO, RM Vi, BH Fr
Osmorhiza	longistylis	Api.	7	N	1	1	BO, BH Vi
Oxalis	dillenii	Oxalid.	5	N	0	0	RM
Oxytropis	campestris	Fab.	8	N	1	1	BO, BH
Oxytropis	lagopus	Fab.	?	N	0	0	BH
Oxytropis	lambertii	Fab.	5	N	1	1	US, BO, RM, BH Vi
Oxytropis	sericea	Fab.	6	N	0	1	BO, RM Vi, BH
Packera	cana	Aster.	8	N	0	0	US, BO, RM, BH
Packera	plattensis	Aster.	6	N	0	0	US, BO, RM,
Panicum	capillare	Po.	0	N	1	1	US, BO, RM Vi, BH Vi
Panicum	virgatum	Po.	5	N	1	1	US, BO, RM Vi, BH Vi, Fr
Parietaria	pensylvanica	Urtic.	3	N	0	0	BO, RM, BH
Paronychia	sesseliflora	Caryophyll.	7	N	1	1	US, BO, RM, BH Vi, Fr
Parthenocissus	vitacea	Vit.	2	N	1	1	US, RM Vi, BH Vi
Pascopyrum	smithii	Po.	4	N	1	1	US, BO, BH Vi, Fr
Pediomelum	argophyllum	Fab.	4	N	1	1	US, BO, RM Vi, BH Vi, Fr
Pediomelum	cuspidatum	Fab.	8	N	0	0	BO, BH
Pediomelum	esculentum	Fab.	9	N	0	0	US, BO, RM, BH
Pellaea	glabella	Pterid.	10	N	1	0	BON, RM, BH
Penstemon	albidus	Plantagin.	7	N	1	1	US, BO, RM Vi, BH Fr
Penstemon	angustifolius	Plantagin.	9	N	1	0	US, BO, RM, BH
Penstemon	cyaneus	Plantagin.	?	N	0	0	RM
Penstemon	eriantherus	Plantagin.	10	N	1	0	US, BO, RM, BH
Penstemon	glaber	Plantagin.	7	N	0	0	US, BO, RM, BH
Penstemon	gracilis	Plantagin.	6	N	0	1	US, BO, RM Vi, BH
Penstemon	nitidus	Plantagin.	7	N	0	0	BO, RM, BH
Perideridia	montana	Api.	?	N	0	1	RM Vi
Peritoma	serrulata	Cleom.	2	N	0	1	US, BO, RM, BH Vi, Fr
Persicaria	amphibia	Polygon.	0	N	1	0	US, BO - Not Seen

Persicaria	lapathifolia	Polygon.	1	N	1	1	US, BO, RM Vi
Persicaria	pensylvanicum	Polygon.	0	N	1	0	US - Not Seen
Phacelia	hastata	Hydrophyll.	5	N	1	1	US, BO, RM Vi, BH Vi
Phacelia	heterophylla	Hydrophyll.	?	N	0	0	RM
Phalaris	arundinacea	Po.	0	N	1	1	US, BO, RM Vi, BH Vi
Phemeranthus	parviflorus	Monti.	8	N	1	1	US, BO, RM
Phleum	pratense	Po.	x	I	1	1	US, BO, RM Vi, BH Vi
Phlox	alyssifolia	Polemoni.	9	N	1	1	US, BO, RM, BH Vi
Phlox	andicola	Polemoni.	7	N	0	1	US, BO, BH Vi
Phlox	hoodii	Polemoni.	6	N	1	0	BO, RM, BH
Phragmites	australis	Po.	0	N/I	0	0	BO, BH
Physalis	heterophylla	Solan.	5	N	1	1	US, BO, RM Vi, BH Vi
Physalis	longifolia	Solan.	0	N	0	1	US, BO, RM Vi
Physaria	arenosa	Brassic.	6	N	1	1	RM Vi, BH
Physaria	brassicoides	Brassic.	8	N	0	0	US, BO, RM Vi, BH Vi
Physaria	ludoviciana	Brassic.	6	N	0	1	US, BO, RM Vi, BH Fr
Physaria	montana	Brassic.	8	N	0	0	BH
Physaria	spatulata	Brassic.	8	N	0	1	BO, RM Vi
Picradeniopsis	oppositifolia	Aster.	2	N	1	1	US, BO, RM Vi, BH Vi, Fr
Pinus	ponderosa	Pin.	6	N	1	1	US, BO, RM Vi, BH
Piptatherum	micranthum	Po.	8	N	0	0	US, BO, RM, BH
Plagiobothrys	scouleri	Boragin.	0	N	0	0	US, BH
Plantago	eriopoda	Plantagin.	5	N	0	0	BO, BH
Plantago	elongata	Plantagin.	3	N	1	0	BO, BH
Plantago	lanceolata	Plantagin.	x	I	1	1	BO, RM Vi
Plantago	major	Plantagin.	x	I	1	1	US, BO, RM, BH Vi, Fr
Plantago	patagonica	Plantagin.	1	N	1	1	US, BO, RM Vi, BH Vi, Fr
Plantago	rugelii	Plantagin.	0	N	1	1	US, BO, RM Vi
Platanthera	aquilonis	Orchid.	9	N	0	1	US, BO, RM, BH Vi
Platanthera	dilatata	Orchid.	?	N	0	1	BO, RM Vi
Poa	arida	Po.	8	N	1	0	US, BO, RM, BH
Poa	bulbosa	Po.	x	I	0	0	RM
Poa	compressa	Po.	x	I	0	0	BO, RM, BH
Poa	fendleriana	Po.	8	N	0	0	BO, RM, BH
Poa	interior	Po.	5	N	1	1	US, RM, BH Fr
Poa	palustris	Po.	4	N	1	0	BO, RM BH
Poa	pratensis	Po.	x	I	0	1	US, BO, RM, BH Vi
Poa	secunda	Po.	8	N	1	1	BO, RM, BH Vi
Polanisia	dodecandra	Cleom.	2	N	1	1	US, BO, RM Vi, BH Vi, Fr
Polygala	alba	Polygal.	5	N	1	1	US, BO, RM Vi, BH Fr

Polygala	verticillata	Polygal.	8	N	1	0	US, BO, RM, BH
Polygonum	achoreum	Polygon.	x	I	0	0	US, BO, BH
Polygonum	aviculare	Polygon.	x	I	1	1	BO, RM Vi, BH Vi
Polygonum	douglasii	Polygon.	3	N	1	1	US, BO, RM Vi, BH Vi
Polygonum	ramosissimum	Polygon.	3	N	1	1	US, BO, RM Vi, BH Vi, Fr
Polygonum	spergulariiforme	Polygon.	?	N	0	0	RM
Populus	x acuminata	Salic.	3	N	0	0	US, BO, RM Vi
Populus	balsamifera	Salic.	6	N	0	0	BO, BH
Populus	deltoides	Salic.	4	N	1	1	US, BO, RM Vi
Populus	tremuloides	Salic.	4	N	1	1	US, BO, RM Vi, BH Vi
Potamogeton	diversifolius	Potamogeton.	4	N	0	1	BH Vi
Potamogeton	pusillus	Potamogeton.	2	N	0	0	BO, BH
Potamogeton	foliosus	Potamogeton.	2	N	0	1	BO, RM Vi
Potentilla	arguta	Ros.	8	N	0	0	US, BO, BH
Potentilla	biennis	Ros.	?	N	1	1	US, BO, BH Vi
Potentilla	bipinnatifida	Ros.	9	N	0	1	RM Vi
Potentilla	concinna	Ros.	8	N	1	1	US, BO - Not Seen
Potentilla	gracilis	Ros.	5	N	0	1	RM Vi
Potentilla	macounii	Ros.	8	N	0	0	RM
Potentilla	norvegica	Ros.	0	N	1	1	US, BO, RM Vi, BH Fr
Potentilla	pensylvanica	Ros.	9	N	1	1	US, BO, RM, BH Vi
Potentilla	rubricaulis	Ros.	9	N	0	1	BH Fr
Prosartes	trachycarpa	Lili.	10	N	1	1	US, BO, RM Vi, BH Vi
Prunus	americana	Ros.	4	N	1	1	US, BO, RM Vi, BH Fr
Prunus	pensylvanica	Ros.	8	N	0	0	BO - Not Seen
Prunus	pumila	Ros.	8	N	1	1	US, BO, RM Vi, BH Vi
Prunus	virginiana	Ros.	4	N	1	1	US, BO, RM Vi, BH Vi
Pseudoroegneria	spicata	Po.	9	N	0	1	BO, BH Fr
Pterospora	andromedea	Eric.	?	N	1	1	US,BO, RM, BH Vi
Puccinellia	nuttalliana	Po.	4	N	1	1	US, BO, BH Vi
Pulsatilla	patens	Ranuncul.	9	N	1	0	US, BO, BH
Pyrola	asarifolia	Pyrol.	8	N	1	1	US, BO, BH Vi
Pyrola	elliptica	Pyrol.	10	N	1	1	US, BO, BH Vi
Ranunculus	abortivus	Ranuncul.	2	N	1	1	US, BO, RM Vi, BH
Ranunculus	aquatilis	Ranuncul.	?	N	0	1	RM Vi
Ranunculus	cymbalaria	Ranuncul.	3	N	1	1	US, RM, BH Vi, Fr
Ranunculus	glaberrimus	Ranuncul.	8	N	0	0	BO, BH
Ranunculus	longirostris	Ranuncul.	7	N	0	1	US, BO, BH Vi
Ranunculus	macounii	Ranuncul.	4	N	1	1	BO, RM Vi, BH Fr
Ranunculus	pensylvanicus	Ranuncul.	4	N	1	0	RM

Ranunculus	sceleratus	Ranuncul.	3	N	1	1	US, BO, RM Vi, BH Vi, Fr
Ratibida	columnifera	Aster.	3	N	1	1	US, BO, RM Vi, BH Fr
Rhus	glabra	Anacardi.	4	N	0	0	BO, BH
Rhus	trilobata	Anacardi.	7	N	1	1	US, BO, RM, BH Fr
Ribes	americanum	Grossulari.	7	N	1	1	US, BO, RM Vi, BH Vi
Ribes	aureum	Grossulari.	6	N	1	1	US, BO, RM Vi, BH Vi
Ribes	cereum	Grossulari.	7	N	1	1	US, BO, RM, BH Fr
Ribes	missouriense	Grossulari.	4	N	0	0	US - Not Seen
Ribes	oxyacanthoides	Grossulari.	5	N	1	1	US, BO, RM Vi, BH Vi
Rorippa	palustris	Brassic.	2	N	1	1	US, BO, RM Vi, BH Vi
Rorippa	sinuata	Brassic.	4	N	1	1	US, BO, RM Vi
Rosa	acicularis	Ros.	8	N	1	1	US, BO, RM Vi, BH Vi
Rosa	arkansana	Ros.	3	N	1	1	US, BO, RM Vi, BH Fr
Rosa	blanda	Ros.	8	N	0	0	BH
Rosa	nutkana	Ros.	?	N	0	0	RM
Rosa	woodsii	Ros.	5	N	1	1	US, BO, RM Vi, BH Vi
Rubus	idaeus	Ros.	5	N/I	1	1	US, BO, RM Vi, BH Vi
Rumex	crispus	Polygon.	x	I	0	0	US, BO, RM, BH
Rumex	fueginus	Polygon.	1	N	0	0	US, RM
Rumex	occidentalis	Polygon.	7	N	1	1	US, BO, RM Vi, BH Vi
Rumex	patientia	Polygon.	x	I	0	0	RM
Rumex	stenophyllus	Polygon.	x	I	0	0	RM
Rumex	triangulivalvis	Polygon.	1	N	0	1	BO, RM Vi, BH Vi
Rumex	utahensis	Polygon.	?	N	0	1	RM Vi
Rumex	venosus	Polygon.	3	N	1	1	US, BO, RM Vi, BH
Sagittaria	cuneata	Alismat.	6	N	1	1	US, BO, RM Vi, BH Vi, Fr
Salix	amygdaloides	Salic.	3	N	1	1	US, BO, RM Vi, BH Vi
Salix	bebbiana	Salic.	8	N	1	1	US, BO, RM Vi, BH Vi
Salix	discolor	Salic.	7	N	0	0	BO, BH
Salix	eriocephala	Salic.	5	N	0	1	RM Vi, BH
Salix	exigua	Salic.	3	N	1	1	BO, RM Vi, BH Vi
Salix	interior	Salic.	3	N	0	0	US, BO, BH
Salix	lutea	Salic.	5	N	0	1	BH Vi
Salsola	collina	Amaranth.	x	I	0	1	BO, BH Fr
Salsola	tragus	Amaranth.	x	I	1	1	US, BO, RM Vi, BH
Sanicula	marilandica	Api.	7	N	1	1	US, BO, RM Vi, BH Vi
Sarcobatus	vermiculatus	Sarcobat.	5	N	0	0	US, BO, BH
Schedonnardus	paniculatus	Po.	1	N	0	1	US, BO, BH Vi
Schizachne	purpurascens	Po.	8	N	0	0	BO, RM, BH
Schizachyrium	scoparium	Po.	6	N	1	1	BO, RM Vi, BH Fr

Schoenoplectus	acutus	Cyper.	5	N	1	0	BO, RM, BH
Schoenoplectus	americanus	Cyper.	4	N	1	1	RM Vi
Schoenoplectus	maritimus	Cyper.	4	N	0	0	BO, BH
Schoenoplectus	pungens	Cyper.	4	N	0	1	US, BO, RM, BH Fr
Schoenoplectus	tabernaemontani	Cyper.	3	N	0	1	US, BO, RM Vi, BH Vi, Fr
Scirpus	atrocinctus	Cyper.	10	N	0	0	BO, BH
Scirpus	pallidus	Cyper.	5	N	0	1	US, BO, RM Vi, BH Vi, Fr
Scrophularia	lanceolata	Scrophulari.	5	N	0	0	RM
Sedum	lanceolatum	Crassul.	9	N	0	0	RM, BH
Selaginella	densa	Selaginell.	6	N	1	1	US, BO, RM Vi, BH Vi
Selaginella	rupestris	Selaginell.	10	N	0	0	BO, BH
Senecio	crassulus	Aster.	?	N	0	0	BH
Senecio	integerrimus	Aster.	7	N	0	0	US, BO, RM, BH
Setaria	italica	Po.	x	I	0	0	BO, BH
Setaria	viridis	Po.	x	I	1	1	US, BO, BH Fr
Shepherdia	argentea	Elaeagn.	5	N	1	1	US, BO, RM Vi, BH Vi, Fr
Shinnersoseris	rostrata	Aster.	10	N	1	1	US, BO, RM Vi
Silene	antirrhina	Caryophyll.	3	N	0	0	US, BO, RM, BH
Silene	drummondii	Caryophyll.	5	N	1	1	US, BO, RM Vi, BH
Silene	latifolia	Caryophyll.	x	I	0	0	BH
Sinapis	arvensis	Brassic.	x	I	1	1	US, BO, RM Vi, BH Vi
Sisymbrium	altissimum	Brassic.	x	I	0	1	US, BO, BH Fr
Sisyrinchium	montanum	Irid.	8	N	0	1	US, BO, RM, BH Vi
Sium	suave	Api.	3	N	0	0	US, BO, BH
Smilax	lasioneura	Smilac.	8	N	1	1	US, BO, RM Vi, BH Vi
Solanum	rostratum	Solan.	0	N	1	1	US, BO, RM, BH Fr
Solanum	triflorum	Solan.	0	N	1	1	US, BO, RM Vi, BH Vi, Fr
Solidago	canadensis	Aster.	8	N	0	1	RM
Solidago	gigantea	Aster.	4	N	1	1	US, BO, RM Vi, BH Fr
Solidago	missouriensis	Aster.	5	N	1	1	US, BO, RM Vi, BH Vi, Fr
Solidago	mollis	Aster.	6	N	1	1	US, BO, RM Vi, BH Vi, Fr
Solidago	nemoralis	Aster.	6	N	1	1	US, BO, RM, BH Vi, Fr
Solidago	ptarmicoides	Aster.	?	N	1	1	US, BO, RM Vi, BH Vi, Fr
Solidago	rigida	Aster.	4	N	1	1	BO, RM Vi, BH Fr
Solidago	speciosa	Aster.	10	N	0	0	US, BO, RM
Solidago	velutina	Aster.	?	N	0	0	BO, BH
Sonchus	asper	Aster.	x	I	1	1	US, BO, RM Vi, BH Vi
Sorghum	bicolor	Po.	x	I	0	0	BO, BH
Spartina	gracilis	Po.	6	N	1	1	US, BO, RM Vi, BH Vi, Fr
Spartina	pectinata	Po.	5	N	0	1	US, BO, RM Vi, BH Fr

Sphaeralcea	coccinea	Malv.	4	N	1	1	US, BO, RM Vi, BH Fr
Sphenopholis	obtusata	Po.	7	N	0	0	BO, RM, BH
Spiranthes	romanzoffiana	Orchid.	9	N	0	0	US, BO, BH
Sporobolus	compositus	Po.	4	N	0	0	BO, BH
Sporobolus	cryptandrus	Po.	6	N	0	0	US, BO, RM, BH
Stenotus	armerioides	Aster.	7	N	0	0	US, BO, RM, BH
Stephanomeria	runciniata	Aster.	?	N	0	0	RM
Stephanomeria	tenuifolia	Aster.	?	N	0	0	RM
Strophostyles	leiosperma	Fab.	6	N	1	0	US, BO - Not Seen
Stuckenia	pectinata	Potamogeton.	0	N	0	0	US, BO, RM, BH
Suaeda	calceoliformis	Amaranth.	2	N	1	1	US,BO, BH Vi, Fr
Suaeda	nigra	Amaranth.	1	N	1	0	US, BO, RM, BH
Symphoricarpos	albus	Caprifoli.	8	N	1	1	BO, RM Vi, BH Vi
Symphoricarpos	occidentalis	Caprifoli.	3	N	1	1	US, BO, RM Vi, BH Vi, Fr
Symphyotrichum	boreale	Aster.	?	N	0	0	RM
Symphyotrichum	ericoides	Aster.	2	N	1	1	US, BO, BH Vi, Fr
Symphyotrichum	falcatum	Aster.	4	N	1	1	US, BO, RM Vi, BH Vi, Fr
Symphyotrichum	laeve	Aster.	5	N	1	1	US, BO, RM Vi, BH Vi, Fr
Symphyotrichum	lanceolatum	Aster.	3	N	1	1	US, BO, RM Vi, BH Fr
Symphyotrichum	oblongifolium	Aster.	8	N	1	1	US, BO, RM Vi, BH Vi, Fr
Syringa	vulgaris	Ole.	x	I	0	0	RM
Taraxacum	erythrospermum	Aster.	x	I	0	0	US, RM, BH
Taraxacum	officinale	Aster.	x	N/I	0	0	BH
Tetraneuris	acaulis	Aster.	6	N	1	1	US, BO, RM Vi, BH Fr
Thalictrum	dasycarpum	Ranuncul.	7	N	1	1	US, BO, RM Vi, BH Fr
Thermopsis	rhombifolia	Fab.	6	N	1	1	US, BO, RM Vi, BH Vi
Thinopyrum	intermedium	Po.	x	I	0	0	BO, RM, BH
Thlaspi	arvense	Brassic.	x	I	0	0	BO, RM, BH
Townsendia	exscapa	Aster.	8	N	0	0	BH
Toxicodendron	rydbergii	Anacardi.	3	N	1	1	US, BO,RM Vi, BH Vi, Fr
Toxicoscordion	venenosum	Melanthi.	7	N	1	1	US, BO, RM Vi,
Tradescantia	bracteata	Commelin.	7	N	0	1	US, BO, BH Vi
Tradescantia	occidentalis	Commelin.	5	N	1	1	US, BO, RM, BH Vi, Fr
Tragopogon	dubius	Aster.	x	I	0	0	US, BO, RM, BH
Tragopogon	pratensis	Aster.	x	I	0	0	RM
Trifolium	fragiferum	Fab.	x	I	0	0	BO, BH
Triglochin	maritima	Juncagin.	5	N	1	1	US, BO, RM Vi, BH Fr
Triglochin	palustris	Juncagin.	8	N	0	0	US, BO, RM, BH Vi
Triodanis	leptocarpa	Campanul.	8	N	0	0	US, BO, RM, BH
Triodanis	perfoliata	Campanul.	6	N	0	0	US, BO, BH

Tripterocalyx	micranthus	Nyctagin.	7	N	0	1	US, BO, BH Vi, Fr
Triticum	aestivum	Po.	x	I	0	0	BH
Turritis	glabra	Brassic.	6	N	0	0	US, BO, BH
Typha	latifolia	Typh.	2	N	1	1	US, BO, RM Vi, BH Vi
Typha	angustifolia	Typh.	x	I	0	0	RM
Ulmus	americana	Ulm.	3	N	0	0	RM
Ulmus	pumila	Ulm.	x	I	0	0	RM
Ulmus	rubra	Ulm.	5	N	1	1	BO, RM Vi, BH Vi
Urtica	dioica	Urtic.	0	N/I	1	1	US, BO, RM Vi, BH Vi
Vaccaria	hispanica	Caryophyll.	x	I	1	1	US, BO, RM Vi, BH Fr
Verbena	bracteata	Verben.	0	N	0	0	US, BO, RM, BH
Verbena	stricta	Verben.	2	N	1	0	BO, BH
Veronica	peregrina	Plantagin.	0	N	0	0	US, BO, BH
Veronica	americana	Plantagin.	5	N	1	1	US, BO, RM Vi, BH Vi,
Viburnum	lentago	Caprifoli.	8	N	1	1	US, BO, RM Vi, BH Vi
Vicia	americana	Fab.	3	N	1	1	US, BO, RM Vi, BH
Viola	adunca	Viol.	8	N	0	0	US, BO, RM, BH
Viola	canadensis	Viol.	4	N	1	1	BO, RM Vi
Viola	nephrophylla	Viol.	8	N	0	1	US, BO - Not Seen
Viola	nuttallii	Viol.	8	N	0	0	US, BO, RM, BH
Viola	pedatifida	Viol.	8	N	0	0	US, BO, RM, BH
Viola	sororia	Viol.	2	N	1	1	BO, RM Vi
Viola	vallicola	Viol.	8	N	1	0	RM
Vitis	riparia	Vit.	3	N	1	1	US, BO, RM Vi, BH Vi
Vulpia	octoflora	Po.	0	N	0	0	US, BO, RM, BH Fr
Woodsia	oregana	Dryopterid.	8	N	0	0	US, BO, RM, BH
Woodsia	scopulina	Dryopterid.	?	N	1	1	US, BO, RM Vi, BH
Xanthisma	grindelioides	Aster.	8	N	1	1	US, BO, RM Vi, BH
Xanthisma	spinulosum	Aster.	7	N	1	1	US, BO, BH Vi
Xanthium	strumarium	Aster.	0	N	1	1	BO, RM, BH Fr
Xylorhiza	glabriuscula	Aster.	4	N	0	0	US, BO, BH
Yucca	glauca	Agav.	6	N	1	1	US, BO, RM Vi, BH Vi
Zannichellia	palustris	Potamogeton.	2	N	0	0	US, BO, BH
Zea	mays	Po.	x	I	0	0	BO, BH

Appendix 2. List of problematic species records. USDA = USDA NRCS (2014); BONAP = Kartesz (2013); RM = Hartman et al. (2009); BHSC = Black Hills State University Herbarium (2014)

Alopecurus pratensis was collected by Visher from adjacent Perkins County in 1912. It has not been seen in Harding County.

Argemone intermedia (*Argemone polyanthemos*) was reported by Visher (1914), but records of more recent collections have not been observed (USDA, BONAP, RM, BHSC).

Atriplex powellii was reported as present in Carter County, MT, adjacent to Harding County, by BONAP.

Carex siccata was listed by Visher, but specimens from Harding County have not been observed. It is present in the Black Hills, approximately 150-200 km to the south.

Carum carvi is found in the Black Hills, but has not been seen in Harding County, nor have specimens from Visher been located.

Chenopodium watsonii specimens from Harding County have not been located. The species is present in the Black Hills.

Crataegus sheridana listed by Visher in Harding County, but specimen not seen, present in Carter Co., MT.

Cyperus squarrosus specimens from Harding County have not been observed. The species is present in other counties in South Dakota.

Epilobium leptocarpum is listed by USDA, BONAP, and RM as occurring only farther west. SDU has a Visher specimen labeled as this species.

Festuca idahoensis has been recorded as present (Bonap 2014). Specimens of the species have not been seen, and Heidel and Dueholm (1995) have suggested that the listing may have been because of an error in identification.

Festuca rubra specimens have not been seen from Harding County, but a Visher specimen is present at RM from adjacent Perkins Co.

Mentzelia nuda is noted by Visher (1914), but no specimens have been seen, and it is not known otherwise from Harding County.

Oxalis stricta is on Visher's list, but no specimens of *O. stricta* have been seen from that county.

Poa cusickii is on Visher's list, but no specimens from Harding County have been seen. BONAP lists as present from adjacent Carter Co., MT

Populus angustifolia specimens have not been seen from Harding County. Visher could have observed *Populus x acuminata.*

Ranunculus rhomboideus was not seen in Harding County, but is present in the Black Hills.

Rorippa curvisiliqua (*R. lyrata*) has not been found in Harding County.

Salix lucida is not known from Harding County. Perhaps the specimens were *Salix amygdaloides.*

IN SILICO ANALYSIS OF CIS-REGULATORY ELEMENTS OF DISEASE RESISTANCE GENES ACROSS SIX PLANT SPECIES

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ABSTRACT

Plant proteins encoded by disease resistance genes (R-genes) are involved in detecting pathogen-attack and subsequently in activating defense mechanisms. Coiled coil Nucleotide binding site Leucine rich region (CNL) type of R-genes are present in all plant species. In this study, we aimed to identify Cis-acting Regulatory Elements (CREs) 2kb upstream of CNL disease resistance genes and elucidate their distribution and diversity across six plant species. Over 900 identified CNL genes from six plant species—*Oryza sativa*, *Glycine max*, *Populus trichocarpa*, *Medicago truncatula*, *Phaseolus vulgaris*, and *Arabidopsis thaliana* were searched using 469 reference CREs available at the PLACE database. Using in-house Perl scripts, we parsed the sequence data to yield 327 of 469 CREs in the described region. Eight of the CREs were common to all genes, including the most-abundant DOFCOREZM, which appeared 27,619 times. Thirteen CREs had a frequency greater than 10 per gene. The only monocot included in this study, *Oryza sativa,* had a significantly lower number of CREs than dicot species. Previous studies have failed to identify a promoter that is universally present in all transcribed plant genes, but the present study identified eight CREs that appeared in the 2kb upstream region of all CNL genes sampled. Since the CREs are involved in initiating transcriptional processes, their identification would have future implication in developing durable resistance genes that are transcribed in predictable ways and that are maintained during the natural processes of reproduction of the plant, thus being useful in crop improvement.

Keywords

Cis-Acting Regulatory Element (CRE), Legumes, Coiled-Coil Nucleotide Binding site Leucine rich region (CNL), Resistance gene (R-gene)

INTRODUCTION

Plants have evolved a sophisticated multi-layered defense system against their pathogens (Hammond-Kosack and Jones 1996). The first layer of plant defense is at least one external physical barrier, such as a waxy cuticle, bark, or trichomes (Metraux et al. 2014). The next layer is the production of chemical compounds that act on fungi or bacteria, or sometimes inhibit the growth of other neighboring plants (Baetz and Martinoia 2014). If a pathogen succeeds in overcoming these two layers of defense, it must then deal with the proteins encoded by one or many resistance genes. The disease resistance gene (R-gene) proteins are classified into eight major groups, of which the largest group comprises the Nucleotide Binding Site-Leucine Rich Repeat (NBS-LRR) proteins (Liu et al. 2014). NBS-LRR proteins are divided into two subfamilies based on the domain structure at the C-terminus: genes with Toll Interleukin Receptor (TIR) are called TIR-NBS-LRR or TNL and those with putative Coiled-Coil (CC) domain are called CC-NBS-LRR or CNL (Marone et al. 2013).

R-genes and their mode of action against pathogen proteins were first proposed by Harold Flor (Flor 1971) much before many of the modern molecular techniques became available. Current wisdom holds that plant NBS-LRR proteins are triggered by a pathogen's elicitors. The sensitized host cells then send a systemic signal to activate defense responses (Gao et al. 2013a). Plant-pathogen interactions may follow the "*Gene-for-Gene Model*" as proposed by Flor (1971), which describes resistance as a function of an individual R-gene that encodes a resistance protein for a single pathogenic elicitor (Liu et al. 2014). Alternatively, NBS-LRR proteins may serve as guards of certain proteins within the signaling pathway of the plant that are targets for pathogen elicitors. When these proteins are disrupted, the guard proteins send their signal as described in the "*Guard Model*" (Jones and Dangl 2006). Regardless of the model followed, plant defense responses to pathogen infections occur through the induction of a large number of host genes. Some directly encode anti-microbial compounds, while others encode proteins with regulatory function in the defense signaling pathways (Rushton and Somssich 1998). The host genes are induced through recognition of Cis-regulatory element (CRE)-binding sites for transcription factors, such as the WRKY zinc–finger motif (Ülker and Somssich 2004). The WRKY binding elements are involved in plant defense response triggered by pathogen elicitors or by salicylic acid when directly applied to the plant (Dong et al. 2003). For example, WRKY binding elements were found to interact with both biotrophic bacterium *Pseudomonas syringae* as well as necrotrophic fungi *Botrytis cinera* and *Alternaria brassicicola* (Zheng et al. 2006) in *Arabidopsis thaliana*. The CREs are located in the non-coding regions of the DNA conserved along with the orthologous genes across species through evolutionary pressure (Kumari and Ware 2013). These elements are involved in recruiting transcription factors and thus have functional significance (Baxter et al. 2012).

Empirical studies have shown that regulation of gene expression serves as a source of evolutionary change (Bryson and Vogel 1965; Britten and Davidson 1969; King and Wilson 1975) and CREs are believed to influence phenotypic divergence (King and Wilson 1975; Stern and Orgogozo 2008). Among the CREs, enhancers are likely to regulate the phenotypic divergence (Brown and Feder 2005), are typically located upstream, downstream or in introns (Kleinjan and van Heyningen 2005), and their genomic locations are often conserved between species (Hare et al. 2008; Cande et al. 2009; Kalay and Wittkopp 2010). A clear understanding of the evolutionary divergence of the CREs requires study of allele-specific expression (Cowles et al 2002;Wittkopp et al. 2004), determination of functionally divergent sites, and their interaction with and among trans-acting elements (Wittkopp and Kalay 2012; Gao et al. 2013b). Previous studies in *Medicago truncatula* showed that there were four over represented regulatory WBOX cassettes associated with the WRKY transcription factors, CBF and DRE boxes, and GCC motif associated with ERF-type transcription factors (Ameline-Torregrosa et al. 2008). Similar studies on the representation of the WRKY transcriptions factors DNA binding elements in *Arabidopsis* and in grape (*Vitis vinifera*) (Marchive et al. 2007; Zheng et al. 2007) have been conducted, while other CREs are yet to be explored. The main objectives of this study were to identify CREs in 2kb upstream of CNL type of R-genes and elucidate their distribution and diversity across six plant species. The resulting identification of the CNL genes and their CREs across species will allow an understanding of the diversity, distribution and evolutionary relationships of these genes.

METHODS

We gathered from Phytozome.net 55 previously-identified CNL genes of *Arabidopsis thaliana* (hereafter AT; Meyers et al. 2003) as reference sequences, and mined the genome sequences of five plant species (*Glycine max, Medicago truncatula, Oryza sativa, Phaseolus vulgaris* and *Populus trichocarpa*, hereafter called GM, OS, PV and PT, respectively. AT CNL gene sequences were used to build a Hidden Markov Model (HMM) profile similar to those employed in *Arabidopsis* (Meyers et al. 2003) and in *Medicago* (Ameline-Torregrosa et al. 2008). Phylogenetic analyses of the NB-ARC (NBS) sequences were performed using *Streptomyces* protein sequence P25941 as outgroup. Phylogenetic analysis was performed in the program MEGA5.2 using Maximum Likelihood method with the best fit model JTT+G. Branch support was estimated for 100 bootstrap replicates. The two thousand base pairs (2kb) upstream region was searched for the CRE regions, and the identification protocol was similar to *Medicago* (Ameline-Torregrosa et al. 2008). SIGNALSCAN program available at PLACE database (Higo et al. 1999) was used for the identification of the CRE regions. Custom Perl scripts were used to parse the output files from the PLACE database. Using the data from PLACE, we estimated the number and abundance of identified CREs across 913 plant CNL genes. One-way ANOVA was conducted to test the statistical difference between the number of CREs in the genomes of monocot and dicot species.

RESULTS AND DISCUSSION

Using *in silico* analysis, we identified 912 genes that included 149, 188, 194, 235 and 94 CNL genes in rice, soybean, poplar, alfalfa, and common bean, respectively (Figure 1). As shown in the figure, these genes were nested into four clades (CNL-A, CNL-B, CNL-C and CNL-D) consistent to those described in *Arabidopsis*. Clade A had two unresolved subgroups each with moderate BS support and Clade B was moderately supported (BS 75%). A previous study (Meyers et al. 2003) described a proportionally-similar number of genes in each group in *Arabidopsis*. Clade A contained between 0% (OS) and10.4% (AT) of the total CNL genes identified within each species. Clade B was moderately supported (BS 75%) containing 2% (OS) and 50% (AT) of the CNL genes within each species. A previous study (Meyers et al. 2003) described a proportionally similar number of genes in each group in *Arabidopsis*. Among the four clades, CNL-C was the most expanded clade. The *Arabidopsis* genome contained only 14.5% CNL-C genes while the other genomes contained a much higher proportion (69.3% [PT] - 97.9% [OS]). The CNL-C clade contained multiple clusters of genes with low clade support, indicating rapidly-evolving genes. The expansion of clade C perhaps provides a source for new CNL sequences while concurrently reducing the risk of auto-activation of the resistance response through the reduction of gene expression. This is consistent with the results reported in *Arabidopsis* (Meyers et al. 2003) and *Medicago* (Ameline-Torregrosa et al. 2008). CNL-D was the least expanded clade, with a strong statistical support (BS 89%). We found that the number of CNL R-genes in GM was closer to the predicted 1.05 gene copy retention after duplication than the 3.1 gene copy number of the overall genome (Ashfield et al. 2012). Despite possessing nearly double the genome size, GM (1.1 Gb genome size and 188 CNL gene) had 1.25 times the number of CNL genes when compared to *Medicago* (500 Mb genome size and 235 CNL genes), suggesting little adherence to the assumption that more duplications or a larger genome would allow for more resistance genes. What affected these genome duplication retention rates, and to what extent were they modified by the effects of selection pressures from pathogens and auto-activation in soybeans are intriguing questions to be investigated in the future. Addressing these questions requires an understanding of how these genes are regulated in the genomes. Below we report our results on Cis-Regulatory Elements (CREs) of the CNL-genes across six plant species.

Among the 469 CREs investigated in the 2kb upstream region of the 913 CNL genes, 327 CREs (~70%) were found across the six plant genomes surveyed. Among these CREs, 253, 264, 271, 281, 283, and 292 CREs were present in AT, PV, GM, MT, PT and OS, respectively. Eight of these CREs (ARR1AT, CAATBOX1, CACTFTPPCA1, DOFCOREZM, GATABOX, GT-1CONSENSUS, POLLEN1LELAT52 and WRKY710S) were found common to all CNL genes, including the most-frequent, DOFCOREZM (DNA binding one finger zinc of *Zea maize*), which appeared a total of 27,619 times. Further analysis of multiple plant genomes at various taxonomic groups is warranted to test a previous claim (Juven-Gershon et al. 2006) that plant genomes lack a universal regulatory element. One of the most commonly occurring CREs in the present study, DOFCOREMZ is the core site required for binding of DOF proteins in maize (*Zea mays*). The DOF proteins are DNA binding proteins with only one zinc finger and are unique to plants (Higo et al. 1999). These single zinc finger domains are related to the WRKY binding site and part of the regulatory network that was previously described (Marchive et al. 2007; Zheng et al. 2007). In the present study, there were six WBOX elements identified: WBBOXPCWRKY1, WBOXATNPR1, WBOXGACAD1A, WBOXHVISO1, WBOXNTCHN48, and WBOXNTERF3. One of these elements appeared in every sequence, with many having three to four different elements appearing in the same sequence. These WBOX elements were found to be involved in R-gene regulation in grape and *Arabidopsis* (Marchive et al. 2007; Zheng et al. 2007). The conservation of the WBOX motif indicates its importance in gene regulation, and the search for less conserved elements involved in gene regulation should continue (Wittkopp and Kalay 2012).

The average occurrence of 13 most common CREs in the present study is shown in Table 1. Evolutionary conservation of these CREs across plant species must have regulatory roles on the gene sequences when they are present. Eighty-one of the 327 CREs were present once per sequence in which they appeared. Further study of the positioning would reveal their functional significance. The average number of CREs in rice (a monocot) was significantly lower ($P < 0.0001$) than that in dicot species (Figure 2). These results correspond to the number of Core Promoters Elements (CPE), a subset of CREs reported previously (Kumari and Ware 2013), where the authors have shown that monocot core promoters

Table 1. ***The most commonly-occurring CREs. These 13 CREs appeared on average greater than 10 times per gene.***

CRE ID	Total Appearances in all 913 Genes	Total Genes the CRE Region Appears	Average Number per Gene
ARR1AT	19,857	913	21.75
CAATBOX1	22,877	913	25.06
CACTFTPPCA1	25,749	913	28.2
DOFCOREZM	27,619	913	30.25
GATABOX	15,478	913	16.95
GT1CONSENSUS	18,124	913	19.85
POLLEN1LELAT52	12,074	913	13.23
WRKY71OS	10,515	913	11.52
GTGANTG10	11,922	912	13.07
ROOTMOTIFTAPOX1	16,589	910	18.23
EBOXBNNAPA	13,052	908	14.37
MYCCONSENSUSAT	13,052	908	14.37
TATABOX5	9,223	905	10.19

had lower DNA free energy than dicot core promoters. The free energy of the DNA sequences of these genes may be associated with GC content which is reported to be less in dicot genomes than that in monocots genomes (Serres-Giardi et al. 2012). Further investigation across multiple dicot and monocot genomes representing major taxonomic groups is required to confirm if this trend holds true as well as to understand the functional correlation.

One of the major caveats of the *in silico* analysis such as presented in this paper is the detection of false positives. For example, in soybean when the start position of the sequences that contained –300CORE (a CRE that has regulatory function when found close to 300 nucleotides before the transcriptional start site) was visualized, we found that the starting position of only 5 of the 23 sequences were found within 50 bases of the reported -300 nucleotide starting position from the transcriptional start site. The detection of potential false positives during any CRE prediction/ identification process may be alleviated by using more rigorous prediction methods that take into account the presence of other genes within the upstream region, by looking at the distance of the CRE from the TSS (Transcriptional Start Site) and by analyzing DNA free energy profiles (Kumari and Ware 2013). These factors are likely to give some insight

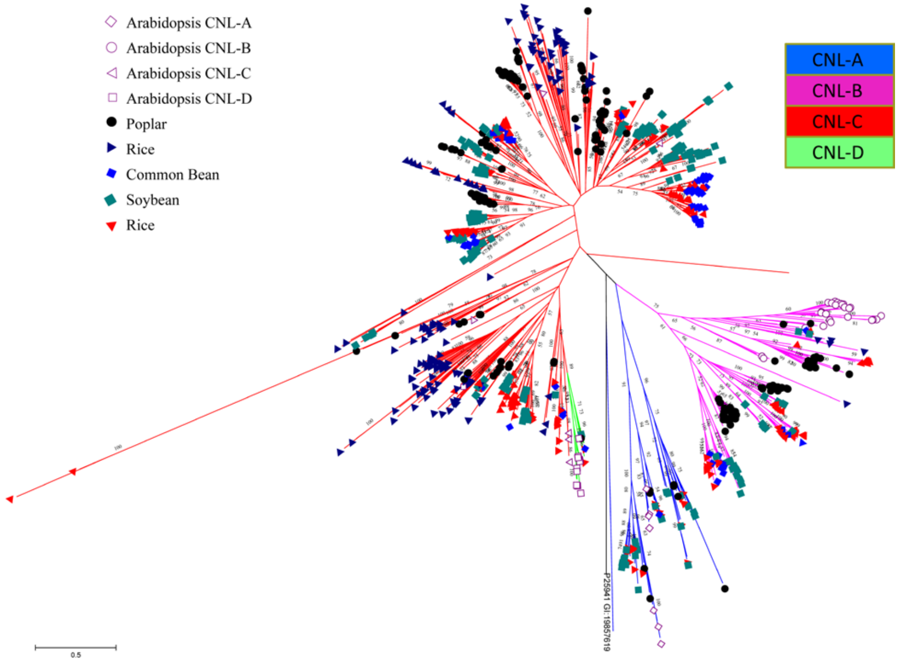

Figure 1. ***Phylogenetic analysis of the CNL genes from six species:*** *A. thaliana O. sativa, M. truncatula, P. vulgaris, P. trichocarpa, and G. max*. ***A Maximum Likelihood tree was constructed using the program MEGA 5. Branch support was estimated using the bootstrap method for 100 replicates.*** *Streptomyces* ***(GBI:19857619) was used as an outgroup. The species are color-coded. Hollow shapes were used to identify the*** *Arabidopsis* ***sequences previously assigned the CNL identifiers. The CNL clades are also color-coded: CNL-A, CNL-B, CNL-C and CNL-D in blue, purple, red and green, respectively.***

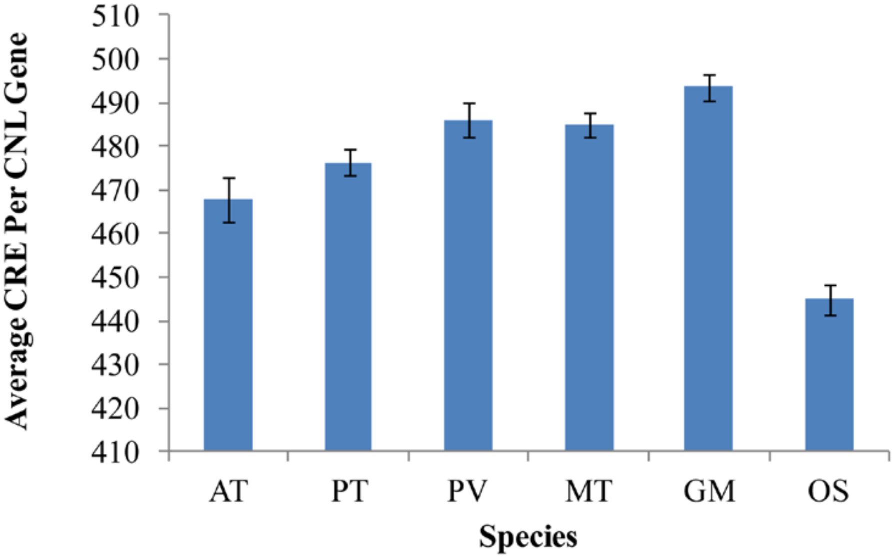

Figure 2. ***Average number of CREs per CNL gene across six species. The species from left to right are AT** = Arabidopsis thaliana**, PT** = Populus trichocarpa, **PV** = Phaseolus vulgaris, **MT** = Medicago trunatula, **GM** = Glycine max, and **OS** = Oryza sativa.*

as to why the monocot and dicot genomes differ beyond the specific nucleotide biases. Identification of CNL R-genes and insights into their regulatory elements presented in this project would have future implication in developing durable resistance genes that are transcribed in predictable ways and maintained during the natural processes of reproduction of the plant thus being useful in crop improvement.

ACKNOWLEDGEMENTS

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INSIGHTS GAINED THROUGH DEVELOPMENT OF PALEONTOLOGY OUTREACH KITS FOR RAPID CITY MIDDLE SCHOOL STUDENTS

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ABSTRACT

Paleontology is a highly effective gateway to science education, making it ideal for outreach. With this in mind, the authors, in collaboration with the South Dakota School of Mines and Technology Paleontology Club, developed teaching kits designed to educate middle-school students about dinosaurs and paleontology.

The self-contained kits, designed to be mobile and easily implemented, include all materials necessary for delivering a lesson illustrating which dinosaurs lived during particular geologic time periods. Scale models of exemplar dinosaur from all Mesozoic time periods are accompanied by fact sheets. The fact sheets, presented in “comic book” style, present aspects of each Mesozoic period, including age, paleogeography, local geology, flora and fauna. Dinosaurs from each period are given a general description and students are prompted to identify example taxa from each time period.

The kits were implemented with 124 eighth-grade students from South Middle School in Rapid City. Cognition of material was measured through concept inventories administered before and after lesson implementation. Student responses to ten questions gauged knowledge of and confidence with the material pre- and post-lesson.

Students showed cognitive improvement in understanding of several paleontologic concepts presented in the kits. Students were initially aware of some paleontologic concepts, such as continent movement, but were generally unaware of changes in dinosaur associations throughout the Mesozoic or showed reduced familiarity with less “charismatic” dinosaur taxa.

Keywords

paleontology, education, outreach, middle-school, dinosaur

INTRODUCTION

Paleontology as a cross-disciplinary endeavor offers many opportunities to introduce students to science. Earth's history offers dynamic and engaging examples from which to draw, and the broad disciplinary nature of paleontology permits application across many scientific disciplines, including geology, biology, chemistry, physics, anatomy, and ecology (Gunckel 1994; Trend 2001; Allmon 2010). Additionally, comprehension of paleontology and Earth history require some fundamental basis in scientific thought, particularly with regard to the vastness of geologic time and understanding of evolutionary change (Smith and Smith 2001; Dodick and Orion 2003a; Dodick and Orion 2006; Dodick 2007). As such, paleontology is filled with opportunities for developing comprehension of scientific knowledge and concepts.

In conjunction with the South Dakota School of Mines and Technology (SDSMT) Paleontology Club, the authors developed a series of teaching kits designed to deliver a lesson on geologic time and evolutionary change among dinosaurs during the Mesozoic Era. The Tech Paleontology Club, established in 1997, has a long and award-winning history of education and outreach involving undergraduate earth science majors and the general public. The club works in cooperation with both the Department of Geology and Geological Engineering and the Museum of Geology at SDSMT to participate in public outreach events.

The kits were targeted at a middle school audience (grades six to eight). This age group was chosen in response to high outreach demand experienced by the club. Additionally, studies suggest that seventh and eighth grade students are at a key developmental point in their ability to comprehend the logical principles of geology and reconstruction of geologic events (Dodick and Orion 2003b; Kozman 2004).

Current South Dakota middle school science standards do not directly address paleontology, even though eighth grade physical science standards place considerable emphasis on the theory of plate tectonics. In addition, life science standards do not emphasize paleontology, changes in diversity through time, or the notion of faunal change. Thus, the teaching kits targeted concepts students were not immediately exposed to.

METHODS

The teaching kits were self-contained, and included all the materials necessary to deliver a lesson on dinosaur paleontology. Materials included scale models of several dinosaur taxa, "fact sheets" with pertinent lesson information, instructional materials, and background information for instructors in both hard copy and on CD ROM. All materials were enclosed in heavy-duty rubberized totes with the intention of quick and easy deployment for outreach events by club participants.

A key component to the kits was the "fact sheets". These sheets provided the necessary information to familiarize each student with specific geologic ages and their associated dinosaur fauna. The sheets were presented in "comic book" style making the material readily accessible in a short amount of time. Each sheet provided a brief description of one of the three periods within the Mesozoic Era. Sections included descriptions of the age, continent position, flora, fauna, and prominent sedimentary rock units in western South Dakota associated with each age. A cursory description of the types of dinosaurs found in each time period helped students find examples among the scale models provided.

Lesson goals were fourfold. Primary goals included instilling knowledge of the names of and temporal order of the three geologic time periods in the Mesozoic Era, and an associated recognition of the types of dinosaurs present during each period. Secondary goals included familiarizing the students with local sedimentary formations from each geologic period, and developing familiarity with paleogeography, flora, and fauna throughout the Mesozoic Era.

The kits and associated lesson were implemented in several eighth-grade classes at South Middle School in Rapid City in October of 2012. A total of 124 students in five classes participated in the initial delivery. Class size ranged from 20-25 students with an approximately 54/46% male/female distribution. Students were divided into groups of four or five individuals. Each group followed the provided directions guiding them through the lesson. Paleontology Club members were on hand to provide assistance when needed. Each class period lasted approximately thirty-five minutes providing ample time for completion of lesson activities.

Approximately one week prior to the lesson, the students were given a concept inventory (CI) to assess their pre-existing knowledge of lesson material. Students assessed ten Likert items (Likert 1932) indicating their level of confidence in each statement's validity. Each item contained five possible responses equating to the following statements: 1) I know this statement is false; 2) I think this statement is false; 3) I am unsure whether this statement is true or false; 4) I think this statement is true; 5) I know this statement is true. For example, the first CI statement was, "The scientific name of the Age of Dinosaurs is the Mesozoic Era." In response, the students could indicate, "No way", "I don't think so", "I don't know", "I think so", or "Absolutely". The number of true versus false statements was randomized to prevent students from recognizing trends. The same concept inventory was administered the day after the lesson was delivered, in order to assess cognition and retention of the material. Each Likert item was coded on a one to five scale to facilitate statistical comparison of students' assessments, the "correct" response for a given question coded as "5" regardless of the true or false nature of each statement.

The pre- and post-lesson inventory results were compared using standard descriptive statistical techniques. Comparisons of total scores and individual item scores were compared using mean, median, mode, and t-tests. The distribution of the scores was assessed using measures of skewness or assessed visually using histograms.

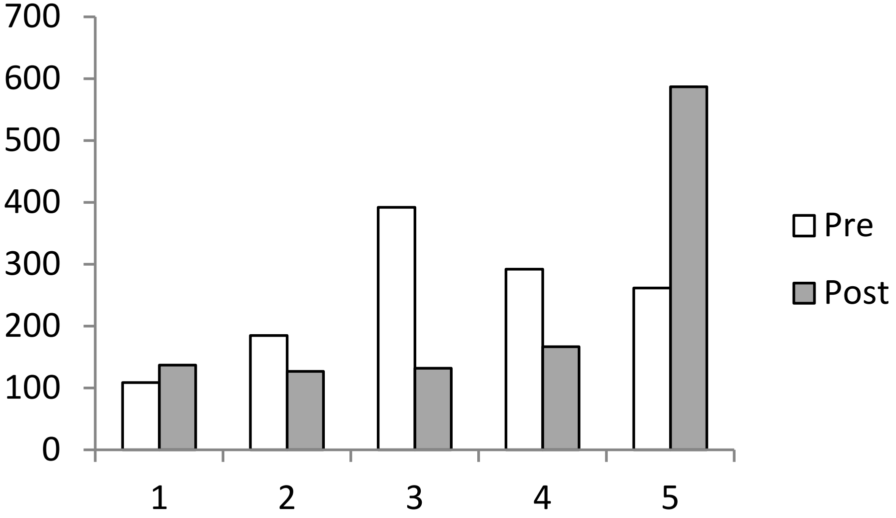

Figure 1. Histogram showing the total Likert scale distribution of scores pre- and post-lesson. The bins, numbered 1 through 5, are as follows: 1= I know this statement is false; 2 = I think this statement is false; 3 = I do not know if this statement is true or false; 4 = I think this statement is true; 5 = I know this statement is true. Responses for individual statements are coded so that the "correct" response is always "5". For false statements, the ordering is reversed, with the statement, "I know this statement is false", coded as "5".

RESULTS

Students showed demonstrable improvement in cognition of the material provided in the lesson. Table 1 illustrates a comparison of the total score on the pre- and post-lesson CI out of a possible score of 50, indicating perfect statement assessment. Male and female students showed no significant difference in pre- and post-lesson scores and equal levels of improvement in cognition. All descriptive statistics indicate an improvement in CI performance and t-test results indicate significant improvement in overall score.

Figure 1 illustrates the distribution of responses based on the modified Likert scale. The apparent normal distribution of the pre-lesson histogram indicates the majority of values on the Likert scale fall within the central bins, suggesting lower confidence in the material (skewness = -0.26632). Figure 1 also shows the distribution of answers following the lesson. Not only do many more values fall in bin "5" indicating correct assessment of each CI statement, it also reveals that students show a much higher level of confidence in their assessment of each statement (skewness = -0.85045).

Table 2 illustrates descriptive statistics for each question in the discussion section, including mean, median, and mode. The results of t-tests are also provided based on individual question differences before and after the lesson.

Answers to Question 3 (Q3), "The same types of dinosaurs lived together throughout the Mesozoic Era" indicated effective cognition of the core concept presented in the lesson. The mean score increased from 3.1 to 4.2 with significance (Table 2). Median and mode values suggest increased confidence in responses as well. The distribution of responses also reveals much greater accuracy and confidence in statement assessment, with many more "correct" responses post lesson (Figure 2). Although both distributions show negative skewness, comparison of pre-lesson (skewness = -0.05467) and post-lesson (skewness = -1.5989) shows a shift toward "correct" responses as well.

Responses to Question 7 (Q7), "Dinosaurs were large and abundant during the Triassic Period" indicated little change. Mean values increased only slightly, but the results were significant (Table 2). Median and mode showed little change. The distribution of responses changed only slightly before and after the lesson (Figure 3a). Dinosaurs were neither large nor abundant in the Triassic; however, most students appeared to be unaware of this fact before the lesson (skewness = 0.548228). Students showed little cognitive improvement in this concept. Most students still delivered an incorrect assessment of this statement after the lesson, and with little confidence, although the number of correct responses did improve slightly (Figure 3a) (skewness = 0.22736).

Conversely, students fared much better with Cretaceous dinosaurs. Responses to Question 9 (Q9), "The largest carnivorous dinosaurs lived during the Cretaceous Period", reveals a great deal of uncertainty before the lesson, with a mean of 3.08 (Table 2). The most well-known carnivorous dinosaur, *Tyrannosaurus*

Table 1. Pre- and Post-Lesson statistics for concept inventory scores out of a total of 50 with t-test results.

	N	Mean	Median	Mode
Pre-Lesson	124	33.306	33	32
Post-Lesson	115	38.139	38	38
	$t = 8.2774$	$P = 9.2034E\text{-}15$		

Table 2. Descriptive statistics and t-test results for concept inventory questions discussed in the results section. Q3: The same types of dinosaurs lived together during the Mesozoic Era; Q7: Dinosaurs were large and abundant during the Triassic Period; Q9: The largest carnivorous dinosaurs lived during the Cretaceous Period; Q10: Earth's continents have always been in the same location.

	Mean	Median	Mode	t	*P*
Q3	3.1209	3	2	-	-
Q3 Post	4.2782	5	5	-7.1134	1.33E-11
Q7	2.3387	2	2	-	-
Q7 Post	2.9043	3	2	-3.3898	6.49E-05
Q9	3.0887	3	3	-	-
Q9 Post	3.5565	4	5	-3.0063	1.15E-07
Q10	4.4032	5	5	-	-
Q10 Post	4.7043	5	5	-2.4614	0.0145

rex, is derived from Cretaceous deposits. While the post lesson mean score for this statement was 3.55, both medial and mode values indicate improvement. Response distribution also revealed little prior knowledge of large Cretaceous carnivores (skewness = -0.24592) (Fig 2b), but showed considerable improvement afterward (skewness = -2.834817) (Figure 3b).

Finally, students showed excellent pre- and post-lesson comprehension of the concepts of continent movement throughout time. Responses to Question 10 (Q10), "The continents have always been in the same location" were correct with confidence during both assessments. Mean, median and mode values (Table 2) were all high or showed slight improvement, and the distribution of responses (Figure 4) changed little.

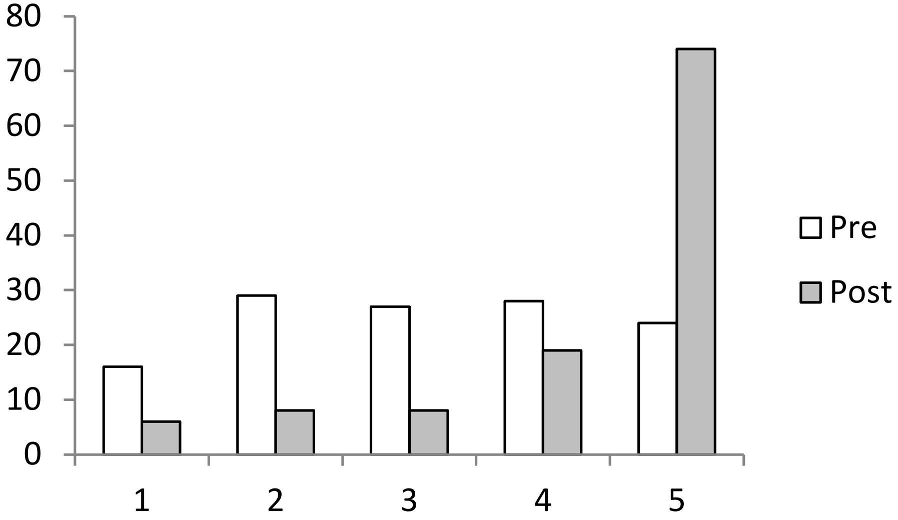

Figure 2. Histogram showing the pre- and post- lesson distribution of responses to the statement, "The same types of dinosaurs lived together throughout the Mesozoic Era." See Figure 1 for explanation of bins.

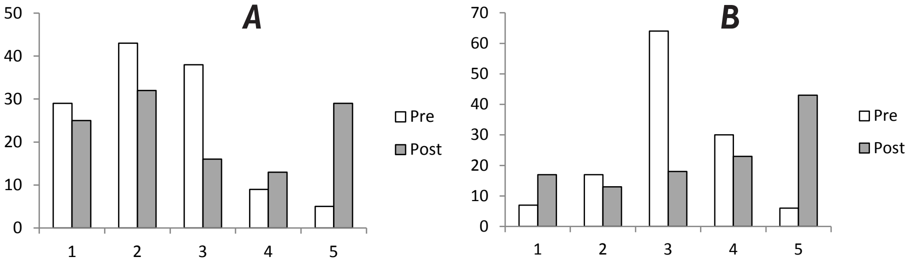

Figure 3. a) Histogram showing the pre- and post- lesson distribution of responses to the statement, "Dinosaurs were large and abundant during the Triassic Period." b) Histogram showing the pre- and post- lesson distribution of responses to the statement, "The largest carnivorous dinosaurs lived during the Cretaceous Period." See Figure 1 for explanation of bins.

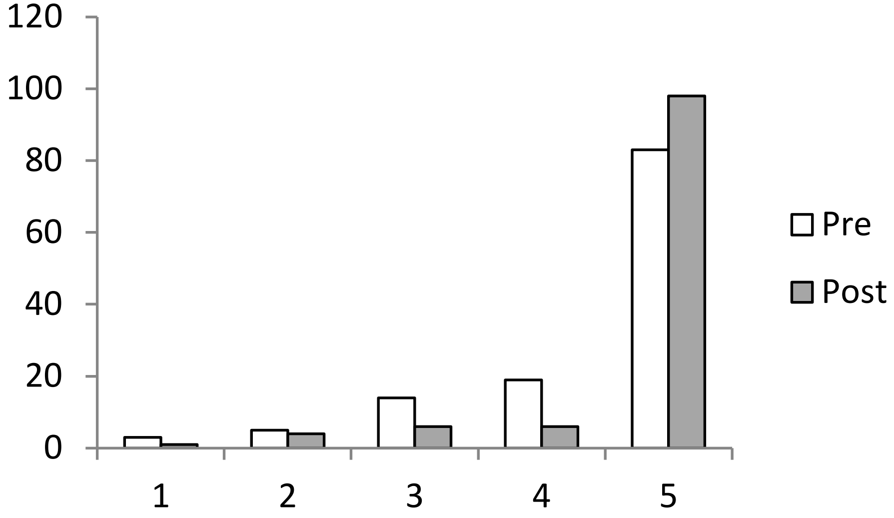

Figure 4: Histogram showing the pre- and post- lesson distribution of responses to the statement, "Earth's continents have always been in the same position." See Figure 1 for explanation of bins.

DISCUSSION

The evaluation results show that, although brief, the lesson appears to have improved students' cognition of some of the key concepts presented through the teaching kits. Students showed demonstrable improvement in both knowledge of and confidence with the material at hand, suggesting that this delivery method is an effective educational tool. Teaching kits can effectively illustrate the concepts of geologic time, changes in biological diversity throughout Earth's past, and plate tectonics.

The responses to specific statements were insightful. The notable increase in "correct" answers to Q3 suggests that students were able to understand, with confidence, the concept of faunal change during the Mesozoic, as presented by the lesson.

Students appeared unable to recognize that dinosaurs were still small and relatively rare during the Triassic Period. The result of this uncertainty may be due to the scale models themselves. The Jurassic and Cretaceous dinosaur models are produced at slightly less than 1:55 scale. The Triassic dinosaur models are produced at 1:10 scale. This likely proved misleading, as all models were similar in size despite the notable differences in size among the actual taxa.

Additionally, Triassic dinosaurs are not morphologically distinctive. As such, they lack some of the "charisma" that dinosaurs from the other time periods exhibit. During the lesson, Triassic dinosaurs may have been overshadowed by more "interesting" dinosaurs from other time periods. Therefore, it would seem more effective to utilize the more recognizable dinosaur taxa to illustrate important concepts as distinct dinosaurs appear to more easily capture students' attention.

Finally, comparatively little attention is paid to Triassic taxa as far less information is readily available. A World Wide Web search for "Triassic dinosaurs" produced 943,000 results, whereas a search for "Cretaceous dinosaurs" produced 2,020,000 results, and one for "Jurassic dinosaurs" produced 15,200,000 results. If less information is available on Triassic dinosaurs, students have less preexisting knowledge.

It was encouraging to note that our sample of students appears to be familiar with the concept that continents have changed position throughout Earth's past. One of the earth science standards in sixth grade at South Middle School requires a comprehension of basic plate tectonics. These concepts are repeated in eighth grade earth science. By middle school, students are equipped with a rudimentary knowledge of a changing and evolving Earth throughout geologic time. Further education could involve outlining events in greater detail.

The impact of these kits and the associated lesson complements some concepts proposed by the Next Generation Science Standards (NGSS). Paleontology can effectively act as a study in scientific synthesis, and many broad scientific concepts can be illustrated through paleontological examples. The need to directly address the cross-disciplinary nature of science was a key factor in development of the NGSS (NGSS Lead States 2013).

Paleontology is specifically utilized in several of the disciplinary core concepts outlined in the NGSS. For example, Middle School-Earth Systems Science practice History of Earth 1-4 (MS-ESS1-4) requires students to "Construct a scientific explanation based on evidence from rock strata for how the geologic time scale is used to organize Earth's 4.6 billion year old history." (NGSS Lead States 2013). One of the primary goals of the teaching kits was to illustrate the differing dinosaur associations from different time periods in the Mesozoic Era, thus allowing students to organize a series of events in Earth history. This study has shown these concepts to be readily accessible to most middle-school students given the proper framework and presentation.

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A MOBILE APPLICATION FOR EDUCATIONAL ASSESSMENT

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ABSTRACT

This paper describes an app which uses mobile, web and database technologies to reduce the time required for compiling and analyzing assessment data. Processing assessment data is the vehicle selected for discussing the broader issues of creating a mobile app to replace a manual process. The first step in converting any process to a mobile app is to map the current process to the functionality provided by the app. The second major step is to choose an operating system and a development language. These two steps are required for any conversion from a manual to an automated process. However, a unique set of constraints must be considered when making the selection for a mobile app. The availability of devices, the uniformity or diversity of the devices, the frequency of updates to the OS, the backward-compatibility of the updates, the restrictions placed on developers by the OS, and the ease of deployment are new factors that must be added to the typical list of software development considerations. This discussion merely provides a snapshot of the issues in a rapidly developing field where standardization is not yet on the horizon. But, using good programming practices, such as following the model-view-controller pattern, and encapsulating pieces of the app as described in the fragment discussion, improves the developer's ability to respond to the rapidly changing mobile environment.

Keywords

ABET, Mobile, Android, Assessment

INTRODUCTION

Assessment activities can be time-consuming and frustrating when the structure for gathering, cross-referencing and analyzing the data is not in place. We developed a web-based system, called MART (Mines Accreditation Rubric Tool) and an accompanying mobile application, to manage assessment data and to generate basic reports from the results. The assessment plan includes programmatic outcomes (student outcomes in ABET terminology), course outcomes and a mapping between the two. The degree to which program outcomes are met can be measured indirectly by combining the student attainment of course outcomes which contribute to the program outcome. The level of student attainment for a particular course can be viewed across multiple semesters, while the degree to which each programmatic outcome has been met can be viewed by combining the results from all course outcomes which support it. MART provides a database for storing the program outcomes, the course outcomes, the mapping, and a rubric for capturing the level of attainment on course outcomes entered each semester by a faculty member. It also generates the summary reports by course across multiple semesters and by program outcome by averaging student attainment on contributing course outcomes. This paper discusses both the product MART and the design trade-offs required to create the system. Issues of extendibility, user interface design, maintainability and ease of integration into existing processes are discussed. A comparison of each platform and development environment is provided, as is an overview of how issues specific to mobile applications, such as battery life and limited storage, were handled.

ABET—The primary accrediting board for engineering and technology programs is ABET. ABET's mission is to recognize and assure quality, while stimulating innovation in applied science, computing, engineering and engineering technology education [1]. Programs at the South Dakota School of Mines and Technology (SDSM&T) typically prepare for ABET accreditation every six years. This process is both time-consuming and arduous, and the correlation of data is not an easy process.

In previous decades, ABET accreditation was characterized by the evaluation of a program against a list of attributes required for all programs. By contrast, the current assessment-based approach requires the institution to determine its constituencies, set its objectives and student outcomes, identify mechanisms for gauging attainment of those objectives and outcomes, and to have a mature self-evaluation process whereby the data collected are used to quantify improvements to the program. This approach is not without problems. Accepted measures are not common and many of those that are evolving are labor-intensive. A lack of comprehensive software solutions to integrate the various measures and provide feedback on attainment is causing universities to divert faculty and staff time to collecting and analyzing data. MART tries to minimize the amount of work needed and maximize the amount of information that can be obtained from the data gathered.

ABET evaluates eight criteria including those focused on students, curriculum, the assessment and continuous improvement mechanism, faculty, facilities, and institutional support. MART and its corresponding database address only one section of the criteria: the Student Outcomes (SOs). Student Outcomes describe what students are expected to know and be able to do by the time of graduation. These relate to the knowledge, skills and behaviors that students acquire as they progress through the program. SDSM&T uses student attainment of Course Outcomes (COs) to assist with the evaluation of SOs across semesters. Each CO can map to one or many SOs and each SO is mapped to by COs from numerous courses. The COs, SOs and mappings for the Computer Science program at SDSM&T are available at the department website [2]. This mapping is an important part of the assessment process and it, with the data gathered, provides information for evaluating the progress of the program in meeting its stated out-

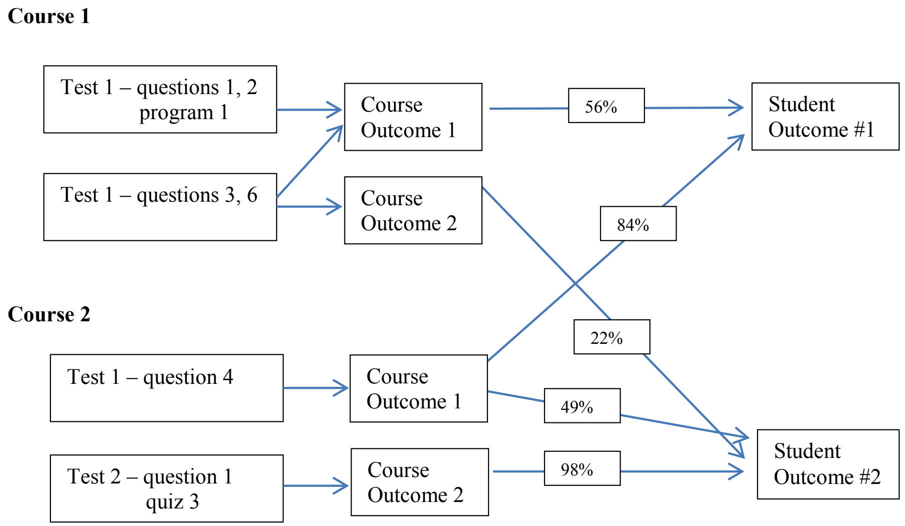


Figure 1. The percentage of students achieving 70% mastery or better on selected graded events is provided to MART for aggregation and analysis.

comes and objectives and in continuously improving. An example of the relationship between individual COs and program-level SOs is provided in Figure 1.

Website and Database—The first step in creating a more elegant system was to develop a website to serve as a control station. The website has many functions, including: add classes and semesters, change or edit SOs, create a list of COs for a certain course and map those outcomes to the SOs selected for the program. Leveraging the power of the website creates an easier process for copying data from year to year since for a given class the COs often change slowly and are repetitive. It also simplifies the process of maintaining the connections between outcomes as well as mapping and correlating trends from semester to semester.

The technology used to create this control station is the industry standard "LAMP" stack. LAMP stands for Linux, the operating system used on the server machine, Apache, the web server used for communication, MySQL, the database used, and PHP, the scripting language used to develop the code which creates the visual consumer-facing part of this system. The LAMP architecture was selected because it is a well-known, frequently-used, and free solution for creating a website to interface with a database.

Mobile Devices—Mobile devices have become ubiquitous. Sensor-packed cellphones connect the entire world and have the ability to access almost any data from virtually anywhere. The connectivity of these devices, coupled with the significant computing power available, makes them ideal for a variety of tasks.

There are certain design decisions that must be made prior to beginning development of a mobile app. One is the selection of a target Operating System (OS). Some developers create solutions for a selected OS while others release for all devices simultaneously, often using third-party software for the conversion.

In the US, there are three major players in mobile device OSs: Google's Android, Apple's iOS and Microsoft's Windows 8. Another notable player is Blackberry, which has been a significant presence in the emerging Smartphone market. All these businesses allow for outside mobile developers to create mobile applications to extend or enhance their OS.

The process of releasing a product to the public can take from hours to weeks, depending on the backlog of submissions and the brevity of the application. The ability to almost instantly distribute a developed product through a centralized marketplace that connects all potential buyers is a significant by-product of mobile development. Software developers in the past had to launch products through established distribution channels that would touch only a fraction of the potential buyers. This new distribution mechanism, largely driven by the creation of the Apple Store, has had the effect of making app development and deployment available to everyone without the need for a large initial capital investment.

Mobile Operating Systems—Choosing an OS can be difficult because each has advantages and disadvantages. One consideration is the number and quality of devices that run a particular OS. The iOS system is run only on Apple iPhones, iPods, and iPads. Apple has highly regulated design philosophies that create an environment where developers can feel safe releasing their products on those machines. Specifications such as screen size, pixel density and which version of the OS a device can run on are controlled to create a unified development procedure. On the negative side, apps which run on Apple devices are built using a programming language that is rarely used except on Apple products. Some developers find the strict control imposed by Apple and the relatively high cost of many Apple devices to be constraining. Google has taken a very different approach with its Android OS. Android apps are created using Java, one of the most often used and well known programming languages. Java has the advantage that it shares similarities with a number of other languages, making it an easy second language for many developers. In addition, Google's laissez-faire attitude toward device specifications has created a robust selection of offerings across all price ranges. Anyone can develop and market an Android phone. Attributes such as screen size and pixel density are uncontrolled. The phone companies themselves can choose which OS their phone will run on. This variability creates a different set of challenges for the app developer. Microsoft, the newest entrant into the mobile device market, currently has the smallest market share, but has adopted positive attributes from both Google and Apple. It semi-regulates screen sizes and pixel densities, creating less fragmentation than Google. The development languages for Microsoft devices are HTML, JavaScript, CSS, and C#, which is a well-known language that shares many similarities with other languages. Microsoft also has the unique feature that its mobile devices can mimic their desktop counterparts, creating a familiar feeling to anyone utilizing Windows 8 as their desktop OS.

Mobile Design—The Android OS was ultimately selected for this project due to the ease of deploying an app to a device, Android's market share, and simplicity of development. While this discussion focuses on Android, there are design constraints common to all the mobile OS choices. Problems such as data storage space, user experience on different screen sizes, battery life, and lack of network access plague all mobile devices and platforms.

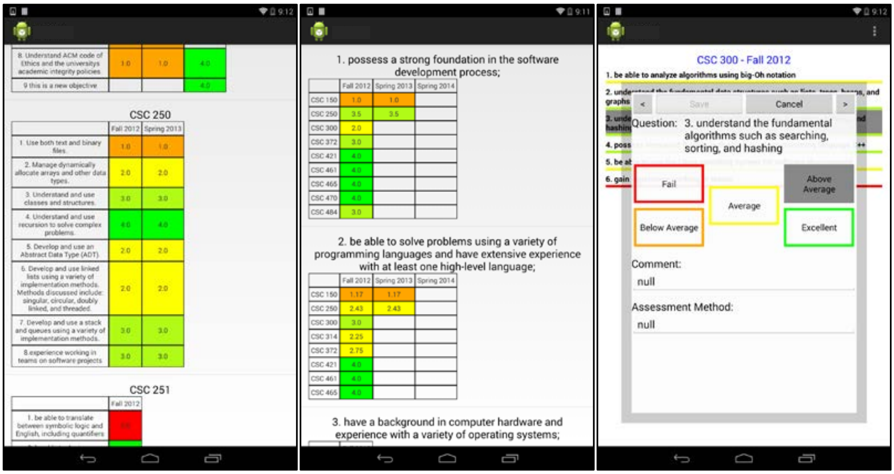
The issues of network connectivity and battery life are inherently linked. The method implemented for keeping data transfers to a minimum, and thus battery life to a maximum, is the use of JSON—a way of organizing data in a lightweight human readable form [3].

There are coding philosophies that need to be evaluated as well, with ever-evolving devices and changes to how mobile devices are used. The code must be easy to maintain and allow for evolution along with the devices. One approach, and the most widely used to create a code base that allows for this, is to use the model-view-controller (MVC) pattern. It insulates each function of the code from the next, thus creating a way to change individual parts without having to reimagine and redesign the rest. The model is the data associated with the application in MART, specifically, the data acquired from the database. The graphical user interface (GUI) is the view the user sees while interacting with the application. The controller is the piece that ties these two parts together and fills the role of interpreter. Using this model, one can make substantial changes to how the data will be viewed without changing how the data are acquired and vice versa.

The Android OS enables the MVC through the use of fragments, pieces of an application's user interface or behavior that can be placed in an Activity. An Activity is the base of any Android application. This means a fragment is a view that can be placed anywhere user interaction is enabled. Fragments are completely independent pieces of the puzzle; the program determines where the fragment resides. The activity, or controller, has no idea what the fragment does; it just passes the data supplied from the model to the fragment, and the fragment creates a view where the controller specified.

The availability of fragments creates an easy-to-use, maintainable and expandable code base. Each fragment can be maintained individually without impacting other parts of the application. It also creates the ability to add new fragments or views without the need to define new ways of accessing or downloading data. At the same time, if the method for gathering information changes, the fragments are not affected as long as the data are translated by the controller into a form the fragment can understand.

Fragments solve many of the problems seen in mobile applications. They create the pure MVC experience, increasing the maintainability and extendibility of the code base. The other issues, such as battery life and network connectivity overlap and are solvable together. There are certain battery costs that are unavoidable in mobile applications, such as keeping the screen lit, changing the contents on a screen or making http requests to pull data out of the Cloud. There are also battery costs that can be avoided, such as preventing http requests when there is no data-capable connection or pulling down data multiple times when no data have changed. Some of these can be handled by making sure the app is connected to a data-capable network before http requests are made. Others can be handled by having the web service push data, thus updating the device only when fresh data are available.



8. Understand ACM code of Ethics and the universitys academic integrity policies	1.0	1.0	4.0
9 this is a new objective			4.0

CSC 250

	Fall 2012	Spring 2013
1. Use both text and binary files.	1.0	1.0
2. Manage dynamically allocate arrays and other data types.	2.0	2.0
3. Understand and use classes and structures.	3.0	3.0
4. Understand and use recursion to solve complex problems.	4.0	4.0
5. Develop and use an Abstract Data Type (ADT).	2.0	2.0
6. Develop and use linked lists using a variety of implementation methods. Methods discussed include: singular, circular, doubly linked, and threaded.	2.0	2.0
7. Develop and use a stack and queues using a variety of implementation methods.	3.0	3.0
8.experience working in teams on software projects	3.0	3.0

CSC 251

	Fall 2012
1. be able to translate between symbolic logic and English, including quantifiers	0.0

1. possess a strong foundation in the software development process;

	Fall 2012	Spring 2013	Spring 2014
CSC 150	1.0	1.0	
CSC 250	3.5	3.5	
CSC 300	2.0		
CSC 372	3.0		
CSC 421	4.0		
CSC 461	4.0		
CSC 465	4.0		
CSC 470	4.0		
CSC 484	3.0		

2. be able to solve problems using a variety of programming languages and have extensive experience with at least one high-level language;

	Fall 2012	Spring 2013	Spring 2014
CSC 150	1.17	1.17	
CSC 250	2.43	2.43	
CSC 300	3.0		
CSC 314	2.25		
CSC 372	2.75		
CSC 421	4.0		
CSC 461	4.0		
CSC 465	4.0		

3. have a background in computer hardware and experience with a variety of operating systems;

CSC 300 - Fall 2012

1. be able to analyze algorithms using big-Oh notation

Question: 3. understand the fundamental algorithms such as searching, sorting, and hashing

Comment: null

Assessment Method: null

Figure 2: Three screens from the MART app. On the left, Figure 2(a), the attainment across multiple semesters for a given course is shown. Figure 2(b) in the center shows attainment for particular SOs as aggregated from all contributing courses. Figure 2(c) on the right shows the data entry screen.

The Assessment App—When designing a course, a faculty member selects the graded events that will be used to evaluate the outcomes identified for that course. The faculty member uses the grades on those items (such as test questions, programs, quizzes, reports or presentations) to determine the extent to which the students in the class have attained sufficient mastery of the material selected for evaluation of the COs. The COs, and their mapping to the programmatic SOs, are determined by the faculty as a group and are reviewed annually.

Final grades and individual test scores did not provide the necessary detail for the assessment plan developed by the Computer Science Department at SDSM&T, hence faculty members track scores on particular questions in a spreadsheet. "Sufficient mastery" is defined by the department as 70% or higher. The faculty member records the percentage of students who met the 70% or higher standard into MART and the app performs the data analysis described above. The app provides reports on attainment of SOs across semesters for a given course as well as attainment of an SO by aggregating data from all courses which contribute to that outcome. While the raw data can be accessed through the database, a simple color-coded display was designed for a quick evaluation. The percentage of students who achieved mastery of 70% or better is converted to the standard 4.0 academic scale. Thus, converting from the traditional grading scale becomes: 90 – 100% = 4.0, 80 – 89% = 3.0, 70 – 79% = 2.0, 60 – 69% = 1.0, and 0 – 60% = 0.0. The blocks are color-coded with 4.0 assigned green, 3.0 light green, 2.0 yellow, 1.0 orange, and 0.0 red to allow for quick visual inspection of the results. Consequently, the app generates a "GPA" for each outcome.

This app has three main data views that either allow the user to input data or to view data. Figure 2(a) and Figure 2(b) are examples of reports that are helpful for assessing a class while Figure 2(c) is a data input screen. Figure 2(a) shows how a class performed across multiple semesters on the SOs to which that class is mapped. Figure 2(b) shows the average performance of all the classes that mapped to that SO for a given semester. Figure 2(c) shows an example of how a user would grade a CO by adding the attainment information and associated comments. The mobile app and website make this process more convenient for the faculty, and the ease of use makes it feasible for the department head to analyze this information on an annual basis.

CONCLUSION

The Computer Science faculty members at SDSM&T have supported the development of this project and are optimistic that it will provide easy, accurate data to support the assessment process. Entering the COs and the mapping between them and the programmatic SOs can be done by a student. This should save each faculty member several hours per semester. The assessment coordinator estimates that collecting, analyzing and formatting the data takes between three and four hours per semester. In the fall semester, these hours must be carved from the same time needed to prepare for the start of the next semester, while in the spring, the task is often delayed until the summer. Removing half a day's effort increases the likelihood that the reports will be completed in a timely manner. The system was launched after the ABET visit in 2013, but is expected to be used in preparation for the next campus-wide visit in 2016. In addition, the department head plans to use the system for the annual assessment report he prepares for the institution.

LITERATURE CITED

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An Introduction to JavaScript Object Notation (JSON) in JavaScript and .NET. Available at http://msdn.microsoft.com/en-us/library/bb299886.aspx [Cited 9 July 2014].

INCORPORATION OF THE DEPARTMENT OF ENERGY "INTERPRET A GEBA GENOME" EDUCATION PROGRAM INTO AN UNDERGRADUATE MOLECULAR BIOLOGY COURSE

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ABSTRACT

Bioinformatics is widely used in molecular biology and proficiency in this discipline has been linked to success in other areas of biology and chemistry. However, acquiring expertise in bioinformatics requires experiential learning with access to a broad spectrum of bioinformatics tools in order for students to comprehend the complexity and value associated with bioinformatics analysis. To increase undergraduate student aptitude in bioinformatics applications, Mount Marty College established collaborations with the Department of Energy Joint Genome Institute (JGI). Faculty collaborators in the "Interpret a GEBA Genome" education program receive training and have access to the Integrated Microbial Genomes Annotation Collaboration Toolkit (IMG-ACT). This program provides a faculty portal which permits the inclusion of a variety of bioinformatics tools along with virtual notebooks and reports that are utilized by the JGI to update their annotation databases based on the manual annotations performed by undergraduate students. In this report, we describe the incorporation of the "Interpret a GEBA Genome" program into an undergraduate molecular biology course and the subsequent increase in student learning in both bioinformatics and associated content. The laboratory component of the molecular biology course was modified such that genome analysis and gene annotation were the predominant content for the laboratory sessions. Students were assessed for knowledge in bioinformatics tools selection, data interpretation, and associated molecular biology content prior to participation and periodically throughout the program. Overall, inclusion of the program has increased student learning in bioinformatics and molecular biology.

INTRODUCTION

As molecular biology has moved from an *in vivo* to *in silico* science, bioinformatics has become a standard technique. At the same time undergraduate students are becoming more tech savvy and expect to have the latest technologies in the classroom. However, there is a divergence between the increasing reliance on bioinformatics in molecular biology and the techniques used to teach molecular biology in the classroom. Bioinformatics is often viewed by the undergraduate student as a discipline solely for the highly educated scientist. The Department of Energy Joint Genome Institute (JGI) developed the "Interpret a GEBA Genome" (IGG) education program to educate undergraduate students in the field of bioinformatics (Ditty et al. 2010). The JGI specifically targeted undergraduate students since they have the capacity to conduct and interpret the bioinformatics results. These data are utilized by the JGI to update their annotation databases based on the manual annotations performed by the undergraduate students (Ditty et al. 2010).

Proficiency in bioinformatics has been identified as an indicator of probable success in other biology and chemistry disciplines (Banta et al. 2012; Dymond et al. 2009; Furge et al. 2009; Shaffer et al. 2014). Acquiring expertise in bioinformatics requires experiential learning with access to a broad spectrum of bioinformatics tools for students to comprehend the complexity and the value associated with bioinformatics analysis. Biology educators have been incorporating bioinformatics analysis into their curricula comprising entire courses dedicated to bioinformatics to special exercises in a wide range of classes (Banta et al. 2012; Beagley 2013; Ditty et al. 2013; Klein and Gulsvig 2012; Newell et al. 2013; Sanders and Hirsch 2014; Wood and Gebhardt 2013). To increase undergraduate student aptitude in bioinformatics applications, Mount Marty College (MMC) established collaborations with the JGI IGG program. This program provided instructor training and access to a faculty portal which permitted the inclusion of a variety of bioinformatics tools along with virtual notebooks and reports.

This report describes the incorporation of the "Interpret a GEBA Genome" program into an undergraduate molecular biology course and the subsequent increase in student learning in both bioinformatics and its associated molecular biology content. The students were assessed based on three components: an online laboratory notebook, an oral presentation, and exam questions. Students were evaluated for acquired knowledge in bioinformatics tools selection, data interpretation, and associated molecular biology content prior to participation and periodically throughout the program. Overall, the inclusion of the program increased student learning in bioinformatics and molecular biology.

METHODS

Gene annotation using the "Interpret a GEBA Genome" toolkit—MMC established collaborations with the Department of Energy Joint Genome Institute (JGI). MMC faculty collaborators in the "Interpret a GEBA Genome" (IGG) education program received training and access to the Integrated Microbial Genomes Annotation Collaboration Toolkit (IMG-ACT). The IGG portal allowed faculty to manage student assignments and harbored the module instructions and links to the annotation tool websites along with the online laboratory notebooks and reports associated with each assigned gene. The professor utilized the portal to customize the genes that the students were assigned and determined which modules were relevant to the learning objectives. Students independently annotated the genes and entered their data from that week's interpretation into the online IGG IMG-ACT laboratory. MMC was assigned *Sphaerochaeta coccoides* DSM 17374 as its GEBA genome to annotate and the data presented are based on annotation of this genome.

The IGG toolkit includes nine annotation modules: Basic Information, Sequence-based Similarity Data, Cellular Localization, Alternative Open Reading Frame, Structure-based Evidence, Enzymatic Function, Gene Duplication and Degradation, Horizontal Gene Transfer, and RNA. These nine modules are composed of 23 sub-modules that directed the student to link to annotation tools which are websites and databases used to collect the pertinent data. The RNA module, which used the Rfam tool, was not included in the course since the selected genes did not include RNA-specific functions such as non-coding RNA genes, regulatory motifs within the transcribed regions of protein-coding genes, and motifs associated with self-splicing RNAs (Griffiths-Jones et al. 2005).

Several of the sub-modules contained overlapping data that the student utilized as evidence to confirm or invalidate the computer annotation. Completion of the module permitted the students to comprehensively annotate the gene of interest by investigating the different properties of the gene and to determine the utility of each of the tools for determining gene function.

Faculty implementation strategy—The laboratory component of the MMC BIO-364 Cell and Molecular Biology course was modified such that genome analysis and gene annotation were the predominant content for the laboratory sessions. Students completed a pre-test which contained questions to determine their previous exposure and knowledge of bioinformatics during the first week of lab. During the following weeks, students were introduced to the IGG's IMG-ACT toolkit and were taught through instructor demonstration one module each week using an example gene. Students were then assigned additional genes to independently annotate and were graded on a mastery based system whereby the student had to correctly complete the module annotation to earn credit.

The IGG material was incorporated with relevant molecular biology content discussed in the lecture portion of the course. For instance, the Basic Information module could be perceived as a "cut and paste" module since the information obtained from the module did not require utilization of specific annotation tools. This module included the sub-modules DNA Coordinates, DNA Sequence, and Protein Sequence. This module was taught first to orient the student to the IMG-ACT portal, including utilization of the online laboratory notebooks and to practice accessing the Gene Details database. This database was regularly utilized for confirmation or invalidation of the computer annotation. To maximize student learning and to negate the "cut and paste" mentality, the professor incorporated concepts such as nucleic acid and protein sequencing, nucleic acid polarity, genome organization, open reading frames, initiation codon utilization, and additional relevant molecular biology content into the class session.

During the incorporation of the IGG program into the course, modules were not taught in the order listed in the IMG-ACT toolkit. The order was modified to correspond with the subjects that were discussed in the lecture portion of the course. In addition, the order was not presented linearly to allow students to understand that the website harbored a collection of tools and that it was flexible enough to meet the needs of the researcher.

The genes for the module lessons were selected to demonstrate that bioinformatic analysis requires data interpretation and proper tool utilization. Genes were also selected that would produce manual annotation data that deviated from the predicted computer annotation. The instructor performed several annotations during course preparation to select different types of results for the student annotations.

After all modules were taught, the students were assigned additional genes in a project format to perform a complete analysis and were also assigned modules to study in depth for presentation at the Natural Science Division Seminar. Data were collected and compiled in accordance with the MMC Internal Review Board guidelines so that no personal identifiers were associated with the data

Assessment instruments—The students were assessed based on three components: an online laboratory notebook, an oral presentation, and exam questions. Each week the students completed an online laboratory notebook regarding the module of interest. Students were required to have accurate data formatted according to IGG program guidelines to complete and earn credit for the module. The notebooks were graded on a mastery system whereby the students were given opportunity to rework the module until the data were entered correctly. After students had learned and completed all of the modules, they were assigned a module to study in depth for an oral presentation at the MMC Natural Science Division Seminar which is attended by community members, science students, and faculty. Students had to prepare a written script for their oral presentation; the script was submitted for grading and revisions prior to the oral presentation. Students were graded based on the rough and final drafts of the script in addition to their oral presentation. They were also assessed on a series of multiple-choice exam questions. Students were tested on identical questions in a pre-test, unit exams, and final exam, each of which assessed knowledge in bioinformatics tools selection, data interpretation, and associated molecular biology content.

RESULTS

After incorporation of the IGG program into the MMC undergraduate molecular biology course, there was an increase in student learning in both bioinformatics and its associated molecular biology content. One module emphasized both the need for data interpretation and confirmation or invalidation of the computer annotation, the Alternative Open Reading Frame module which utilized the IMG Six-Frame Viewer. I describe the student annotation of three *S. coccoides* open reading frames (ORFs) in the following paragraphs.

The students utilized the IMG Six-Frame Viewer to analyze the ORF of *S. coccoides* gene Spico_0417, which depicted a blue-highlighted Shine-Dalgarno sequence but had two potential start codons including the computer annotated codon (Figure 1, highlighted in yellow with red text). Students had to determine which of the two start codons, which were in proximity to the Shine-Dalgarno sequence, was the correct one based on the properties of the ORF. The students

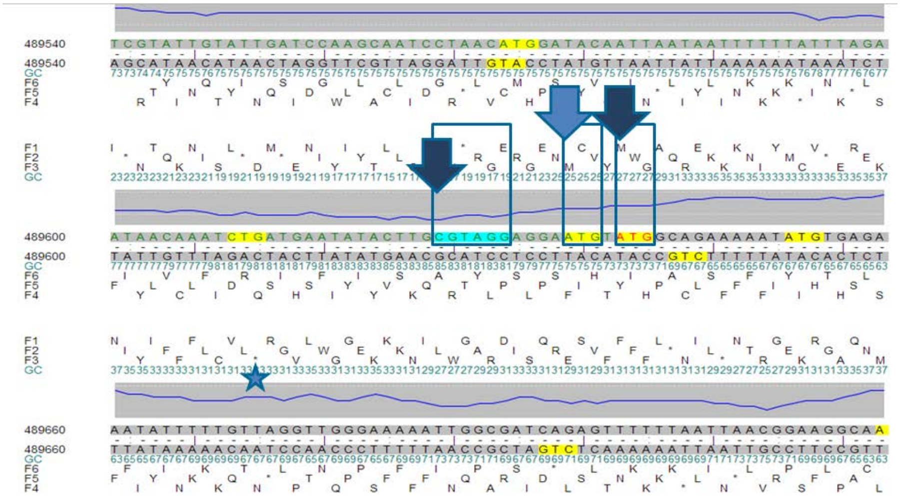


Figure 1. Spico_0417 Six-Frame Viewer .** S. coccoides **gene Spico_0417 was analyzed using the IMG Six-Frame Viewer to analyze the ORF. This resulted in a blue highlighted Shine-Dalgarno sequence and two potential start codons including the computer annotated codon highlighted in red text. The students determined that the computer annotated Reading Frame 1 start codon (dark blue arrow) was correctly identified since the reading frame did not contain an in-frame stop codon close to the start site compared to Reading Frame 3 (star).

determined that the computer annotated Reading Frame 1 start codon was correctly identified since the reading frame did not contain an in-frame stop codon close to the start site compared to Reading Frame 3 (Figure 1) and also coded for the predicted translated protein sequence (data not shown).

IMG Six-Frame Viewer analysis of Spico_0630 required the students to compare two Shine-Dalgarno and start codon pairs, both of which were in the proper reading frame. The computer-predicted start site did not have a blue-highlighted Shine-Dalgarno sequence in the tool output; however, the students were informed during their initial training that the tool prioritizes start codons over Shine-Dalgarno sequences. The students compared their gene sequence to a consensus WebLogo sequence for Shine-Dalgarno that they had previously created. The students had utilized the 55 blue-highlighted sequences obtained from analyzing 90 base-pairs upstream and downstream of Spico_0332 Reading Frame 1 (Figure 2, inset; Crooks et al. 2014). The students then identified a putative Shine-Dalgarno sequence and start codon pair for Spico_0630 (Figure 2, dark blue arrows). The students also located an additional putative Shine-Dalgarno sequence and start codon pair that was in the same reading frame (Figure 2, light blue arrows). After comparing both Shine-Dalgarno sequences to the consensus sequence, the students determined that both pairs were potentially valid and proceeded with the remainder of the annotation modules analyzing with and without the first four amino acids.

Analysis of the ORF for Spico_0409 required the students to utilize both the graphic and text outputs of the IMG Six-Frame Viewer tool in addition to the Chromosome GC Viewer tool. The IMG Six-Frame Viewer graphic output did

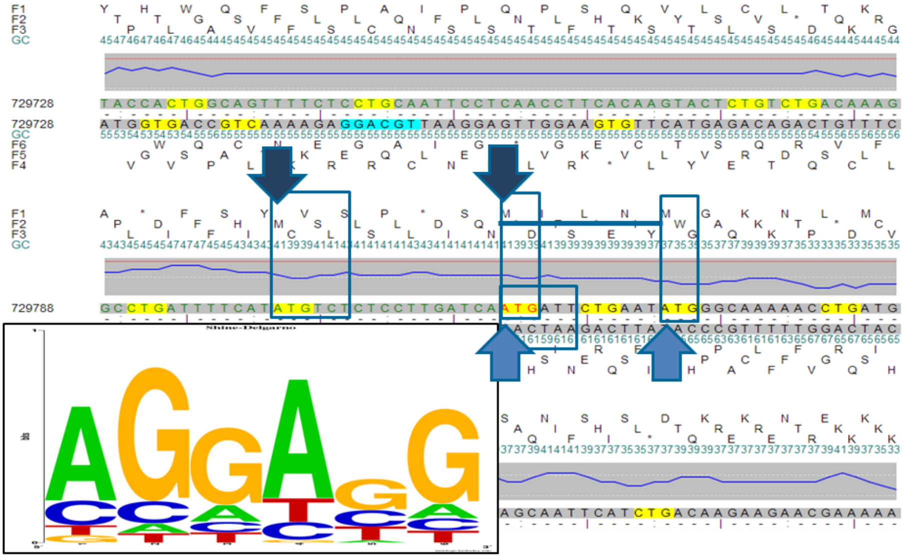


Figure 2. Spico_0630 Six-Frame Viewer . *S. coccoides* **gene Spico_0630 was analyzed using the IMG Six-Frame Viewer . The computer predicted start site did not have a highlighted blue Shine-Dalgarno sequence in the tool output since the tool prioritized start codons over Shine-Dalgarno sequences. The students identified a putative Shine-Dalgarno sequence and start codon pair (dark blue arrows). The students also located an additional putative Shine-Dalgarno sequence and start codon pair that was in the same reading frame (light blue arrows). The students compared both Shine-Dalgarno sequences to a consensus WebLogo sequence produced by the students (inset; Crooks et al. 2014).**

not have a coded methionine at the computer-predicted start site (Figure 3, dark blue arrow). The students subsequently performed the analysis utilizing the text output and a threshold value of 80 amino acids. The students then determined that the computer-predicted sequence was truncated and, in reality, could harbor an additional nine amino acids at the amino terminus (Figure 4). Since there was no putative Shine-Dalgarno sequence upstream of either start site, the students utilized the Chromosome GC Viewer tool and determined that the gene was potentially a co-transcribed/translated gene due to the proximity of the upstream gene (Figure 5).

Reinforcement through group projects and oral presentations—The students were assigned complete analyses of additional genes in a project format to increase student comprehension and retention of the bioinformatics techniques and data interpretation. The first group project was to conduct an IMG Pathway Analysis for a pathway that had an unknown assertion status designated by the JGI. The assertion status was categorized as unknown since the initial automatic IMG annotation was uncertain that the complete pathway existed because the genes for one or more enzymes in the pathway appeared to be absent from the genome. This project required identification of the missing gene and annotations to confirm all of the genes identified along the pathway. The *S. coccoides* Standard Embden-Meyerhoff Pathway was missing the initial enzyme of the

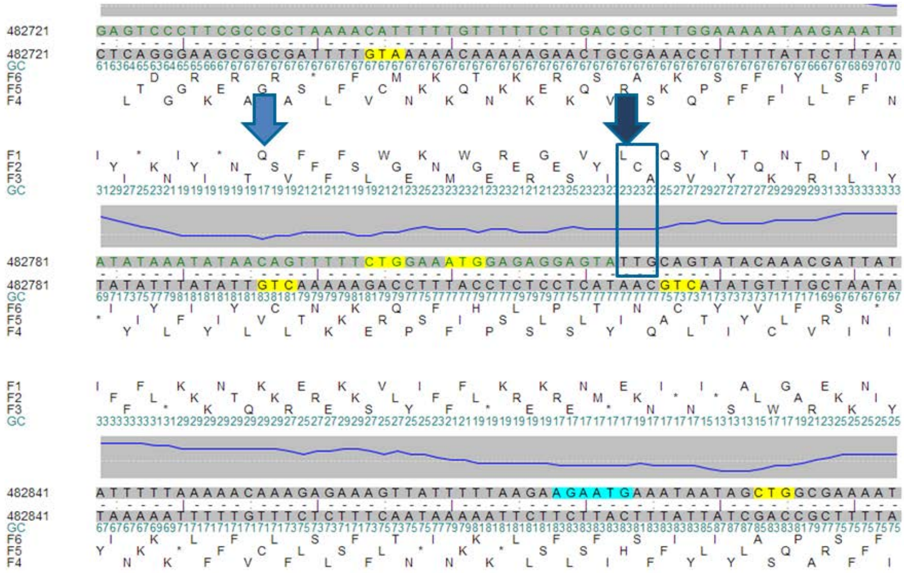
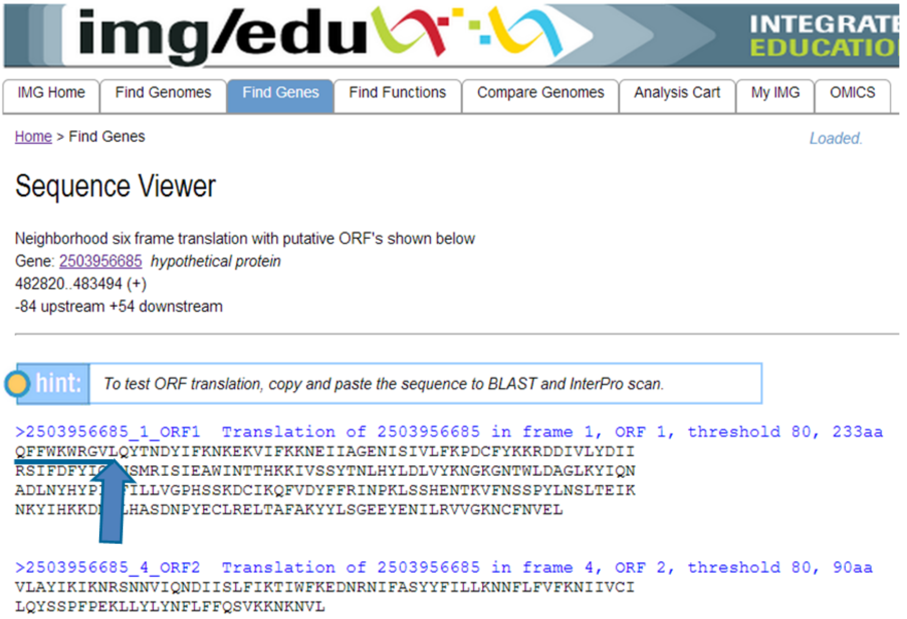


Figure 3. ***Spico_0409 Six-Frame Viewer.*** *S. coccoides* ***gene Spico_0409 was analyzed using the IMG Six-Frame Viewer . The graphic output did not have a coded methionine at the computer predicted start site (dark blue arrow). The subsequently performed text output analysis (Figure 4) predicted an additional nine amino acids at the amino terminus (light blue arrow).***



Sequence Viewer

Neighborhood six frame translation with putative ORF's shown below
Gene: 2503956685 *hypothetical protein*
482820..483494 (+)
-84 upstream +54 downstream

To test ORF translation, copy and paste the sequence to BLAST and InterPro scan.

```
>2503956685_1_ORF1  Translation of 2503956685 in frame 1, ORF 1, threshold 80, 233aa
QFFWKWRGVLQYTNDYIFKNKEKVIFKKNEIIAGENISIVLFKPDCFYKKRDDIVLYDII
RSIFDFYI[illegible]SMRISIEAWINTTHKKIVSSYTNLHYLDLVYKNGKGNTWLDAGLKYIQN
ADLNYHYP[illegible]FILLVGPHSSKDCIKQFVDYFFRINPKLSSHENTKVFNSSPYLNSLTEIK
NKYIHKKD[illegible]LHASDNPYECLRELTAFAKYYLSGEEYENILRVVGKNCFNVEL

>2503956685_4_ORF2  Translation of 2503956685 in frame 4, ORF 2, threshold 80, 90aa
VLAYIKIKNRSNNVIQNDIISLFIKTIWFKEDNRNIFASYYFILLKNNFLFVFKNIIVCI
LQYSSPFPEKLLYLYNFLFFQSVKKNKNVL
```

Figure 4. ***Spico_0409 ORF Text Viewer.*** *S. coccoides* ***gene Spico_0409 was analyzed using the IMG Six-Frame Viewer text output and a threshold value of 80 amino acids. The students determined that the computer predicted sequence was truncated and could harbor an additional nine amino acids at the amino terminus (underlined).***

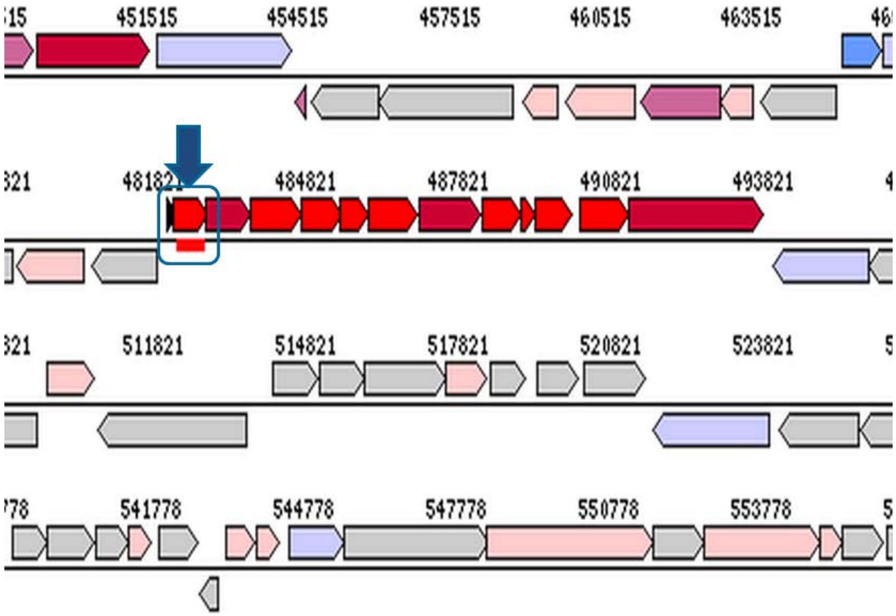
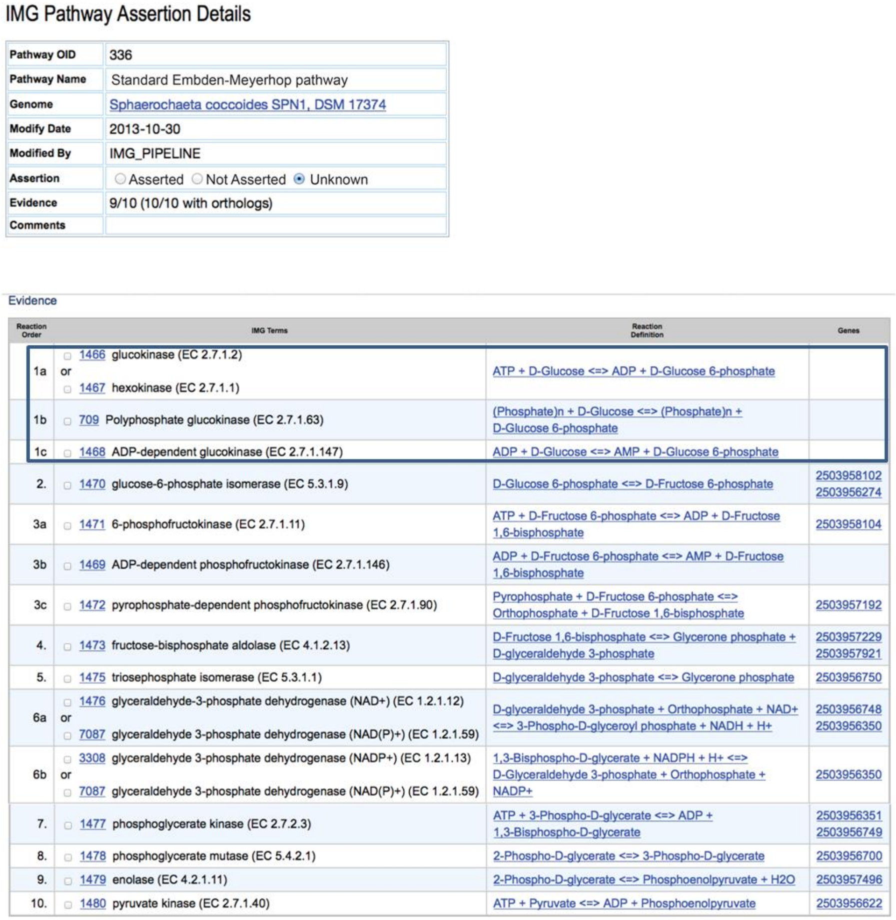


Figure 5. Spico_0409 Chromosome GC Viewer.** S. coccoides* ***gene Spico_0409 was analyzed using the Chromosome GC Viewer tool since IMG Six-Frame Viewer analysis did not detect a putative Shine-Dalgarno sequence upstream of either start site (Figure 3). The students determined that the Spico_0409 gene (red underline) was potentially a co-transcribed/translated gene due to the proximity of the upstream gene (blue box).

pathway (Figure 6, blue box). The students determined through their annotations, including Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, that both a glucokinase and hexokinase were encoded in the genome, but had not yet been associated with the pathway (Figure 7, left panel, light blue arrows). However, the original KEGG analysis was missing the third enzyme of the pathway (Figure 7, left panel, dark blue arrows) which students confirmed was encoded in the *S. coccoides* genome. This information was later used by Kanehisa Labs, who manage the KEGG database, to revise the KEGG pathway annotation (Figure 7, right panel; Kanehisa Labs 2012 and 2014).

A second group project was to annotate a group of genes that the computer had annotated as hypothetical proteins. This project required a comprehensive annotation of the genes and interpretation of the results to determine the possible functions of the putative proteins. The computer had identified these genes as hypothetical proteins due to weak, minimal, or conflicting results from individual annotation tools. Students analyzed their results from the 22 tools and determined the overall annotation based on the strongest evidence (Table 1). Students were given the same gene set in two sequential courses since the databases that are utilized for the tools are dynamic and would likely result in additional hits due to sequences added to the databases. Seven out of eight genes were identified with a function and several genes were identified with the same function by both groups of students independently.



IMG Pathway Assertion Details

Pathway OID	336
Pathway Name	Standard Embden-Meyerhop pathway
Genome	Sphaerochaeta coccoides SPN1, DSM 17374
Modify Date	2013-10-30
Modified By	IMG_PIPELINE
Assertion	○ Asserted ○ Not Asserted ● Unknown
Evidence	9/10 (10/10 with orthologs)
Comments	

Evidence

Reaction Order	IMG Terms	Reaction Definition	Genes
1a	☐ 1466 glucokinase (EC 2.7.1.2) or ☐ 1467 hexokinase (EC 2.7.1.1)	ATP + D-Glucose <=> ADP + D-Glucose 6-phosphate	
1b	☐ 709 Polyphosphate glucokinase (EC 2.7.1.63)	(Phosphate)n + D-Glucose <=> (Phosphate)n + D-Glucose 6-phosphate	
1c	☐ 1468 ADP-dependent glucokinase (EC 2.7.1.147)	ADP + D-Glucose <=> AMP + D-Glucose 6-phosphate	
2.	☐ 1470 glucose-6-phosphate isomerase (EC 5.3.1.9)	D-Glucose 6-phosphate <=> D-Fructose 6-phosphate	2503958102 2503956274
3a	☐ 1471 6-phosphofructokinase (EC 2.7.1.11)	ATP + D-Fructose 6-phosphate <=> ADP + D-Fructose 1,6-bisphosphate	2503958104
3b	☐ 1469 ADP-dependent phosphofructokinase (EC 2.7.1.146)	ADP + D-Fructose 6-phosphate <=> AMP + D-Fructose 1,6-bisphosphate	
3c	☐ 1472 pyrophosphate-dependent phosphofructokinase (EC 2.7.1.90)	Pyrophosphate + D-Fructose 6-phosphate <=> Orthophosphate + D-Fructose 1,6-bisphosphate	2503957192
4.	☐ 1473 fructose-bisphosphate aldolase (EC 4.1.2.13)	D-Fructose 1,6-bisphosphate <=> Glycerone phosphate + D-glyceraldehyde 3-phosphate	2503957229 2503957921
5.	☐ 1475 triosephosphate isomerase (EC 5.3.1.1)	D-glyceraldehyde 3-phosphate <=> Glycerone phosphate	2503956750
6a	☐ 1476 glyceraldehyde-3-phosphate dehydrogenase (NAD+) (EC 1.2.1.12) or ☐ 7087 glyceraldehyde 3-phosphate dehydrogenase (NAD(P)+) (EC 1.2.1.59)	D-glyceraldehyde 3-phosphate + Orthophosphate + NAD+ <=> 3-Phospho-D-glyceroyl phosphate + NADH + H+	2503956748 2503956350
6b	☐ 3308 glyceraldehyde 3-phosphate dehydrogenase (NADP+) (EC 1.2.1.13) or ☐ 7087 glyceraldehyde 3-phosphate dehydrogenase (NAD(P)+) (EC 1.2.1.59)	1,3-Bisphospho-D-glycerate + NADPH + H+ <=> D-Glyceraldehyde 3-phosphate + Orthophosphate + NADP+	2503956350
7.	☐ 1477 phosphoglycerate kinase (EC 2.7.2.3)	ATP + 3-Phospho-D-glycerate <=> ADP + 1,3-Bisphospho-D-glycerate	2503956351 2503956749
8.	☐ 1478 phosphoglycerate mutase (EC 5.4.2.1)	2-Phospho-D-glycerate <=> 3-Phospho-D-glycerate	2503956700
9.	☐ 1479 enolase (EC 4.2.1.11)	2-Phospho-D-glycerate <=> Phosphoenolpyruvate + H2O	2503957496
10.	☐ 1480 pyruvate kinase (EC 2.7.1.40)	ATP + Pyruvate <=> ADP + Phosphoenolpyruvate	2503956622

Figure 6. *S. coccoides* ***Standard Embden-Meyerhoff IMG Pathway Assertion. The JGI IMG Pathway Analysis for the*** *S.coccoides* ***Standard Embden-Meyerhoff pathway had an unknown assertion status pathway since it was missing the initial enzyme of the pathway (blue box). The remaining downstream enzymes in the*** *S. coccoides* ***pathway had previously been associated during assertion.***

After students had learned all of the modules, they were assigned a module to study in depth for an oral presentation at the MMC Natural Science Division Seminar which was attended by community members, science students, and faculty. Student learning increased as a result of preparation and participation in the seminar since students earned an average of 84% of the points on the preliminary written draft of the presentation and 92% of the points in the final draft and oral presentation. This increase was likely from knowledge gained from peer teaching in addition to retention of material presented by classmates. Since the group projects and peer teaching were simultaneously conducted, the specific contribution to increased student learning due to each project cannot be discerned.

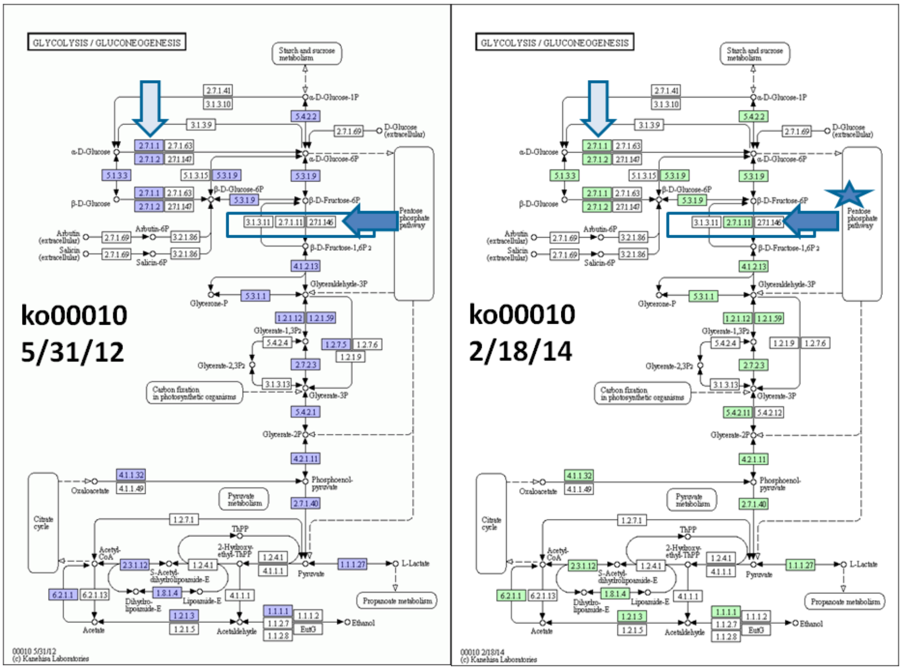


Figure 7. *S.coccoides* ***Standard Embden-Meyerhoff KEGG analysis. The Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis for the*** *S.coccoides* ***Standard Embden-Meyerhoff Pathway included both a glucokinase and hexokinase (light blue arrows) which were missing in the JGI IMG Pathway analysis (Figure 6). However, the KEGG analysis was missing the third enzyme of the pathway (left panel; dark blue arrows; Kanehisa Labs, 2012) which the students confirmed during their assertion and was later updated by Kanehisa Labs who manages the KEGG database (right panel; Kanehisa Labs, 2014).***

Table 1. Computer and Student Manual Hypothetical Protein Annotation. Putative gene functions of computer annotated hypothetical proteins following student manual annotation. Students analyzed their results from the 22 IGG tools and determined the overall annotation based on the strongest evidence. Seven out of eight genes were identified with a function and several genes were identified by both groups of students independently with the same function.

Locus Tag	Computer Annotation	2013 Student Manual Annotation	2014 Student Manual Annotation
Spico_0409	hypothetical protein	Cytoplasmic protein	Zinc finger protein
Spico_0412	hypothetical protein	Nucleotidetransferase	Nucleotidyl transferase
Spico_0413	hypothetical protein	Aminotransferase	Nucleotidyl transferase
Spico_0416	hypothetical protein	DNA polymerase	Nucleotidyl transferase
Spico_0417	hypothetical protein	Coenzyme PQQ synthesis protein D	Coenzyme PQQ synthesis protein
Spico_0490	hypothetical protein	Cytoplasmic membrane protein	Cytoplasmic transmembrane protein
Spico_0491	hypothetical protein	Small-conductance mechanosensitive channel protein	Small-conductance mechanosensitive channel protein
Spico_0630	hypothetical protein	Hypothetical protein	Hypothetical protein

Test assessment for correct tool selection and data interpretation—Students were assessed for knowledge in bioinformatics tools selection based on a series of multiple-choice exam questions. Students were tested on identical questions in a pre-test, unit exams, and final exam (Figure 8). Overall, 10-40% of the students correctly identified the tools in the pre-test. The low pre-test percentage in tool selection was likely due to minimal exposure to methods of collecting bioinformatics data. The percentage of students correctly identifying the tools increased 50-80% overall for all five tools. The percentage of students correctly answering the questions increased to maximum levels at unit exams for three of the tools (Pfam, WebLogo, and Paralog). The percentage identifying the correct tool increased between unit and final exams for two tools (KEGG and PSORT-B). These two modules contained several tools that produced similar data outputs. A likely reason that students required additional time to correctly answer the question was that the initial exposure to each module was not sufficient to help them distinguish between tools. The percentage of students correctly identifying the tool for gene duplication decreased 10% between unit and final exams while the other tools either increased or plateaued.

Students were also assessed for their ability to interpret bioinformatics data through the pre-test, unit exams, and final exam (Figure 9). Overall, 30-80% of the students correctly interpreted the data in the pre-test. The wide range of data

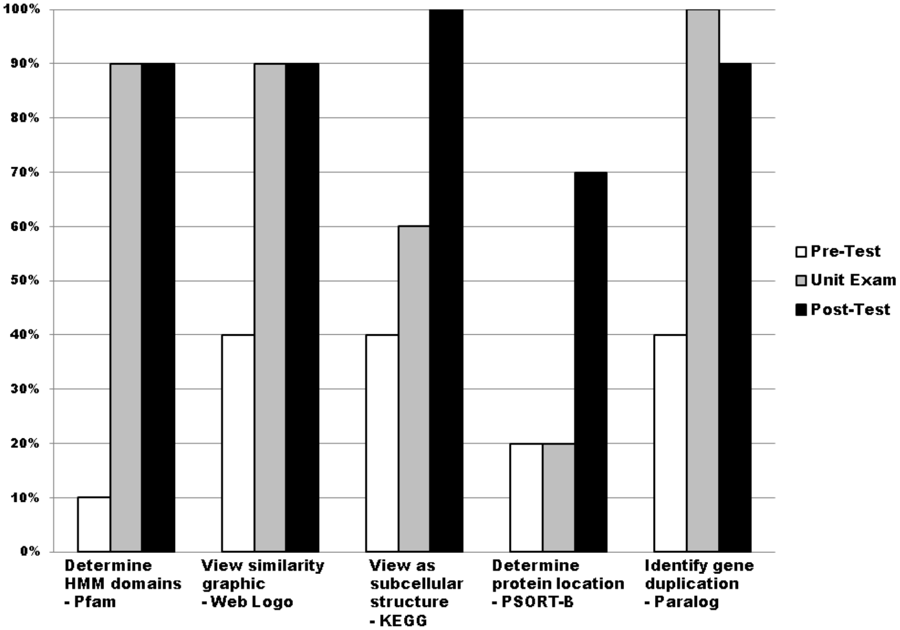


Figure 8. Test Assessment for Correct Tool Selection. Students were assessed for knowledge in bioinformatics tools selection based on a series of identical multiple-choice exam questions on a pre-test (white filled), unit exams (grey filled), and final exam (black filled). The learning objective and bioinformatics tool assessed in each question are listed on the x-axis. The percentage of students correctly answering the question is indicated on the y-axis (n=10).

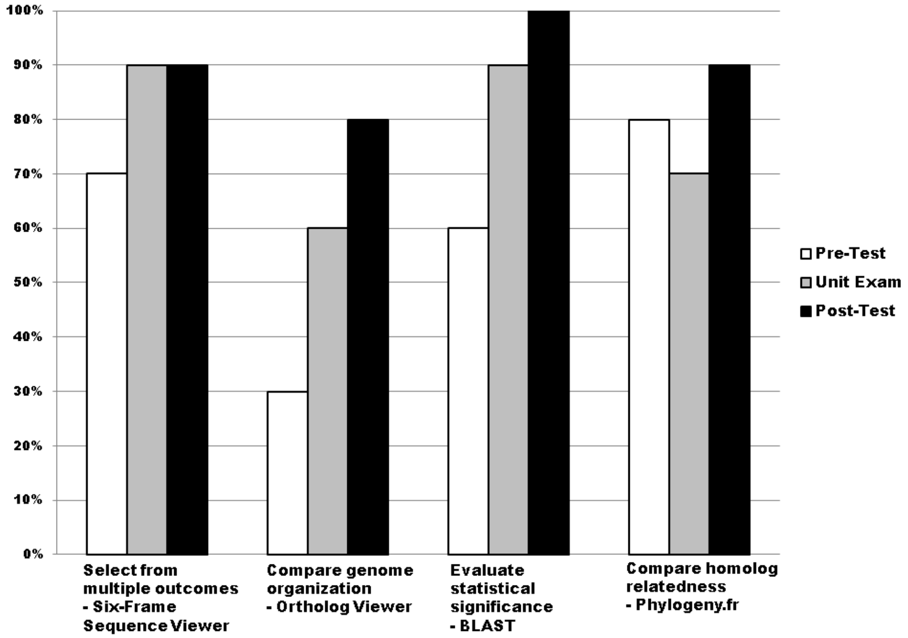


Figure 9. Test Assessment for Correct Data Interpretation. Students were also assessed for their ability to interpret bioinformatics data based on a series of identical multiple-choice exam questions on a pre-test (white filled), unit exams (grey filled), and final exam (black filled). The learning objective and bioinformatics tool assessed in each question are listed on the x-axis. The percentage of students correctly answering the question is indicated on the y-axis (n=10).

interpretation likely came from a mixed exposure to bioinformatics data. Many students in the course had attended the bioinformatics seminar in previous years which exposed them to bioinformatics concepts, but they may not have recalled the tool that was used to obtain the result. All of the students had taken Introductory Biology and Microbiology which would have exposed them to the concepts of open reading frames and phylogenetic trees which 70-80% of the students correctly interpreted in the pre-test (Figure 9). The percentage of students correctly interpreting the data increased 10-50% overall for all four tools. The percentage of students correctly answering the questions increased to maximum levels at the unit exam for one of the tools (Six-Frame Sequence Viewer) while the percentage increased between unit and final exams for two tools (Ortholog Viewer and BLAST). The percentage of students correctly interpreting the data for homolog relatedness (Phylogeny.fr) decreased 10% between the pre-test and the unit exam but increased 20% overall by the final exam.

DISCUSSION

To enhance experiential learning of bioinformatics tools, the professor modified the laboratory component of the undergraduate, MMC BIO-364 (Cell and Molecular Biology) course such that genome analysis and gene annotation were the predominant content for the laboratory sessions through the incorporation of the JGI "Interpret a GEBA Genome" (IGG) Education program. Students were assessed for their knowledge in bioinformatics tools selection, data interpretation, and associated molecular biology content prior to participation and periodically throughout the program.

To increase student knowledge of bioinformatics and the tools that are available to acquire and analyze these data, students utilized the JGI IMG-ACT toolkit. The IGG program utilized the JGI IMG-ACT website until May 2014, after which it was changed to the GENI-ACT website. This next generation website was funded by the NSF and features the educational components of IMG-ACT with the ability for collaborators to collectively suggest changes to an existing genome with supporting evidence. These changes can be ported back to GenBank by exporting to a sequin file format (Welch 2014). The advantage of this upgrade is that the undergraduate student research results can more easily impact the publicly available database system since the faculty member can make available to the public the student notebook, coordinate changes, and gene product annotations, whereas IMG-ACT relied on multiple independent analysis and approval by JGI personnel. On the other hand, faculty members will need to use discretion when making the results public since the verification of the data has been decentralized. Faculty members will need to exercise caution when balancing the students' satisfaction from making a "real" impact on the research community and determining the validity of their annotations.

During the incorporation of the IGG program into the course, modules were not taught in the order listed in the IMG-ACT toolkit but corresponded with the subjects that were discussed in the lecture portion of the course. Abstract concepts such as the prokaryotic Shine-Dalgarno sequence can be easily dismissed as irrelevant details to the undergraduate molecular biology student. However, after the student utilizes the IMG Six-Frame Viewer to determine the presence and proper spacing of the Shine-Dalgarno sequence, the student appreciates the knowledge of this sequence to gene function. As such, the IGG material was incorporated with relevant molecular biology content that was discussed in the lecture portion of the course. For instance, the Basic Information module did not require utilization of specific annotation tools so it could be perceived as a "cut and paste" module if not presented with the supplemental content. An additional benefit of not conducting the modules in the order listed was that students perceived the flexibility of the toolkit and demonstrated their understanding of the non-linearity of the toolkit during subsequent group project annotations. For example several groups broke the annotation into individual components that they regarded themselves as experts in, rather than a continuous annotation that a single student had to complete.

The genes of interest for the module lessons were selected to demonstrate proper tool utilization, data interpretation, and computer annotation error. Genes were selected that resulted in a variety of manual annotation results as well as data that deviated from the predicted annotation. For example, students were able to correct possible annotation errors in three *S. coccoides* ORFs after analyzing potential alternate Shine-Dalgarno sequences and start codons (Figures 1, 2, and 3).

To increase student comprehension and retention of the bioinformatics techniques and data interpretation, the professor assigned the students complete analyses of additional genes in a project format and modules to present at a seminar. In the first project, the students analyzed the genes of the *S. coccoides* Standard Embden-Meyerhoff Pathway, demonstrating the existence of the complete pathway by locating the gene for an enzyme previously thought to be absent (Figure 6). In the second group project, students annotated and assigned probable functions to a group of genes that the computer had identified as hypothetical proteins (Table 1). As the student groups completed the sub-modules, they comprehensively annotated the genes and also determined the utility of each of the tools for determining gene function. Similarly, students likely comprehended the nuances of the tools through peer teaching during preparation and participation in the bioinformatics seminar.

Overall, inclusion of the "Interpret a GEBA Genome" program increased student learning in bioinformatics and molecular biology, as measured by student completion of online notebooks, pre- and post-activity exams, and presentation of student annotation results at seminars. Students continued to increase their knowledge of bioinformatics between unit and final exams. A probable explanation is that the initial exposure to each module was not sufficient to help them distinguish between tools and scrutinize data results. This supports the need for long-term exposure reinforced through group projects and module presentations when incorporating bioinformatics into an undergraduate biology course.

ACKNOWLEDGEMENTS

The author is grateful to Cheryl Kerfeld, Seth Axen, and Brad Goodner for the support of the IMG-ACT and GENI-ACT websites. The Microbial Genome Annotation Network (funded by NSF DBI 0954829) provided travel support to attend the National DOE-JGI IMG-ACT Training Workshop and travel support and training in the use of the DOE-JGI's IMG-ACT platform. The author would also like to thank Lori Scott for her assistance in coordinating the regional faculty training workshop for the South Dakota Genomics Consortium. The author thanks Jane Venes for her graphic design assistance. This work would not have been possible without the students enrolled in BIO-364 for their collective contributions.

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SAFETY ON SITE: DEVELOPING A SCIENCE LAB SAFETY PROTOCOL FOR SCHOOLS

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ABSTRACT

In South Dakota, science instruction now includes more inquiry-based, hands-on experiments at all educational levels. Implementing science safety training becomes vitally important as more students learn from teachers who may not have been safety-trained (Singer et al. 2005). A wide-spread lack of knowledge exists about how to handle, store and depose of deteriorating chemicals that populate science storerooms and labs, chemicals that may create a "ticking time bomb" of materials ready to explode. Schools are responsible for OSHA-mandated "standards of care" when designing labs and securing equipment for science students. Districts also must create policies that clearly state how to provide for regular maintenance and oversight of these materials. The expectation is that science teachers, as licensed professionals, will be "reasonably prudent" and take precautions to prevent lab accidents from occurring. Students also must be provided with proper safety expectations, protection and precautions. As a means to alleviate these problems, a safety protocol was developed for teachers and schools. An excerpt from this Safety on Site (SOS) guide was presented at the April 2014 meeting of the South Dakota Academy of Science in Rapid City. In May 2014, a detailed safety audit was undertaken of the science facilities in a small South Dakota school district. This paper discusses some important safety issues, citing data from a case study that includes the results of a safety inspection. Suggestions and solutions are presented so that districts may act to prevent science lab-related accidents in their science classrooms and laboratories.

Keywords

Safe lab facilities, science safety, laboratory safety protocol, school safety, safe science teaching

INTRODUCTION

"A danger foreseen is half avoided."—James Wood, comp.
Dictionary of Quotations, 1899.

Laboratory experiments are essential for the effective learning of science. These experiments provide students with opportunities to interact directly with natural phenomena or with collected data, using tools, materials, lab techniques, and models. Inherent in most laboratory-based activities is the potential for injury. And, because all lab experiments hold some risk, science teachers must closely comply with safety regulations for storage, use and labeling of materials used in science experiments and demonstrations. Safety rules, established by the Occupational Safety and Health Administration (OSHA), the National Science Teachers Association (NSTA) and the American Chemical Society (ACS) as well as state and local regulating agencies outline procedures for lab safety at all instructional levels. According to NSTA, science experiments must also be reasonably appropriate for the type and size of the classroom as well as for the level of students (NSTA 2003). Laboratory workers (including science teachers) have the legal right to a safe workplace. OSHA, as established by the Occupational Safety and Health Act of 1970, enforces protective safety and health standards in all United States workplaces. It is unfathomable that many regulations in place are commonly ignored—most commonly in schools (Stroud and Roy 2009). Ignoring regulations and guidelines has often led to disastrous consequences as reported in the 14 January, 2014 New York Times.

> Teacher's Conduct Scrutinized In 'Rainbow' Chem Experiment That Burned Students*: An investigation continues into the Manhattan high school chemistry demonstration-gone-wrong that left one student with extremely serious third-degree burns and another with first-degree burns. The demonstration, which is used to show how mineral salts burn at different colors, has injured numerous other students in the past, alarming a federal agency enough to issue a warning. Beacon School teacher Anna Poole was starting the "rainbow experiment" in her 10th grade chemistry class. The demonstration included mineral salts being involved with extremely flammable methanol.—January 14, 2014, New York Times, Science Section*

The U.S. Environmental Protection Agency (EPA) also administers laws and regulations affecting safety in high school science laboratories. These include the Resource Conservation and Recovery Act, the Emergency Planning and Right-to-Know laws and regulations, and the Toxic Substances Control Act. In order to carry out these provisions, EPA issues regulations and guidelines governing safe storage of laboratory chemicals, equipment, and supplies (U.S. Consumer Safety Product Commission 2006). The National Science Teachers Association guidelines emphasize competent science safety knowledge and have recommended that teachers should model science safety procedures, training students explicitly during instruction, rather than leaving this important issue to chance (NSTA 2003). Data indicate that large class sizes may pose a threat to safety in school laboratories—many science classes exceed the NSTA standard of 24 students per teacher. It may be extremely difficult for teachers in classes of 30 students to perform the "duty of supervision" and maintain safety during laboratory experiences (Stephenson et al. 2003).

In 2009 and again in 2012, a South Dakota Science Safety Survey was disseminated by the Department of Education to teachers on the science listserv (Ezrailson 2013). Results from that study and results from other research formed the basis for the safety audit and subsequent recommendations to remediate unsafe lab conditions in schools. Facilities are an important place to start as they dictate how many students can perform experiments safely and the procedures and precautions that must be instituted. Studies have documented that the smaller the lab space and the larger the class, the higher the risk for accidents (NSTA 2003; Stephenson et al. 2003). Properly stored and labeled chemicals and inventoried materials allow for an orderly, well-documented procedure for lab and classroom (U.S. General Accounting Office 1996).

This paper will outline hazards associated with science lab facilities, outline best practices for safe science teaching, present data from a science facility audit and recommend solutions to hazards and problems found in science labs and classrooms.

METHODS

In May 2014, an inspection was performed of the science and storage facilities, chemical closets and preparation rooms at a middle and high school in South Dakota. The high school had been a college previously, is 100 years old, and in some disrepair. The middle school was connected to the high school and was several decades newer. This audit also included an examination of seven science classrooms and interviews with science teachers (7), school and central office administrators (3) and custodial staff (2). initiated by the district superintendent and curriculum coordinator. A review of safety procedures ascertained the status of science teacher safety training and lab procedures. Three research questions formed the basis of the Case Study that was drawn from the audit data. These were: Which factors contribute to “safe” school lab environments? How can the potential for lab accidents be identified? What positive steps should be taken to resolve “unsafe” conditions and implement safe practices?

Based on previous studies in South Dakota and elsewhere, best practices of safety materials/laws and standards of performance were also compiled for this study. Ten Essential Questions were constructed, posed and answered for the audit. Using qualitative data, I developed a case study from the audit to illustrate a snapshot of one typical school setting in which science lab hazards were found. The following three steps were then taken.

The First Step: A checklist of safe procedures and best practices—The following **e**ssential questions were asked of schools developing safety implementation plans:

1. How current is the schools’ science safety training program?
2. How safe are the lab facilities, stockroom, classroom, and prep room? What constitutes “safe?”
3. What precautionary safety equipment is present? Is the quantity sufficient?
4. Is there a written procedure for storage and inventory of lab materials?
5. How knowledgeable about safe lab practice are the instructors? Students?
6. Have the safety hazards been identified in each experiment/demonstration performed?

7. Are lab procedures spelled out for students – specifically, including safety?
8. Is there regular inspection and inventory of lab materials, including toxic chemicals?
9. What provisions have been taken for the safe disposal of toxic wastes and old or inappropriate chemicals?
10. Who is responsible, besides the teacher and administration, for developing a Safety Plan?

The Second Step: Determining accident potential—lab inspection/audit—The reason for implementing a laboratory inspection/audit program should include periodic inspections that can identify and prevent problems before they occur. When problems do arise, a methodical safety protocol should be closely followed and outside audit help secured for emergencies and problems that may have difficult solutions (U.S. Department of Transportation 2012).

> *Purposes*: To ensure that the physical space, processes, and practices used in the science lab, classroom and storeroom are safe. This includes assessment of facilities (including stockrooms, prep rooms, classrooms and labs), verifying that safeguards and safety equipment are within acceptable guidelines for position, condition, and that approved safety practices are being followed. Areas where materials may be stored outside the storeroom, such as closets, basement storage areas, cabinets and other areas not readily recognized as housing problems, should be regularly checked.
>
> *Responsibility for Safety:* The teacher has the most intimate knowledge of the safety issues involved in any particular experiment, demonstration or activity. Any unsafe work practices observed during an inspection/audit should become an opportunity for training. The inspection/audit protocol should follow the format and requirements for all safety elements in a science classroom, stockroom or lab or anywhere science labs and activities are conducted. Checklists are used in most laboratories as an effective way to keep track of maintenance and safety issues. The science teacher should develop one or use either the "Five-Minute Safety Inspection" provided by Flinn Scientific (Flinn Scientific 2014), guidelines provided by the Council of Chief State Science Supervisors (CCSSS No date), or adopt the Safety on Site program outlined here.

The Third Step: Instituting a Safety on Site inventory and protocol—Personnel compiling an inventory should be prepared to find substances that might be explosive, highly flammable, highly toxic, or unlabeled. If needed, then expert advice should be sought prior to attempting to remove or dispose of such items (Ezrailson 2013). Once the inventory is compiled, all items should be evaluated to determine if they are justified for safe use in the school lab and classroom.

Copies of the inventory should be *on file* with each teacher who uses and/or orders chemicals, with the school principal, and with central administration. The inventory should be updated regularly – no less often than annually. Inclusion of the following information is essential:

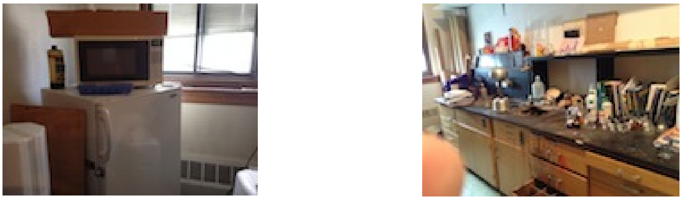
1. School name, address, telephone number
2. Name of person(s) compiling inventory
3. Date of inventory
4. Alphabetical listing of all chemicals
5. Amount
6. Storage location (room number)
7. Storage category (based on chemical compatibilities)
8. Hazardous class information
9. Date purchased and by whom
10. Date disposed of and by whom

RESULTS

The safety audit of the middle school and high school revealed areas of concern. This included problems of degrading chemicals and containers, inappropriate materials (for high school science), inadequate ventilation, improper storage, and inconsistent labeling. Most importantly, it revealed an urgent need for further comprehensive science safety training for teachers and students. The results and recommendations are given below. Findings for the high school problem areas included the lab rooms, prep rooms, chemical storerooms and classrooms and are given in Table 1, with photographs of some of the hazards encountered (Figure 1):

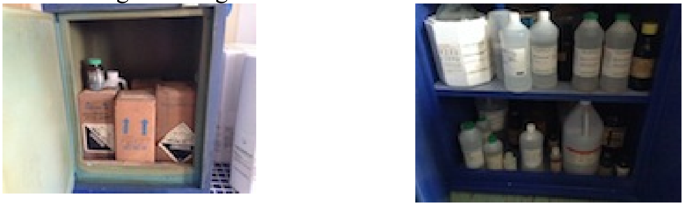
Table 1. Hazards and Problem Areas

- The chemical closet was unlocked, opening directly into the classroom.
- Not all chemicals were stored per SDS and OSHA requirements.
- Chemicals inappropriate to high school classrooms were present and should be discarded.
- Hazardous materials—e.g. elemental mercury, lithium, potassium, and sodium were present and should not be stored—especially in large quantities and long-term
- All appropriate safety instructions for students were not displayed at all times.
- Heavy containers were stored above eye level with chemicals on top of "un-lipped" cabinets
- Some containers were not marked—many were corroding and leaking in cardboard boxes on floors.
- Teachers and students were not formally trained in science safety procedures.
- Large amounts of chemicals were stored in unvented cabinets, (only enough for one school year should be ordered, if possible).
- Safety precautions were not visible on lab sheets and should be taught to students before any lab.
- Nitric acid was not stored separately from other acids in a vented storage cabinet (unvented cabinets were present).
- There was no list of incoming and outgoing chemicals/specimens with amounts and dates.
- Chemicals (dates on unopened packages with shipping date in 1950s) were not discarded.
- Uranium ore was not stored properly, although it was a small amount, it had no warning label.
- Some students not supervised in stockroom, which was not vented with strong odors from leaking containers.
- A safe exit needs to be established! The classroom exit lies next to the chemical closet and prep room.
- The fire extinguisher was mounted on the wall 20 feet away. Classroom and lab areas located on a second floor with no fire escape except through classroom door to hall located next to chemical stockroom.



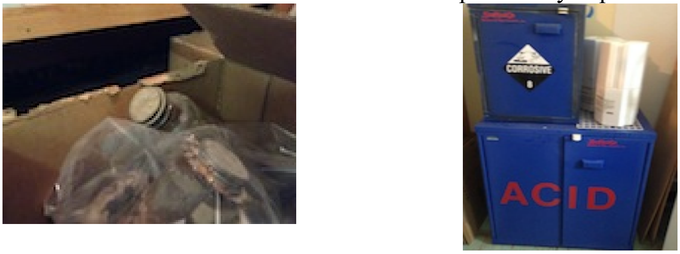
Chemical refrigerator not labeled. NO FOOD sign missing.

Sinks eroded, cabinets swelled, not vented.



Old disintegrating boxes of acids in unvented acid cabinet.

Old or unmarked chemicals in corrosive cabinet. Some potentially explosive.



Leaking containers of unmarked chemicals stored together.

Acid cabinets not locked nor vented.

Figure 1. Examples of high school prep/storeroom hazardous areas.

The middle school science classrooms and prep room were also audited. The findings for these areas are given in Table 2 along with photographs of some of the hazardous areas.

Table 2. Middle school hazardous areas.

Science Rooms	Stockrooms
• No flushing eyewash at sink • No broken glass or chemical waste containers • Plumbing in sinks dripping. • No drains in floor • No exhaust fan • No fire blanket	• Large amounts of undiluted acids stored in a flimsy metal cabinet in unvented storeroom • Nitric acid stored with other acids. • Storeroom not vented • No drains in floor. • No sink or exhaust fan

Figure 2. Middle school prep/storeroom hazardous areas. An "un-lipped" top shelf storage above eye level with old chemicals in eroding containers.

DISCUSSION

Factors were identified that increased the potential for lab accidents. There was inconsistent oversight of students and implementation of proper procedures, uneven awareness of regulations and best practices for safety in labs and classrooms, low budget for facilities and supplies, no repair or replacement plan, and lack of consistent documentation and training. All of these issues had, over many years, added up to an unsafe facility causing potential harm for students and their teachers. The answers to the following survey questions provided valuable insight into existing problems for these schools:

1. **How current is the science safety-training program?**
   When interviewed, all five science teachers had indicated that they had some working knowledge of safety, although none had had any formal safety training, neither during their teacher preparation programs nor on the job.
2. **How safe are the lab facilities, stockroom, classroom, and preparation room?**
   All administrators and teachers knew that safety problems existed, but were unsure about what steps to take to resolve the issues. One of the staff had researched and contacted agencies for help but was given only general information. When she contacted the government offices, she received no concrete help about how to proceed. Subsequently, a private safety auditor was hired to tie information together, inspect the facilities and make recommendations.
3. **What precautionary safety equipment is present? Is the quantity sufficient?**
   All classrooms had adequate numbers of safety goggles, but no sanitation equipment was present. Many chemical aprons in the physics and chemistry area were in shreds and the lab facilities in general were in extreme disrepair.
4. **Is there a written procedure for storage and inventory of lab materials?**
   A piecemeal inventory put together for the purposes of ordering chemicals lacked sufficient information, e.g., dates, amounts and location data. The

amounts to be ordered were in excess of safe amounts for long-term storage. The teachers expressed frustration with the procedure that included ordering chemicals every other year because of budget constraints. Storage facilities were not adequate and large amounts of chemicals were also stored in classrooms.

5. **How knowledgeable about safe lab practice are the instructors? Students?**
   When questioned, the teachers responded with adequate knowledge about most safety procedures and equipment. However, they requested training and workshop availability in this important area.
6. **Have the safety hazards been identified in each experiment/demonstration performed?**
   During the audit, overall hazards were identified (see Tables 1 and 2). Recommendations included the procedures for safety for each lab/demo performed.
7. **Are lab procedures spelled out for students—specifically including safety?**
   Safety procedures were not always specifically addressed. There was no safety quiz that students had to pass in order to be allowed in lab, no letter home to parents or safety contract entered into for science students—all best safety practices specified by NSTA (2003).
8. **Are there regular inspection and inventory of lab materials including toxic chemicals?**
   Inspections were left to the staff to undertake and some records did exist. Some of the problems with decaying chemical containers had been pointed out and some efforts at finding out how to dispose of those were documented. But, no consistent plan or solution had been formulated.
9. **What provisions have been taken for the safe disposal of toxic wastes and old or inappropriate chemicals?**
   A private auditor was called in to assess the situation and to recommend procedures for disposal and for proper care and storage of chemicals.
10. **Who is responsible, besides the teacher and administration, for developing a Safety Plan?**
    Ultimately, the school district began to comply with regulations and rules for employee safety and proper procedures as they were made aware. Teachers and custodial staff were given the responsibility of standards of care that they must enforce to keep teachers and students safe during lab experiments. School administrators implemented procedures to dispose of chemicals deemed unsafe and to begin a redesign of the facilities.

What positive steps should be taken to resolve issues and “unsafe” lab facilities? First, it is important to determine what constitutes a safe environment using guides and best practices that have been proven over time. A safety inspection or audit should uncover issues in the environment that have potential to create hazards. Uncovering hazards takes a willful effort and determination to “leave no stone unturned.” Paramount to the process is to correct the issues found in a proper manner, using guidelines outlined in this paper and elsewhere. Failure to act to resolve unsafe conditions could be harmful for students and their teachers.

The two schools that comprised this case study have taken steps to correct the issues uncovered by their inspection and safety audit. They have raised teacher and administrator awareness, taken steps to become more informed, and called in experts to help them dispose of chemicals and equipment that could have wrought disastrous consequences. As it turns out, they found that they were able to dispose of small amounts of their non-poisonous, non-radioactive, noxious solids and their liquids by solidifying them with kitty litter. And, with the permission of the sanitary landfills in their area, they were able to dispose of these chemicals properly (Committee on Hazardous Substances in the Laboratory 1981).

Implementing Safe Science—The problem of school and lab science safety is an extremely urgent one—teachers and administrators MUST communicate regularly and fully on the essentials of proper procedures reflecting the latest research on chemical storage, protective equipment and safety training. To help teachers and schools meet regulations and standards of care, several organizations provide safety materials (U.S. General Accounting Office 1996; Flinn Scientific 2013; Ezrailson 2013). Some materials are readily accessible on the Internet. Unfortunately, no national repository of information on school science accidents exists. What data there are have typically been gathered by individual states, public entities or researchers, which make a larger picture of the state of safety in schools difficult. The lack of publicly available data on laboratory accidents and injuries may be due in part to the fact that many legal cases are settled before trial. As a result, there are few articles discussing legal precedents and findings in cases related to laboratory science (Standler 1999).

An understanding of where we are as a nation on protecting our students and their science teachers amounts to a serious issue as it affects every community school, teacher and student in this country. To the extent that government agencies, states, school districts, and professional associations notify schools about safety policies and practices, evidence suggests that schools tend to react only to an immediate accident when it occurs, rather than taking preventative action to avoid catastrophe. This paper has outlined a safety protocol that consists of procedures for schools to follow. Highlighted was the case study of one school district that has resolved to do what it takes to be safe while conducting science in their classrooms and labs. The problems that individual school districts face, whether rural or urban, small or large, are very similar. School storerooms all over the USA house degrading chemicals in eroding containers that create hazardous and explosive conditions that could cause injury and death according to the Montana School Labs Waste Management protocols (2014). A coordinated initiative and concerted efforts at ALL levels of government--states, school districts and local communities--is needed in order to solve this problem. The question isn't "if" unsafe conditions exist, but a recognition "that" they do and that there is a will to develop a uniform plan for action. And, because educators and their students have not only a duty, but a right to a safe learning environment during science instruction, this duty must be shared equally among school leaders, district administrators, school boards, parents, and students themselves. Thus, a little training and a whole lot of education are in order.

> "To be prepared against surprise is to be trained.
> To be prepared for surprise is to be educated."
> —James P. Carse, *Finite and Infinite Games:*
> *A Vision of Life as Play and Possibility*

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Abstracts of Senior Research Papers

presented at the

99th Annual Meeting

of the

South Dakota Academy of Science

A LARGE UNDERGROUND LIQUID ARGON TIME PROJECTION CHAMBER AT SURF FOR NEUTRINO PHYSICS AND BEYOND

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ABSTRACT

The proposed far detector of the Long-Baseline Neutrino Experiment (LBNE), a massive liquid argon time-projection chamber (LArTPC), is planned to be at the 4850 feet underground at the Sanford Underground Research Facility (SURF), South Dakota. Once built, it will detect neutrinos from an intense wide-band neutrino source at Fermi National Accelerator Laboratory (FNAL) and other astronomical sources. The experiment will support cutting-edge research programs in neutrino physics, particle astrophysics, the study of symmetries in weak interactions, and frontiers beyond the Standard Model of particle physics, such as proton decay as predicted by the Grand Unified Theory (GUT). In this presentation, we explain the principle, configuration and scientific potentials of the massive LArTPC. The R&D progress made by the LBNE collaboration, including research and educational programs in South Dakota's universities, and the status at SURF in facilitating the detector are reported.

THERMOCHEMICAL WATER-SPLITTING FOR HYDROGEN GENERATION USING MODIFIED $M1_XM2_YO_Z$

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ABSTRACT

This study investigates the thermochemical water-splitting process for hydrogen generation using ferrite nanoparticles. Thermochemical water-splitting involves a two-step cyclic process. In step-1 (regeneration), a redox material (e.g. ferrite) is heated at high temperatures creating oxygen vacancies in a crystal lattice; whereas in step-2 (water-splitting), partially reduced redox material scavenges oxygen from steam producing hydrogen. Because of thermal cycling, the redox materials undergo thermal stresses leading to grain growth thereby reducing the specific surface area.. Consequently, the diffusional distance for oxygen transport within grains increases, which results in lower hydrogen volume generation with increase in number of thermochemical cycles. Additionally, thermal cycling leads to spalling of redox materials in thin-film thermal reactors that can possibly increase pressure drop. It is possible to control the grain growth of the nanoparticles by the addition of inhibitors such as ZrO_2 and SiO_2. In particular, ferrite nanoparticles were synthesized using the sol-gel method and utilized further to prepare porous core-shell nanoparticles. As-calcined ferrite core-shell nanoparticles were loaded into an Inconel reactor, where five consecutive thermochemical cycles were performed at 900 ° – 1100 °C. Hydrogen and oxygen volumes were monitored during multiple thermochemical cycles, which indicate higher hydrogen volume generation using core-shell ferrite nanoparticles. Characterization of core-shell nanoparticles and results obtained on the hydrogen generation ability of these materials were presented.

THE PRODUCTION OF A NANOTRIANGLE LATTICE FOR USE IN METAL-ENHANCED UPCONVERSION OF LIGHT

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ABSTRACT

To improve the efficiency of photo-activated processes reliant on solar radiation, which consists of a broad spectrum of wavelengths spanning the infrared and visible range, we used the process of metal-enhanced upconversion to spectrally convert light from infrared to visible wavelengths. Metal-enhanced upconversion involves the fabrication of a noble metal periodic nanostructure. For this process, arrays of nanotriangles were made. These nanostructures can be produced using several methods. Among these methods, nanosphere lithography was used in lieu of electron beam lithography, due to its low cost and relative ease. The process involves the surface crystallization of a nanosphere monolayer or bilayer and subsequent deposition of a thin metallic film onto this surface. The basic process involved a drop cast of nanosphere solution, which allows crystallization of the spheres during the evaporation of the water. This process was optimized in terms of surface preparation, deposition of metallic thin-film and characterization. The deposition of the metal, either Ag or Au, was optimized for different thicknesses and shapes. Using this process, we were able to create a substantial area (>100 μm^2) of nanotriangles for use in metal-enhanced upconversion applications. Detailed characterization was performed using an optical microscope combined with both atomic force microscopy (AFM) and scanning electron microscopy (SEM). The results obtained from surface characterization are presented.

DISPERSION AND ORIENTATION OF ORGANICALLY MODIFIED MONTMORRILONITE IN NYLON 6 MATRIX BY A NOVEL FABRICATION METHOD

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ABSTRACT

In this research, organically modified Montmorillonite (oMMT) was dispersed and oriented within Nylon 6 utilizing a new processing technique to create nano-engineering films. Nylon 6 (N6) nanocomposites (NCs) containing 0.1wt% to 5wt% of oMMT were directly prepared in the High Shear Thin Film Machine (HSTFM) which we designed and fabricated. Using the HSTFM, we produced NC films with good dispersion and simultaneously good orientation. Transmission Electron Microscopy (TEM) was used to characterize the dispersion and orientation of the oMMT in the Nylon6 matrix. Mechanical properties of the NCs were studied through tensile tests and Dynamic Mechanical Analysis (DMA). At 5% oMMT in N6 Young modulus is increased by 66% when compared to neat Nylon 6. XRD analysis result shows that the dispersed 5wt% oMMT/N6 NCs exhibit predominantly γ_2 crystal form; however when this sample was processed to include orientation, the α_2 crystal form became predominant. Results from these analyses will be presented.

COMPARATIVE ANALYSIS OF ATMOSPHERIC THERMAL INVERSION CONDITIONS REPORTED BY SOUTH DAKOTA WRAN NETWORK

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ABSTRACT

Atmospheric thermal inversions (ATI) are a leading contributor to air quality degradation; therefore, a more acute understanding of their conditions is necessary. In this study, the extent of ATI events in central South Dakota is explored. Traditional knowledge suggests ATI events are local scale phenomena that may vary over a region owing to localized differences in environmental and land surface parameters. Since large scale atmospheric forcing can modulate ATI development, it is hypothesized that inversions can be continuous over broad areas.

In this study, temperature data from the South Dakota Wind Resource Assessment Network (WRAN) were compiled from a network of three 80 meter observing towers to identify variations in inversion strength and duration across a mesoscale 300 kilometer region. The inversion data were compared to observed surface meteorological conditions including the locations of high and low pressure centers, surface boundaries, and cloudiness.

The degree of variation in inversion strength and duration between observing stations was assessed, with special attention paid to the reasons for the variation. Specifically, a combination of siting and meteorological differences were determined to be the most likely causes of the reported fluctuation in ATI intensity($\Delta T/\Delta Z$) and duration (total time inversion conditions are recorded in each 24-hour period).

This knowledge will lead to improved accuracy in air quality alerts by forming a basis to more accurately assess the extent of ATI conditions under given synoptic situations. If adapted by industry and policy makers, these finds will lead to fewer air quality exceedances to regions downwind of heavy polluters.

THE APPLICATION OF MICRO-COMPUTER TOMOGRAPHY AND 3D PRINTING TO THE STUDY OF FOSSILS

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ABSTRACT

The union of several technologies has enabled a new format for studying fossils. Traditional preparation methods require the fossil to be extracted from the surrounding matrix, often resulting in minute but cumulative physical damage to the specimen. Facilitating collaborative research often requires transporting the fossil with the obvious associated hazards. New imaging equipment allows for digital reconstruction of a fossil encased within matrix, and 3D printing provides a mechanism for recreating the fossil shape in plastic. SDSM&T's new Micro-Computed Tomography (MCT) machine, Xradia XCT-400, is utilized to image the specimen into layered x-ray images. Visualization Science Group's Avizo Fire and 3DS's SolidWorks are used to render and transform layers of interest and remove others. Finally, Makerbot's 3D printer and software print the replicated sample by extruding thin strips of plastic on a movable platform. Difficulties with using these technologies include: fossil quality, the MCT's sensitivity to fossil densities in lithified samples, software compatibility to data formats, and resolution of the sample to be printed. The techniques developed to overcome these obstacles are also applicable to tasks requiring a non-destructive evaluation of manufacturing quality control or counterfeit analysis.

INTRODUCTION INTO THE STABILIZATION AND PRESERVATION OF THE PREPARATION AND LITHOLOGY OF PAPER SHALE FOSSILS OF THE FLORISSANT FOSSIL BEDS NATIONAL MONUMENT, COLORADO

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ABSTRACT

Florissant Fossil Beds National Monument and the Museum of Geology, South Dakota School of Mines and Technology are conducting a study to determine which consolidants are best suited for stabilizing the fossils preserved in paper shale. The deposits have been dated to the late Eocene (34.8 mya) and originated in a lacustrine setting. The paper shale itself is smectite clay weathered from volcanic ash and is interlayered with diatomite. Each layer is 0.05-2 mm in thickness and contains carbonized fossils of plants and insects, preserved with microscopic details, such as compound eyes, antennae, coloration and venation intact. These fragile fossils are susceptible to cracking and loss from even minor environmental changes, so stabilization is vital. The consolidants tested were Butvar B-76, Butvar B-98, Paraloid B-72, Primal WS24, Paleobond pre-prep field penetrant, Paleobond penetrating consolidant, and Devcon 2-ton epoxy. The Butvars and Paraloid were dissolved into acetone, ethanol and toluene at concentrations of 2, 5 and 10% (weight/weight ratio). Initial research suggests that the best consolidant would be a Butvar or Paraloid in a 5 to 10% solution of ethanol. Acetone evaporates too quickly, possibly contributing to warping and flaking of the shale, while toluene is toxic and creates a solution that has a high viscosity and doesn't penetrate the shale well. Epoxy doesn't penetrate the shale, but instead remains on the surface in a thick layer that obscures any detail of the specimen. Primal WS24 dries to a glossy finish, inhibiting examination of a specimen under bright light.

AN UNKNOWN UNGULATE: IDENTIFYING A LATE PLEISTOCENE MAMMOTH CO-HABITANT AT THE MAMMOTH SITE OF HOT SPRINGS, SOUTH DAKOTA

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ABSTRACT

The Mammoth Site documents a Late Pleistocene (26-27 Kya) megafauna in the southern Black Hills of South Dakota. This site is interpreted as a sinkhole resulting from a karstic landscape that became a behaviorally selective trap for adolescent to adult male mammoths (*Mammuthus columbi* and *M. primigenius*). Several other large mammalian taxa have been collected from this locality, including: *Camelops hesternus, Hemiauchenia macrocephala, Euceratherium collinum, Arctodus simus,* and *Canis lupus*.

However, the fauna recovered from the site is depauparate in regards to characteristic Rancholabrean taxa. Large predators are typically uncommon, so absence of these taxa in not unexpected. Large herbivores are far more common, particularly those that herd, such as *Equus* and *Bison*. However, these taxa are conspicuously absent at the Mammoth Site. Recently, perusal of collections noted the presence of a disarticulated proximal femoral epiphysis. The specimen, originally found on site in 1976, is far too small to be *Mammuthus* yet large enough to identified as megafauna. Visual assessment of the epiphysis has ruled out camelid, equid, ursid, and felid taxa and suggests bovid affinities, resembling *Bison* sp., most likely *B. antiquus*. The specimen is currently being compared through morphometric analyses to large artiodactyls, including a wide variety of bovids and cervids.

TAPHOFACIES OF SELECTED FOSSIL SITES WITHIN THE EARLY CRETACEOUS CEDAR MOUNTAIN FORMATION IN EAST-CENTRAL UTAH

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ABSTRACT

Taphofacies were delineated from a comparison between the sedimentology of rock units and the preservational features of fossils (primarily dinosaurs) found within them from the lower Cretaceous Cedar Mountain Formation in a localized study area north and west of Arches National Park, Utah. Selected paleontological sites on federal- and state-owned land with established fossil collections were targeted, including: Doelling's Bowl (DB), Dalton Wells (DW), and Gaston Quarry (GQ) (Yellow Cat Member); Tony's Bone Bed (TBB) (Poison Strip Sandstone); and Lorrie's Site (LS) (Ruby Ranch Member). Analysis of taphonomic variables (fossil preservational features) was conducted, focusing on weathering, abrasion, fracture, and breakage conditions. Utilizing several statistical methods (F- and T-testing, chi-square randomization, and linear trend analysis), we compared these taphonomic characters 1) between localities, 2) between element classes (ex. rod-shaped bones vs. flat bones), and sometimes 3) between taxa within each locality. These associations between the preservational characteristics and sedimentology have yielded a basis for a definitive description of five taphofacies, which are mapped intervals of rock defined by their own specific preservational characteristics. Taphofacies DB showed moderate weathering and abrasion in its fossilized specimens, whereas Taphofacies DW showed greater average abrasion with a bias towards flat elements. Taphofacies TBB revealed yet higher averages in abrasion and weathering, and Taphofacies LS showed the highest average weathering.

SOUTH DAKOTA SPIDER SURVEY: UPDATED FINDINGS AND SUMMARY

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ABSTRACT

The South Dakota Spider Survey (SDSS) was established in 2010 to inventory and catalogue the abundance, diversity, and distribution of spider species within the state. Previous to this current survey effort, only three publications directly examined spiders (Araneae) within the state, and a few additional publications included records from the state. Since the survey was established, we have increased sampling efforts within targeted areas of the state, most notably within the Fort Pierre National Grassland and areas of the Black Hills. To date, more than 300 species have been documented from South Dakota from either direct field sampling or from previously published literature, with more species added as sampling efforts have expanded. An online list is maintained, and digital imaging of the dorsal and ventral general habitus, relevant views of genitalia, and key identification characteristics (e.g., eye arrangement, spinnerets) has started, as well as DNA barcodes collected for as many species as possible.

MOVEMENT AND HIBERNATION OF ORNATE BOX TURTLES IN SOUTH DAKOTA

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ABSTRACT

We studied the ecology of ornate box turtles (*Terrapene ornata*) on the Pine Ridge Reservation to examine movement, home range (HR), and hibernation. Since 2010 more than 200 ornate box turtles were recorded and yearly 25 individuals were radio-tracked twice a week during the turtles' active season (March - October). Annual HR was calculated using Minimum Convex Polygons (MCP) for total area, and Local Convex Hull (LoCoH) Nonparametric Kernel Method for utilization areas. MCP calculations varied greatly among turtles, ranging from 0.35 - 89 ha, with males having a larger HR size then females. LoCoH results demonstrated that females have more concentrated, smaller core areas than males. Most of the hibernation sites occurred within the core areas with stronger association amongst males then females. In our study area, most of the turtles hibernated in clusters, and hibernation site fidelity was observed with turtles overwintering in the same vicinity in 2010-2011. Because ornate box turtles cannot withstand sustained freezing temperatures, hibernation sites at higher latitudes must protect turtles from freezing conditions for longer periods of time. Consequently, fewer locations are likely to be suitable than at lower latitudes, and hibernation site availability may restrict the northern distribution of box turtles. Therefore, protecting suitable hibernation areas is important for the species survival. This research was supported by State Wildlife Grant T-44-R-1, Federal Aid Study # 2452, administered through the USFWS/SDGFP - Wildlife Action Plan Competitive Grant (PI A. Higa), NSF/TCUP - Research Initiation Awards (PI A. Higa), and Oglala Lakota College.

HABITAT CHARACTERISTICS OF ORNATE BOX TURTLES IN SOUTH DAKOTA

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ABSTRACT

The ornate box turtle (*Terrapene ornata*) is a species of greatest conservation need in South Dakota. Habitat loss through agricultural development and fragmentation is the main threat to the species throughout its range, which extends from Wisconsin and northern Indiana through the central Great Plains from southern South Dakota to Arizona, northern Mexico, and the Gulf Coast of Texas. We studied box turtle habitat characteristics on the Pine Ridge Reservation, South Dakota, documenting macro- and microhabitat use during 2010-2011. For both years, based on a modified Robel pole method, turtles selected habitats with greater visual obstruction readings (VOR; a function of vegetation height and density) than at a stratified random distance of 10 m from the turtle ($P < 0.10$), with means of 22 cm and 15 cm, respectively. Higher VOR values indicate greater vegetation height and/or density. Canopy cover results indicated a preference (38%) for sand sagebrush (*Artemisia filifolia*) coverage to grasses and forbs. Shrubs, such as sand sagebrush, are an important component of box turtle microhabitat, as they facilitate thermoregulation by providing cool areas during the summer and favorable hibernation sites during the winter. Therefore, shrub coverage should be taken into consideration when developing an effective conservation plan for the ornate box turtle in the sand hills of South Dakota. This research was supported by State Wildlife Grant T-44-R-1, Federal Aid Study # 2452, administered through the USFWS/SDGFP - Wildlife Action Plan Competitive Grant (PI A. Higa), NSF/TCUP - Research Initiation Awards #1153443 (PI A. Higa), and Oglala Lakota College.

TURBIDITY OF OVARIAN FLUID, AND OVARIAN FLUID COMBINED WITH DIFFERENT WATER SOURCES, IN RELATION TO LANDLOCKED FALL CHINOOK SALMON EYED EGG SURVIVAL

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ABSTRACT

This study assessed the turbidity of ovarian fluid, and ovarian fluid mixed with lake water, distilled water, and well water, as potential indicators of egg survival in landlocked fall Chinook Salmon *Oncorhynchus tshawytscha.* Twenty six females with a total length ranging from 587 to 768 mm were spawned, with survival to the eyed egg stage ranging from 0 to 61%. Ovarian fluid turbidity ranged from 23.1 to 101.7 (NTU), with turbidity increasing with the addition of any of the water sources. Egg survival was not significantly correlated with ovarian fluid turbidity or the turbidities of any of the ovarian fluid and water combinations. Ovarian fluid turbidity was significantly correlated with all of the ovarian fluid and water combinations (r = 0.502, 0.426, and 0.505 for distilled water, lake water, and hatchery water, respectively). Extremely high correlations (r = 0.898 and higher) occurred among the turbidities of the ovarian fluid combined with either lake water, distilled water, or well water. The turbidity of ovarian fluid mixed with either lake, distilled, or well water was significantly and positively correlated with post-spawn female weight, and negatively correlated with the total number of eggs per spawning female. Based on these results, the use of ovarian fluid turbidity, or turbidity of ovarian fluid combined with water, cannot be used to accurately predict subsequent egg survival in landlocked fall Chinook Salmon.

ALGAL BIOMASS PREDICTION USING A STELLA MODEL

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ABSTRACT

Non-point source nutrient loading is associated with a majority of threatened and impaired streams across the Nation. Elevated nutrients increase algal biomass, which in turn reduces dissolved oxygen concentration in streams. Stream assimilative capacity to nutrient loading may vary according to watershed physiography. I developed a STELLA model to evaluate the potential for stream health declines given the physiographic variation of three major ecoregions of the Pine Ridge reservation (PRR). The model predicts daily algal biomass accumulation from nitrate and soluble reactive phosphorus concentration, high stream flow frequency, and canopy cover. I ran the model for 22 stream segments on the PRR using average monthly nitrate and total phosphorus concentrations from 2008-2012 and the average number of high flows per year. The model predicts: 1) PRR streams are nitrogen limited, 2) the most important predictor of algal biomass is the frequency of high stream flow ($R^2 = 0.945$), and 3) the second most important predictor of chlorophyll-a concentration is nitrate concentration for Badlands and Sandhills stream segments ($R^2 = 0.912$) and canopy cover for Tablelands stream segments ($R^2 = 0.230$). Model outputs are greater than the expected algal biomass concentration for Sandhills stream segments. Future work includes: 1) development of a scour sub-routine by modeling insipient motion of non-cohesive particles or modeling flow competence of the channel, and 2) running the model using daily stream flow and monthly nutrient concentrations to evaluate wet-dry cycles on algal accrual. Research funded by NSF TCUP grant #0903686.

FLORISTIC SURVEY AND VEGETATION ANALYSIS OF LANDS ADJACENT TO THE MISSOURI RIVER ON THE LOWER BRULE SIOUX RESERVATION

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ABSTRACT

We conducted a floristic survey and vegetation analysis of lands adjacent to the Missouri River on the Lower Brule Sioux Reservation. Selected areas were explored and inventoried multiple times during the growing seasons of 2011 through 2013. Efforts focused on land recently returned to the Lower Brule Sioux Tribe by the U.S. Army Corp of Engineers. An annotated checklist of native and naturalized vascular flora includes 442 species in 78 families, including 182 species previously unrecorded for Lyman County. Approximately 17% of the flora is naturalized species. Floristic Quality Analysis was used to evaluate the ecological condition of four survey areas of particular interest, including (1) Medicine Creek (= 2.63, FQI = 21.00), (2) Cedar Creek (= 2.79, FQI = 20.85), (3) a series of short loamy drainages adjacent to the Missouri River near Fort Hale (= 3.43, FQI = 25.66), and (4) a remnant sandy prairie (= 4.60, FQI = 48.66) on the Little Bend of the Missouri River. A comparison of woodland communities in the Ft. Hale drainages and Medicine Creek riparian corridor was made using Non-metric Multidimensional Scaling (NMS). Community data were acquired using 12, 50-m transects, including four along the Medicine Creek drainage and eight from individual Ft. Hale drainages. Modified Daubenmire cover classes were used to estimate cover of understory species based 1-m^2 quadrats placed at 3.3-m intervals along each transect. Cover values per species were averaged for each transect. Trees were sampled in variable width belt transects. DBH values of individual trees 5 cm or larger in diameter were converted to basal area and summed by species for each transect and divided by the total area to give a measure of relative dominance. Density was recorded for individuals smaller than 5 cm DBH. NMS analysis of understory and tree data together produced a 2-axes solution with a minimum stress of 0.071. Incremental r^2 values for axes 1 and 2 were 0.722 and 0.133, respectively (cumulative r^2 = 0.854). Differences in community composition appear to be driven mainly by flooding regimes and geomorphological factors.

IDENTIFICATION OF DIFFERENTIAL AGRONOMIC TRAITS IN EARLY STAGE TEOSINTE, FLINT, DENT, AND SUGAR (SWEET) CORN VARIETIES IN COMPETITION WITH WEEDS

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ABSTRACT

Weed competition reduces corn yield. Today's corn monoculture relies heavily on herbicide inputs to maintain yield. However, teosinte, corn's ancestor, was successfully grown in mixed production systems. Harnessing genes and traits that allow teosinte to be a better competitor but which may have been lost during corn domestication could help producers reduce herbicide inputs and maintain yield. The first step to finding those genes is to identify varieties of corn and lines of teosinte that have a higher tolerance of weeds or greater weed suppressive ability. Five introductions of teosinte and 14 varieties of corn (including dent, heritage, and sweet corn types) were cultivated with and without weed pressure. Early and end of season growth characteristics including leaf area, plant height, stem diameter, biomass, and yield, when possible, indicated a wide range of weed tolerance. Differences between weedy and weed-free treatments within a type ranged from 1-10% in corn height, 3-20% in leaf area, 1-27% in corn biomass (July), 0.4-28% in top collar height (September), and 2-17% in grain yield on a per cob basis at harvest (October). Varieties demonstrating the greatest and least deviations in these measurements between treatments have been selected for preliminary molecular analysis (data not available at this time). Identifying early season growth characteristics and gene expression associated with maintaining high yield under weed stress conditions can, in the long term, lead to better understanding the mechanisms of crop tolerance, its heritability, and reducing weed control inputs.

COMPARING BUD PHENOLOGY AND DEVELOPMENT OF SMOOTH BROME AND WESTERN WHEATGRASS

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ABSTRACT

The dynamics and persistence of perennial grass populations depend on tiller production from the bud bank, and the maintenance of a bud bank has important demographic consequences for perennial grasses. Bud and tiller production depend on each other and are affected by many factors, one of them being interspecific competition. Invasive smooth brome (SB) (*Bromus inermis*) and native western wheatgrass (WW) (*Pascopyrum smithii*) are rhizomatous, sod-forming, cool-season perennial grasses. They may compete for limited resources in a similar fashion. However the outcome of the interaction could be affected by bud phenological development and tiller recruitment, which unfortunately is unexplored. In this study we compared the two species' bud development and tiller production related to growth stage. Seedlings of both species with the same growth stage were transplanted into pots (11-cm dia. X 10-cm depth) and placed in a greenhouse with ambient light. Each pot had one seedling of each species. Samples were taken at each growth stage with 6 replications. Numbers of bud and tiller were recorded, and variability of buds was tested. At two-leaf stage, 100% sampled SB seedlings produced up to 3 buds and only 75% WW produced up to 2 buds. At 5 (WW) to 6 (SB) leaf stage, buds on the seedlings developed into first generation tillers, which produced subsequent buds. Most SB produced second generation tillers and buds at the elongation stage, but none of WW produced second generation tillers during the experimental period. Numbers of total bud and active bud on parent tillers increased from two-leaf stage, peaked on four-leaf stage, then declined as numbers of tillers increased.

INTERPRETING NATIVE UPLAND TALLGRASS PRAIRIE COMMUNITIES OF SOUTH DAKOTA'S PRAIRIE COTEAU USING MULTIVARIATE METHODS

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ABSTRACT

Vegetation documentation and classification are essential to biological conservation and management. Meaningful classification of plant communities can be revealed by the interpretation of species composition, physiognomic groups, disturbance effects and environmental gradients. South Dakota currently lacks a standardized classification of prairie communities. In order to produce a standardized classification of South Dakota upland tallgrass prairie associated with Dakota skipper habitat, we conducted a survey on a 58,275 ha portion of the South Dakota Prairie Coteau in 2013. Vegetation sampling followed a protocol developed by the Minnesota County Biological Surveys. Ten by ten meter relevé plots were subjectively placed in uniform native plant communities. Within each plot, vegetation was separated by strata. Within each stratum, cover by species was estimated using Braun-Blanquet cover classes. On each site, environmental factors (e.g. elevation, aspect, slope, soil texture, etc.) and management types (e.g. grazed, hayed or rested) were recorded. Sixty-three plots were sampled from July 8 until August 20 and then ordinated using Nonmetric Multidimensional Scaling (NMS) to explore patterns for classification. The NMS analysis indicated a 2-dimensional solution with axis 1 explaining 43% of the variability and axis 2 explaining 26% of the variability (69% cumulative). Examination of species composition showed a strong moisture gradient on the first axis, while management type and time of sampling also influenced plot location in ordination space along the second axis. These results will lead to a descriptive and quantitative classification of northeastern South Dakota upland tallgrass prairie communities.

MOB GRAZING AS A METHOD OF WEED CONTROL IN SOUTH DAKOTA

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ABSTRACT

Mob grazing is a grazing system that uses very high stocking densities of 100,000 pounds per acre or more for short durations of a few hours to one day in small paddocks. Mob grazing has been suggested to increase vegetation usage and minimize selective grazing behavior compared with lower stocking densities in rotational systems. Decreased selectivity increases grazing pressure on plants, such as spiny thistles and tough, woody brush, typically avoided by herbivores. The objective of this project was to determine effects of mob grazing cattle on the selected invasive weeds: musk thistle (*Carduus nutans*), absinth wormwood (*Artemisia absinthium*), and buckbrush (*Symphoricarpos occidentalis*). Cooperating producers in Hayti, Selby, and Chamberlain, South Dakota named their most problematic pasture weeds, listed above. The Hayti site consisted of mob grazed, rotationally grazed, and spray/rotationally grazed treatments; Selby of rotationally grazed and mob grazed treatments; and Chamberlain of ungrazed and mob grazed treatments. The specific problematic weed at each site was permanently tagged along transects. Measurements of surrounding vegetation and weeds were taken before and after grazing. Initial results quantified a decrease in weed volume and height in mob grazed systems; a decrease in surrounding vegetation height, but not weed volume in rotational pastures; and an increase in vegetation height and weed volume for the ungrazed pasture. These data indicate that mob grazing may be a viable method of weed control in South Dakota grazinglands.

IMPACT OF BIOCHAR APPLICATION ON SOIL PROPERTIES AND HERBICIDE SORPTION

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ABSTRACT

Biochars are the byproduct of anaerobic combustion (pyrolysis) of organic materials. Three biochars (switchgrass, cornstover, and Ponderosa pine woodchip) were created by burning the materials under anaerobic conditions for four hours at maximum temperatures of 850 °C (fast pyrolysis). Biochar samples were sorted by size (< 2 mm, 2-4 mm, and > 4 mm) and electrical conductivity (EC) and pH characteristics were determined in 1:5 (w/v) water and 0.01M $CaCl^2$. Each biochar type and size was added at 1 and 10% (w/w), to two South Dakota soils, Barnes (loamy) or Maddock (loamy fine sand). Atrazine sorption and changes in soil pH and EC were measured in slurry experiments (1:2 w/v). Biochar pH values were higher than soil pH values; however, the addition of biochar had minimal influence on soil pH. Biochar size affected soil EC values; the smallest sized chars at the 10% addition increased the soil EC. Atrazine sorption from solution increased from about 35% in soil only to almost 99% with each biochar treatment. Targeted biochar addition to soil may be warranted. If atrazine carryover is suspected, addition of biochar may reduce unwanted affects; however, higher sorption may require higher application rates to provide weed control similar to that of nonamended soil.

IMPROVEMENT OF SOIL STRENGTH USING AN AGRICULTURAL BY-PRODUCT– PRELIMINARY RESULTS

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ABSTRACT

Acquiring good quality soil is one of the major factors contributing to higher construction costs. Improving substandard soils with stabilizing agents has proven to be more economical and environmentally friendly for many types of earth works. Industry presents a multitude of options for chemical stabilization of soils and some of them have shown successful applications in engineering projects. However, due to the fact that chemicals are used, it may not be environmentally friendly and may cause secondary issues such as corrosion of contacting materials and leaching of chemicals into soil and ground water. Thus, developing new stabilizing agents that increase mechanical behavior of problematic soils but have minimal environmental impact has been a goal for many researchers. The primary goal of this study is to identify the stabilizing effects of the agricultural by-product, pea hulls, on coarse and local fine-grained soils. This study shows preliminary results on the improved shear strength of the sand and local soils with different by-product mixture ratios and curing periods, and presents the potentials of further research on the applications of agricultural by-products in construction applications.

EXAMINATION OF THE EFFICACY OF SELECTED CLEANING PRODUCTS USED IN A LONG TERM CARE FACILITY AGAINST TWO BACTERIAL ISOLATES AND *E. COLI* W3104

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ABSTRACT

In long-term care facilities, the potential for microorganisms to develop resistance to cleaning agents, or to colonize after the cleaning agents have become ineffective, is of concern. Six different cleaning products are used daily at approximately 9:00-10:00 am in a facility in eastern South Dakota. With permission, we sampled from various surfaces morning, mid-day, or evening. Sterile moistened filter paper was used to swab each surface. The filter paper was then swabbed across nutrient agar and incubated at 25 °C. After two days the plates were observed, photographed, and recorded. Two bacterial colonies that appeared different in color, pink and yellow, were selected and then transferred to nutrient broth and incubated at 25 °C. To examine the efficacy of cleaning products, we applied the products separately to sterile paper discs and then added them either immediately or over a time course to freshly plated broth suspensions, incubated, and observed for a zone of inhibition. Results from sampling surfaces at the facility indicated that there appears to be an increase in numbers of various bacteria and fungi detected over time. A pilot study examined the efficacy of the cleaning products against the bacterial samples and *E. coli* W3104. A time course was set up to investigate the efficacy of three cleaning products against the two bacterial isolates from the facility and *E. coli.* Preliminary results indicate that the efficacy of the three cleaning products was detectable after one hour but not after twenty four hours. Additional investigations are ongoing.

EXAMINATION OF ANTIBIOTIC RESISTANCE IN *E. COLI* FROM REDFIELD SLOUGH LAKE COUNTY, SD AND LAB STRAIN W3140

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ABSTRACT

Antibiotic resistance is a major concern in the medical field. Antibiotic resistance occurs with incorrect or over use of antibiotics, and when strains of bacteria become unaffected by the antibiotics used originally against them. This study examined *E. coli* collected from six sites in Lake County, SD, and lab strain W3140 against a series of eleven antibiotics that work in different ways to kill bacteria. *E. coli* was isolated from water samples utilizing Coliscan Easy Gel®. The Kirby-Bauer method was used to determine initial susceptibility. Selected isolates from Redfield Slough were further examined for an increased potential for resistance to specific antibiotics. Under our treatment conditions, we observed that 1) the six isolates of *E. coli* exhibited variable resistance; 2) for specific *E. coli*/antibiotic combinations, some *E. coli,* after challenge, became more resistant, however for other combinations no detectable change was observed and, 3) additional challenge by four specific antibiotics against *E. coli* resulted in increased resistance. Additional investigations are ongoing.

IN VIVO AND *IN VITRO* ANTHELMINTIC ACTIVITY OF *MELILOTUS ALBA* (WHITE SWEET CLOVER) FROM THE UNITED STATES NORTHERN GREAT PLAINS ON *HAEMONCHUS CONTORTUS* NEMATODES

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ABSTRACT

Anthelmintic resistance in the gastrointestinal nematode, *Haemonchous contortus*, is a growing health problem for commercially-raised sheep and goats, and so alternatives to the commonly-used anthelmintics are needed. A previous study showed that methanol extracts from *Melilotus alba* sweet clover possessed anthelmintic activity to *H. contortus* infective juveniles under *in vitro* conditions. For this reason, the anthelmintic activity of *M. alba* was evaluated under *in vivo* conditions in 18 yearling ewes naturally infected with *H. contortus*. Heavily-infected ewes were selected and divided into 2 experimental groups and 1 control group. One of the experimental groups was fed 0.68 kg of fresh-frozen *M. alba* sweet clover (harvested from local plants in late fall) per day for 2 weeks. This group was also given a mixed Garrison/sweet clover hay *ad lib*. The second group received only Garrison/sweet clover hay, while the control group received only Garrison hay. *Haemonchus* fecal-egg- counts were determined daily after the 2 weeks to estimate the post-treatment worm loads. Under these conditions, the results did not show any decrease in egg shedding within either experimental groups compared to the controls. To evaluate the anthelmintic activity of each grazed component of the sweet clover plant (i.e. leaves, stems, pods), we extracted the components in 100% methanol and measured their activity using a larval migration assay involving excysted third-stage *H. contortus* juveniles. Stems and pods showed no anthelmintic activity, while the leaf extract possessed 97.3% migration inhibition at 30 mg/ml. Similar inhibition activity was also measured when leaves were extracted in water.

NITROGEN FIXING ENDOPHYTES WITHIN *PEDIOMELUM ESCULENTUM*

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ABSTRACT

Prairie turnip (*Pediomelum esculentum* (Pursh) Rydb. [Fabaceae]), an indigenous legume, is a traditional food for Native Americans of the Great Plains, and an excellent source of protein due to its high nitrogen content. We investigated endophytic diazotrophs as a potential source of nitrogen for the large protein stores contained within the edible tuber. Genomic bacterial DNA was isolated from lower root, bark, tuber, stem, and leaf tissue of a surface sterilized plant. PCR amplification of *nifH*, a gene that codes for a nitrogenase subunit, was performed to detect nitrogen fixers. Denaturing gradient gel electrophoresis of the *nifH* PCR product revealed the composition and diversity of these endophytes. The presence of the *nifH* gene from multiple bacterial strains was confirmed within all examined tissues. The identification of symbiotic nitrogen fixers inside prairie turnip increases the potential of this unique plant to be utilized for novel crop endeavors.

METAGENOMIC ANALYSIS OF MICROBIAL COMMUNITIES IN THE SANFORD UNDERGROUND RESEARCH FACILITY (SURF) IN THE FORMER HOMESTAKE MINE, LEAD, SD

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ABSTRACT

Metagenome data were obtained by 454 pyrosequencing of genomic DNA from three microbial communities at a depth of 1478 m in the former Homestake mine: pelagic cells in a pool of fracture water, a biofilm in the same pool, and pelagic cells in a drainage ditch about 100 m from the pool. In addition, a detailed phylogenetic analysis of the rock pool biofilm was compiled from a plasmid library of 16S rDNA amplicons. All three communities are composed almost entirely of Bacteria; few sequences from Archaea were found. The rock pool biofilm was a diverse community with 105 probable species noted from 197 16S rDNA sequences, with the genera *Thiobacillus, Thiothrix*, and *Nitrospira* being most abundant. Pelagic cells in the rock pool water were primarily *Thiothrix*, while pelagic cells in the drainage ditch were dominated by several taxa of beta-Proteobacteria, especially *Methylotenera*. Metagenome data suggest the importance of chemoautotrophy based on sulfide and nitrite oxidation in the rock pool community and the utilization of methanol as a substrate for growth in the drainage ditch community.

REU SITE: BRINGING US TOGETHER, IMPROVING COMMUNICATIONS AND LIVES

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ABSTRACT

This recently awarded REU Site is a summer program with the primary theme, *Bringing Us Together, Improving Communications and Lives.* This title is meant to convey the role that electrical and computer engineering (ECE) have played in developing technologies that improve how people communicate and live. The focus of the research projects will reflect the strengths of the ECE department at the South Dakota School of Mines & Technology (SDSM&T). The REU Site is meant to spur research interest in undergraduate students and equip them to pursue careers in engineering or science, and encourage the pursuit of graduate degrees. Two key student populations will be targeted for recruitment- women and veterans. However, members of all underrepresented groups will be sought out. In addition to hands-on research, the REU Site will feature weekly professional development, research, and historical development seminars. The purpose of the seminars is to develop collegiality and the skills needed to pursue higher level work, as well as to foster an appreciation for the historical development and current applications of ECE and the role they play in improving communications and lives in our global society. Quantitative assessments will be made of REU Site activities and students including post-REU tracking, assessment of communication skills and student motivation, etc.

SCIENCE, HUMANITIES, AND THE CHALLENGES OF FRESHMAN MULTIDISCIPLINARY STUDIES

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ABSTRACT

From 2007 through 2013, the South Dakota School of Mines and Technology (SDSMT) offered a required two-credit multidisciplinary course (IS 110) for all first-time freshman science majors. Entitled *Explorations*, the course was taught jointly by Atmospheric Sciences, Chemistry, Geology, Math, and Humanities faculty. Designed to provide a theme-based approach for studying the relationships between science and society, the course consisted of lectures, breakout sessions, field work, organized trips, and mentoring activities to help students fulfill seven course goals and objectives. During the first two years, faculty also led summer field trips to national parks, where interested students participated in workshops offered by the Glacier and Yellowstone Institutes, and learned about a variety of scientific topics, including the effects of climate change on mountain ecosystems. During its seven-year run, IS 110 largely met the expectations of the faculty; unfortunately, most of the students did not share the same view and responded poorly to the pedagogical aims of the course. Although the goals and objectives of the course were defined clearly, the blending of mentoring activities and scientific content caused conceptual confusion for many students. In addition, most saw the course as an obstacle rather than a conduit to their future development. This presentation outlines some of the lessons learned from this experience.

UNDERGRADUATE RESEARCH-BASED NATURAL SCIENCE PROGRAM AT OGLALA LAKOTA COLLEGE, PINE RIDGE RESERVATION, SOUTH DAKOTA

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ABSTRACT

In 2008 Oglala Lakota College's (OLC's) Department of Math, Science, & Technology (MST) realigned its BS in Natural Science to emphasize undergraduate research and a constructivist andragogy. OLC's 1800 students are 90% Native, 60% female, and 50% non-traditional. MST has 90 declared majors including 20 paid interns. MST's approach is a significant departure from other programs at OLC, and is partly based on a progressive increase in research emphasis in courses: 1) 100-level classes emphasize basic content knowledge and a survey of our existing research programs; 2) 200-level classes emphasize the scientific method, technical writing, and guided research experiences; 3) 300-level classes emphasize mentor-selected research projects and in-house dissemination; and 4) 400-level classes emphasize self-selected research projects and professional dissemination. Also, our students are encouraged and supported in the pursuit of both individual and team-oriented research projects. We currently support undergraduate research in landscape analysis, geologic mapping, freshwater invertebrate, box turtle, and Bison ecology, radionuclide contamination pathways, volcaniclastic mineralogy and geochemistry, Paleogene stratigraphy, and the pharmacology of native plants. Following the realignment, OLC raised its retention rate from 20% to 60%, quadrupled its number of annual graduates (2 to 8), and overall has placed 96% of its students in jobs on the reservation or in graduate school. OLC MST's STEM curriculum and undergraduate research is supported by funding from the NSF/TCUP Phase III (Tinant & LaGarry), NSF/PEEC (Tinant & LaGarry), NSF/EPSCoR RII T1 (LaGarry), USDA/NIFA Tribal Research Program (Higa), NSF/TUCP RIA (Higa), NIH/BRIN (Sandoval), and NSF/EPSCoR Biofuels (Sandoval).

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Green, J.C., and R.B. Janssen. 1975. Minnesota birds: where, when, and how many. University of Minnesota Press, Minneapolis, MN.
Stewart, A.V., A. Joachimiak and N. Ellison. 2009. Genomic and geographic origins of timothy (Phleum sp.) based upon ITS and chloroplast sequences. Pages71-81in T. Yamada and G. Spangenberg, editors. Molecular breeding of forage and turf. Springer, New York, NY.
Svihovec, L.K. 1967. A comparison study of the ecological distribution of small mammals in southwestern North Dakota. Thesis. University of North Dakota, Grand Forks, ND.

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